14| Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway

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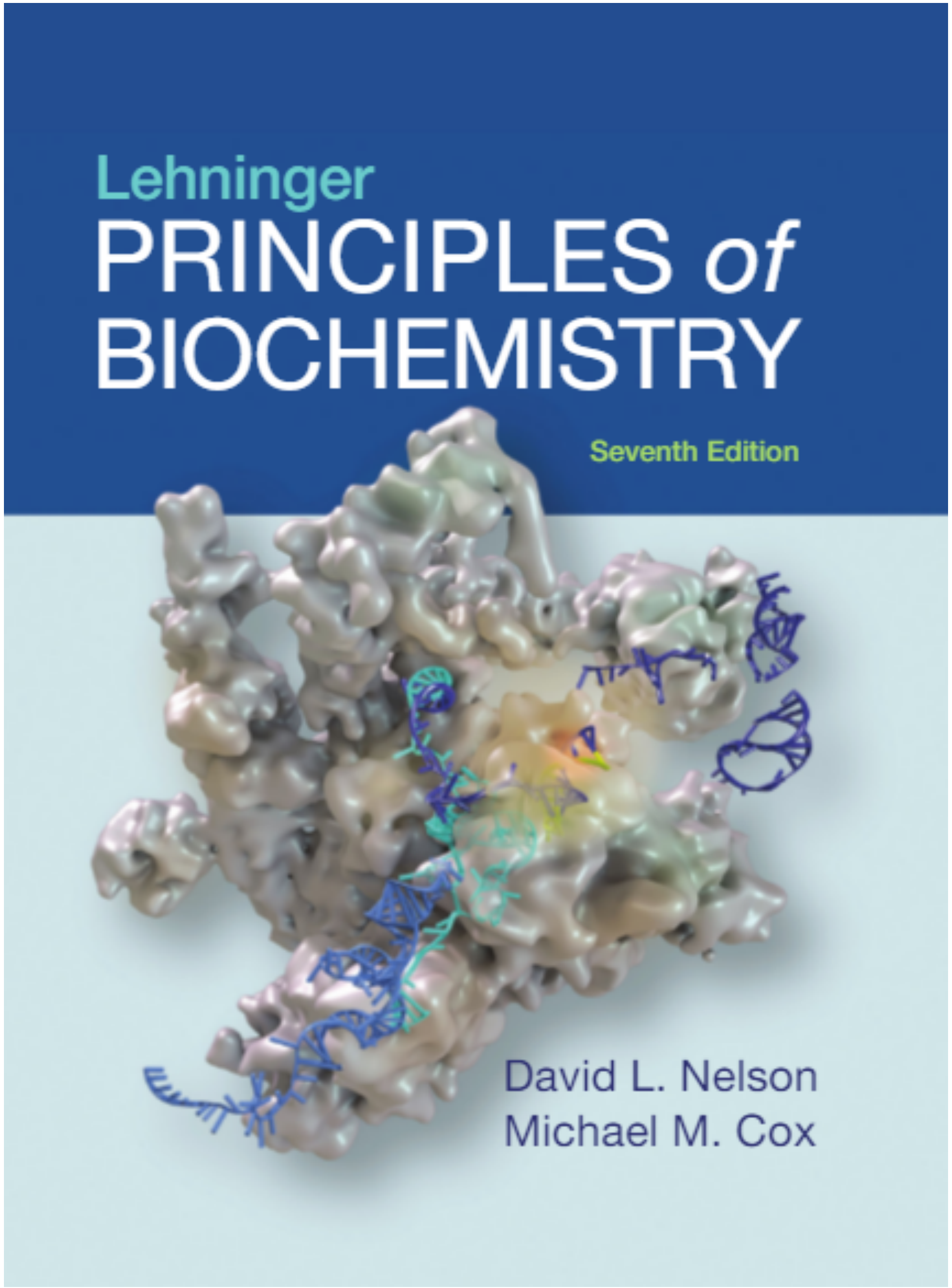


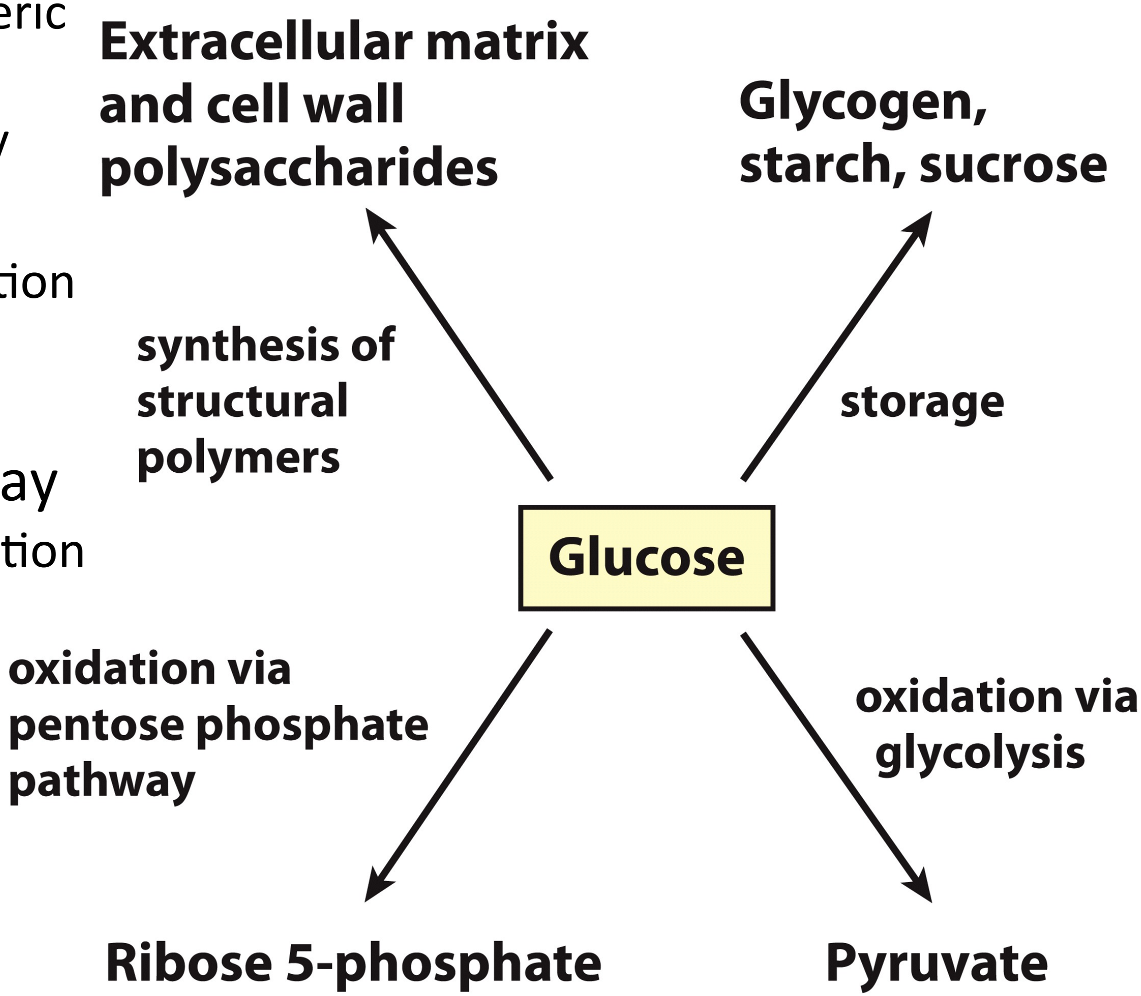
Central Importance of Glucose

- Glucose is an excellent fuel
  - Yields good amount of energy upon oxidation ($\Delta G_{\text{complete oxidation}} = -2840$ kJ/mol)
  - 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
  - Many organisms can use glucose to build the carbon skeletons of:
    - Some or all the amino acids
    - Membrane lipids
    - Nucleotides in DNA and RNA
    - Cofactors needed for the metabolism

Four Major Pathways of Glucose Utilization

- Storage
  - Can be stored in the polymeric form (starch, glycogen)
  - When there's excess energy
- Glycolysis
  - Generates energy via oxidation of glucose
  - Short-term energy needs
- Pentose Phosphate Pathway
  - Generates NADPH via oxidation of glucose
  - For detoxification and the biosynthesis of lipids and nucleotides
- Synthesis of Structural Polysaccharides
  - For example, in cell walls of bacteria, fungi, and plants



