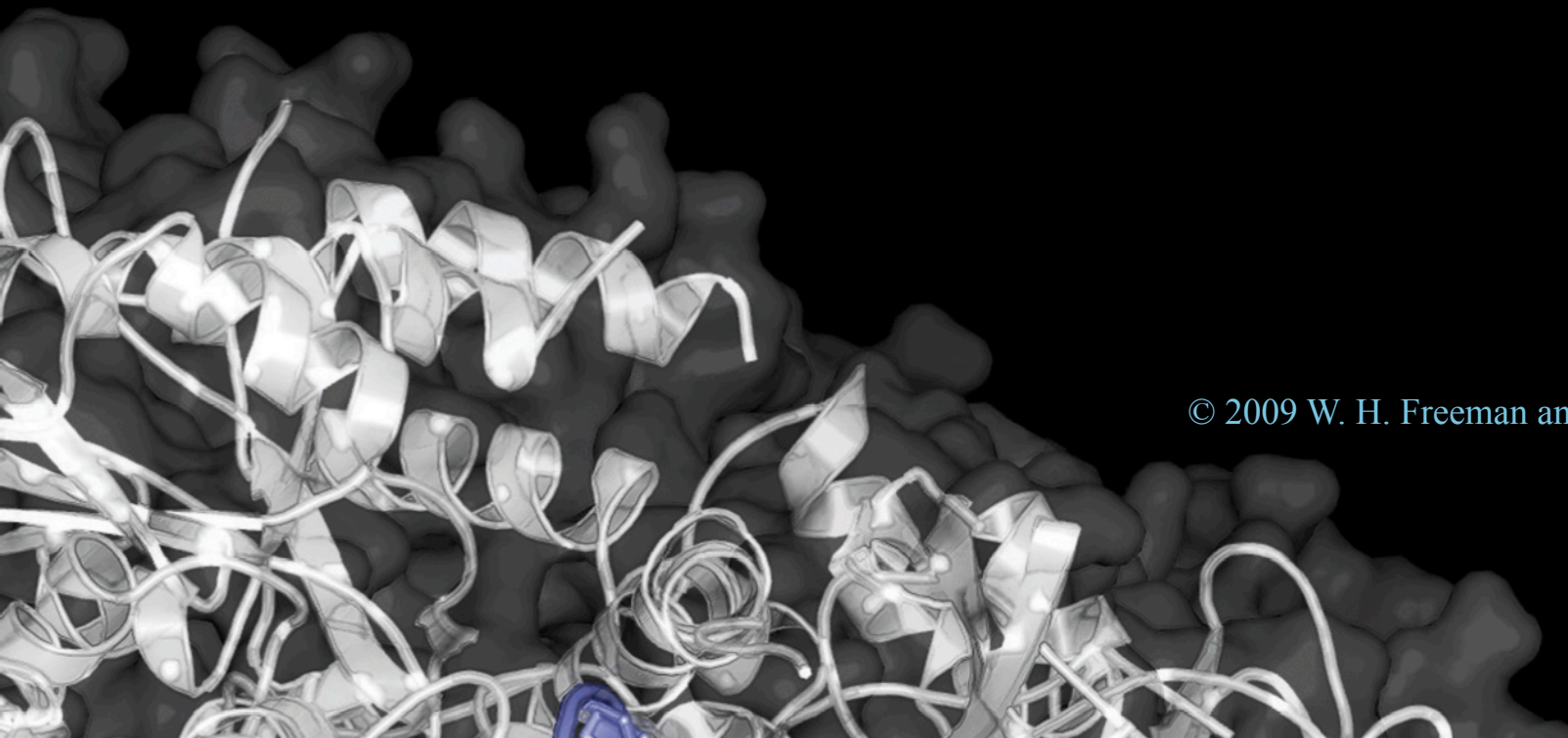
LEHNINGER
PRINCIPLES OF BIOCHEMISTRY

David L. Nelson
Michael M. Cox

FIFTH EDITION

Lecture Connections

14+16+19 | Introduction to Metabolism



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CHAPTER 14, 16 & 19
Introduction to Metabolism

Key topics:

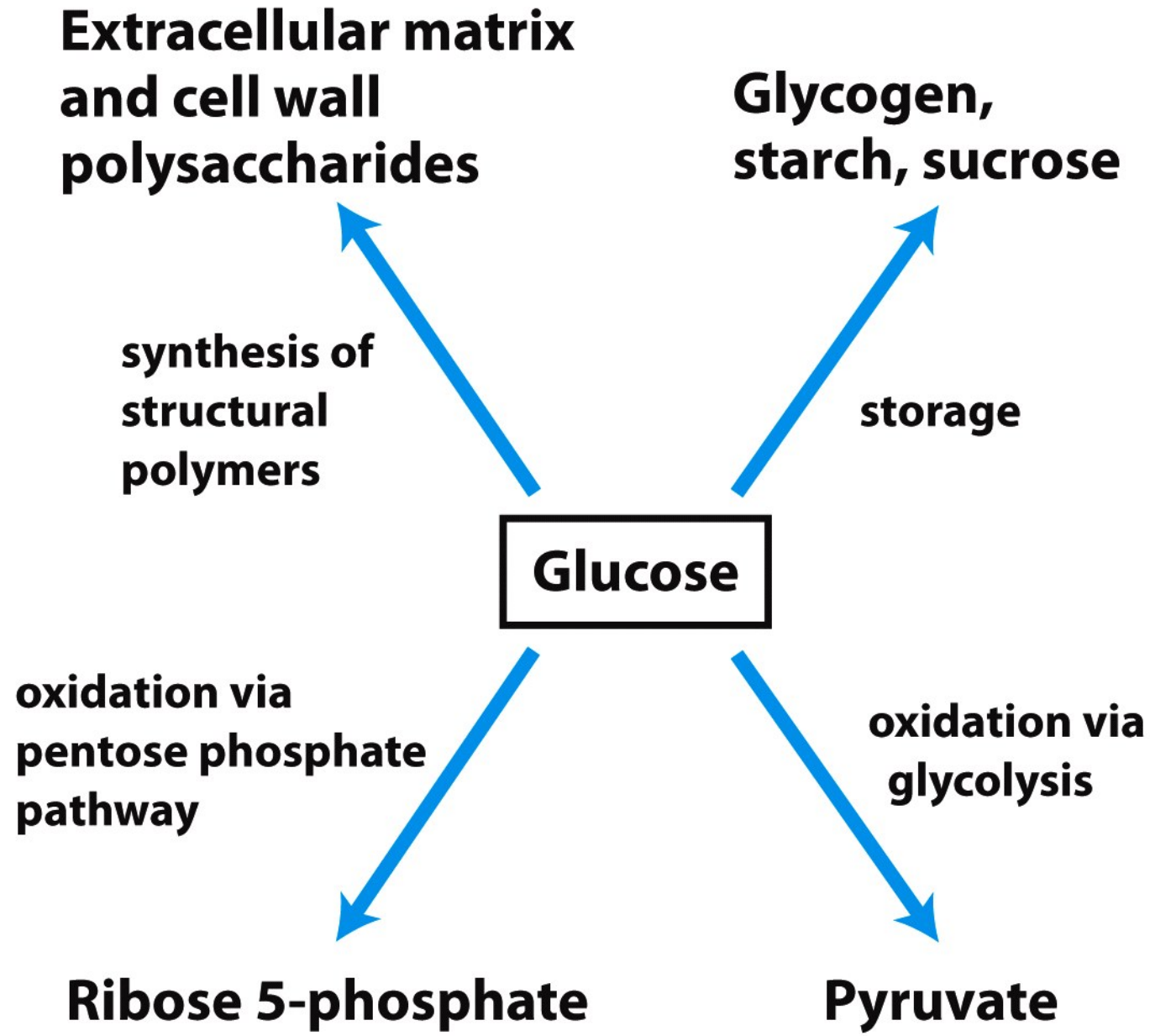
- Harnessing energy from glucose via glycolysis
- Fermentation under anaerobic conditions
- Gluconeogenesis
- Cellular respiration
- Conversion of pyruvate to activated acetate
- Reactions of the citric acid cycle
- Electron transport chain in mitochondria
- Building up the proton-motive force
- Synthesis of ATP in mitochondria

Central Importance of Glucose

- Glucose is an excellent fuel
  - Yields good amount of energy upon oxidation
  - Can be efficiently stored in the polymeric form
  - Many organisms and tissues can meet their energy needs on glucose only
- Glucose is a versatile biochemical precursor
  - Bacteria can use glucose to build the carbon skeletons of:
    - All the amino acids
    - Membrane lipids
    - Nucleotides in DNA and RNA
    - Cofactors needed for the metabolism

Four Major Pathways of Glucose Utilization

- When there's plenty of excess energy, glucose can be stored in the polymeric form (starch, glycogen)
- Short-term energy needs are met by oxidation of glucose via glycolysis
- Pentose phosphate pathway generates NADPH that is used for detoxification, and for the biosynthesis of lipids and nucleotides
- Structural polysaccharides (e.g. in cell walls of bacteria, fungi, and plants) are derived from glucose



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Glycolysis: Importance

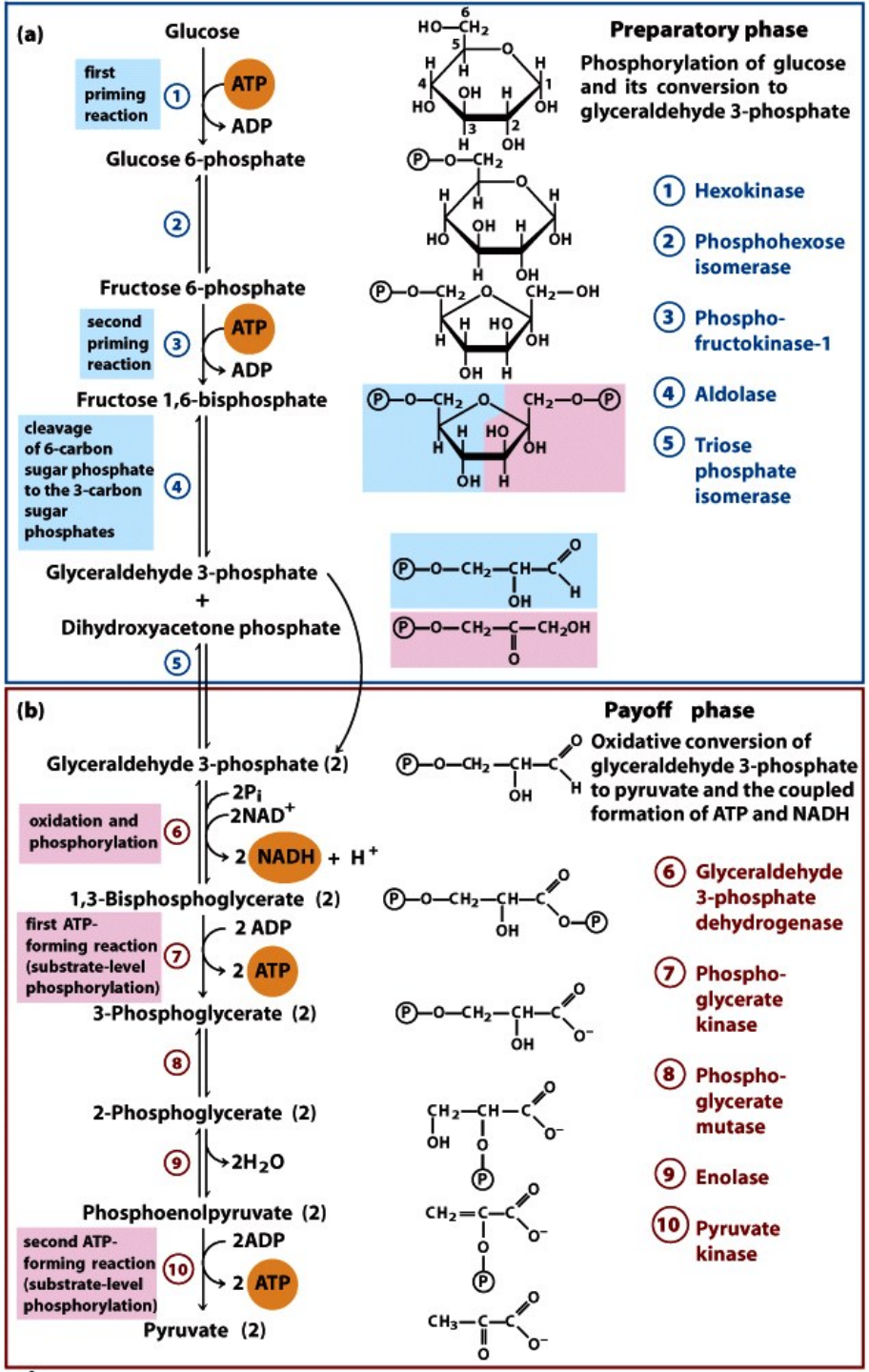
- Glycolysis is a sequence of enzyme-catalyzed reaction by which glucose is converted into pyruvate
  - Pyruvate can be further aerobically or anaerobically oxidized
  - Pyruvate can be used as a precursor in biosynthesis

- In the process, some of the oxidation free energy in captured by the synthesis of ATP and NADH

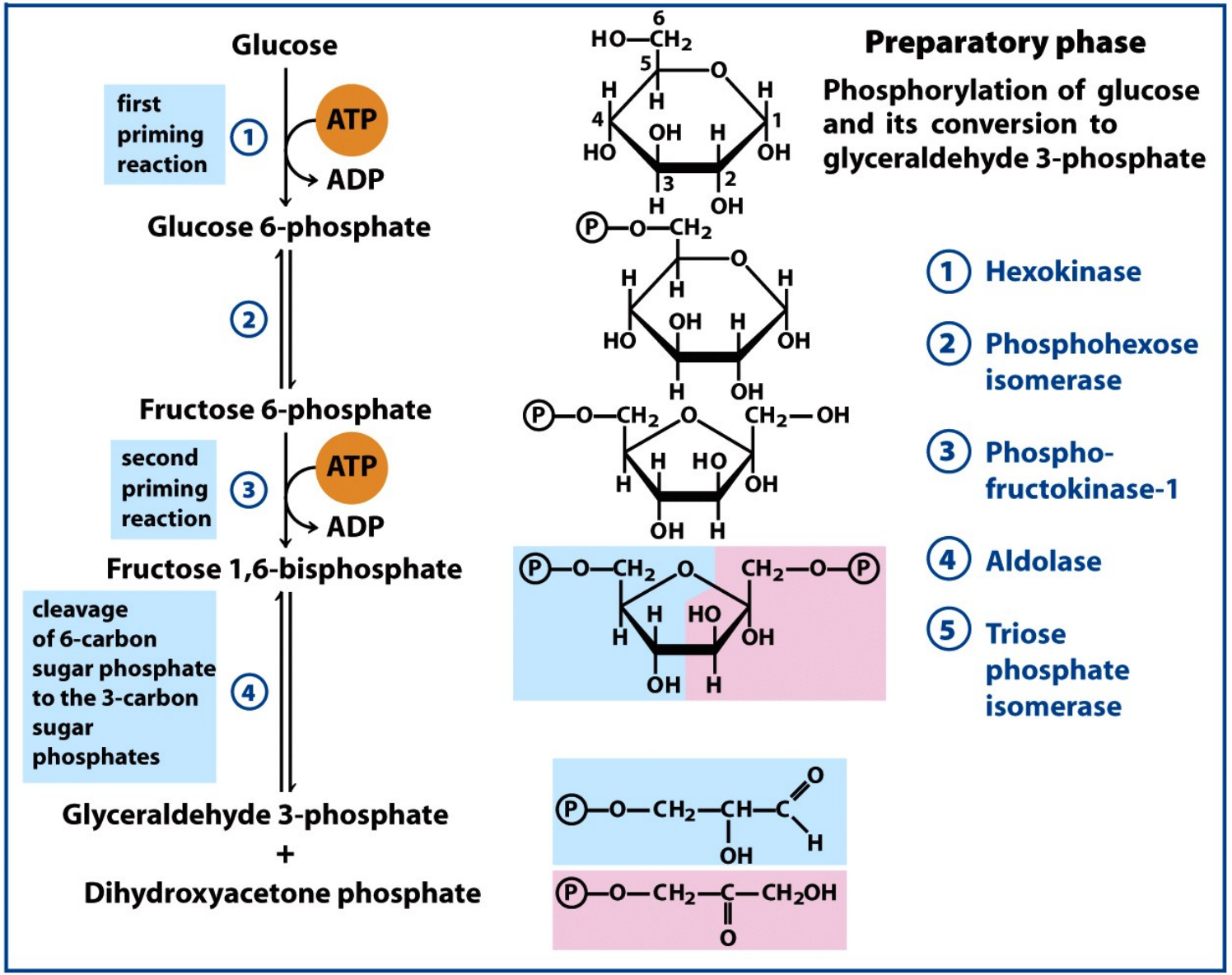
The two phases of glycolysis.

For each molecule of glucose that passes through the **preparatory phase (a)**, two molecules of glyceraldehyde 3-phosphate are formed; both pass through the **payoff phase (b)**. Pyruvate is the end product of the second phase of glycolysis.

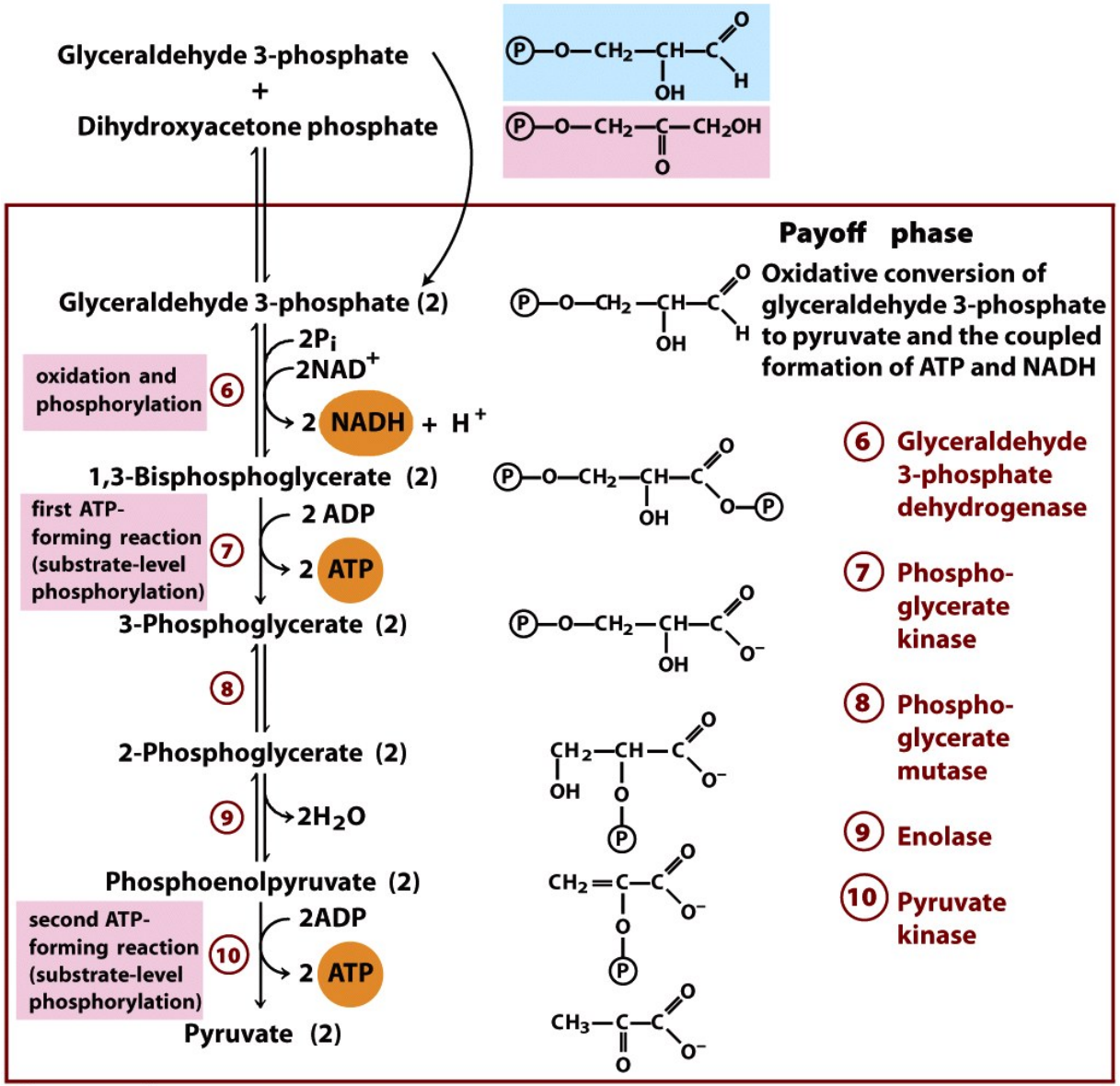
For each glucose molecule, 2 ATP are consumed in the preparatory phase and 4 ATP are produced in the payoff phase.



