King Saud University
College of Sciences
Biochemistry Department

# الأيض (١)
# Metabolism (1)
# BCH 340

## Lecture 3: Glycolysis, TCA cycle and glyoxylate cycle

Prepared by Dr. Atekah Alshammari

# Intended learning outcomes (ILOs)

**By the end of this lecture, students will be able to:**

- Describe the step-by-step process of glycolysis.
- Illustrate the fate of pyruvate under aerobic and anaerobic conditions.
- Comprehend TCA cycle reactions and appreciate its regulation mechanism.
- Calculate the net ATP yield from glycolysis and TCA cycle.
- Describe the pathway of the glyoxylate cycle.

# Carbohydrate metabolism

**Major pathways in carbohydrate metabolism:**

- Glycolysis
- Citric acid cycle
- Electron transport system
- Gluconeogenesis
- Glycogenesis
- Glycogenolysis
- Pentose phosphate pathway

# Glycolysis

- Glycolysis is a fundamental metabolic pathway that lays at the center of carbohydrate metabolism because nearly all sugars (whether arising from the diet or from catabolic reactions in the body) can ultimately be converted to glucose.
- This pathway is employed by all tissues for the breakdown of glucose to generate energy (in the form of ATP) and intermediates to serve as precursors in other metabolic pathways.
- Glycolysis consists of ten enzyme-catalyzed reactions in which one molecule of glucose (6 carbon) is degraded to yield two molecules of pyruvate (3 carbon).

# Glycolysis (cont.)

**Site of glycolysis:**

- Glycolysis takes place in the cytoplasm of all cells of most living organisms, from bacteria to humans.
- It is an oxygen-independent metabolic pathway and it operates in both aerobic and anaerobic conditions.
- Some tissues have particularly high rates of glycolytic activity, these tissues include:
  - Muscle tissues
  - Liver
  - Adipose tissue
  - Brain
  - Red blood cells

# Glycolysis (cont.)

- Glycolysis is a complex biochemical pathway that can be divided into two main stages:

## I. Energy investment (preparatory) phases:

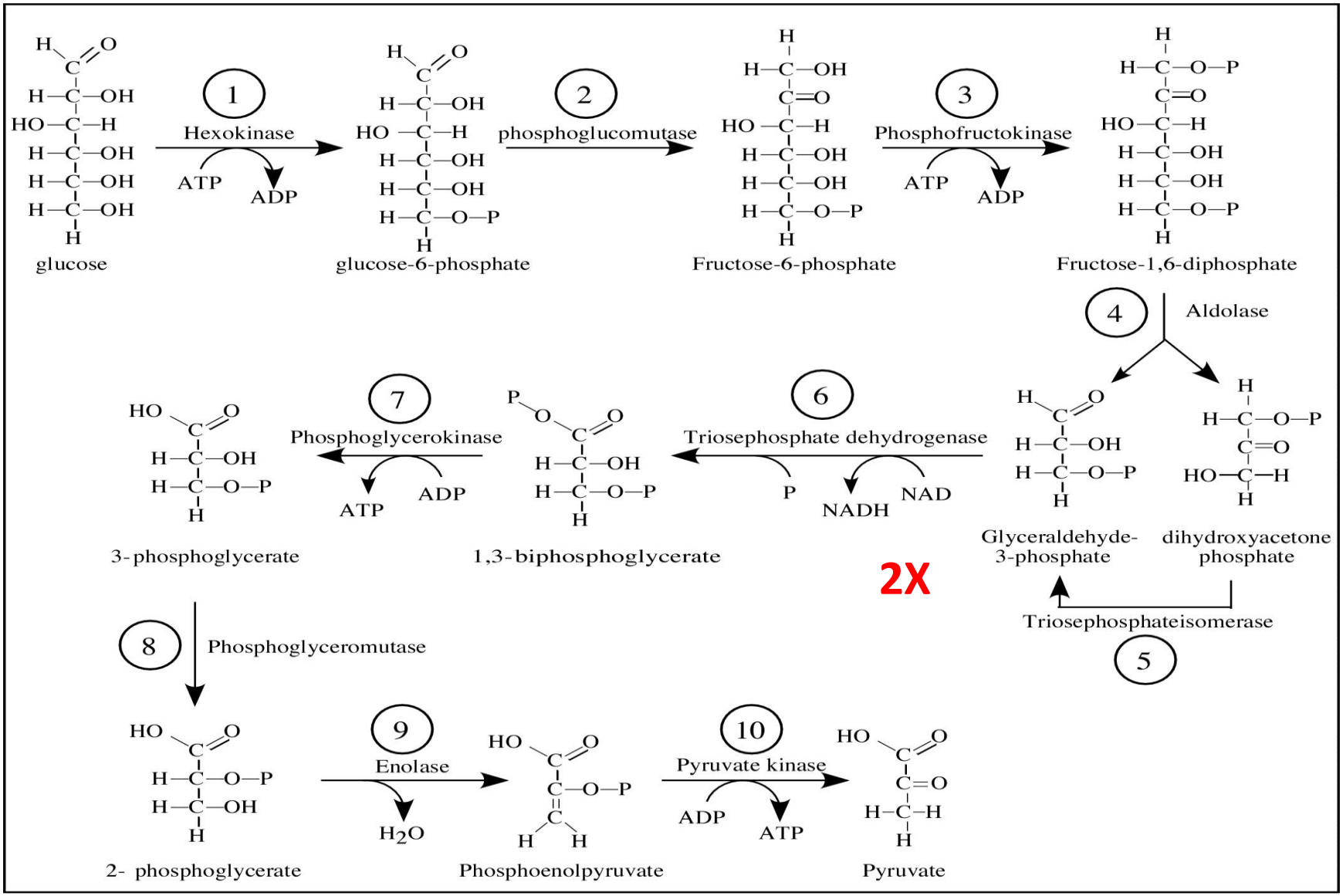
- The first five enzymatic reactions of glycolysis correspond to an energy consumption phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP.
- It starts with glucose (6 carbons) and ends with two molecules of glyceraldehyde 3-phosphate (3 carbons).

# Glycolysis (cont.)

## II. Energy generation (pay-off) phases:

- The last five enzymatic reactions constitute the energy generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation per one glucose molecule metabolized.
- During these reactions, some of the free energy released from glucose is conserved in the form of NADH.

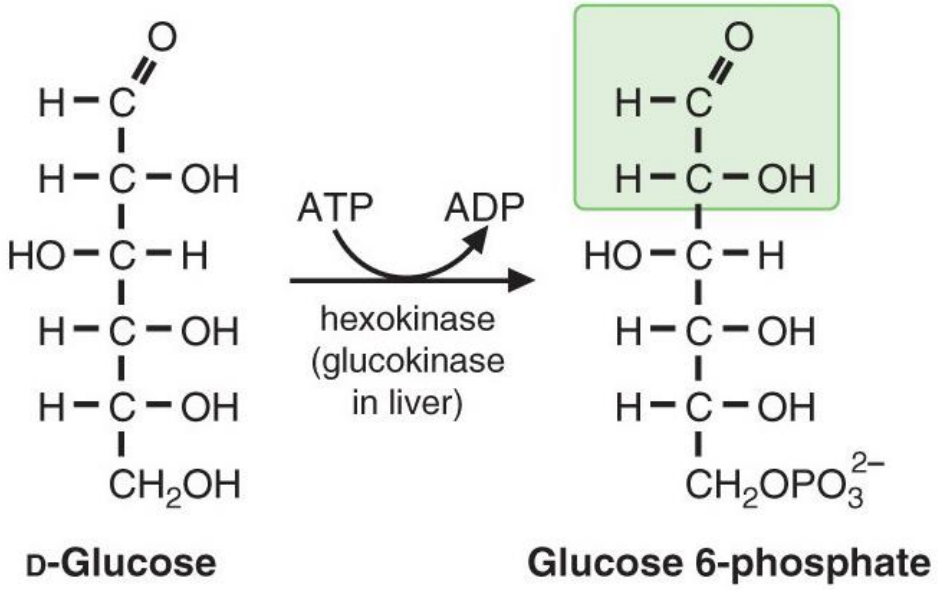
# Glycolysis (cont.)



# Steps of glycolysis

## Step 1: Phosphorylation of glucose

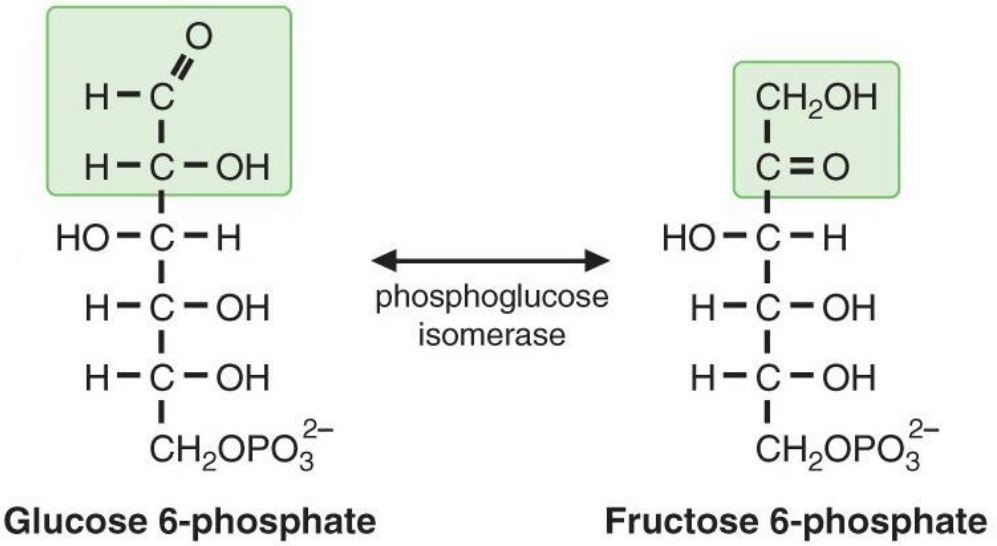
- Hexokinases catalyze the irreversible phosphorylation of glucose to form glucose-6-phosphate using one molecule of ATP.
- Hexokinase requires $Mg^{2+}$ for substrate formation (Mg-ATP) and enzyme activity.
- This reaction is a rate- limiting step in glycolysis.



# Steps of glycolysis (cont.)

## Step 2: Isomerization of glucose-6-P

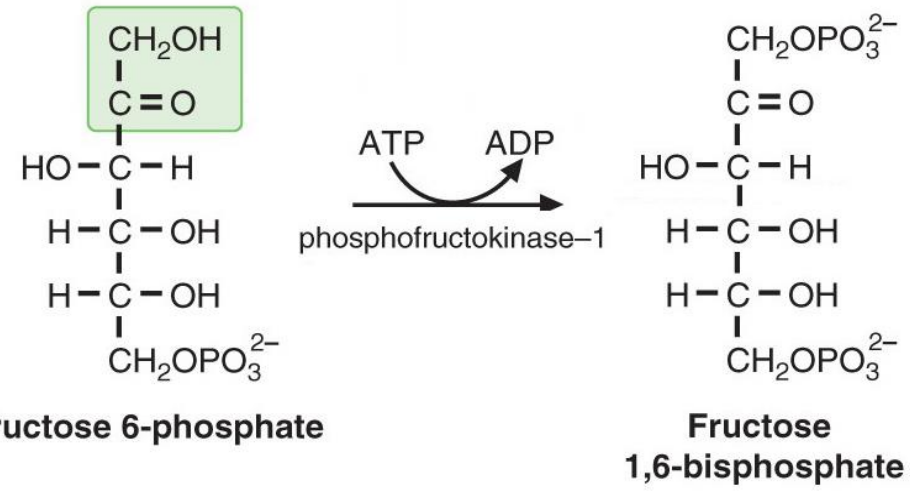
- Phosphoglucose isomerase catalyzes rearranged of glucose-6-phosphate into fructose-6-phosphate.
- This is a reversible reaction under normal cellular conditions.



# Steps of glycolysis (cont.)

## Step 3: Phosphorylation of fructose-6-P

- In this step, fructose-6-phosphate is phosphorylated to form fructose-1,6-bisphosphate, consuming one molecule of ATP by the action of phosphofructokinase-1 (PFK-1).
- PFK-1 is an allosteric enzyme, it is inhibited allosterically by elevated levels of ATP.
- This reaction is a rate- limiting step in glycolysis.

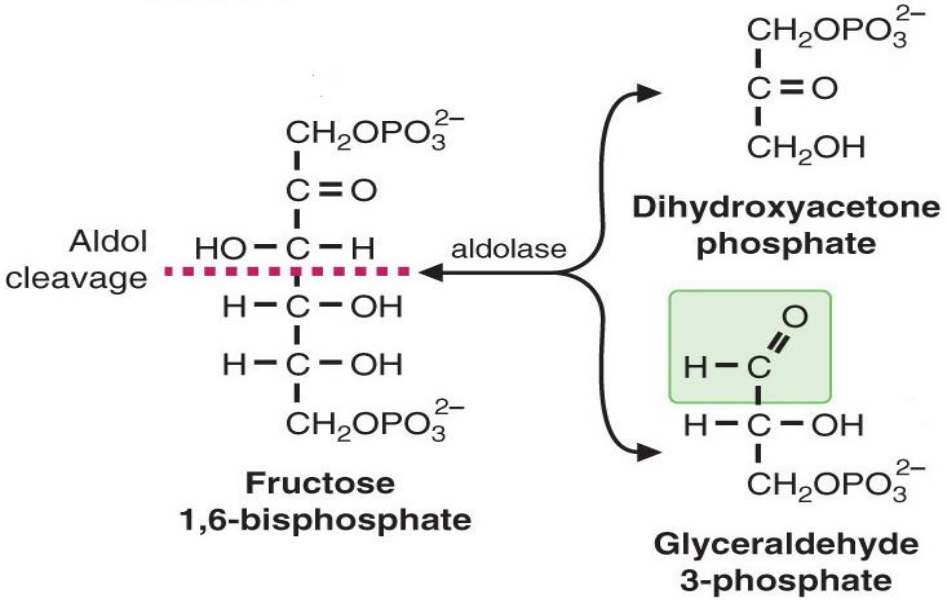


# Steps of glycolysis (cont.)

## Step 4: Cleavage of fructose-1,6-bisphosphate

- The enzyme aldolase catalyzes the cleavage of fructose-1,6-bisphosphate into two three-carbon sugars:
  - Dihydroxyacetone phosphate (DHAP)
  - Glyceraldehyde-3-phosphate (G3P)

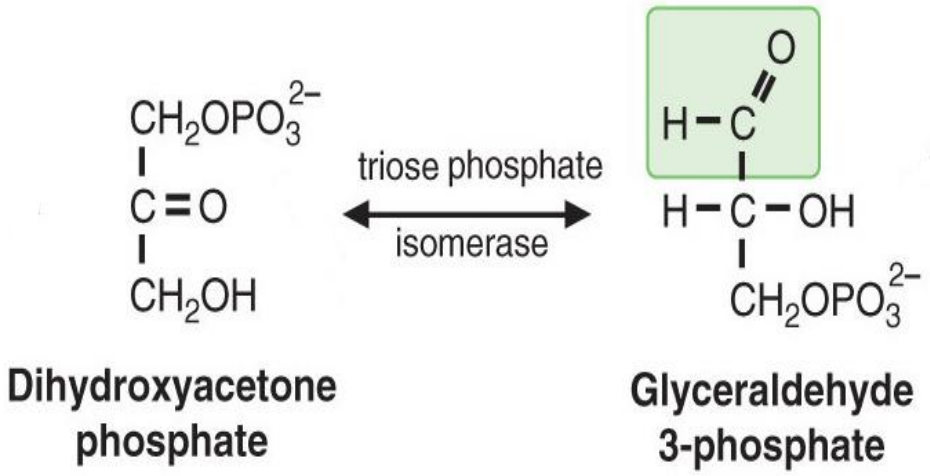
} isomers to each other



# Steps of glycolysis (cont.)

## Step 5: Isomerization of dihydroxyacetone phosphate

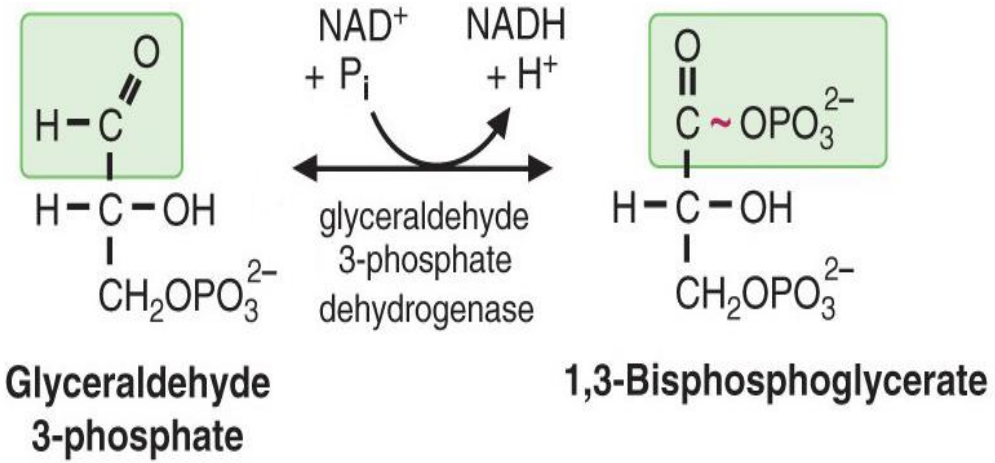
- In this step, the enzyme triose phosphate isomerase interconverts the dihydroxyacetone phosphate to glyceraldehyde phosphate.
- The end product of the preparatory phase is two molecules of glyceraldehyde 3-phosphate (G3P).