Extracellular matrix and cell wall polysaccharides

Glycogen, starch, sucrose

synthesis of structural polymers

storage

Glucose

oxidation via pentose phosphate pathway

oxidation via glycolysis

Ribose 5-phosphate

Pyruvate

Figure 14-1
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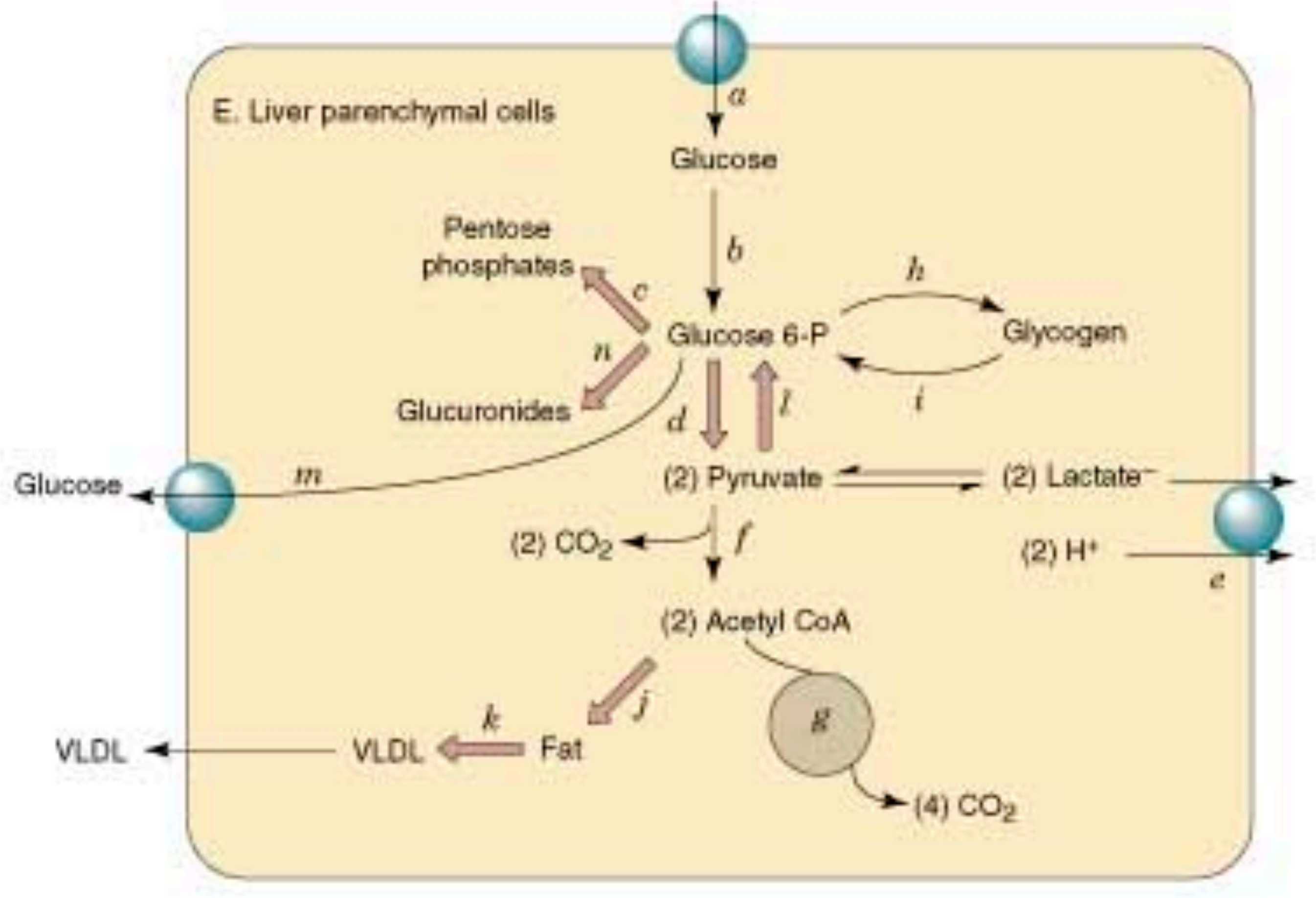
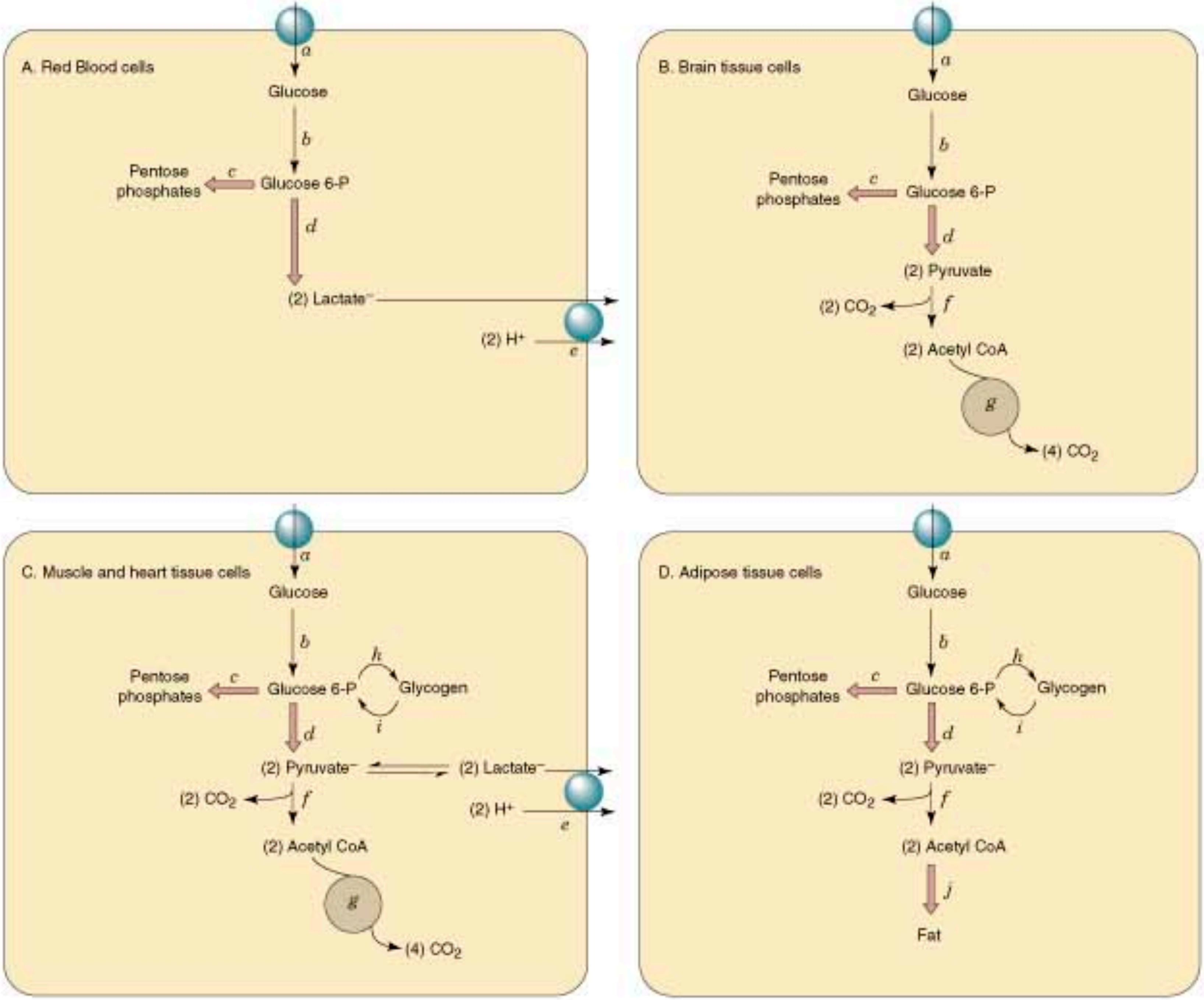
Glucose is metabolized differently in different cells

Our study in this course is on the liver and hepatocytes…



However, there are many more cells in the body…



Textbook of Biochemistry with Clinical Correlations, 7e edited by Thomas M. Devlin © 2011 John Wiley & Sons, Inc.

Glycolysis: Importance

- Almost universal central pathway of glucose catabolism
- Glycolysis occurs in ALL human cells
- Sequence of enzyme-catalyzed reactions by which glucose is converted into pyruvate
  - Pyruvate can be further aerobically oxidized
  - Pyruvate can be used as a precursor in biosynthesis

- Some of the oxidation-free energy is captured by the synthesis of ATP and NADH

- Research of glycolysis played a large role in the development of modern biochemistry
  - Understanding the role of coenzymes
  - Discovery of the pivotal role of ATP
  - Development of methods for enzyme purification

Glycolysis: Overview

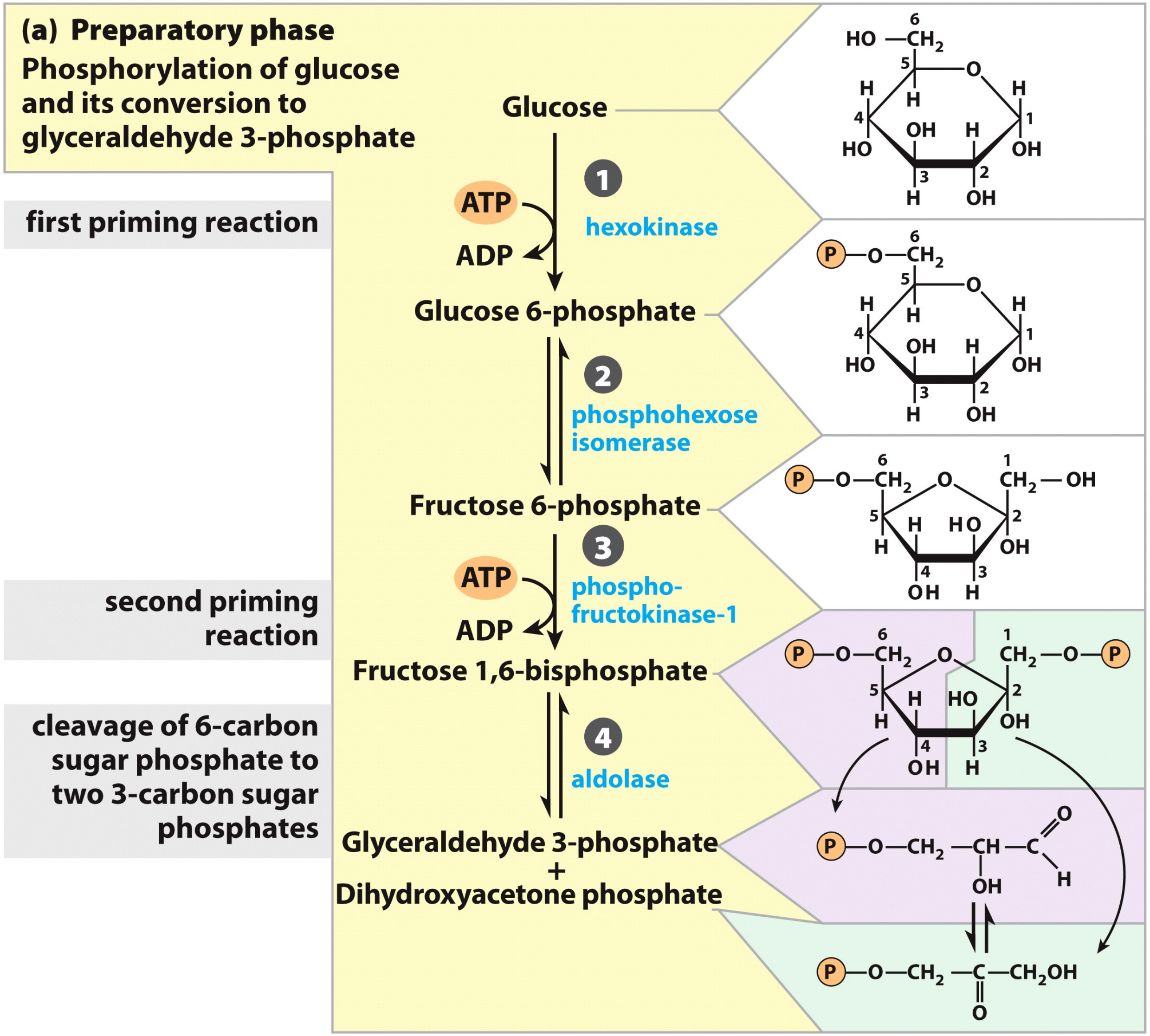
- In the evolution of life, glycolysis probably was one of the earliest energy-yielding pathways
- It developed before photosynthesis, when the atmosphere was still anaerobic

- Thus, the task upon early organisms was:
  How to extract free energy from glucose anaerobically?
- The solution:
  - First: Activate it by phosphorylation
  - Second: Collect energy from the high-energy metabolites
- Glycolysis is a sequence of 10 reactions, 5 are preparatory and 5 are energy-yielding *(two phases of glycolysis)*