Glycolysis: The Preparatory Phase

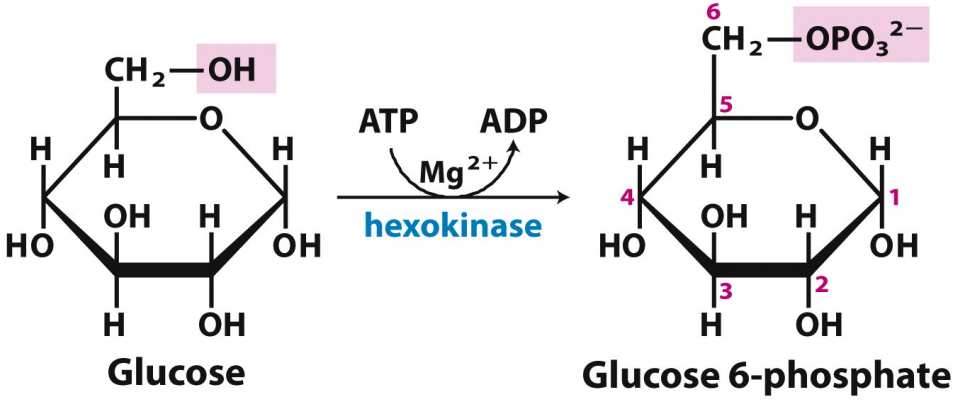


Glycolysis: The Payoff Phase



(1) The First Priming Reaction; The Hexokinase Reaction

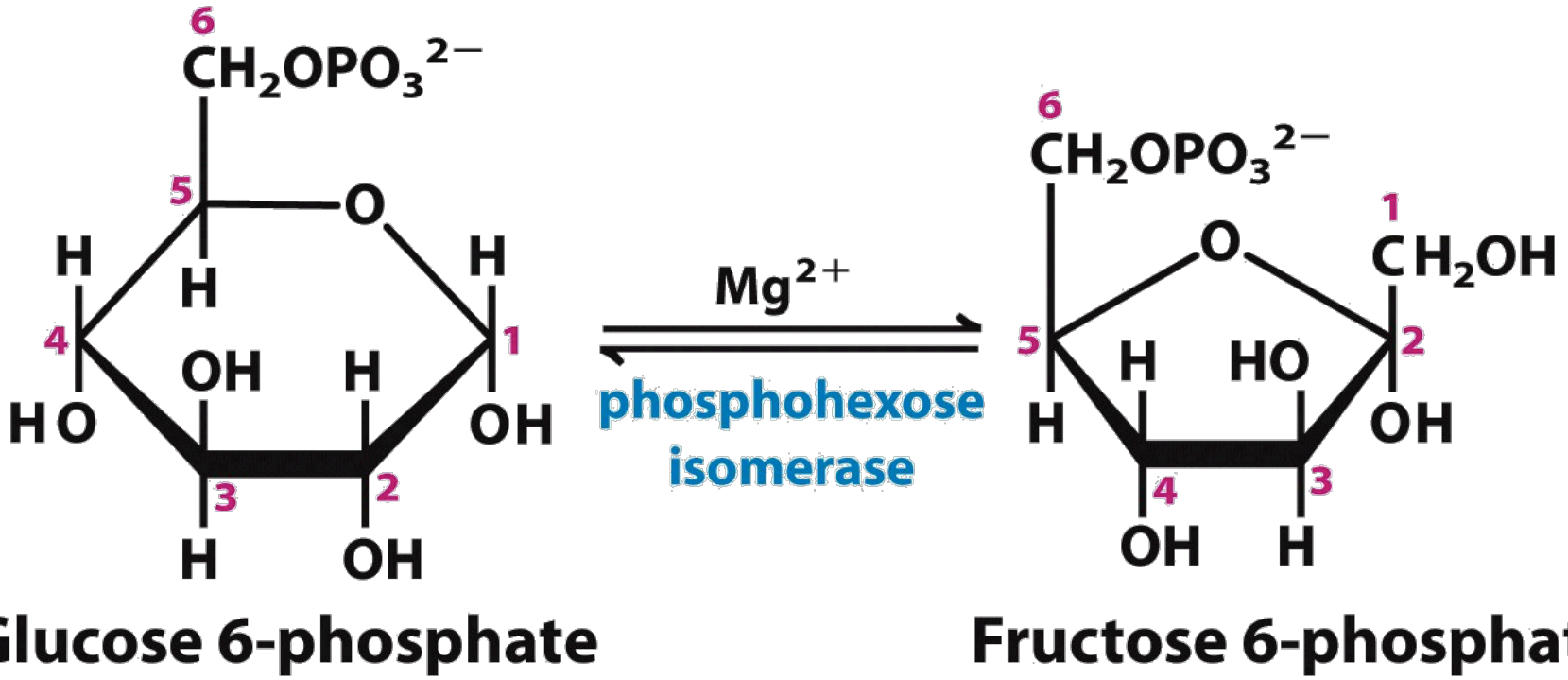
- The first step, phosphorylation of glucose, is catalyzed by hexokinase in eukaryotes, and by glucokinase in prokaryotes
- Nucleophilic oxygen at C6 of glucose attacks the last (γ) phosphorous of ATP
- Bound Mg^{2+} facilitates this process by stabilizing the negative charge in the transition state
- *This process uses the energy of ATP*



$\Delta G'^{\circ} = -16.7$ kJ/mol

(2) Phosphohexose Isomerization

- An aldose can isomerize into a ketose



Glucose 6-phosphate **Fructose 6-phosphate**

$$\Delta G'^{\circ} = 1.7 \text{ kJ/mol}$$

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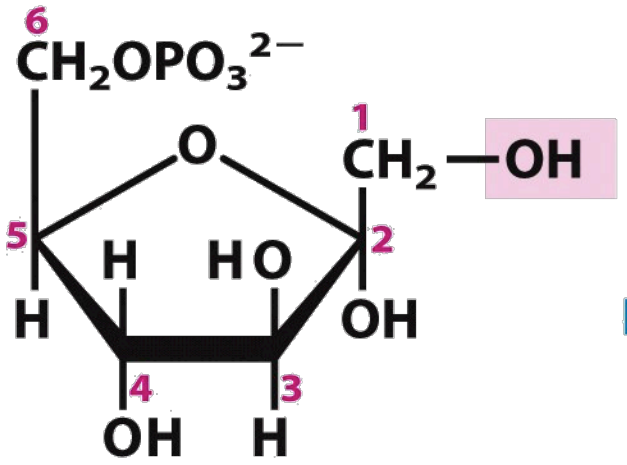
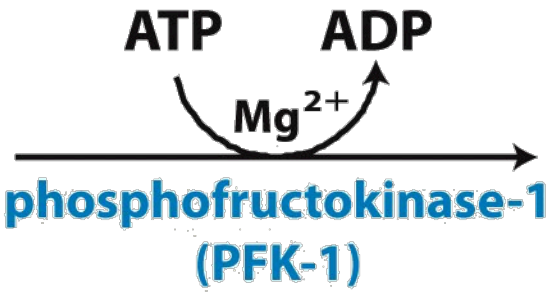
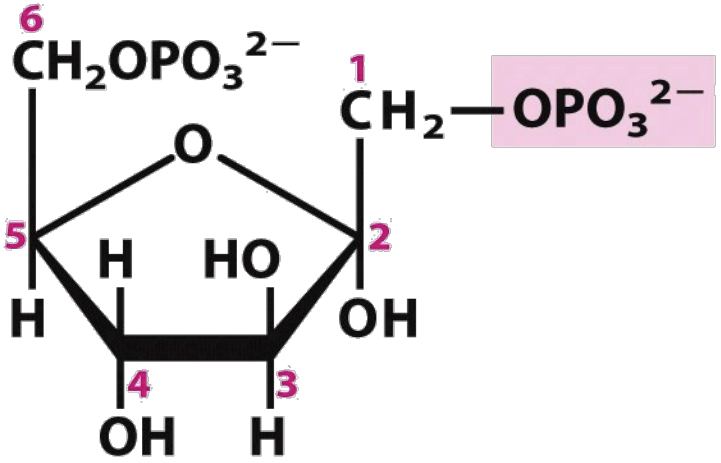
(3) The Second Priming Reaction; The First Commitment

- ATP is the donor of the second phosphate group
- This is an irreversible step
- The product, fructose 1,6-bisphosphate is committed to become pyruvate and yield energy
- Phosphofructokinase-1 is negatively regulated by ATP
  - Do not burn glucose if there is plenty of ATP





Fructose 6-phosphate



Fructose 1,6-bisphosphate

$$\Delta G'^{\circ} = -14.2 \text{ kJ/mol}$$

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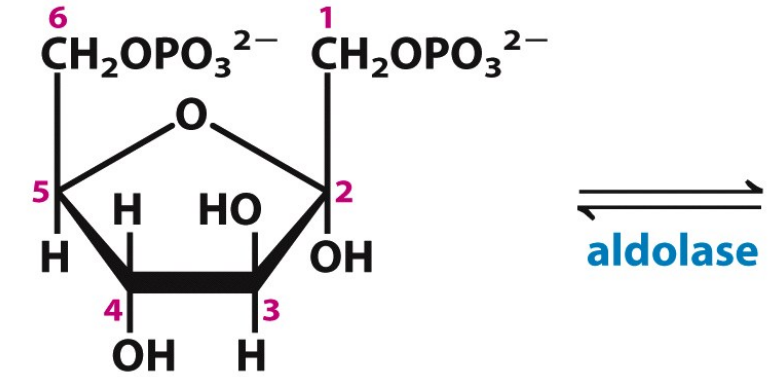
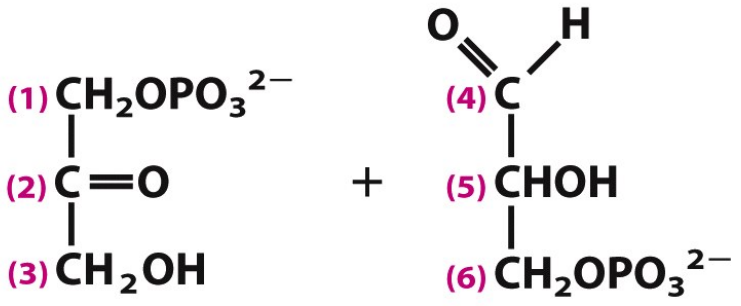
(4) Aldolases Cleave 6-Carbon Sugars

- The reverse process is the familiar aldol condensation



Fructose 1,6-bisphosphate

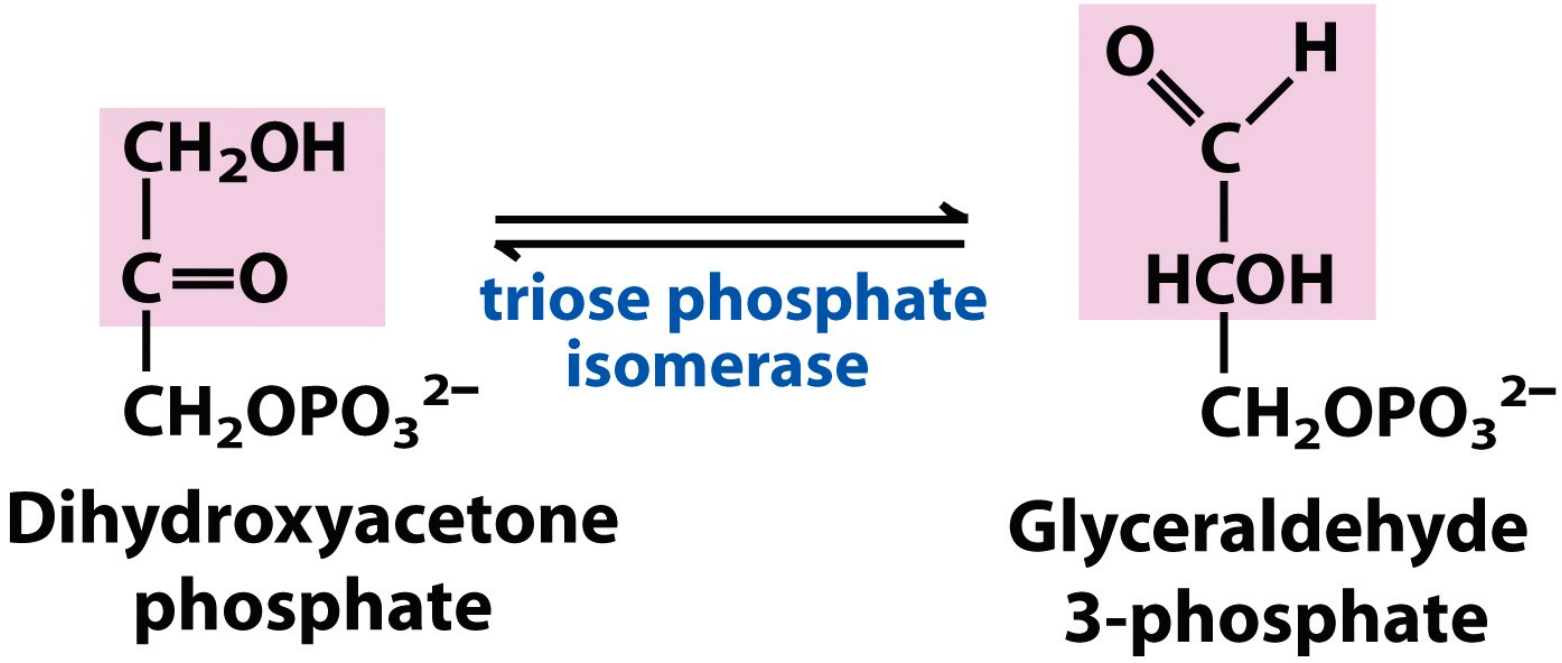


Dihydroxyacetone phosphate + **Glyceraldehyde 3-phosphate**

$\Delta G'^{\circ} = 23.8$ kJ/mol

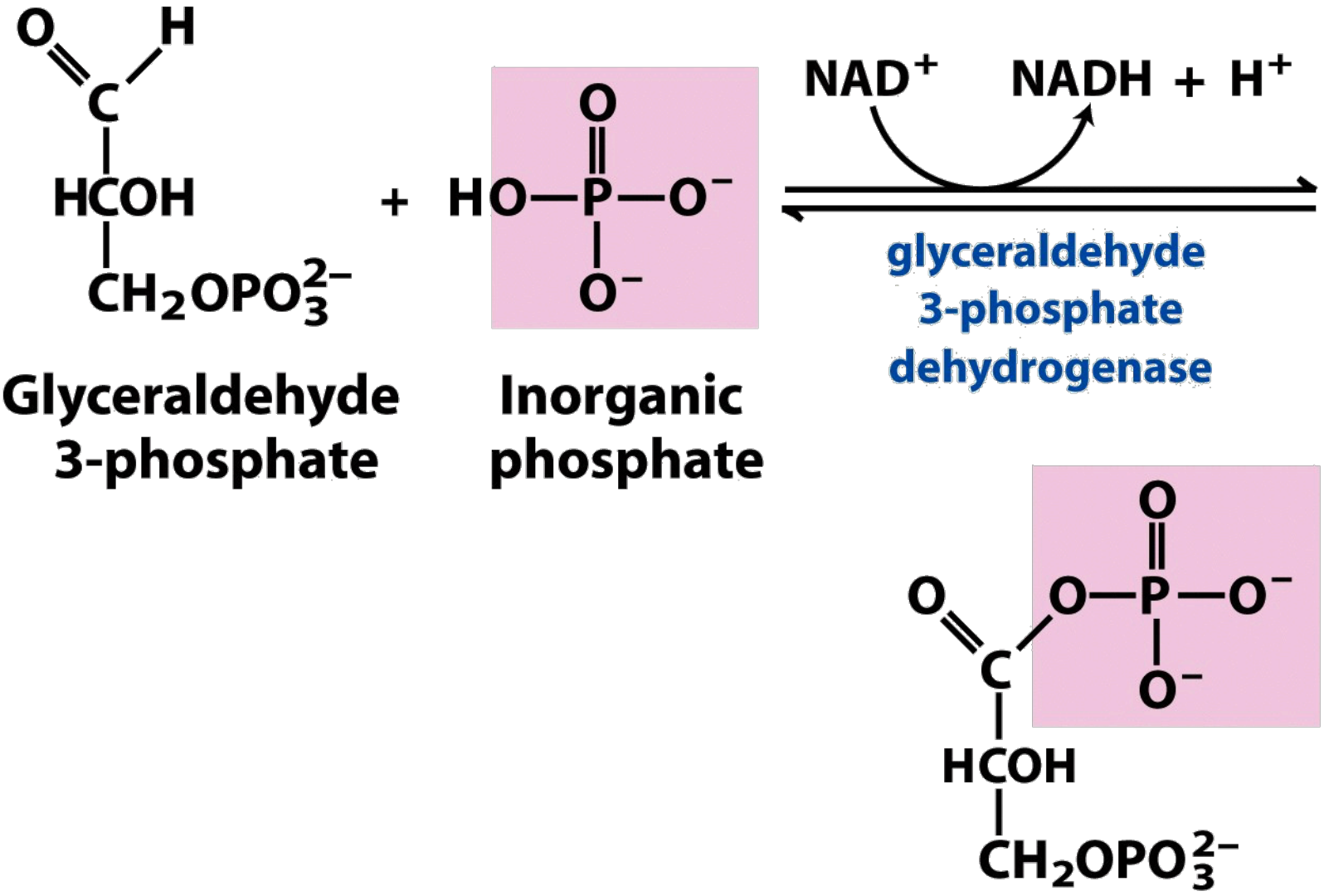
(5) Triose Phosphate Interconversion

- Aldolase creates two triose phosphates: DAP and GAP
- Only GAP is the substrate for the next enzyme
- DAP is converted enzymatically to GAP



(6) Glyceraldehyde 3-Phosphate Dehydrogenase Reaction

- *First energy-yielding step in glycolysis*
- Oxidation of aldehyde with NAD^+ gives NADH
- Phosphorylation yields an high-energy reaction product



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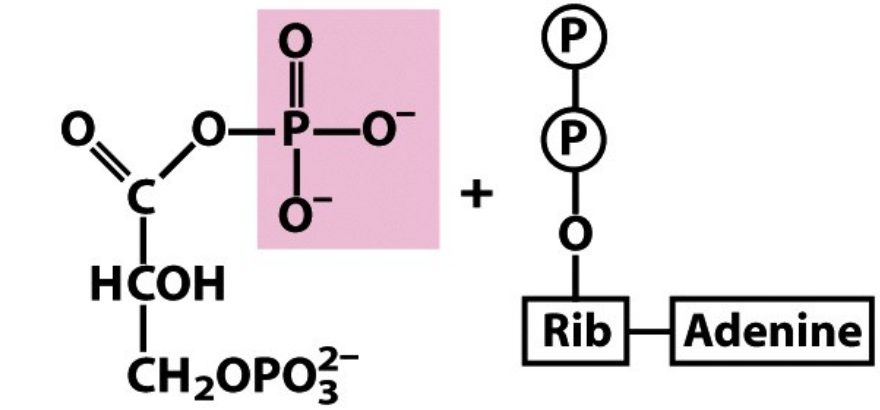
(7) First Substrate-Level Phosphorylation

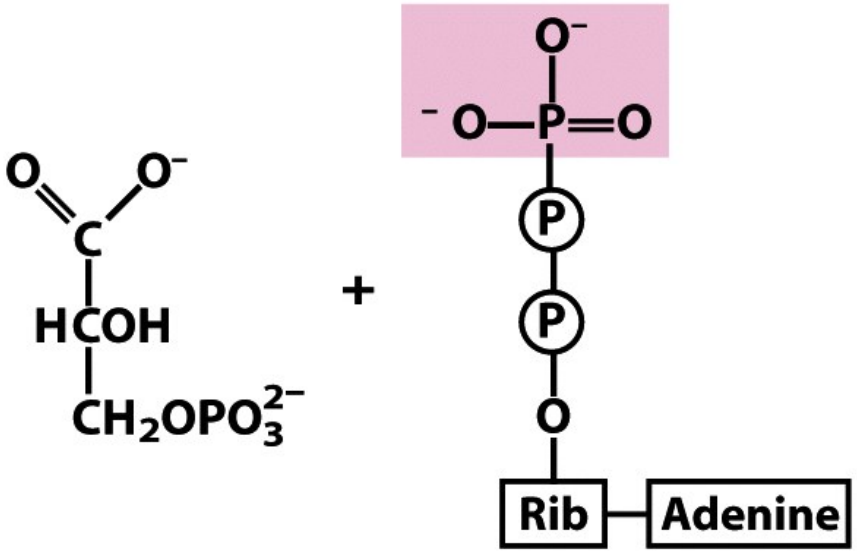
- 1,3-bisphosphoglycerate is a high-energy compound that can donate the phosphate group to ADP to make ATP
- The reaction is reversible, the reverse process transfer of phosphate from ATP to phosphoglycerate
- Kinases are enzymes that transfer phosphate groups from molecules like ATP to various substrates



1,3-Bisphosphoglycerate ADP

Mg^{2+}

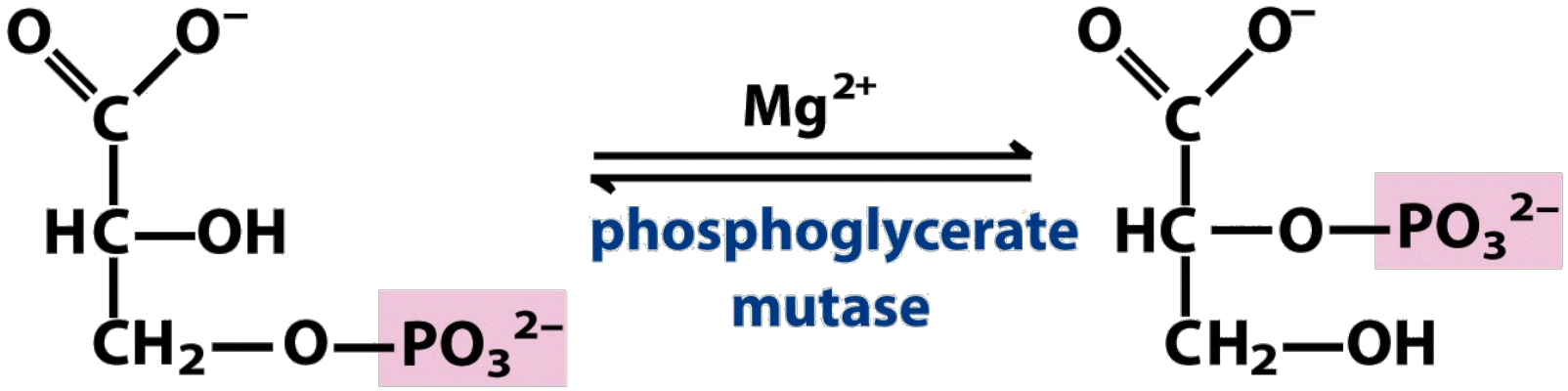
phosphoglycerate kinase



3-Phosphoglycerate ATP $\Delta G'° = -18.5$ kJ/mol

(8) Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

- This is a reversible isomerization reaction
- Enzymes that shift functional groups around are called mutases



