

CHEM 153A Week 9

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March 8, 2025

Glycolysis (Continued)

Importance of Phosphorylated Intermediates

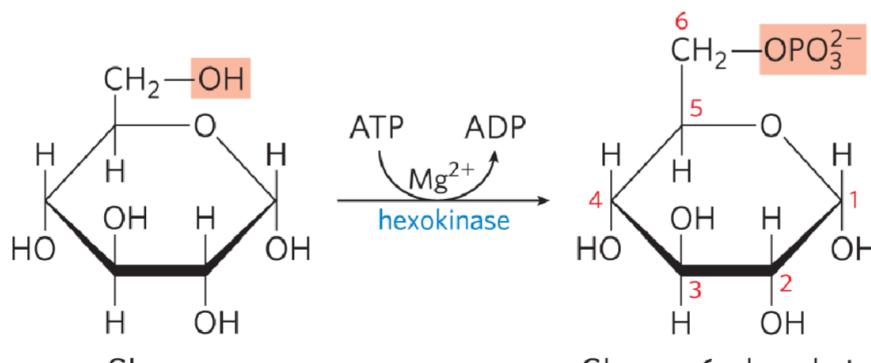
- All nine intermediates are phosphorylated
- Functions of the phosphoryl groups:
 - Prevent glycolytic intermediates from leaving the cell
 - Serve as essential components in the enzymatic conservation of metabolic energy
 - Lower the activation energy and increase the specificity of the enzymatic reactions

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



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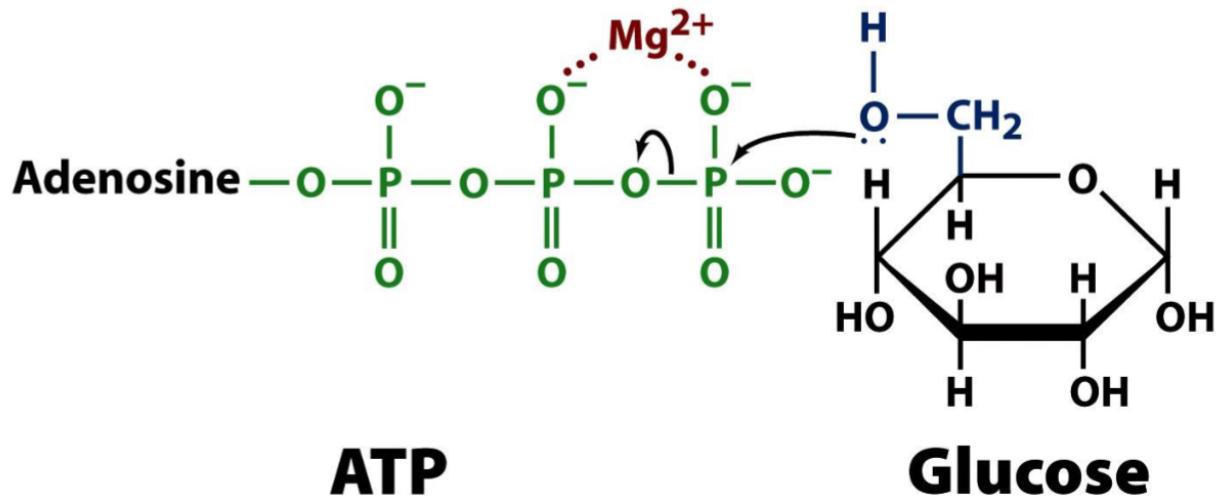
$$\Delta G'^\circ = -16.7 \text{ kJ/mol}$$

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

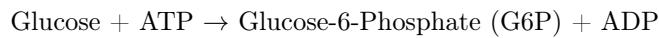
Hexokinase mechanism basics

- 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:

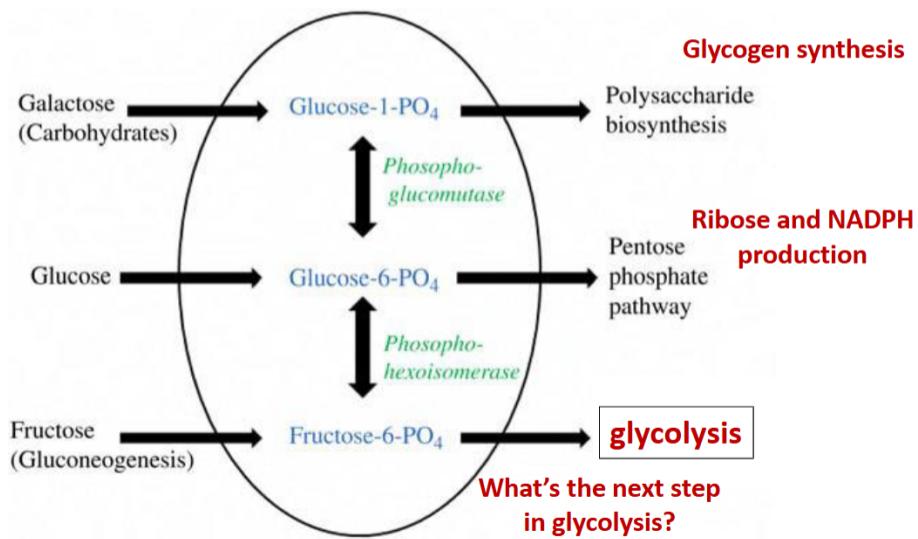


Commitment to Metabolic Pool:

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

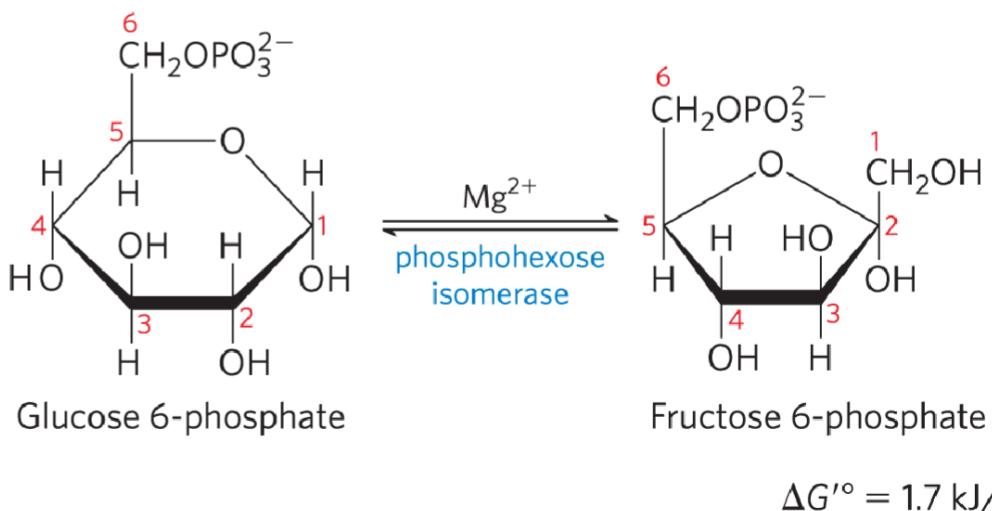
The pool of hexoses

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



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

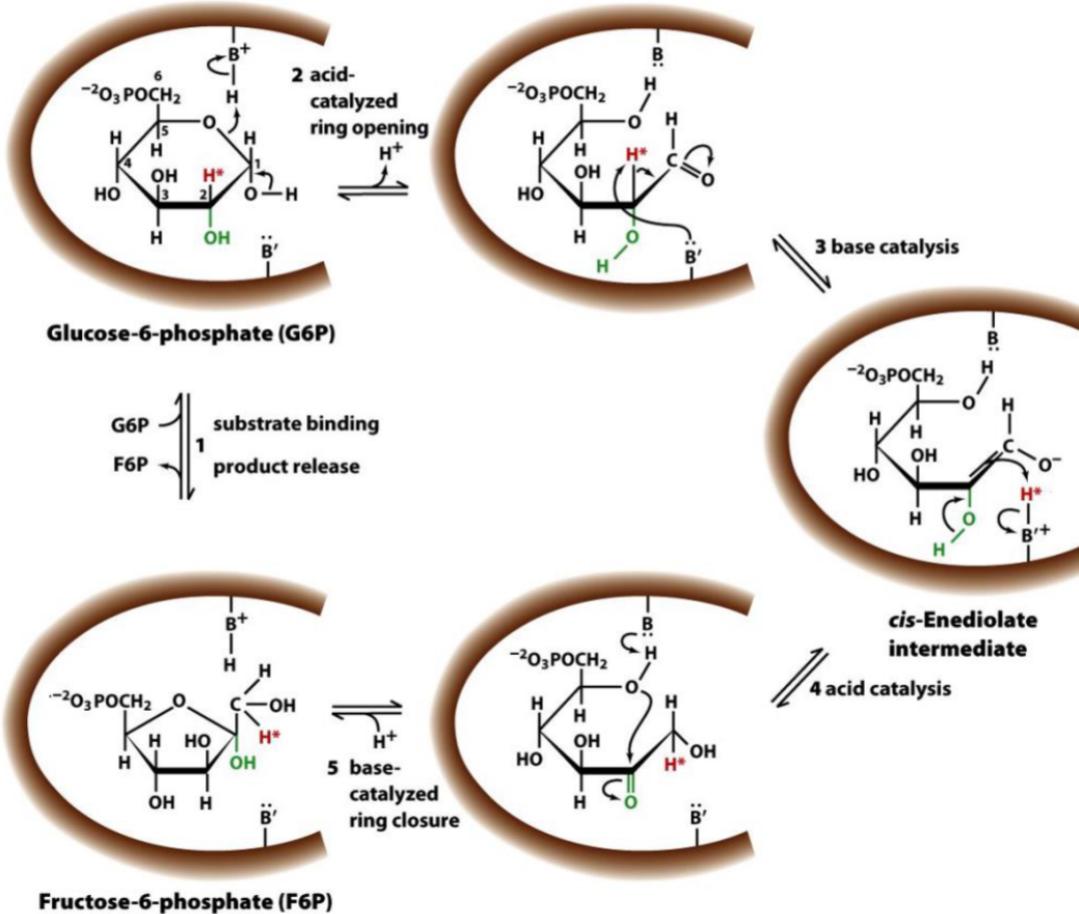
- Phosphohexose isomerase (phosphoglucose isomerase) catalyzes the reversible isomerization of glucose 6-phosphate to fructose 6-phosphate
 - mechanism involves an enediol intermediate
 - reaction readily proceeds in either direction



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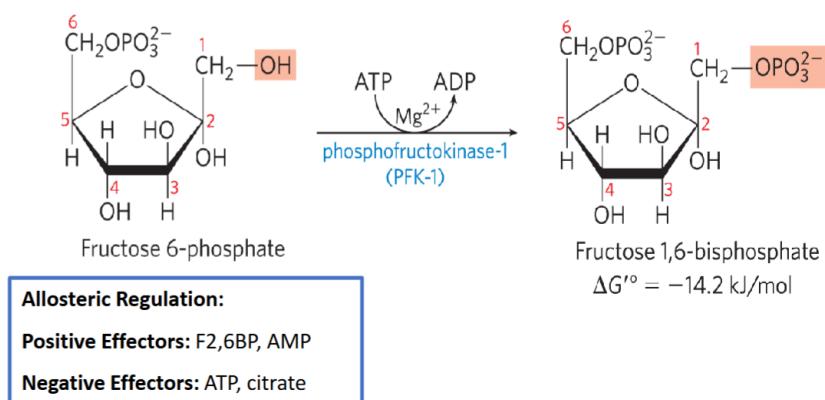
- The rearrangement of G6P to F6P is critical for the efficient progression of glycolysis. It ensures compatibility with downstream enzymes, facilitates the symmetrical cleavage of the sugar, and prepares the molecule for the energy-investment step catalyzed by PFK-1. Without this rearrangement, glycolysis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

Phosphohexose isomerase mechanism



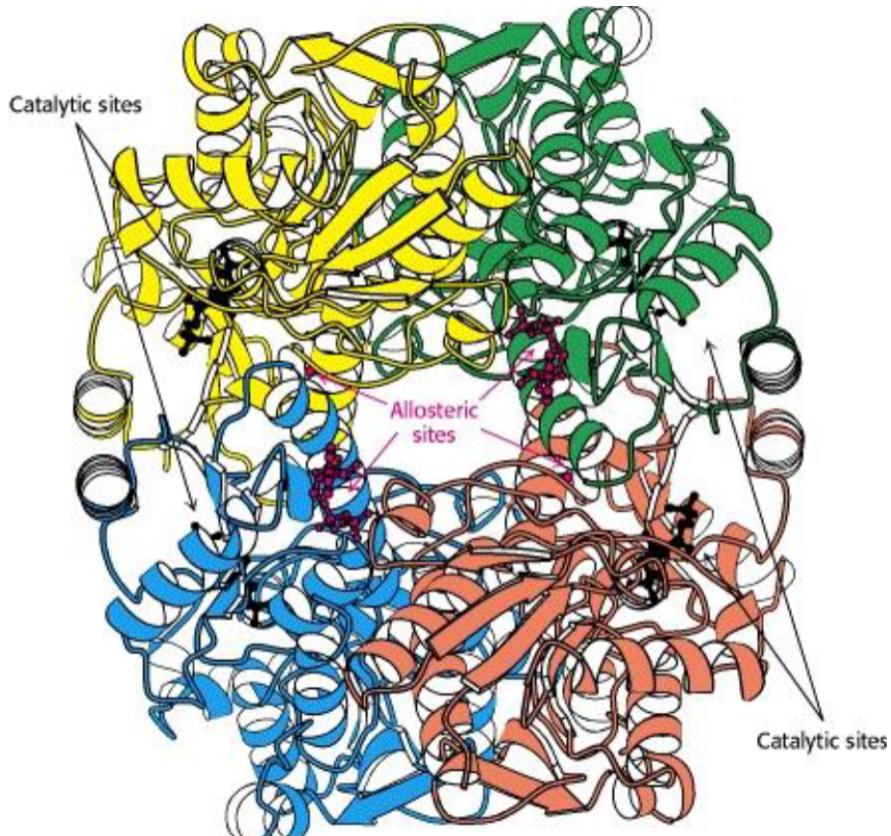
(Step 3) Phosphorylation of Fructose 6-Phosphate to Fructose 1,6-Bisphosphate

- 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

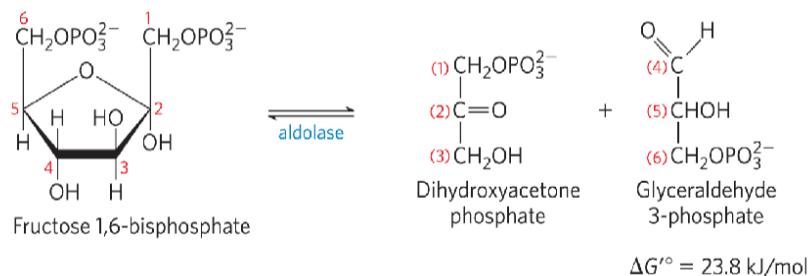
- Activity increases when:
 - ATP supply is depleted
 - ADP and AMP accumulate
- Fructose 2,6-bisphosphate is a potent allosteric activator
- PFK-1 acts as a metabolic "gatekeeper", integrating signals from the cell's energy status and hormonal environment. This regulation allows glycolysis to be precisely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

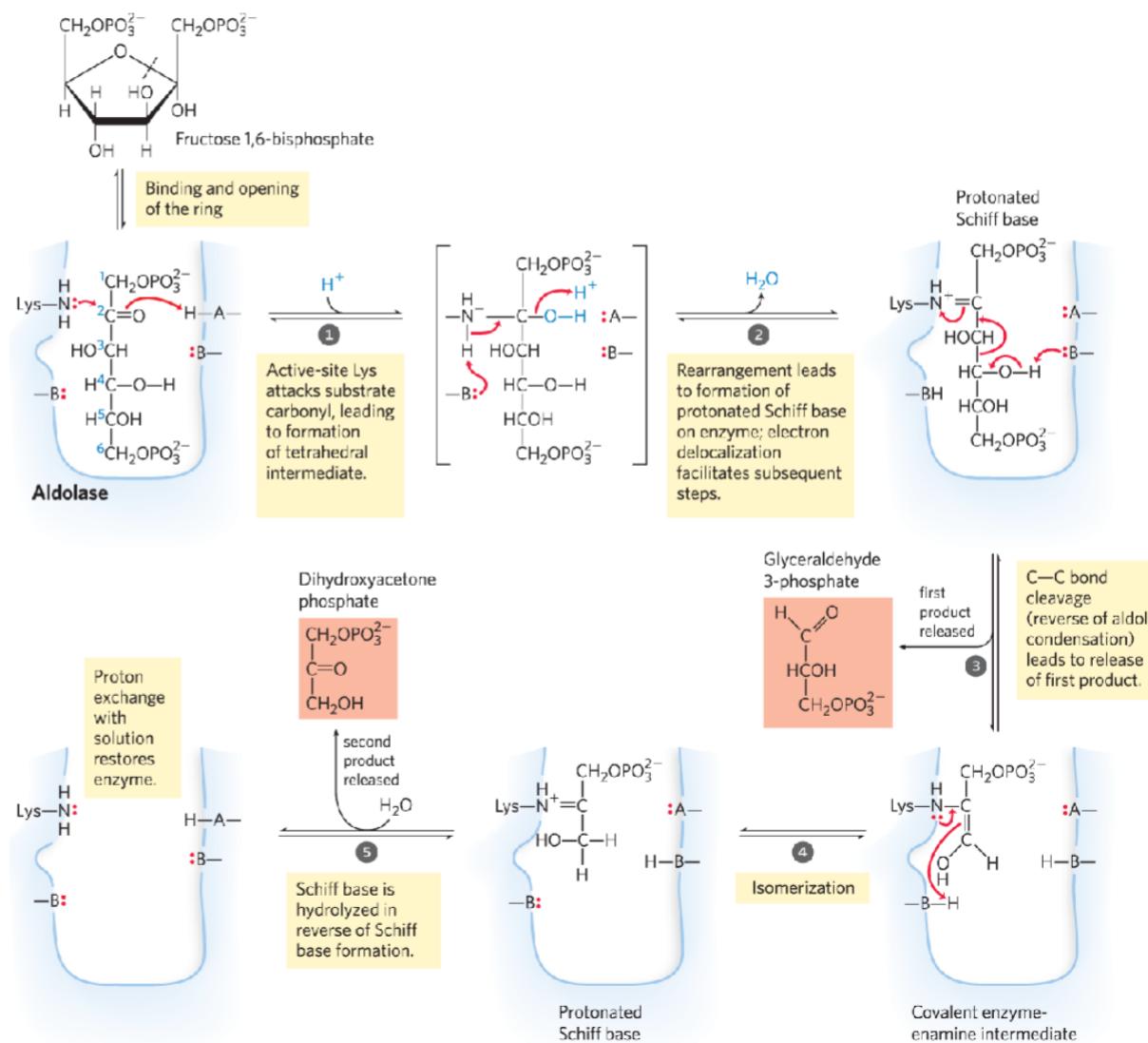
(Step 4) Cleavage of Fructose 1,6-Bisphosphate

- 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.



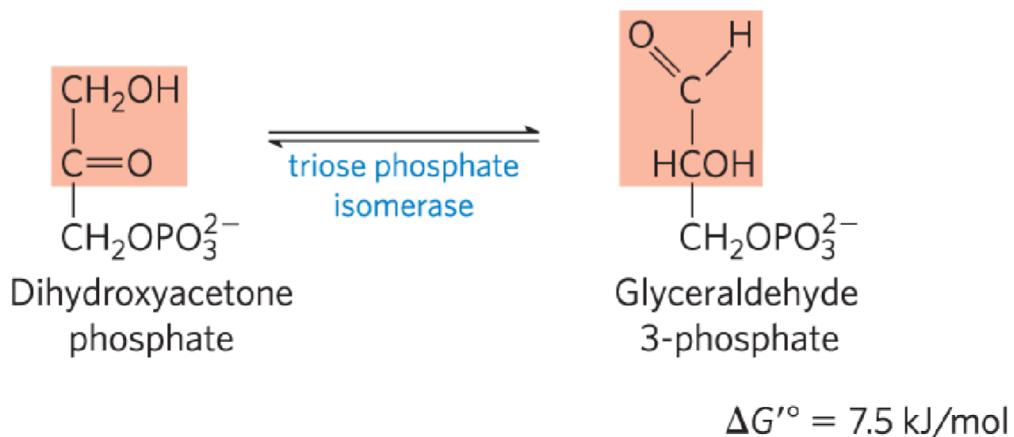
The Class I Aldolase Reaction

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



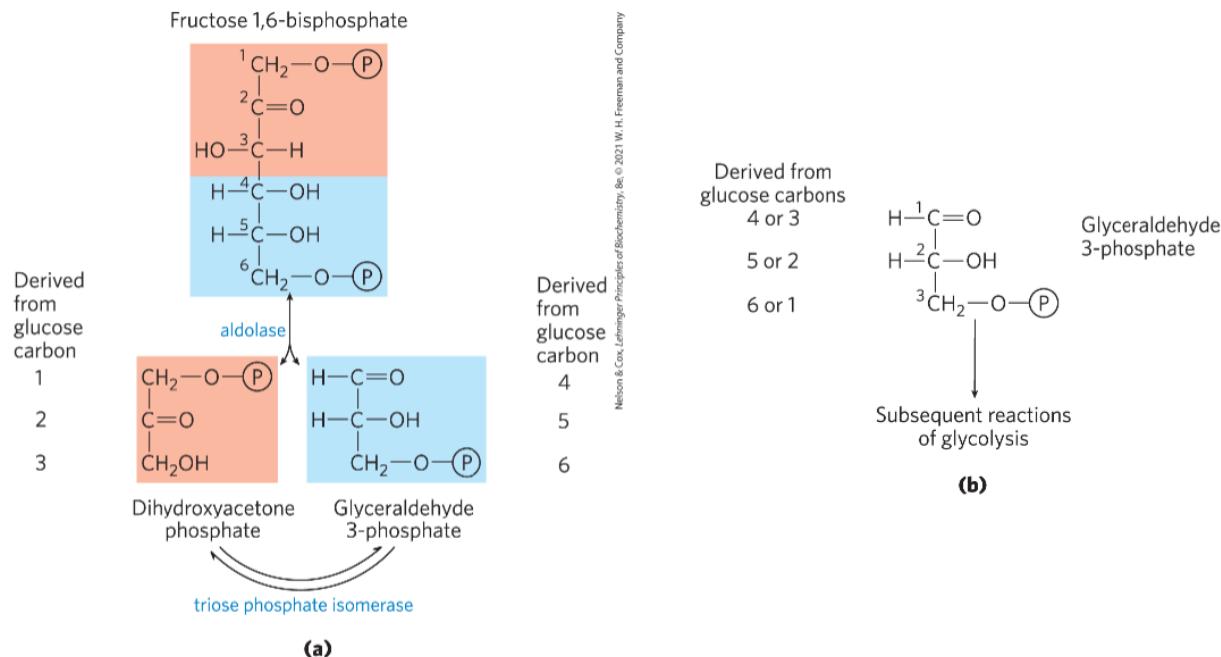
(Step 5) Interconversion of the Triose Phosphates