Glycolysis: The Preparatory Phase

2 ATP molecules are used to raise the free energy of the intermediates

*For **each molecule of glucose** that passes through the preparatory phase, **two molecules of glyceraldehyde 3-phosphate** are formed.*



(a) Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate

first priming reaction

second priming reaction

cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates

Glucose

① ATP → ADP, **hexokinase**

Glucose 6-phosphate

② **phosphohexose isomerase** (reversible)

Fructose 6-phosphate

③ ATP → ADP, **phospho-fructokinase-1**

Fructose 1,6-bisphosphate

④ **aldolase** (reversible)

Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate

The "*lysis*" step of glycolysis

Figure 14-2 part 1
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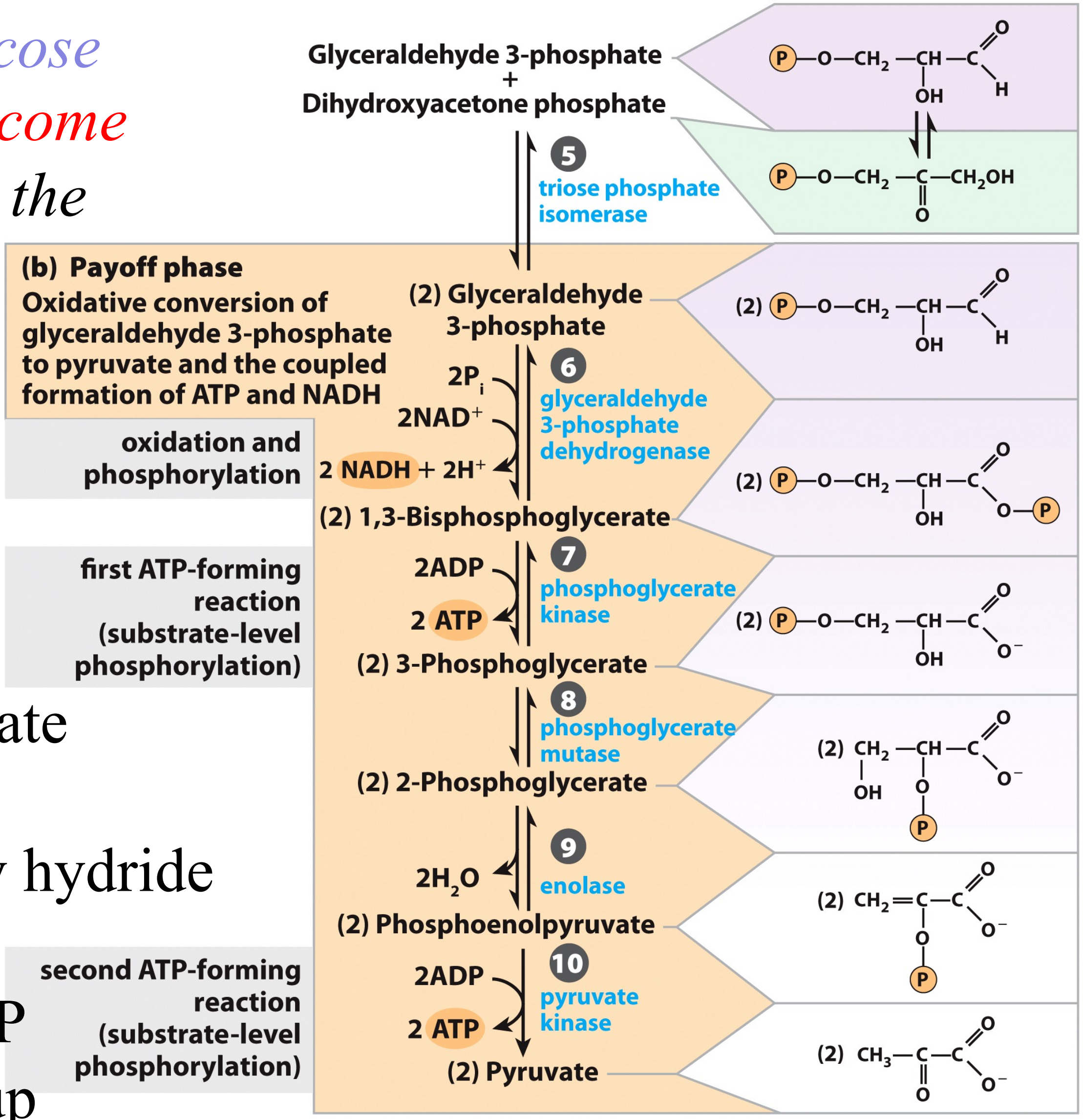
Glycolysis: The Payoff Phase

4 ATP are produced per glucose

2 ATP/glucose is the net outcome

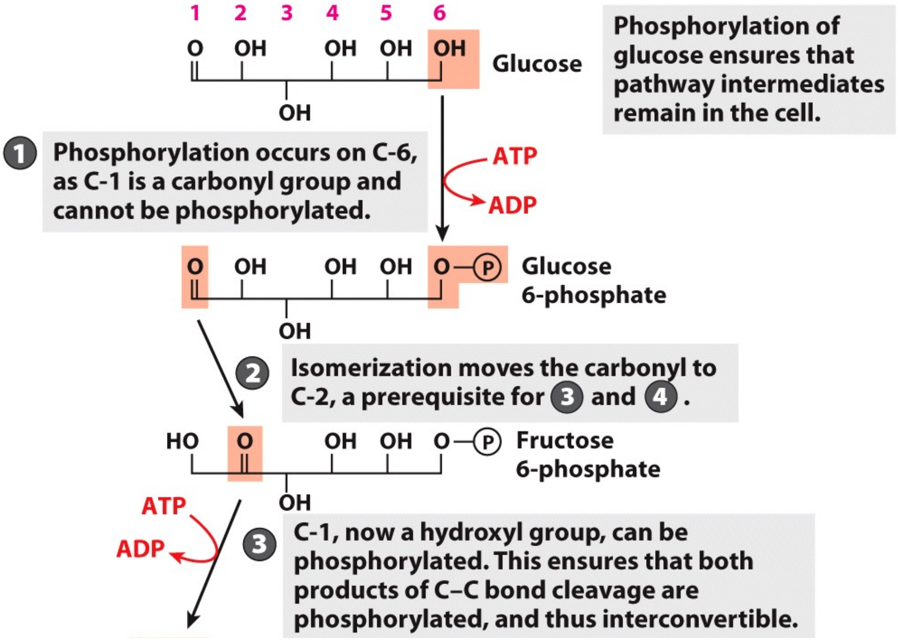
Energy is also conserved by the formation of 2 NADH molecules



3 types of chemical transformations:

1. **Breakage** of glucose backbone to yield pyruvate (6C → 2x 3C)
2. **Formation** of NADH by hydride transfer to NAD^+
3. **Phosphorylation** of ADP by high phosphoryl group potential compounds to make ATP

Chemical Logic of Glycolysis

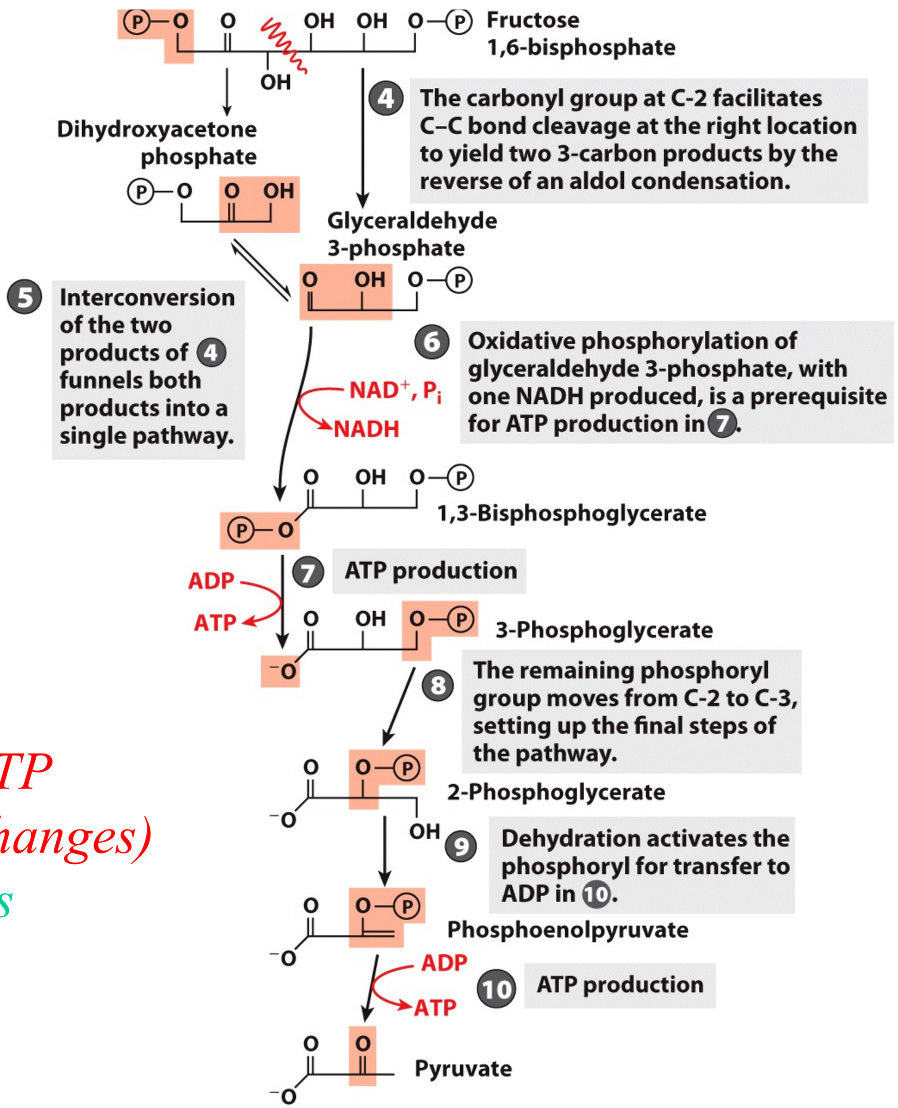


Phosphorylation of glucose ensures that pathway intermediates remain in the cell.

① Phosphorylation occurs on C-6, as C-1 is a carbonyl group and cannot be phosphorylated.

② Isomerization moves the carbonyl to C-2, a prerequisite for ③ and ④.

③ C-1, now a hydroxyl group, can be phosphorylated. This ensures that both products of C–C bond cleavage are phosphorylated, and thus interconvertible.



④ The carbonyl group at C-2 facilitates C–C bond cleavage at the right location to yield two 3-carbon products by the reverse of an aldol condensation.