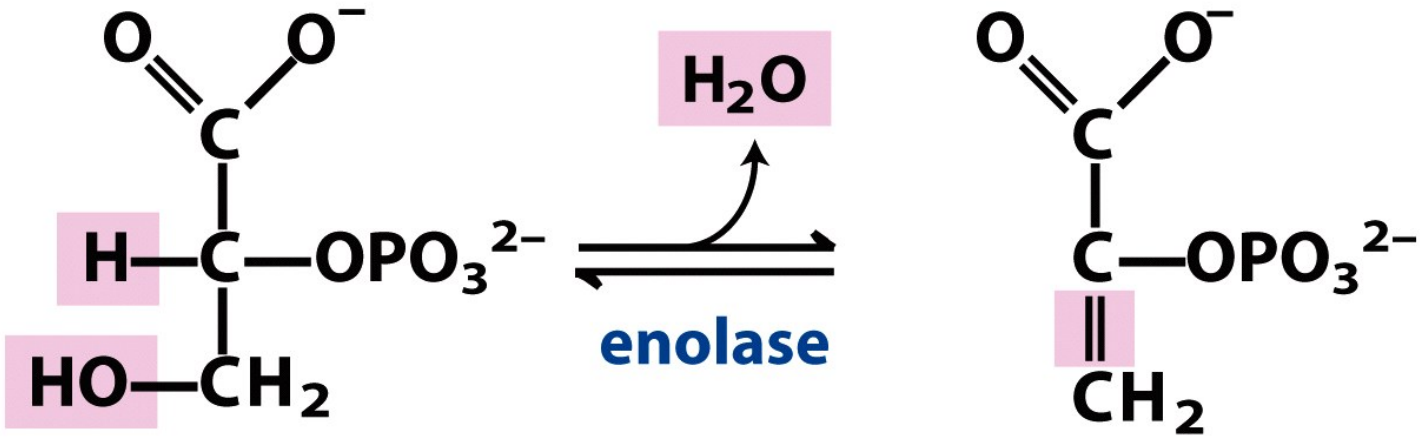
3-Phosphoglycerate

2-Phosphoglycerate

$\Delta G'^{\circ} = 4.4$ kJ/mol

(9) Dehydration of 2-Phosphoglycerate

- The goal here is to create a better phosphoryl donor
- Loss of phosphate from 2-phosphoglycerate would just give a secondary alcohol with no further stabilization …

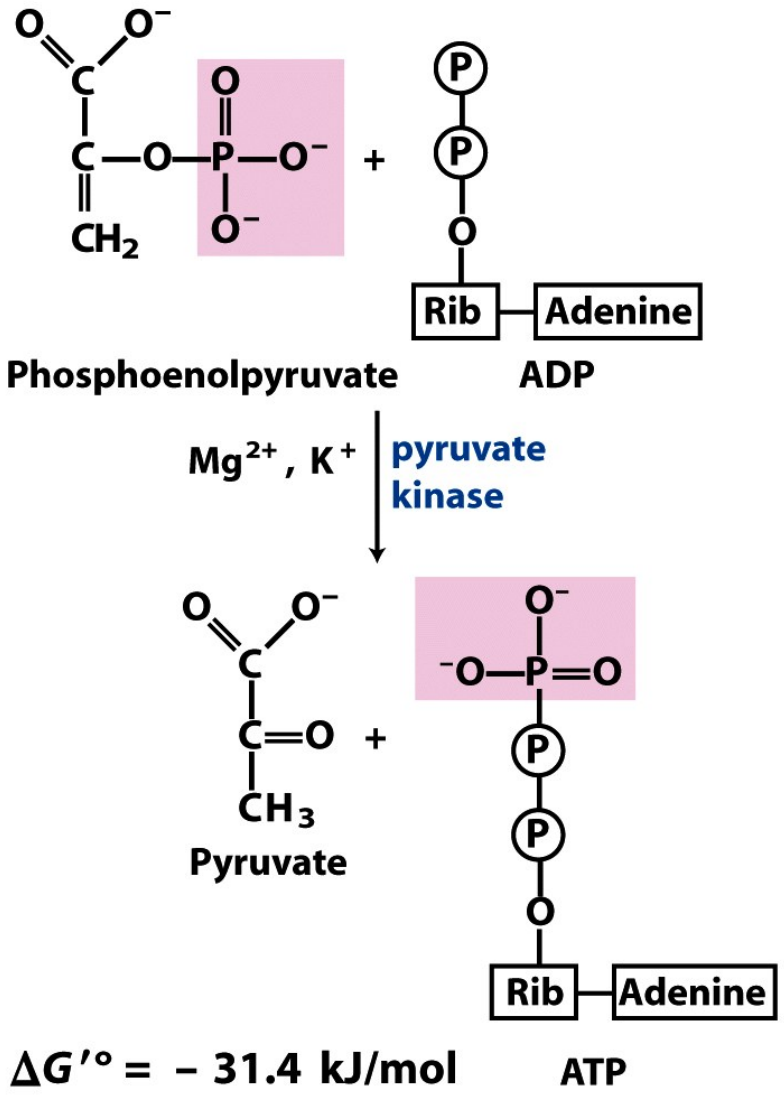


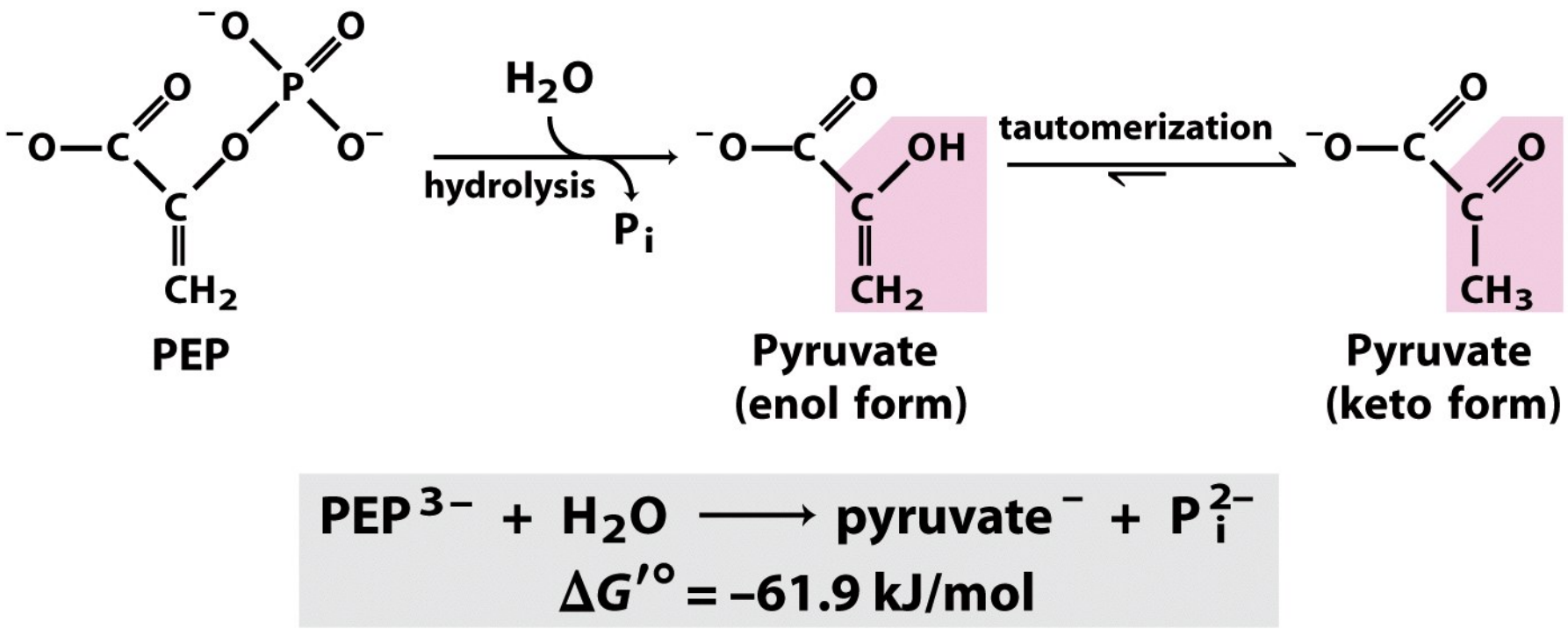
2-Phosphoglycerate **Phosphoenolpyruvate**

$\Delta G'^{\circ} = 7.5$ kJ/mol

(10) Second Substrate-Level Phosphorylation

- … but loss of phosphate from phosphoenolpyruvate yields an enol that tautomerizes into ketone
- The tautomerization effectively lowers the concentration of the reaction product and drives the reaction toward ATP formation





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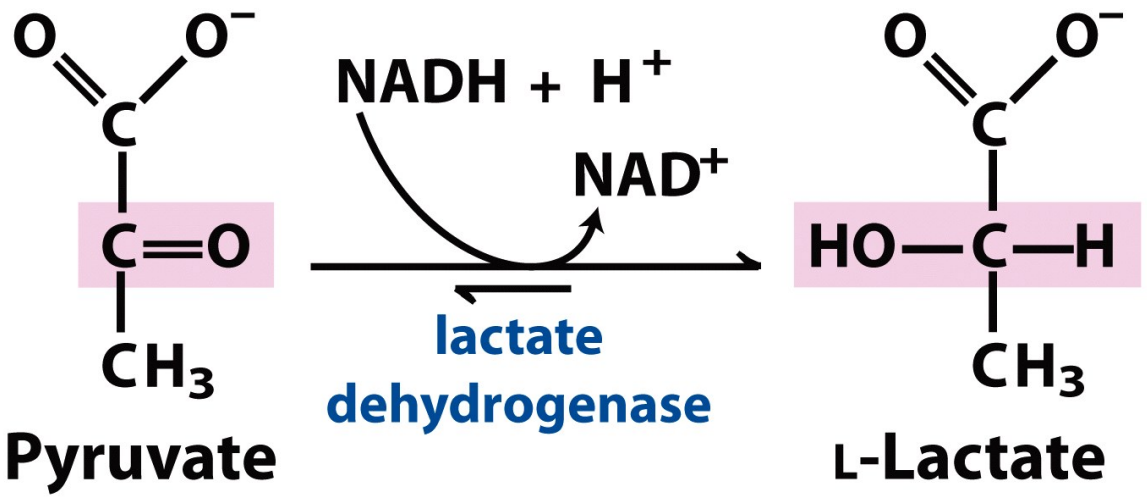
Tautomerization is not possible in PEP, and thus the products of hydrolysis are stabilized relative to the reactants. Resonance stabilization of P_i also occurs.

Pyruvate Kinase is Subject to Regulation

- Pyruvate kinase requires divalent metals (Mg^{2+} or Mn^{2+}) for activity
- Under physiological conditions, the activity of pyruvate kinase is limited by the level of Mg^{2+}
- When there is plenty of ATP, the Mg ions are sequestered by ATP; this slows down pyruvate kinase
- Increased concentration of metabolites in the glycolytic pathway slows down glucose utilization

Under Anaerobic Conditions, Animals Reduce Pyruvate to Lactate

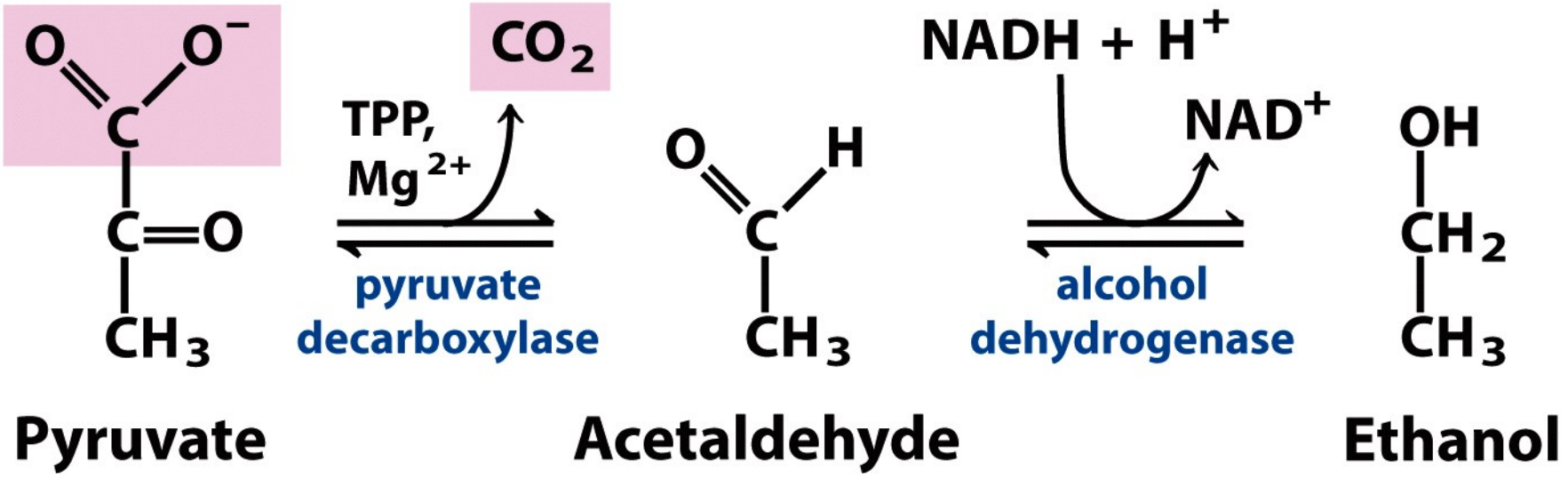
- During strenuous exercise, lactate builds up in the muscle
- The acidification of muscle prevents its continuous strenuous work
- The lactate can be transported to liver and converted to glucose there



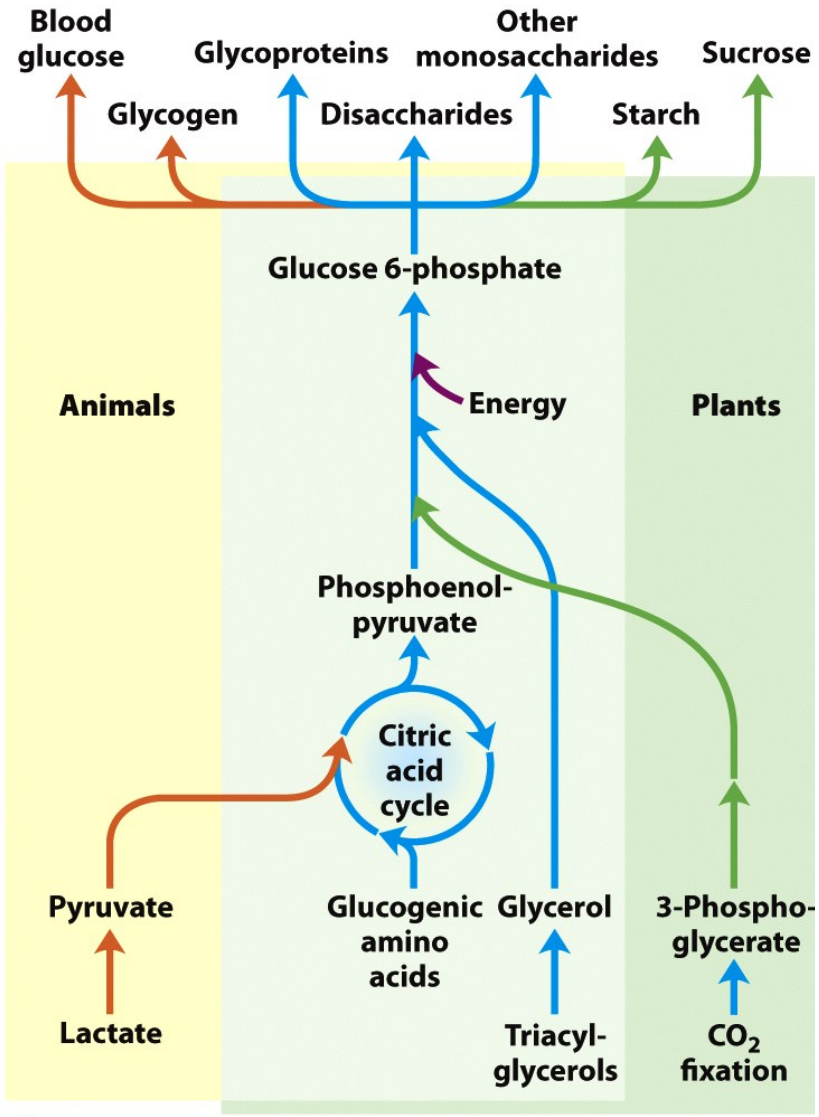
$\Delta G'° = -25.1$ kJ/mol

Under Anaerobic Conditions, Yeast Ferments Glucose to Ethanol

- Both steps require cofactors
  - Mg^{2+} and thiamine pyrophosphate (TPP) in pyruvate decarboxylase
  - Zn^{2+} and NAD^+ in alcohol dehydrogenase



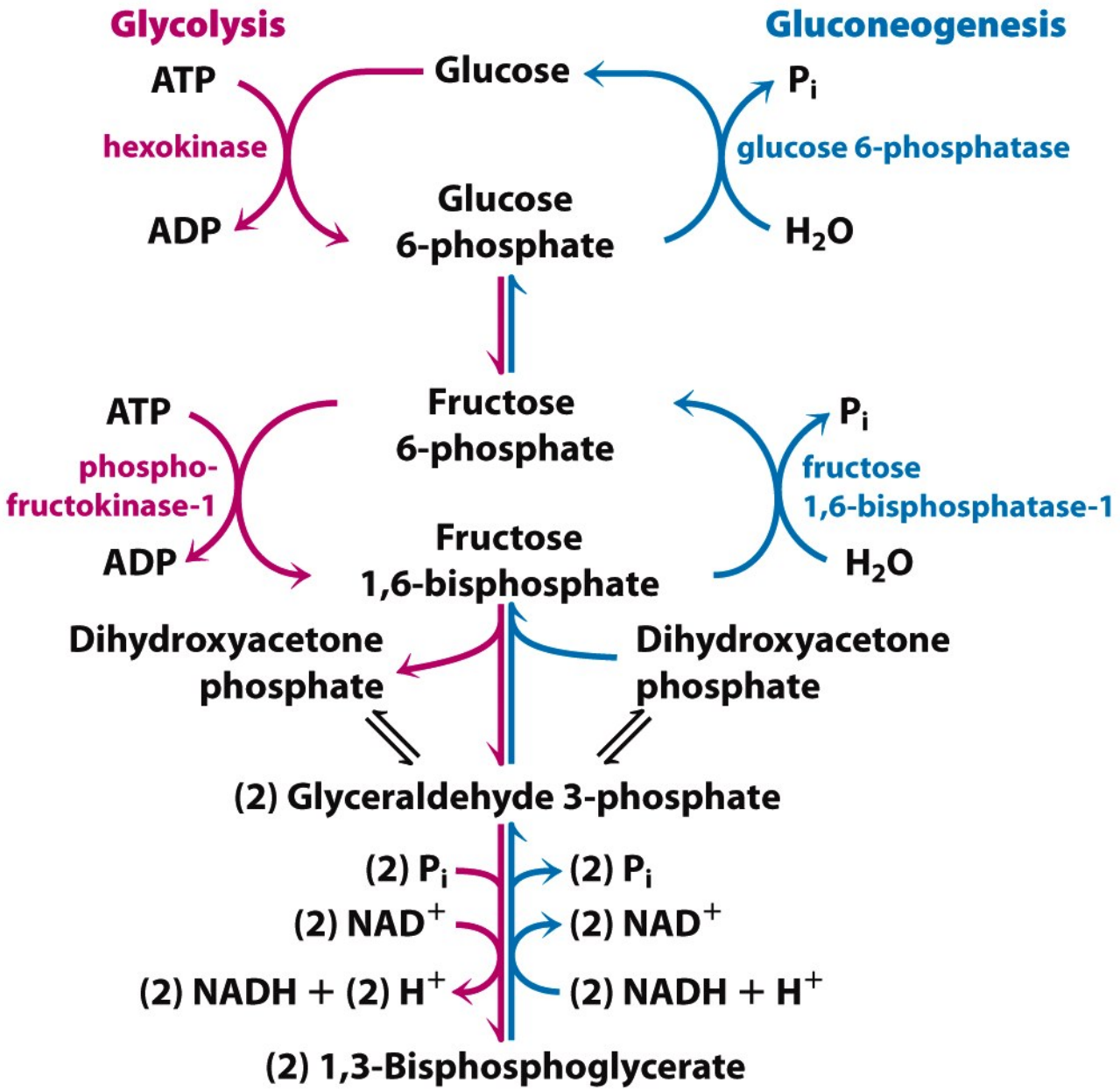
Gluconeogenesis: Precursors for Carbohydrates



- Notice that mammals cannot convert fatty acids to sugars
- The pathway from phosphoenolpyruvate to glucose 6-phosphate is common to the biosynthetic conversion
- The path from pyruvate to phosphoenolpyruvate leads through oxaloacetate
- Any compound that can be converted to either pyruvate or oxaloacetate can serve as starting material for gluconeogenesis

Glycolysis vs. Gluconeogenesis

- Glycolysis occurs mainly in the muscle and brain
- Gluconeogenesis occurs mainly in the liver