# Steps of glycolysis (cont.)

## Step 6: Oxidation of glyceraldehyde-3-phosphate

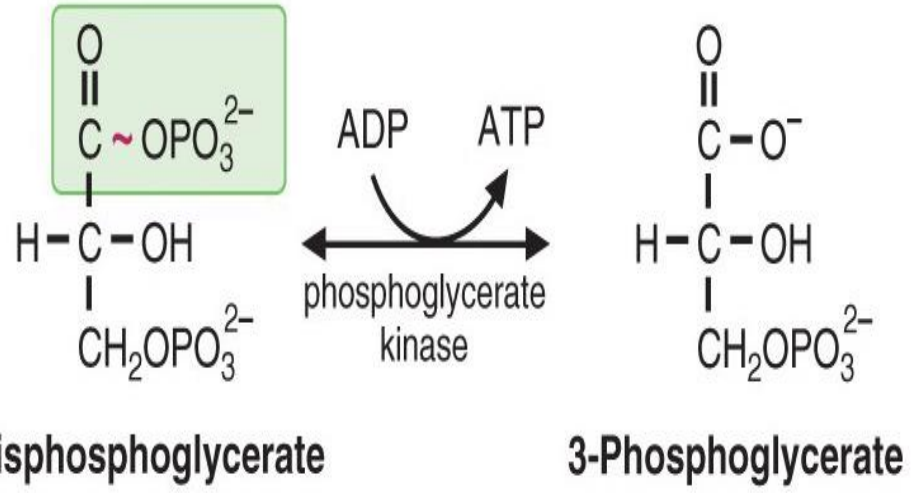
- The enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) catalyzes the oxidation of each G3P molecule by:
  1. The addition of an inorganic phosphate to G3P forming 1,3-bisphosphoglycerate (high energy compound "~").
  2. The reduction of $NAD^+$ to NADH.



# Steps of glycolysis (cont.)

## Step 7: Formation of ATP from 1,3-BPG

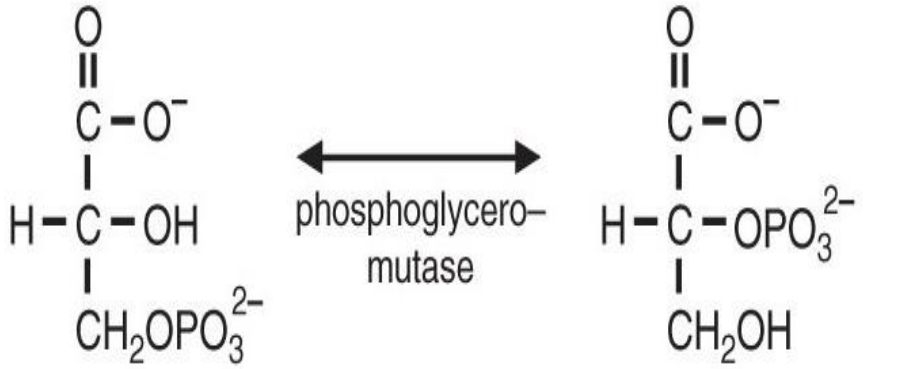
- The enzyme phosphoglycerate kinase transfers a phosphate group from each 1,3-bisphosphoglycerate to one molecule of ADP forming 3-phosphoglycerate and generating ATP.
- This reaction produces two molecules of ATP by substrate-level phosphorylation.



# Steps of glycolysis (cont.)

## Step 8: Shift of the P group from C3 to C2

- The enzyme phosphoglyceromutase relocates the phosphate group from the third carbon position to the second carbon position of the 3-phosphoglycerate molecule resulting in the formation of 2-phosphoglycerate.

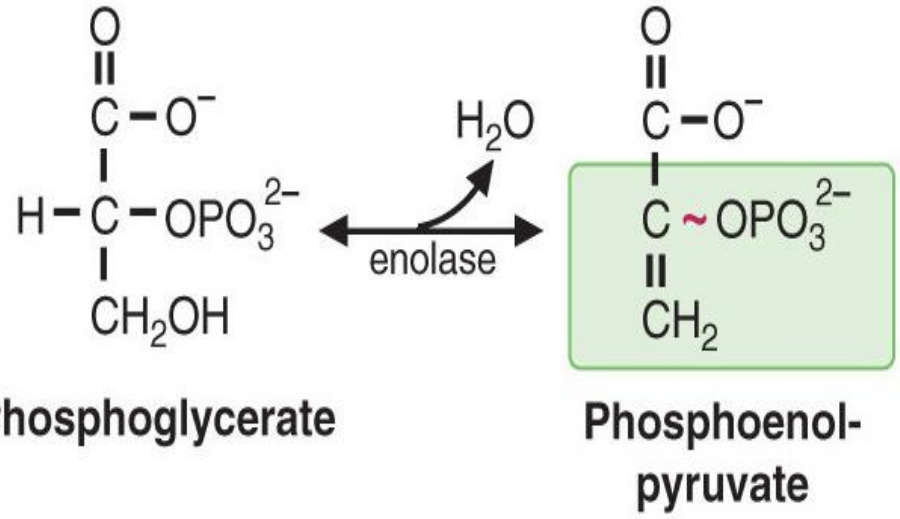



3-Phosphoglycerate 2-Phosphoglycerate

# Steps of glycolysis (cont.)

## Step 9: Dehydration of 2-phosphoglycerate

- In the presence of enolase enzyme, a water molecule is removed from 2-phosphoglycerate to form phosphoenolpyruvate (PEP; high energy compound).
- Enolase reaction is **$Mg^{2+}$**-dependent and can be inhibited by fluoride.



# Steps of glycolysis (cont.)

## Inhibition of glycolysis for glucose estimation in plasma:

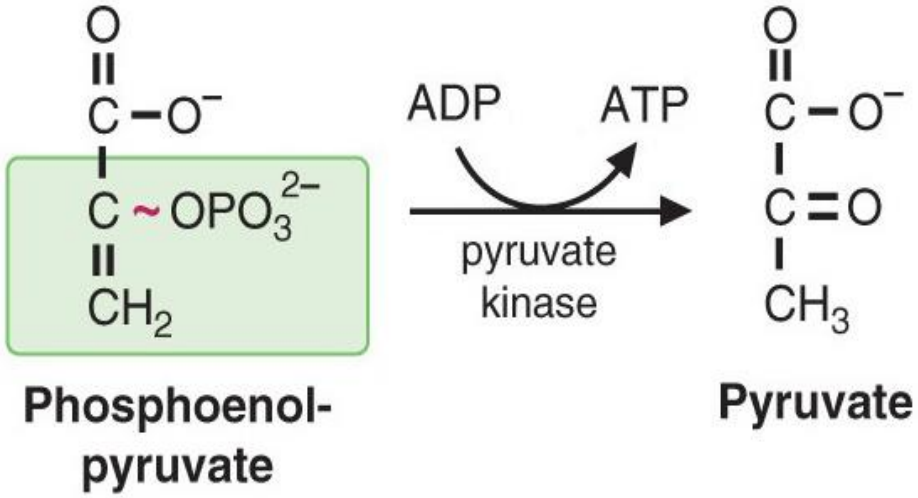
- Sodium fluoride (NaF) is commonly used to inhibit the enolase enzyme (which catalyzes a down-stream step of glycolysis).
  - Other up-stream step enzymes remain active which delays glycolysis inhibition for about 4 hours until all substrates for up-stream enzymes are depleted.

- In contrast, the acidification of whole blood with citrate buffer seems to immediately inhibit the up-stream enzymes of glycolytic cascade (hexokinase and phosphofruttokinase).

# Steps of glycolysis (cont.)

## Step 10: Formation pyruvate

- Pyruvate kinase catalyzes the transfer of an inorganic phosphate from phosphoenolpyruvate to ADP forming pyruvic acid and ATP.
- This enzyme requires $Mg^{2+}$ and $K^{+}$.
- This reaction produces ATP by substrate-level phosphorylation.
- This reaction is a rate-limiting step in glycolysis.



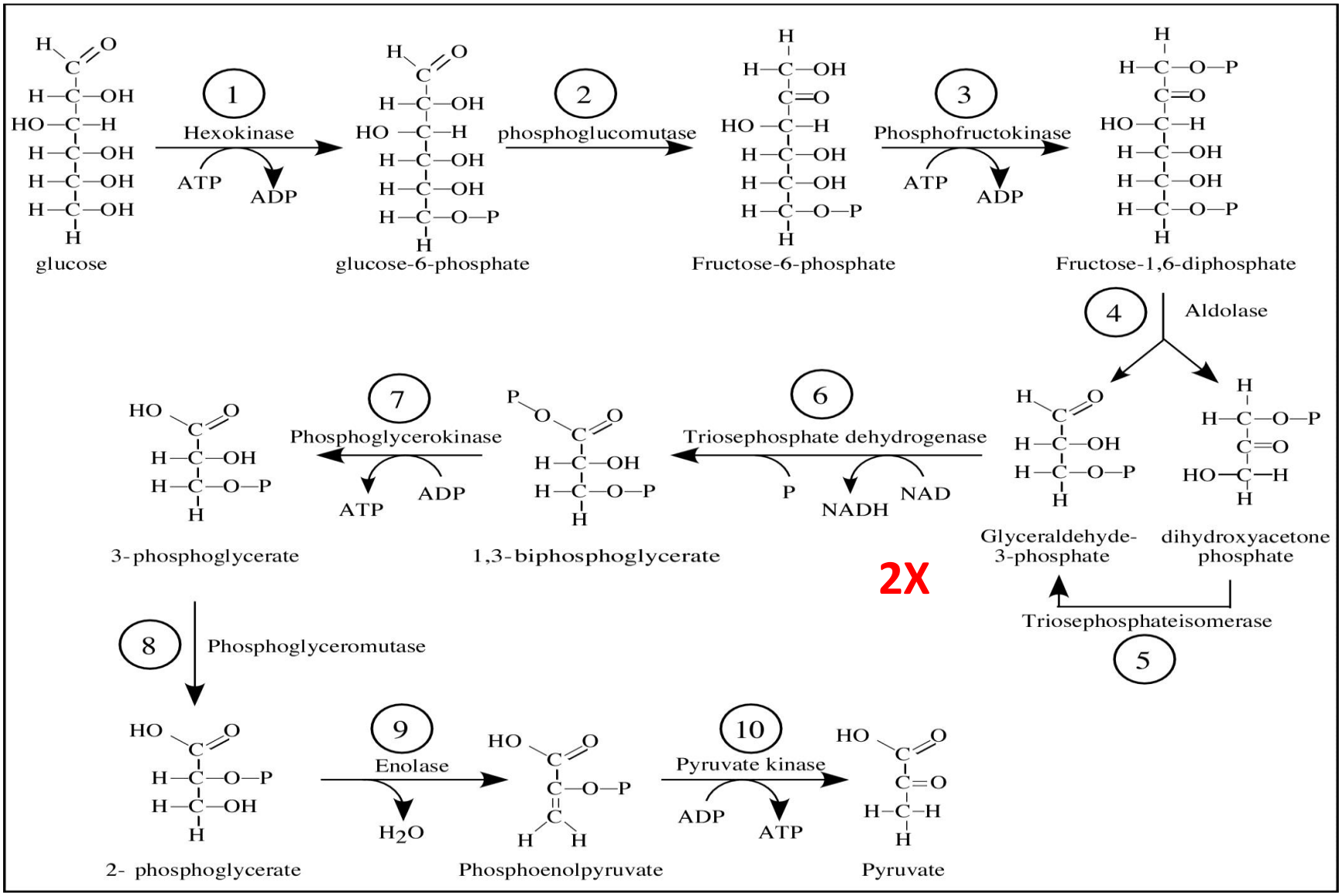
# Glycolysis: in summary

- Glycolysis occurs in the cytoplasm and provides the cell with a modest amount of ATP and precursor molecules for other metabolic pathways.
- The net gain from glycolysis is:
  - The conversion of one molecule of glucose into two molecules of pyruvate
  - The production of four molecules of ATP (two were consumed) and two molecules of NADH.
- In the presence of oxygen, the NADH produced during glycolysis can be transferred to the mitochondria where the oxidation of one molecule of NADH leads to the synthesis of three molecules of ATP.

# Glycolysis: energy production

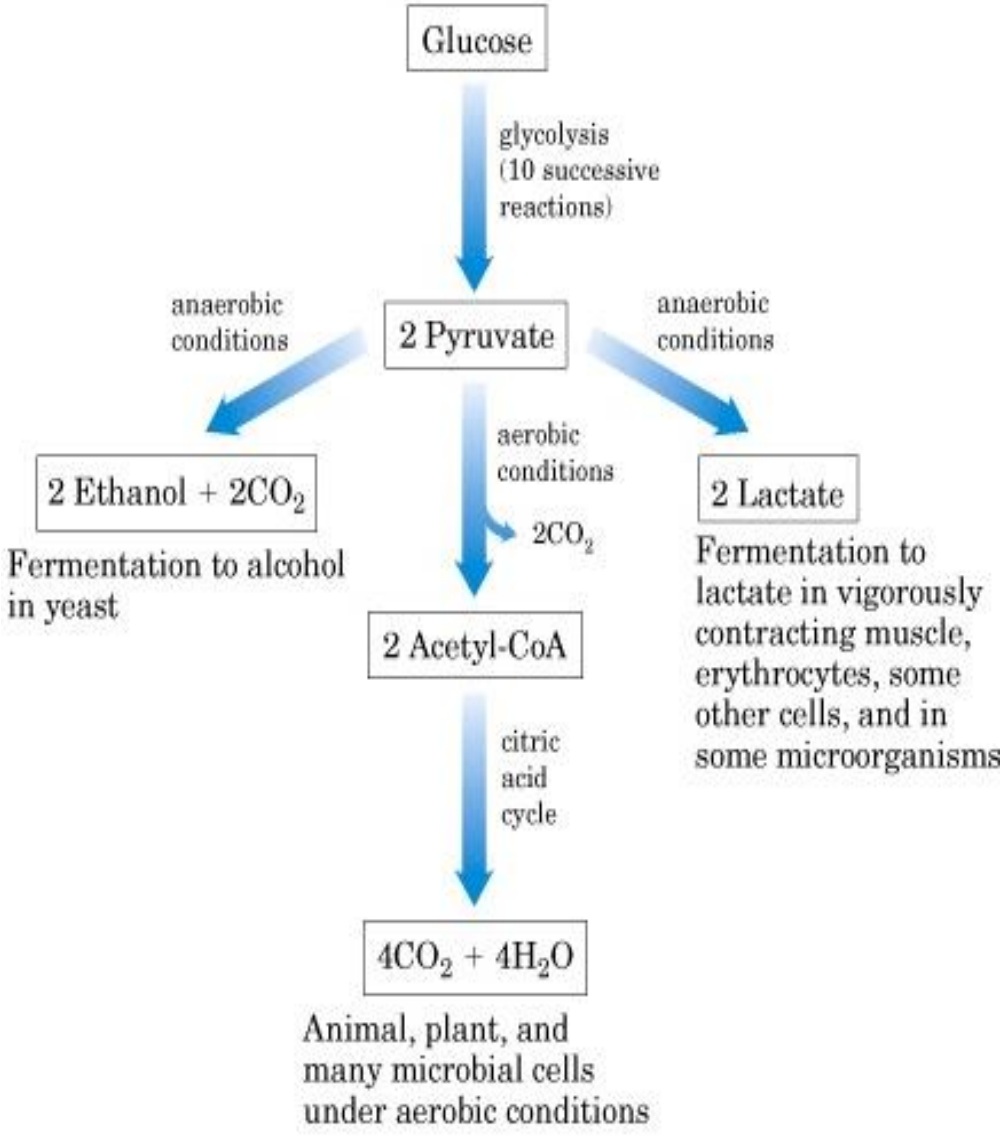
**Question:** Calculate the net ATP/NADH yield from glycolysis of one molecule of glucose?

| Glycolysis Reaction | Yield of ATP/NADH |
|---|---|
| Hexokinase | - 1 ATP |
| Phosphofructokinase | - 1 ATP |
| GAPDH | + 2 NADH |
| Phosphoglycerate kinase | + 2 ATP |
| Pyruvate kinase | + 2 ATP |
| Total energy production | 2 ATP + 2 NADH = 8 ATP |


glucose
1
Hexokinase
ATP
ADP
glucose-6-phosphate
2
phosphoglucomutase
Fructose-6-phosphate
3
Phosphofructokinase
ATP
ADP
Fructose-1,6-diphosphate
4
Aldolase
Glyceraldehyde-3-phosphate
dihydroxyacetone phosphate
Triosephosphateisomerase
5
6
Triosephosphate dehydrogenase
P
NADH
NAD
1,3-biphosphoglycerate
7
Phosphoglycerokinase
ATP
ADP
3-phosphoglycerate
2X
8
Phosphoglyceromutase
2- phosphoglycerate
9
Enolase
H2O
Phosphoenolpyruvate
10
Pyruvate kinase
ADP
ATP
Pyruvate

# The fate of pyruvate

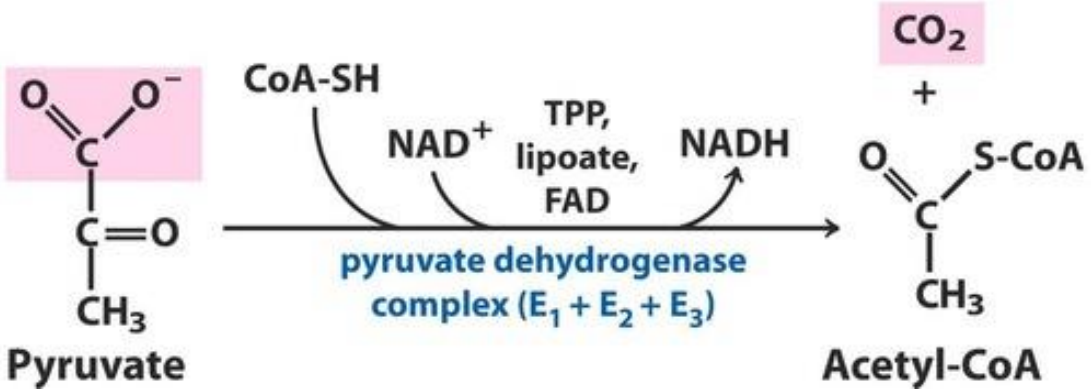
- The metabolic fate of pyruvate (product of glycolysis) depends on the presence or absence of oxygen and the type of organism (aerobic or anaerobic).



