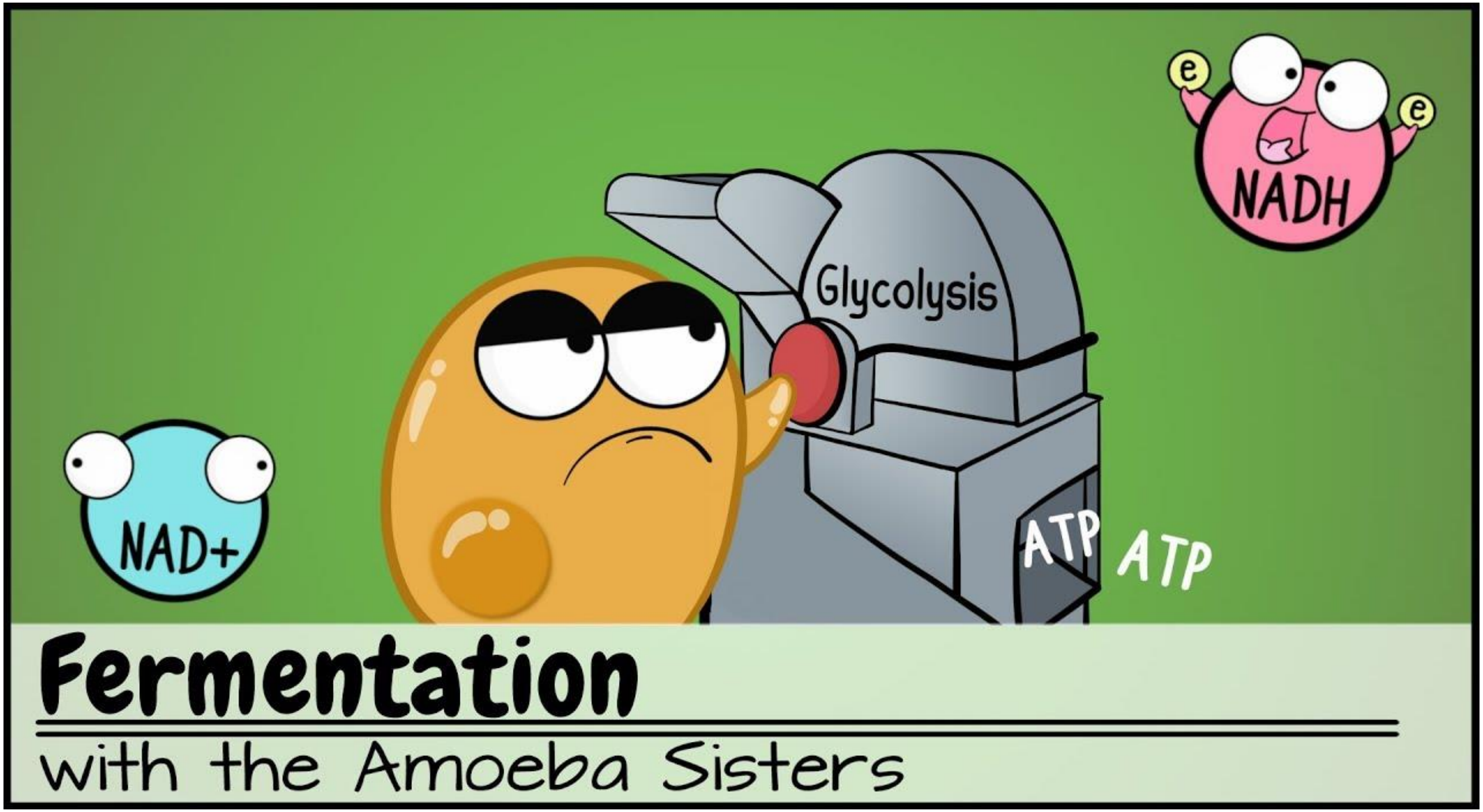
GLYCOLYSIS


NADH
Glycolysis
NAD+
ATP
ATP
Fermentation
with the Amoeba Sisters

Glycolysis

Glycolysis (from glycose, an older term for glucose + -lysis degradation) is the metabolic pathway that converts glucose $C_6H_{12}O_6$, into **pyruvate, CH_3COCOO^-, and a hydrogen ion, H^+.**

The free energy released in this process is used to form ATP molecules and NADH (reduced nicotinamide adenine dinucleotide).

Glycolysis is a sequence of **ten** enzyme-catalyzed reactions. The intermediates of these reactions may also be directly useful…… For example, the intermediate dihydroxyacetone phosphate (DHAP) is a source of the glycerol that combines with fatty acids to form fat.