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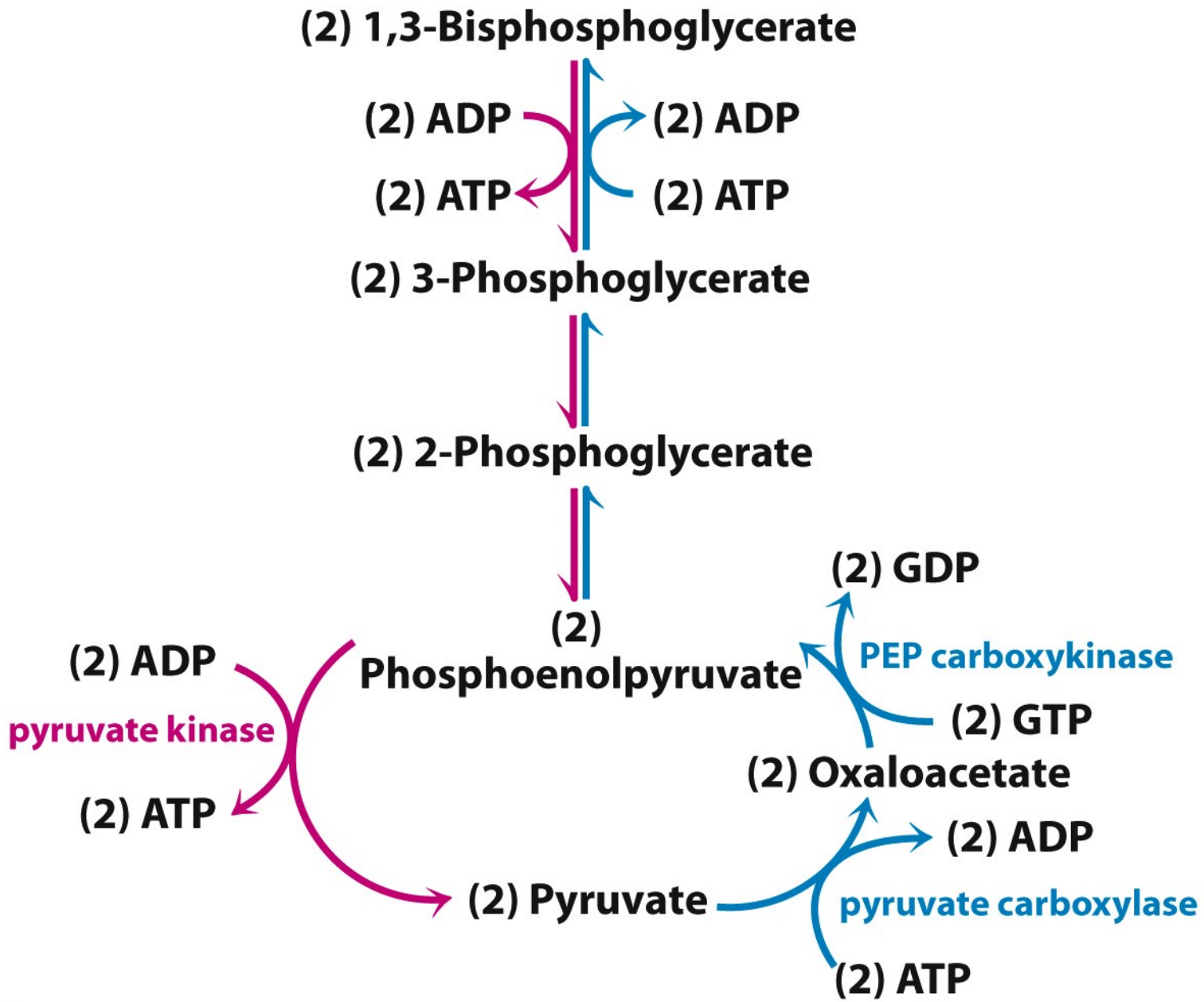

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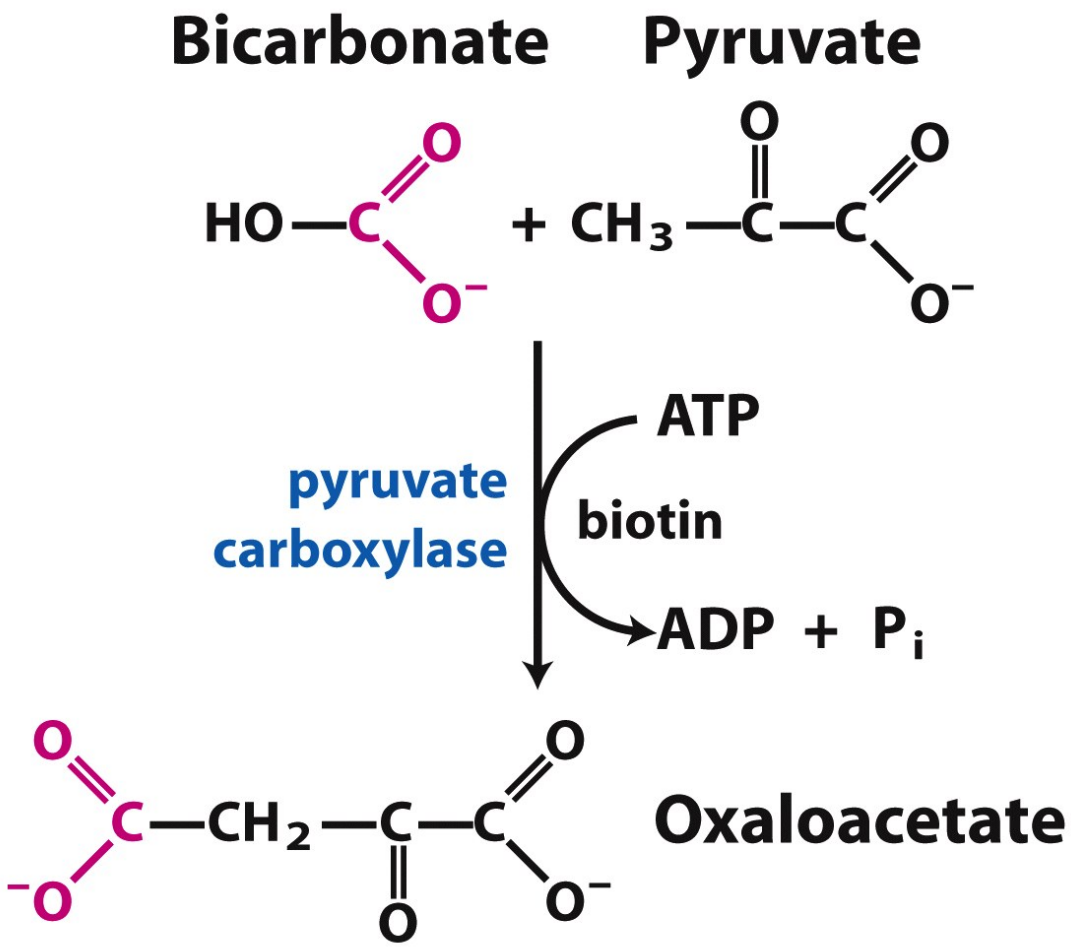
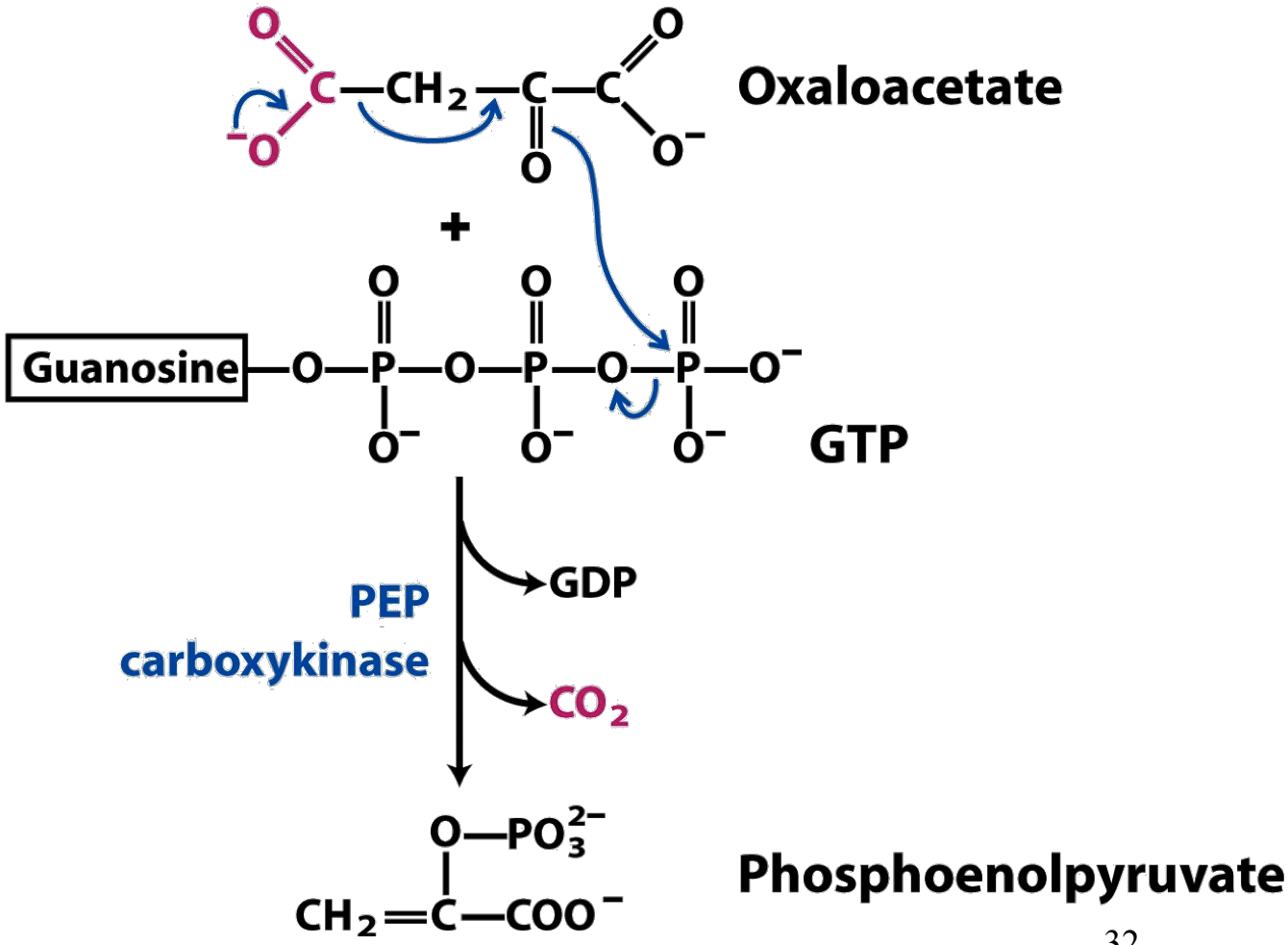
Synthesis of Oxaloacetate

- Conversion of pyruvate to energy-rich phosphoenolpyruvate requires two energy-consuming steps
- In the first step, pyruvate is transported into mitochondria and converted into oxaloacetate by pyruvate carboxylase

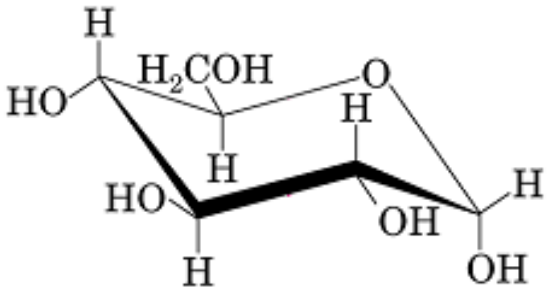


Oxaloacetate Picks Up Phosphate from GTP

- The phosphoenolpyruvate carboxykinase reaction occurs either in the cytosol or the mitochondria



Only a Small Amount of Energy Available in Glucose is Captured in Glycolysis

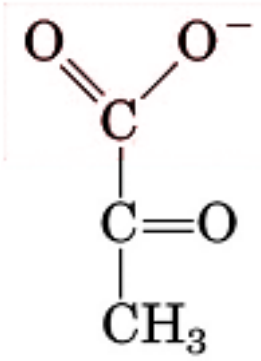


GLUCOSE

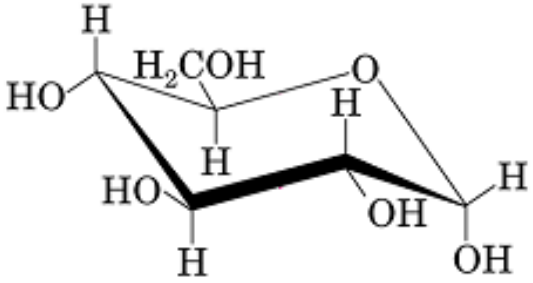
Glycolysis

$\Delta G'^{\circ} = -146$ kJ/mol

2



Pyruvate



Full oxidation (+ 6 O_2)

$\Delta G'^{\circ} = -2{,}840$ kJ/mol

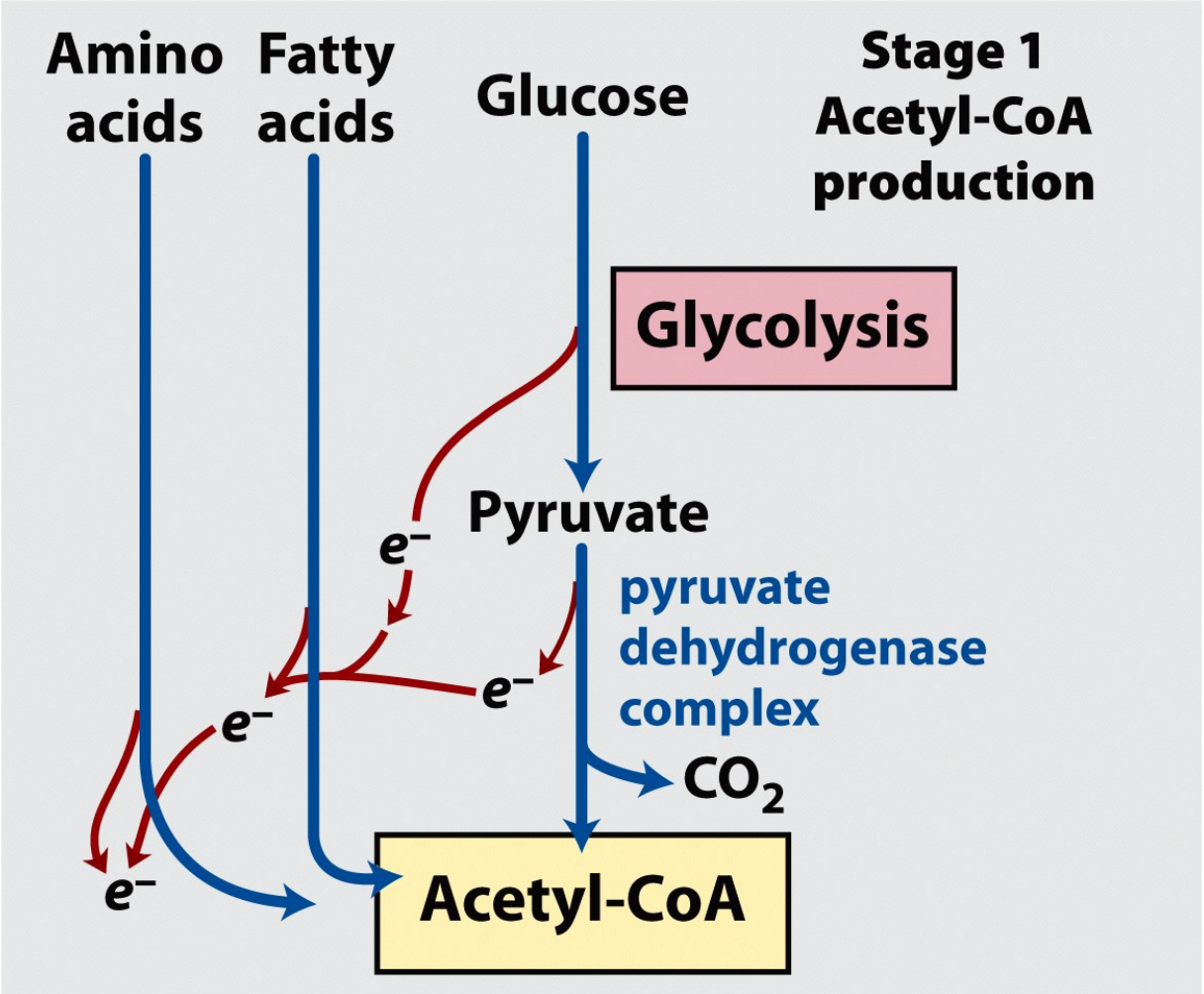
$6\,CO_2 + 6\,H_2O$

Cellular Respiration

- process in which cells consume O_2 and produce CO_2
- provides more energy (ATP) from glucose than glycolysis
- also captures energy stored in lipids and amino acids
- evolutionary origin: developed about 2.5 billion years ago
- used by animals, plants, and many microorganisms
- occurs in three major stages:
  - acetyl CoA production
  - acetyl CoA oxidation
  - electron transfer and oxidative phosphorylation