# The fate of pyruvate (cont.)

## Under aerobic conditions:

- In the presence of oxygen, pyruvate enters the mitochondria, where it is converted to acetyl-coenzyme A (acetyl-CoA) by the action of pyruvate dehydrogenase complex.

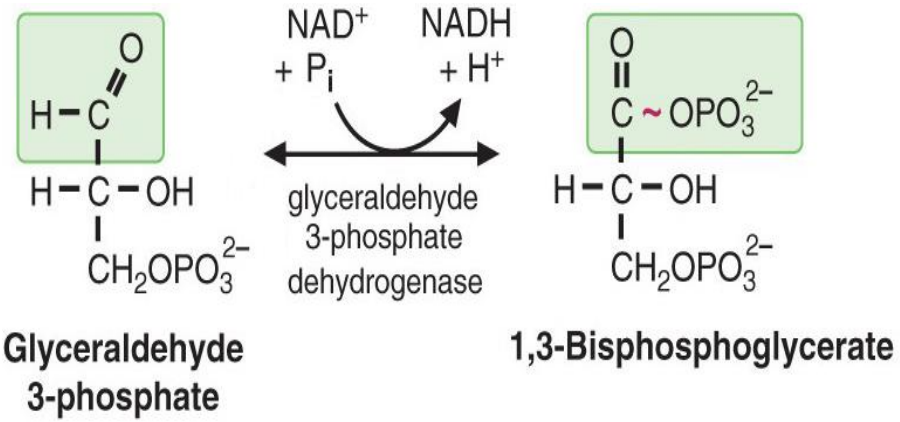



- Acetyl-CoA then undergoes further oxidation by entering the citric acid cycle, generating NADH, FADH2 and GTP (which can be used to generate ATP) and releasing carbon dioxide as a byproduct.

# The fate of pyruvate (cont.)

## Under anaerobic condition:

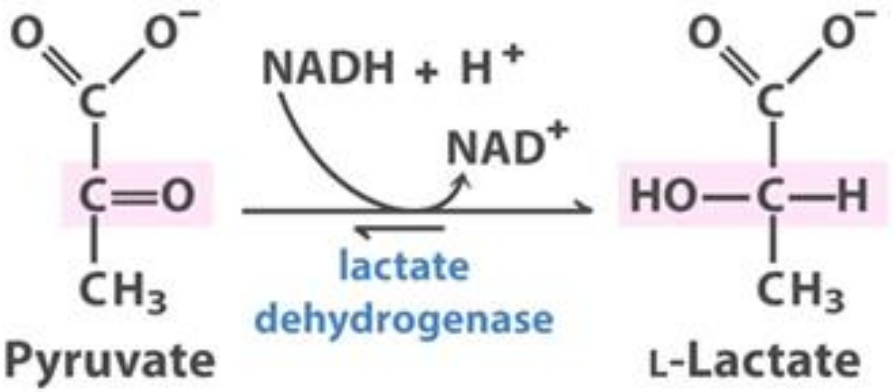
- In the absence of oxygen, pyruvate can undergo fermentation, producing lactic acid (homolactic fermentation) or ethanol (alcohol fermentation in yeast).
- The process of pyruvate fermentation helps to regenerate $NAD^+$ that is used (as a cofactor for GAPDH reaction, Step 6) to ensure the continuity of glycolysis (in the absence of oxygen).



# The fate of pyruvate (cont.)

## Homolactic fermentation:

- In skeletal muscles, the fermentation of pyruvate to lactate occurs during intense exercise or when there is an increased demand for energy and oxygen cannot be supplied rapidly enough to meet that demand.
- lactate dehydrogenase (LDH) catalyzes the reversible conversion of pyruvate to lactate coupled with the oxidation of NADH to $NAD^+$.
- $NAD^+$ is needed for glycolysis to continue, as it is a cofactor required for GAPDH reaction (Step 6).

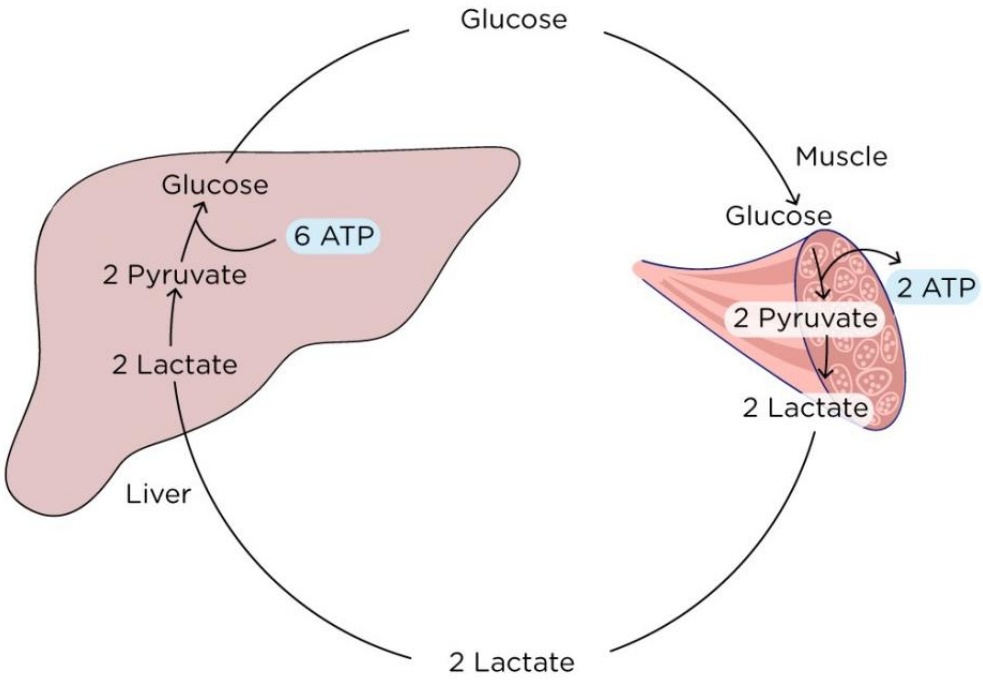


# The fate of pyruvate (cont.)

## Homolactic fermentation (cont.):

- Lactic acid can be transported out of the muscles into the bloodstream, where it can be taken up by the liver and converted back into glucose in a process known as the Cori cycle.

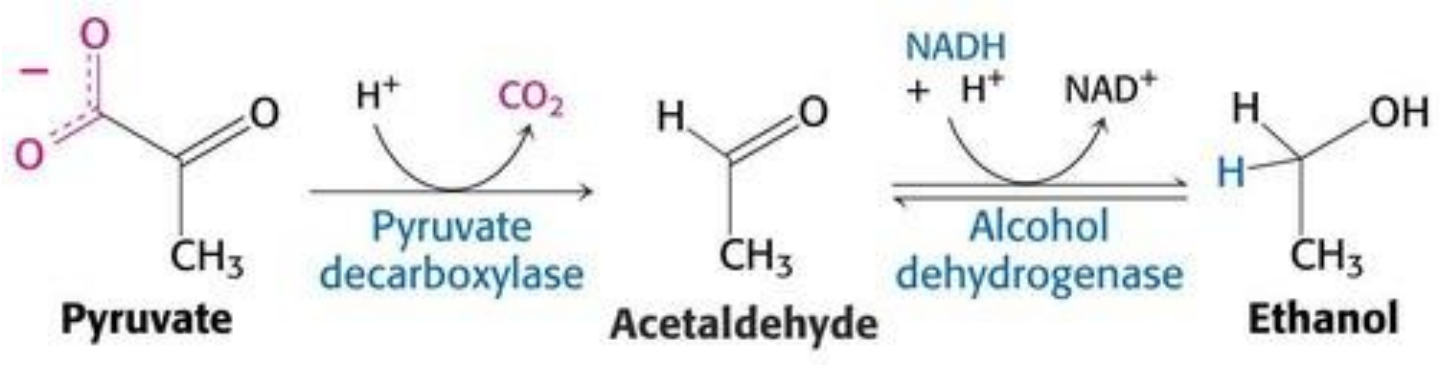
- Lactate serves as an important energy source for cardiac muscles as well as brain (comprises 57% of the total brain energy source).



# The fate of pyruvate (cont.)

## Alcoholic fermentation:

- Certain microorganisms (such as yeast) convert pyruvate into ethanol (alcohol) and carbon dioxide.
- Alcoholic fermentation is essential in these organisms to regenerate $NAD^+$, which will be used to sustain glycolysis in the absence of oxygen.



# The fate of pyruvate (cont.)

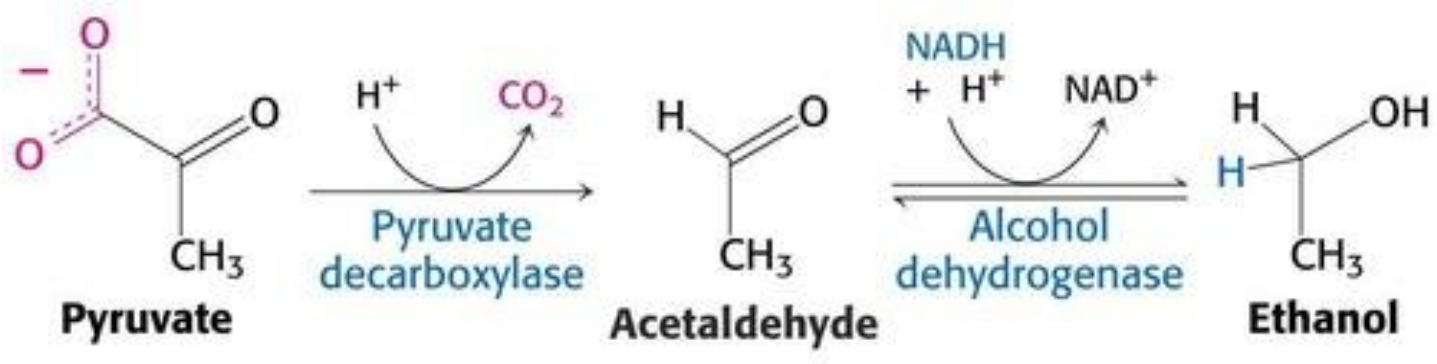
## Alcoholic fermentation is a two step process:

1. **Pyruvate decarboxylase (PDC) reaction:**

- This enzyme is $Mg^{2+}$-dependent and requires an enzyme-bound cofactor, thiamine pyrophosphate (TPP).
- In this reaction carbon dioxide is released as a byproduct and acetaldehyde is formed.

2. **Alcohol dehydrogenase reaction:**

- It involves the reduction of acetaldehyde to ethanol.
- It uses NADH as a reducing agent, thus regenerating $NAD^+$.



# Regulation of glycolysis

- Glycolysis, the metabolic pathway that breaks down glucose into pyruvate.
- It plays a vital role in cellular energy production and is central to various physiological processes.
- Therefore, this pathway is tightly regulated by both enzymatic and hormonal mechanisms to ensure that cells can respond to changes in energy demand and maintain homeostasis.

# Enzymatic regulation

- The regulation of glycolysis is crucial for maintaining energy homeostasis within the cell.
- The main regulatory steps involve enzymes that catalyze irreversible reactions (rate-limiting steps) in the pathway. These enzymes include:
    - Hexokinase/glucokinase (HK)
    - Phosphofructokinase-1 (PFK-1)
    - Pyruvate kinase (PK)
- These enzymes are regulatory enzymes which are regulated by the level of ATP in the cell.

# Enzymatic regulation (cont.)

**Hexokinase/glucokinase:**

- Hexokinase activates an irreversible phosphorylation of glucose by ATP to glucose 6-phosphate (Step1).
- Hexokinase has a high affinity (low Km) for glucose. This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low.
- It is inhibited by its product (feedback inhibition), which prevents unnecessary phosphorylation of glucose when cellular energy needs are met.

# Enzymatic regulation (cont.)

## Hexokinase/glucokinase (cont.):

- Glucokinase is ONLY found in the liver and pancreas (β-cells).
- It has a low affinity for glucose. Therefore, it is very active after a meal when concentrations of glucose are high and it is relatively inactive during fasting when concentrations of glucose are low.
- Glucokinase is regulated by an inhibitory protein (glucokinase regulatory protein), which binds to glucokinase at low glucose concentrations and sequesters the enzyme in the nucleus.
- When glucose concentrations increase, the glucokinase dissociates from its regulatory protein and brought back to the cytoplasm where it can phosphorylate glucose.

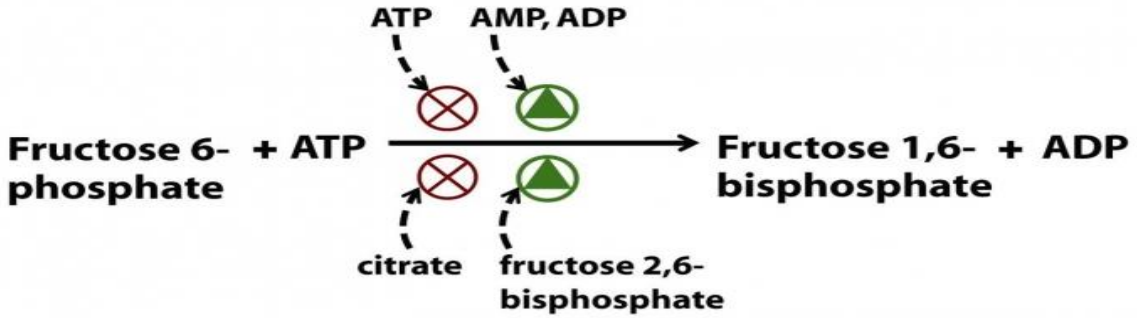
# Enzymatic regulation (cont.)

| | Hexokinase | Glucokinase |
|---|---|---|
| **Site** | All tissues | Liver cells mainly |
| **Substrate(s)** | Glucose and other hexoses | Glucose only |
| **Affinity to glucose** | High affinity (low Km) | Low affinity (high Km) |
| **Effect of glucose-6-P** | Allosteric feed-back inhibition | No effect |
| **Effect of insulin** | No effect | Induces synthesis of the enzyme |
| **Carbohydrate fed/fasting state** | No effect | Increases/decreases activity of the enzyme |
| **Function** | It phosphorylates glucose inside the body cells at low glucose concentrations, ensuring a continuous supply of glucose for tissues. | It is only active at high glucose concentration.<br>In the liver, it phosphorylates glucose ONLY after meals. |

# Enzymatic regulation (cont.)

## Phosphofructokinase-1:

- Phosphofructokinase-1 (PFK-1) is a key regulatory enzyme which catalyzes the second irreversible reaction unique to the glycolytic pathway (the committed step, Step 3).
- It functions at a rapid rate in the liver when blood glucose levels are high or in cells such as muscle when there is a need for ATP.
- PFK-1 is allosterically regulated by several factors; including:
  - ATP
  - AMP
  - Citrate
  - Fructose 2,6-BP



# Enzymatic regulation (cont.)

## Phosphofructokinase-1 (cont.):

- When ATP levels are high, ATP acts as an allosteric inhibitor of PFK-1, signaling that the cell has sufficient energy. Thus, decreasing PFK-1 affinity for its substrate (fructose 6-phosphate).
  - However, low levels of ATP activates PFK-1, increasing its affinity for fructose 6-phosphate to form fructose 1,6-bisphosphate.
- High AMP/ADP levels activate PFK-1, indicating energy deficit.

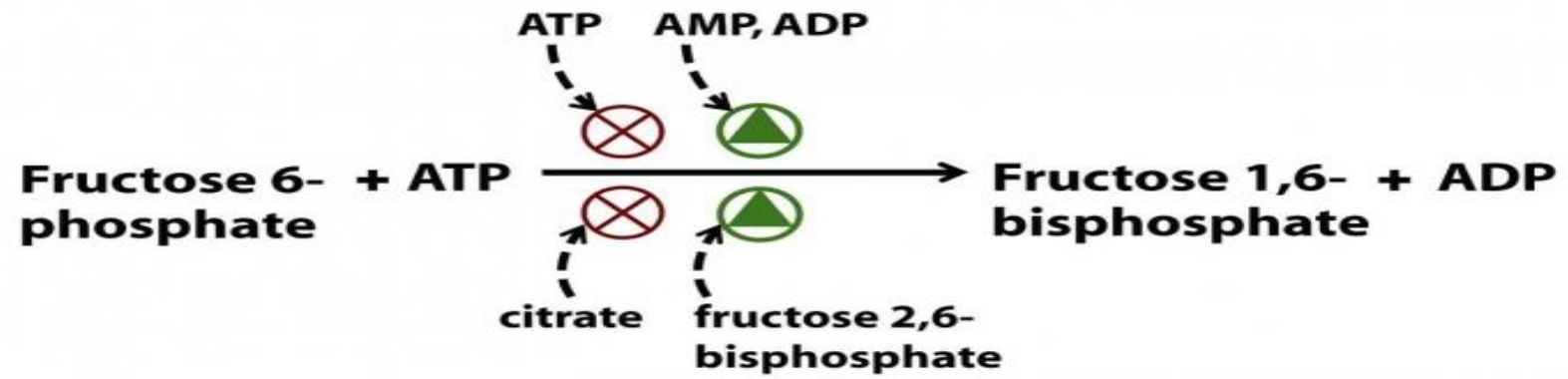
# Enzymatic regulation (cont.)

