

## Glycolysis (Continued)

### Importance of Phosphorylated Intermediates

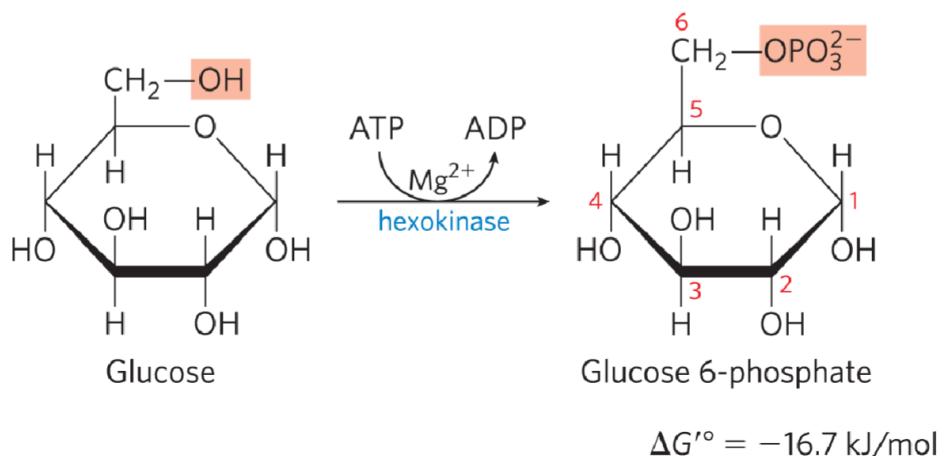
- All nine intermediates are phosphorylated
- Functions of the phosphoryl groups:
  - Prevent glycolytic intermediates from leaving the cell
  - Serve as essential components in the enzymatic conservation of metabolic energy
  - Lower the activation energy and increase the specificity of the enzymatic reactions

### The Preparatory Phase of Glycolysis Requires ATP

- In the preparatory phase of glycolysis:
  - Two molecules of ATP are invested to activate **glucose** to **fructose 1,6-bisphosphate**
  - The bond between C-3 and C-4 of fructose 1,6-bisphosphate is then broken to yield two molecules of triose phosphate

#### (Step 1) Phosphorylation of Glucose

- Hexokinase activates glucose by phosphorylating at C-6 to yield **glucose 6-phosphate**
  - ATP serves as the phosphoryl donor
  - hexokinase requires  $Mg^{2+}$  for its activity
  - irreversible under intracellular conditions

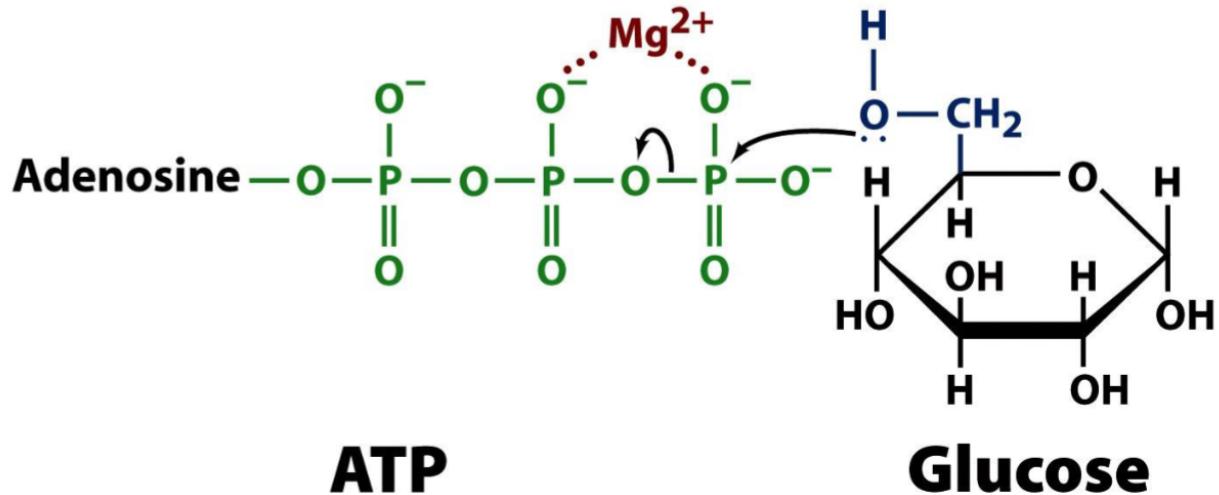


Hexokinase commits glucose to the hexose phosphate pool by converting glucose to glucose-6-phosphate (G6P)

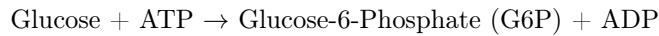
#### Hexokinase mechanism basics

- Hexokinase relies on magnesium for stabilizing triphosphate
- Shielding the negative phosphate charges allows for nucleophilic attack by hydroxyl
- Example in metal-ion catalysis

## partial mechanism:



### Hexokinase Reaction:

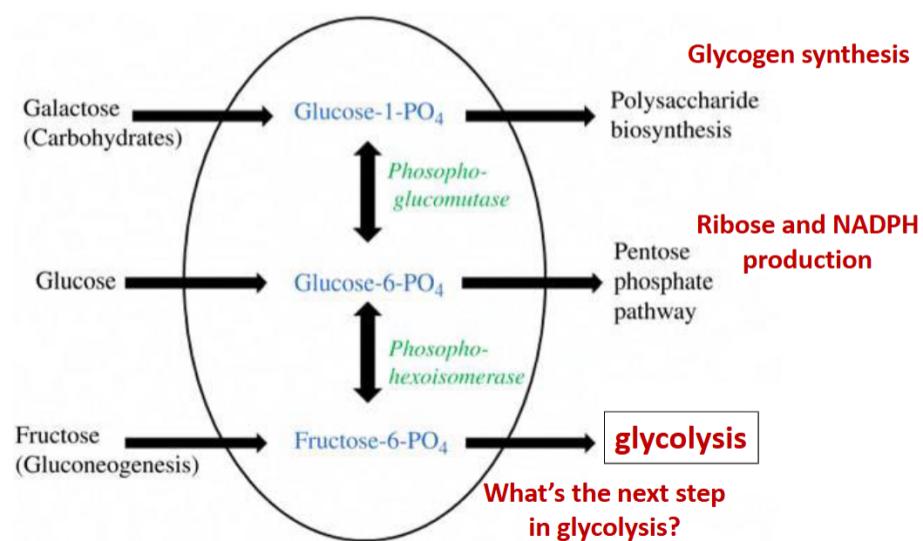


### Commitment to Metabolic Pool:

- The hexose phosphate pool
- Significance:
  - Traps glucose inside the cell (G6P cannot cross the cell membrane)
  - Commits glucose to further metabolism within the cell

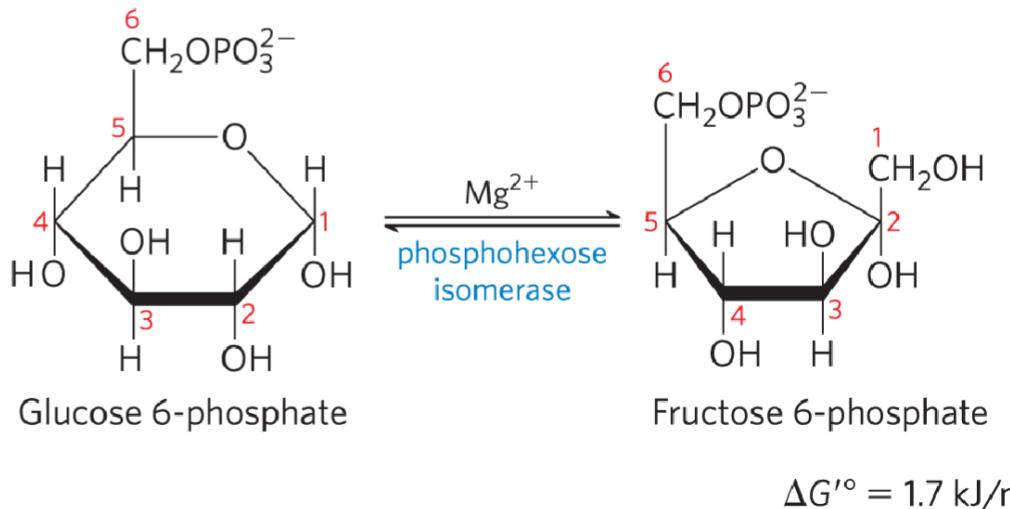
### The pool of hexoses

**These intermediates aren't just shared by glycolysis and gluconeogenesis...**



## (Step 2) Conversion of Glucose 6-Phosphate to Fructose 6-Phosphate

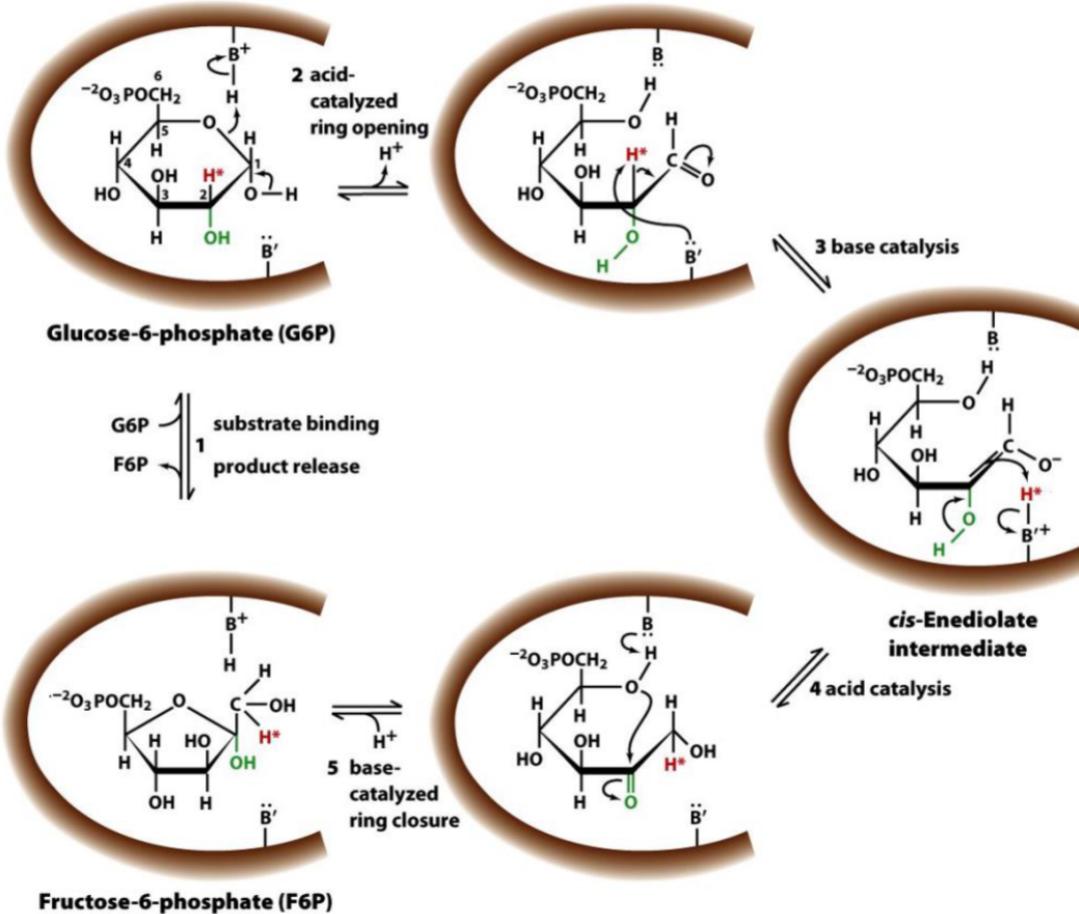
- Phosphohexose isomerase (phosphoglucose isomerase) catalyzes the reversible isomerization of glucose 6-phosphate to fructose 6-phosphate
  - mechanism involves an enediol intermediate
  - reaction readily proceeds in either direction



Nelson & Cox, Lehninger Principles of Biochemistry, 8e, © 2021  
W. H. Freeman and Company

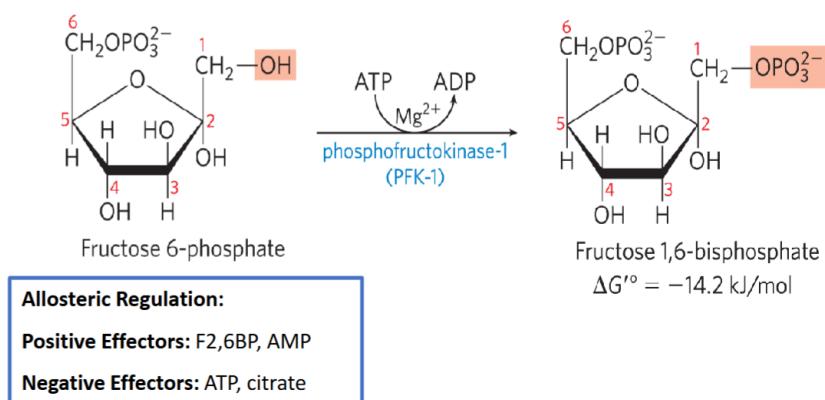
- The rearrangement of G6P to F6P is critical for the efficient progression of glycolysis. It ensures compatibility with downstream enzymes, facilitates the symmetrical cleavage of the sugar, and prepares the molecule for the energy-investment step catalyzed by PFK-1. Without this rearrangement, glycolysis could not proceed in a coordinated or efficient manner.