- 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

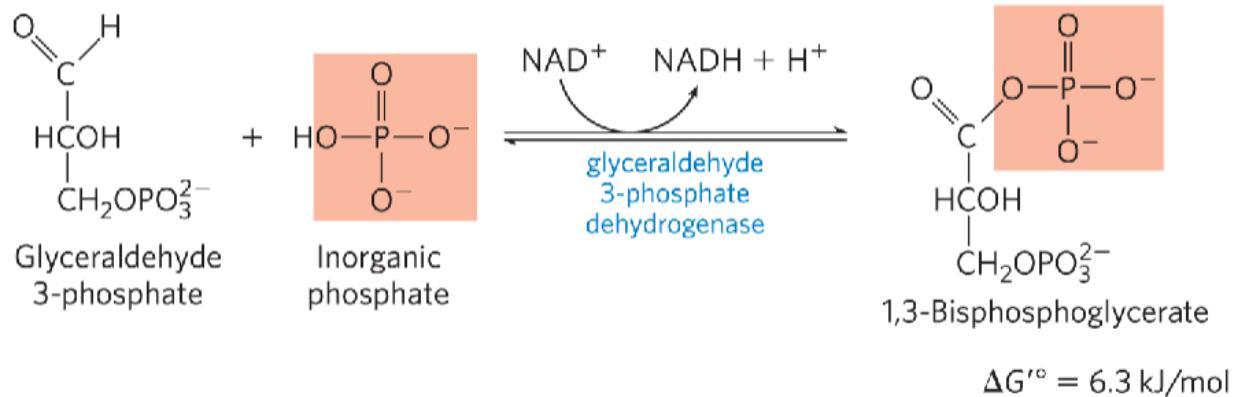
In the payoff phase of glycolysis:

- 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

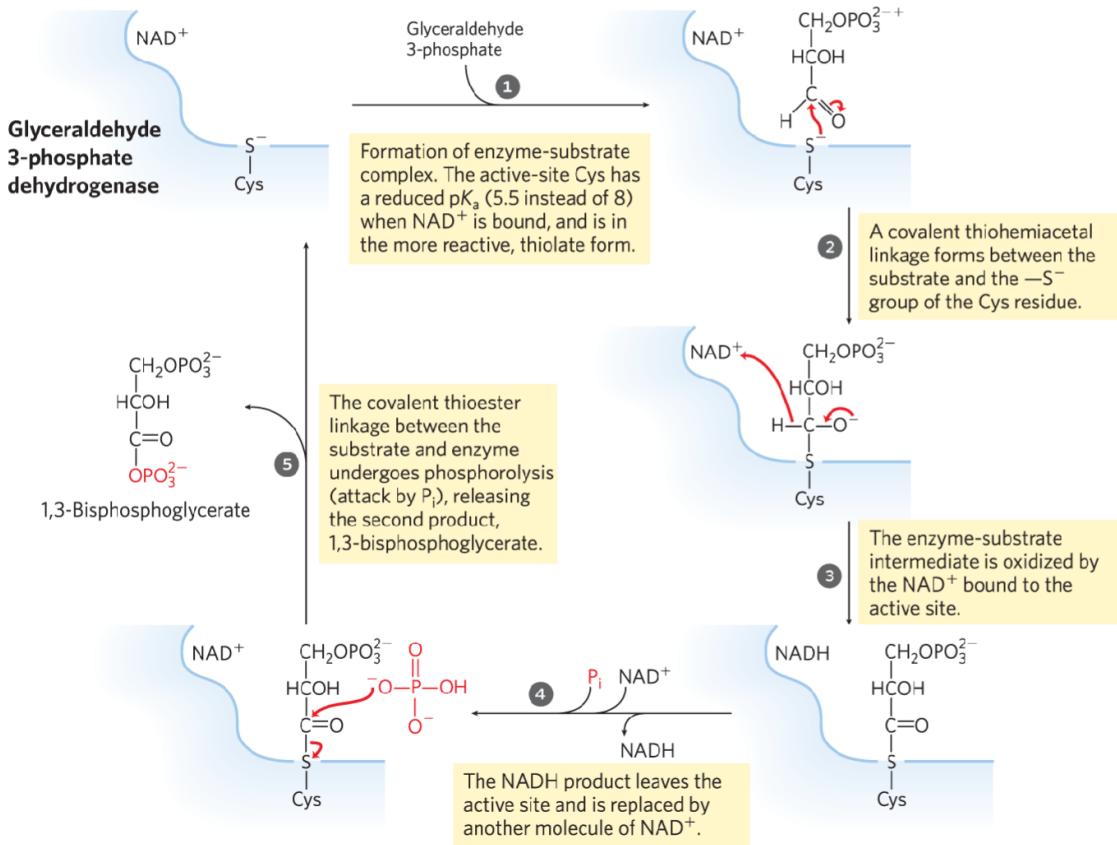


This reduction step stores energy with the formation of the acyl phosphate and in the form of high-energy electrons within NADH

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 ($\Delta G'^\circ = -49.3 \text{ kJ/mol}$)

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 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 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.

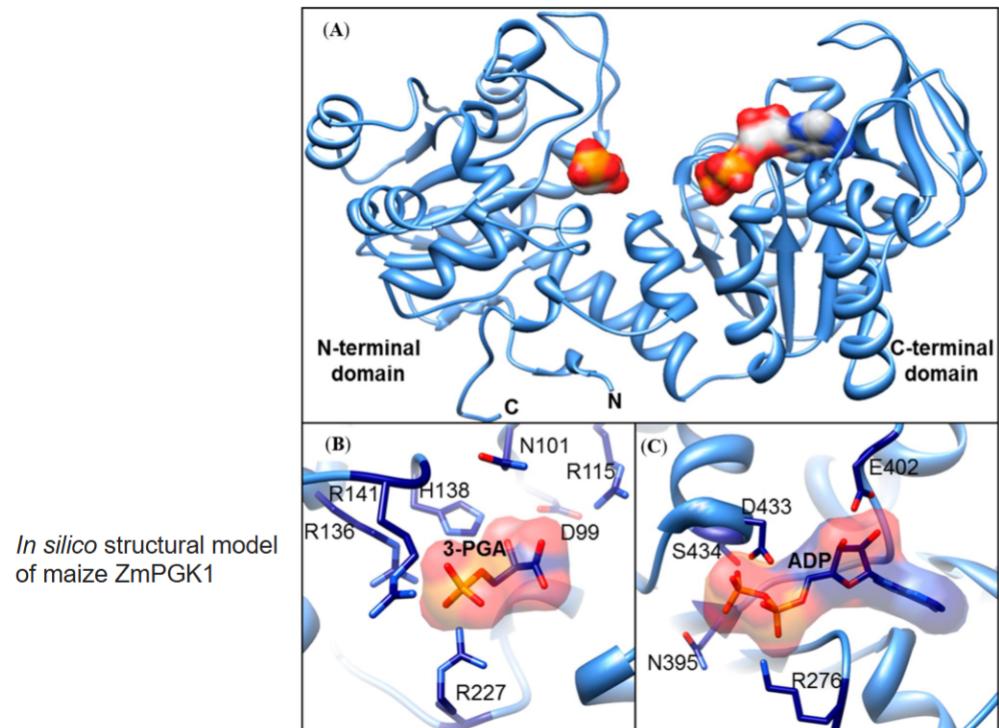
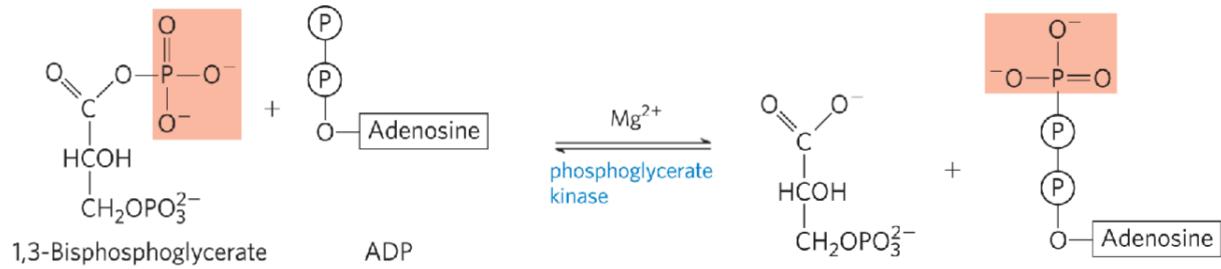
Why This Process Works

- 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
- 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

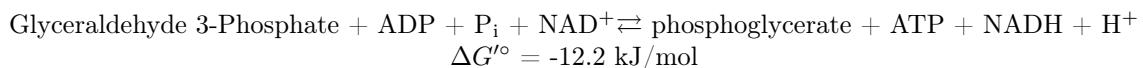
- Phosphoglycerate Kinase transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and **3-phosphoglycerate**

- substrate-level phosphorylation



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

- The sum of the two reactions is:



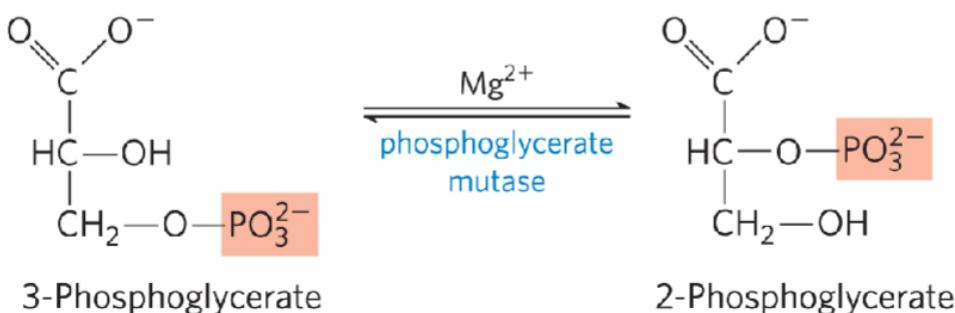
- substrate-level phosphorylation** = the formation of ATP by phosphoryl group transfer from a substrate different from **respiration-linked phosphorylation**
- G3P dehydrogenase is coupled to phosphoglycerate kinase
 - G3P dehydrogenase is forming a high energy phosphate while phosphoglycerate kinase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

		ΔG°
	GAPDH GAP + NAD ⁺ + P _i → 1,3-BPG + NADH + H ⁺	+6.3
first substrate-level phosphorylation	PGK + 1,3-BPG + ADP → 3-PG + ATP	-18.8
	GAP + NAD ⁺ + ADP + P _i → 3-PG + ATP + NADH + 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

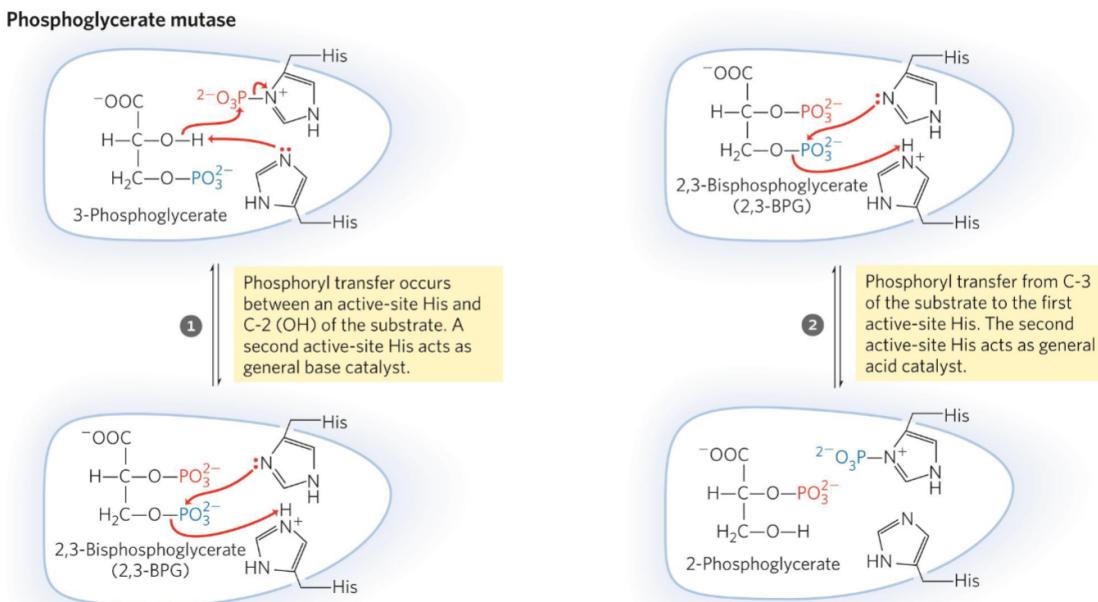
- requires Mg^{2+}



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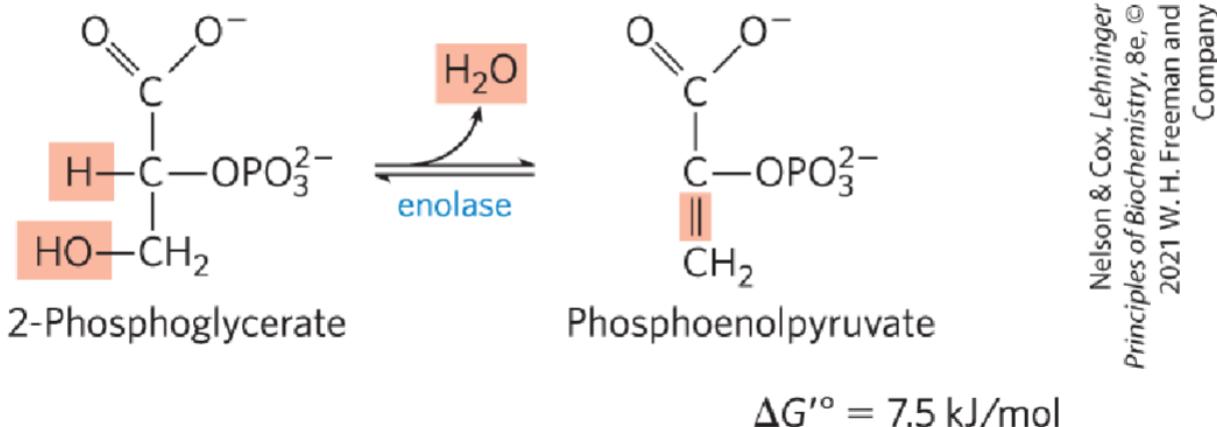
$$\Delta G'^\circ = 4.4 \text{ kJ/mol}$$

The Phosphoglycerate Mutase Reaction



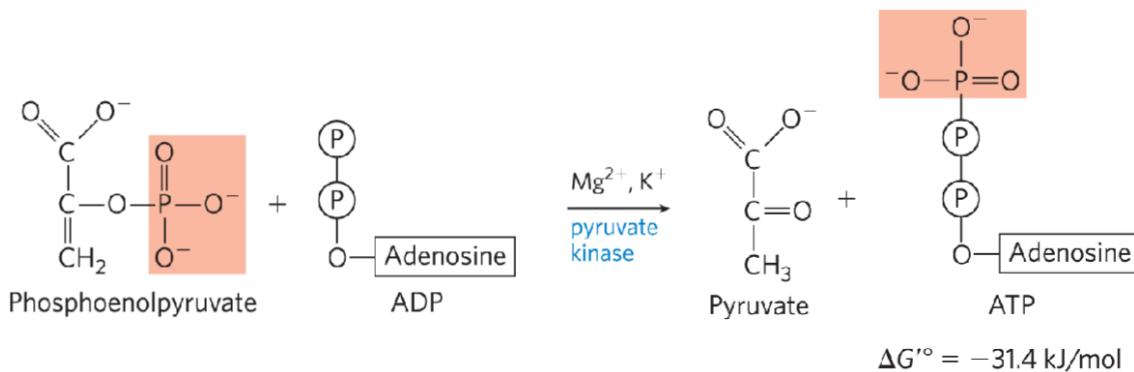
(Step 9) Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

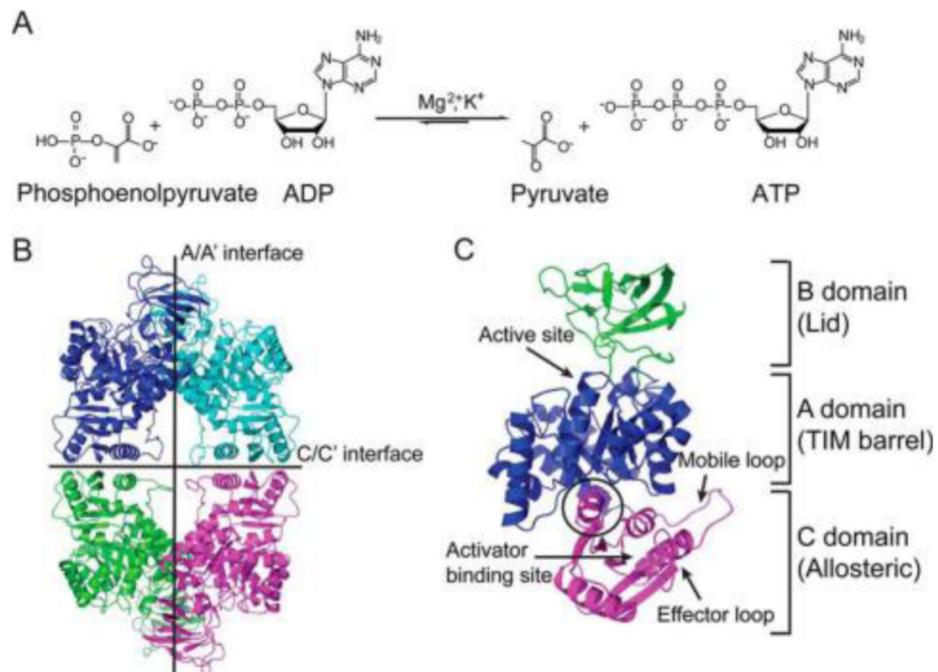
- **enolase** promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield **phosphoenolpyruvate (PEP)**
 - energy-conserving reaction
 - mechanism involves a Mg^{2+} -stabilized enolic intermediate



(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**

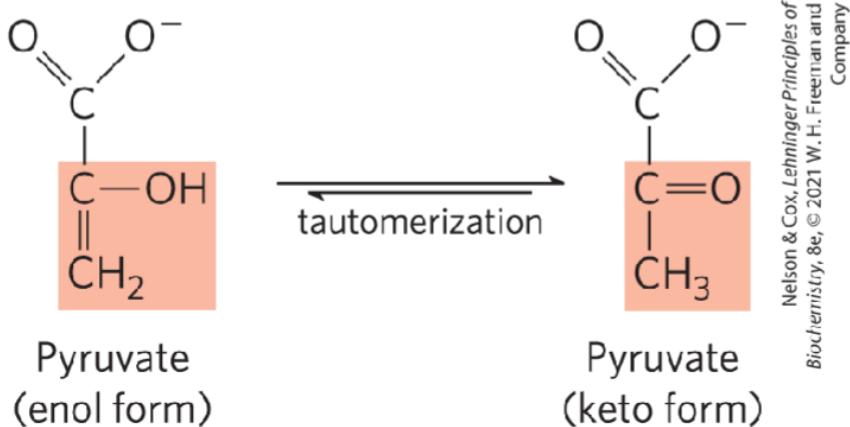




A, the reaction catalyzed by pyruvate kinase. B, structure of *E. coli* pyruvate kinase type 1 tetramer with the tetrameric A/A' and C/C' interfaces labeled. C, pyruvate kinase type 1 monomer showing the active site and allosteric binding site. The monomer is colored by domain, and the helix that connects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

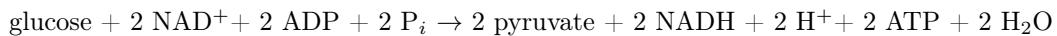
Pyruvate in its Enol Form Spontaneously Tautomerizes to its Keto Form

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

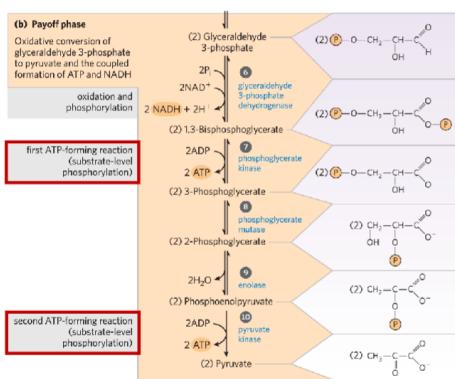


Table 15-1 ΔG° and ΔG for the Reactions of Glycolysis in Heart Muscle^a

Reaction	Enzyme	ΔG° (kJ · mol ⁻¹)	ΔG (kJ · mol ⁻¹)
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

^aCalculated 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

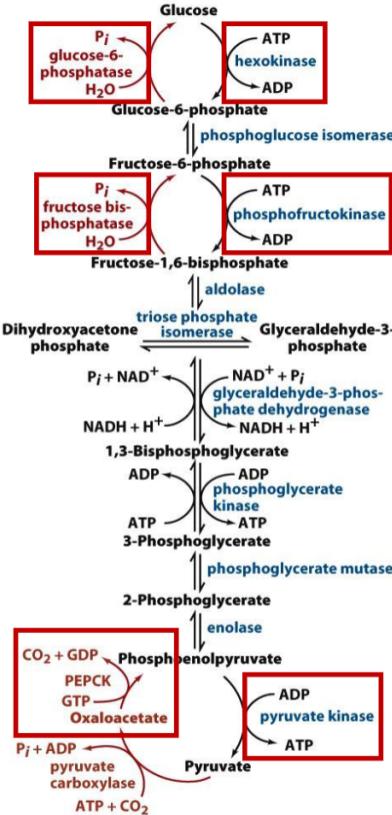
ΔG° vs. ΔG in Glycolysis

- ΔG° (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 ΔG° (unfavorable under standard conditions)
- ΔG (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 ΔG to drive the pathway forward

Steps with large, negative ΔG (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

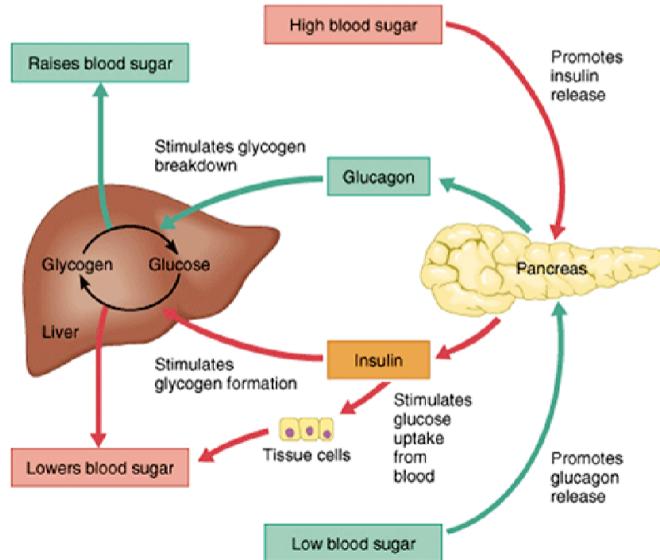
- Hexokinase Isoforms:
 - Type II (muscle): Inhibited by glucose-6-phosphate (G6P), which prevents the wasteful use of glucose when energy isn't needed
 - Type IV (glucokinase, liver): Not inhibited by G6P, has a higher Km (works at higher glucose concentrations), and is induced by insulin, helping the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