**Phosphofructokinase-1 (cont.):**

- Fructose 2,6-bisphosphate activates PFK-1 and enhances glycolysis.

  - Fructose 2,6-bisphosphate is formed by the hormone-stimulated phosphorylation of fructose 6-phosphate.

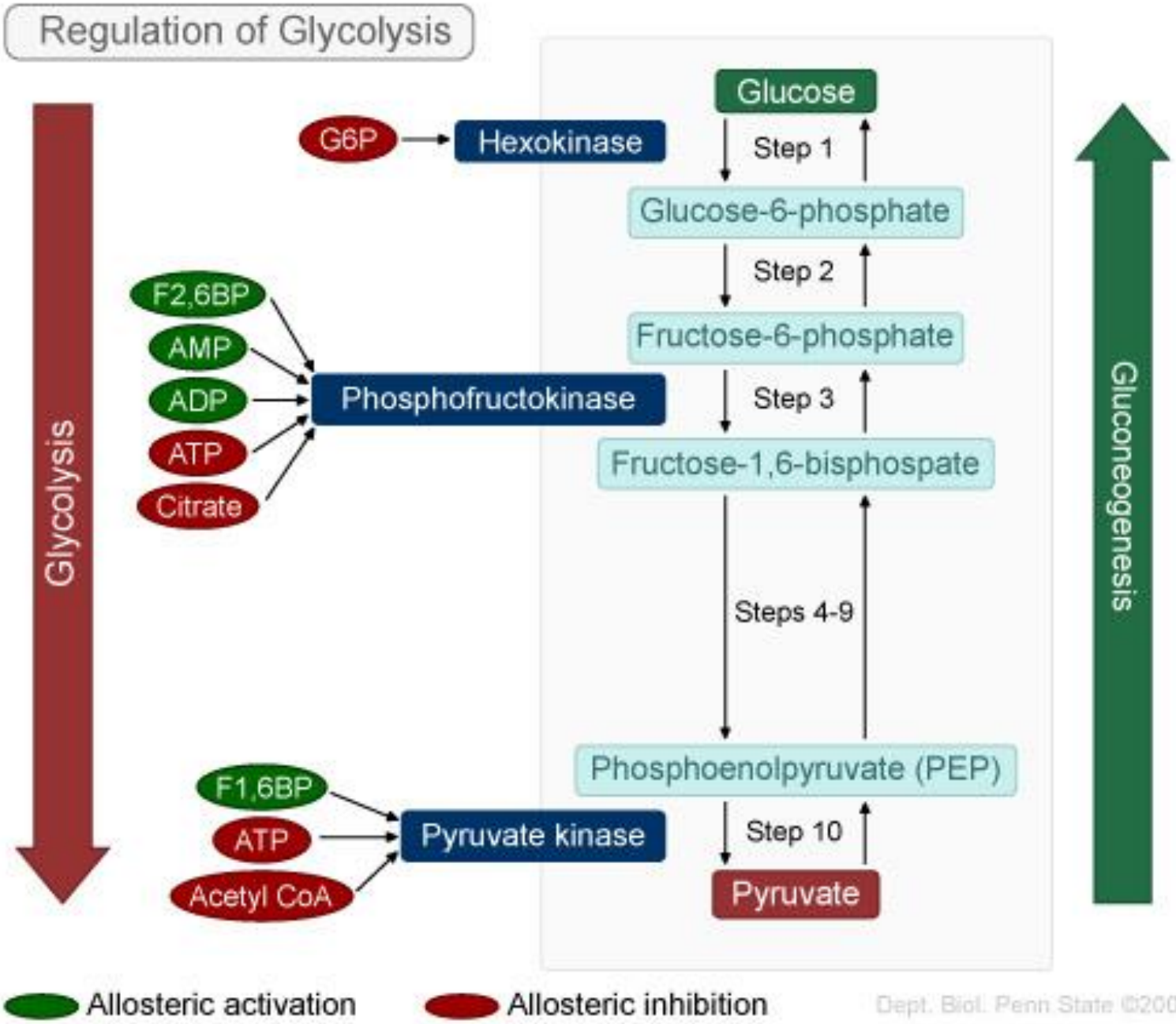
- High levels of citrate (an intermediate in TCA cycle) inhibits PFK-1, linking glycolysis to the overall energy status of the cell.



# Enzymatic regulation (cont.)

**Pyruvate kinase:**

- Pyruvate kinase is an important regulatory enzyme of glycolysis as it ensures the formation of pyruvate in the last step of glycolysis (Step 10).
- It is allosterically inhibited when the cellular levels of ATP is high, indicating a surplus of energy.
- The activity of pyruvate kinase can also inhibited by high concentrations of acetyl-CoA and long chain fatty acids. Explain why??
- Fructose 1,6-bisphosphate (formed in Step 3) acts a allosteric feedforward activator of the pyruvate kinase reaction, enhancing glycolytic flux.


Regulation of Glycolysis
Glycolysis
Gluconeogenesis
Glucose
G6P
Hexokinase
Step 1
Glucose-6-phosphate
Step 2
F2,6BP
AMP
ADP
ATP
Citrate
Phosphofructokinase
Fructose-6-phosphate
Step 3
Fructose-1,6-bisphospate
Steps 4-9
Phosphoenolpyruvate (PEP)
F1,6BP
ATP
Acetyl CoA
Pyruvate kinase
Step 10
Pyruvate
Allosteric activation
Allosteric inhibition
Dept. Biol. Penn State ©2004

# Hormonal regulation

- Hormonal regulation plays a significant role in the control of glycolysis, influencing the activity of the key enzymes and the overall rate of glucose metabolism.
- The two primary hormones involved in the regulation of glycolysis are insulin and glucagon.
- These hormones are secreted by the pancreas and act in opposition (antagonistic) to each other to help maintain glucose homeostasis in the body.

# Hormonal regulation (cont.)

**Insulin:**

- Insulin is secreted from the β-cells of the islet of Langerhans in response to hyperglycemia to promote:
  - Glucose uptake by cells (especially in muscle and adipose tissue).
  - Glucose availability for glycolysis.
  - Synthesis and activation of key enzymes involved in glycolysis, including hexokinase and phosphofructokinase.
  - Activation of protein phosphatase 1 that dephosphorylate (activate) a number of enzymes involved in glycogen and lipid metabolism.

# Hormonal regulation (cont.)

## Fructose 2,6-bisphosphate:

- Fructose 2,6-bisphosphate (F26BP) is an activator of glycolysis. This molecule increases the activity of the important rate-limiting enzyme phosphofructokinase-1 (PFK-1) by acting as an allosteric activator.

- The concentration of F26BP is directly linked to the activity of the unique bifunctional enzyme, known as phosphofructokinase-2/fructose-2,6-bisphosphatase (PFK-2/FBPase).
  - When the kinase activity is high, F26BP concentrations (and consequently glycolytic flux) increase.
  - On the other hand, when the bisphosphatase activity is dominant, the concentration of F26BP decreases, removing the allosteric activator of PFK-1, and decreasing glycolytic flux.

# Hormonal regulation (cont.)

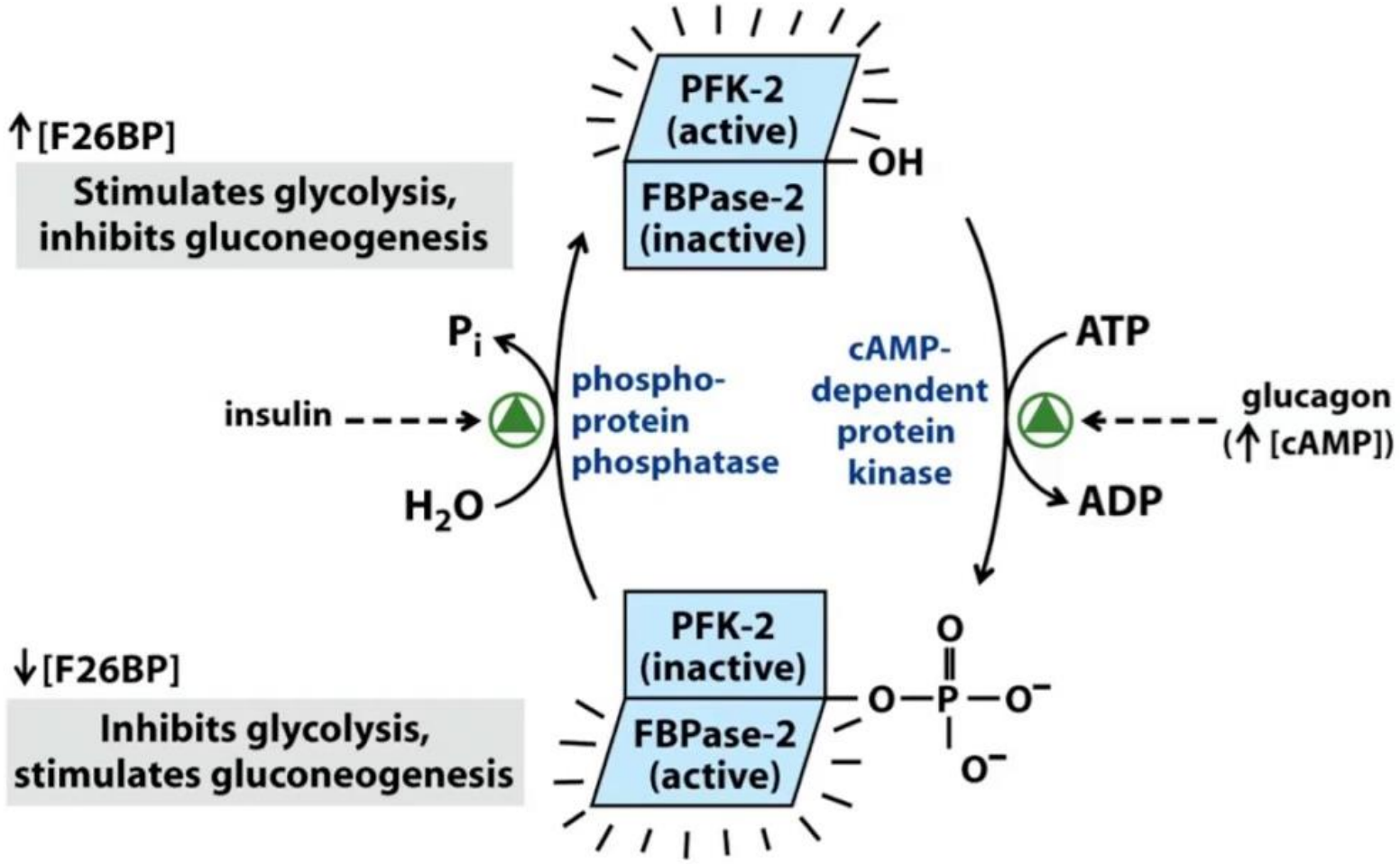
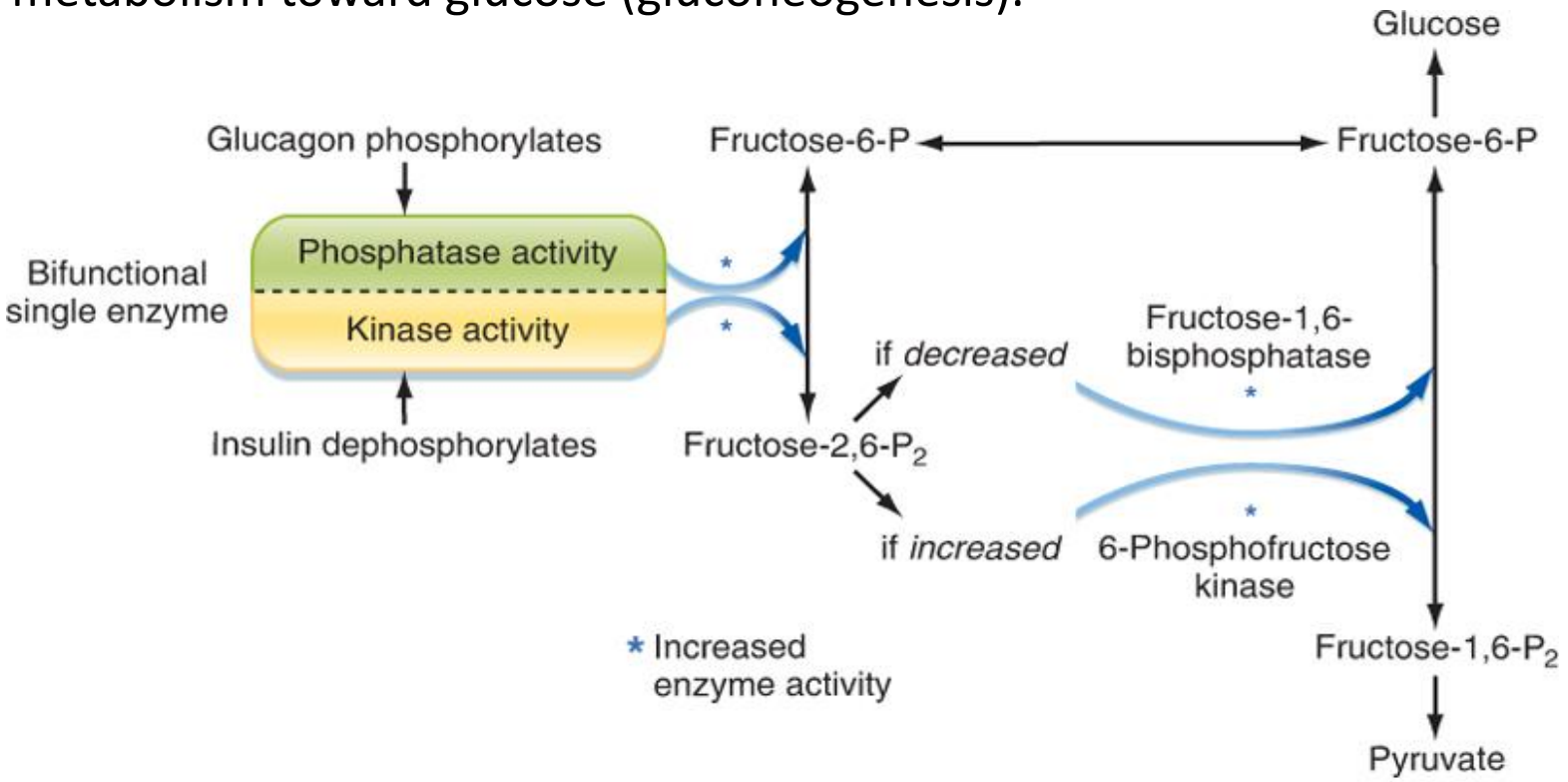



Figure 15-17b
*Lehninger Principles of Biochemistry, Fifth Edition*

## Regulation of the relative rates of gluconeogenesis and glycolysis by the actions of islet hormones on a single bifunctional enzyme.

- Insulin causes dephosphorylation of the enzyme, thus making it a kinase, which raises the level of fructose-2,6-bisphosphate.
- This intermediate stimulates the activity of 6-phosphofructose kinase and shifts metabolism toward pyruvate (glycolysis).
- Phosphorylation of the bifunctional enzyme by glucagon makes it a phosphatase, which lowers the level of fructose-2,6-bisphosphate and thereby increases the activity of fructose-1,6-bisphosphatase and shifts metabolism toward glucose (gluconeogenesis).



# Hormonal regulation (cont.)

## Glucagon:

- Glucagon acts to increase blood glucose levels during periods of fasting (hypoglycemia) or low energy availability.
- Glucagon inhibits key enzymes in glycolysis, including glucokinase, phosphofructokinase, and pyruvate kinase (opposes the action of insulin).
- Glucagon stimulates gluconeogenesis (the synthesis of glucose from non-carbohydrate sources) and promotes the release of glucose into the bloodstream.

# Inhibitors of glycolysis

Examples of inhibitors targeting different steps in the glycolytic pathway include:

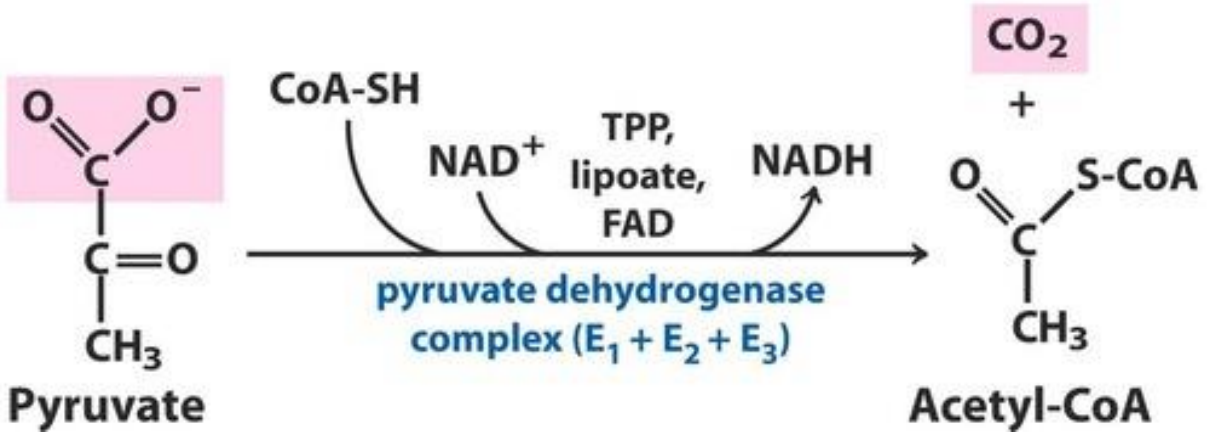
- **2-Deoxyglucose (2-DG):** it is a glucose analog that competitively inhibits hexokinase.
- **Fluoride:** it can inhibit enolase and pyruvate kinase by forming complexes with $Mg^{2+}$ (a cofactor required for the enzymes' activity).
- **Sodium arsenate:** It interferes with the formation of ATP by substrate-level phosphorylation.