Glycolysis

- Breaking down glucose“:**glyco – lysis**” **(splitting sugar)**

glucose → → → → → pyruvate

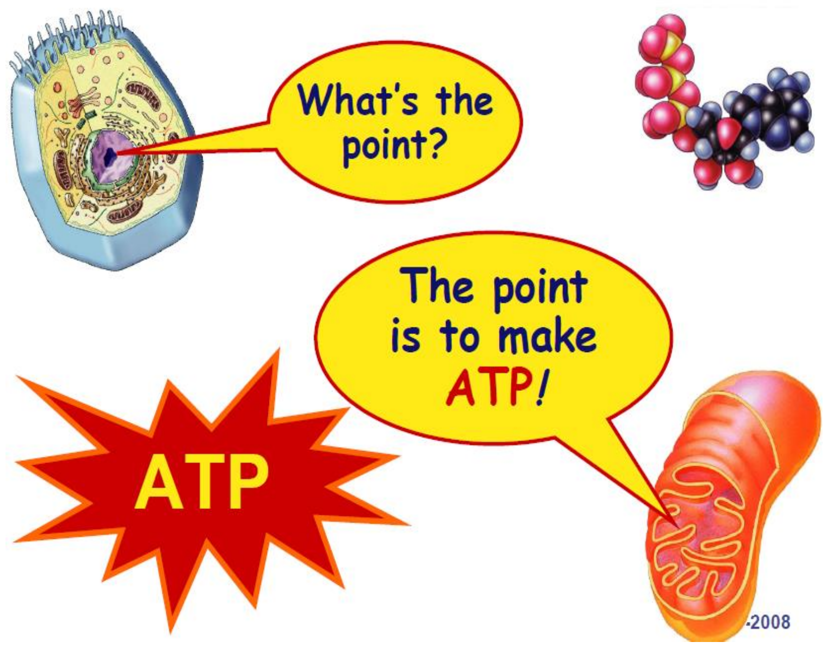
6C **2x 3C**

- **transfer energy from organic molecules to ATP**
- **still is starting point for ALL cellular respiration**