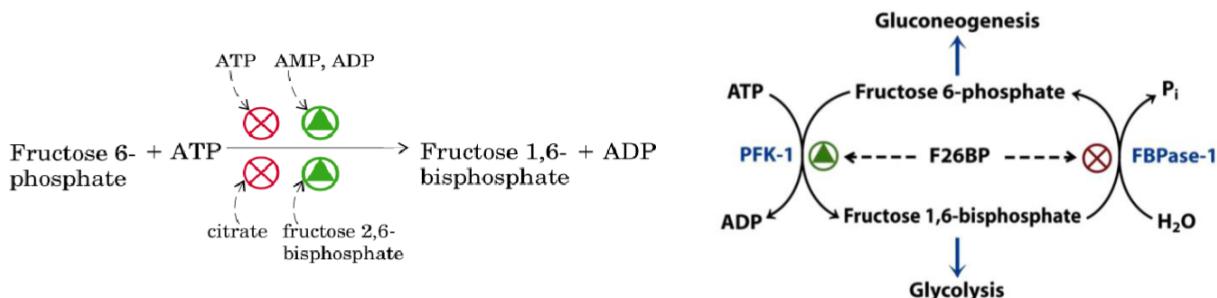


Things we're missing from the diagram:

- **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

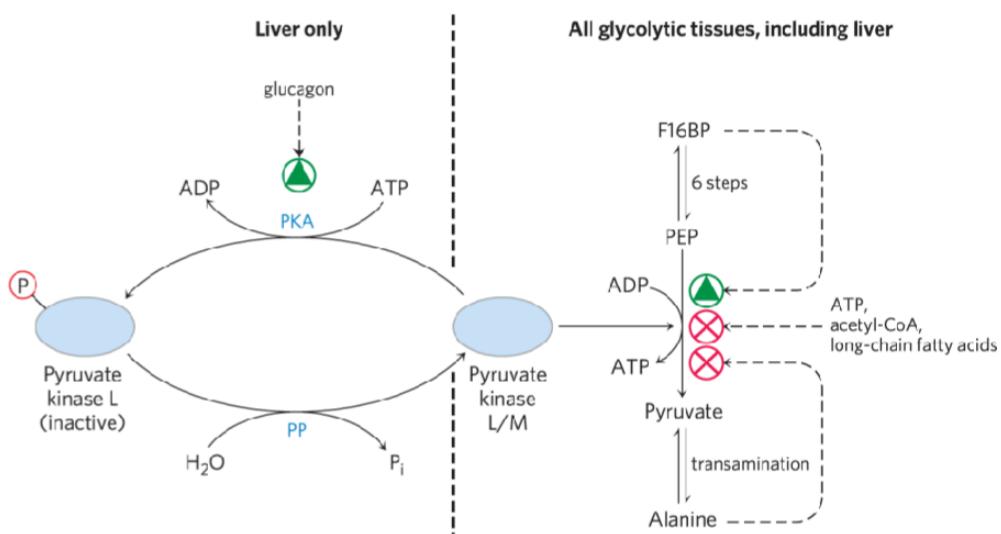
 - Inhibited by:

 - * **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

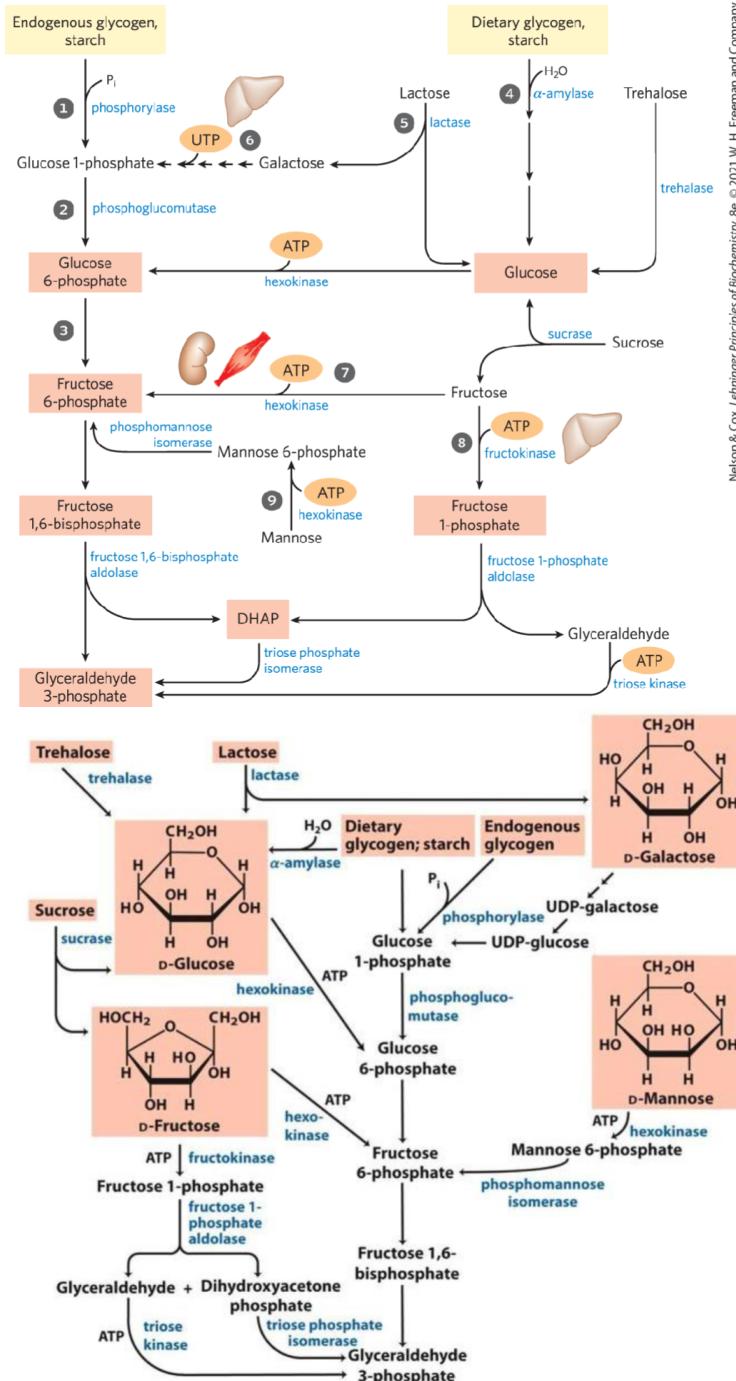
- Big Picture:

 - 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.



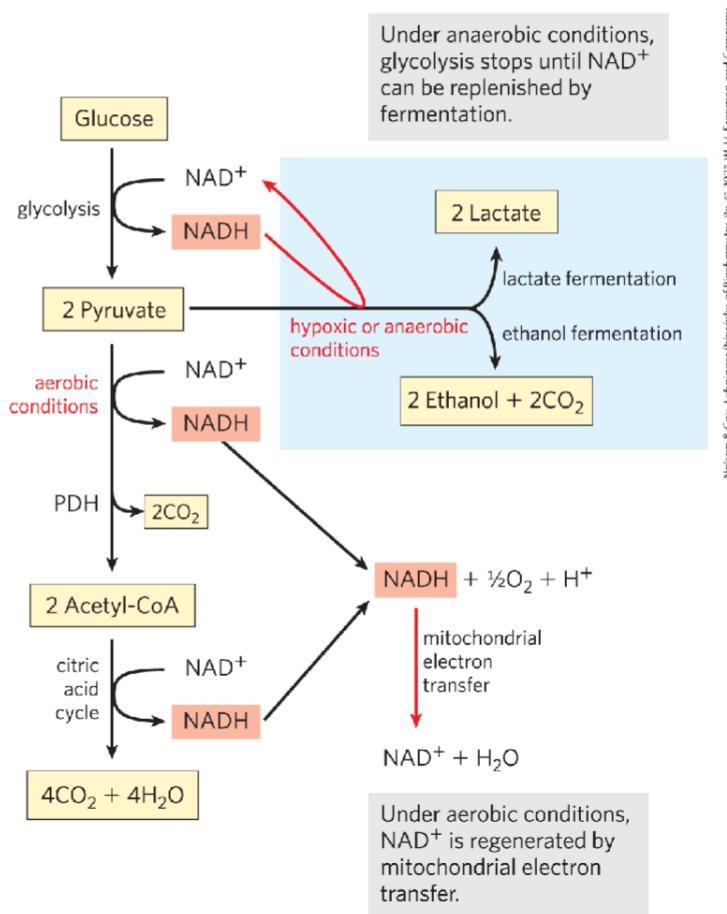
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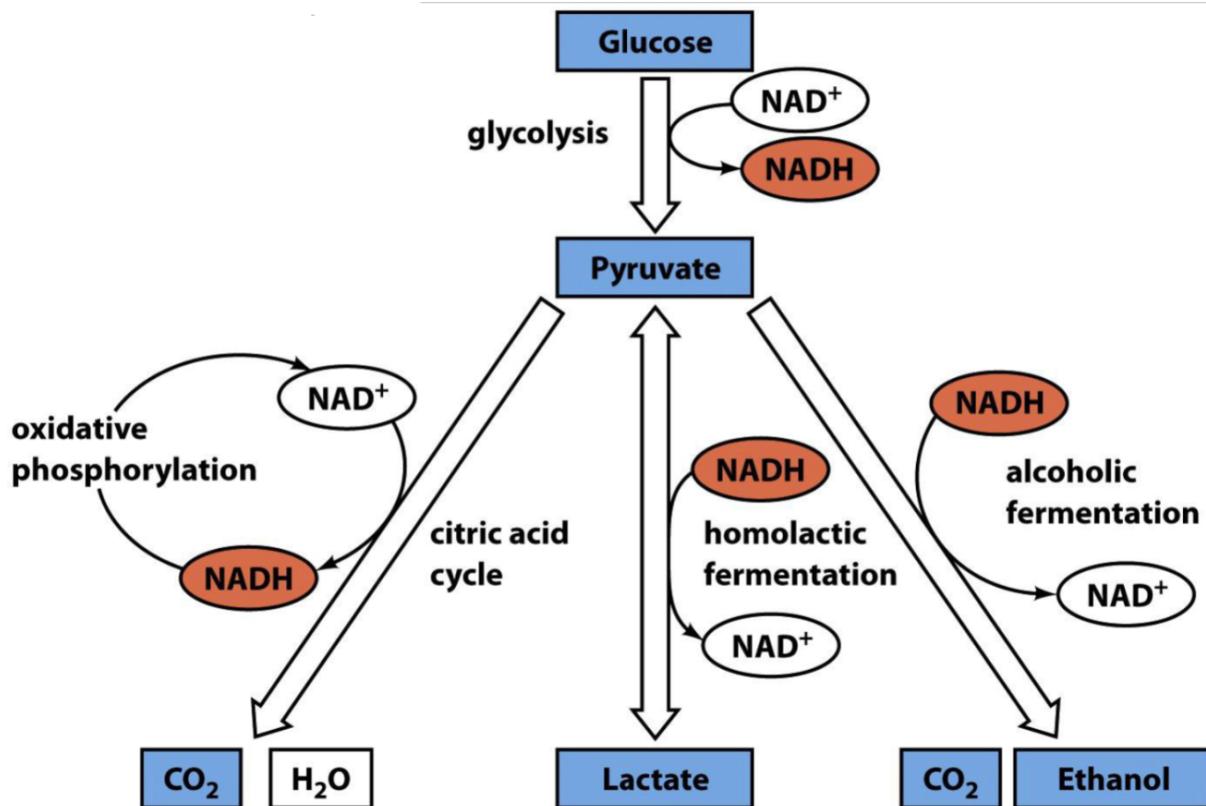
Energy Remaining in Pyruvate

- 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⁺
- 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⁺, and allowing continued glycolysis in the processes of lactate or alcohol fermentation

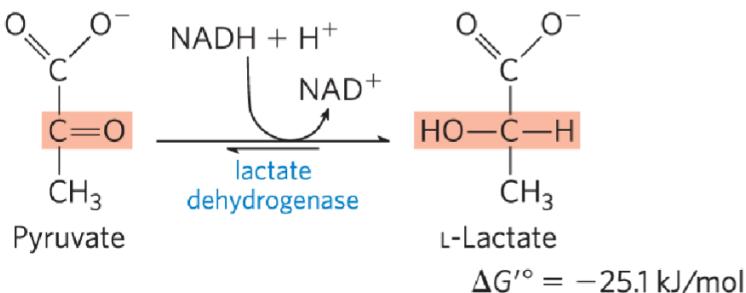
Fermentation

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

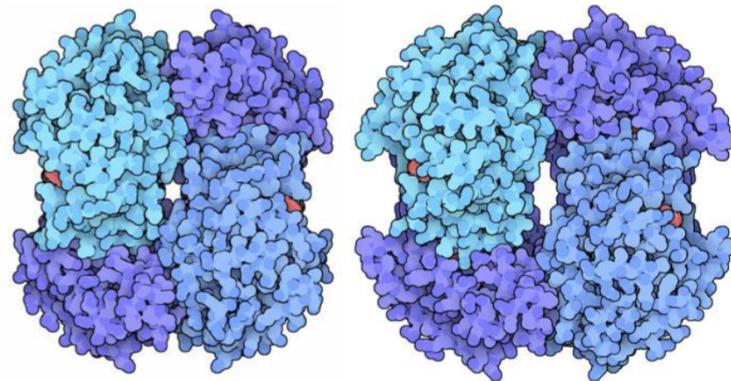
- **lactic acid fermentation** = pyruvate accepts electrons from NADH and is reduced to lactate (one step) while regenerating the NAD⁺ necessary for glycolysis
- **ethanol (alcohol) fermentation** = pyruvate is further catabolized (two steps) to ethanol

Pyruvate is the Terminal Electron Acceptor in Lactic Acid Fermentation

- Organisms can regenerate NAD⁺ 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.

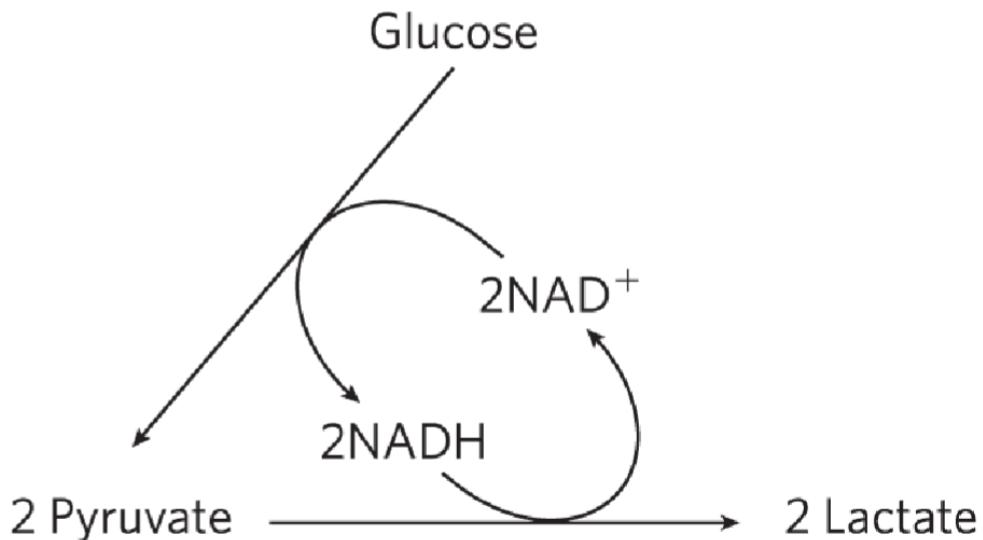


Active (left) and inactive (right) lactate dehydrogenase
NAD in red

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

Reduction of Pyruvate to Lactate Regenerates NAD^+

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

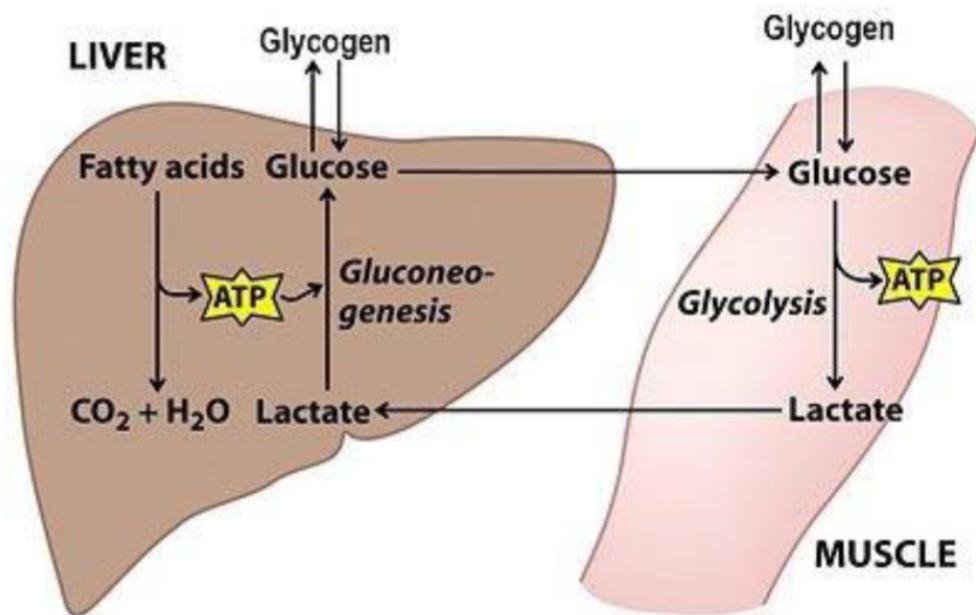


Lactate can be Recycled

- 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?

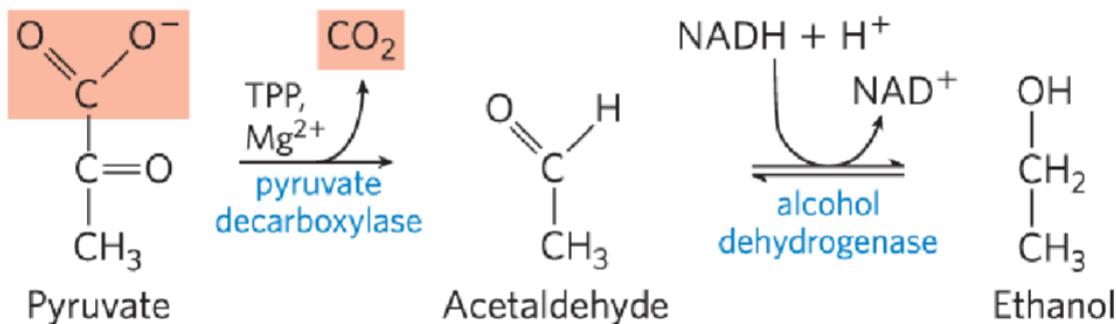
- The brain, nervous system, and red blood cells rely exclusively on glucose for ATP production
- Prolonged fasting or intense exercise depletes glycogen, **requiring glucose synthesis through gluconeogenesis**
- The liver upregulates gluconeogenesis to synthesize glucose and export it to meet the energy 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⁺ by reducing pyruvate to ethanol and CO₂



- The overall equation is:



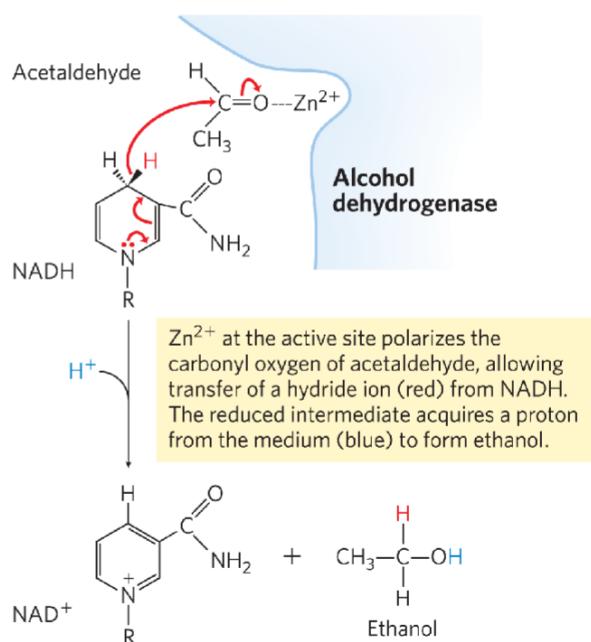
- Yeast have evolved to thrive in high-sugar, low-oxygen environments by using fermentation. The production of ethanol during fermentation is toxic to many competing organisms, giving yeast a competitive advantage
- Fermentation is much faster than aerobic respiration, though less efficient. In high-glucose 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 CO₂ for leavening. In alcohol production, ethanol is the desired product, and fermentation under anaerobic conditions ensures its accumulation.

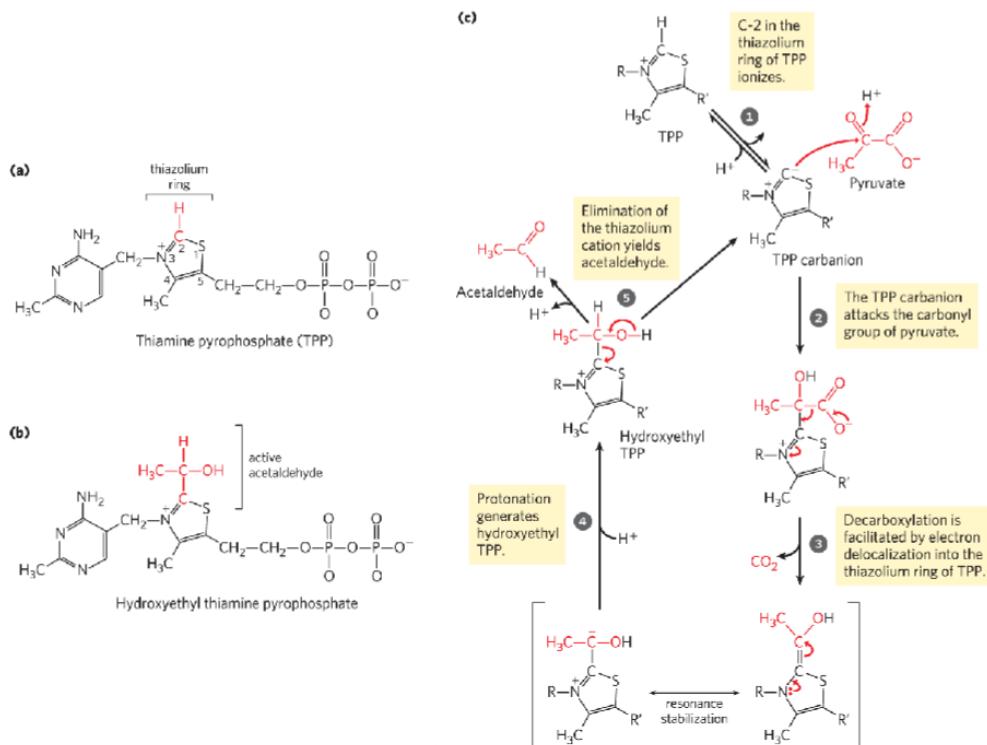
Pyruvate Decarboxylase and Alcohol Dehydrogenase Reactions

- **Pyruvate decarboxylase:** catalyzes the irreversible decarboxylation of pyruvate to acetaldehyde
 - requires Mg²⁺ 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 B₁.