# Pasteur effect

- The Pasteur effect refers to the phenomenon observed by French scientist Louis Pasteur, where the rate of fermentation (anaerobic glycolysis) is reduced in the presence of oxygen.
- In the absence of oxygen, cells can generate ATP (in small amounts) through glycolysis.
- However, the presence of oxygen activates the oxidative phosphorylation that provides a higher yield of ATP.
- The phenomenon is particularly relevant to cells that can switch between aerobic and anaerobic metabolism (e.g. muscle cells).

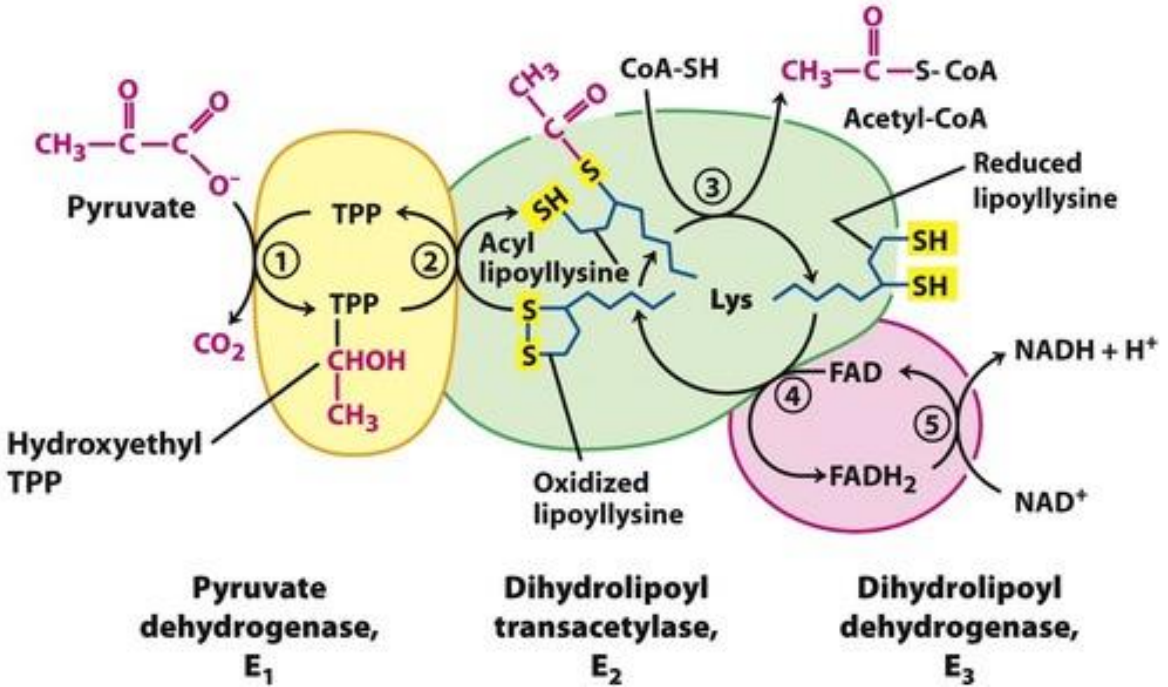
# Oxidation of pyruvate

- The oxidation of pyruvate is a crucial step in cellular respiration that ultimately leads to the production of ATP.
- The process takes place in the mitochondria, and it involves the conversion of pyruvate (3 carbons) into acetyl-CoA (2 carbons).
- The overall reaction is catalyzed by the enzyme pyruvate dehydrogenase complex (PDC), which is a multi enzyme complex.



# Oxidation of pyruvate (cont.)

- The enzyme pyruvate dehydrogenase complex is composed of three enzymes:
  - Pyruvate dehydrogenase (E1)
  - Dihydrolipoyl transacetylase (E2)
  - Dihydrolipoyl dehydrogenase (E3)



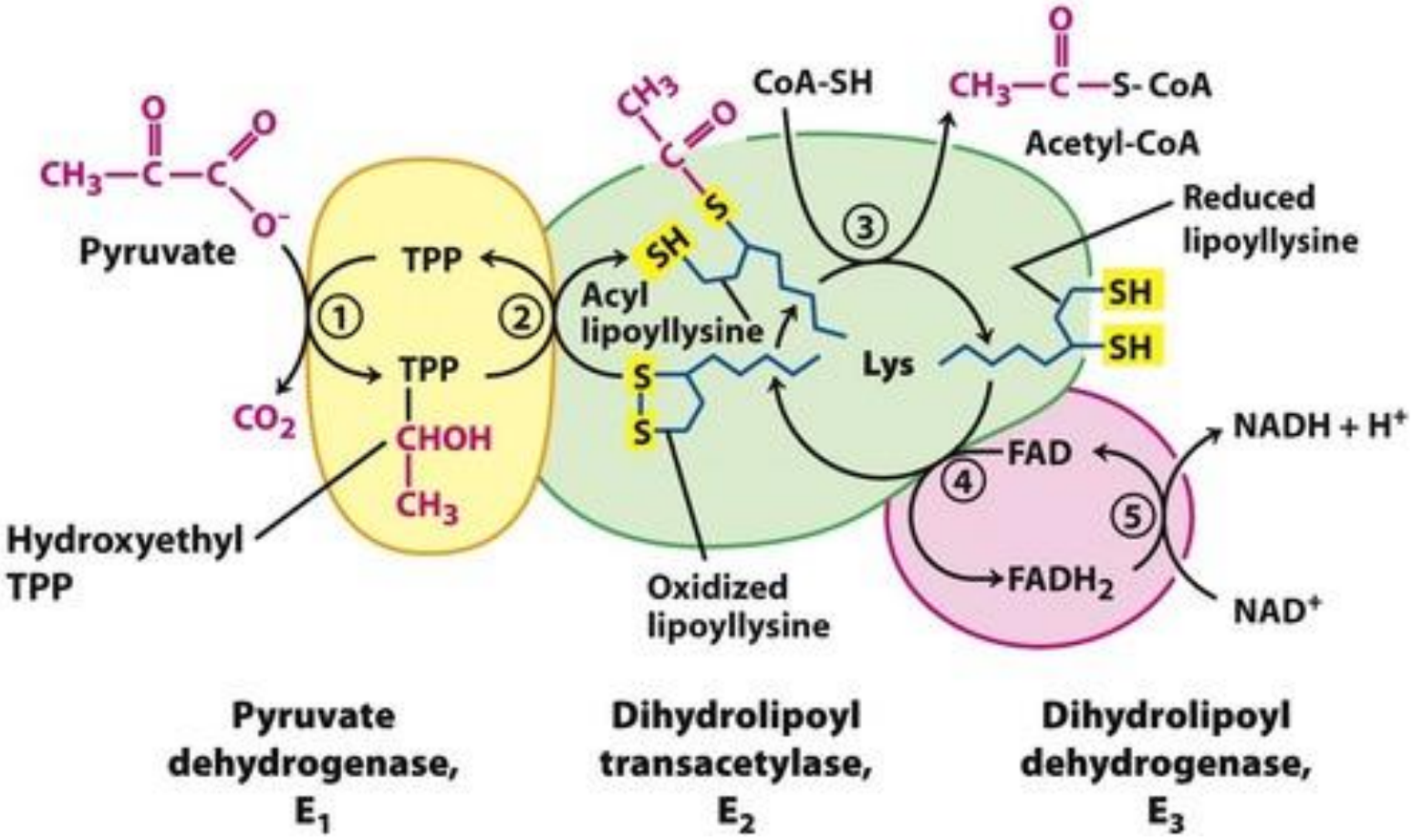
# Oxidation of pyruvate (cont.)

- Pyruvate dehydrogenase complex also requires five cofactors, they include:
  - Thiamine pyrophosphate (TPP; vitamin B1)
  - Coenzyme A (CoASH; vitamin B5)
  - Lipoic Acid
  - Nicotinamide adenine dinucleotide ($NAD^+$; vitamin B3)
  - Flavin adenine dinucleotide (FAD; vitamin B2)

# Oxidation of pyruvate (cont.)

- The enzyme pyruvate dehydrogenase complex is composed of three enzymes:
  - Pyruvate dehydrogenase (E1):
    - Catalyzes the decarboxylation of pyruvate, removing a carboxyl group and producing carbon dioxide.
  - Dihydrolipoyl transacetylase (E2):
    - Receives the acetyl group from pyruvate and transfers it to coenzyme A (CoA), forming acetyl-CoA.
  - Dihydrolipoyl dehydrogenase (E3)
    - Regenerates NADH from $NAD^+$ by oxidizing the reduced lipoamide cofactor.

# Oxidation of pyruvate (cont.)



# Oxidation of pyruvate (cont.)

## Regulation of the pyruvate dehydrogenase complex:

- The activity of the pyruvate dehydrogenase complex is tightly regulated by the following factors:
  - **Allosteric inhibition:** high levels of ATP, acetyl-CoA, and NADH (indicative of sufficient energy production).
  - **Allosteric activation:** high levels of AMP (or ADP), CoA, and $NAD^+$ .

*Note:*

*The three following ratios are important for the regulation of pyruvate dehydrogenase:*

- ATP/ADP
- $NADH/NAD^+$
- Acetyl-CoA/CoA.

# Oxidation of pyruvate (cont.)

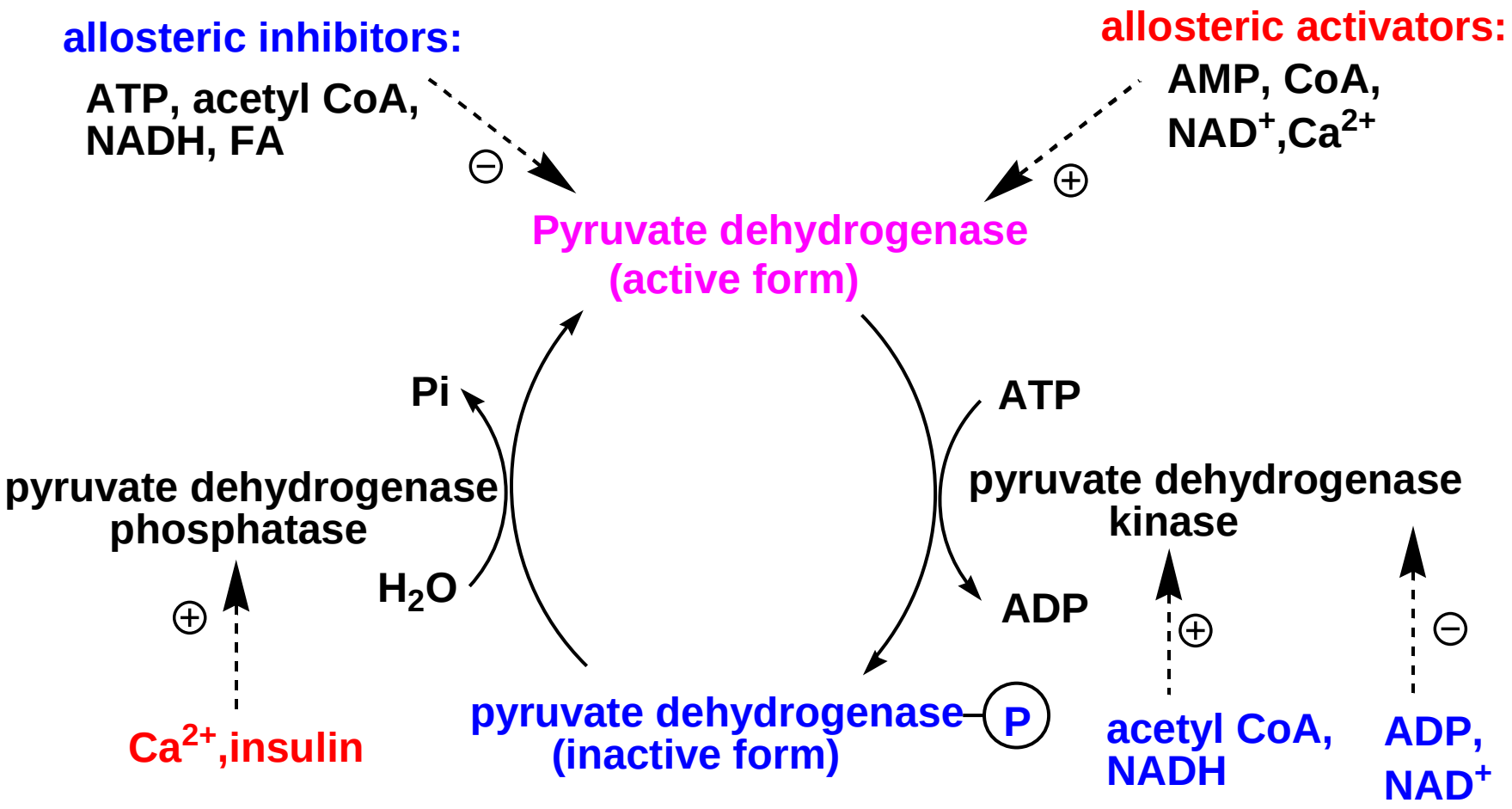
## Regulation of the pyruvate dehydrogenase complex (cont.):

- **Covalent modification:** through the phosphorylation and dephosphorylation of the E1 subunit of pyruvate dehydrogenase complex.

- Pyruvate dehydrogenase kinase (PDK) phosphorylates the PDH complex and inhibits its activity.

- While pyruvate dehydrogenase phosphatase (PDP) dephosphorylates and activates the PDH complex.

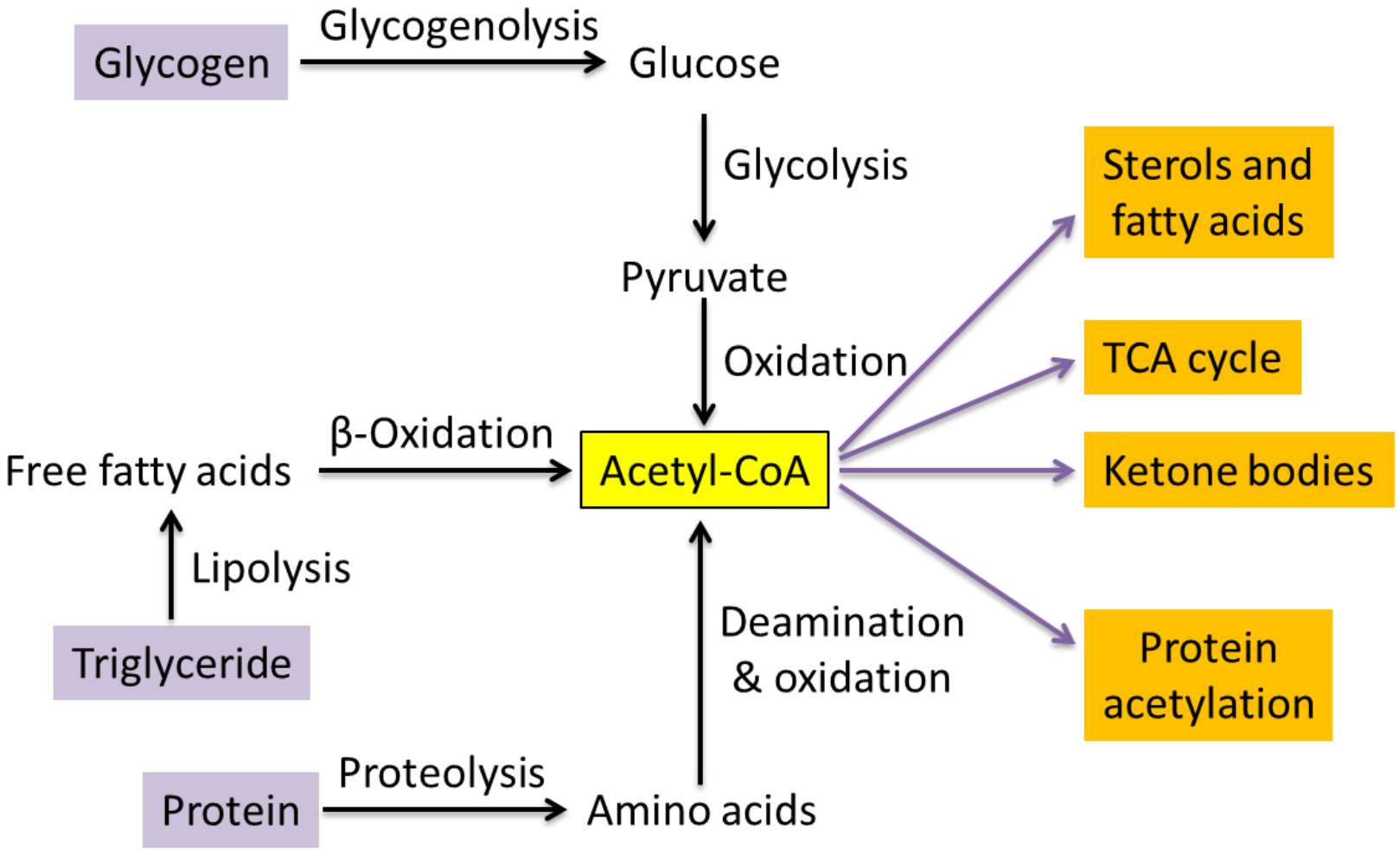
# Regulation of E1 by covalent modification through phosphorylation/dephosphorylation



# Fates of Acetyl Co-A

- Acetyl-CoA is a central metabolic intermediate with versatile roles in cellular metabolism.
- It can be derived by combustion of glucose, fatty acids, proteins or amino acids, and alcohol.
- Its fate depends on the specific metabolic demands of the cell and the prevailing conditions:
  - Channeled into the citric acid cycle (Krebs cycle)
  - Fatty acid synthesis (lipogenesis)
  - Cholesterol synthesis
  - Ketone body formation (ketogenesis)
  - Amino acid synthesis
  - Acetylation of various metabolites

# Fates of Acetyl Co-A (cont.)



# Krebs cycle

- Krebs cycle is also known as:
  - Citric acid cycle (CAC)
  - Tricarboxylic acid (TCA) cycle
  - Catabolism of acetyl-CoA (CAC)
- TCA cycle is a series of enzyme-catalyzed chemical reactions in which acetyl-CoA is oxidized to produce energy.
- Most of the body's catabolic pathways converge on TCA cycle as it is the final pathway for a complete oxidation of carbohydrates, amino acids, and fatty acids, where their carbon skeletons being converted to carbon dioxide.