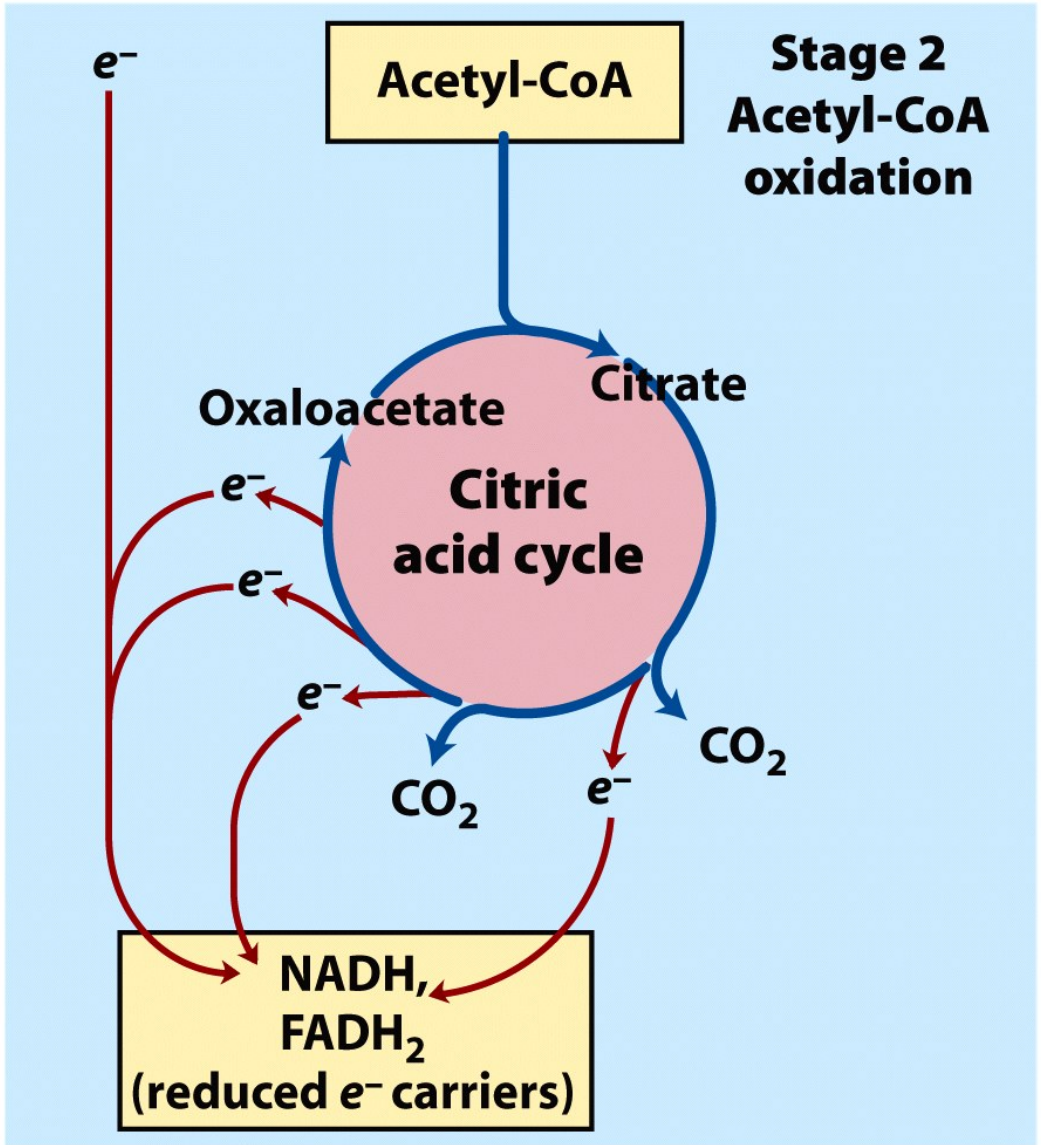
Respiration: Stage 1

Generates some:
ATP, NADH, $FADH_2$



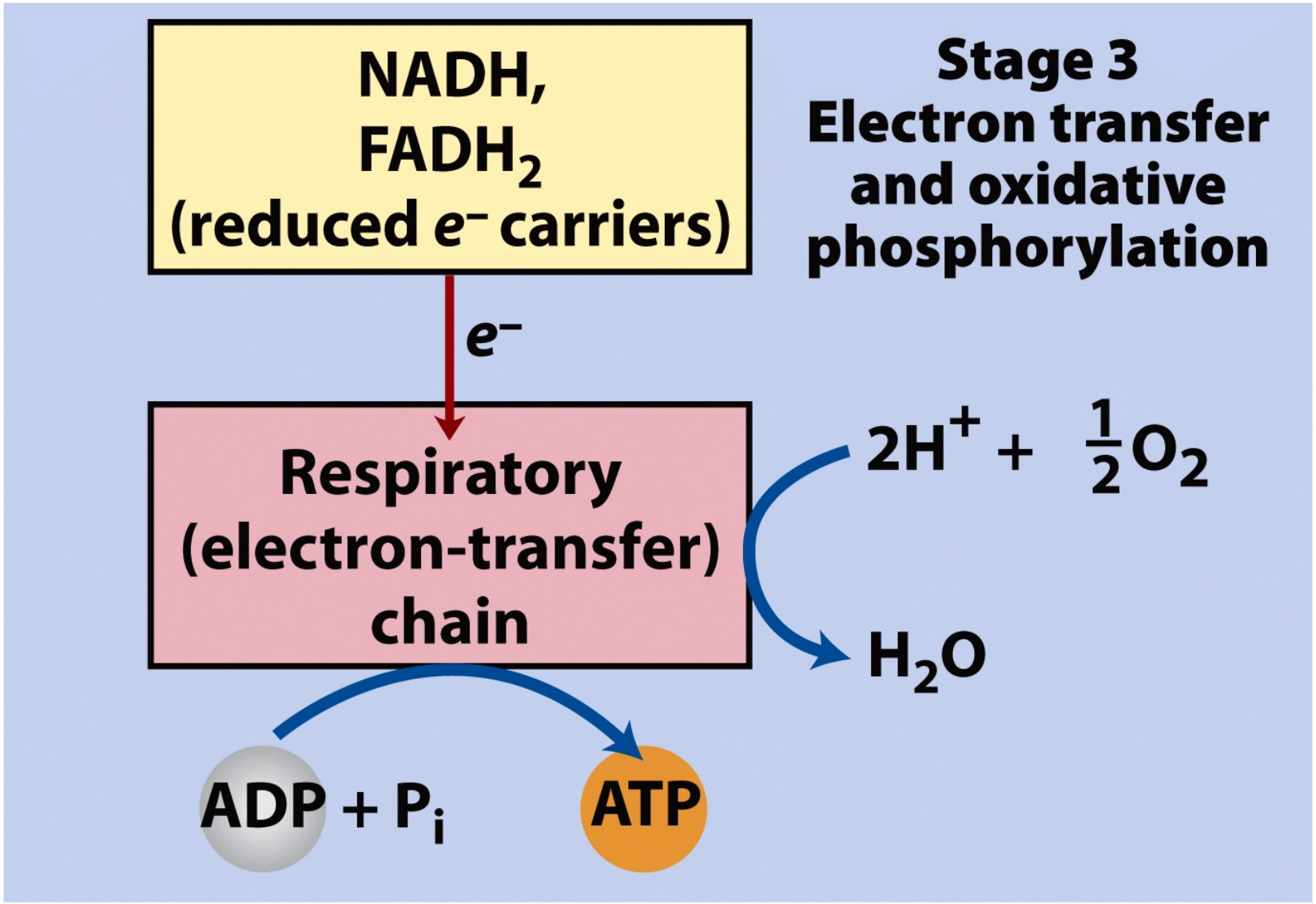
Respiration: Stage 2

Generates more NADH, $FADH_2$ and one GTP



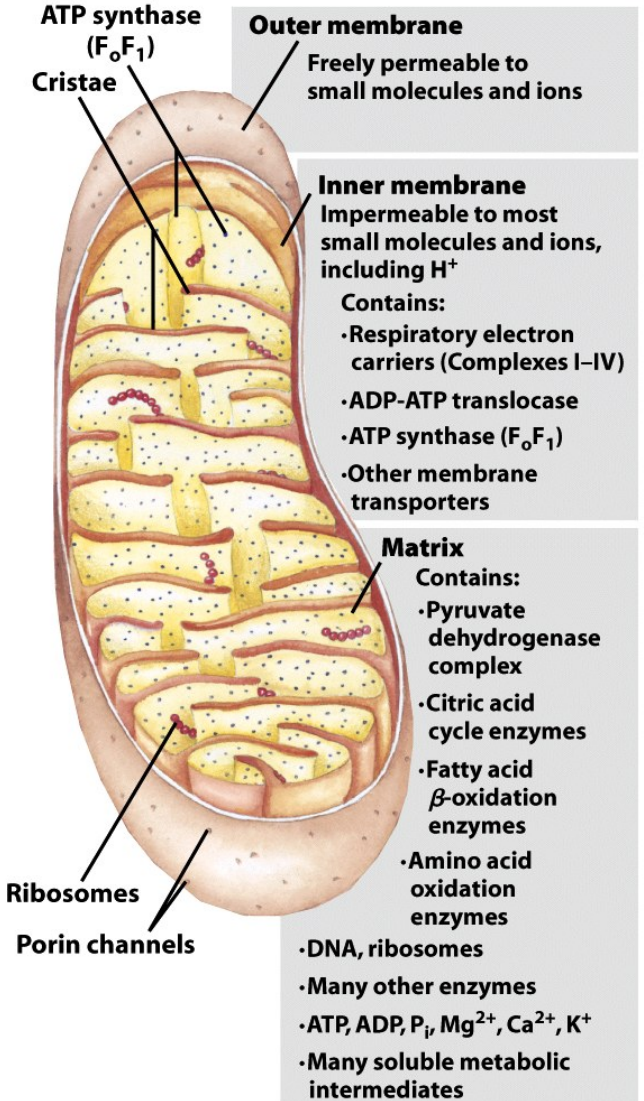
Respiration: Stage 3

Makes lots of **ATP**



In Eukaryotes, Citric Acid Cycle Occurs in Mitochondria

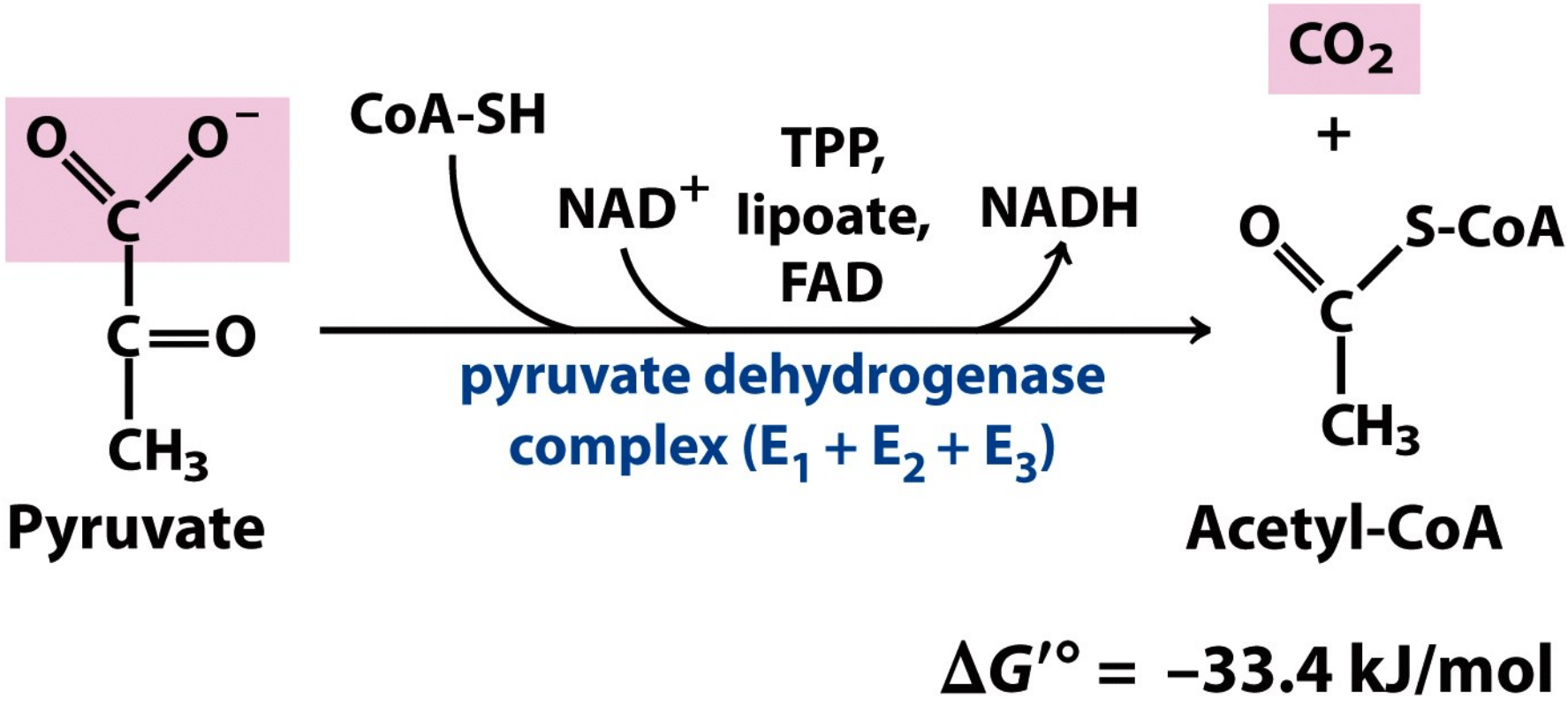
- Glycolysis occurs in the ***cytoplasm***
- Citric acid cycle occurs in the ***mitochondrial matrix***†
- Oxidative phosphorylation occurs in the ***inner membrane***



† Except succinate dehydrogenase, which is an integral inner membrane protein

Conversion of Pyruvate to Acetyl-CoA

- net reaction: oxidative decarboxylation of pyruvate
  - acetyl-CoA can enter the citric acid cycle and yield energy
  - acetyl-CoA can be used to synthesize storage lipids
- requires five coenzymes
- catalyzed by the pyruvate decarboxylase complex



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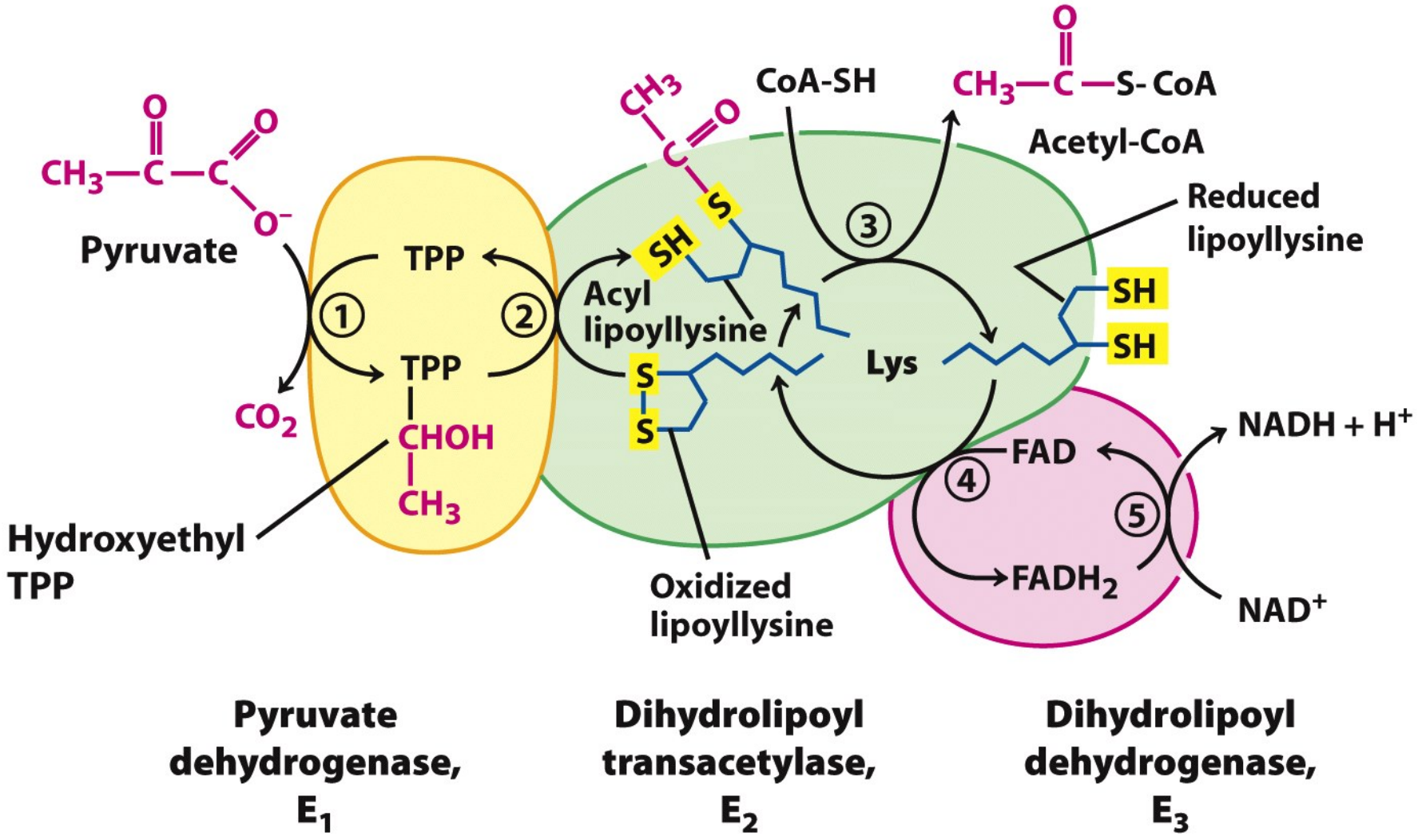
Pyruvate Dehydrogenase Complex (PDC)

- PDC is a large (M_r = 7.8 × 10^6 Da) multienzyme complex
  - pyruvate dehydrogenase (E_1)
  - dihydrolipoyl transacetylase (E_2)
  - dihydrolipoyl dehydrogenase (E_3)
- short distance between catalytic sites allows channeling of substrates from one catalytic site to another
- channeling minimizes side reactions
- activity of the complex is subject to regulation (ATP)

Chemistry of Oxidative Decarboxylation of Pyruvate

- NAD^+ and CoA-SH are co-substrates
- TPP, lipoyllysine and FAD are prosthetic groups
- *Coenzymes or co-substrates are not a permanent part of the enzymes' structure; they associate, fulfill a function, and dissociate*
- The function of CoA is to accept and carry acetyl groups
- *Prosthetic groups are strongly bound to the protein*
- Lipoic acid is covalently linked to the enzyme via a lysine residue



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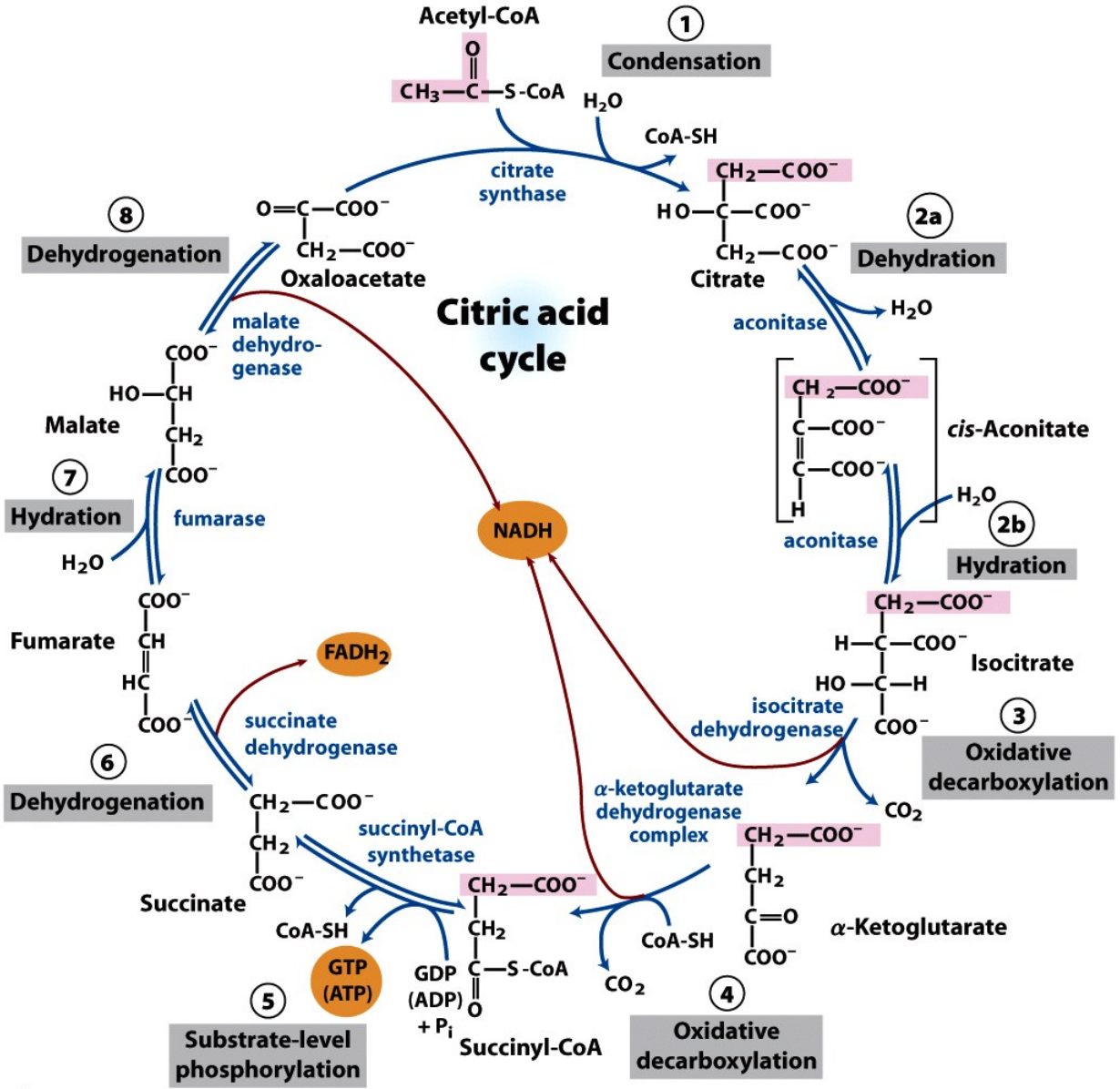
The Citric Acid Cycle

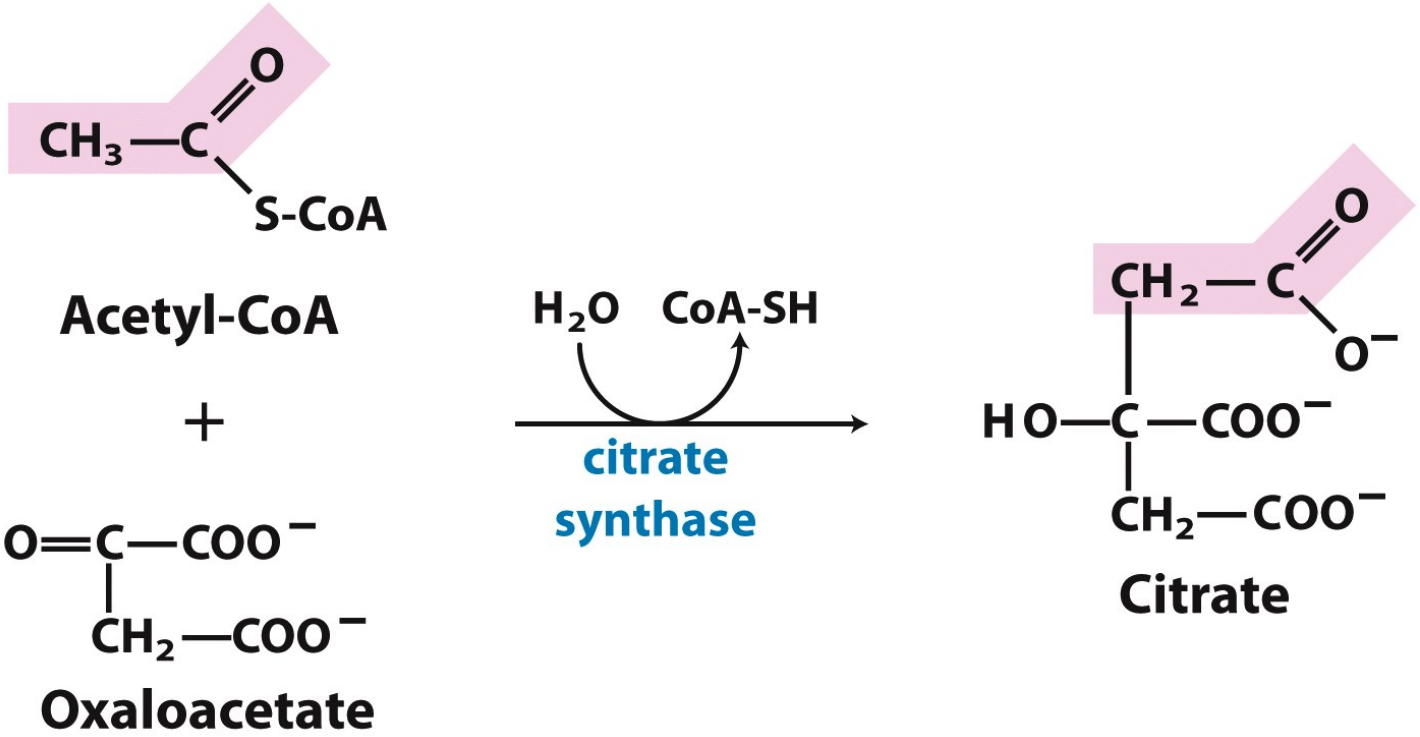


Sequence of Events in the Citric Acid Cycle

- **Step 1:** C-C bond formation to make citrate
- **Step 2:** Isomerization via dehydration, followed by hydration
- **Steps 3-4:** Oxidative decarboxylations to give 2 NADH
- **Step 5:** Substrate-level phosphorylation to give GTP
- **Step 6:** Dehydrogenation to give reduced $FADH_2$
- **Step 7:** Hydration
- **Step 8:** Dehydrogenation to give NADH