- **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, CO₂ 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 α -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	$\text{R}^3-\overset{\text{O}}{\underset{\text{C}}{\text{ }}}-\overset{\text{OH}}{\underset{\text{C}}{\text{ }}}-\text{R}^4$	$\text{R}^3-\overset{\text{O}}{\underset{\text{C}}{\text{ }}}-\overset{\text{OH}}{\underset{\text{C}}{\text{ }}}-\text{R}^5$

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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 O₂ \rightarrow 6 CO₂ + 6 H₂O

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

- 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 ₂)	CO ₂ + H ₂ O
Energy Efficiency	Low	High

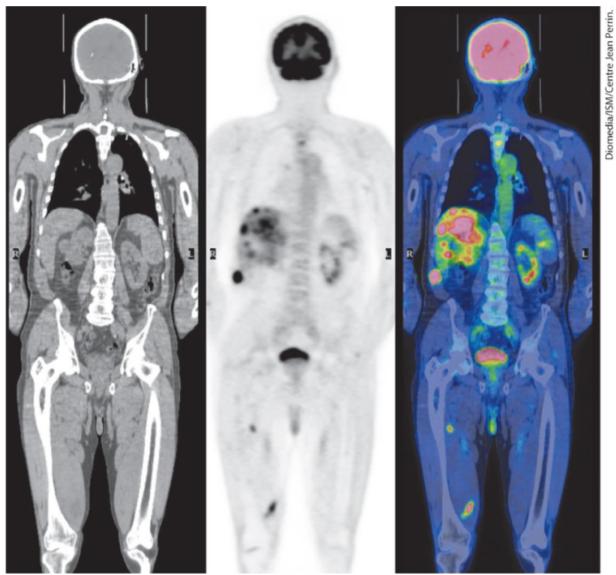
The Pasteur and Warburg Effects are due to the Dependence on Glycolysis Alone for ATP Production

- 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 CO₂ (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

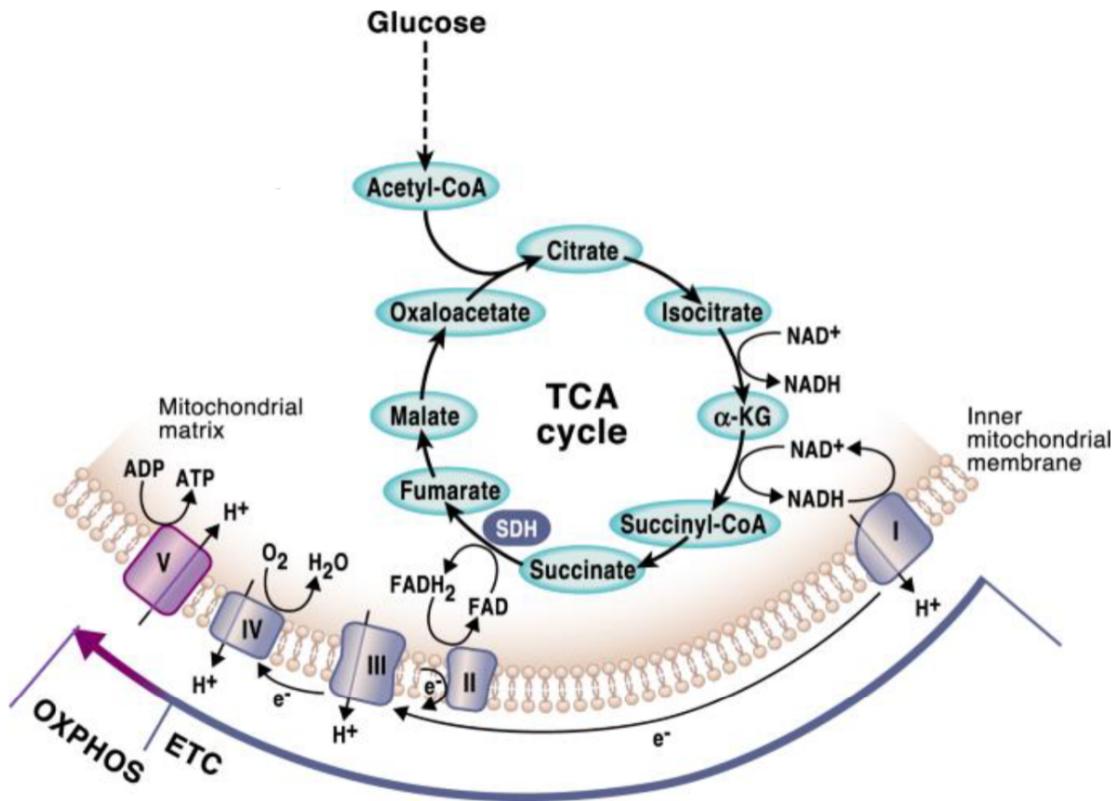
- The basis of PET scanning used to diagnose tumors
- Tumor cells often grow away from arteries, so they naturally have less access to oxygen.



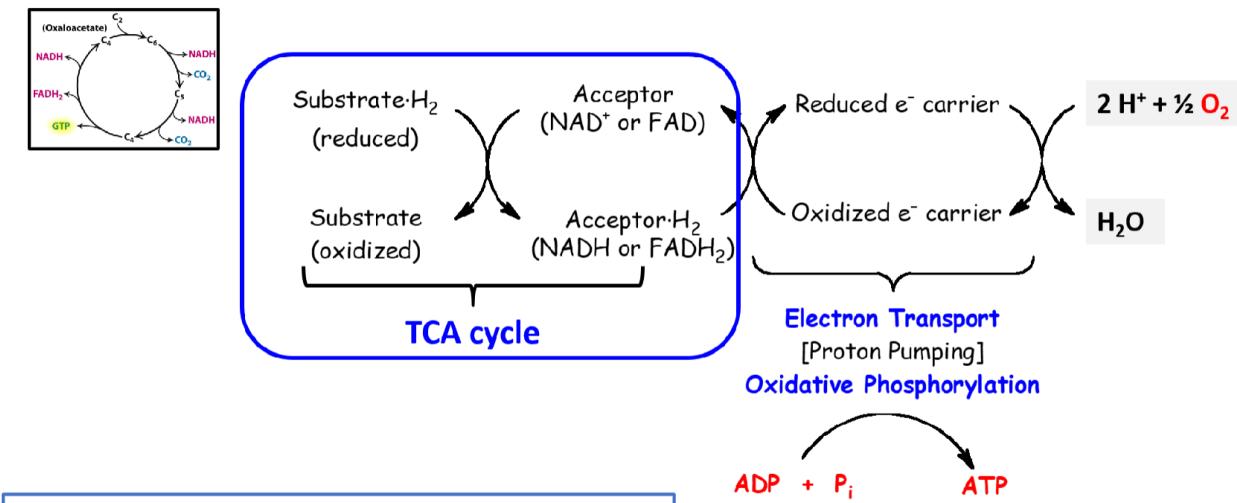
Tricarboxylic Acid Cycle

Also known as...

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



The Goal of the TCA cycle

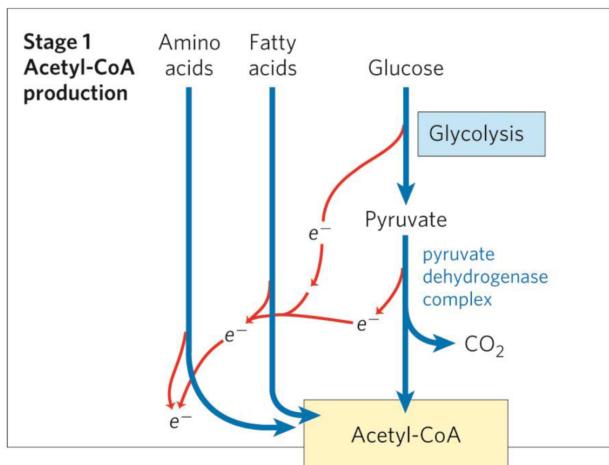


Cellular Respiration

cellular respiration = process by which the pyruvate produced by glycolysis is further oxidized to H₂O and CO₂

Stage 1 of Cellular Respiration

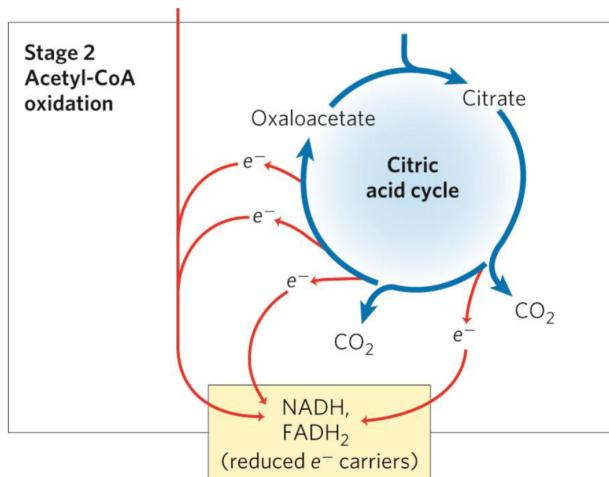
- Stage 1: Oxidation of fuels to acetyl-CoA
 - generates ATP, NADH, FADH₂



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Stage 2 of Cellular Respiration

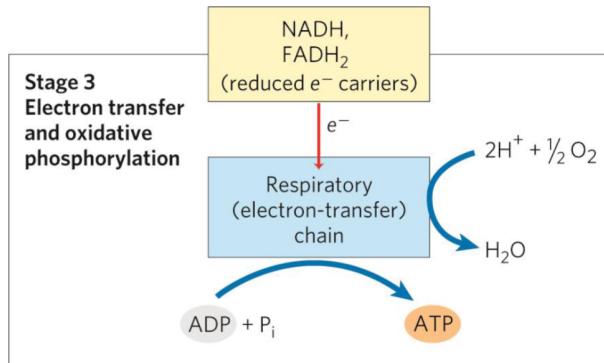
- Stage 2: oxidation of acetyl groups to CO_2 in the **citric acid cycle (tricarboxylic acid (TCA) cycle, Krebs cycle)**
 - generates NADH, FADH₂, and one GTP



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Stage 3 of Cellular Respiration

- Stage 3: electron transfer chain and oxidative phosphorylation
 - generates the vast majority of ATP from catabolism



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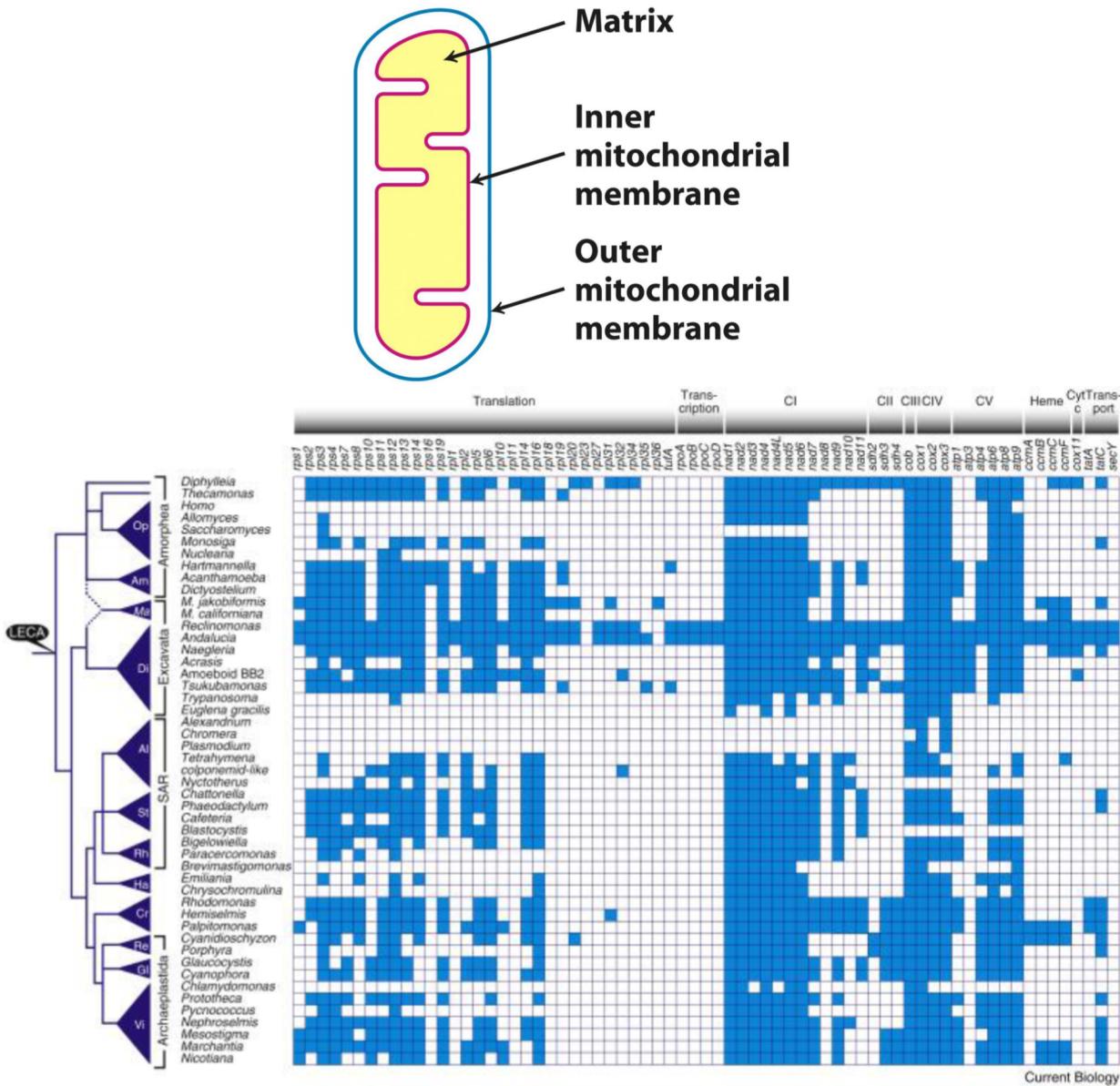
Pyruvate is the metabolite that links two central catabolic pathways, glycolysis, and the citric acid cycle. It is therefore a logical point for regulation that determines the rate of catabolic activity and the partitioning of pyruvate among its possible uses.

Pyruvate is Oxidized to Acetyl-CoA and CO₂

- **Mitochondrial pyruvate carrier (MPC)** = an H⁺-coupled pyruvate specific symporter in the inner mitochondrial membrane
- **pyruvate dehydrogenase (PDH) complex** = highly ordered cluster of enzymes and cofactors that oxidizes pyruvate in the mitochondrial matrix to acetyl-CoA and CO₂
 - the series of chemical intermediates remain bound to the enzyme subunits
 - regulation results in precisely regulated flux

The Mitochondrion

- Energy production: Site of **aerobic respiration**, oxidizing pyruvate to CO₂ and generating ATP
- Diverse biochemical processes:
 - Protein synthesis
 - Amino acid and nucleotide metabolism
 - Fatty-acid catabolism
 - Lipid, quinone, and steroid biosynthesis
 - Iron-sulfur (Fe/S) cluster biogenesis
 - Apoptosis (programmed cell death)
- The mitochondrial proteome contains over 1000 proteins, all (ETC subunits) contributing to many cellular pathways beyond ATP synthesis



The Endosymbiotic Origin of Mitochondria

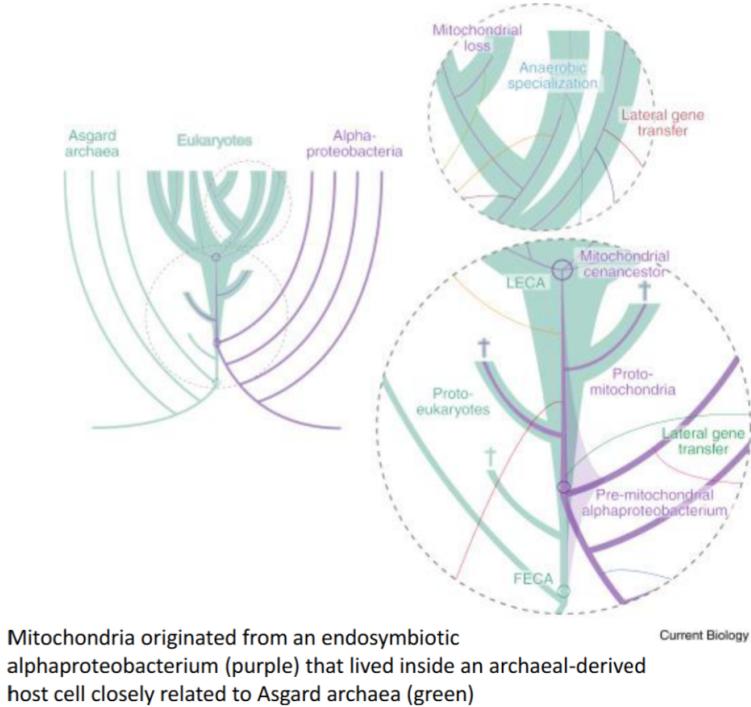
Lynn Margulis and the Endosymbiotic Theory (1967)

- Mitochondria evolved from an **endosymbiotic relationship** with an ancestral organism
- Phylogenetic analyses confirmed:
 - Mitochondria originated from a lineage related to **alphaproteobacteria**
 - The host lineage is closely related to **Asgard Archaea**
- Early controversy turned into widespread acceptance with advances in sequencing and proteomics

Prokaryotic Feature of Mitochondria:

- Double membrane
- Circular DNA similar to bacteria

- Prokaryote-like ribosomes



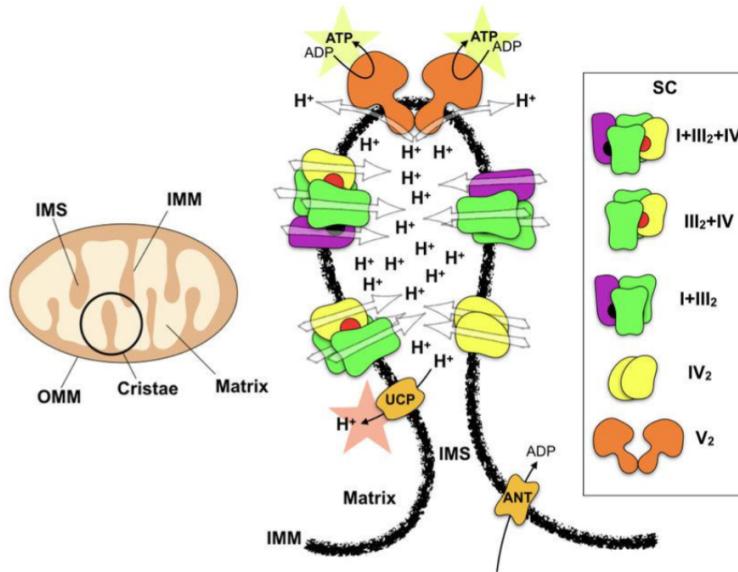
Mitochondria and the Evolution of Eukaryotes

- The Last Eukaryotic Common Ancestor (LECA)
 - All modern eukaryotes are descended from a mitochondrion-containing ancestor
 - LECA had many features of modern eukaryotes, including a fully functional mitochondrion
- Mitochondria's Evolutionary Role:
 - Enabled eukaryotes to thrive in oxygen-rich environments through efficient ATP production
 - Supported the evolution of multicellularity and cellular complexity
- Ongoing Research:
 - Genomic and cell biology studies reveal diversity in mitochondrial structure and function across
 - Controversy remains regarding the exact bacterial lineage that gave rise to mitochondria

The Mitochondrion

- Double Membrane Structure
 - The mitochondrion is enclosed by an **outer membrane** and an **inner membrane (IMM)**
 - Both membranes are **semi-permeable**, with the IMM being **impermeable to charged molecules** like protons, ensuring the separation of compartments necessary for energy production
- Cristae - Maximizing Efficiency:
 - Cristae are the **folded structures of the IMM**, significantly increasing its surface area
 - This expanded surface area accommodates more **Electron Transport Chain (ETC) complexes** and **ATP synthase**, enhancing the mitochondrion's capacity for ATP production

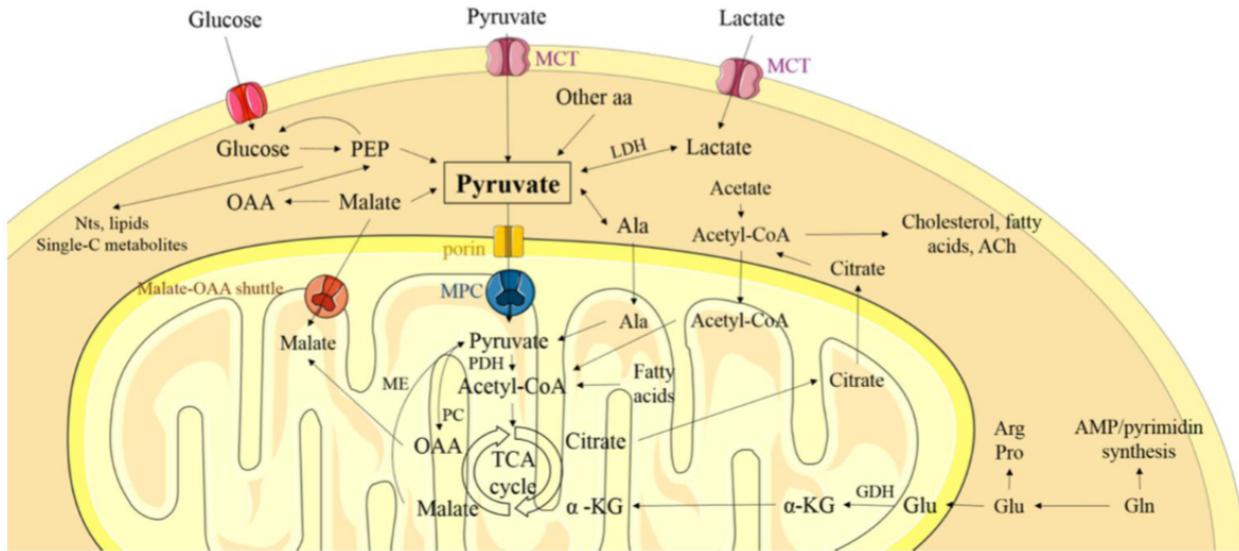
- Cristae also facilitate the **compartmentalization and concentration of protons**, creating a stronger electrochemical gradient for ATP synthesis
- **Mitochondrial Matrix:** The **matrix** is the internal space of the mitochondrion. It houses the enzymes of the **TCA cycle (Krebs cycle)**, which generate NADH and FADH₂, essential electron carriers for the electron transport chain.