# Krebs cycle (cont.)

- TCA cycle is an aerobic pathway that occurs in the matrix of the mitochondria
  - It is in close proximity to the reactions of electron transport, which oxidize the reduced coenzymes produced by the cycle (namely; NADH and FADH2).

**Remember:**

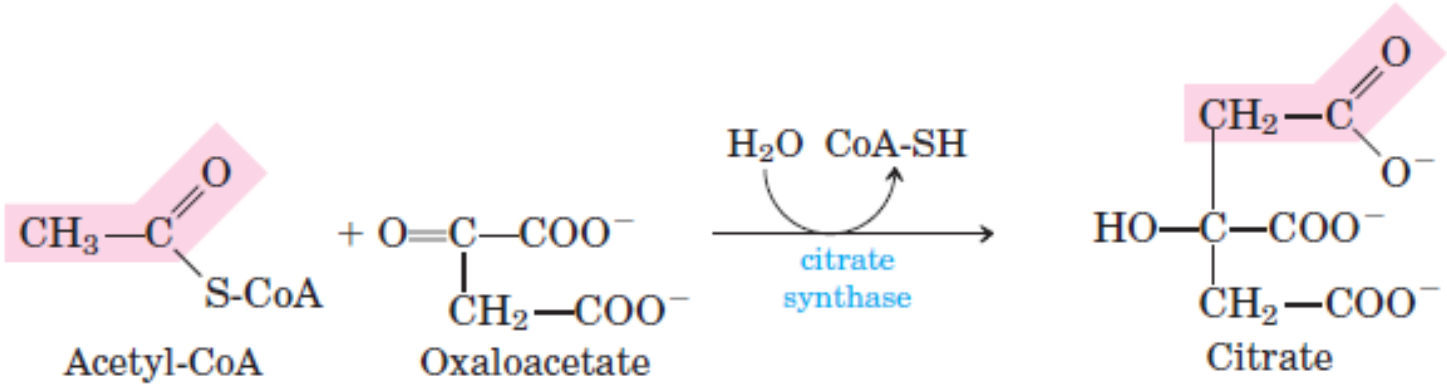
- In the presence of oxygen pyruvate(the end product of aerobic glycolysis) is transported into the mitochondrion.
- Once in the matrix, pyruvate is converted to acetyl-CoA by the pyruvate dehydrogenase complex.

*Note:* The conversion of pyruvate to acetyl-CoA is NOT part of TCA cycle.

# Steps of Krebs cycle

## Step 1: Formation of citrate

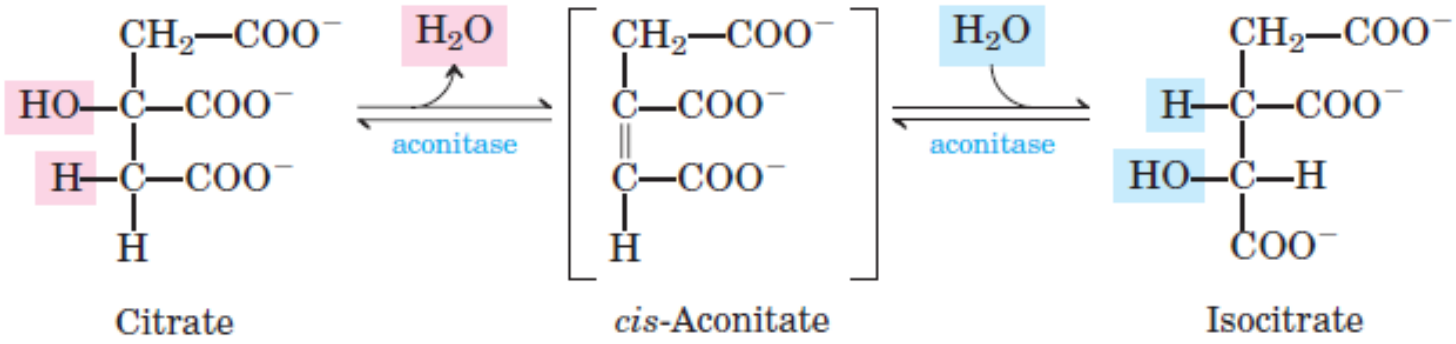
- The condensation of acetyl-CoA and oxaloacetate to form citrate (a tricarboxylic acid) catalyzed by citrate synthase.
- This reaction is inhibited by:
  - ATP
  - NADH
  - Citrate (competitive inhibitor for oxaloacetate)



# Steps of Krebs cycle (cont.)

## Step 2: Isomerization of citrate to isocitrate

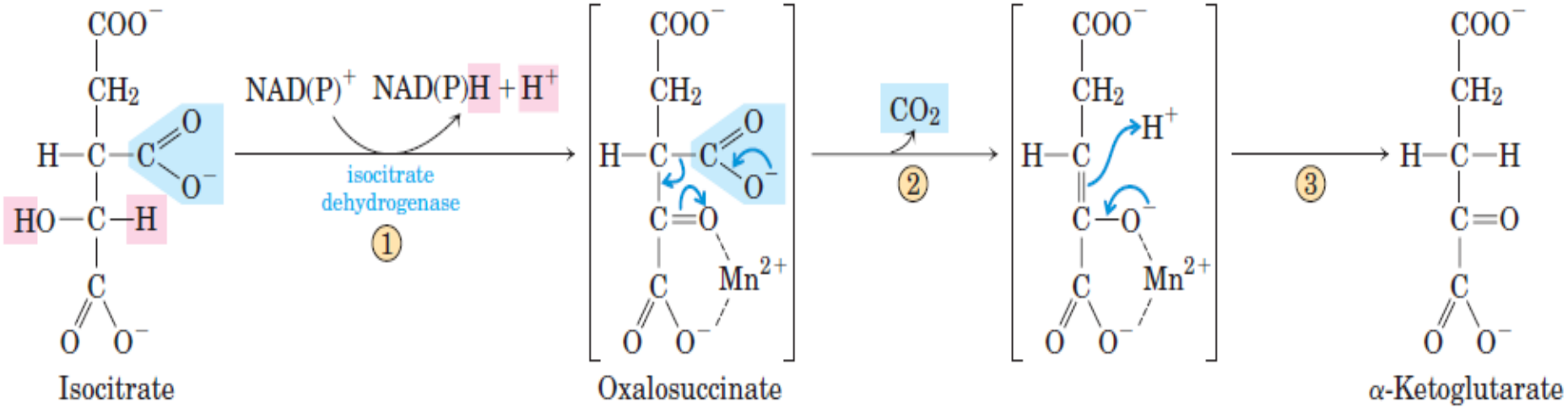
- Aconitase catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of the tricarboxylic acid cis-aconitate.
- Aconitase is inhibited by fluoroacetate.



# Steps of Krebs cycle (cont.)

## Step 3: Oxidation and decarboxylation of isocitrate

- Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate, yielding the first of the three NADH molecules produced by this cycle, and the first release of carbon dioxide.
- This reaction is a rate-limiting step of TCA cycle.



# Steps of Krebs cycle (cont.)

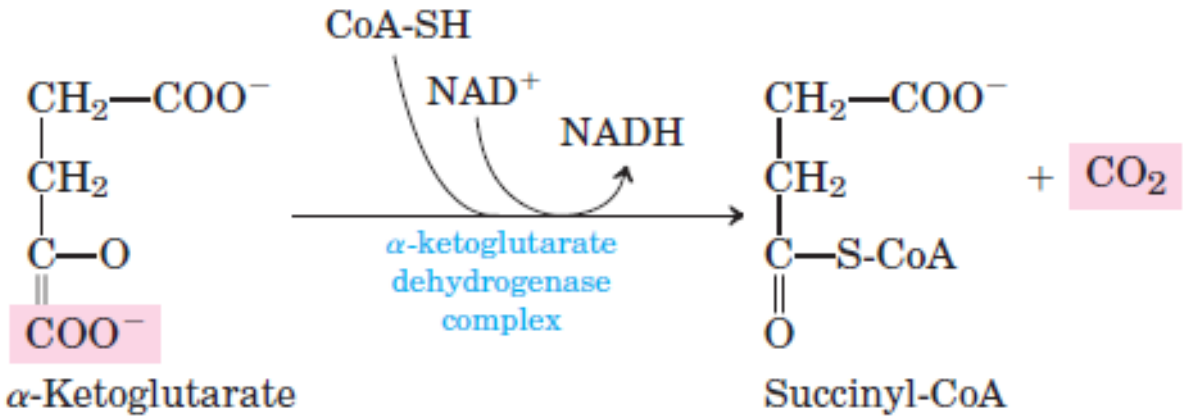
## Step 3: Oxidation and decarboxylation of isocitrate (cont.):

- Regulation of the isocitrate dehydrogenase enzyme:
  - It is allosterically activated by ADP (a low-energy signal) and calcium ions.
  - It is inhibited by ATP and NADH, whose levels are elevated when the cell has abundant energy stores.

# Steps of Krebs cycle (cont.)

## Step 4: Oxidation and decarboxylation α-ketoglutarate

- The conversion of α-ketoglutarate to succinyl-CoA is catalyzed by the α-ketoglutarate dehydrogenase complex.
- The reaction releases the second carbon dioxide and produces the second NADH of the cycle.
- α-Ketoglutarate dehydrogenase complex is inhibited by ATP, NADH, and succinyl-CoA, while calcium ions activate it.

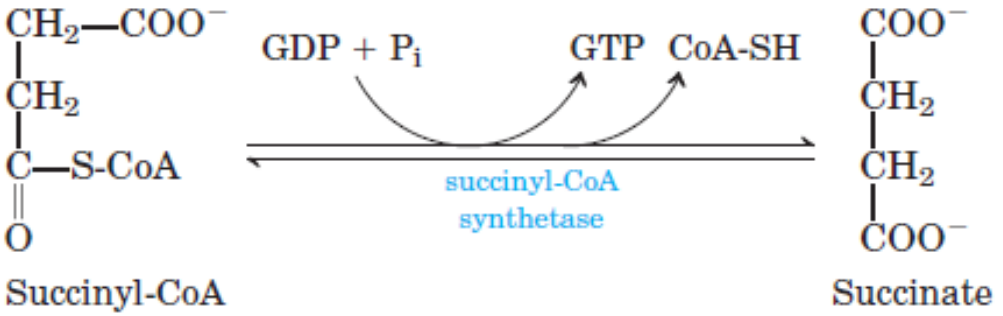


# Steps of Krebs cycle (cont.)

## Step 5: Cleavage of succinyl-CoA

- Succinate thiokinase (also called succinyl-CoA synthetase) cleaves the high-energy thioester bond of succinyl-CoA.
- This reaction is coupled with the substrate-level phosphorylation of GDP to GTP.
- GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction:

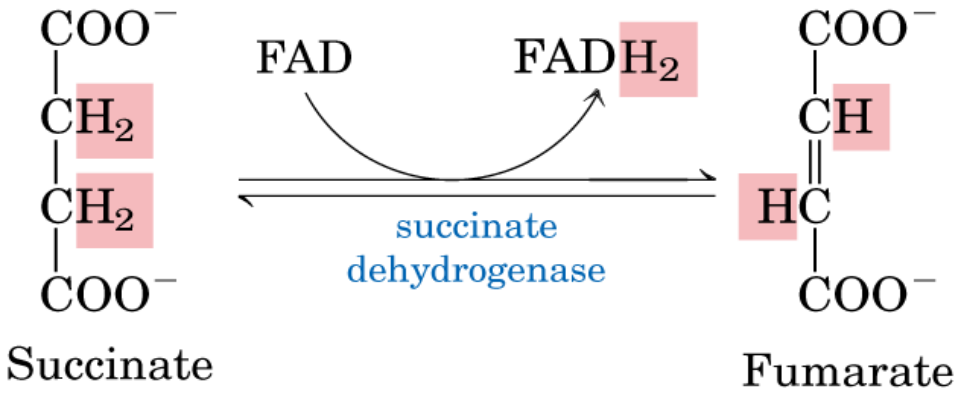
**GTP + ADP ↔ GDP + ATP**



# Steps of Krebs cycle (cont.)

## Step 6: Oxidation of succinate to fumarate

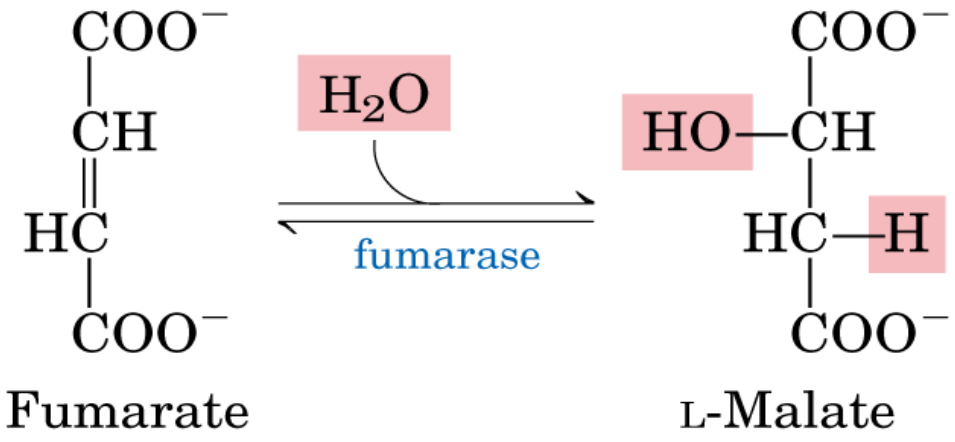
- Succinate is oxidized to fumarate by succinate dehydrogenase, as FAD (its coenzyme) is reduced to FADH2.
- Succinate dehydrogenase is embedded in the inner mitochondrial membrane and is part of both TCA cycle and the electron transport chain.



# Steps of Krebs cycle (cont.)

## Step 7: Hydration of fumarate to malate

- Fumarate is hydrated to malate in a freely reversible reaction catalyzed by fumarase (also called fumarate hydratase).