(1) The Citrate Synthase Reaction

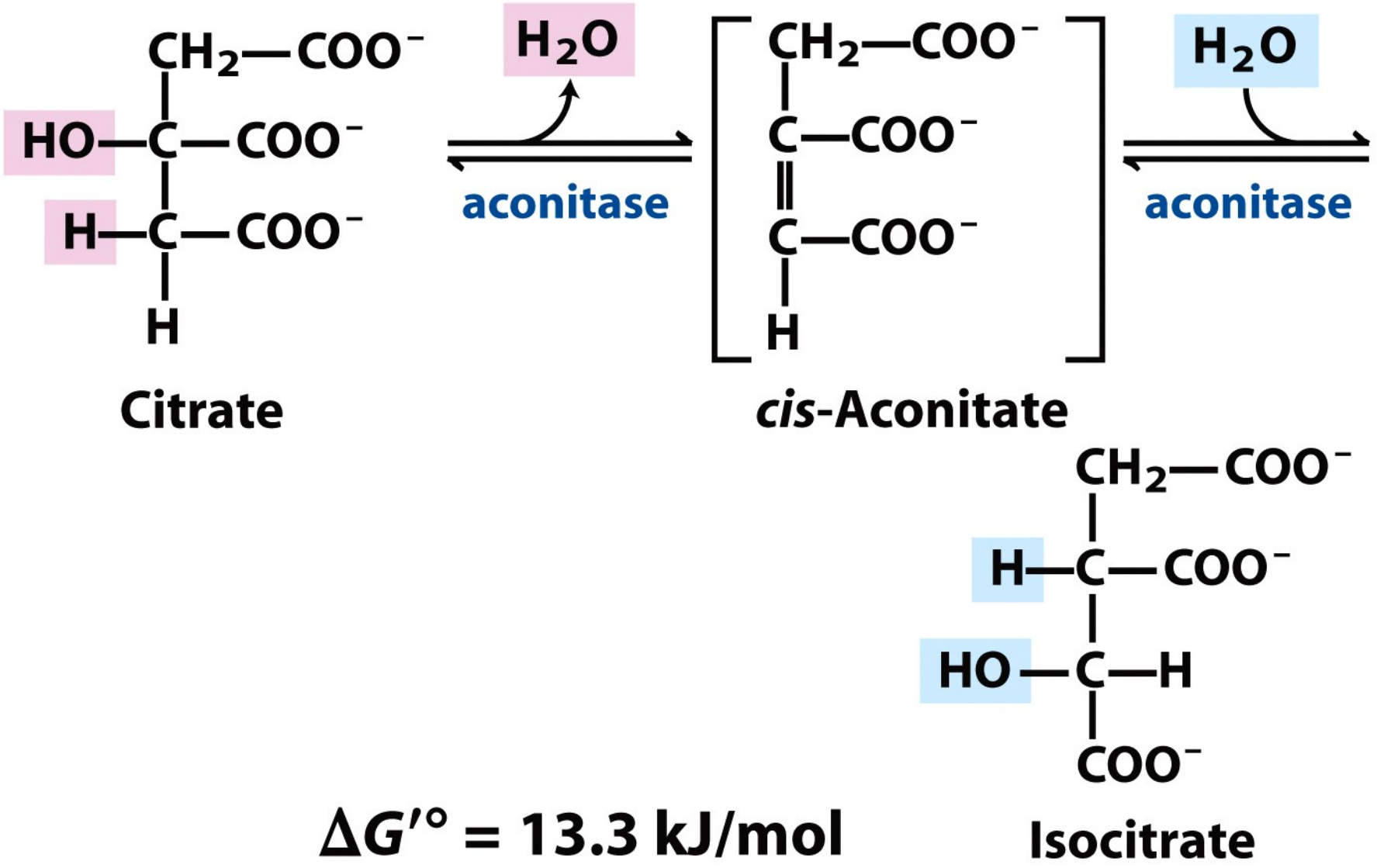
- The only cycle reaction with C-C bond formation
- Essentially *irreversible* process



$\Delta G'^{\circ} = -32.2$ kJ/mol

(2) Isomerization of Citrate by Aconitase

- Citrate, a tertiary alcohol, is a poor substrate for oxidation
- Elimination of H_2O from citrate gives a *cis* C=C bond
- Addition of H_2O to *cis*-aconitate is stereospecific
- Isocitrate, a secondary alcohol, is a good substrate for oxidation



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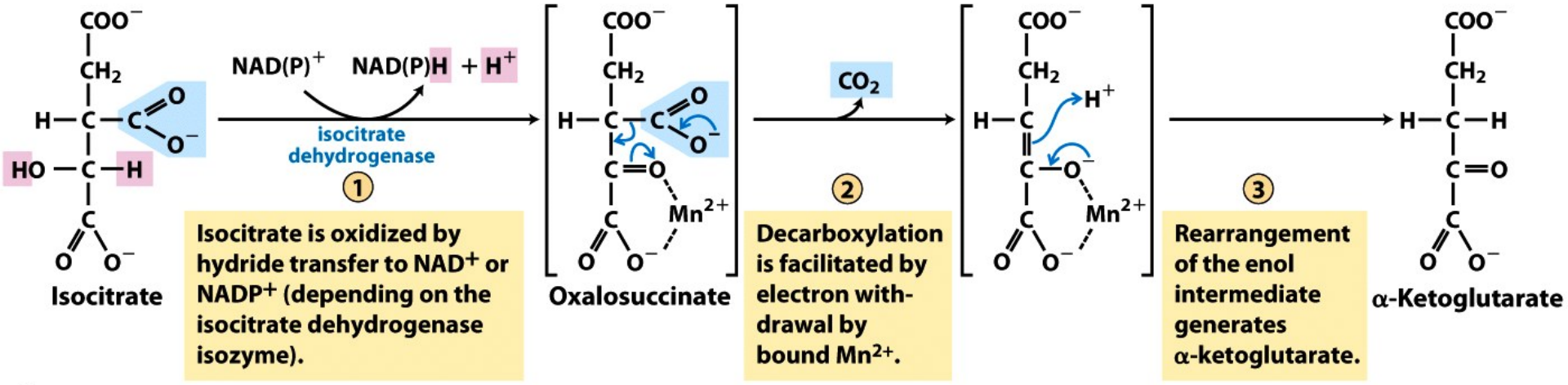
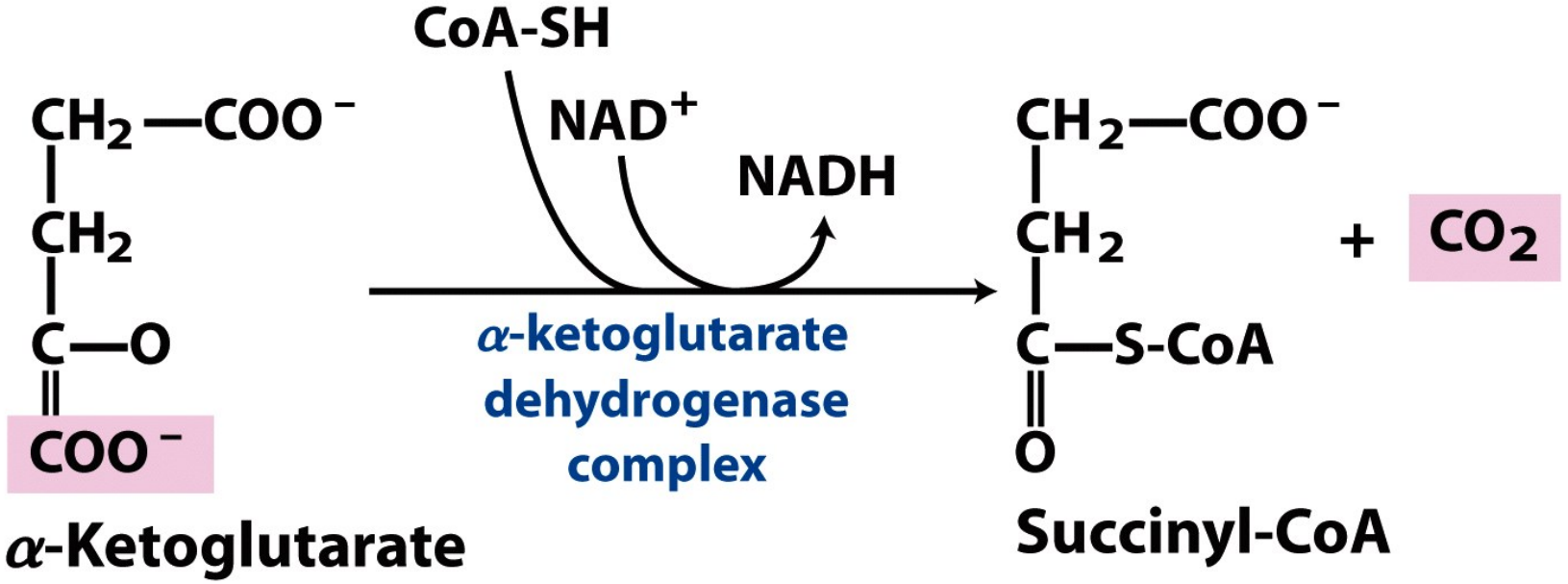
(3) The Isocitrate Dehydrogenase Reaction

Oxidation of the alcohol to ketone involves the transfer of a hydride from the C-H of the alcohol to the nicotinamide cofactor



(4) Oxidation of α-ketoglutarate

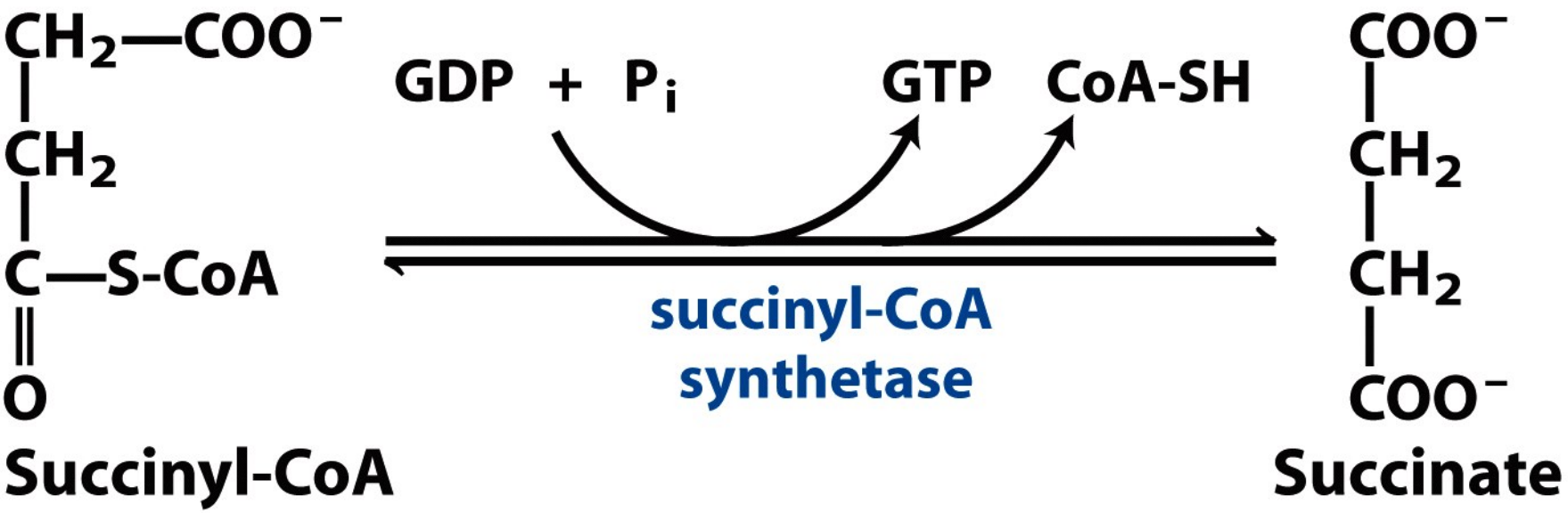
- Enzyme: α-ketoglutarate dehydrogenase complex
- Similar to pyruvate dehydrogenase complex
- Same coenzymes, identical mechanisms



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

(5) Substrate-Level Phosphorylation

Produces GTP, which can be converted to ATP



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

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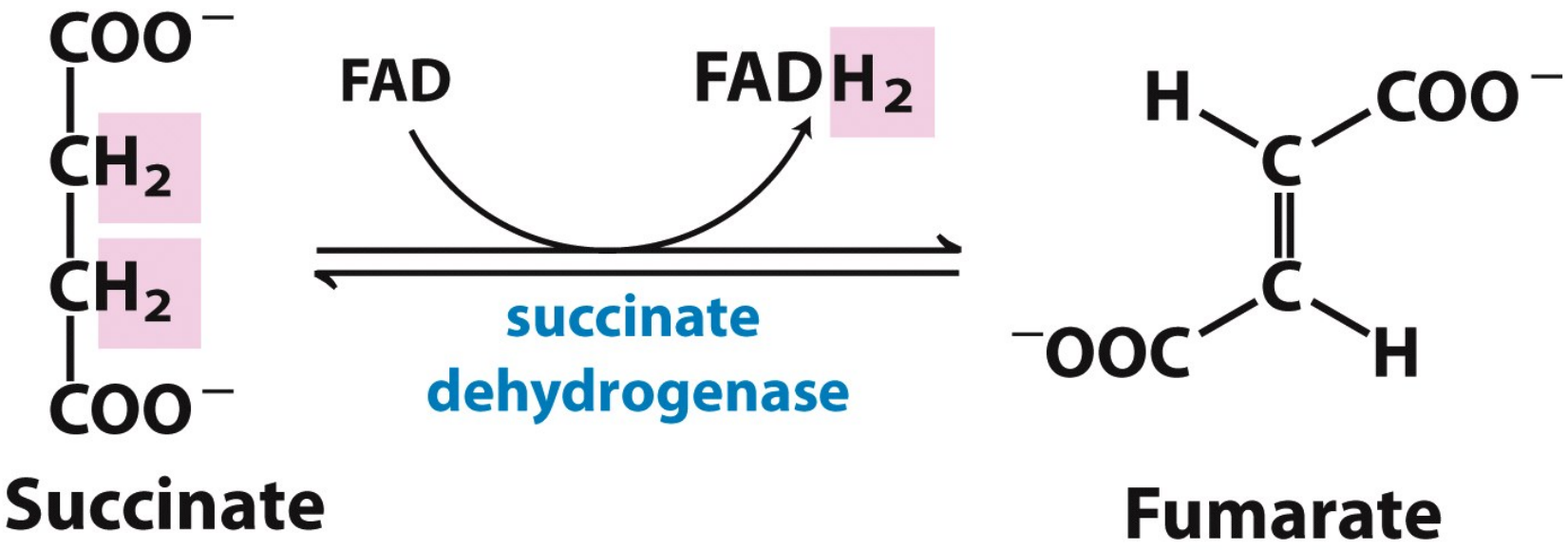
(6) Succinate Dehydrogenase

- Covalently bound FAD is reduced to $FADH_2$
- $FADH_2$ passes electrons to coenzyme Q
- Reduced coenzyme (QH_2) can be used to make ATP

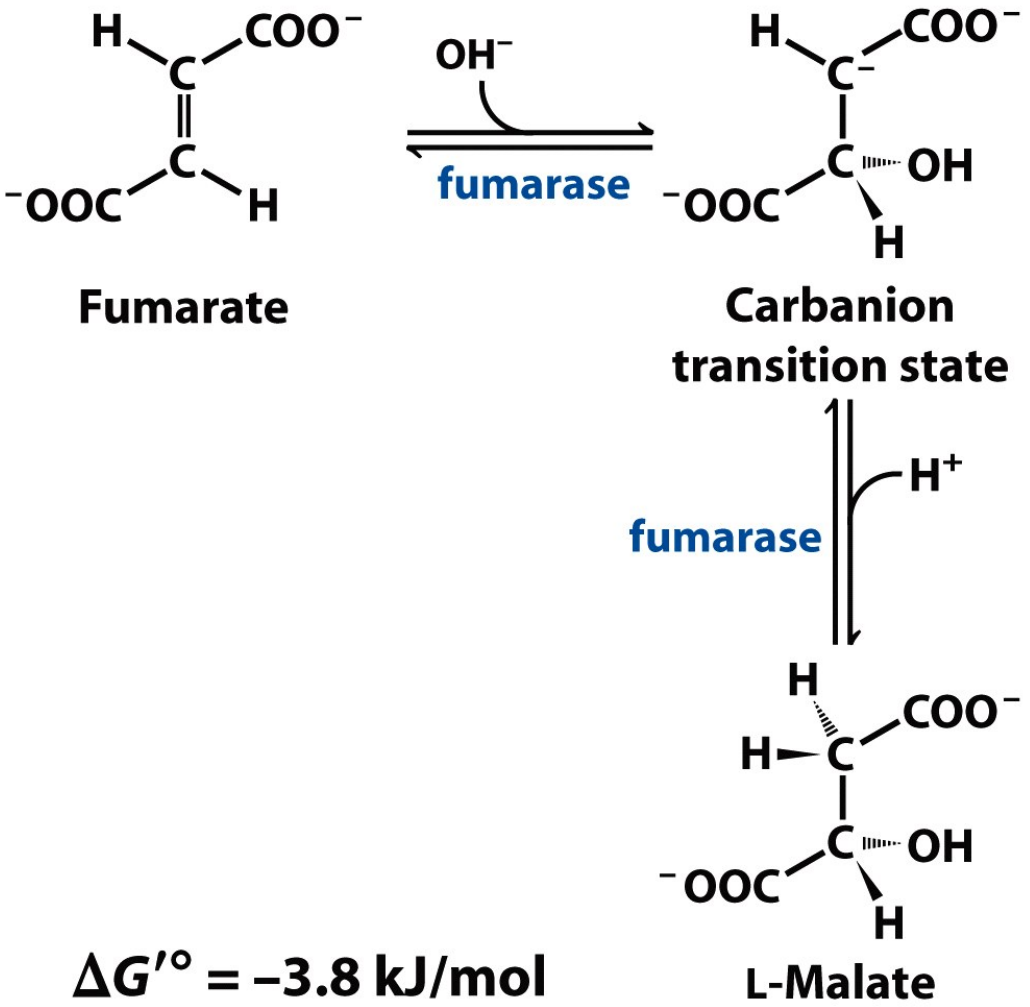


Succinate **Fumarate**

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

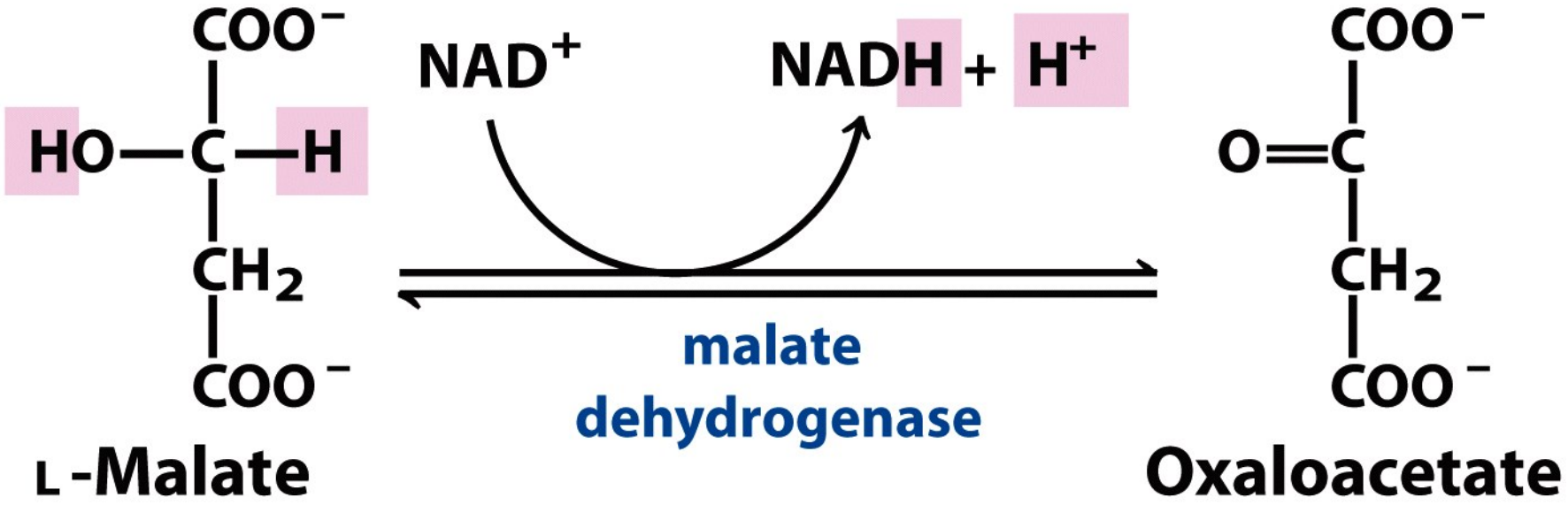
(7) Hydration of Fumarate to Malate

- Fumarase is highly stereospecific
- OH^- adds to fumarate …
  then H^+ adds to the carbanion
- Net effect: trans addition of water
- Reversible reaction