## Phosphohexose isomerase mechanism



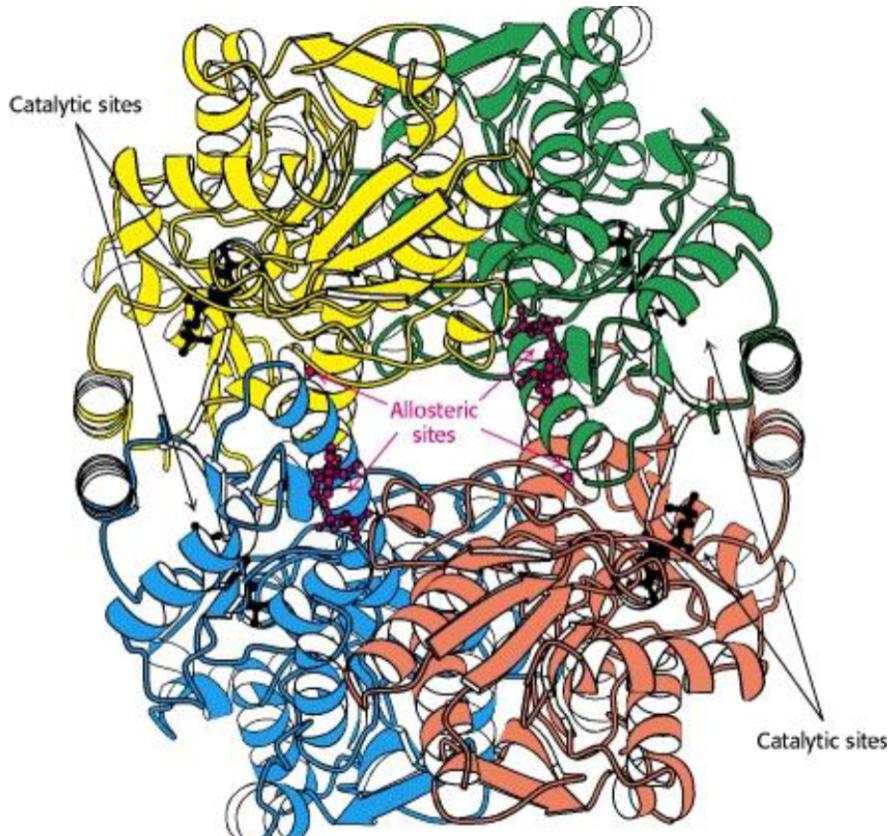
### (Step 3) Phosphorylation of Fructose 6-Phosphate to Fructose 1,6-Bisphosphate

- Phosphofructokinase-1 (PFK-1) is a key regulatory enzyme in glycolysis
- Catalyzes the transfer of a phosphoryl group from ATP to fructose 6-phosphate to yield fructose 1,6-bisphosphate
  - Essentially irreversible under cellular conditions
  - The first "committed" step in the glycolytic pathway



## Allosteric Regulation of PFK-1

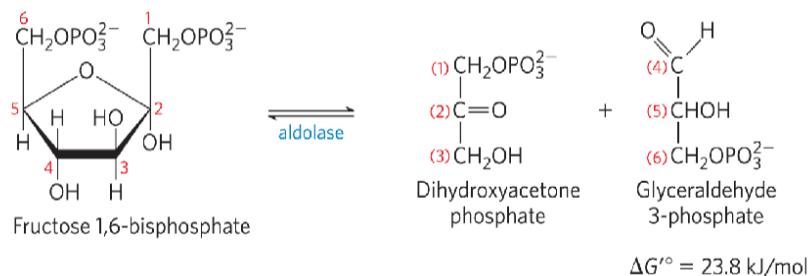
- Activity increases when:
  - ATP supply is depleted
  - ADP and AMP accumulate
- Fructose 2,6-bisphosphate is a potent allosteric activator
- PFK-1 acts as a metabolic "gatekeeper", integrating signals from the cell's energy status and hormonal environment. This regulation allows glycolysis to be precisely tuned to the cell's energy demands, maintaining metabolic balance and energy homeostasis



- Fructose 6-Phosphate (F6P), an intermediate of glycolysis, is phosphorylated by phosphofructokinase-2 (PFK-2) to form Fructose 2,6-bisphosphate (F2, 6BP). F2,6BP is not an intermediate in glycolysis or gluconeogenesis but acts as a potent allosteric regulator of PFK-1, stimulating glycolysis and inhibiting gluconeogenesis.

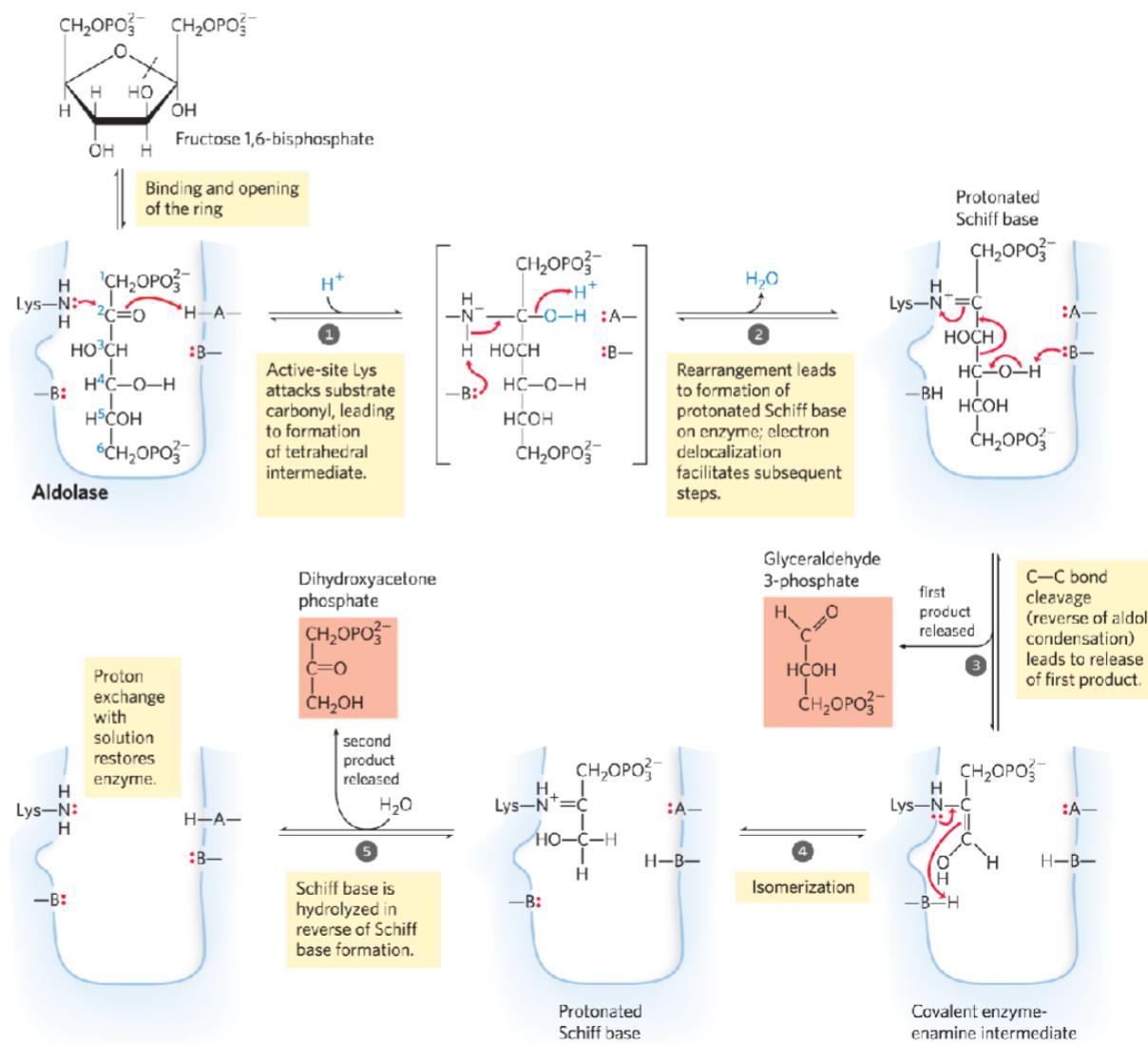
## (Step 4) Cleavage of Fructose 1,6-Bisphosphate

- Fructose 1,6-Bisphosphate aldolase (aldolase) catalyzes a reverse aldol condensation and cleaves fructose 1,6-bisphosphate to yield **glyceraldehyde 3-phosphate** and **dihydroxyacetone phosphate**
- Reversible because reactant concentrations are low in the cell.



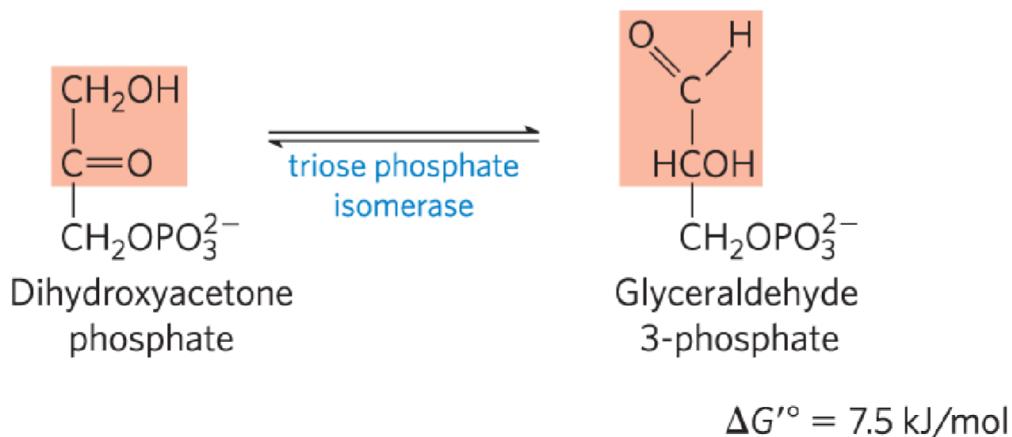
## The Class I Aldolase Reaction

- Class I = found in animals and plants
- Class II = found in fungi and bacteria
  - Do not form the Schiff base intermediate



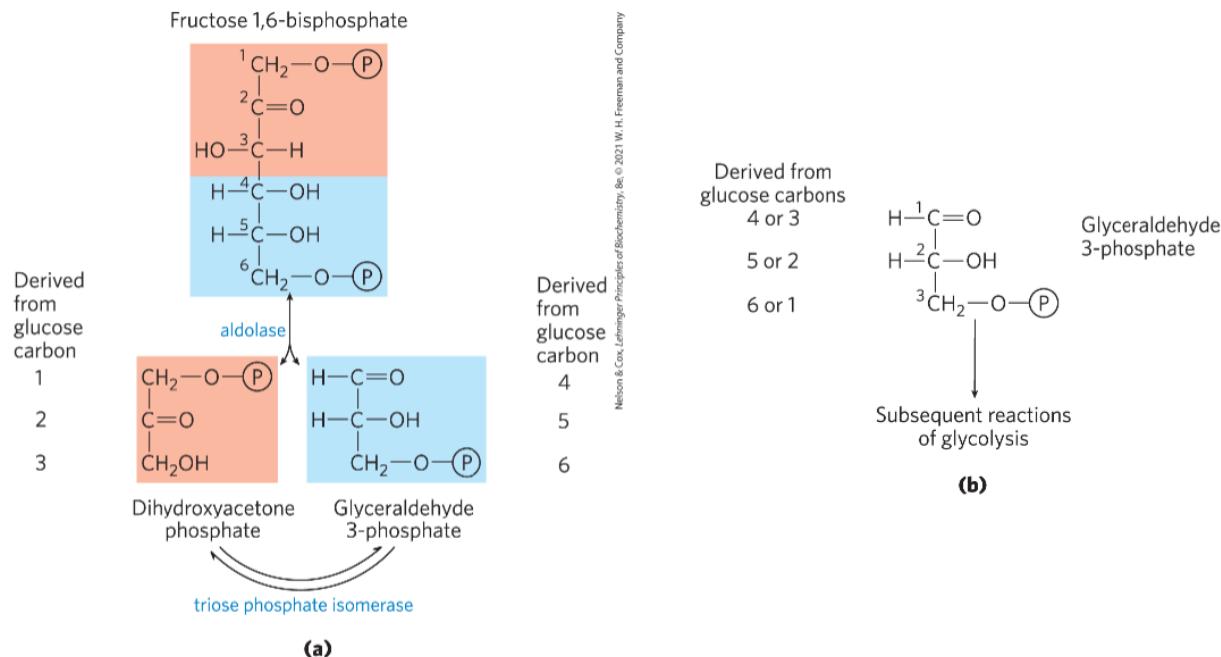
## (Step 5) Interconversion of the Triose Phosphates