⑤ Interconversion of the two products of ④ funnels both products into a single pathway.

⑥ Oxidative phosphorylation of glyceraldehyde 3-phosphate, with one NADH produced, is a prerequisite for ATP production in ⑦.

⑦ ATP production

⑧ The remaining phosphoryl group moves from C-2 to C-3, setting up the final steps of the pathway.

⑨ Dehydration activates the phosphoryl for transfer to ADP in ⑩.

⑩ ATP production

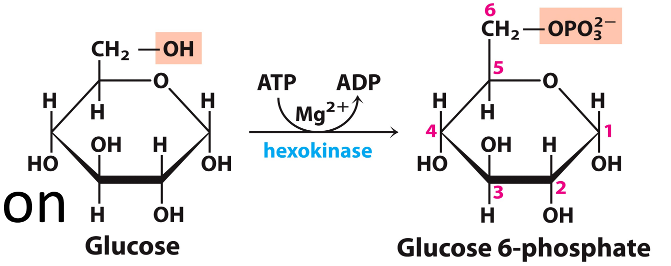
- *How is the formation of NADH and ATP coupled to glycolysis? (Free energy changes)*
- *Why are phosphorylated intermediates important in glycolysis?*

The Preparatory Phase

Step 1: Phosphorylation of Glucose

- Rationale
  - Traps glucose inside the cell
  - Lowers intracellular glucose concentration to allow further uptake
- This process uses the energy of ATP
- The first “priming” reaction
- Hexokinase in eukaryotes, and glucokinase in prokaryotes and liver (**isozymes**: 2 or more enzymes encoded in different genes but catalyze the same reaction)
- Soluble cytosolic enzyme (like all other glycolytic enzymes)
- Nucleophilic oxygen at C6 of glucose attacks the last (γ) phosphate of ATP
- ATP-bound Mg^{2+} facilitates this process by shielding the negative charges on ATP

- Regulated mainly by substrate inhibition



$\Delta G'° = -16.7$ kJ/mol

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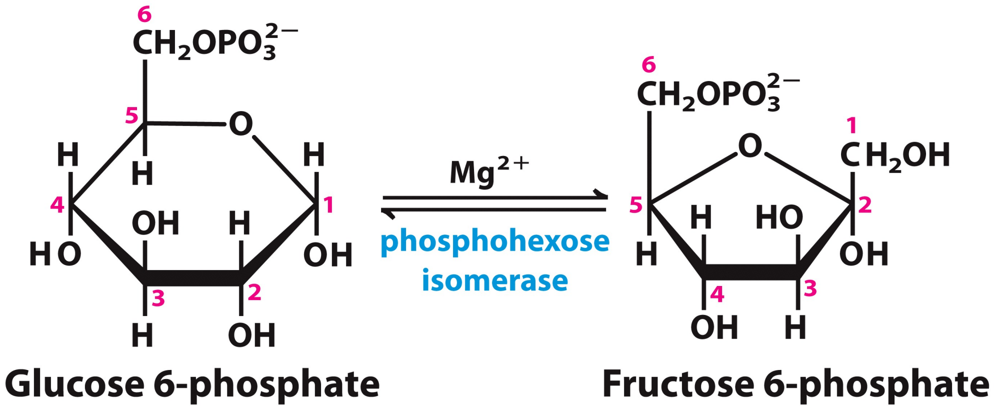
Step 2: Phosphohexose Isomerization



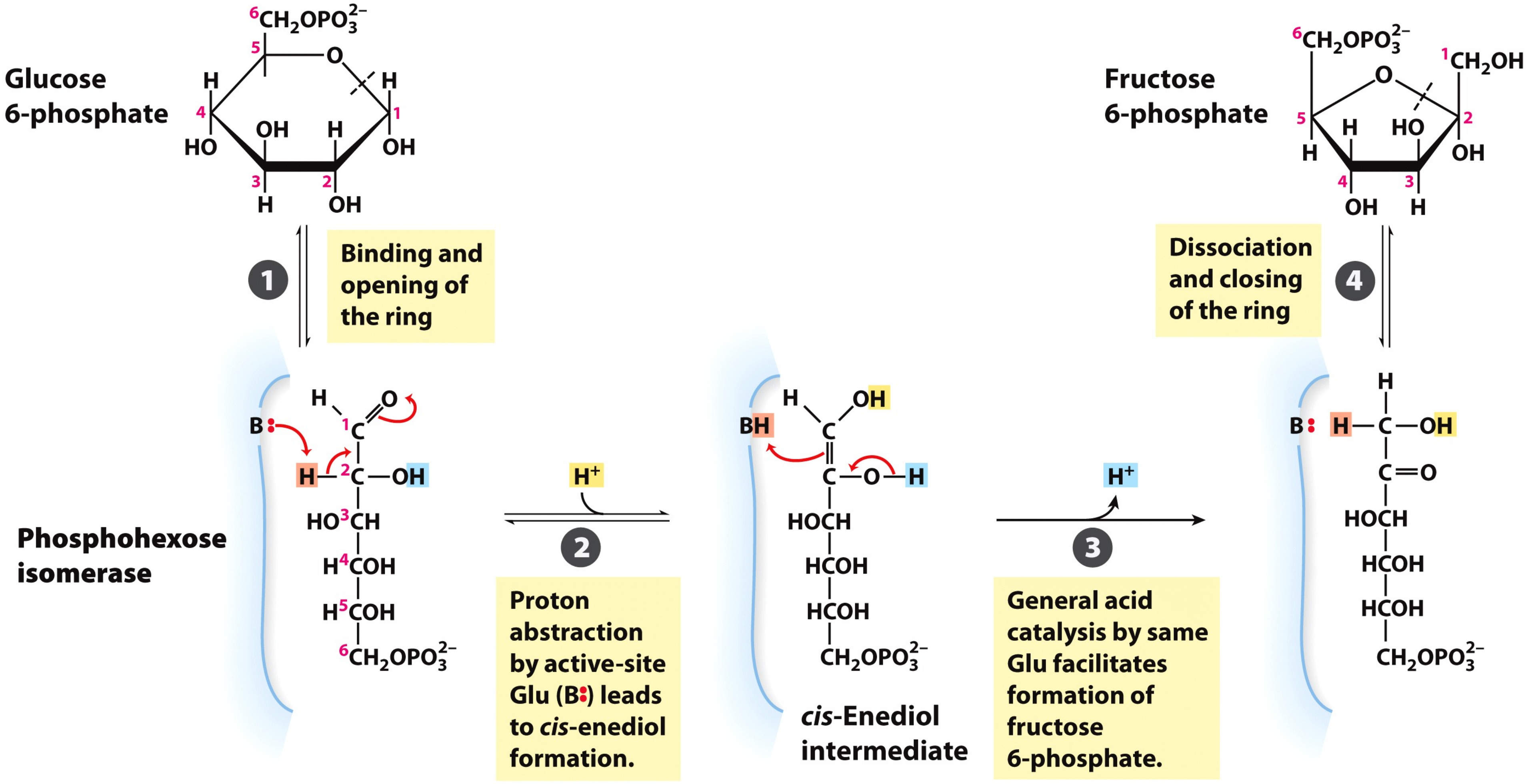
- Rationale
  - C1 of fructose is easier to phosphorylate by PFK
  - Allows for symmetrical cleave by aldolase

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

- An aldose (glucose) can isomerize into a ketose (fructose) via an *enediol* intermediate
- The isomerization is catalyzed by the active-site glutamate, via general acid/base catalysis

- Product concentration kept low to drive forward

Mechanism of Phosphohexose Isomerase



Glucose 6-phosphate

Fructose 6-phosphate

Phosphohexose isomerase

① **Binding and opening of the ring**

② **Proton abstraction by active-site Glu (B:) leads to *cis*-enediol formation.**

***cis*-Enediol intermediate**

③ **General acid catalysis by same Glu facilitates formation of fructose 6-phosphate.**

④ **Dissociation and closing of the ring**

Figure 14-5

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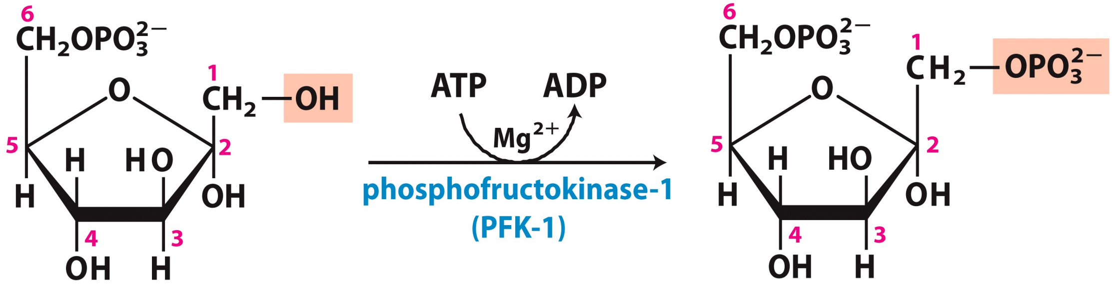
Step 3: 2nd Priming Phosphorylation



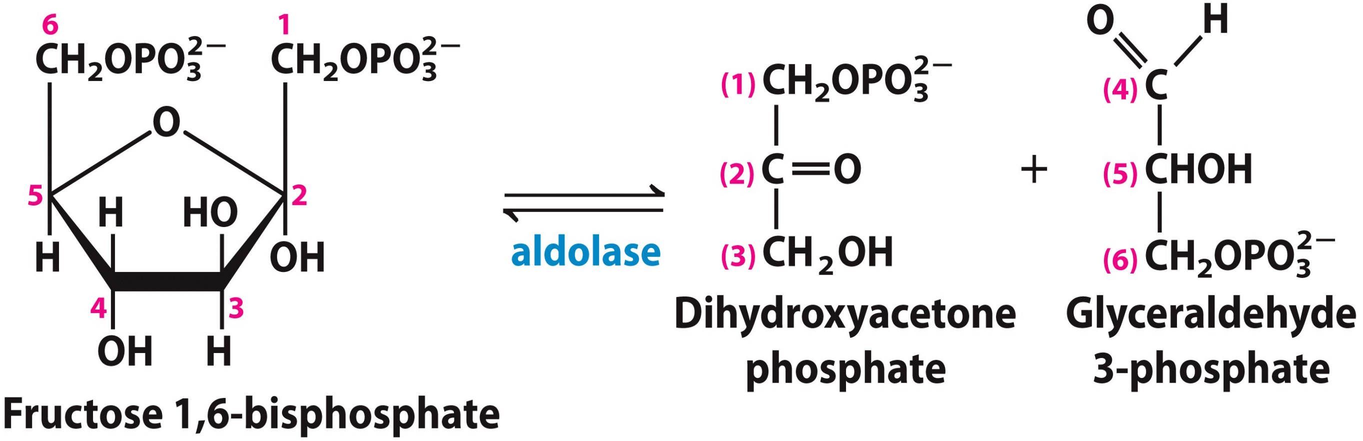
Fructose 6-phosphate

Fructose 1,6-bisphosphate

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

- Rationale
  - Further activation of glc
  - Allows for 1 phosphate/ 3-carbon sugar after step 4
- First Committed Step of Glycolysis
  - fructose 1,6-bisphosphate is committed to become pyruvate and yield energy whereas g-6-p and f-6-p have other possible fates
- This process uses the energy of ATP
- Phosphofructokinase-1 is highly regulated
  - By ATP, ADP, AMP, fructose-2,6-bisphosphate, and other metabolites (detailed next chapter)
  - Do not burn glucose if there is plenty of ATP