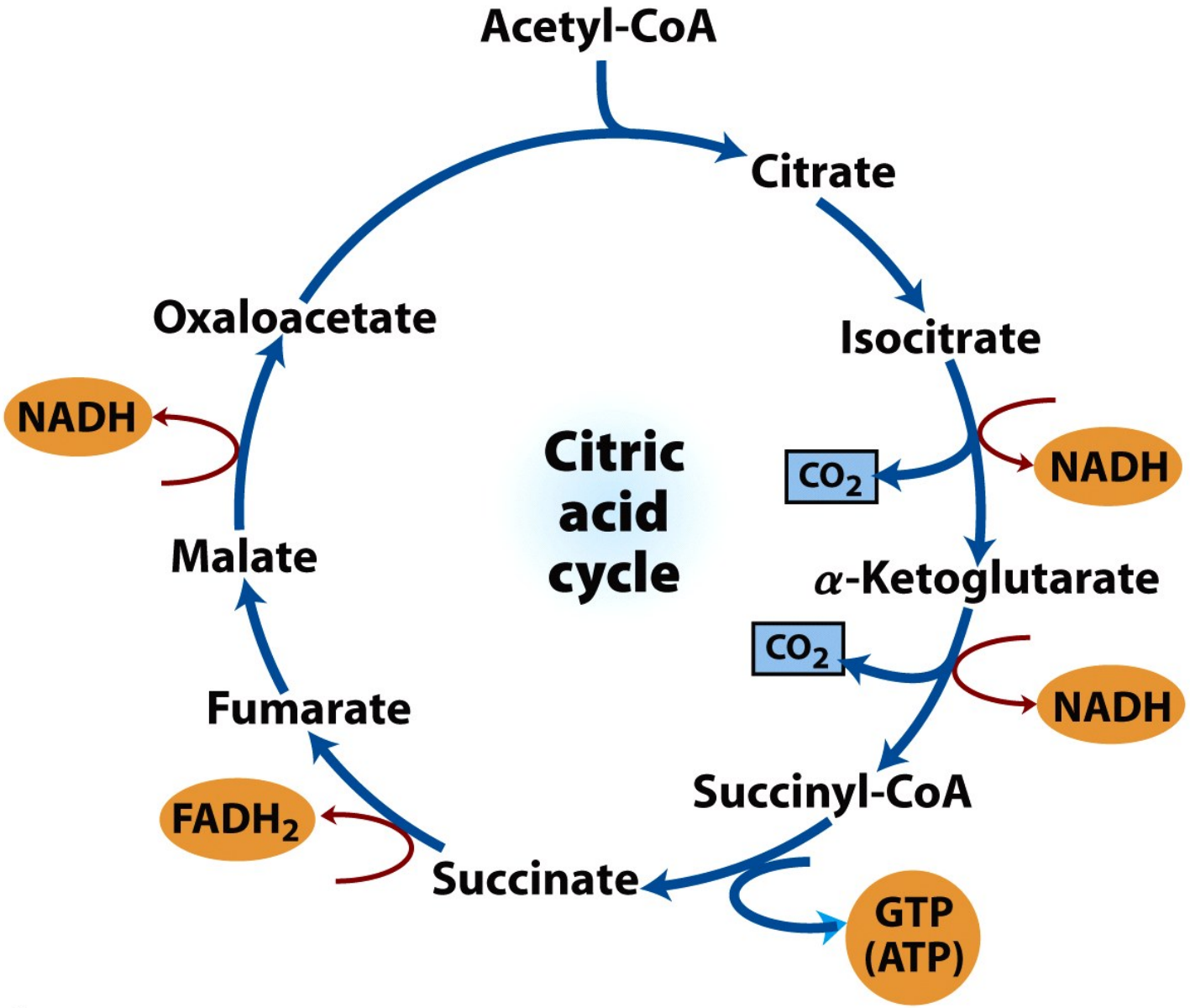
(8) Oxidation of Malate to Oxaloacetate

- Thermodynamically unfavorable reaction
- Oxidation occurs because oxaloacetate concentration is very low as it is continuously used to make citrate



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

Products from One Turn of the Cycle



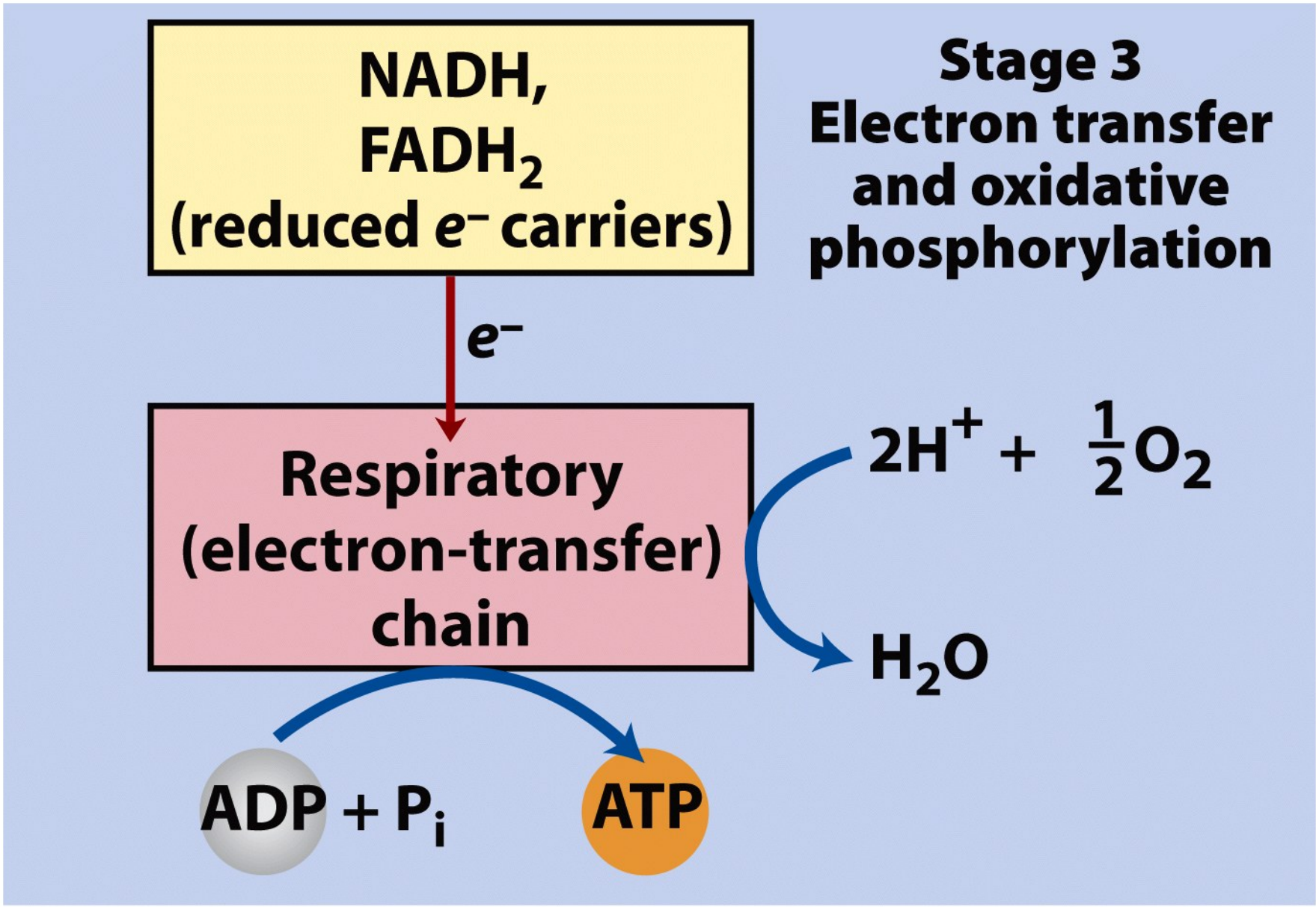
Net Effect of the Citric Acid Cycle

$$\text{Acetyl-CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{P}_i + 2\ \text{H}_2\text{O} \longrightarrow 2\text{CO}_2 + 3\text{NADH} + \text{FADH}_2 + \text{GTP} + \text{CoA} + 3\text{H}^+$$

- carbons of acetyl groups in acetyl-CoA are oxidized to CO_2
- electrons from this process reduce NAD^+ and FAD
- one GTP is formed per cycle, this can be converted to ATP
- intermediates in the cycle are not depleted

Oxidative Phosphorylation

- Electrons from the reduced cofactors NADH and $FADH_2$ are passed to proteins in the respiratory chain
- In eukaryotes, oxygen is the ultimate electron acceptor for these electrons
- Energy of oxidation is used to phosphorylate ADP



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Chemiosmotic Theory

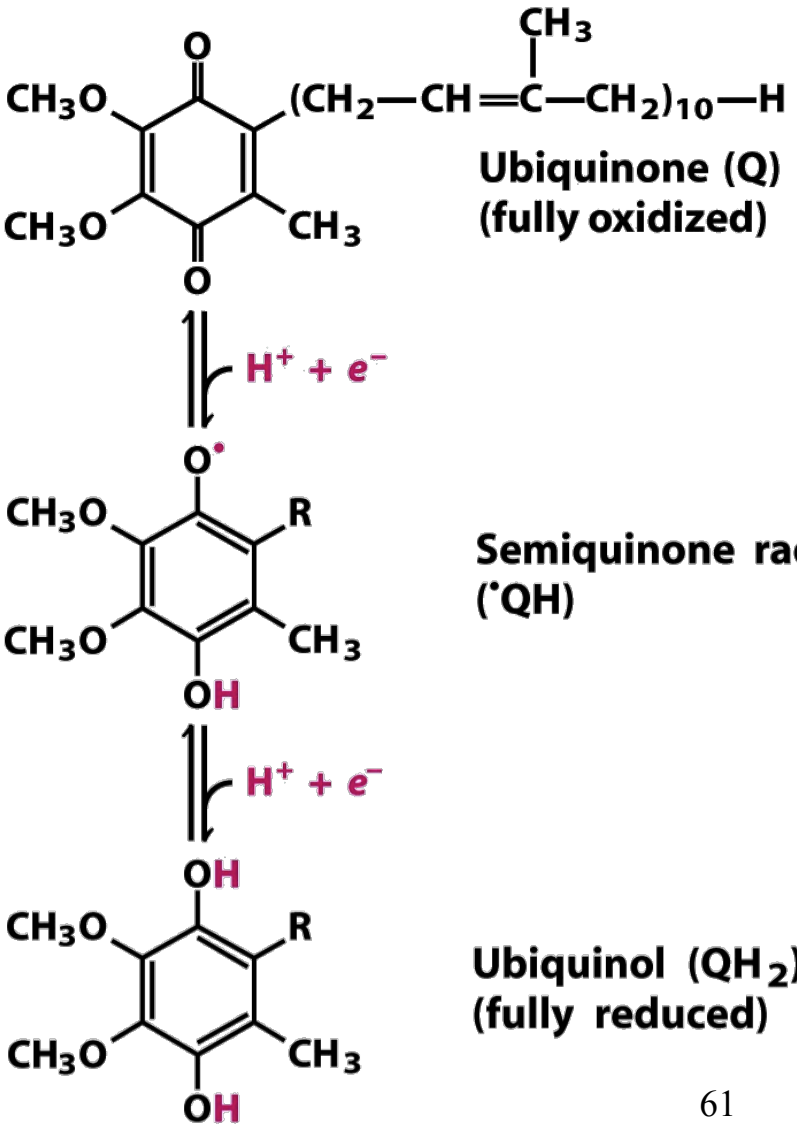
- How to make an unfavorable ADP + P_i → ATP possible?
- Phosphorylation of ADP is not a result of a direct reaction between ADP and some high energy phosphate carrier
- Energy needed to phosphorylate ADP is provided by the flow of protons down the electrochemical gradient
- The electrochemical gradient is established by transporting protons against the electrochemical gradient during the electron transport

Chemiosmotic Energy Coupling Requires Membranes

- The proton gradient needed for ATP synthesis can be stably established across a topologically closed membrane
  - Plasma membrane in bacteria
  - Cristae membrane in mitochondria
  - Thylakoid membrane in chloroplasts
- Membrane must contain proteins that couple the "downhill" flow of electrons in the electron transfer chain with the "uphill" flow of protons across the membrane
- Membrane must contain a protein that couples the "downhill" flow of proton to the phosphorylation of ADP

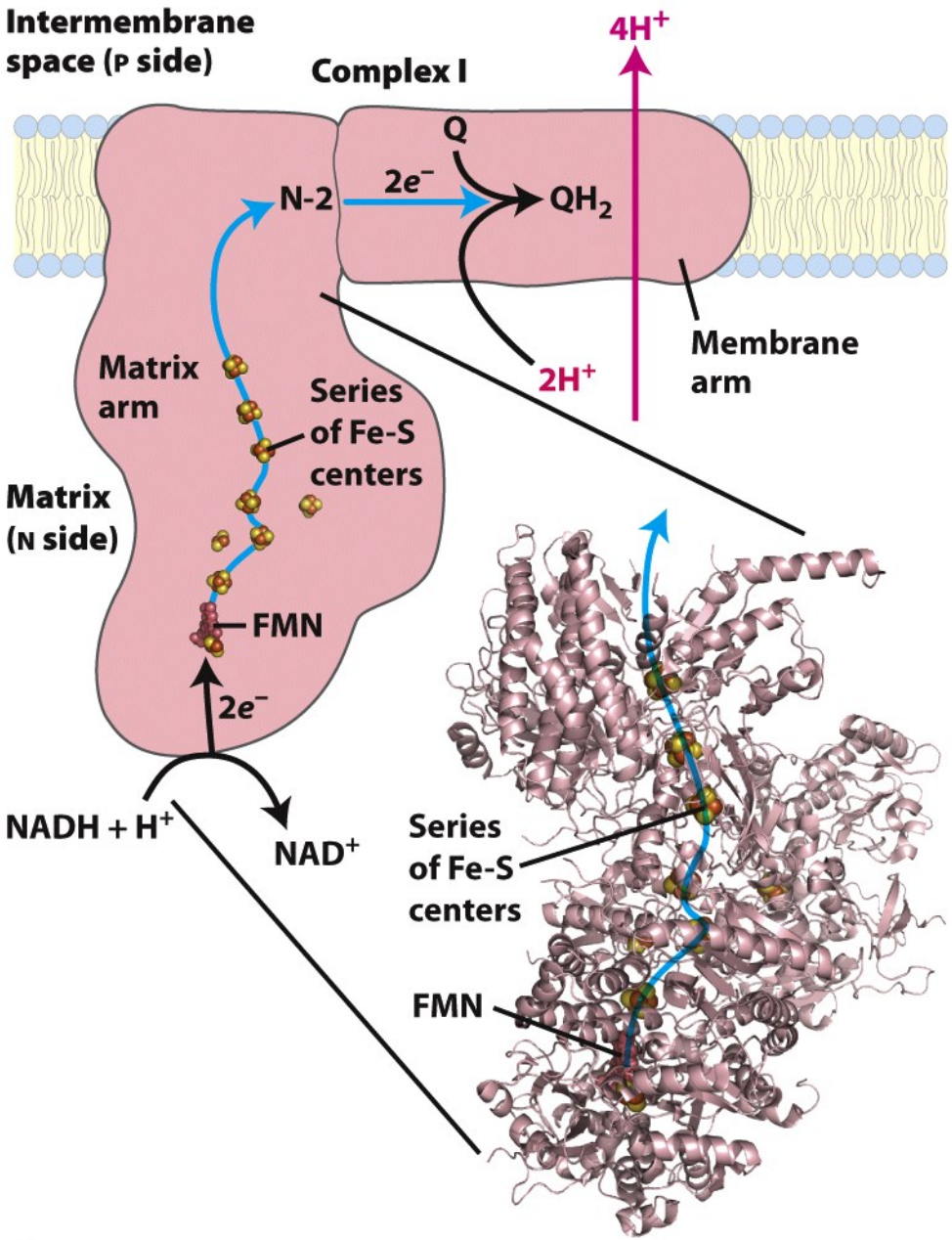
Coenzyme Q or Ubiquinone

- Ubiquinone is a lipid-soluble conjugated dicarbonyl compound that readily accepts electrons
- Upon accepting two electrons, it picks up two protons to give an alcohol, ubiquinol
- Ubiquinol can freely diffuse in the membrane, carrying electrons with protons from one side of the membrane to another side



NADH:Ubiquinone Oxidoreductase (Complex I)

- One of the largest macro-molecular assemblies in the mammalian cell
- Over 40 different polypeptide chains, encoded by both nuclear and mitochondrial genes
- NADH binding site in the matrix side
- Non-covalently bound flavin mononucleotide (FMN) accepts two electrons from NADH
- Several iron-sulfur centers pass one electron at the time toward the ubiquinone binding site



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NADH:Ubiquinone Oxidoreducase is a Proton Pump

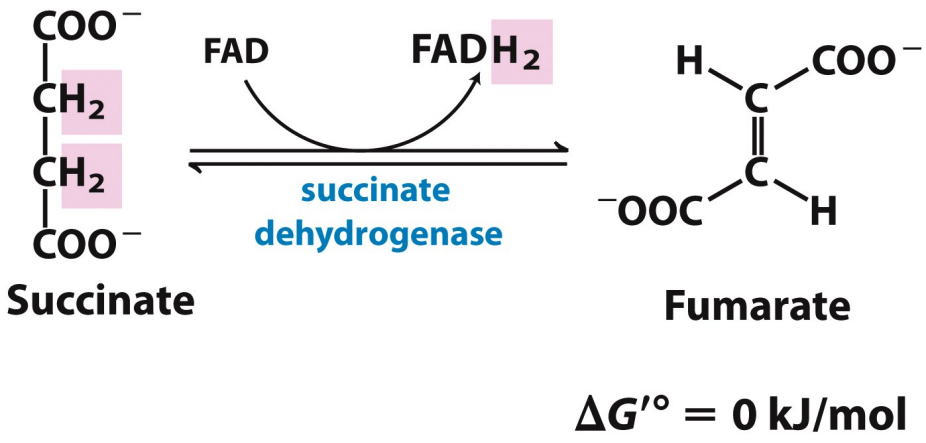
- Transfer of two electrons from NADH to uniquinone is accompanied by a transfer of protons from the matrix (N) to the inter-membrane space (P)
- Experiments suggest that about four protons are transported per one NADH

$$NADH + Q + 5H^+_N = NAD^+ + QH_2 + 4\ H^+_P$$

- Reduced coenzyme Q picks up two protons
- Despite 50 years of study, it is still unknown how the four other protons are transported across the membrane

Succinate Dehydrogenase (Complex II)

- FAD accepts two electrons from succinate
- Electrons are passed, one at a time, via iron-sulfur centers to ubiquinone that becomes reduced QH_2



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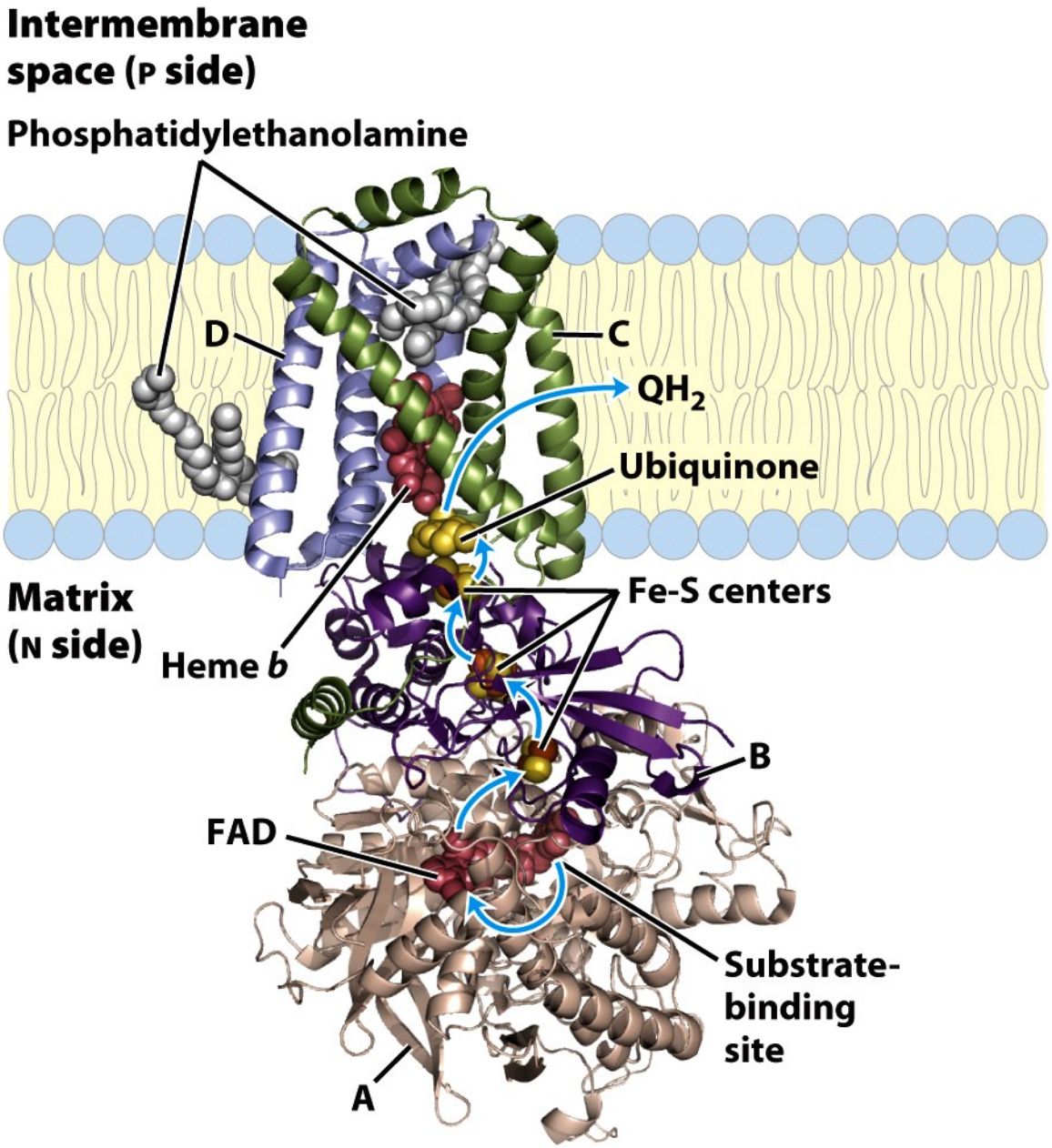