The ETC complexes and accessory proteins are organized into individual **complexes** and **supercomplexes** embedded in the IMM. These complexes pump **protons (H⁺)** from the **matrix** into the **intermembrane space (IMS)**, creating an **electrochemical gradient**. The electrochemical gradient drives **Complex V (ATP synthase)** to produce ATP (lime-colored stars) from ADP.

Pyruvate Transport

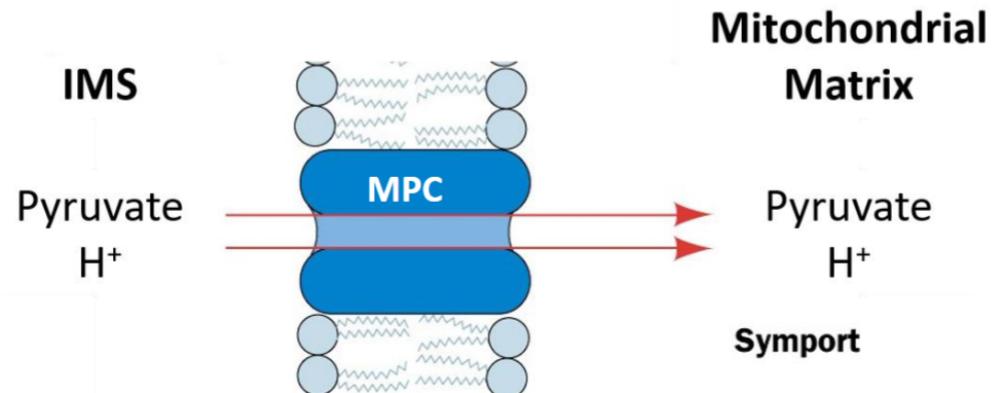
- The transport of pyruvate into the mitochondria involves crossing **two membranes**: the **outer mitochondrial membrane (OMM)** and the **inner mitochondrial membrane (IMM)**
 - The OMM contains **porins**, which are large, non-selective protein channels. These porins allow **small molecules like pyruvate** (and other metabolites up to ~5 kDa) to diffuse freely between the cytosol and the **intermembrane space (IMS)**
 - **Mechanism:** Pyruvate diffuses through the porins in a passive manner, driven by its concentration gradient



- The IMM is impermeable to charged or polar molecules, including pyruvate, so it requires a **specific transporter** for pyruvate to enter the matrix. Transport is mediated by the **Mitochondrial Pyruvate Carrier (MPC)**, a protein complex embedded in the IMM:
- Pyruvate is transported into the mitochondrial matrix together with a proton (H^+) via the MPC
- This symport is powered by the **proton gradient** across the IMM:
 - The **intermembrane space (IMS)** has a lower pH (7.0-7.4), while the **matrix** has a higher pH (7.8)
 - Protons moving down their gradient into the matrix drive the transport of pyruvate into the matrix

Energy Source:

- The transport is **secondary active transport**, as it indirectly uses the energy from the proton gradient created by the electron transport chain (ETC)



Pyruvate is imported into the mitochondrial matrix for oxidation by the TCA cycle

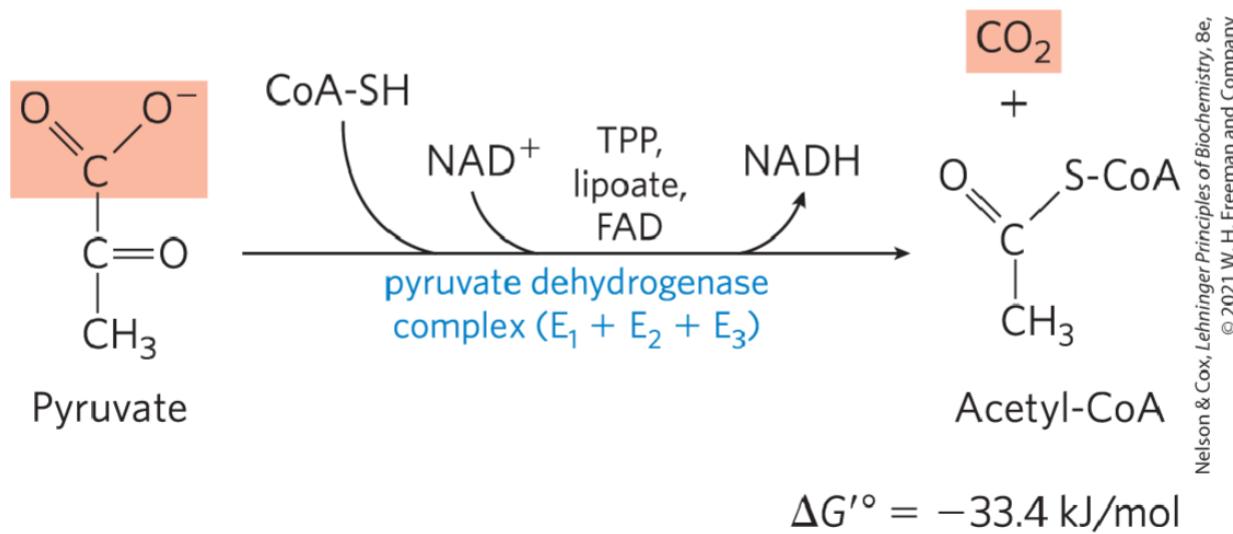
- **What happens?** Pyruvate and H^+ symport into the matrix via mitochondrial pyruvate carrier (MPC)
- **What powers the transport?** Driven by the pH gradient: matrix (pH 7.8) vs. IMS (pH 7.0-7.4)
- **Why is it important?** Essential for TCA cycle and ATP production

Symport mechanism:

- Pyruvate is transported into the matrix together with a proton (H^+) via the MPC in a process called **symport**
- The inward flow of protons (driven by the gradient) provides the energy to "pull" pyruvate into the matrix, even if the pyruvate concentration is higher inside the matrix than in the IMS. This pH gradient (a component of the proton-motive force) is the **driving force** for this transport, leveraging the natural movement of protons down their gradient to "power" the symport of pyruvate

The PDH Complex Catalyzes an Oxidative Decarboxylation

- **oxidative decarboxylation:** an irreversible oxidation process in which the carboxyl group is removed, forming CO_2 .



The PDH Complex Employs Three Enzymes and Five Coenzymes to Oxidize Pyruvate

Three enzymes:

- pyruvate dehydrogenase, E_1
- dihydrolipoyl transacetylase, E_2
- dihydrolipoyl dehydrogenase, E_3

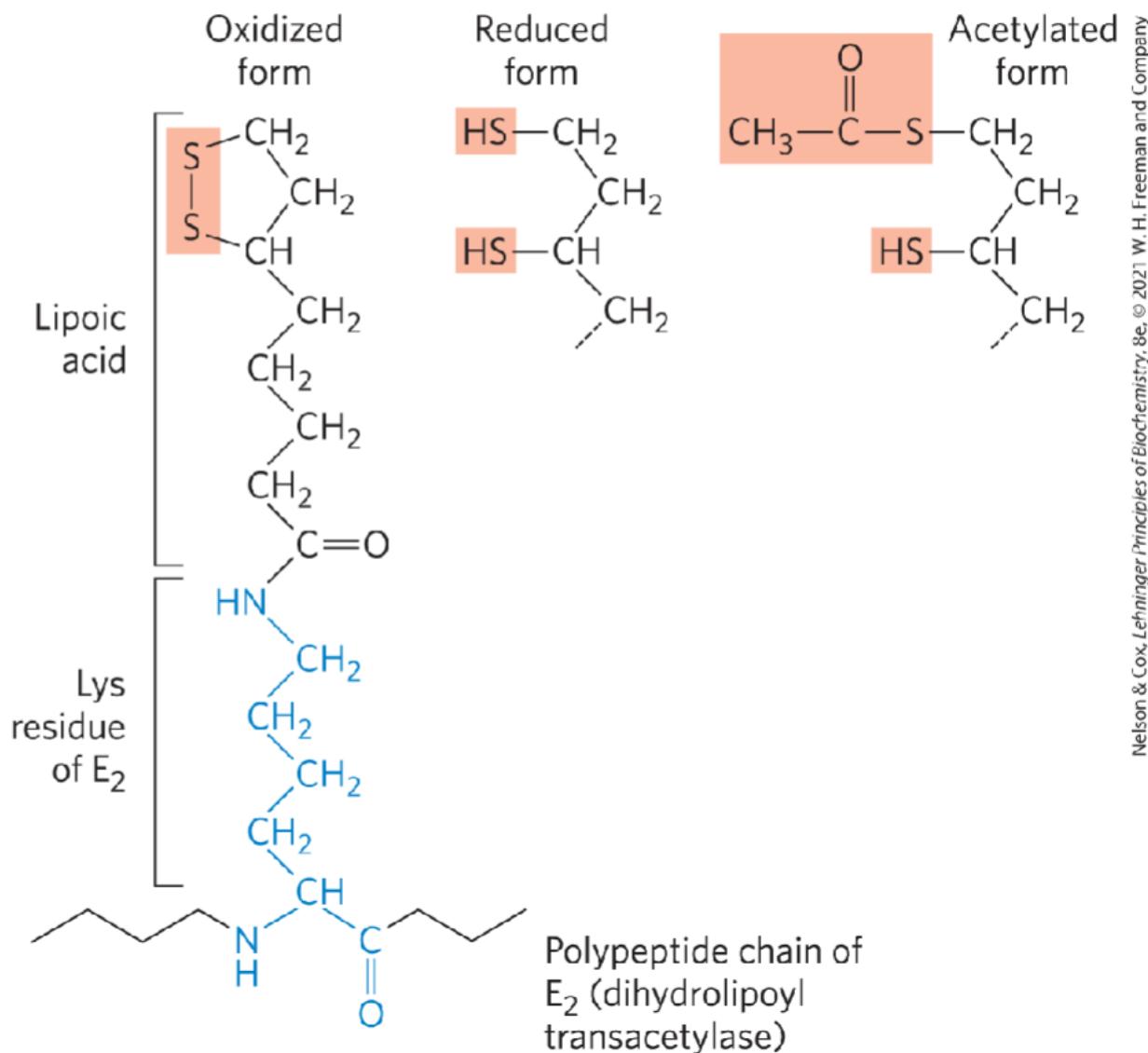
Five coenzymes:

- thiamine pyrophosphate (TPP)
- lipoate
- coenzyme A (CoA, CoA-SH)
- flavin adenine dinucleotide (FAD)
- nicotinamide adenine dinucleotide (NAD)

Lipoate

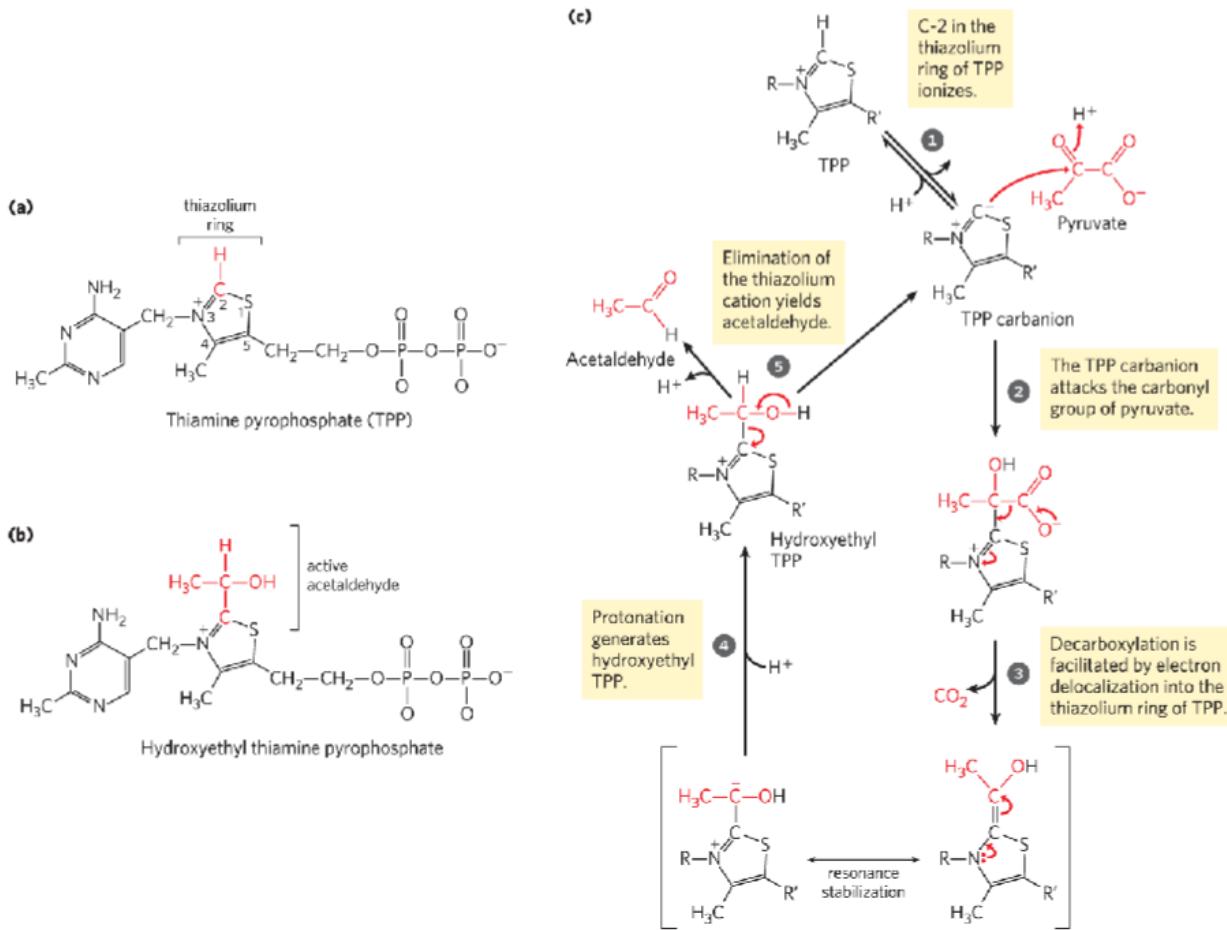
Lipoate is a coenzyme with two thiol groups that can undergo reversible oxidation to a disulfide bond (-S-S-)

- serves as an electron (hydrogen) carrier and an acyl carrier
- covalently linked to E₂ via a lysine residue



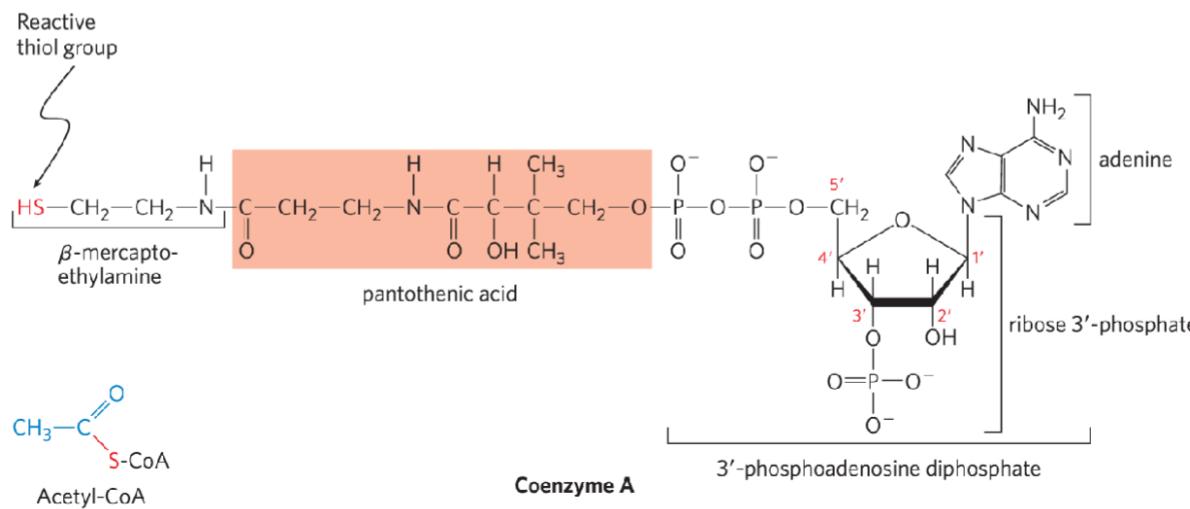
Thiamine Pyrophosphate (TPP)

- **thiamine pyrophosphate:** coenzyme derived from vitamin B₁
 - the thiazolium ring plays an important role in the cleavage of bonds adjacent to a carbonyl group

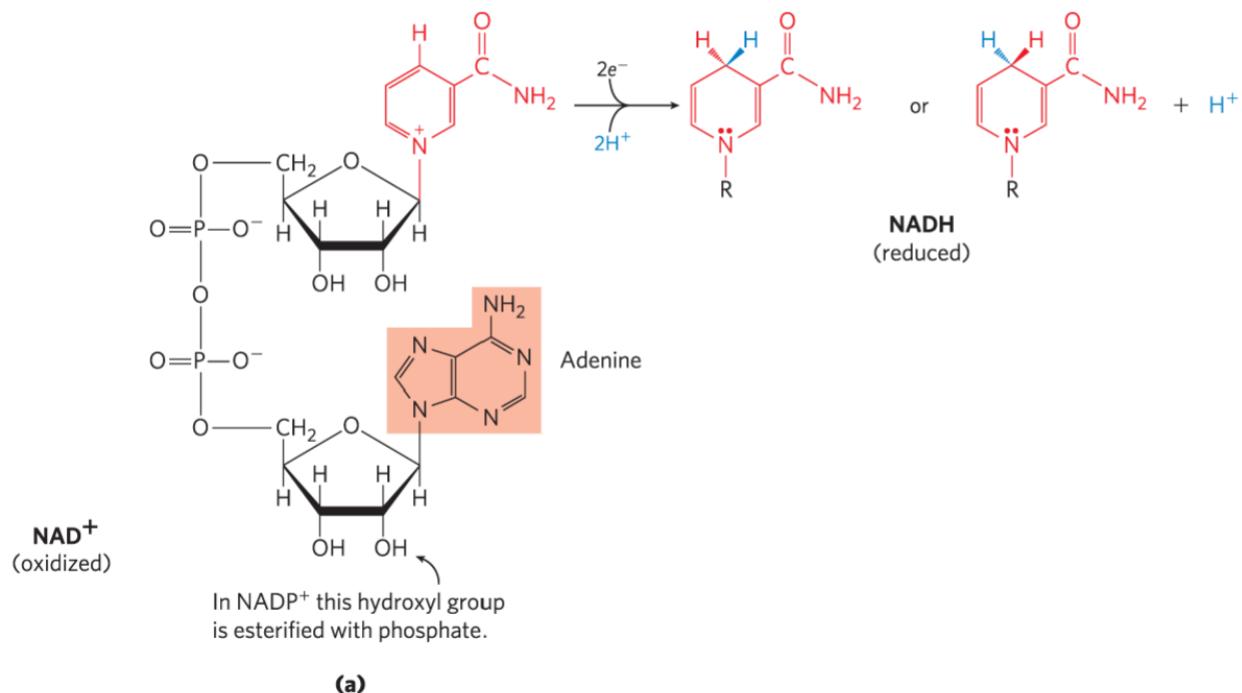


Coenzyme A (CoA-SH)

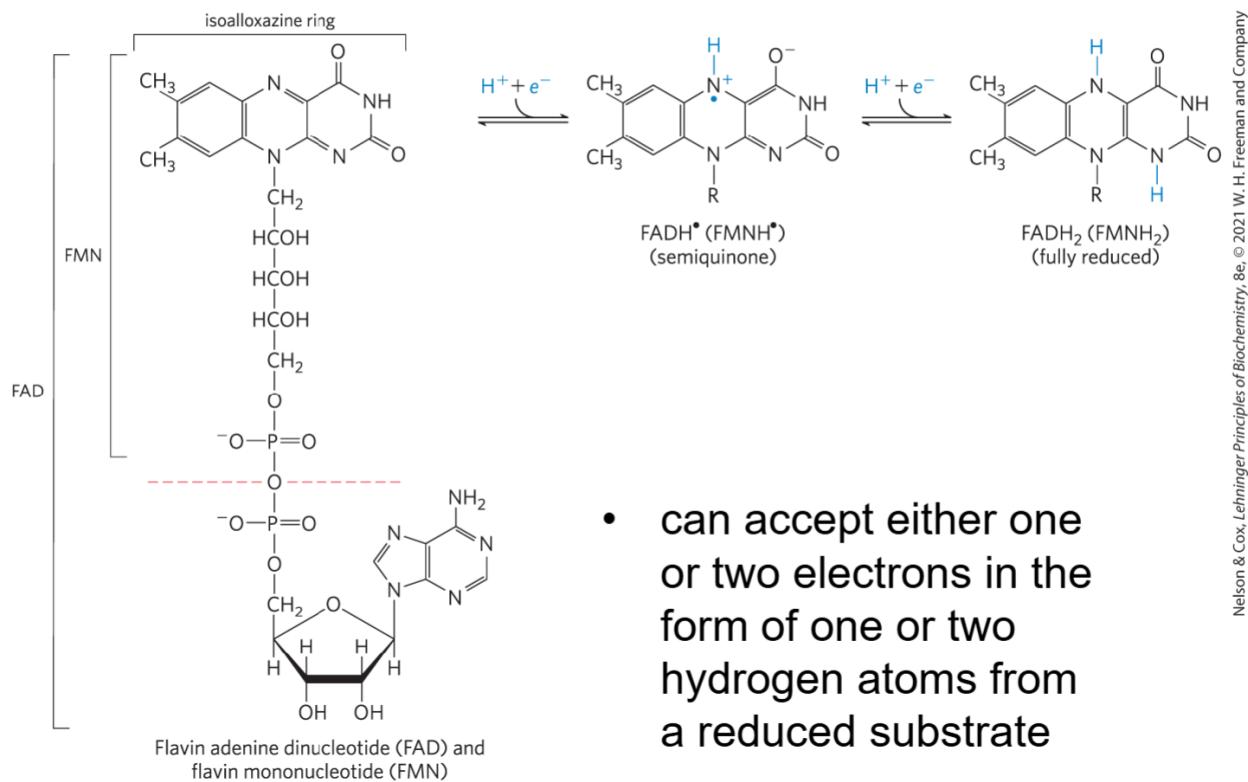
- coenzyme A has a reactive thiol (-SH) group that is critical to its role as an acyl carrier
 - the -SH group forms a **thioester** with acetate in acetyl-CoA



NAD and NADP Undergo Reversible Reduction of the Nicotinamide Ring



Oxidized and Reduced FAD and FMN



Coenzyme and Prosthetic Group Roundup

Table 17-1

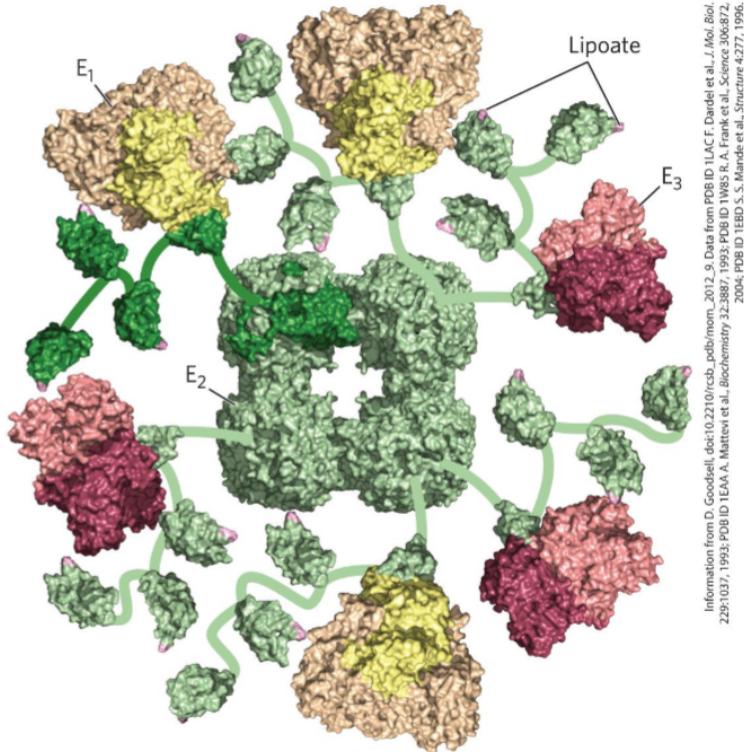
The Coenzymes and Prosthetic Groups of Pyruvate Dehydrogenase

Cofactor	Location	Function
Thiamine pyrophosphate (TPP)	Bound to E ₁	Decarboxylates pyruvate yielding a hydroxyethyl-TPP carbanion
Lipoic acid	Covalently linked to a Lys on E ₂ (lipoamide)	Accepts the hydroxyethyl carbanion from TPP as an acetyl group
Coenzyme A (CoA)	Substrate for E ₂	Accepts the acetyl group from lipoamide
Flavin adenine dinucleotide (FAD)	Bound to E ₃	Reduced by lipoamide
Nicotinamide adenine dinucleotide (NAD ⁺)	Substrate for E ₃	Reduced by FADH ₂

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The PDH Complex Enzymes

- the PDH complex contains multiple copies of:
 - pyruvate dehydrogenase (E₁)
 - dihydrolipoyl transacetylase (E₂)
 - dihydrolipoyl dehydrogenase (E₃)
- an E₂ core (of 24-60 copies) is surrounded by multiple and variable numbers of E₁ and E₃ copies



Enzymes have evolved to form complexes to efficiently achieve a series of chemical transformations without releasing the intermediates into the bulk solvent. This strategy, seen in the pyruvate dehydrogenase complex of the metabolons of the citric acid cycle, is ubiquitous in other pathways of metabolism, in respiration, and in the many complexes.