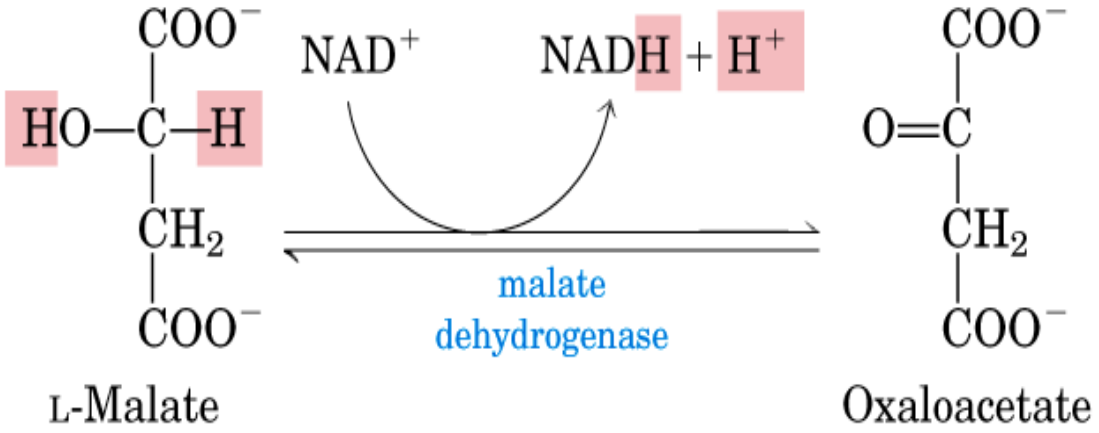



Fumarate L-Malate

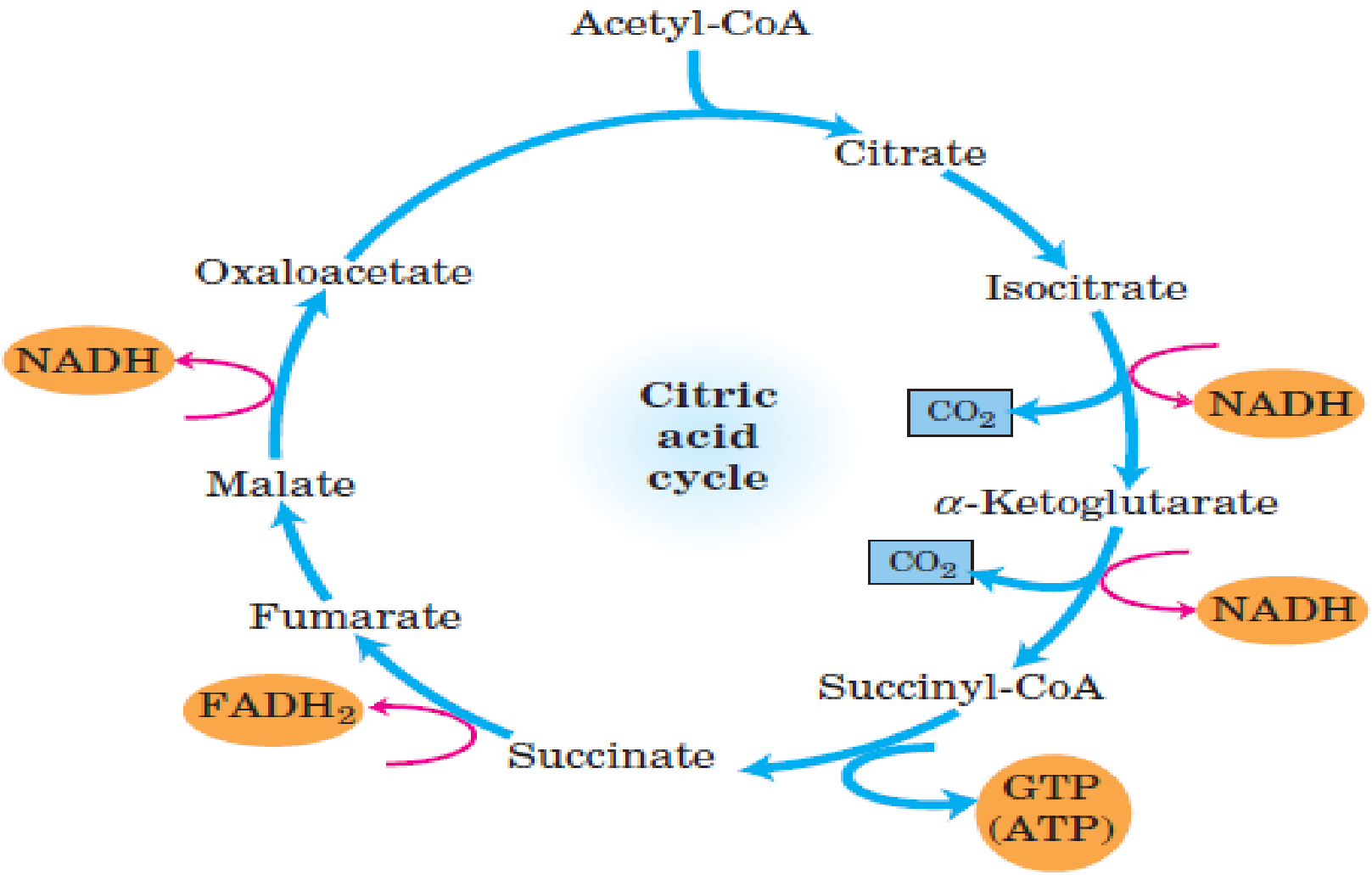
# Steps of Krebs cycle (cont.)

## Step 8: Regeneration of oxaloacetate

- Malate is oxidized to oxaloacetate by malate dehydrogenase.
- This reaction produces the third and final NADH.
- The oxaloacetate produced in this step (final step) can combine with another molecule of acetyl-CoA to start a new turn of TCA cycle.



# Krebs cycle: in summary



# Krebs cycle: energy production

- Two carbon atoms enter TCA cycle as acetyl-CoA and leave as carbon dioxide.

**One cycle of TCA cycle produces:**

- Three molecules of NADH
- One molecules of FADH2
- One molecule of GTP
- Two molecules of carbon dioxide are released in the oxidative decarboxylation reactions.

**Remember:**

- Oxidation of one NADH by the electron transport chain leads to formation of three ATP, whereas oxidation of FADH2 yields two ATP.

# Krebs cycle: energy production (cont.)

**One cycle of TCA cycle produces:**

- Three molecules of NADH
- One molecules of FADH2
- One molecule of GTP
- Two molecules of carbon dioxide are released in the oxidative decarboxylation reactions.

**Remember:**

- One molecule of glucose produces two molecules of acetyl-CoA.

# Krebs cycle: energy production

**Question:** Calculate the total ATP yield from the oxidation of one molecule of acetyl-CoA?

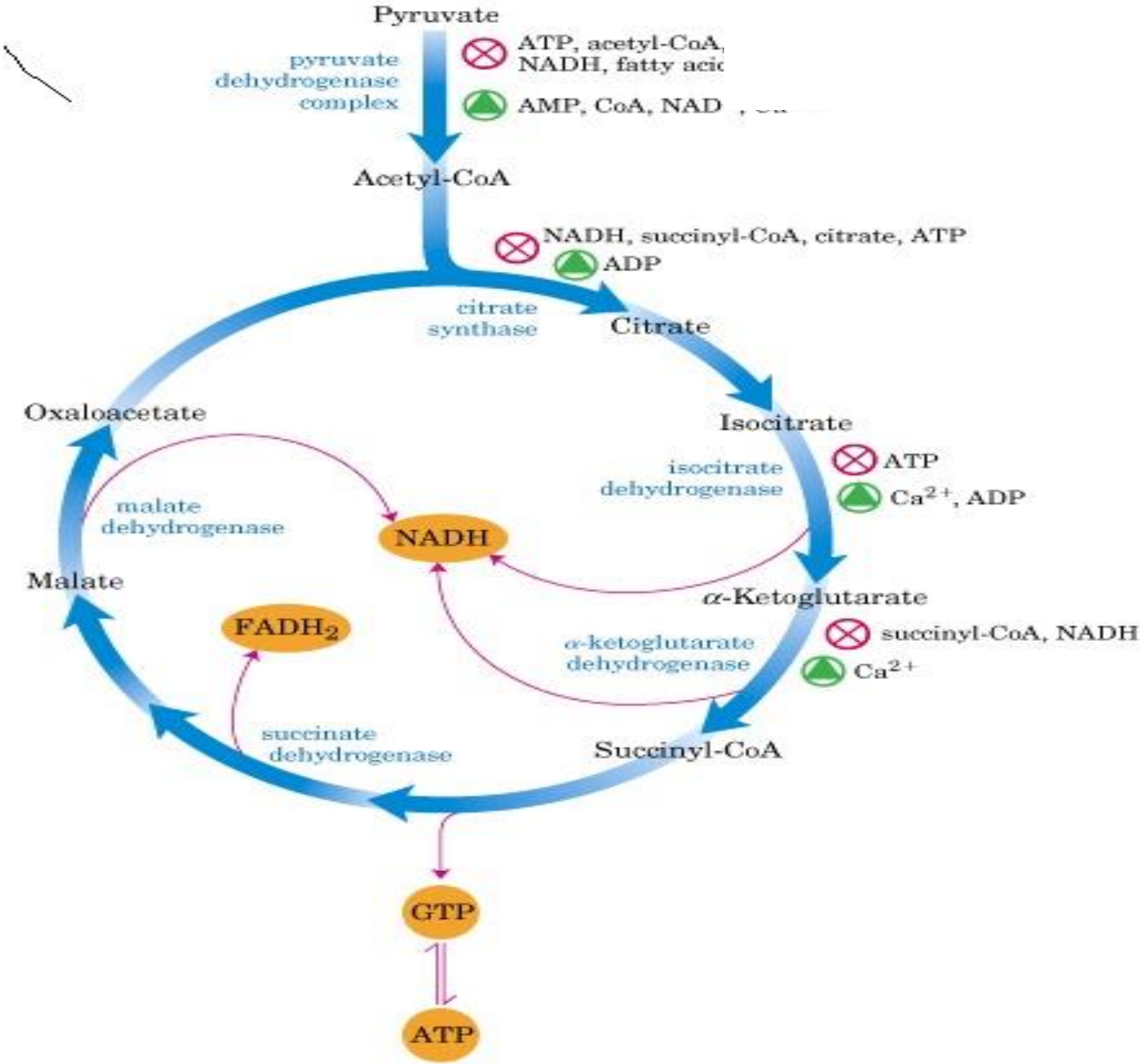
| TCA cycle reaction | Yield of ATP/electron carriers |
|---|---|
| Isocitrate dehydrogenase | +1 NADH |
| α-Ketoglutarate dehydrogenase | +1 NADH |
| Succinate thiokinase | +1 GTP |
| Succinate dehydrogenase | +1 FADH2 |
| Malate dehydrogenase | +1 NADH |
| Total energy production | 12 ATP |

# Regulation of Krebs cycle

- In contrast to glycolysis (regulated primarily by phosphofructokinase), the regulation of TCA cycle occurs through the allosteric control of enzyme activity.

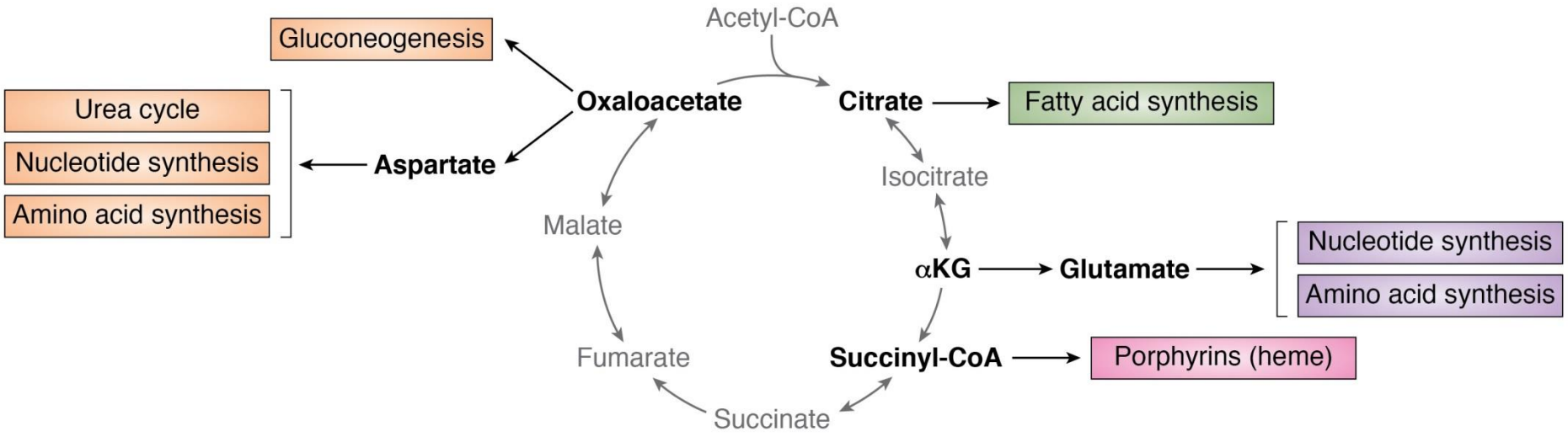
- The most important regulated enzymes are:

- Citrate synthase: inhibited by ATP, NADH, succinyl-CoA, and citrate-feedback inhibition.
- Isocitrate dehydrogenase: inhibited by high levels of ATP and NADH.
- α-Ketoglutarate dehydrogenase: inhibited by high levels of ATP, NADH, and succinyl-CoA-feedback inhibition.


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

# Krebs cycle: amphibolic pathway

- **TCA cycle is an amphibolic pathway because it serves as:**
  - Catabolic pathway: generating energy and reducing agents.
  - Anabolic pathway: generating metabolic intermediates for biosynthesis.
- This pathway allows the cell to coordinate energy production with the synthesis of essential biomolecules.



# The Glyoxylate Cycle

# The glyoxylate cycle

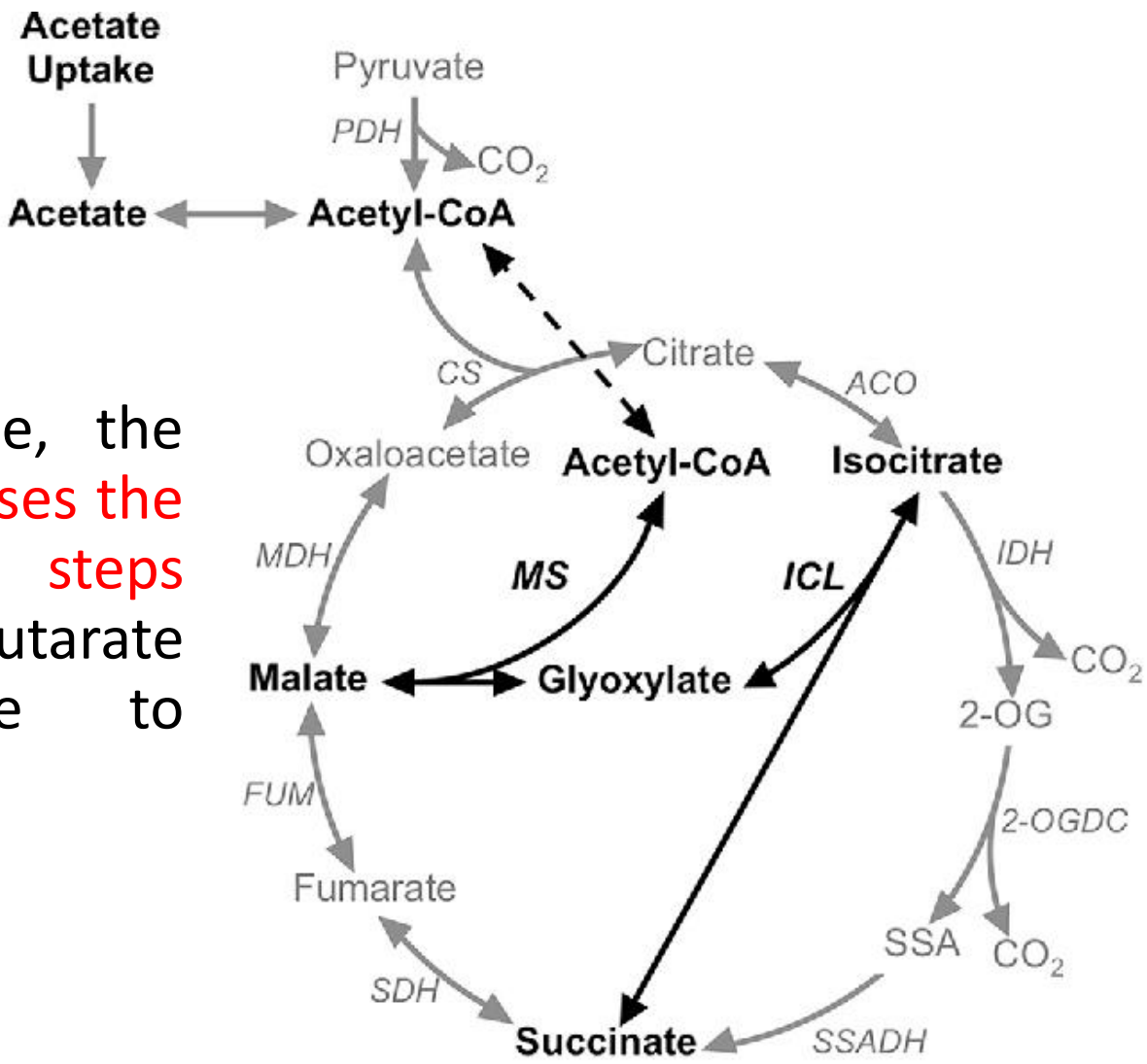
- The glyoxylate cycle is a metabolic pathway that operates in certain microorganisms, plants, and some fungi.
- It is **an anaplerotic pathway**, meaning that it replenishes intermediates that are taken out of the TCA cycle for biosynthetic purposes.
- This pathway **allows the net synthesis of carbohydrates from acetyl-CoA** (derived from β-oxidation of fatty acids).

**Note:**

- The glyoxylate cycle occurs in the peroxisome and cytoplasm, while the TCA cycle only occurs in the mitochondria.

# The glyoxylate cycle (cont.)

Unlike the TCA cycle, the glyoxylate cycle bypasses the two decarboxylation steps (isocitrate to α-ketoglutarate and α-ketoglutarate to succinate).