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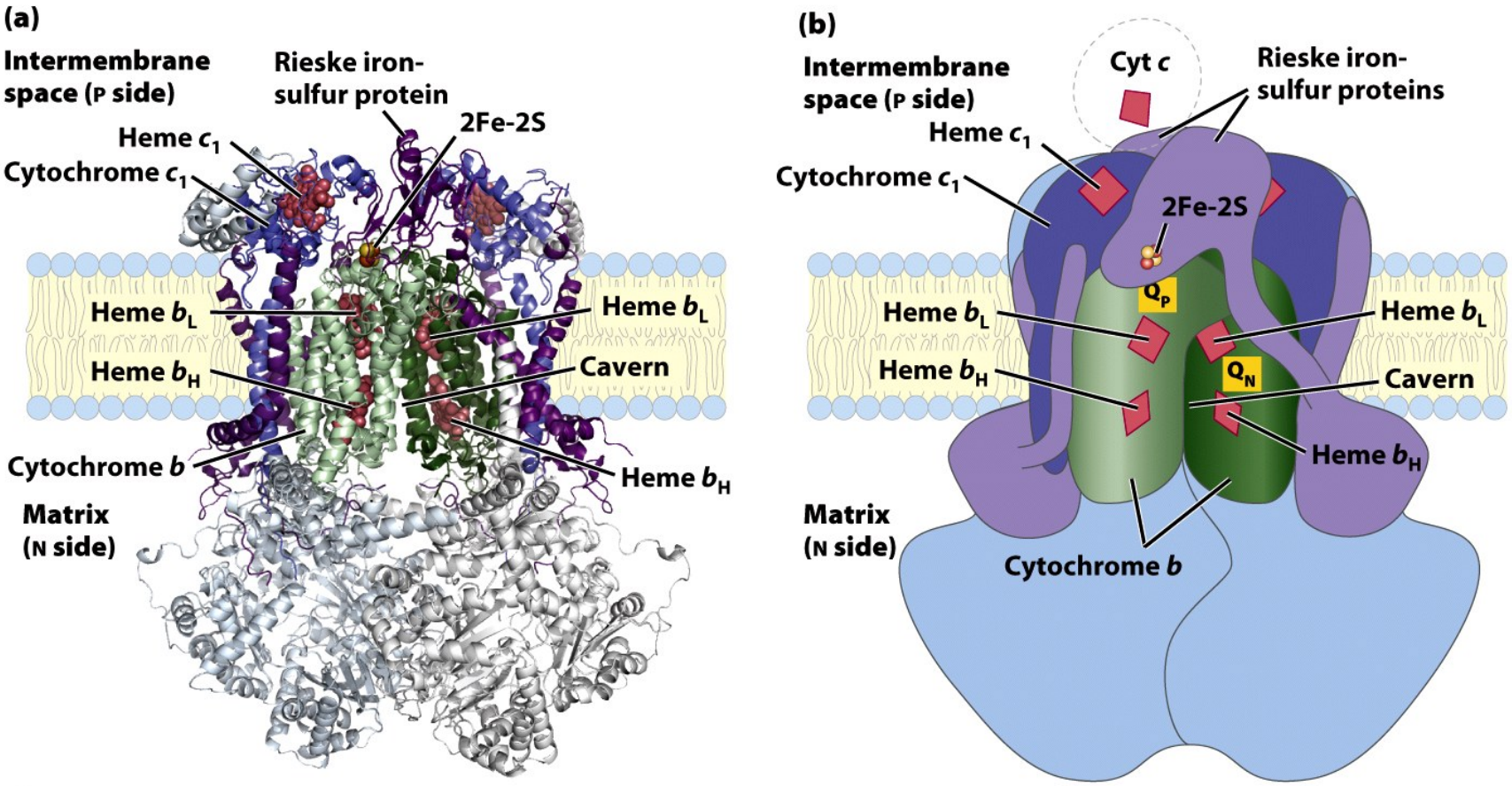
Cytochrome bc_1 Complex (Complex III)

- Uses two electrons from QH_2 to reduce two molecules of cytochrome c



Cytochrome *c*

- Cytochrome *c* is a soluble heme-containing protein in the intermembrane space
- Heme iron can be either ferrous(Fe^{3+}, oxidized) or ferric(Fe^{2+}, reduced)
- Cytochrome *c* carries a single electron from the cytochrome bc_1 complex to cytochrome oxidase

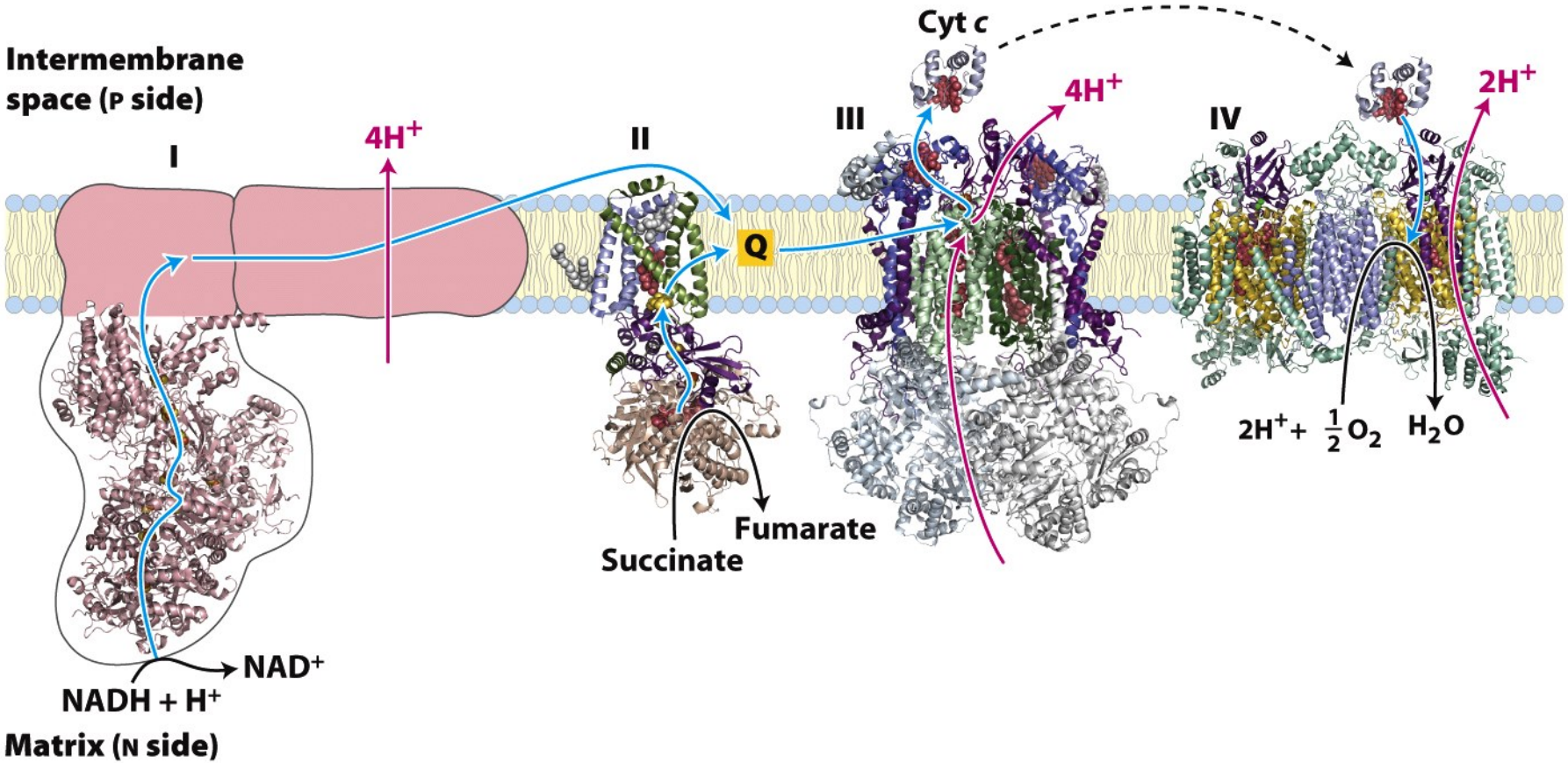
Cytochrome Oxidase (Complex IV)

- Mammalian cytochrome oxidase is a membrane protein with 13 subunits
- Contains two heme groups
- Contains copper ions
  - Two ions (Cu_A) form a binuclear center
  - Another ion (Cu_B) bonded to heme forms Fe-Cu center

Cytochrome Oxidase Passes Electrons to O_2

- Four electrons are used to reduce one oxygen molecule into two water molecules
- Four protons are picked up from the matrix in this process
- Four additional protons are passed from the matrix to the inter-membrane space by an unknown mechanism

Summary of the Electron Flow in the Respiratory Chain



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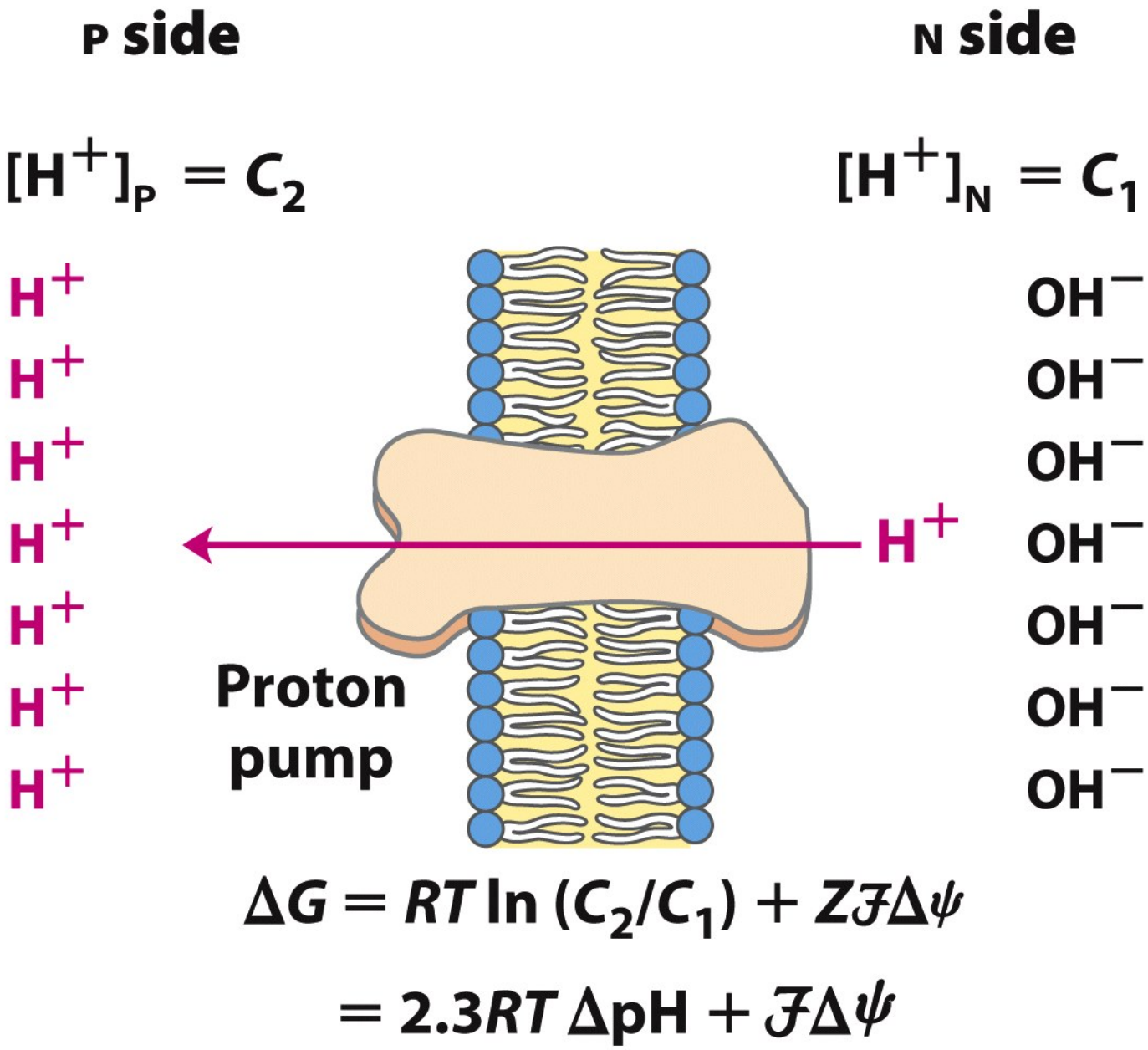
Proton-motive Force

- The proteins in the electron transport chain created the electrochemical proton gradient by one of the three means:
  - actively transported protons across the membrane via poorly understood mechanisms
  - passed electrons to coenzyme Q that picked up protons from the matrix
  - took electrons from QH_2 and released the protons to the inter-membrane side



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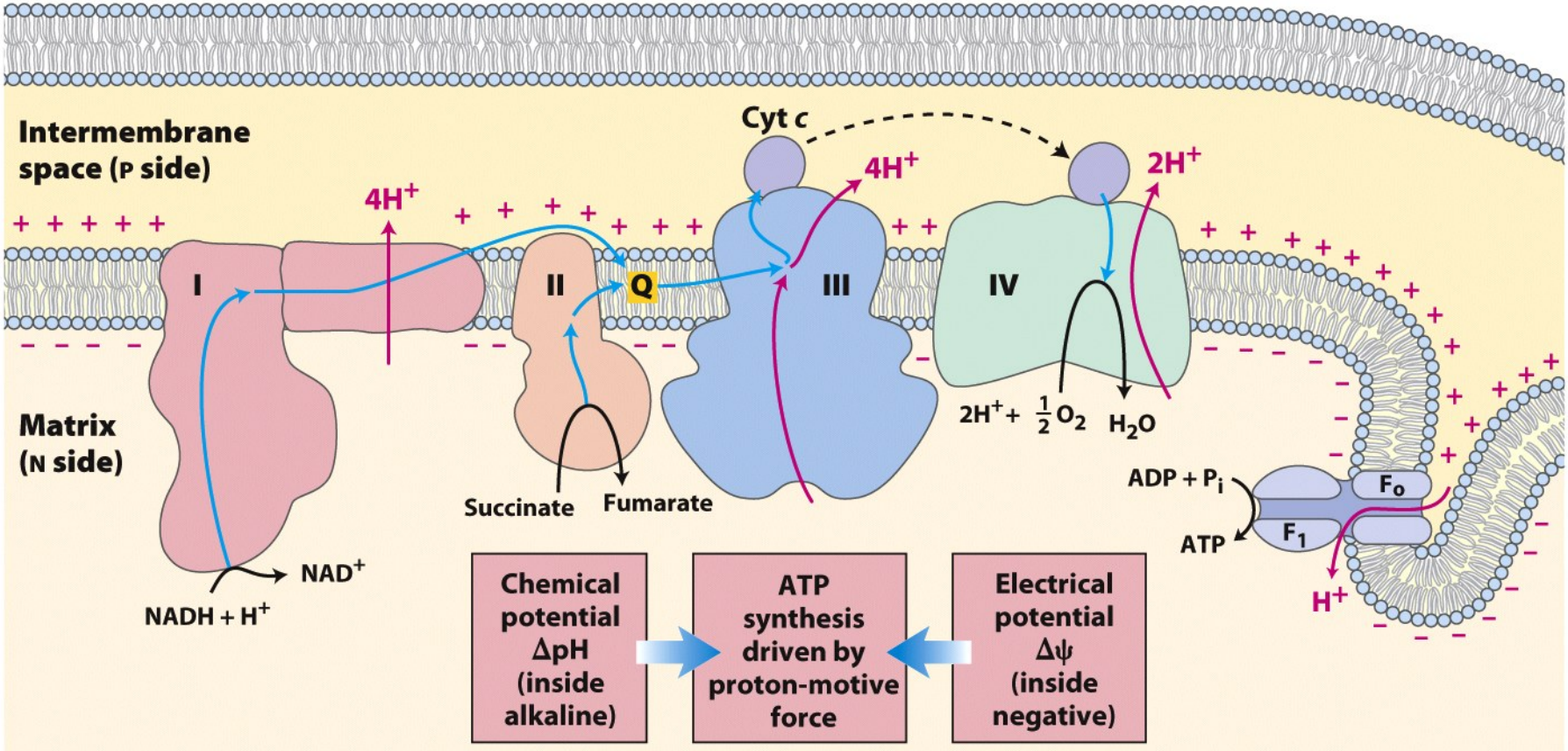
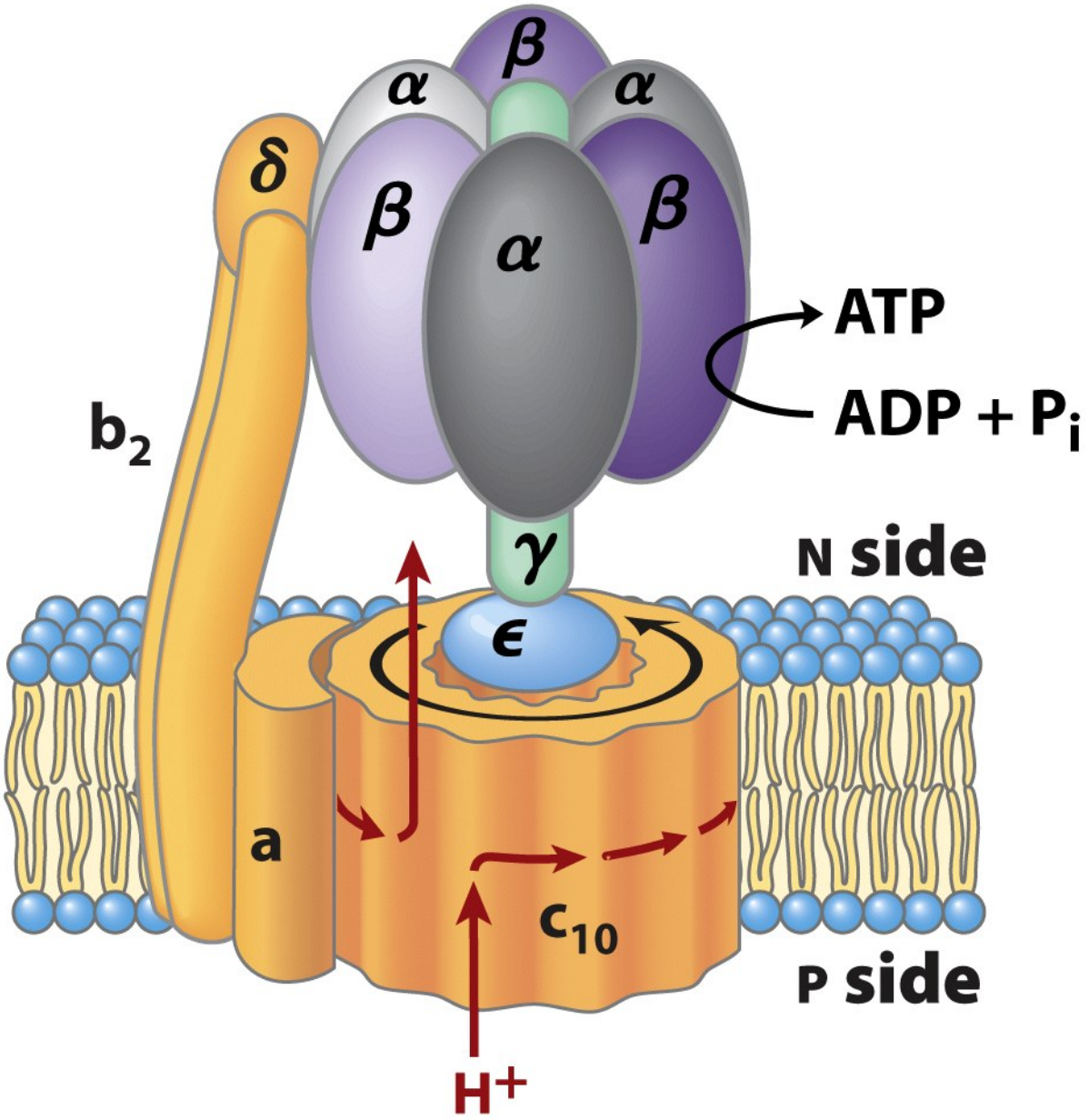
Chemiosmotic Model for ATP Synthesis

- Electron transport sets up a proton-motive force
- Energy of proton-motive force drives synthesis of ATP



Mitochondrial ATP Synthase Complex

- The proton-motive force causes rotation of the central shaft γ
- This causes a conformational change within all the three $\alpha\beta$ pairs
- The conformational change in one of the three pairs promotes condensation of ADP and P_i into ATP



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ATP Yield From Glucose

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.

Table 19-5
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Intro to Metabolism: Summary

- Glycolysis, a process by which cells can extract a limited amount of energy from glucose under anaerobic conditions
- Gluconeogenesis, a process by which cells can use a variety of metabolites for the synthesis of glucose
- Citric acid cycle is an important catabolic process: it makes GTP, and reduced cofactors that could yield ATP
- Citric acid cycle plays important anabolic roles in the cell
- A large multi-subunit enzyme, pyruvate dehydrogenase complex, converts pyruvate into acetyl-CoA
- Several cofactors are involved in reactions that harness the energy from pyruvate
- The reduced cofactors pass electrons into the electron transport chain in mitochondria
- Stepwise electron transport is accompanied by the directional transport of protons across the membrane against their concentration gradient
- The energy in the electrochemical proton gradient drives synthesis of ATP by coupling the flow of protons via ATP