- Triose phosphate isomerase converts dihydroxyacetone phosphate to glyceraldehyde 3-phosphate
  - reversible
  - final step of the preparatory phase of glycolysis



## Fate of the Glucose Carbons in the Formation of Glyceraldehyde 3-Phosphate

- After Step 5 of glycolysis, the carbon atoms derived from C-1, C-2, and C-3 of the starting glucose are chemically indistinguishable from C-6, C-5, and C-4, respectively



## The Payoff Phase of Glycolysis Yields ATP and NADH

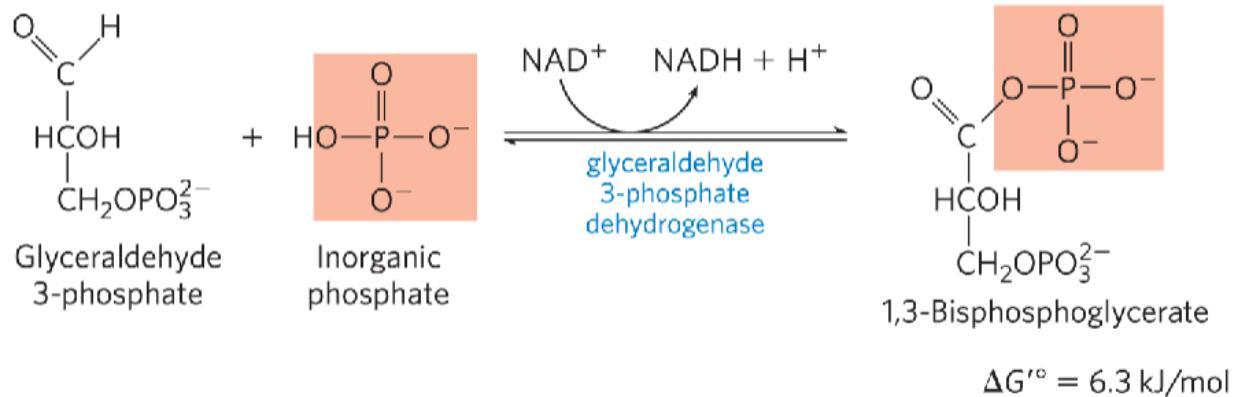
In the payoff phase of glycolysis:

- Each of the two molecules of glyceraldehyde 3-phosphate undergoes **oxidation at C-1**

- Some energy from the oxidation reaction is conserved in the form of one **NADH** and two ATP per triose phosphate oxidized

### (Step 6) Oxidation of Glyceraldehyde 3-Phosphate to 1,3-Bisphosphoglycerate

- Glyceraldehyde 3-Phosphate Dehydrogenase** catalyzes the oxidation of glyceraldehyde 3-phosphate to **1,3-bisphosphoglycerate**
- This is an energy-conserving reaction

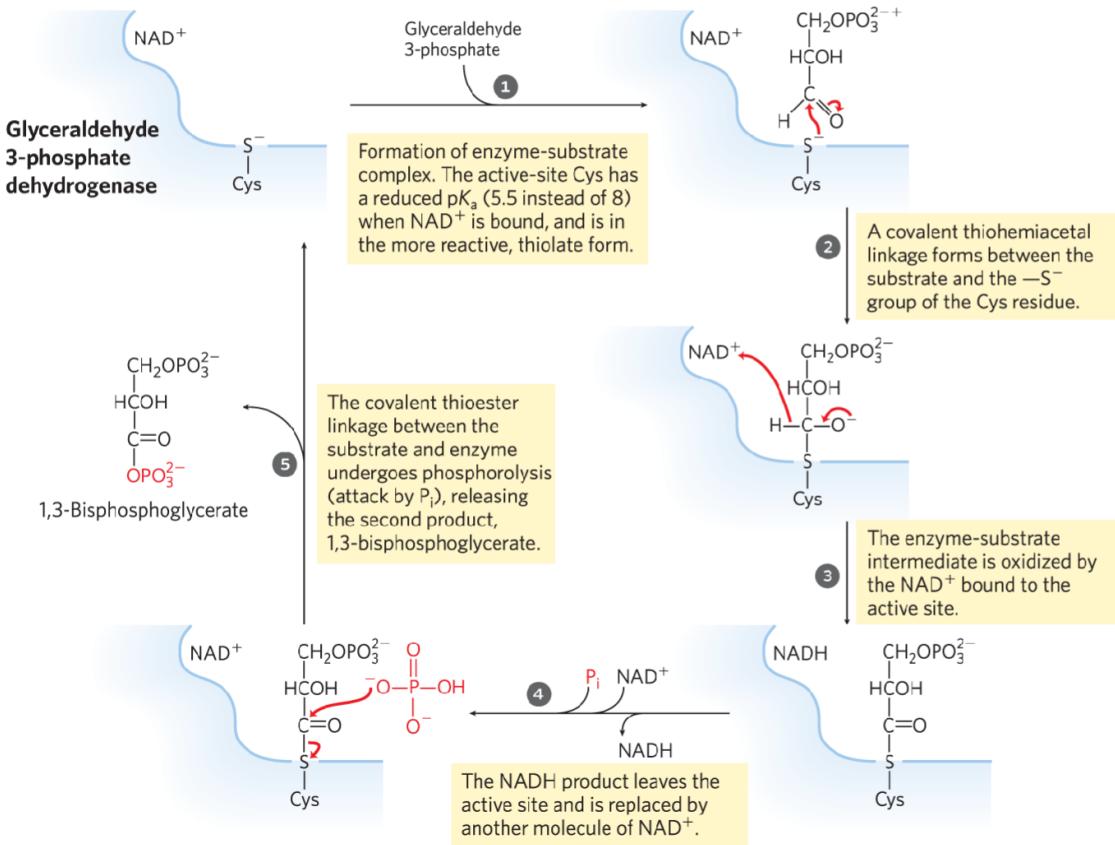


This reduction step stores energy with the formation of the acyl phosphate and in the form of high-energy electrons within NADH

### The First Step of the Payoff Phase is an Energy-Conserving Reaction

- Formation of the **acyl phosphate** group at C-1 of 1,3-bisphosphoglycerate conserves the free energy of oxidation
- acyl phosphates have a very high standard free energy of hydrolysis ( $\Delta G'° = -49.3 \text{ kJ/mol}$ )

## The Glyceraldehyde 3-Phosphate Dehydrogenase Reaction



- First, the thiolate ion attacks the carbonyl group of the substrate to form a thiohemiacetal, which is then oxidized to a thioester by transfer of a hydride ion (a hydrogen with two electrons,  $H^-$ ) to an enzyme-bound  $\text{NAD}^+$ , with concurrent release of a proton ( $H^+$ ). Thus, in effect, two hydrogen atoms are removed from the substrate.
- Once NADH is formed, its affinity for the enzyme decreases, so that a free  $\text{NAD}^+$  displaces this NADH. The thioester is an energy-rich intermediate, and by phosphorolysis the high-energy 1,3-bisphosphoglycerate is generated with the release of the free enzyme. Thus, the substrate aldehyde group is oxidized to a carboxylic acid group, with conservation of most of the energy of oxidation in formation of the anhydride bond between carboxylic and phosphoric acids.

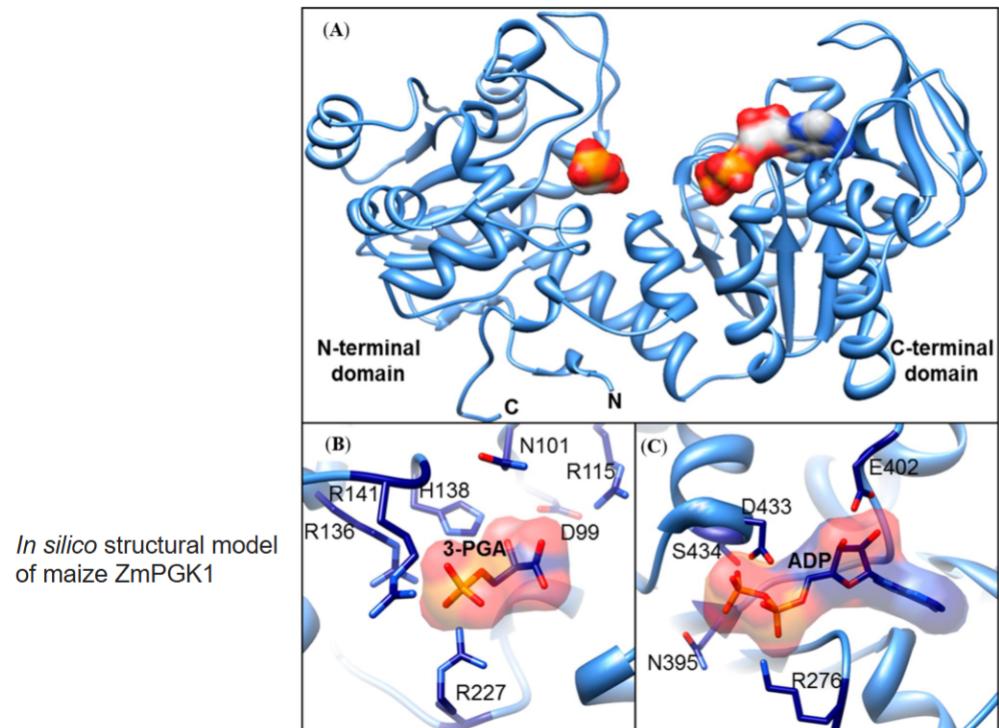
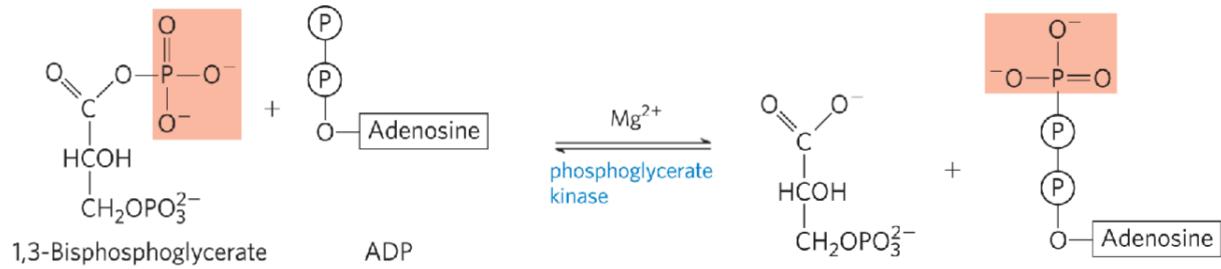
### Why This Process Works

- The **thioester intermediate** serves as a critical energy-rich intermediate that conserves the energy released during the oxidation of G3P. This conserved energy is then used to drive the unfavorable phosphorylation step
- $\text{NAD}^+$  not only acts as an electron acceptor, forming NADH, but also activates the cysteine residue for catalysis
- The release of NADH ensures that the enzyme is ready to catalyze subsequent reactions efficiently

### (Step 7) Phosphoryl Transfer from 1,3-Bisphosphoglycerate to ADP

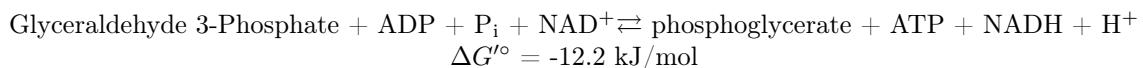
- Phosphoglycerate Kinase transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and **3-phosphoglycerate**