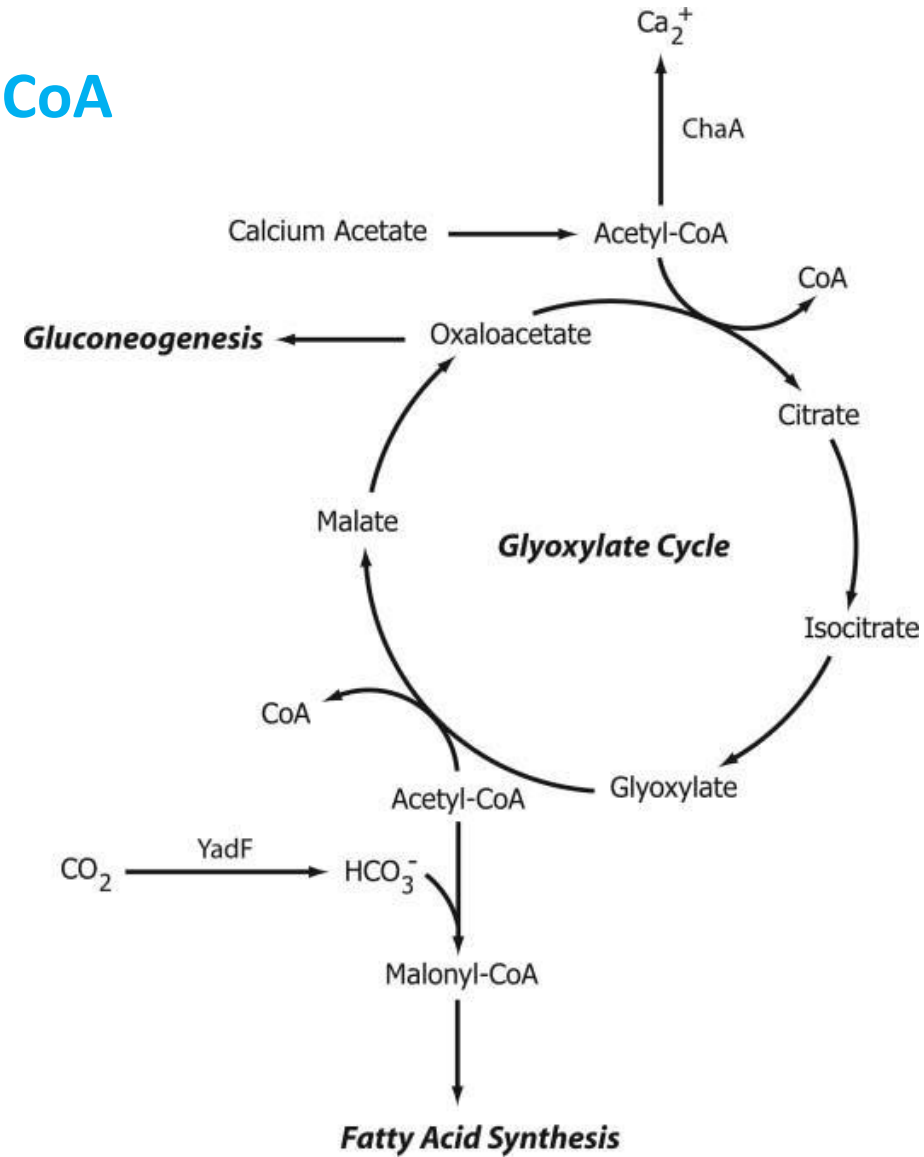
# Steps of the glyoxylate cycle

## Step 1: Condensation of Acetyl-CoA

- The cycle begins with the condensation of one molecule of acetyl-CoA with oxaloacetate to form citrate. This reaction is catalyzed by citrate synthase.

## Step 2: Formation of isocitrate

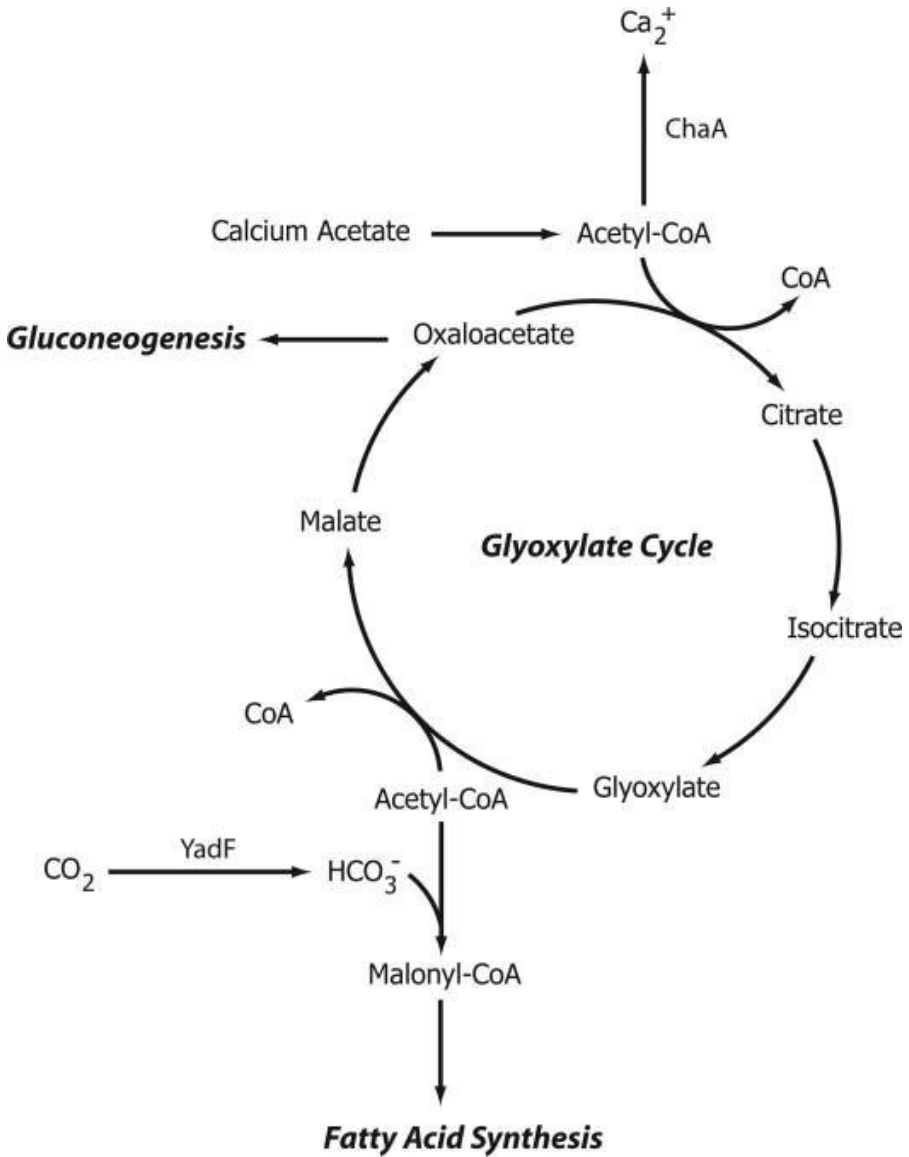
- Citrate is isomerized to isocitrate in a reaction catalyzed by aconitase.



# Steps of the glyoxylate cycle (cont.)

## Step 3: Cleavage of isocitrate

- Isocitrate is cleaved by the enzyme isocitrate lyase into glyoxylate and succinate.
- This step is unique to the glyoxylate cycle and allows the preservation of the acetyl-CoA carbons.

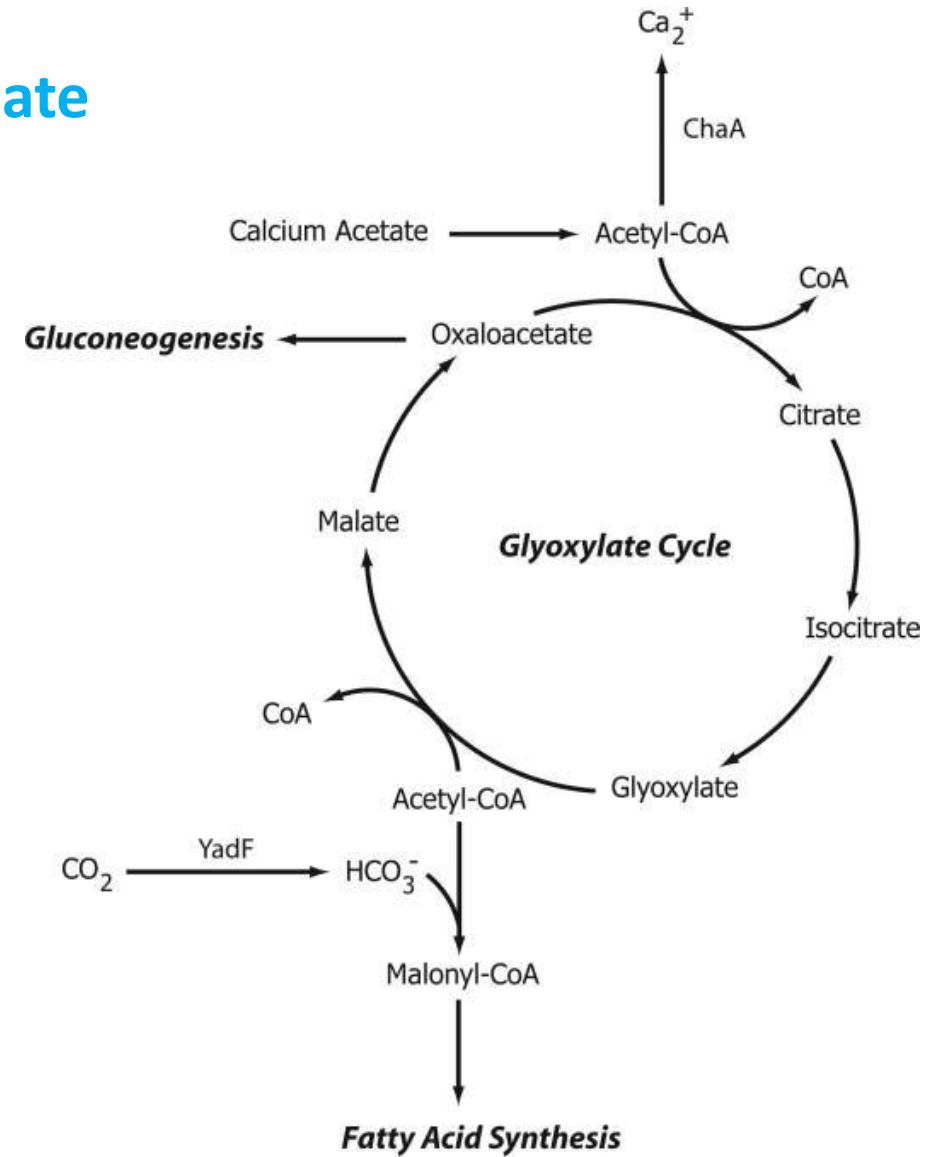


# Steps of the glyoxylate cycle (cont.)

## Step 4: Condensation of glyoxylate

- Glyoxylate reacts with another molecule of acetyl-CoA, forming malate. This reaction is catalyzed by malate synthase.

- This step is unique to the glyoxylate cycle.



# Steps of the glyoxylate cycle (cont.)

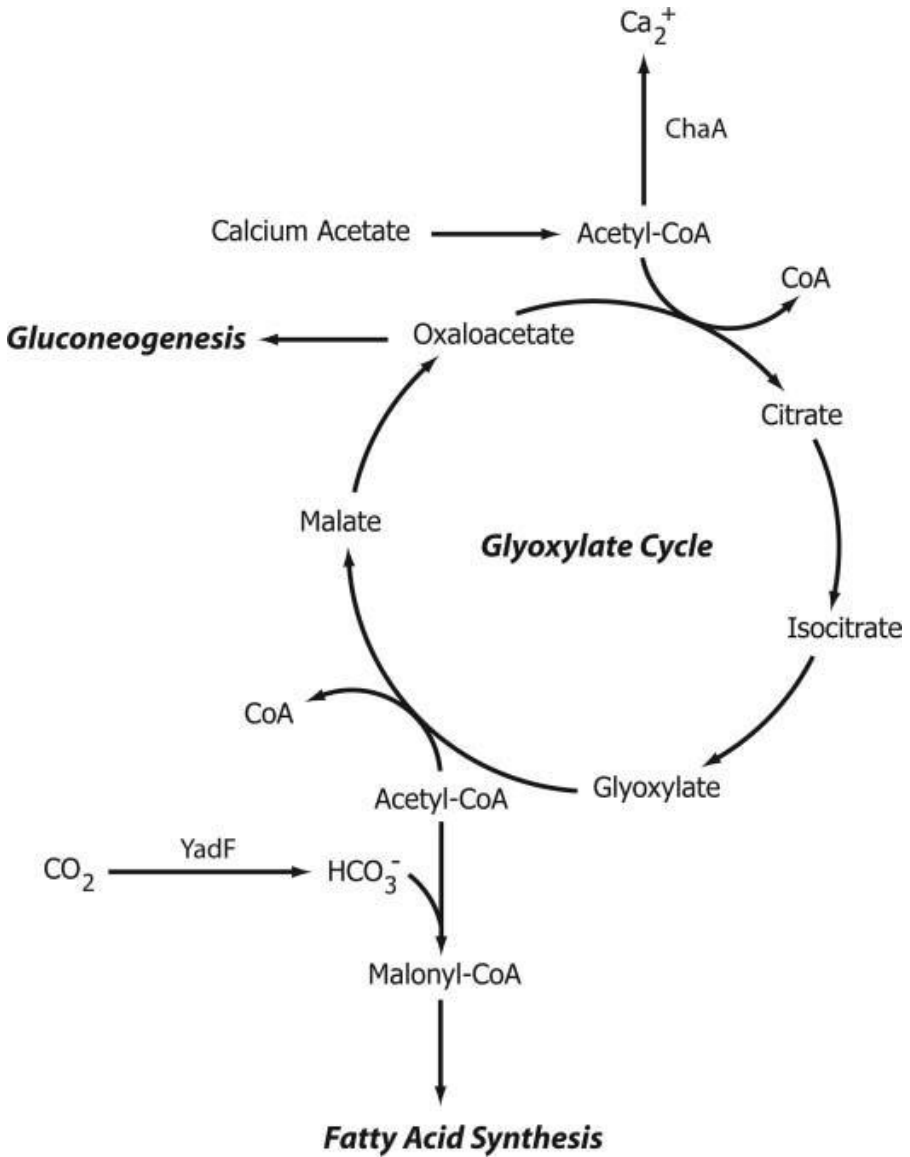
## Step 5: Oxidation of malate

- Malate is oxidized to oxaloacetate by malate dehydrogenase.

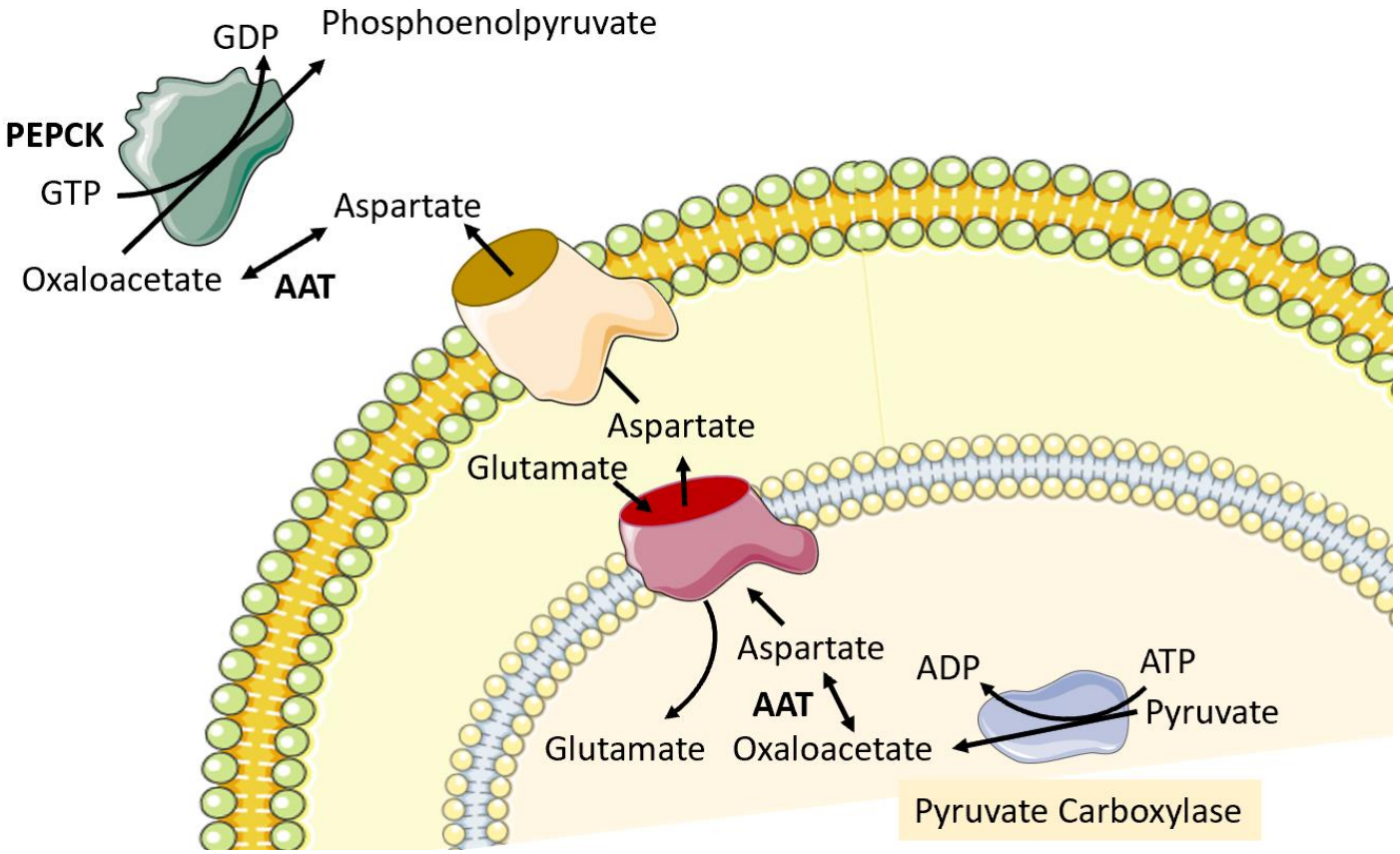
**Fates of oxaloacetate produced in this step:**

- Condenses with another molecule of acetyl-CoA to start a new turn of glyoxylate cycle.

- Converts to aspartate (by AST) and exits to the cytosol where it can be converted back to oxaloacetate (a precursor of glucose vis gluconeogenesis).



AST/AAT = aspartate transaminase



# Summary

- Glycolysis is a central metabolic pathway occurring in the cytoplasm that breaks down one molecule of glucose into two molecules of pyruvate, producing ATP and NADH. It serves as a universal energy-yielding process in cells, initiating both aerobic and anaerobic respiration.
- The TCA cycle (located in the mitochondria) completes the oxidative breakdown of acetyl-CoA generated from glucose, fatty acids, and amino acids. It produces NADH, FADH2, GTP, and carbon dioxide, feeding electrons into the electron transport chain for ATP synthesis.
- The glyoxylate cycle (unique to certain microorganisms and plants) enables the net synthesis of carbohydrates from acetyl-CoA by bypassing decarboxylation steps.