but it’s still *inefficient*

- **generates only 2 ATP for every 1 glucose**

◆ **occurs in cytosol**



It functions either

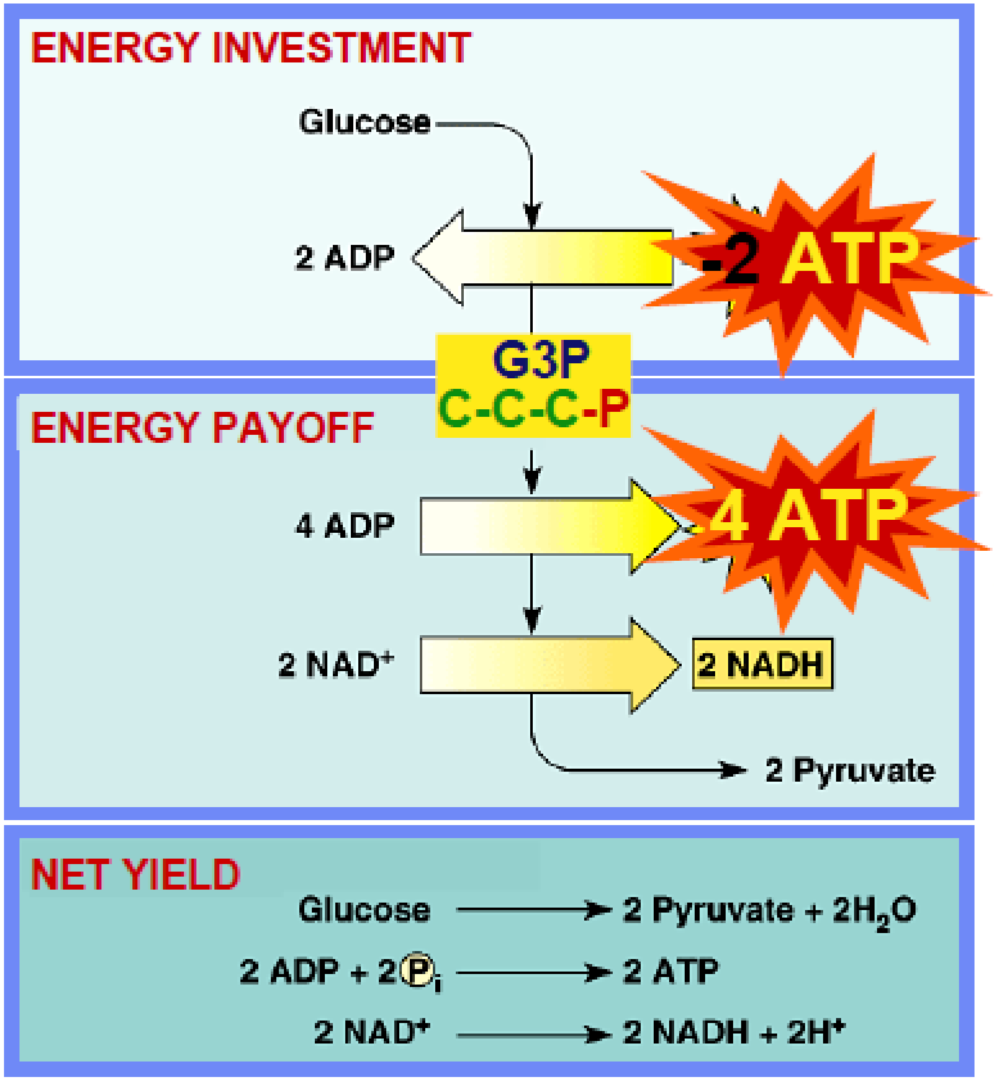
aerobically or anaerobically

Overview of Glycolysis

Glycolysis consists of two phases:

- In the first, a series of five reactions, glucose is broken down to two molecules of **glyceraldehyde-3phosphate.**
- In the second phase, five subsequent reactions convert these two molecules of glyceraldehyde-3phosphate into two molecules of **pyruvate**.
- Phase 1 consumes *two molecules of ATP*.
- The later stages of glycolysis result in the production of *four molecules of ATP*.
- The net is 4 – 2 = 2 molecules of ATP produced per molecule of glucose.