Step 4: Aldol Cleavage of F-1,6-bP



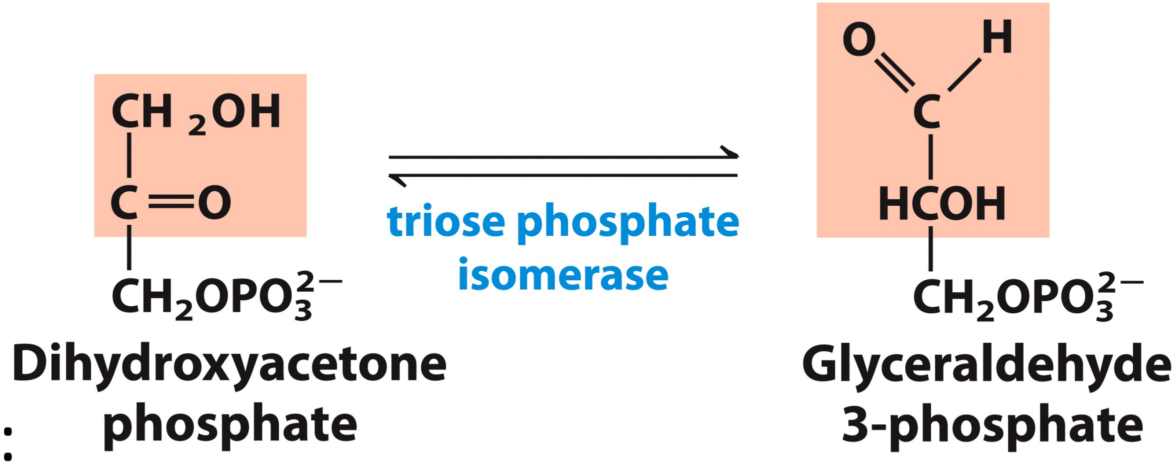
$\Delta G'^{\circ} = 23.8$ kJ/mol

- Rationale
  - Cleavage of a 6-C sugar into two 3-C sugars
  - High-energy phosphate sugars are 3-C sugars

- The reverse process is the familiar aldol condensation
- Animal and plant aldolases employ covalent catalysis
- Fungal and bacterial aldolases employ metal ion catalysis

- *What is the mechanism of aldolase (class I)?*

Step 5: Triose Phosphate Interconversion



Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

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

- Rationale:
  - Allows glycolysis to proceed by one pathway

- Aldolase creates two triose phosphates:
  - Dihydroxyacetone Phosphate (DHAP)
  - Glyceraldehyde-3-Phosphate (GAP)
- Only GAP is the substrate for the next enzyme
- DHAP must be converted to GAP

- Similar mechanism as phosphohexose isomerase
- Completes preparatory phase

Glucose Carbons in GAP

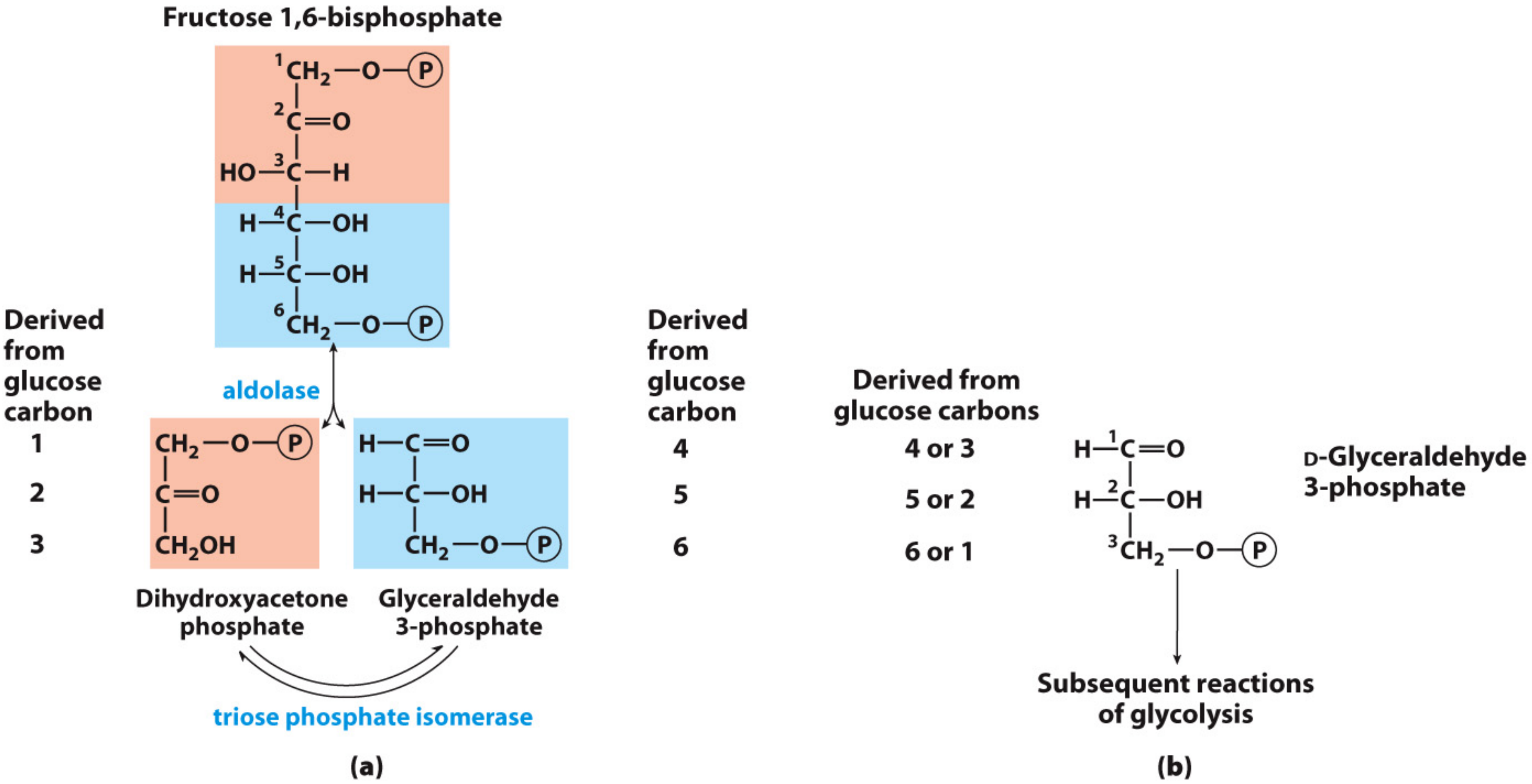


Figure 14-7

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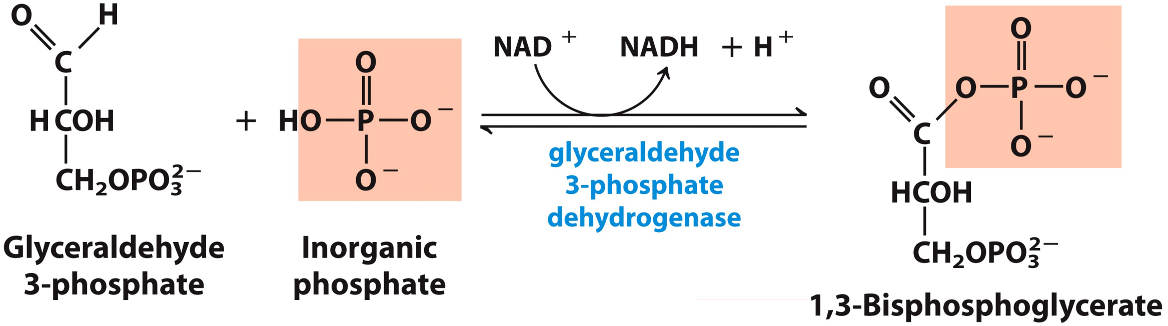
The Payoff Phase

Step 6: Oxidation of GAP



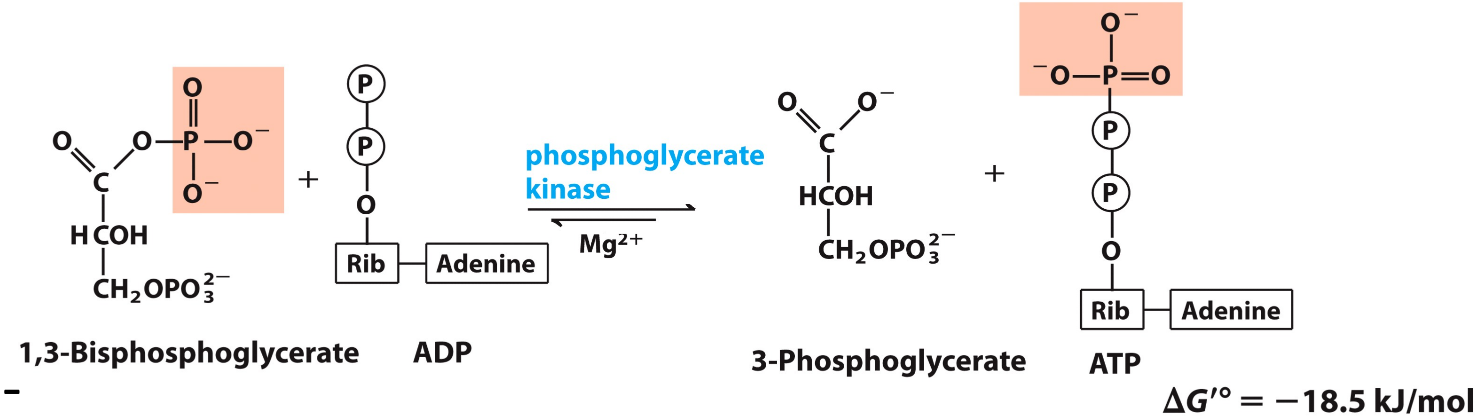
$\Delta G'^{\circ} = 6.3$ kJ/mol

- Rationale:
  - Generation of a high-energy phosphate cpd
  - Incorporates inorganic phosphate **which allows for net production of ATP via glycolysis!**