- substrate-level phosphorylation



### Steps 6 and 7 of Glycolysis Consistute an Energy-Coupling Process

- The sum of the two reactions is:



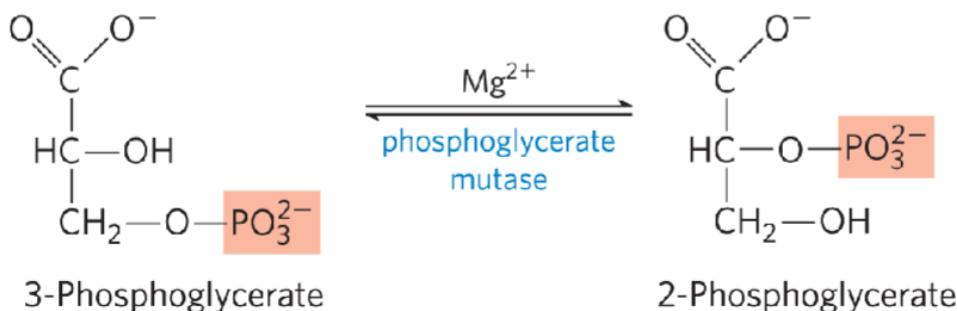
- substrate-level phosphorylation** = the formation of ATP by phosphoryl group transfer from a substrate different from **respiration-linked phosphorylation**
- G3P dehydrogenase is coupled to phosphoglycerate kinase
  - G3P dehydrogenase is forming a high energy phosphate while phosphoglycerate kinase is removing the phosphoryl group and adding it to ADP ( $\Delta G < 0$  overall)

		$\Delta G^{\circ'}$
GAPDH	$\text{GAP} + \text{NAD}^+ + \text{P}_i \rightarrow 1,3\text{-BPG} + \text{NADH} + \text{H}^+$	+6.3
first substrate-level phosphorylation	<b>PGK</b> + $1,3\text{-BPG} + \text{ADP} \rightarrow 3\text{-PG} + \text{ATP}$	-18.8
	$\text{GAP} + \text{NAD}^+ + \text{ADP} + \text{P}_i \rightarrow 3\text{-PG} + \text{ATP} + \text{NADH} + \text{H}^+$	-12.5

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

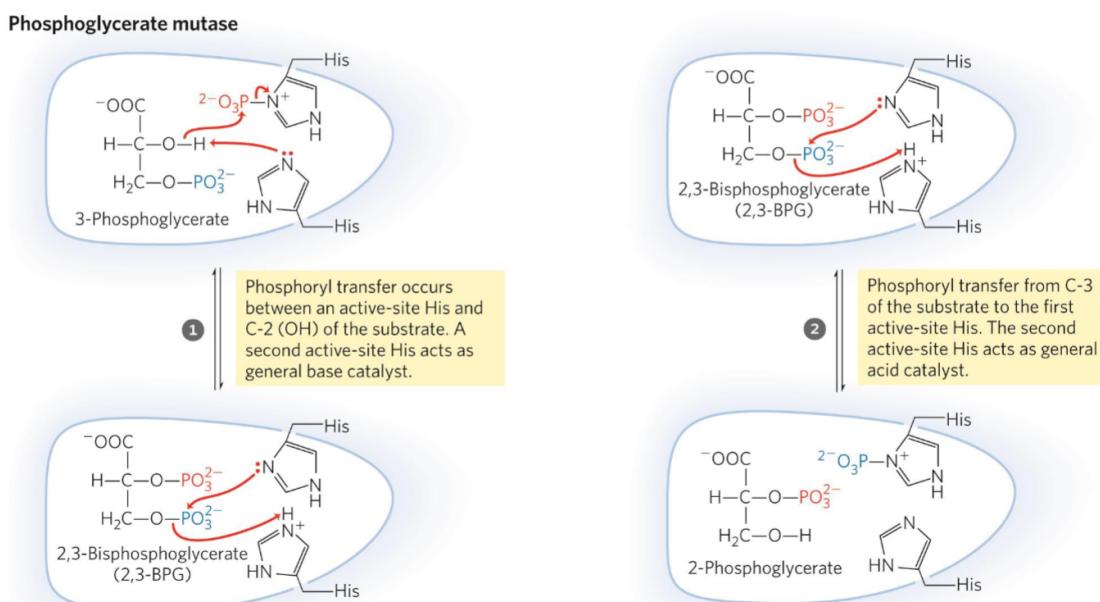
- phosphoglycerate mutase catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate

– requires  $\text{Mg}^{2+}$



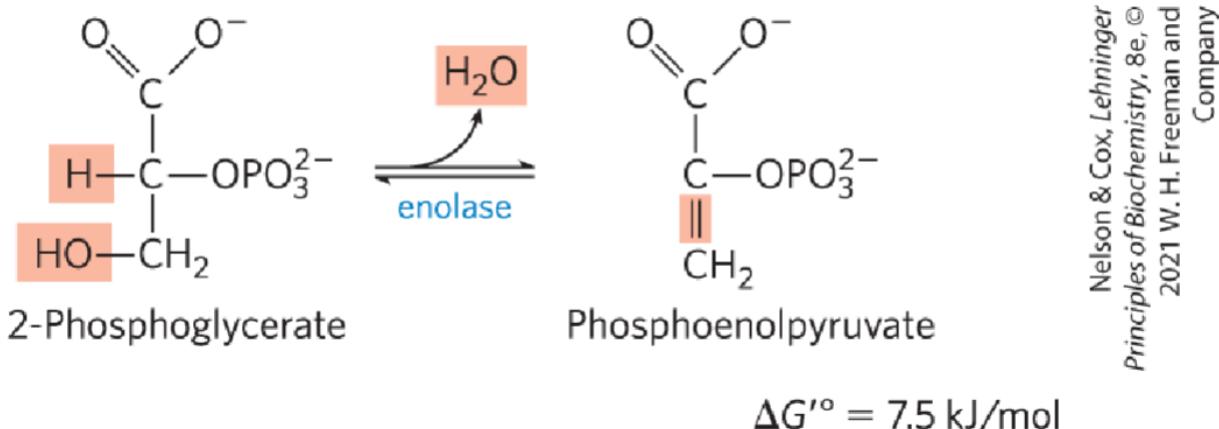
$$\Delta G'^{\circ} = 4.4 \text{ kJ/mol}$$

### The Phosphoglycerate Mutase Reaction



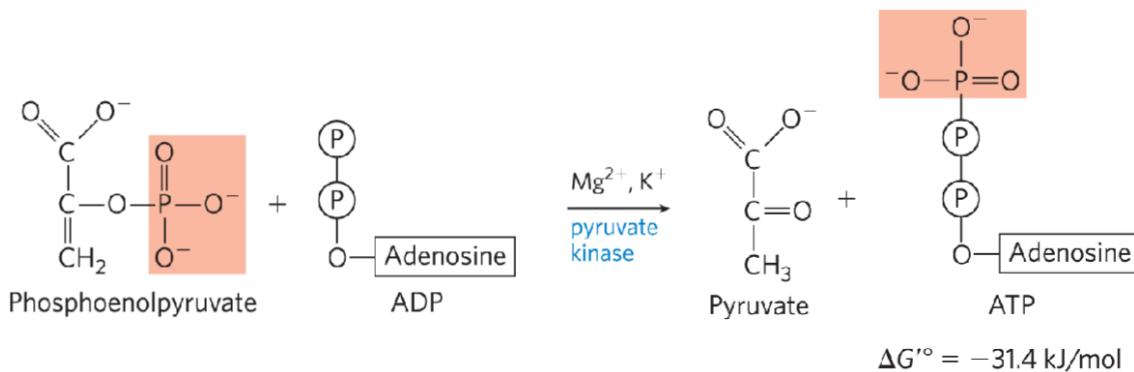
### (Step 9) Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

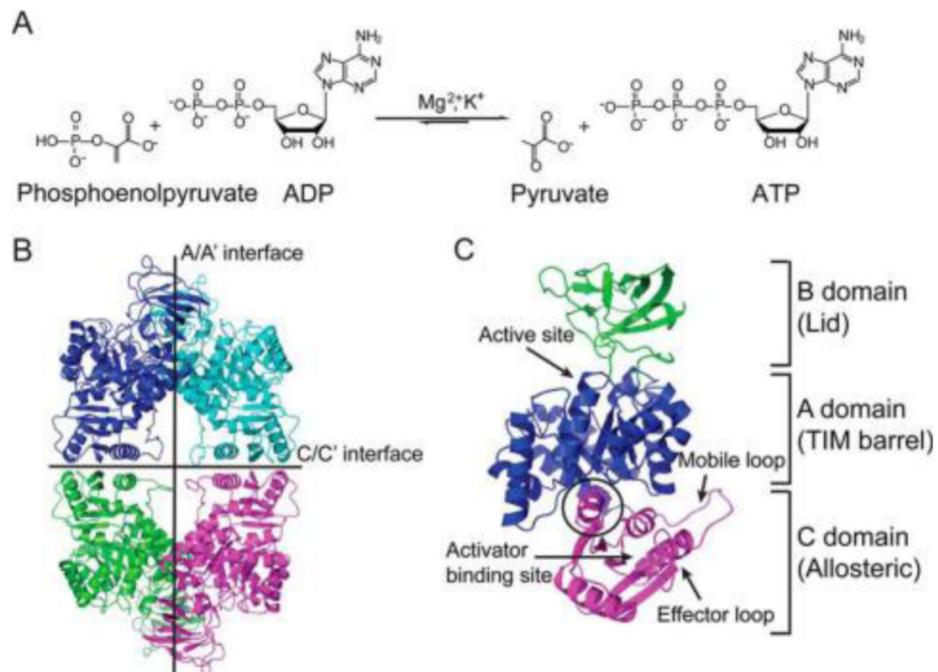
- **enolase** promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield **phosphoenolpyruvate (PEP)**
  - energy-conserving reaction
  - mechanism involves a  $Mg^{2+}$ -stabilized enolic intermediate



### (Step 10) Transfer of the Phosphoryl Group from Phosphoenolpyruvate to ADP

- **pyruvate kinase** catalyzes the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, yielding **pyruvate**
- Requires  $K^+$  and either  $Mg^{2+}$  or  $Mn^{2+}$
- **substrate-level phosphorylation** - the formation of ATP by phosphoryl group transfer from a substrate different from **respiration-linked phosphorylation**

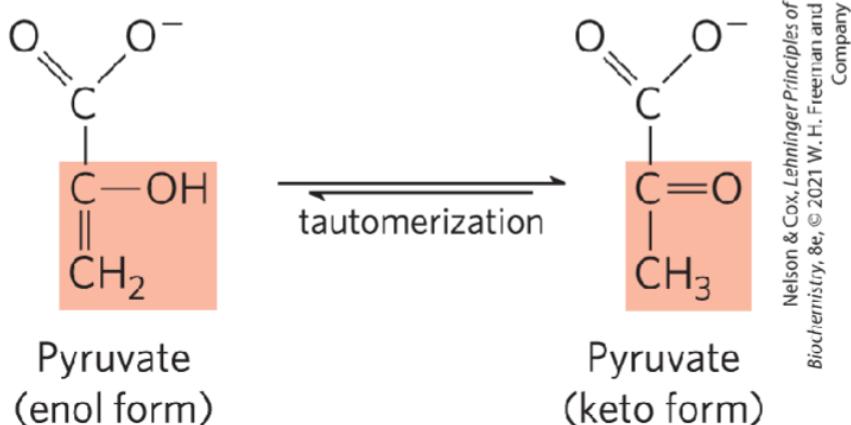




A, the reaction catalyzed by pyruvate kinase. B, structure of *E. coli* pyruvate kinase type 1 tetramer with the tetrameric A/A' and C/C' interfaces labeled. C, pyruvate kinase type 1 monomer showing the active site and allosteric binding site. The monomer is colored by domain, and the helix that connects the allosteric domain with the active site domain is circled

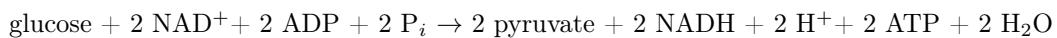
### Pyruvate in its Enol Form Spontaneously Tautomerizes to its Keto Form

- **pyruvate kinase** catalyzes the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, yielding **pyruvate**
  - requires  $\text{K}^+$  and either  $\text{Mg}^{2+}$  or  $\text{Mn}^{3+}$

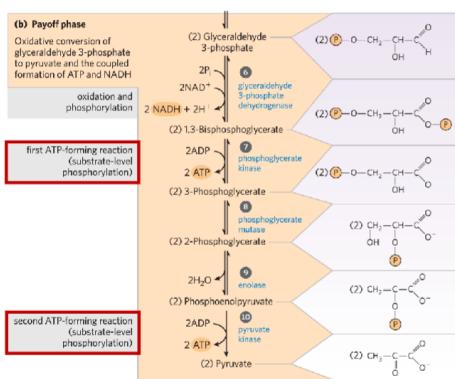


### The Overall Balance Sheet Shows a Net Gain of Two ATP and Two ADH per Glucose

- Subtracting the two ATP spent in the preparatory phase, the net equation for the overall process is:



## Glycolysis Overview



**Table 15-1**  $\Delta G^\circ$  and  $\Delta G$  for the Reactions of Glycolysis in Heart Muscle<sup>a</sup>

Reaction	Enzyme	$\Delta G^\circ$ (kJ · mol <sup>-1</sup> )	$\Delta G$ (kJ · mol <sup>-1</sup> )
1	Hexokinase	-20.9	-27.2
2	PGI	+2.2	-1.4
3	PFK	-17.2	-25.9
4	Aldolase	+22.8	-5.9
5	TIM	+7.9	~0
6 + 7	GAPDH + PGK	-16.7	-1.1
8	PGM	+4.7	-0.6
9	Enolase	-3.2	-2.4
10	PK	-23.0	-13.9

<sup>a</sup>Calculated from data in Newsholme, E.A. and Start, C., *Regulation in Metabolism*, p. 97, Wiley (1973).