Glycolysis summary



endergonic

invest some ATP

exergonic

harvest a little ATP & a little NADH

net yield

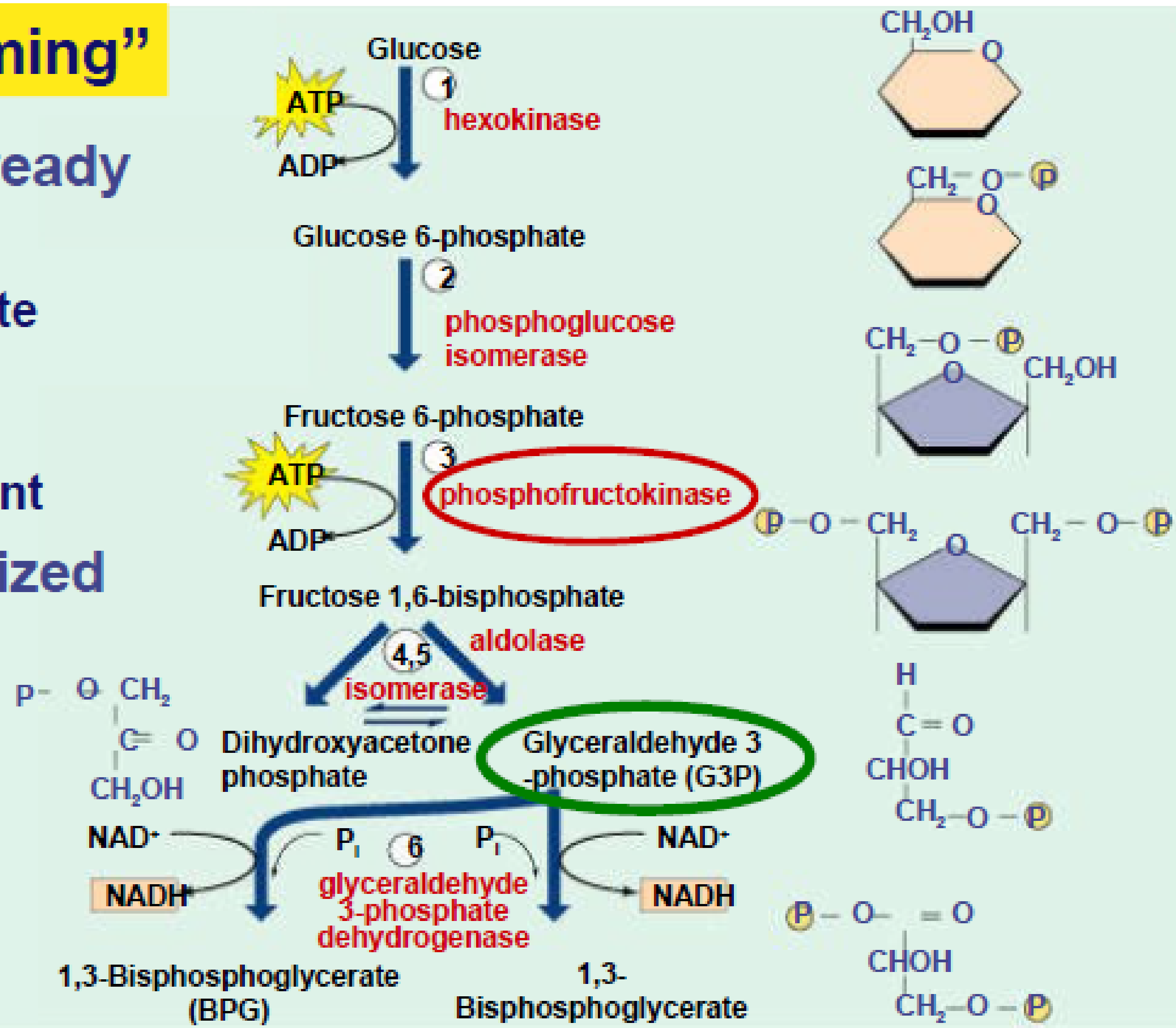
✓2 ATP

✓2 NADH

1st half of glycolysis (5 reactions)

Glucose "priming"

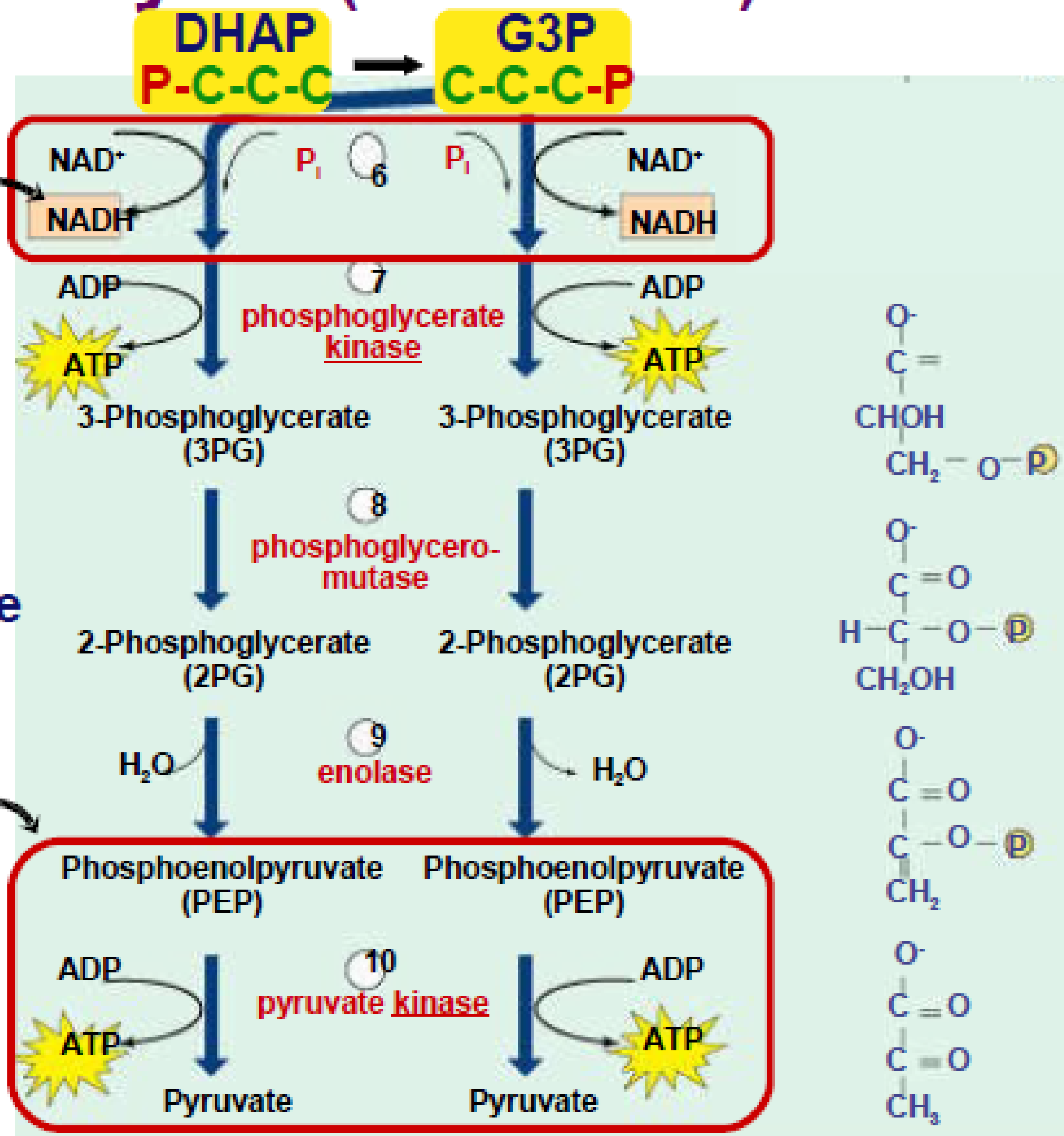
- get glucose ready to split
  - phosphorylate glucose
  - molecular rearrangement
- split destabilized glucose