- First energy-yielding step in glycolysis
- Oxidation of aldehyde with NAD^+ gives NADH and an **acyl phosphate**
- Active site cysteine
  - Forms high-energy thioester intermediate
  - Subject to inactivation by oxidative stress

- Thermodynamically unfavorable/reversible
  - Coupled to next reaction to pull forward
- *What is the mechanism of GAPDH mechanism?*

Step 7: 1st Production of ATP

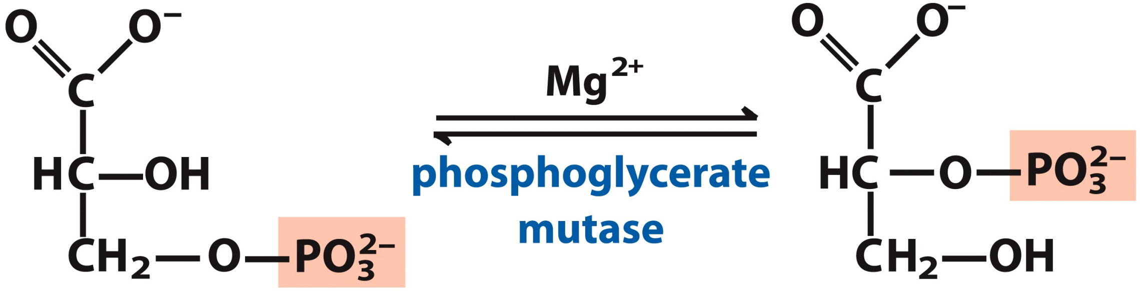


- Rationale:
  - Substrate-level phosphorylation to make ATP
- 1,3-bisphosphoglycerate is a high-energy compound
  - can donate the phosphate group to ADP to make ATP
- The enzyme is named after the reverse reaction
- **Substrate-level phosphorylation**: the formation of ATP by group transfer from a substrate
  - Is reversible because of coupling to GAPDH reaction
  - Steps 6 and 7 are strongly coupled:

    Glyceraldehyde 3-P + ADP + P_i + NAD^+ $\rightleftharpoons$ 3-phosphoglycerate + ATP + NADH + H^+

    $\Delta G'^{\circ} = -12.2$ kJ/mol

Step 8: Migration of the Phosphate



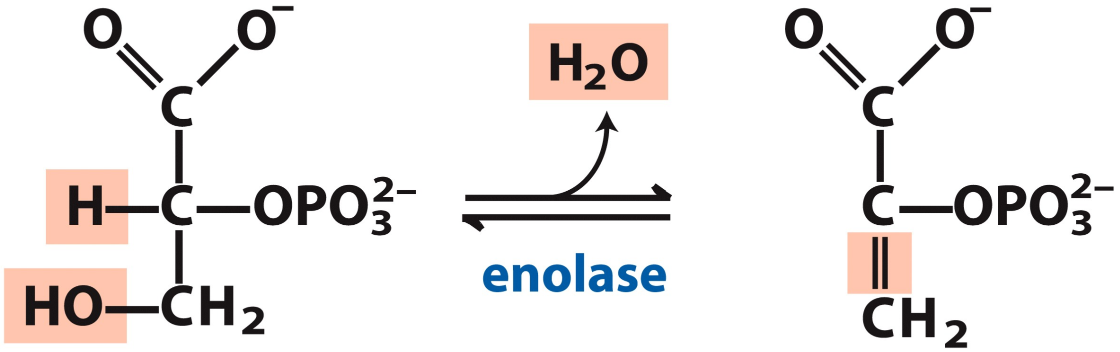
3-Phosphoglycerate

2-Phosphoglycerate

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

- Rationale:
  - Be able to form high-energy phosphate compound
- Mutases catalyze the (apparent) migration of functional groups
- One of the active site histidines is post-translationally modified to phosphohistidine
- *What is the mechanism of PG mutase?*

Step 9: Dehydration of 2-PG to PEP



2-Phosphoglycerate

Phosphoenolpyruvate

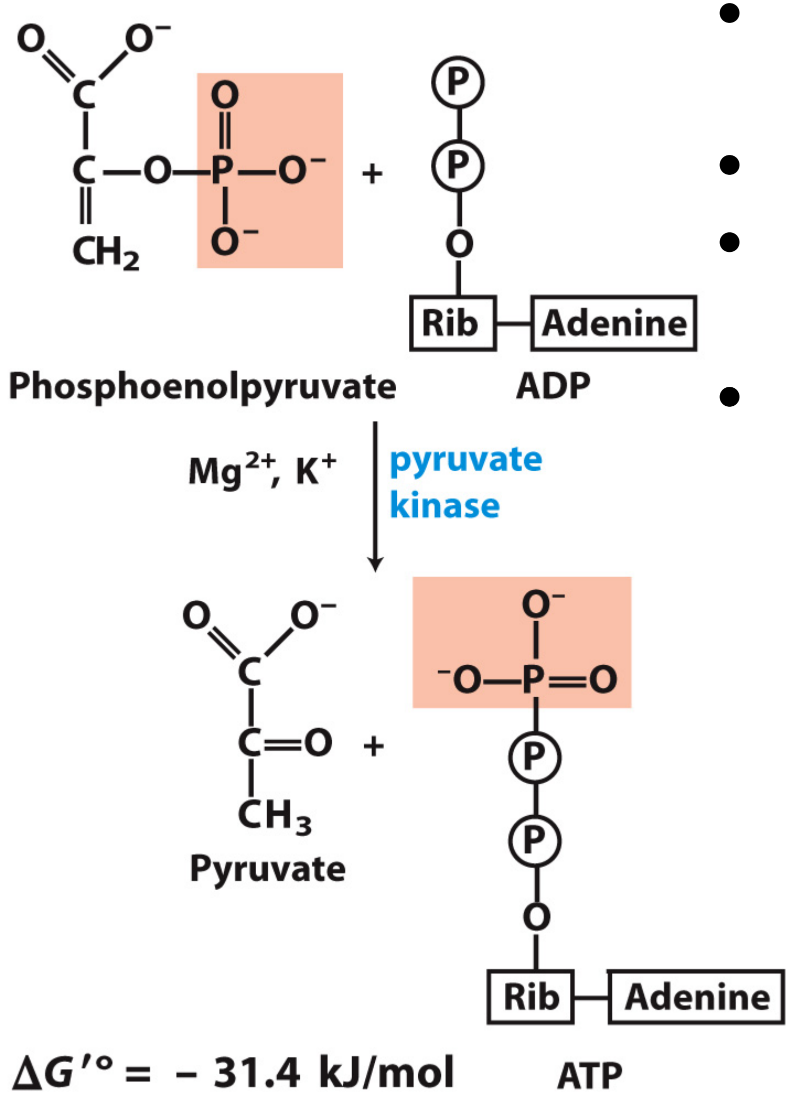
$\Delta G'° = 7.5$ kJ/mol

- Rationale
  - – Generate a high-energy phosphate compound

- 2-Phosphoglycerate is not a good enough phosphate donor ($\Delta G'^{o} = -17.6$ kJ/mol; $\Delta G'^{o}_{PEP} = -61.9$ kJ/mol)

  - Product concentration kept low to pull forward

Step 10: 2nd Production of ATP



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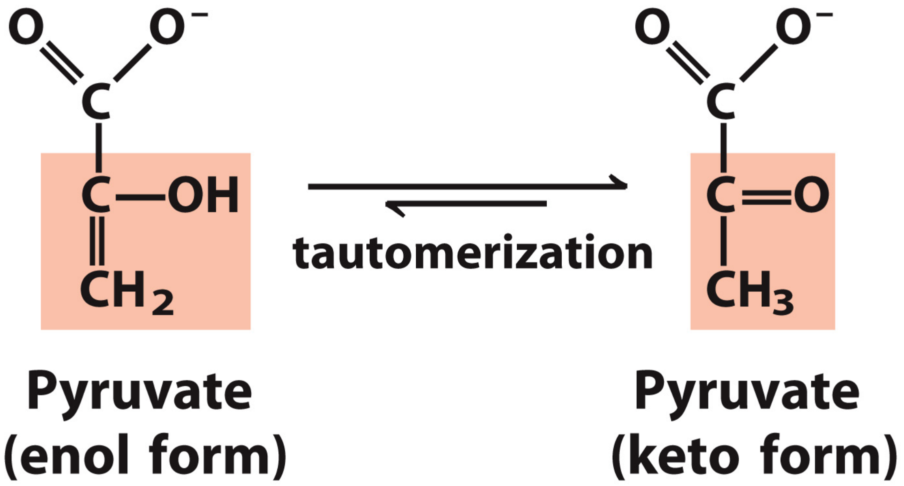
- Substrate-level phosphorylation to make ATP
- net production of 2 ATP/glucose
- Loss of phosphate from PEP yields an enol that tautomerizes into ketone.
- Tautomerization
  - effectively lowers the concentration of the reaction product
  - drives the reaction toward ATP formation



Summary of Glycolysis

Glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 Pyruvate + 2 NADH + 2 H^+ + 2 H_2O+ ***2 ATP***

- Used:
  - 1 glucose; 2 ATP; 2 NAD^+
- Made:
  - 2 pyruvate
    - Various different fates
  - 4 ATP
    - Used for energy-requiring processes within the cell
  - 2 NADH
    - Must be reoxidized to NAD^+ in order for glycolysis to continue
- Glycolysis is heavily regulated
  - Ensure proper use of nutrients
  - Ensure production of ATP only when needed
  - Under anaerobic conditions, both the rate and the total amount of glucose consumption are many times greater than with oxygen present, **why???**