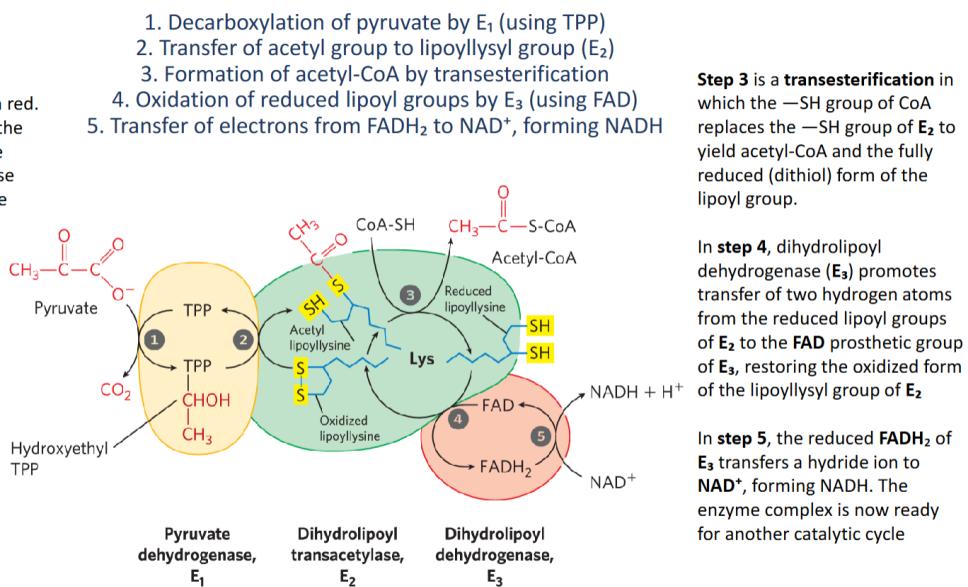
The PDH Complex Enzymes

- **E1: Pyruvate Dehydrogenase (Decarboxylase)**
 - Catalyzes the decarboxylation of pyruvate, releasing CO₂ and forming hydroxyethyl-TPP (a covalent intermediate with thiamine pyrophosphate, TPP)
 - Association: E1 binds to E2 non-covalently, allowing close interaction with E2's lipoyl domain
- **Dihydrolipoyl Transacetylase**
 - Transfers the acetyl group from hydroxyethyl-TPP to coenzyme A (CoA) forming acetyl-CoA
 - Structure: E2 forms the **core structure** of the complex, providing a scaffold for E1 and E3 to associate
 - The flexible **lipoyl arms** of E2 (with covalently attached lipoic acid cofactor) shuttle intermediates between the active sites of E1, E2, and E3
- **Dihydroilipoyl Dehydrogenase**
 - Reoxidizes the reduced lipoyl group of E2 and transfers electrons to NAD⁺, forming NADH
 - Association: E3 is non-covalently attached to the E2 core and interacts with the lipoyl domain during electron transfer

The PDH Complex Integrates Five Reactions to Convert Pyruvate into Acetyl-CoA

The fate of pyruvate is traced in red. In step 1, pyruvate reacts with the bound thiamine pyrophosphate (TPP) of pyruvate dehydrogenase (E_1) and is decarboxylated to the hydroxyethyl derivative

Pyruvate dehydrogenase also carries out step 2, the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group of the core enzyme, dihydrolipoyl transacetylase (E_2), to form the acetyl thioester of the reduced lipoyl group



Oxidative Decarboxylation of Pyruvate

Pyruvate dehydrogenase, E_1 , with bound TPP catalyzes:

- Step 1: decarboxylation of pyruvate to the hydroethyl derivative
 - Rate-limiting step
- Step 2: Oxidation of the hydroethyl derivative to an acetyl group
 - Electrons and the acetyl group are transferred from TPP to the lipoyllysyl group of E_2

Dihydrolipoyl Transacetylase, E_2 , catalyzes:

- Step 3: esterification of the acetyl moiety to one of the lipoyl-SH groups, followed by transesterification to CoA to form acetyl-CoA

Dihydrolipoyl dehydrogenase, E_3 , catalyzes:

- Step 4: Electron transfer to regenerate the oxidized form of the lipoyllysyl group
- Step 5: Electron transfer to regenerate the oxidized FAD cofactor, forming NADH

The Five-Reaction Sequence of the PDH Complex is an Example of Substrate Channeling

- **Substrate Channeling** = the passage of intermediates from one enzyme directly to another enzyme without release
- the long lipoyllysyl arm of E_2 channels the substrate from the active site of E_1 to E_2 to E_3
 - tethers intermediates to the enzyme complex
 - increases the efficiency of the overall reaction
 - minimizes side reactions

Regulation of the PDH Complex Ensures Cellular Energy Balance

- Activation by Dephosphorylation (via PDP)
- Inactivation by Phosphorylation (via PDK)

Activators of PDC (Promote Dephosphorylation):

- Ca^{2+} : Directly activates PDP; important in muscle contraction and energy demand
- Insulin: Stimulates PDP; promotes glucose utilization in the fed state
- Pyruvate: Inhibits PDK, allowing PDC to stay active
- ADP: Inhibits PDK, signals low energy, promoting PDC activation
- NAD^+ : Competes with NADH to inhibit PDK, favoring PDC activation

Inhibitors of PDC (Promote Phosphorylation):

- ATP: Activates PDK; signals high energy, reducing pyruvate usage
- NADH: Activates PDK; indicates reduced state, suppressing PDC
- Acetyl-CoA: Activates PDK; signals sufficient TCA cycle substrate

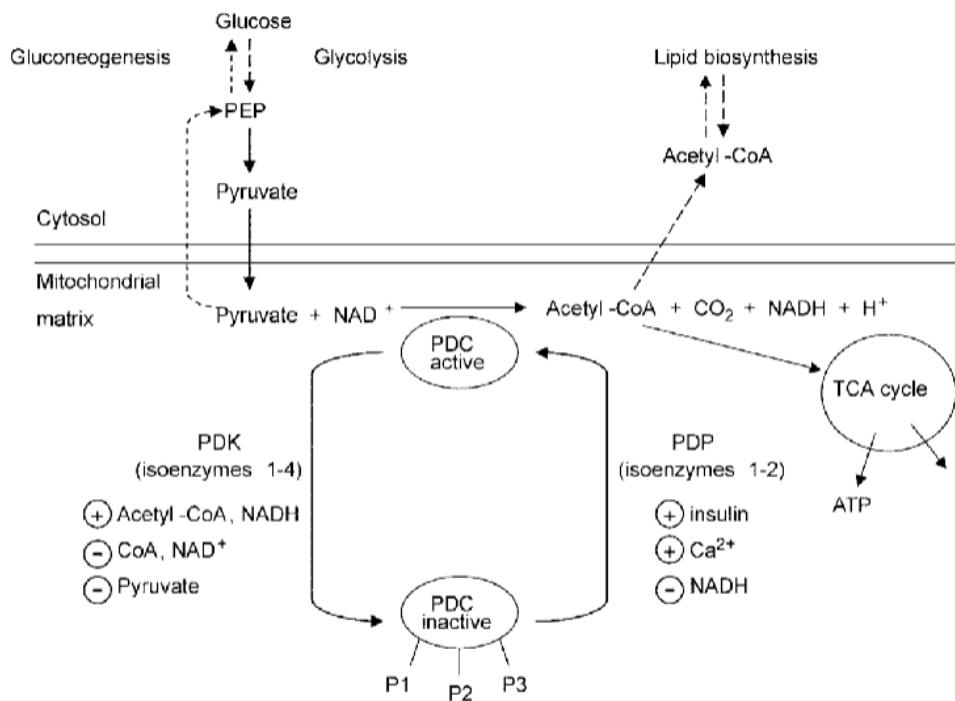


Figure 1. Regulation of PDC activity by interconversion between active (unphosphorylated) and inactive (phosphorylated) forms catalyzed by PDPs and PDKs.

Regulation ensures that the PDH Complex (PDC in this figure) integrates signals from the cell's energy status, substrate availability, and metabolic demands. It allows:

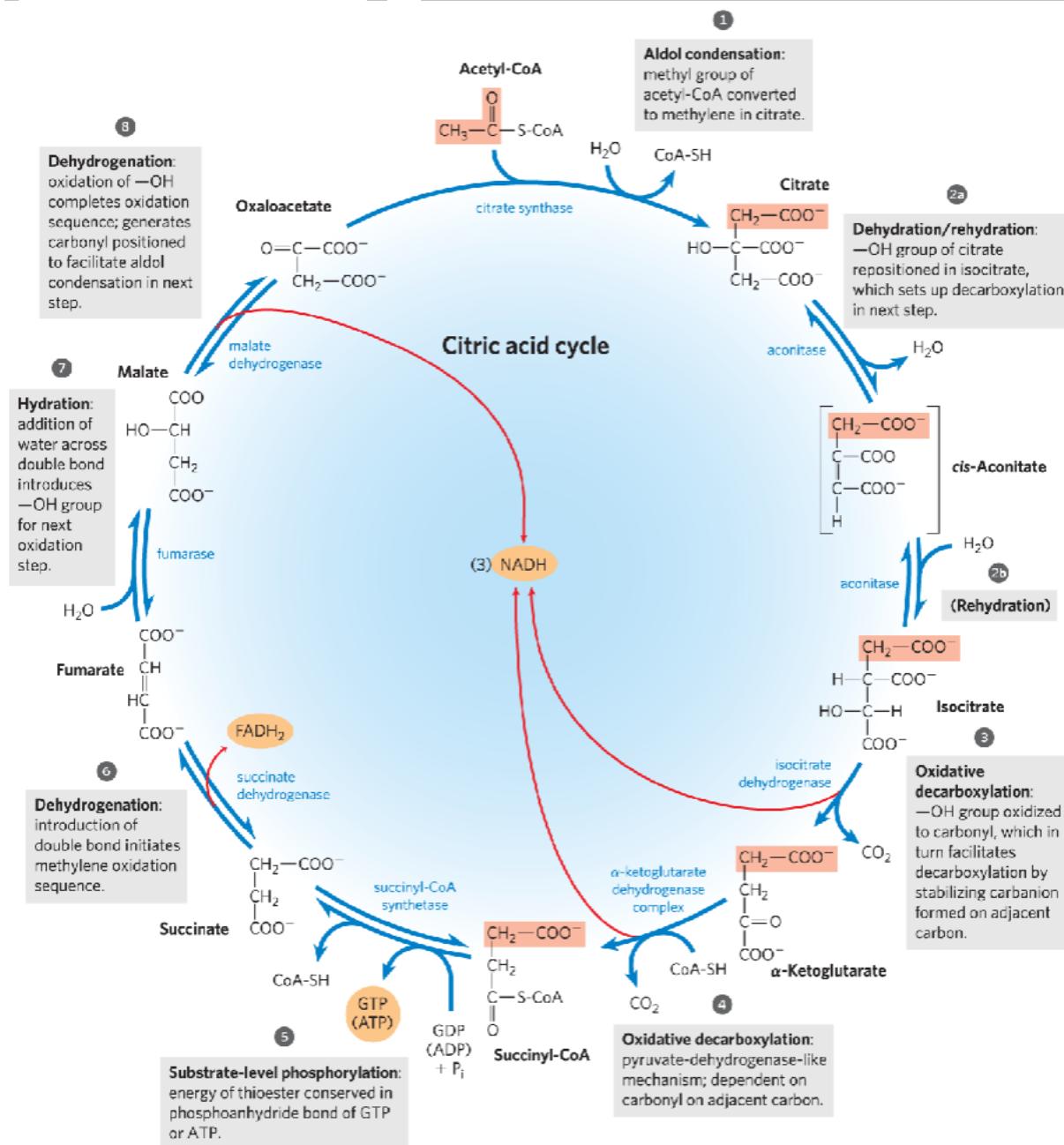
activation in conditions of high energy demand or substrate availability (e.g., pyruvate, ADP, NAD^+ , insulin), and **inhibition** in conditions of sufficient energy or biosynthetic precursor abundance (e.g., acetyl-CoA, NADH , ATP).

Reactions of the Citric Acid Cycle

Reactions of the citric acid cycle follow a chemical logic: In its catabolic role, the citric acid cycle oxidizes acetyl-CoA to CO₂ and H₂O. Energy from the oxidations in the cycle drives the synthesis of ATP. The chemical strategies for activating groups for oxidation and for conserving energy in the form of reducing power and high-energy compounds are used in many other biochemical pathways

The Citric Acid Cycle oxidizes acetyl-CoA to CO₂ and conserves energy:

- Produces 3 NADH, 1 FADH₂, and 1 GTP (or ATP) per cycle
- Regenerates oxaloacetate, allowing continuous substrate oxidation
- Feeds electrons into the electron transport chain for ATP production
- Citrate formed from acetyl-CoA and oxaloacetate is oxidized to yield:
 - CO₂
 - NADH
 - FADH₂
 - GTP or ATP
- energy from the **four** oxidations is conserved as NADH and FADH₂



The cycle enables the sequential oxidation of acetyl-CoA carbons, capturing high-energy electrons as NADH and FADH₂. Regenerating oxaloacetate ensures that the process can continue indefinitely as long as acetyl-CoA is available, maximizing the energy yield from substrates like glucose and fatty acids.

In Eukaryotes, the Mitochondrion is the Site of Energy-Yielding Oxidative Reactions and ATP Synthesis

- Isolated mitochondria contain all enzymes, coenzymes, and proteins needed for:
 - the citric acid cycle
 - electron transfer and ATP synthesis by oxidative phosphorylation

- (and also:)
 - oxidation of fatty acids and amino acids to acetyl-CoA
 - oxidative degradation of amino acids to citric acid cycle intermediates

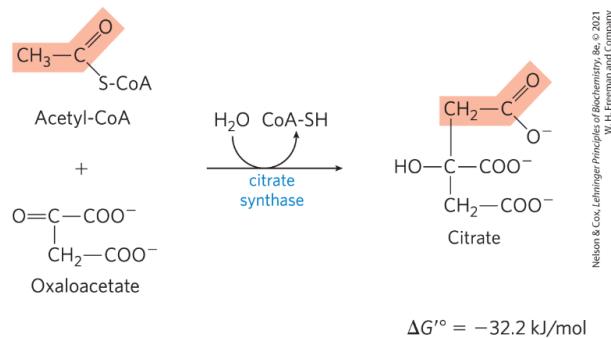
The Sequence of Reactions in the Citric Acid Cycle Makes Chemical Sense

- complete oxidation of acetyl-CoA and CO₂ extracts the maximum potential energy
- direct oxidation to yield CO₂ and CH₄ is not biochemically feasible because [most] organisms cannot oxidize CH₄
- carbonyl groups are more chemically reactive than a methylene group or methane
- each step of the cycle involves either:
 - an energy-conserving oxidation
 - placing functional groups in position to facilitate oxidation or oxidative decarboxylation

The Citric Acid Cycle has Eight Steps

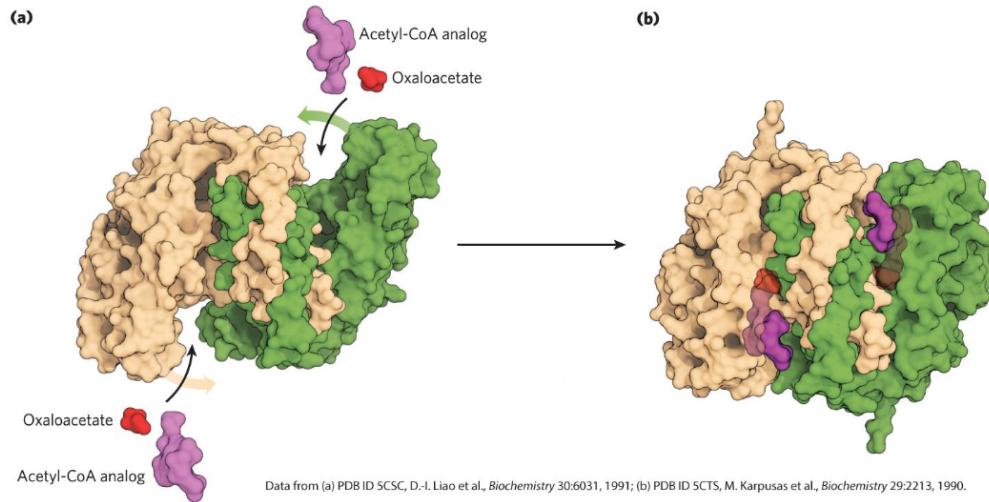
(Step 1) Formation of Citrate

- **Citrate Synthase** = catalyzes the condensation of acetyl-CoA with **oxaloacetate** to form **citrate**
 - involves the formation of a transient intermediate, citroyl-CoA
 - large, negative ΔG° is needed because [oxaloacetate] is normally very low.