## Energy Remaining in Pyruvate

- Energy stored in pyruvate can be extracted by:
  - **aerobic processes:**
    - \* oxidative reactions in the citric acid cycle (TCA cycle)
    - \* oxidative phosphorylation
  - **anaerobic processes:**
    - \* reduction to lactate
    - \* reduction to ethanol
- pyruvate can provide the carbon skeleton for alanine synthesis or fatty acid synthesis

## $\Delta G^\circ$ vs. $\Delta G$ in Glycolysis

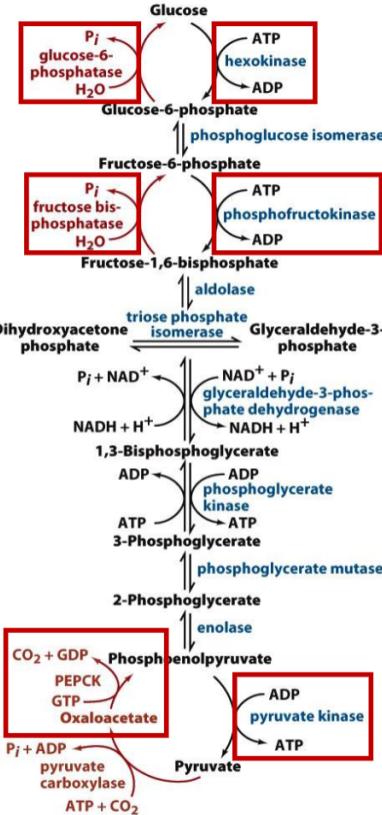
- $\Delta G^\circ$  (Standard Free Energy Change):
  - Measured under standard conditions (1 M concentrations, pH 7.0, 25°C)
  - Reflects theoretical favorability of reactions
  - Some reactions, like **aldolase**, have positive  $\Delta G^\circ$  (unfavorable under standard conditions)
- $\Delta G$  (Actual Free Energy Change):
  - Reflects real cellular conditions with regulated metabolite concentrations
  - **Le Chatelier's Principle:** Substrate and product levels shift equilibrium to make reactions favorable
  - Enzymes tightly control  $\Delta G$  to drive the pathway forward

Steps with large, negative  $\Delta G$  (marked in red boxes) are **irreversible and regulate glycolysis**:

- Hexokinase (Step 1)
- PFK-1 (Step 3)
- Pyruvate Kinase (Step 10)

These steps ensure glycolysis flows in one direction and are critical control points in the pathway

- Irreversible steps regulated differently to define direction of metabolic flux
  - 3 steps in glycolysis, 3 steps in gluconeogenesis
- Reversible steps follow ratios of reactants:products



## Regulation of Hexokinase

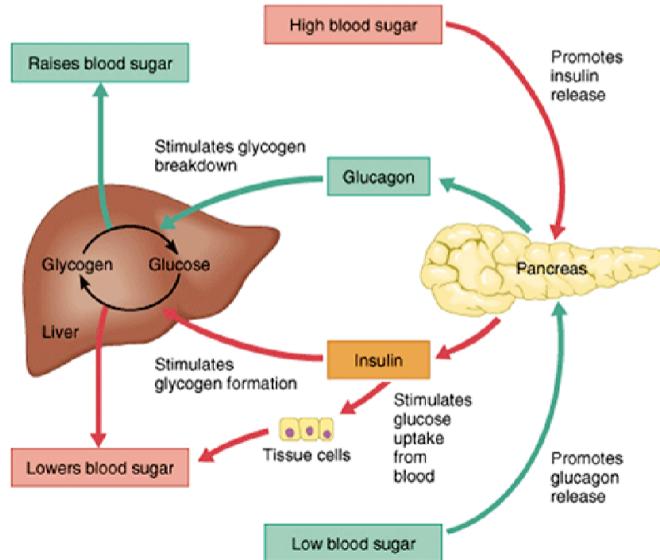
- Hexokinase Isoforms:
  - Type II (muscle): Inhibited by glucose-6-phosphate (G6P), which prevents the wasteful use of glucose when energy isn't needed
  - Type IV (glucokinase, liver): Not inhibited by G6P, has a higher Km (works at higher glucose concentrations), and is induced by insulin, helping the liver store glucose as glycogen

Muscle cells tightly regulate glucose usage to prioritize immediate energy production. The liver adapts to blood glucose levels to balance storage (glycogen) and supply (to other tissues)

## Regulation by Glucose Levels

- High Glucose Levels ( $\uparrow$  Insulin):
  - Activates glucokinase to increase glucose uptake and storage (glycogen synthesis)
  - Enhances glycolysis to process excess glucose
- Low Glucose Levels ( $\uparrow$  Glucagon):
  - Promotes gluconeogenesis and glycogen breakdown in the liver to release glucose into the bloodstream

## Glucose Homeostasis (Insulin/Glucagon)

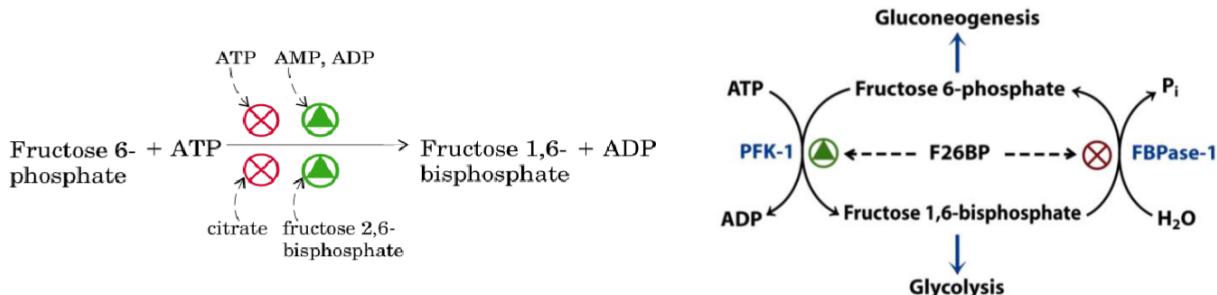


Things we're missing from the diagram:

- **Low glucose levels ( $\uparrow$ glucagon)**
  - Increased gluconeogenesis  $\rightarrow$  release of blood glucose
- **High glucose levels ( $\uparrow$ insulin)**
  - Stimulation of glycogen formation involves **upregulation of glucokinase**

## Regulation of Phosphofructokinase-1 (PFK-1)

- PFK-1: The "Gatekeeper" of Glycolysis
  - Activated by:
    - \* AMP/ADP: Signals low energy, stimulating glycolysis to make ATP
    - \* F2,6BP: Feed-forward signal that boosts glycolysis when glucose is abundant
  - Inhibited by:
    - \* ATP: Signals high energy, slowing glycolysis
    - \* Citrate: Indicates sufficient energy from the TCA cycle
- Fructose 2,6-Bisphosphate (F2, 6BP)
  - Coordinates glycolysis and gluconeogenesis
    - \* High F2,6BP  $\rightarrow$  Activates PFK-1 (glycolysis) and inhibits gluconeogenesis
    - \* Low F2,6BP  $\rightarrow$  Slows glycolysis and releases gluconeogenesis inhibition
  - Regulated by PFK-2 (in turn, regulated by insulin and glucagon)
    - \* **Insulin:** Increases F2, 6BP (promotes glycolysis)
    - \* **Glucagon:** Decreases F2, 6BP (promotes gluconeogenesis)



- PFK-1 ensures glycolysis runs only when energy is needed, or glucose is abundant
- F2,6BP acts as a "metabolic switch" to balance energy needs

## Why does PFK-2 Exist?

- From an evolutionary perspective, phosphofructokinase-2 (PFK-2) and its product, fructose 2,6-bisphosphate (F2,6BP), provide an additional layer of regulation that allows cells to fine-tune glycolysis and gluconeogenesis based on broader metabolic and hormonal signals. This control mechanism outside the core glycolytic pathway likely evolved to optimize energy balance and metabolic flexibility in response to environmental and physiological changes
- **Integration of metabolic and hormonal signals:**
  - Unlike PFK-1, which is directly regulated by ATP, AMP, and citrate, PFK-2 allows glycolysis to respond to hormonal signals such as insulin and glucagon
  - This enables systemic control over metabolism, ensuring glucose utilization aligns with the organism's energy needs rather than just local cellular conditions
- **Fine-tuned control of glycolysis and gluconeogenesis:**
  - F2,6BP is a potent activator of PFK-1, enhancing glycolysis when energy is needed
  - Simultaneously, F2,6BP inhibits fructose-1,6-bisphosphatase (FBPase-1), suppressing gluconeogenesis when glucose breakdown is required
  - This dual action prevents futile cycling and ensures efficient energy management
- **Rapid and reversible adaptation to nutritional states:**
  - PFK-2 activity can be quickly modulated by phosphorylation (e.g., by PKA in response to glucagon), allowing immediate metabolic shifts
  - This regulatory mechanism is particularly crucial for organisms that experience fluctuating nutrient availability
- **Evolutionary advantage in multicellular organisms:**
  - As organisms evolved from unicellular to multicellular forms, systemic control over energy metabolism became essential
  - Hormone-driven regulation via PFK-2/F2,6BP allows coordination between tissues (e.g., liver vs. muscle) to maintain blood glucose homeostasis

## Why Control Glycolysis via an External Regulator Like F2,6BP?

- **Separation of Immediate Energy Sensing and Long-Term Metabolic Regulation:**
  - PFK-1 responds to local energy levels (ATP, AMP), ensuring rapid adjustments
  - PFK-2/F2,6BP introduces an additional control point that responds to hormonal and systemic energy states, optimizing metabolism beyond individual cell needs
- **Prevention of Metabolic Imbalance:**
  - If glycolysis and gluconeogenesis were regulated solely by direct feedback loops, they might operate inefficiently in dynamic environments
  - F2,6BP provides a fail-safe mechanism to ensure that energy production and consumption remain synchronized across different physiological conditions

Overall, PFK-2 and F2,6BP likely evolved as a sophisticated regulatory adaptation, allowing multicellular organisms to maintain metabolic homeostasis efficiently in response to both internal energy demands and external environmental changes

## Regulation of Pyruvate Kinase

- Pyruvate Kinase: The Final Step

  - Activated by:

    - \* **Fructose 1,6-Bisphosphate (F1,6BP):** Feed-forward activation ensures glycolysis flows efficiently, linking upstream reactions to downstream energy production

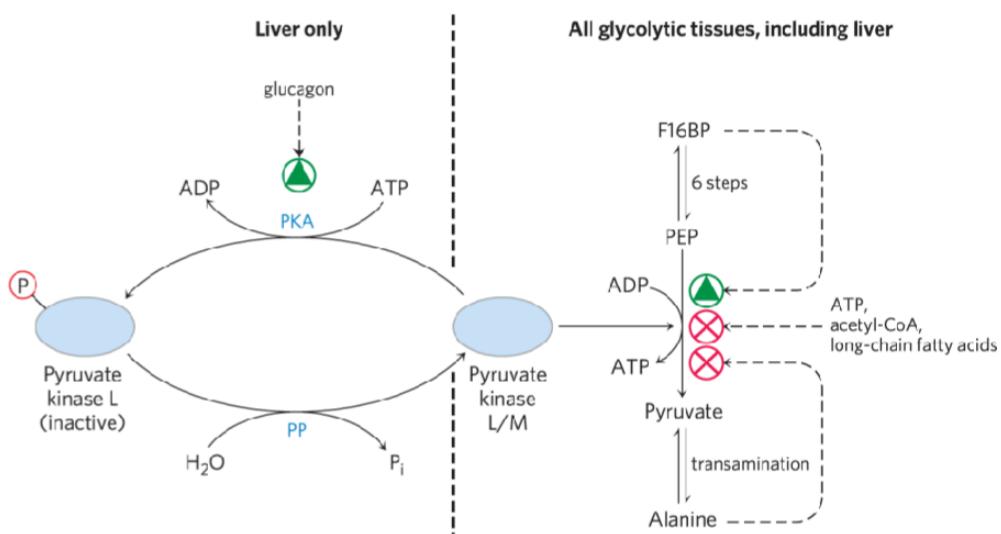
  - Inhibited by:

    - \* **ATP:** Signals a high energy state, reducing unnecessary glycolysis
    - \* **Acetyl-CoA and Long-Chain Fatty Acids:** Energy-rich molecules from fatty acid oxidation signal sufficient energy, repressing glycolysis
    - \* **Alanine:** Indicates amino acid sufficiency, reducing the need for glycolysis.