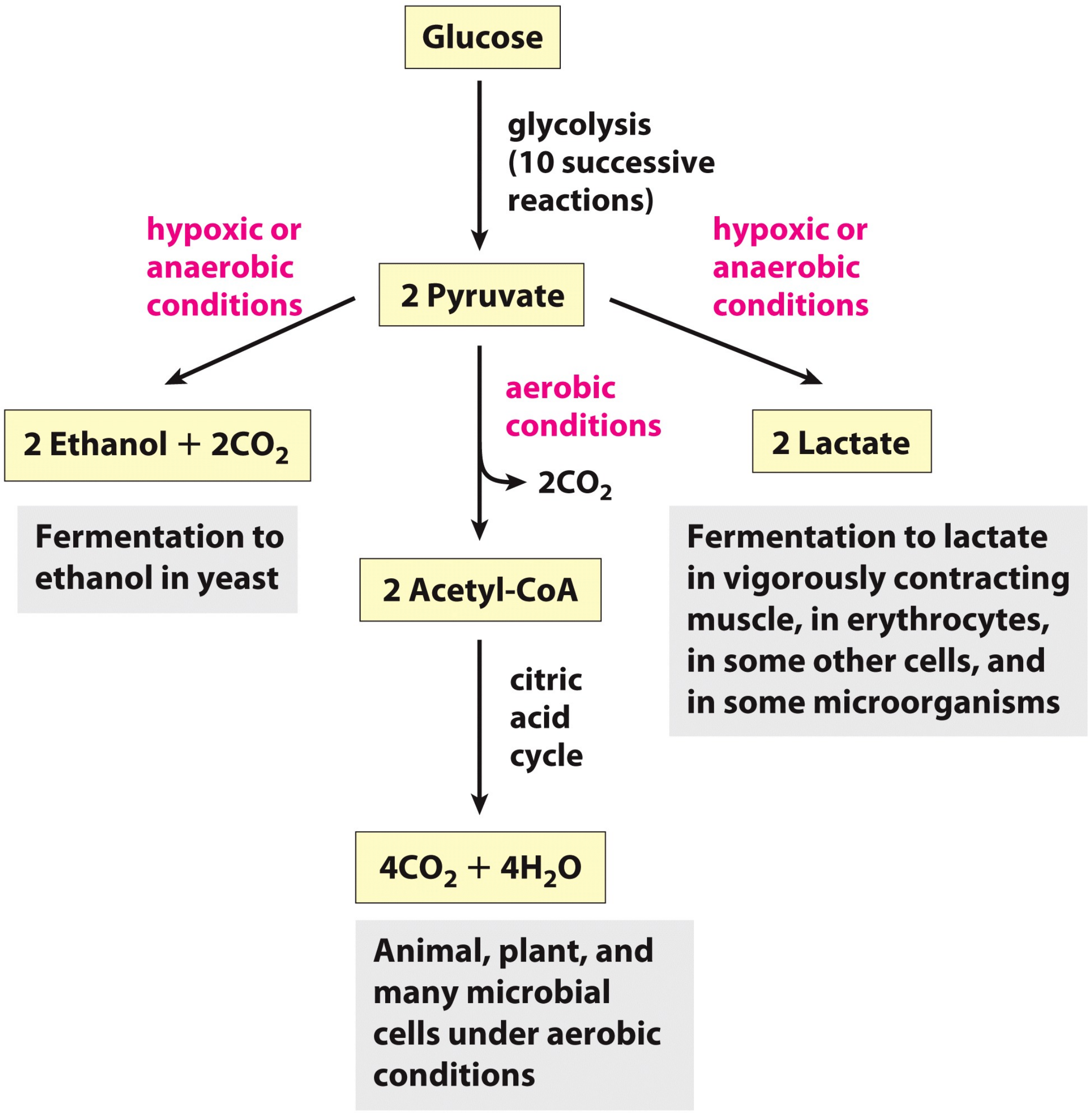
Glycolysis: Fates of Pyruvate

- In most organisms pyruvate is metabolized via one of three catabolic routes:

1. *Citric acid cycle:* pyruvate is oxidized and decarboxylated to release CO_2 (the electrons that are moving go through ETC in mito and are used to make ATP; aerobic conditions)
2. *Lactic acid fermentation:* after vigorous exercise, $[O_2]$ in muscles is low (**hypoxia**) NADH cannot be reoxidized to NAD^+ for glycolysis to continue → pyruvate is reduced to lactate accepting electrons from NADH (regenerating NAD^+). Certain tissues (RBC and retina) ferment pyruvate into lactate even under aerobic conditions
3. *Alcohol fermentation:* some yeasts and plants can ferment pyruvate into ethanol and CO_2 (important for beverage production and baking)

- Pyruvate also some anabolic fates (can produce a.a. alanine or fatty acids)

Fates of Pyruvate



Glucose

glycolysis
(10 successive
reactions)

2 Pyruvate

hypoxic or
anaerobic
conditions

$2\ \text{Ethanol} + 2CO_2$

Fermentation to
ethanol in yeast

aerobic
conditions

$2CO_2$

2 Acetyl-CoA

citric
acid
cycle

$4CO_2 + 4H_2O$

Animal, plant, and
many microbial
cells under aerobic
conditions

hypoxic or
anaerobic
conditions

2 Lactate

Fermentation to lactate
in vigorously contracting
muscle, in erythrocytes,
in some other cells, and
in some microorganisms

Figure 14-4
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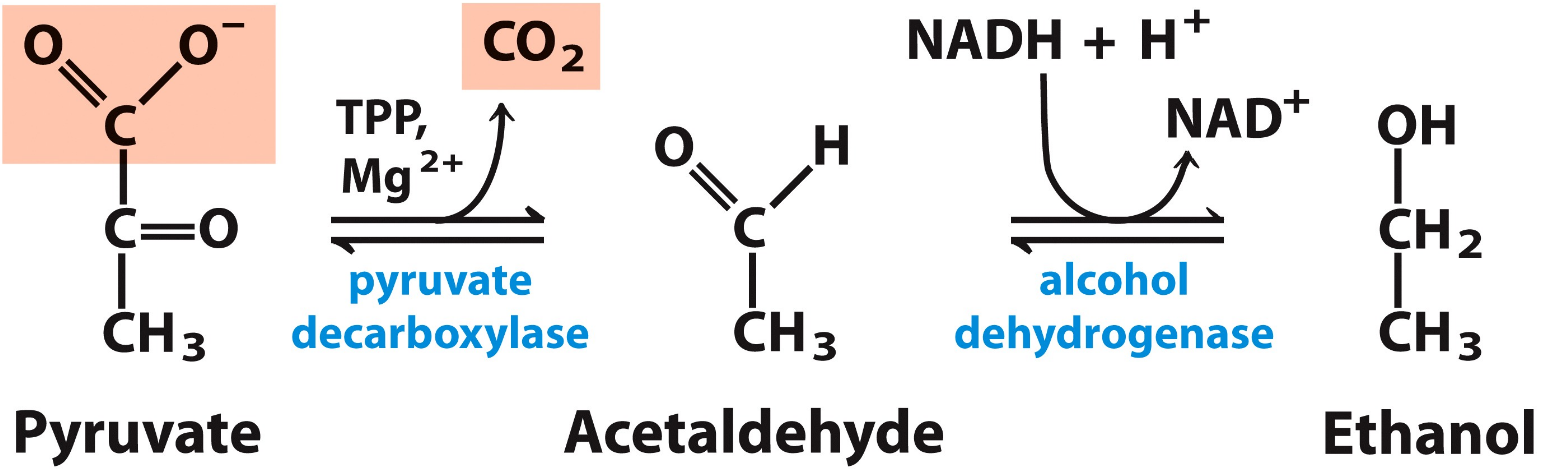
Anaerobic Glycolysis: Fermentation

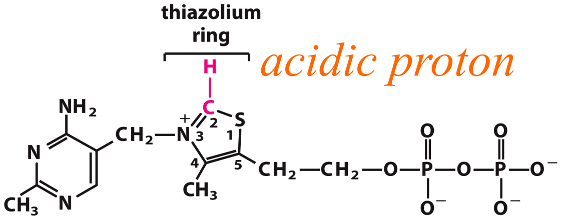
- *Generation of energy (ATP) without consuming oxygen or NAD$^+$*
- No net change in oxidation state of the sugars
- Reduction of pyruvate to another product
- Regenerates NAD$^+$ for further glycolysis under anaerobic conditions
- The process is used in the production of food from beer to yogurt to soy sauce

Yeast undergo Ethanol Fermentation



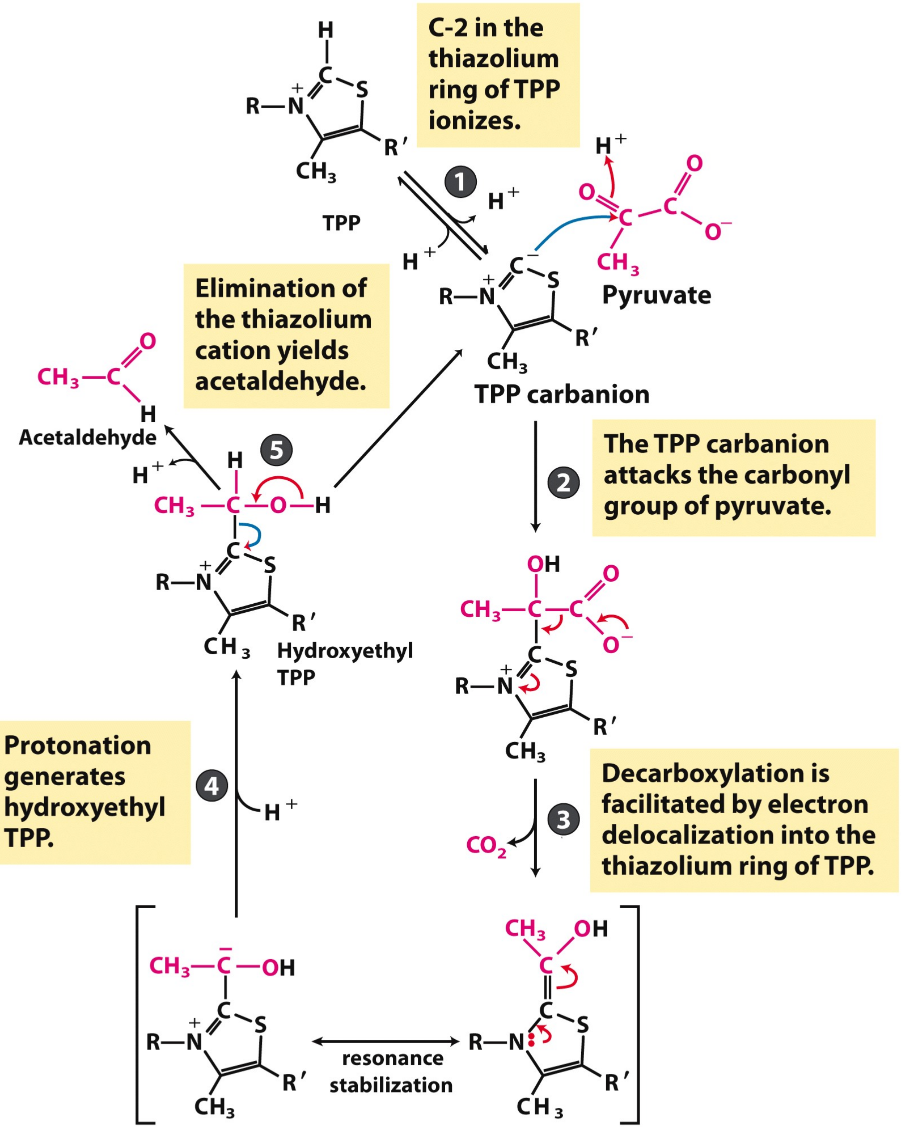
- Two-step reduction of pyruvate to ethanol, irreversible
- Humans do not have *pyruvate decarboxylase*
- Humans do express *alcohol dehydrogenase* for ethanol metabolism
- CO_2 produced in the first step is responsible for:
  - carbonation in beer
  - dough rising when baking bread
- Both steps require cofactors
  - Pyruvate decarboxylase: Mg^{2+} and thiamine pyrophosphate (TPP)
  - Alcohol dehydrogenase: Zn^{2+} and NADH

TPP is a Common Acetaldehyde Carrier



Thiamine pyrophosphate (TPP)

- Coenzyme derived from vitamin B_1 (thiamine)
- Lack of B_1 → *beriberi* (swelling, pain, paralysis and death)
- Cleavage of bonds adjacent to carbonyl groups
- Thiazolium ring of TPP stabilizes carbanion intermediates by providing an electrophilic structure into which the carbanion electrons can be delocalized by resonance “electron sinks”



C-2 in the thiazolium ring of TPP ionizes.