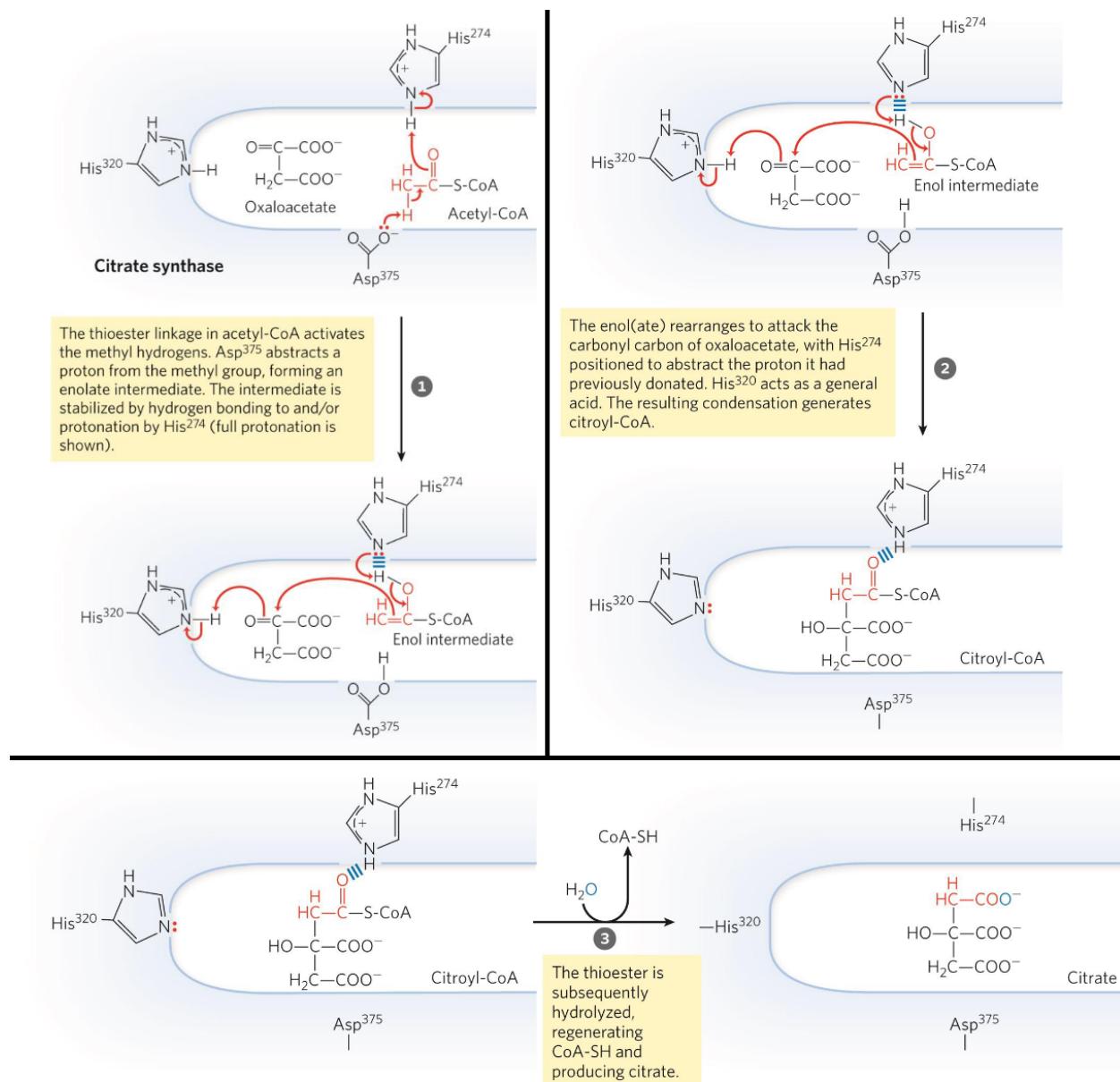
Structure of Citrate Synthase

- binding of oxaloacetate creates a binding sites for acetyl-CoA
- induced fit decreases the likelihood of premature cleavage of the thioester bond of acetyl-CoA



- Regulated by energy charge and need for TCA intermediates/products
 - Inhibited by ATP
 - Inhibited by NADH
 - Inhibited by citrate and succinyl-CoA (both downstream)

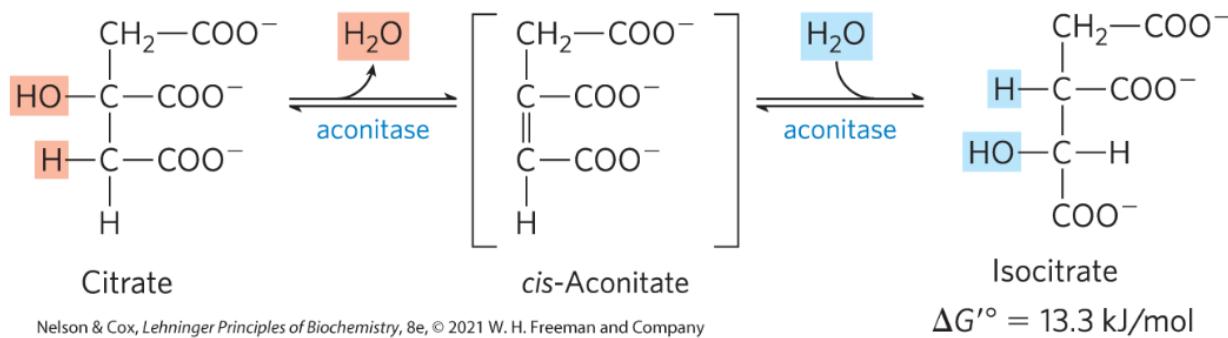
Mechanism of Citrate Synthase: Acid/Base Catalysis



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(Step 2) Formation of Isocitrate via *Cis*-Aconitate

- aconitase (aconitate hydratase) = catalyzes the reversible transformation of citrate to **isocitrate** through the intermediate ***cis*-aconitate**
 - addition of H₂O to *cis*-aconitate is stereospecific
 - low [isocitrate] pulls the reaction forward
 - aconitase is a **metalloprotein**



Stereochemical Control in the Citric Acid Cycle

- Citrate's Symmetry and Enzymatic Specificity

- Citrate is a symmetric molecule, meaning its two carboxymethyl ($-\text{CH}_2\text{COO}^-$) groups are chemically equivalent.
- Despite this symmetry, aconitase binds citrate asymmetrically due to a **three-point attachment** to its active site.
- This binding allows aconitase to distinguish between the two ends of citrate, enforcing stereochemical control

- The Role of Aconitase in Stereospecific Transformation

- Aconitase catalyzes the conversion of citrate to isocitrate through an intermediate, **cis-aconitate**
- It removes water specifically from the pro-R arm of citrate to form **cis-aconitate**, which is a prochiral intermediate
- Water is then **re-added stereospecifically**, generating a single enantiomer of **isocitrate**

- Why the Same Carbons Follow a Fixed Pathway

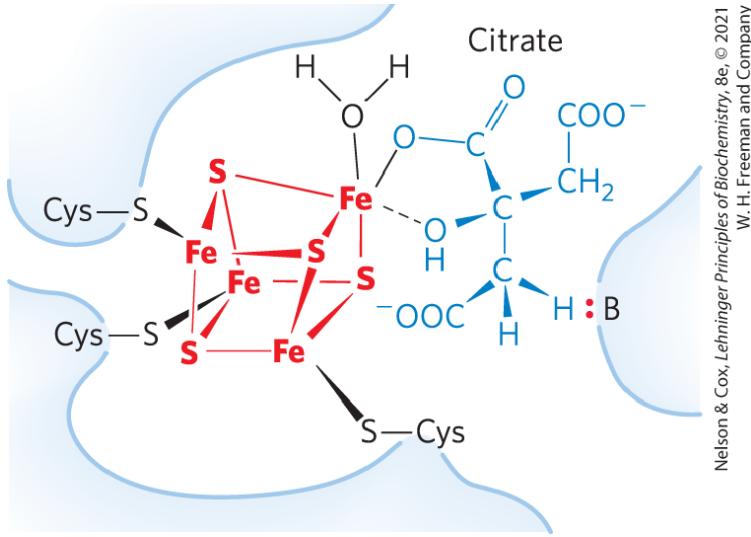
- The stereospecificity of aconitase ensures that the same carbons from acetyl-CoA (originally from pyruvate) always appear in the same positions in subsequent metabolites
- This is why, even though citrate appears symmetric, the carbon atoms consistently follow a specific trajectory through the cycle, influencing molecules like **α -ketoglutarate** and **succinyl-CoA**

- Enzymes and Molecular Symmetry

- This process highlights how enzymes **exploit molecular symmetry while still imposing stereochemical control** over metabolic pathways
- Aconitase ensures that the citric acid cycle maintains a consistent and regulated flow of metabolites, crucial for cellular respiration and metabolism

Iron-Sulfur Center in Aconitase

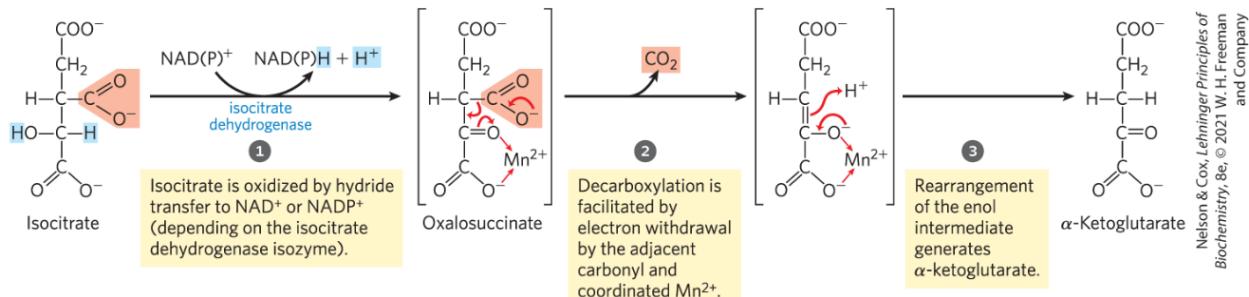
- **Iron-sulfur center** = acts both in the binding of the substrate to the active site and in the catalytic addition or removal of H_2O
- Since this step requires iron, people with iron deficiencies often have aching muscles and tiredness



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(Step 3) Oxidation of Isocitrate to α -Ketoglutarate and CO_2

- **isocitrate dehydrogenase** = catalyzes the oxidative decarboxylation of isocitrate to α -ketoglutarate
 - Mn^{2+} interacts with the carbonyl group of the oxalosuccinate and stabilizes the transiently-formed enol
 - specific isozymes for NADP^+ (cytosolic and mitochondrial) or NAD^+ (mitochondrial)

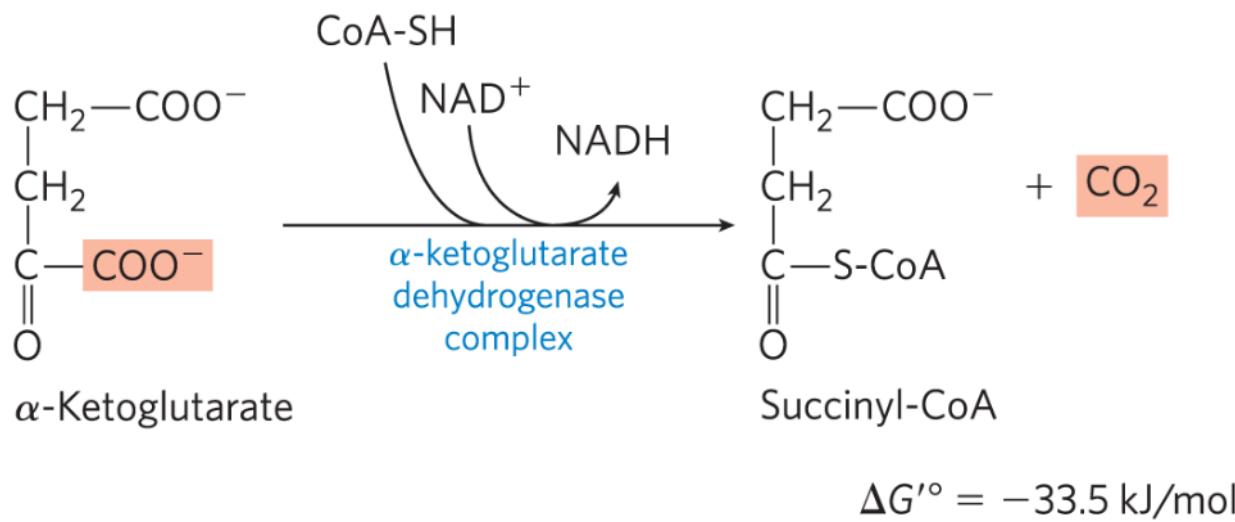


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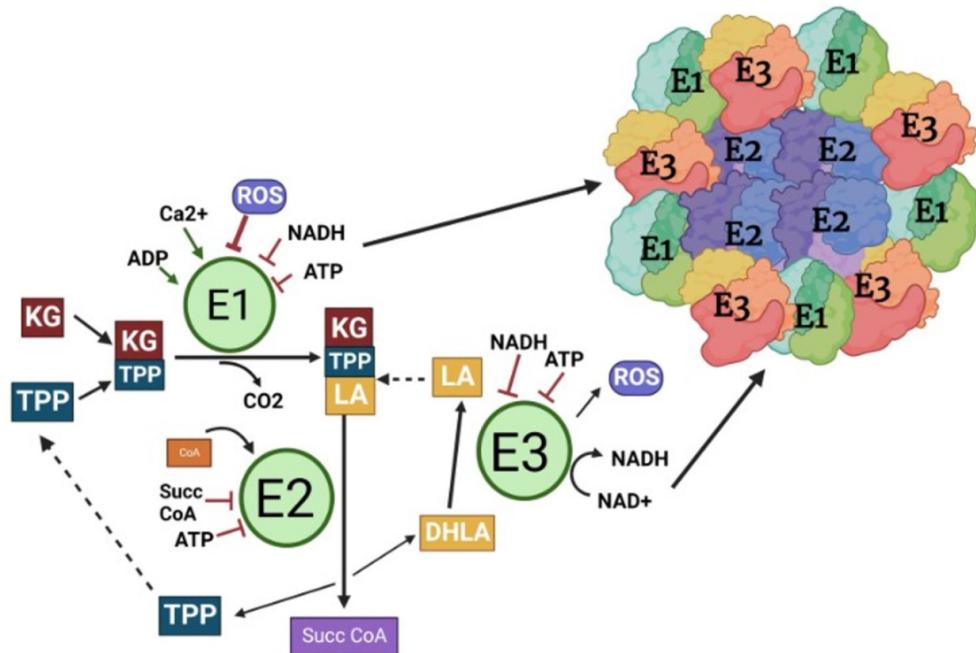
- This is the rate-limiting step of TCA

(Step 4) Oxidation of α -Ketoglutarate to Succinyl-CoA and CO_2

- **α -ketoglutarate dehydrogenase complex** = catalyzes the oxidative decarboxylation of α -ketoglutarate to succinyl-CoA and CO_2
 - energy of oxidation is conserved in the thioester bond of succinyl-CoA

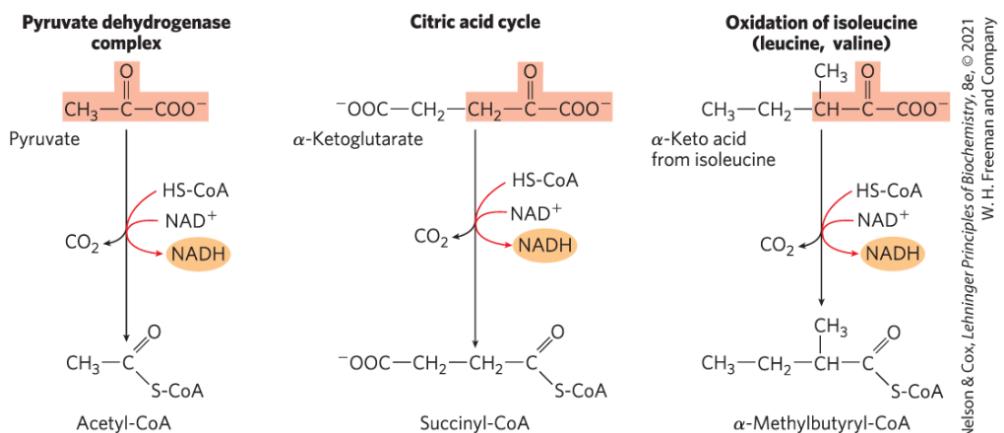


Overall Reaction Catalyzed by KGDHC



A Conserved Mechanism for Oxidative Decarboxylation

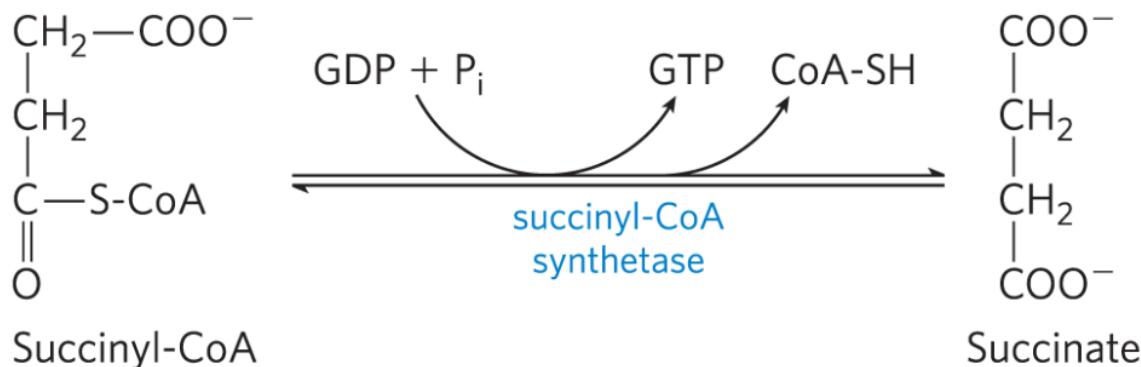
- pathways use the same five cofactors, similar to multienzyme complexes, and the same enzymatic mechanism
 - have homologous E₁ and E₂ and identical E₃
 - example of gene duplication and divergent evolution



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(Step 5) Conversion of Succinyl-CoA to Succinate

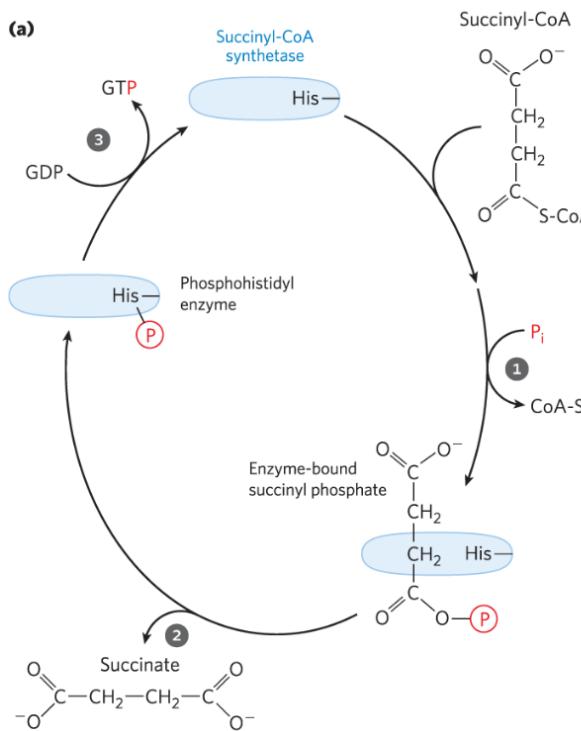
- **Succinyl-CoA synthetase (succinic thiokinase)** = catalyzes the breakage of the thioester bond of succinyl-CoA to form succinate
 - energy released drives the synthesis of a phosphoanhydride bond in either GTP or ATP (substrate level phosphorylation)



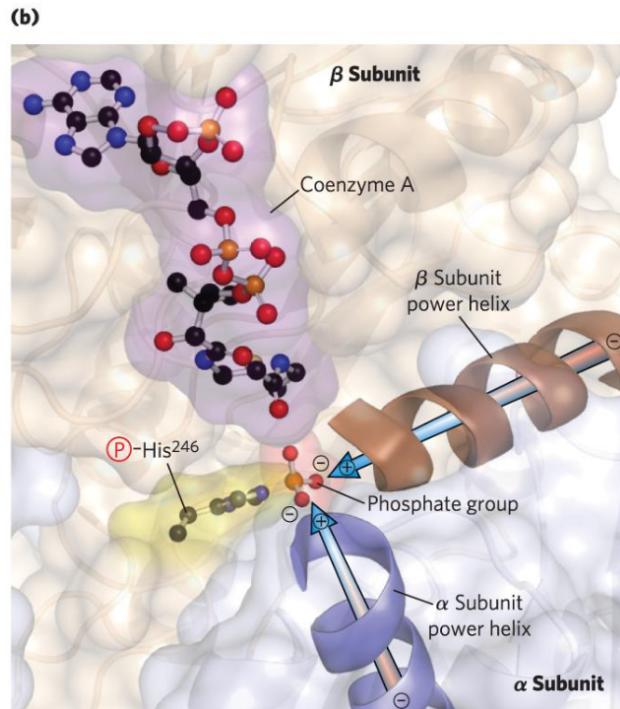
$$\Delta G'^\circ = -2.9 \text{ kJ/mol}$$

The Succinyl-CoA Synthase Reaction

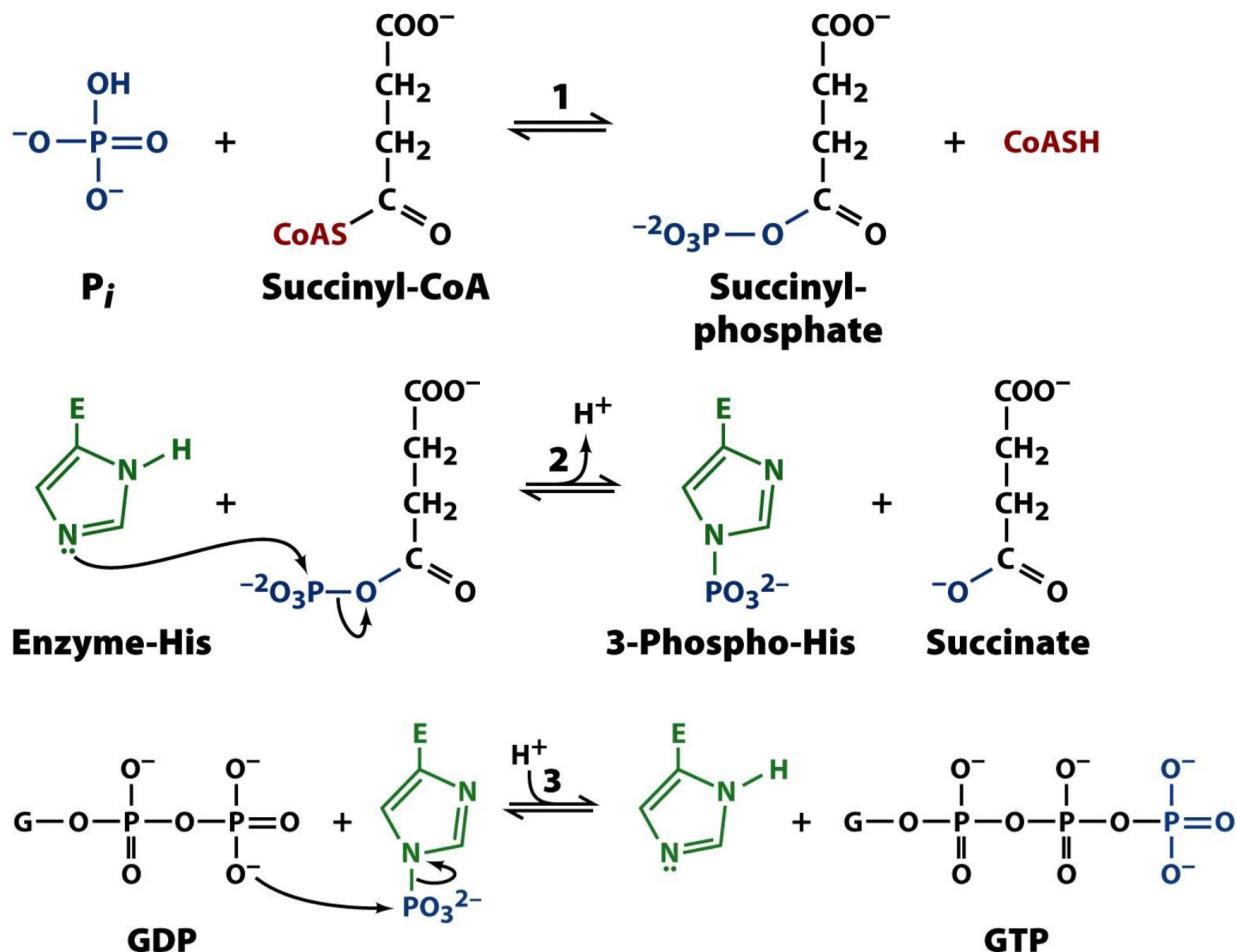
- Enzyme molecule becomes phosphorylated at a His residue in the active site
- phosphoryl group is then transferred to ADP or GDP to form ATP or GTP
 - animal cells have specific isozymes for ADP and GDP



- power helices place the partial positive charges of the helix dipole near the phosphate group of the α chain phosphorylated His²⁴⁶ to stabilize the phosphoenzyme intermediate