- Hormonal Regulation (Liver-Specific):

  - **Insulin:** Activates pyruvate kinase via dephosphorylation (promotes glycolysis)

  - **Glucagon:** Inhibits pyruvate kinase via phosphorylation (slows glycolysis)



Pyruvate kinase balances energy production with resource availability. Feed forward activation ensures that glycolysis is efficient when glucose is being processed upstream.

## Regulation of Glycolysis

- Allosteric Regulators (AMP, ATP, citrate, F2,6BP)

  - Activators ramp up glycolysis when energy is needed
  - Inhibitors slow glycolysis to conserve energy when it's abundant

- Hormonal Regulation (Insulin, Glucagon)

  - Insulin promotes glucose use and storage during energy abundance.
  - Glucagon mobilizes glucose during energy scarcity

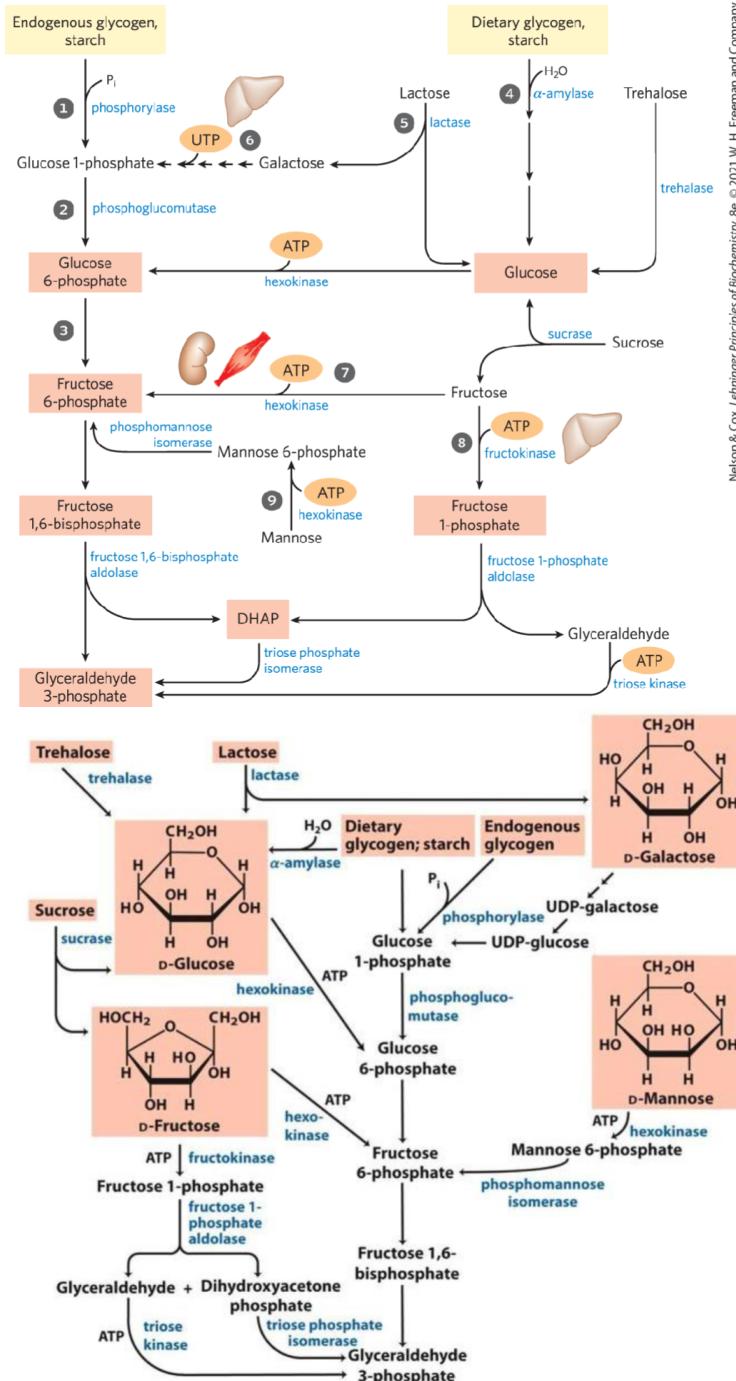
- Big Picture:

  - Glycolysis adapts to cellular and systemic needs:

    - \* Muscle prioritizes energy for contraction
    - \* The liver balances glucose storage and release, regulating blood sugar for the whole body

## Entry of Dietary Glucogen, Starch, Disaccharides, and Hexoses into the Preparatory Stage of Glycolysis

- Glucose and other hexoses and hexose phosphates obtained from stored polysaccharides or dietary carbohydrates feed into the glycolytic pathway
- By using a common pathway for a number of enzymes that must be synthesized and simplifies the regulation of the common pathway.

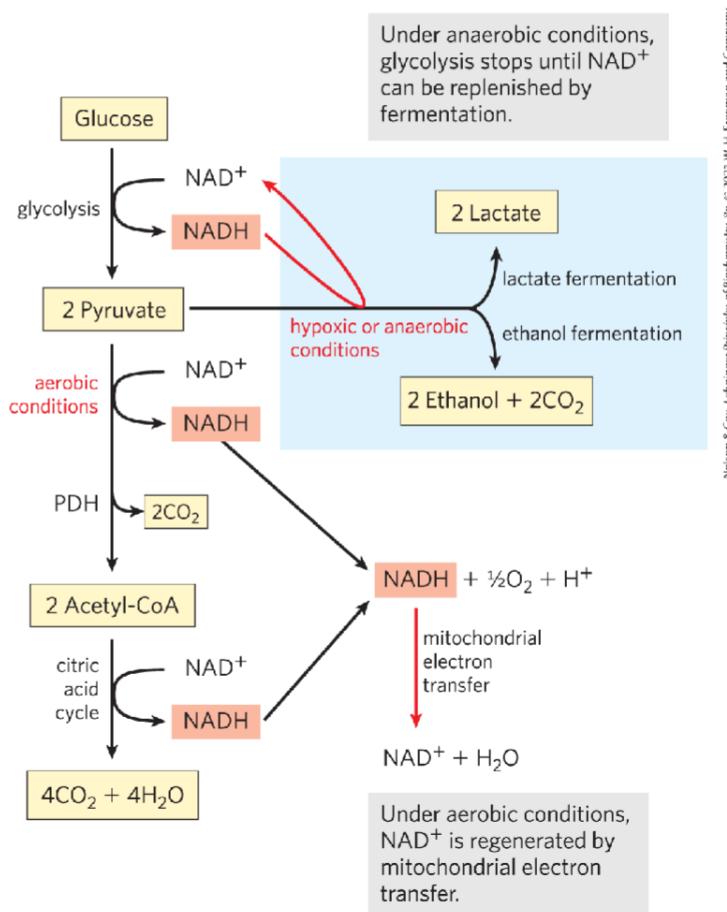


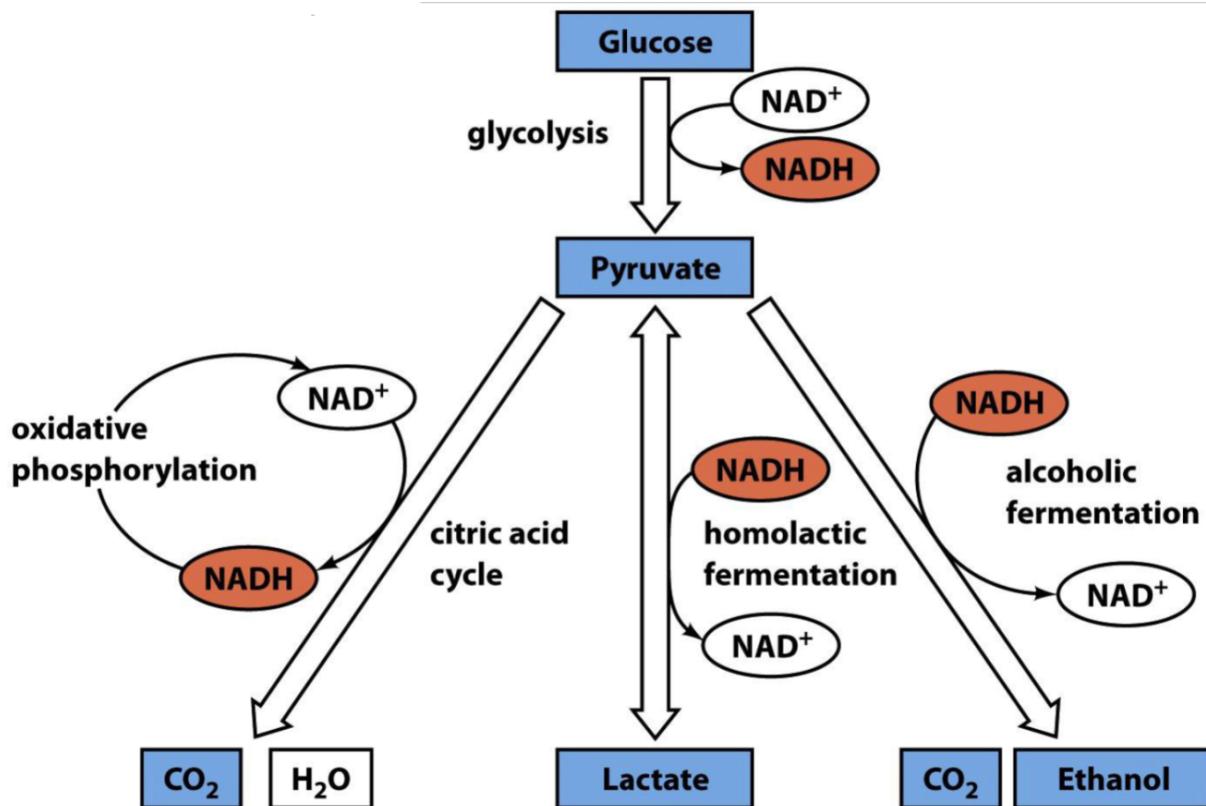
## Energy Remaining in Pyruvate

- Energy stored in pyruvate can be extracted by:
  - Aerobic processes:
    - \* oxidative reactions in the citric acid cycle (TCA cycle)
    - \* oxidative phosphorylation
  - Anaerobic processes:
    - \* reduction to lactate
    - \* reduction to ethanol
- pyruvate can provide the carbon skeleton for alanine synthesis or fatty acid synthesis

## Three Catabolic Fates of Pyruvate

- NADH must be recycled to regenerate NAD<sup>+</sup>
- under **anaerobic** conditions or low oxygen condition (**hypoxia**), pyruvate is **reduced to lactate or ethanol**
- under **aerobic** conditions, **pyruvate is oxidized to acetyl-CoA**





Pyruvate formed under anaerobic conditions is reduced to lactate with electrons from NADH, recycling NADH to NAD<sup>+</sup>, and allowing continued glycolysis in the processes of lactate or alcohol fermentation