The TPP carbanion attacks the carbonyl group of pyruvate.

Decarboxylation is facilitated by electron delocalization into the thiazolium ring of TPP.

Protonation generates hydroxyethyl TPP.

Elimination of the thiazolium cation yields acetaldehyde.

Figure 14-15c

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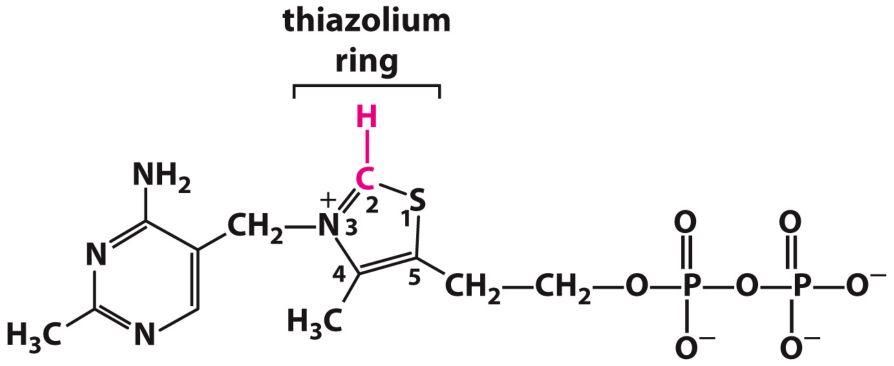
TPP Is a Common Acetaldehyde Carrier



Thiamine pyrophosphate (TPP)

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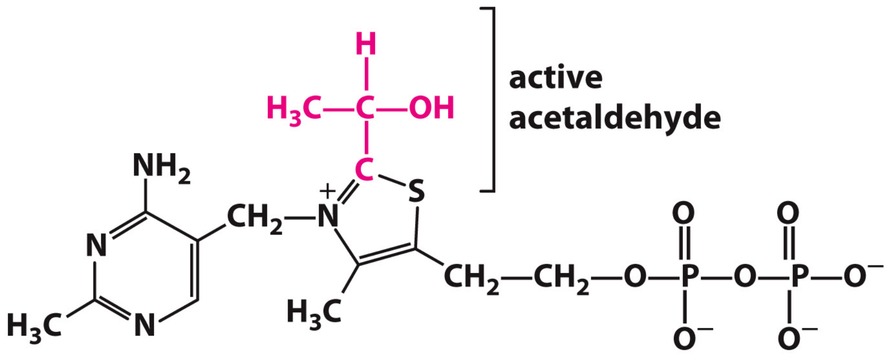

Hydroxyethyl thiamine pyrophosphate

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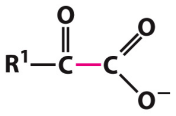
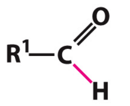
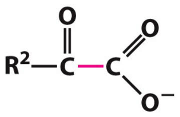
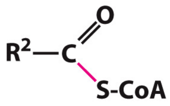
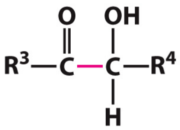
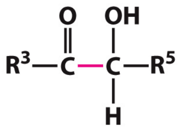
TABLE 14-1 Some TPP-Dependent Reactions

Enzyme	Pathway(s)	Bond cleaved	Bond formed
Pyruvate decarboxylase	Ethanol fermentation		
Pyruvate dehydrogenase α-Ketoglutarate dehydrogenase	Synthesis of acetyl-CoA Citric acid cycle		
Transketolase	Carbon-assimilation reactions Pentose phosphate pathway		

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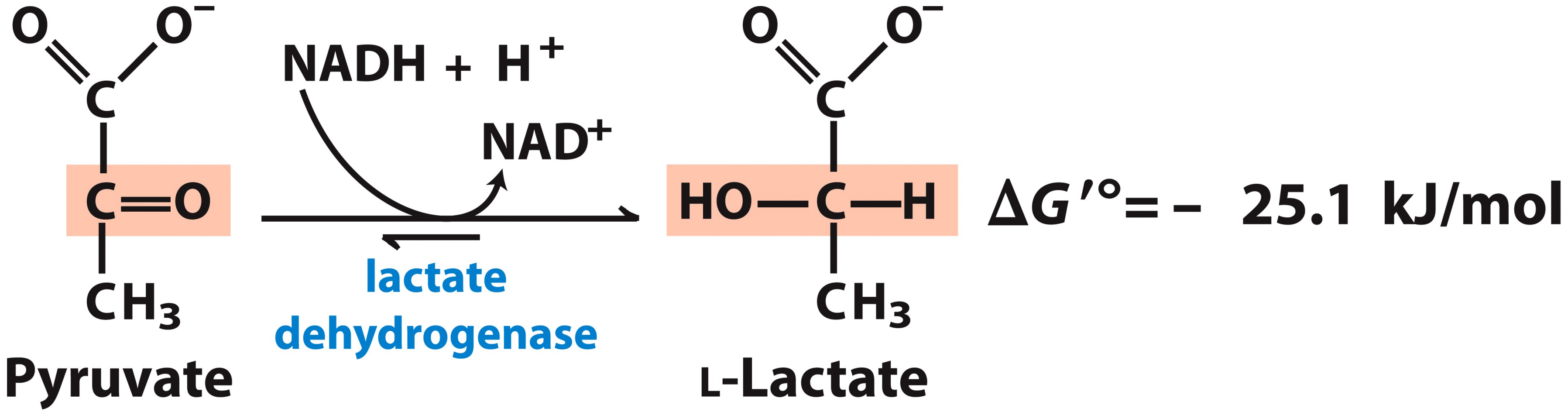
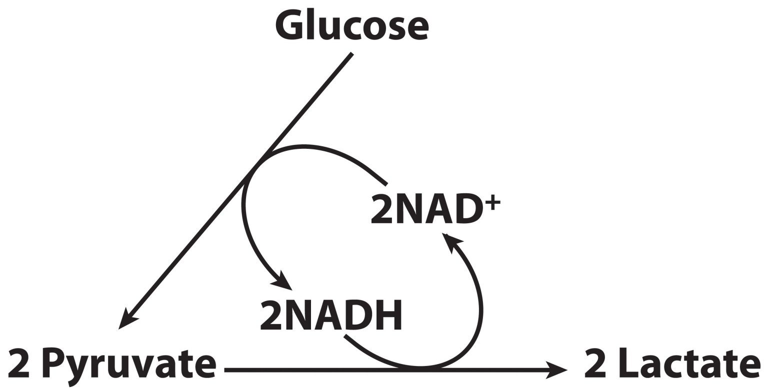
Animals undergo lactic acid fermentation



- Reduction of pyruvate to lactate, reversible
- Equilibrium favors lactate formation
- During strenuous exercise, lactate builds up in the muscle
  - Generally less than 1 minute (even most toned athletes cannot sprint at highest speeds for more than a minute!)
- The acidification of muscle prevents its continuous strenuous work

Lactic Acid Fermentation

- *No net change in NAD^+ or NADH levels*



Unnumbered 14 p563b
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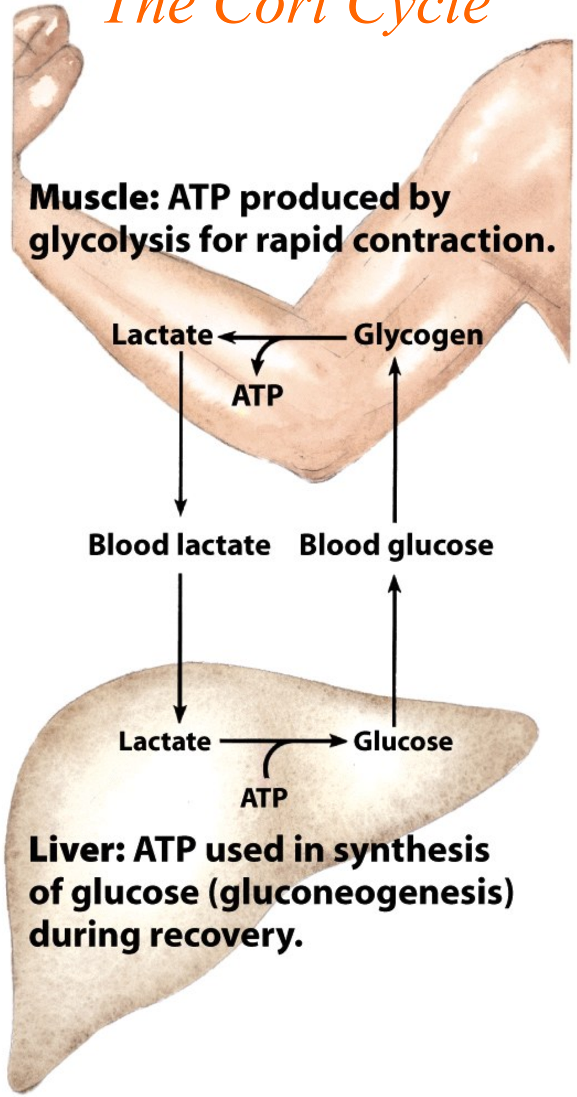
- Lactate can be transported to the liver to be converted to glucose (the Cori cycle)
- Requires a recovery time
  - High amount of oxygen consumption to fuel gluconeogenesis
  - Restores muscle glycogen stores
  - Heavy breathing is required to replenish oxygen to repay the “oxygen debt”

The Cori Cycle



Muscle: ATP produced by glycolysis for rapid contraction.

Liver: ATP used in synthesis of glucose (gluconeogenesis) during recovery.

Figure 23-20
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