Succinyl-CoA synthetase mechanism



Nucleoside Diphosphate Kinase

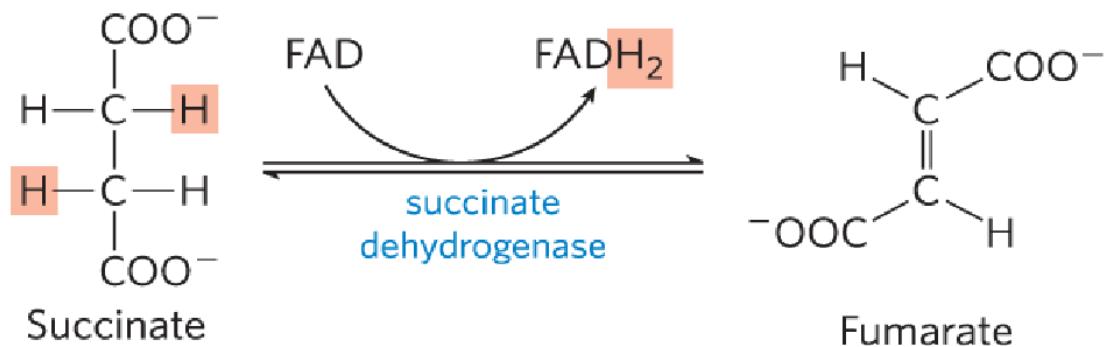
- nucleoside diphosphate kinase = catalyzes the reversible conversion of GTP and ATP



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

(Step 6) Oxidation of Succinate to Fumarate

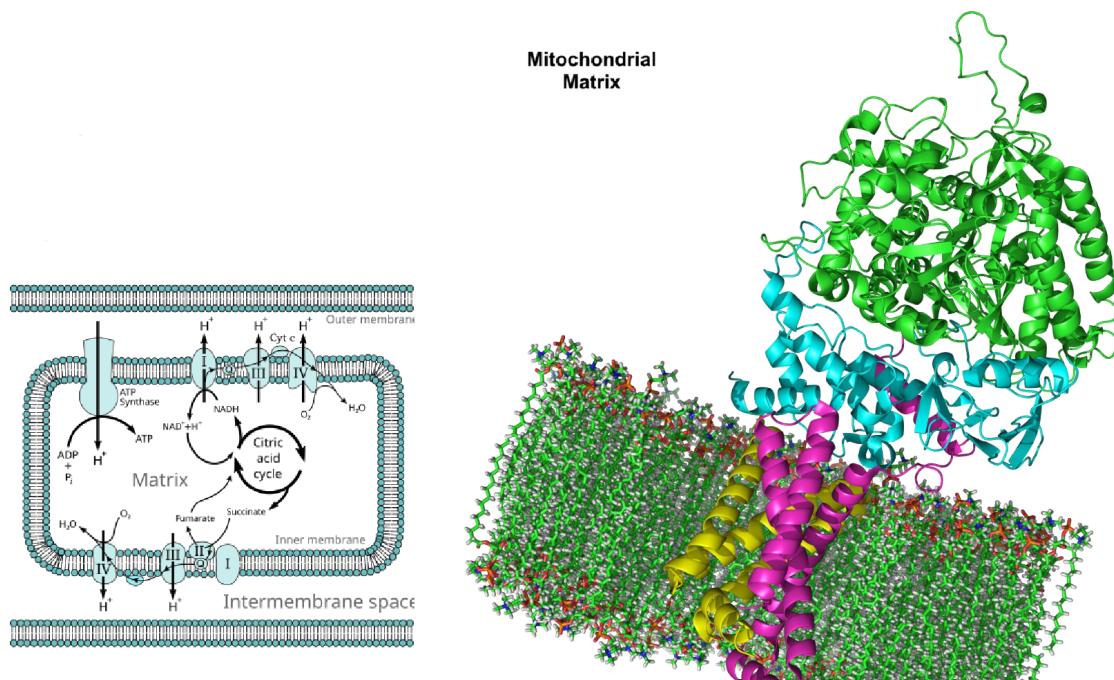
- succinate dehydrogenase = flavoprotein that catalyzes the reversible oxidation of succinate to fumarate
 - integral protein of the mitochondrial inner membrane in eukaryotes
 - contains three iron-sulfur clusters and covalently bound to FAD

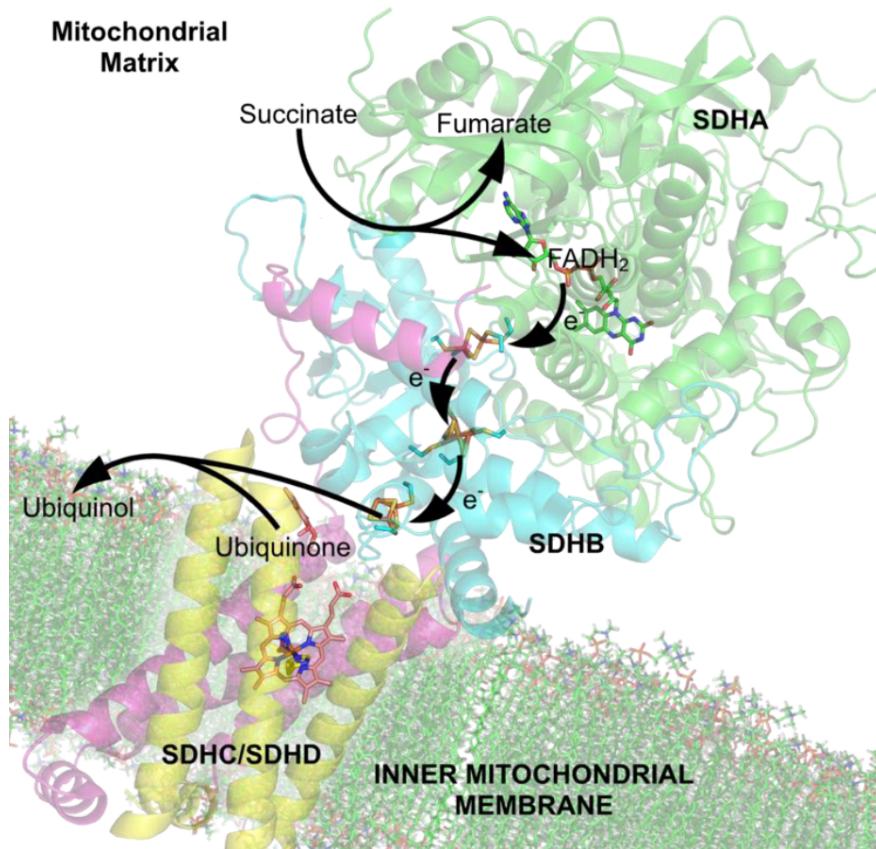


$$\Delta G'^\circ = 0 \text{ kJ/mol}$$

Succinate Dehydrogenase

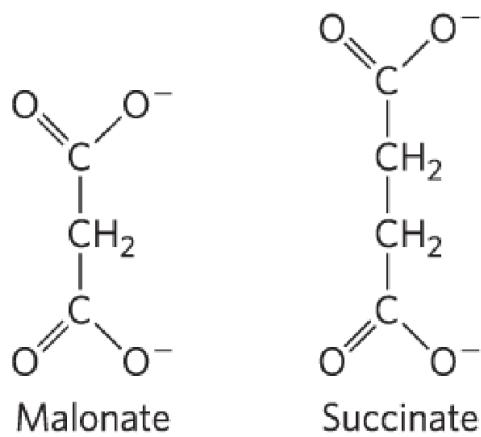
- the only enzyme that participates in both the citric acid cycle and oxidative phosphorylation (complex II)





Malonate is a Strong Competitive Inhibitor of Succinate Dehydrogenase

- malonate = an analog of succinate
 - not normally present in cells
 - addition to mitochondria *in vitro* blocks citric acid cycle activity

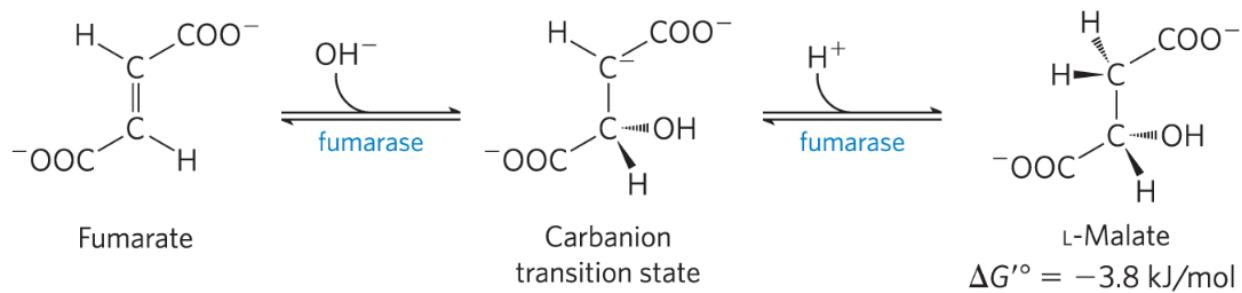


Malonate competitively inhibits succinate dehydrogenase, blocking the TCA cycle

(Step 7) Hydration of Fumarate to Malate

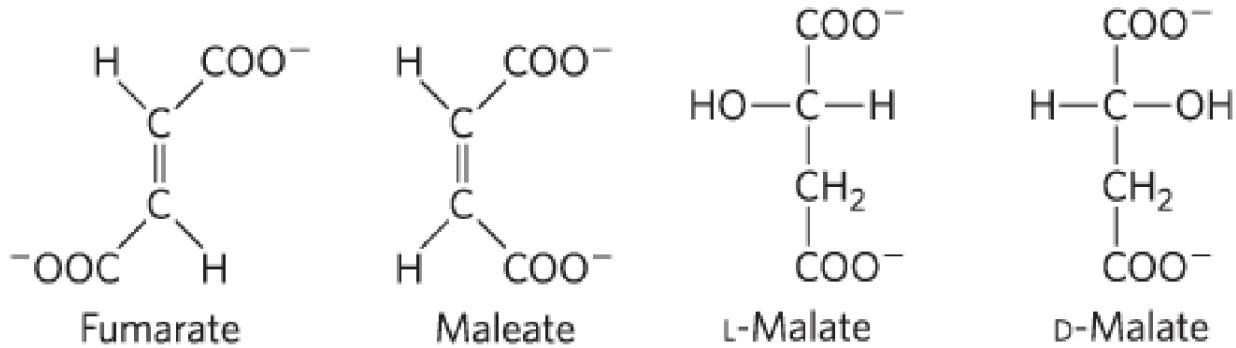
- **fumarase** = catalyzes the reversible hydration of fumarate to **L-malate**

- transition state is a carbanion



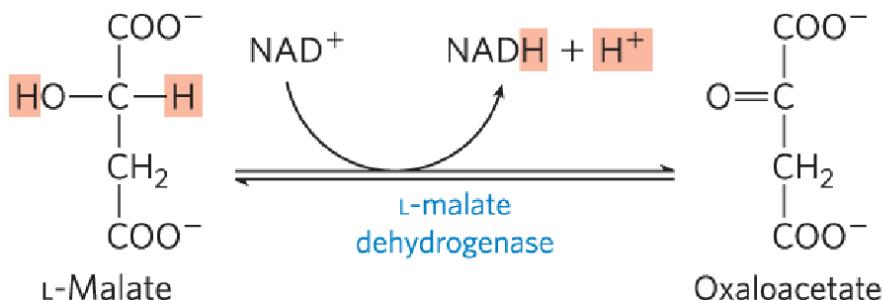
Fumarase is Highly Stereospecific

- In the forward direction, fumarase catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate
- In the reverse direction, fumatase is equally stereospecific



(Step 8) Oxidation of Malate to Oxaloacetate

- **L-malate dehydrogenase** = catalyzes the oxidation of **L-malate** to oxaloacetate, coupled to the reduction of NAD^+
 - low [oxaloacetate] pulls the reaction forward
 - regenerates oxaloacetate for citrate synthesis

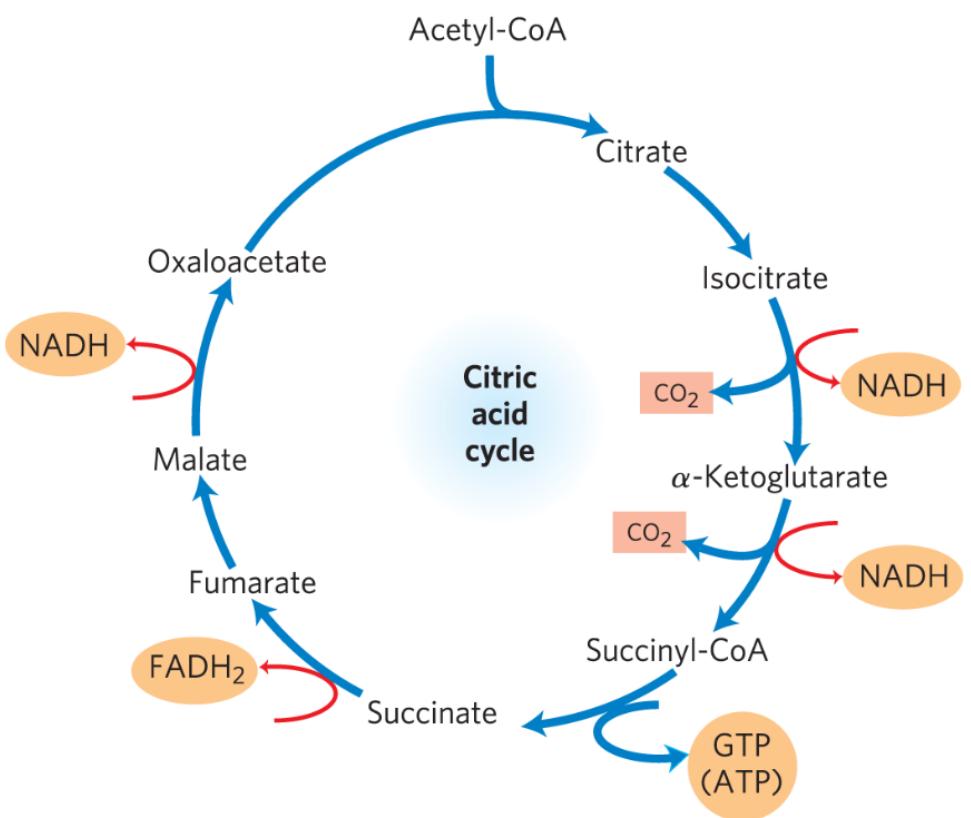


$$\Delta G'^\circ = 29.7 \text{ kJ/mol}$$

Products of TCA

The Energy of Oxidations in the Cycle is Efficiently Conserved

- Energy released by oxidation is conserved in the production of:
 - 3 NADH
 - 1 FADH₂
 - 1 GTP (or ATP)



Electrons from NADH and FADH₂ Enter the Respiratory Chain

- The citric acid cycle directly generates only one ATP per turn
- the large flow of electrons into the respiratory chain via NADH and FADH₂ leads to formation of almost 10 times more ATP during oxidative phosphorylation
 - each NADH drives formation of ~2.5 ATP
 - each FADH₂ drives the formation of ~1.5 ATP

Stoichiometry of Coenzyme Reduction and ATP Formation in Aerobic Oxidation of Glucose

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

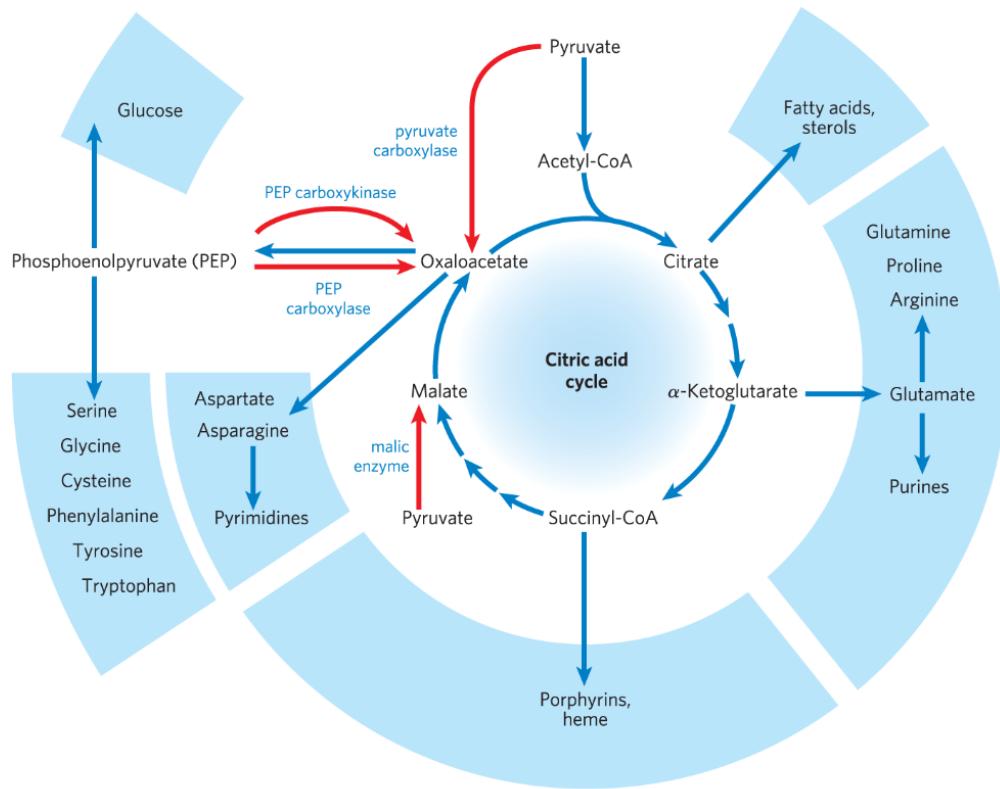
Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed
Glucose → glucose 6-phosphate	-1 ATP	-1
Fructose 6-phosphate → fructose 1,6-bisphosphate	-1 ATP	-1
2 Glyceraldehyde 3-phosphate → 2 1,3-biphosphoglycerate	2 NADH	3 or 5
2 1,3-Biphosphoglycerate → 2 3-phosphoglycerate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

The Citric Acid Cycle Serves in Both Catabolic and Anabolic Processes

- **amphibolic pathway** = one that serves in both catabolic and anabolic processes
 - Besides its role in the oxidative catabolism of carbohydrates, fatty acids, and amino acids, the cycle provides precursors for many biosynthetic pathways
- The citric acid cycle is a hub of metabolism, with catabolic pathways leading in and anabolic pathways leading out
- Acetate groups (acetyl-CoA) from the catabolism of various fuels are useful in the synthesis of such metabolites as amino acids, fatty acids, and sterols. The breakdown products of many amino acids and nucleotides are intermediates of the cycle, and they can be fed in or siphoned off as needed by the cell

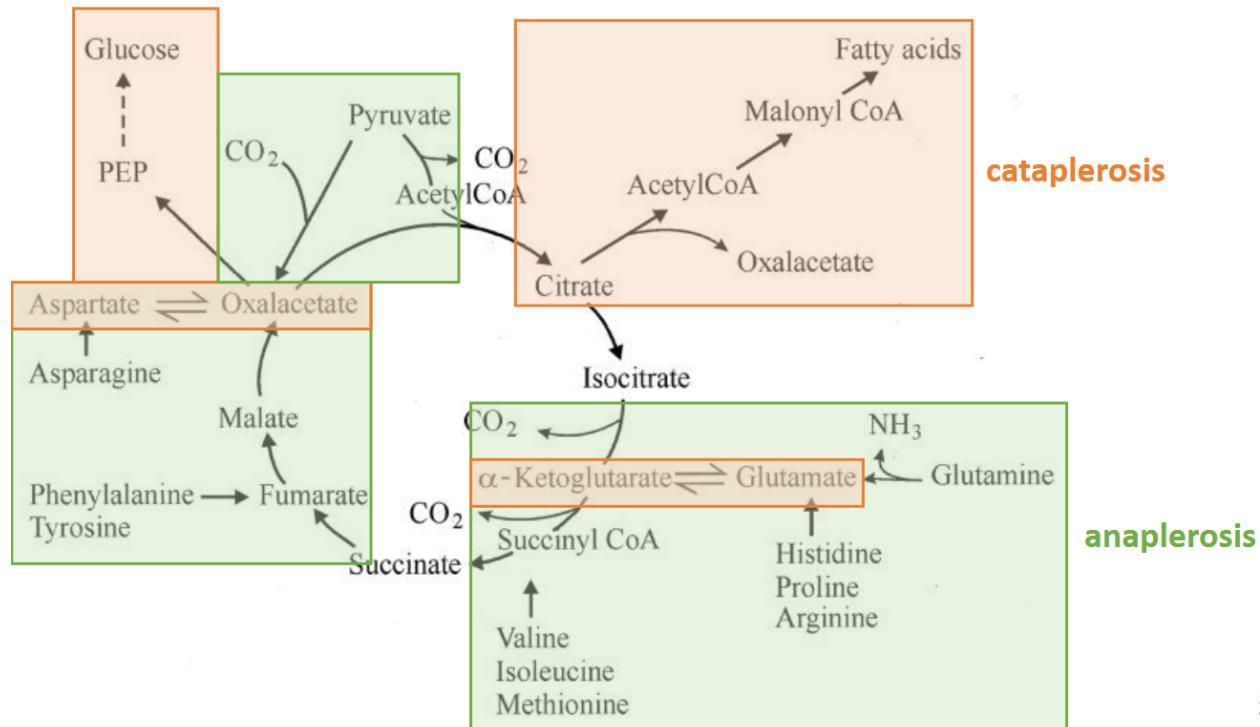
Role of the Citric Acid Cycle in Anabolism

- **Cataplerosis** describes the series of enzymatic reactions that draw down pools of metabolic intermediates
- **Anaplerosis** describes the series of enzymatic reactions or pathways that replenish pools of metabolic intermediates in the TCA cycle
- As intermediates of the citric acid cycle are removed to serve as biosynthetic precursors, they are replenished by **anaplerotic reactions**



Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways. Shown in red are four anaplerotic reactions that replenish depleted cycle intermediates

Anaplerosis and Cataplerosis



Anaplerotic Reactions Replenish Citric Acid Cycle Intermediates

- when intermediates are shunted from the citric acid cycle to other pathways, they are replenished
- anaplerotic reactions** = chemical reactions that replenish intermediates

TABLE 16-2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
Pyruvate + HCO ₃ ⁻ + ATP $\xrightleftharpoons{\text{pyruvate carboxylase}}$ oxaloacetate + ADP + P _i	Liver, kidney
Phosphoenolpyruvate + CO ₂ + GDP $\xrightleftharpoons{\text{PEP carboxykinase}}$ oxaloacetate + GTP	Heart, skeletal muscle
Phosphoenolpyruvate + HCO ₃ ⁻ $\xrightleftharpoons{\text{PEP carboxylase}}$ oxaloacetate + P _i	Higher plants, yeast, bacteria
Pyruvate + HCO ₃ ⁻ + NAD(P)H $\xrightleftharpoons{\text{malic enzyme}}$ malate + NAD(P) ⁺	Widely distributed in eukaryotes and bacteria

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