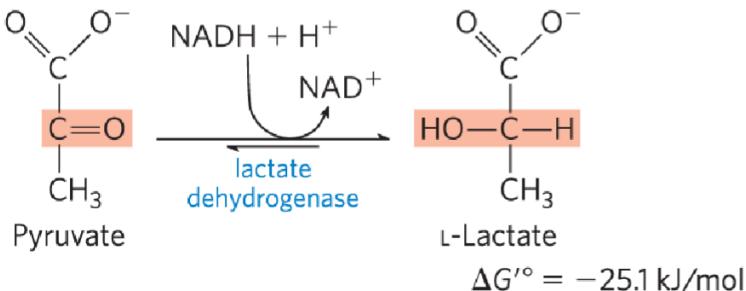
## Fermentation

**Fermentation** = general term for processes that extract energy (as ATP) but do not consume oxygen or change the concentrations of NAD<sup>+</sup> or NADH

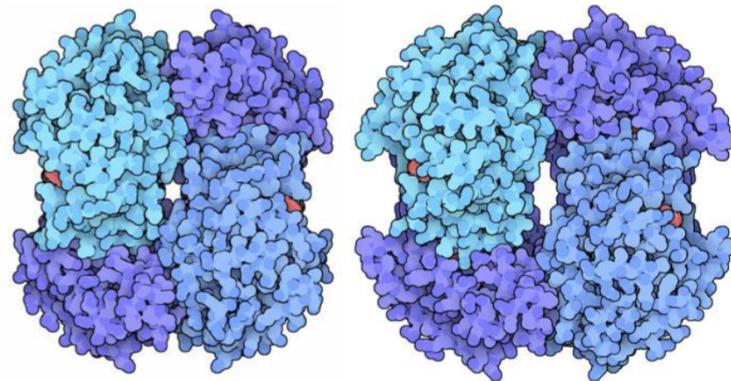
- **lactic acid fermentation** = pyruvate accepts electrons from NADH and is reduced to lactate (one step) while regenerating the NAD<sup>+</sup> necessary for glycolysis
- **ethanol (alcohol) fermentation** = pyruvate is further catabolized (two steps) to ethanol

## Pyruvate is the Terminal Electron Acceptor in Lactic Acid Fermentation

- Organisms can regenerate NAD<sup>+</sup> by transferring electrons from NADH to pyruvate, forming **lactate**
- **lactate dehydrogenase** = catalyzes the reduction of pyruvate to lactate



Binding of fructose 1,6-bisphosphate causes the enzyme to change into an active shape.

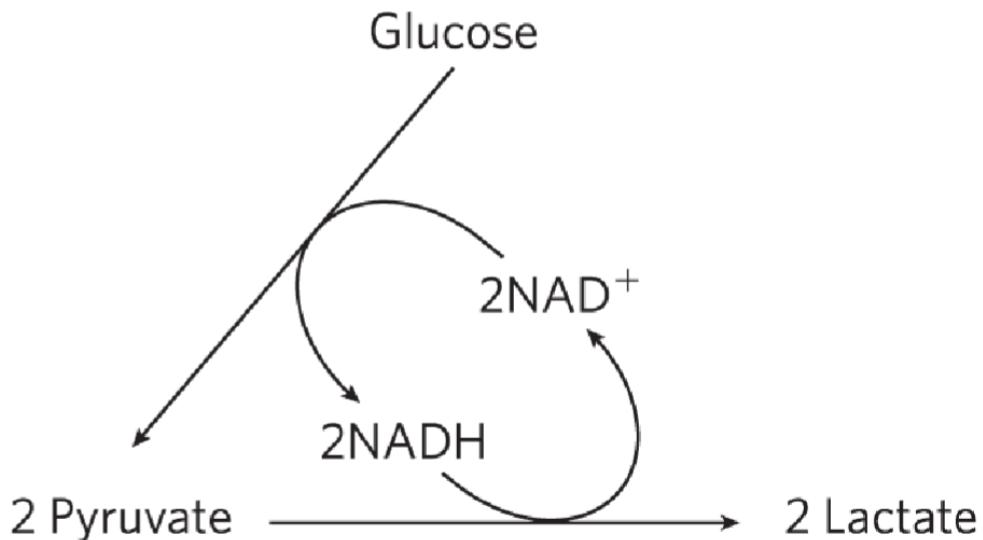


Active (left) and inactive (right) lactate dehydrogenase  
NAD in red

<https://pdb101.rcsb.org/motm/102>

### Reduction of Pyruvate to Lactate Regenerates $\text{NAD}^+$

- glycolysis converts  $2\text{NAD}^+$  to  $2\text{NADH}$
- reduction of pyruvate to lactate regenerates  $2\text{NAD}^+$
- there is no net change in  $\text{NAD}^+$  or  $\text{NADH}$

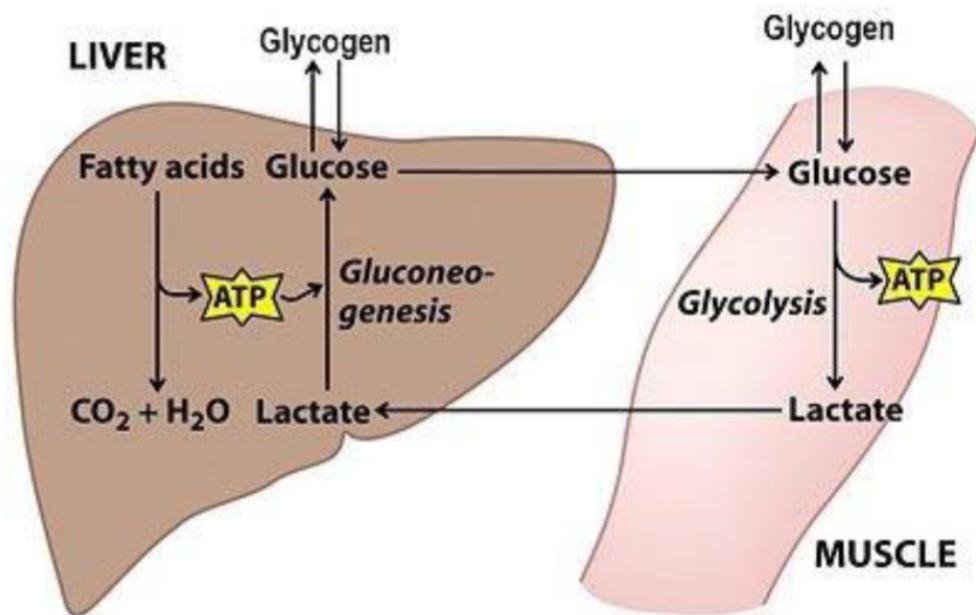


### Lactate can be Recycled

- Anaerobic catabolism of glucose to lactate occurs during short bursts of extreme muscular activity - for example, in a sprint - during which oxygen cannot be carried to the muscles fast enough to oxidize pyruvate
- **lactate is carried in blood to the liver, where it is converted to glucose during recovery**
- acidification resulting from ionization of lactic acid in muscle and blood limits the period of vigorous activity

## Why do we need Gluconeogenesis?

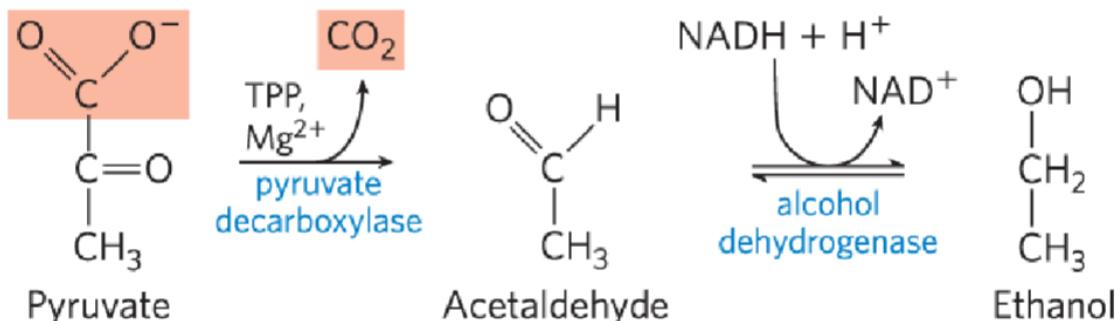
- The brain, nervous system, and red blood cells rely exclusively on glucose for ATP production
- Prolonged fasting or intense exercise depletes glycogen, **requiring glucose synthesis through gluconeogenesis**
- The liver upregulates gluconeogenesis to synthesize glucose and export it to meet the energy demands of other tissues
- Lactate recycling:** The liver converts lactate (via reversible lactate dehydrogenase, LDH), to pyruvate, which then enters the gluconeogenesis pathway to be converted into glucose, which is exported back into the bloodstream to maintain blood sugar levels - a process known as the Cori cycle



- ATP for gluconeogenesis is generated through fatty acid oxidation in the liver, ensuring a continuous supply of glucose even during energy scarcity

## Ethanol is the Reduced Product in Ethanol Fermentation

- yeast and other microorganisms regenerate NAD<sup>+</sup> by reducing pyruvate to ethanol and CO<sub>2</sub>



- The overall equation is:



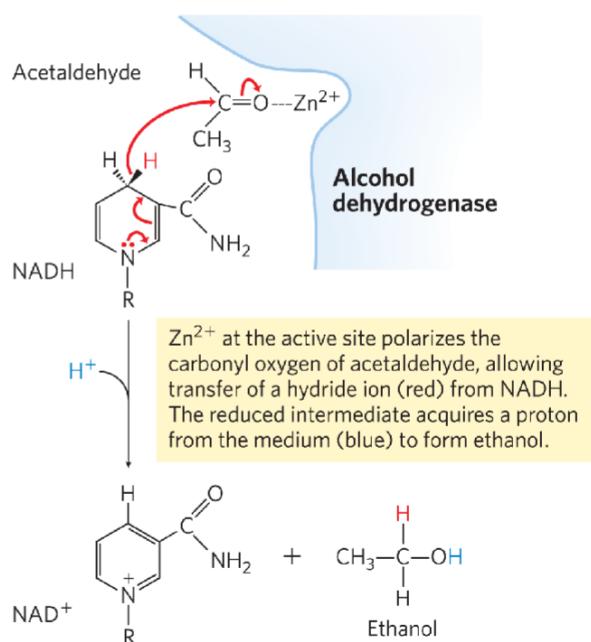
- Yeast have evolved to thrive in high-sugar, low-oxygen environments by using fermentation. The production of ethanol during fermentation is toxic to many competing organisms, giving yeast a competitive advantage
- Fermentation is much faster than aerobic respiration, though less efficient. In high-glucose environments, yeast prioritize speed over efficiency, allowing rapid growth and competition with other microorganisms. For example, fermentation produces ATP quickly to support immediate cellular needs, even if it yields only 2 ATP per glucose molecule
- Even in the presence of oxygen, yeast may favor fermentation when glucose is abundant. This phenomenon, known as the Crabtree Effect, occurs because the fermentation pathway is energetically beneficial for yeast to grow and divide rapidly under high-sugar conditions. Mitochondrial respiration is activated once glucose levels drop
- Humans manipulate the oxygen levels to control yeast metabolism, promoting fermentation to achieve specific outcomes - rising bread in baking or alcohol production in brewing and winemaking. This is an intentional application of yeast's metabolic flexibility.

### Why Anaerobic Conditions are Essential:

1. **Promotes Fermentation:** Anaerobic conditions ensure that yeast performs fermentation rather than aerobic respiration, which would fully oxidize glucose to carbon dioxide and water without producing ethanol
2. **Maximizes Desired Products:** In baking, the focus is on CO<sub>2</sub> for leavening. In alcohol production, ethanol is the desired product, and fermentation under anaerobic conditions ensures its accumulation.

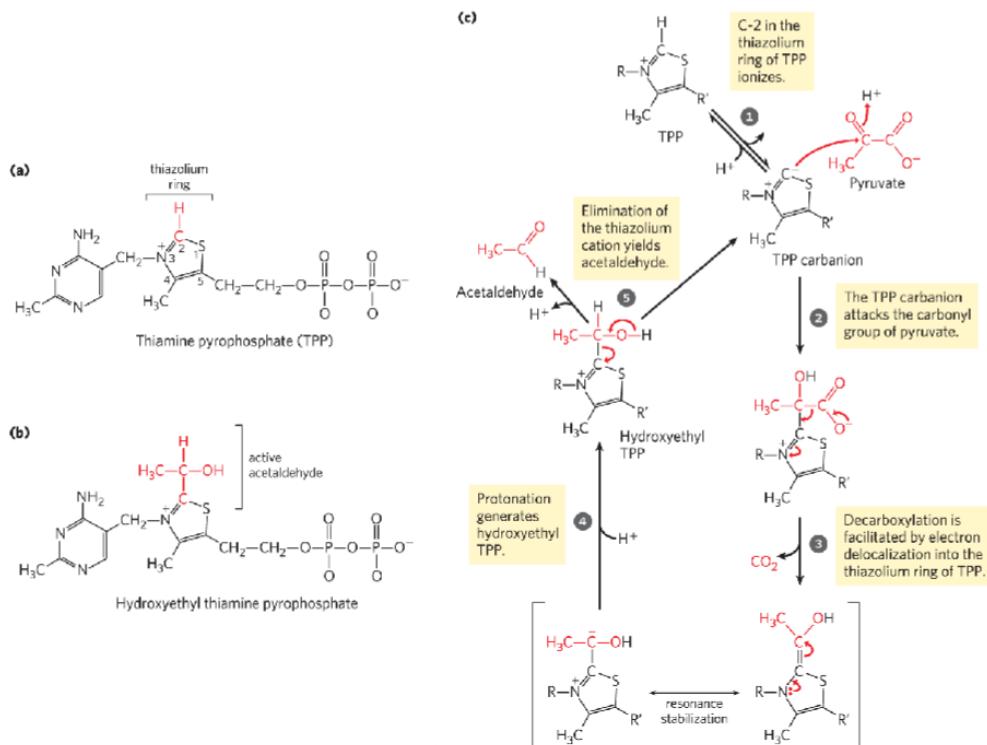
### Pyruvate Decarboxylase and Alcohol Dehydrogenase Reactions

- **Pyruvate decarboxylase:** catalyzes the irreversible decarboxylation of pyruvate to acetaldehyde
  - requires Mg<sup>2+</sup> and the coenzyme thiamine pyrophosphate
- **alcohol dehydrogenase:** catalyzes the reduction of acetaldehyde to ethanol



## Thiamine Pyrophosphate (TPP) in Pyruvate Decarboxylase

**Thiamine Pyrophosphate:** coenzyme derived from vitamin B<sub>1</sub>.



- **Nucleophilic Attack:** The **thiazolium ring** in TPP acts as a nucleophile, specifically the carbon between the sulfur and nitrogen in the thiazolium ring (a highly reactive position due to resonance stabilization of the positive charge on the nitrogen), forming a covalent intermediate that allows the decarboxylation to proceed efficiently
- **Stabilization of Carbanions:** During decarboxylation of the covalent intermediate, CO<sub>2</sub> is released, leaving behind a highly unstable **carbanion**. TPP has a thiazolium ring with a positively charged nitrogen atom that stabilizes the negatively charged carbanion intermediate formed during decarboxylation of pyruvate. The positively charged nitrogen in the thiazolium ring of TPP **stabilizes this carbanion** via resonance

## Some TPP-Dependent Reactions

**TABLE 14-1 Some TPP-Dependent Reactions**

Enzyme	Pathway(s)	Bond cleaved	Bond formed
Pyruvate decarboxylase	Ethanol fermentation	$\text{R}^1-\overset{\text{O}}{\underset{\text{C}}{\text{  }}}-\overset{\text{O}}{\underset{\text{C}}{\text{  }}}-\text{O}^-$	$\text{R}^1-\overset{\text{O}}{\underset{\text{C}}{\text{  }}}-\text{H}$
Pyruvate dehydrogenase $\alpha$ -Ketoglutarate dehydrogenase	Synthesis of acetyl-CoA Citric acid cycle	$\text{R}^2-\overset{\text{O}}{\underset{\text{C}}{\text{  }}}-\overset{\text{O}}{\underset{\text{C}}{\text{  }}}-\text{O}^-$	$\text{R}^2-\overset{\text{O}}{\underset{\text{C}}{\text{  }}}-\text{S-CoA}$
Transketolase	Carbon-assimilation reactions Pentose phosphate pathway	$\text{R}^3-\overset{\text{O}}{\underset{\text{C}}{\text{  }}}-\overset{\text{OH}}{\underset{\text{C}}{\text{  }}}-\text{R}^4$	$\text{R}^3-\overset{\text{O}}{\underset{\text{C}}{\text{  }}}-\overset{\text{OH}}{\underset{\text{C}}{\text{  }}}-\text{R}^5$

Nelson & Cox, Lehninger Principles of Biochemistry, 8e, © 2021 W. H. Freeman and Company

## Anaerobic Glycolysis vs. Aerobic Respiration



This process is essential under anaerobic conditions, but it barely extracts the energy available from glucose. To maximize energy yield, additional pathways are needed to fully oxidize glucose beyond pyruvate

**Glucose  $\rightarrow$  2 Lactate**

$\Delta G^\circ' = -200 \text{ kJ/mol}$

**Glucose + 6 O<sub>2</sub>  $\rightarrow$  6 CO<sub>2</sub> + 6 H<sub>2</sub>O**

$\Delta G^\circ' = -2866 \text{ kJ/mol}$

- Aerobic respiration releases approximately **14 times more energy** ( $\Delta G^\circ'$ ) than anaerobic glycolysis, highlighting the efficiency advantage of oxygen in energy production

## Anaerobic Glycolysis vs Aerobic Respiration

	Anaerobic Glycolysis	Aerobic Respiration
Oxygen Requirement	No	Yes
ATP Yield per Glucose	2 ATP	30-32 ATP
End Products	Lactate (or ethanol + CO <sub>2</sub> )	CO <sub>2</sub> + H <sub>2</sub> O
Energy Efficiency	Low	High

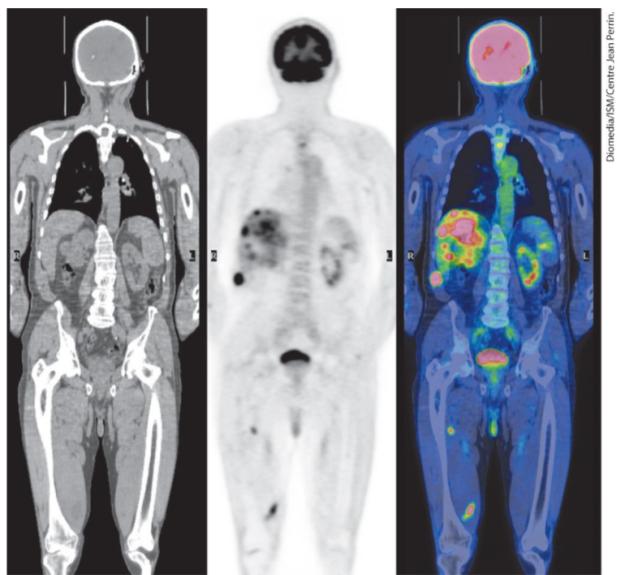
## The Pasteur and Warburg Effects are due to the Dependence on Glycolysis Alone for ATP Production

- The "Pasteur effect" = effect by which the rate and total amount of glucose consumption under anaerobic conditions is many times greater than under aerobic conditions
  - Occurs because the ATP yield from glycolysis alone is much smaller (2 ATP per glucose) than complete oxidation to CO<sub>2</sub> (30 or 32 ATP per glucose)

## The Warburg Effect

- The "Warburg effect" = observation that tumor cells have high rates of glycolysis, with fermentation of glucose to lactate, even in the presence of oxygen

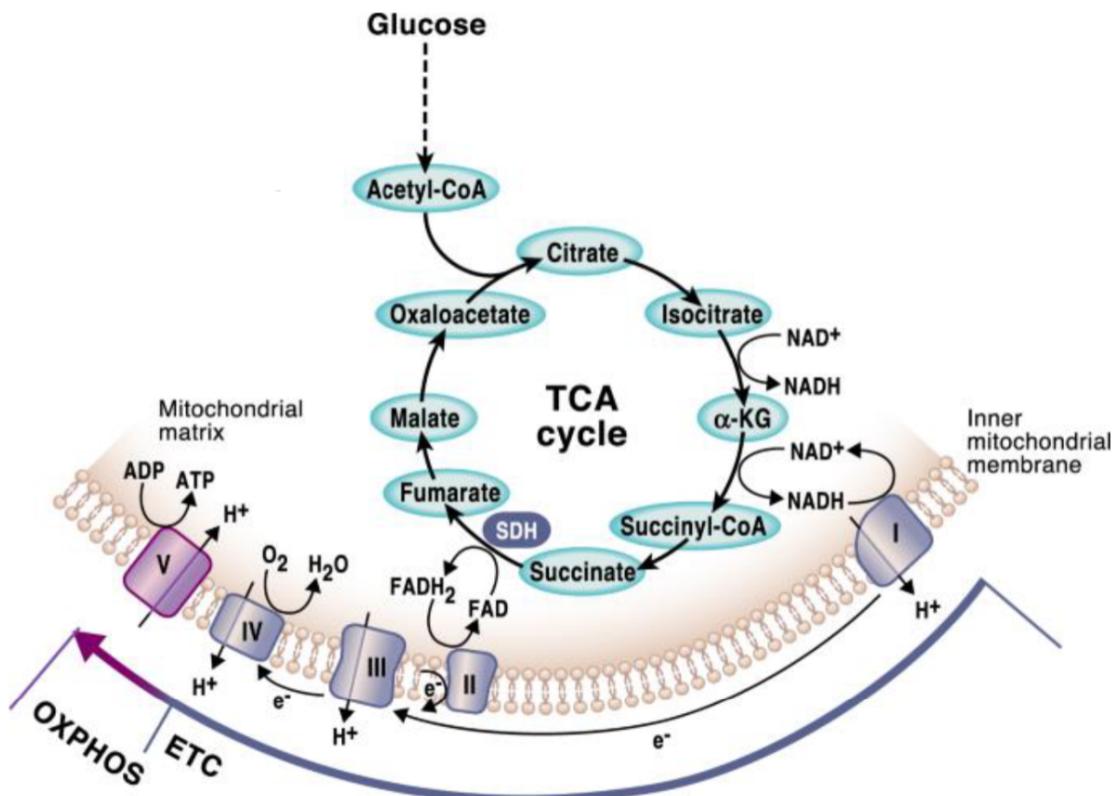
- The basis of PET scanning used to diagnose tumors



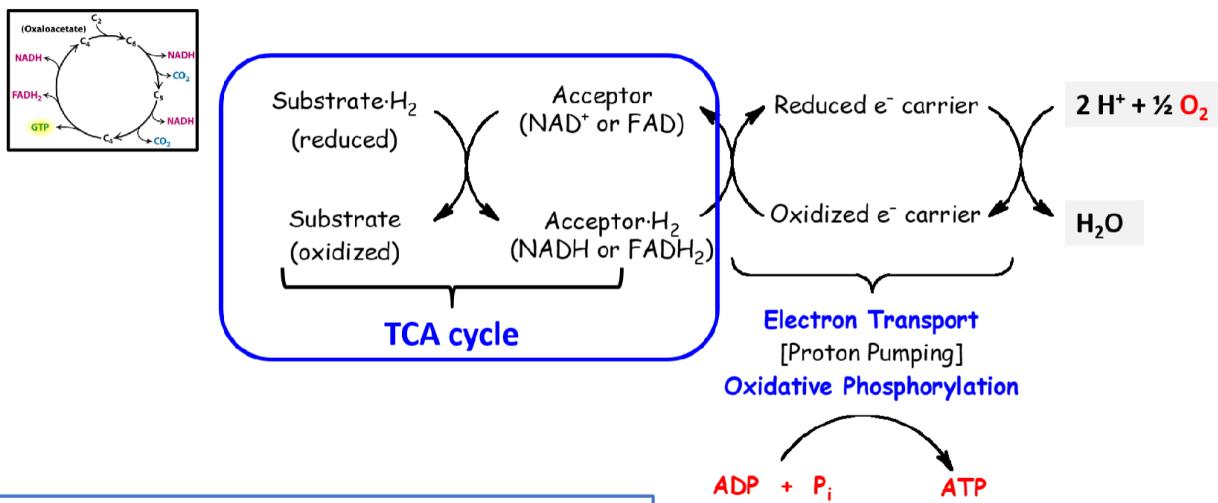
## Tricarboxylic Acid Cycle

Also known as...

- Citric Acid Cycle
- Krebs Cycle
- Szent-Györgyi-Krebs Cycle



## The Goal of the TCA cycle



Extract high-energy electrons from fuel molecules (like acetyl-CoA) and transfer them to electron carriers (NAD<sup>+</sup> and FAD) for ATP production through oxidative phosphorylation.