2nd half of glycolysis (5 reactions)

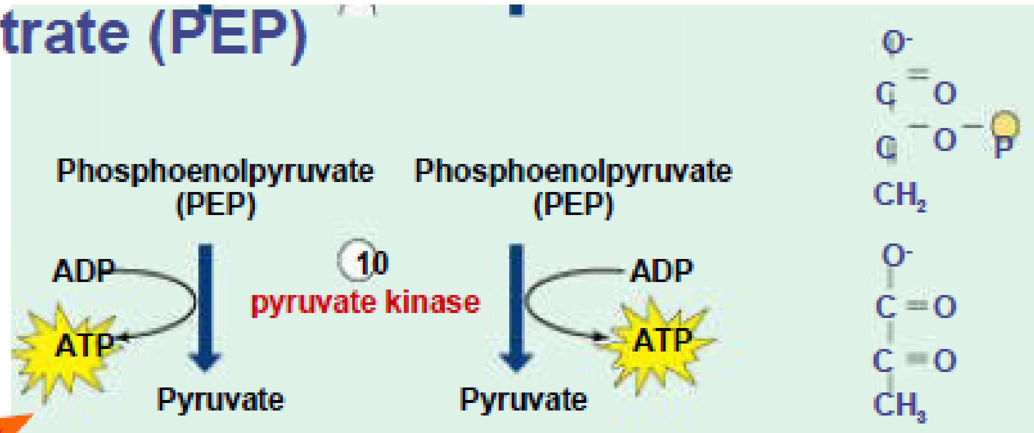
Energy Harvest

- NADH production
  - G3P donates H
  - oxidizes the sugar
  - reduces NAD^+
  - $NAD^+ \rightarrow$ NADH
- ATP production
  - G3P $\rightarrow \rightarrow \rightarrow$ pyruvate
  - PEP sugar donates P
    - "substrate level phosphorylation"
  - ADP $\rightarrow$ ATP

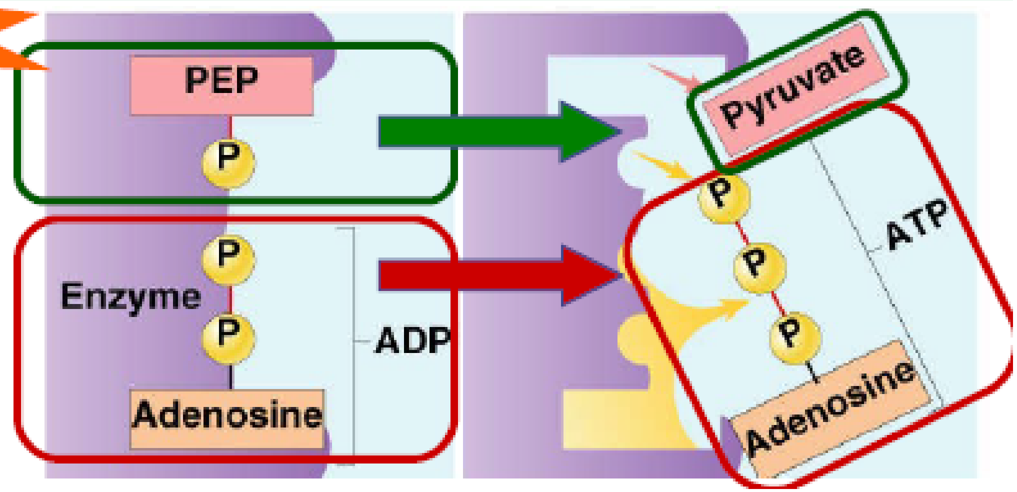


Substrate-level Phosphorylation

- In the last steps of glycolysis, where did the P come from to make ATP?
  - the sugar substrate (PEP)



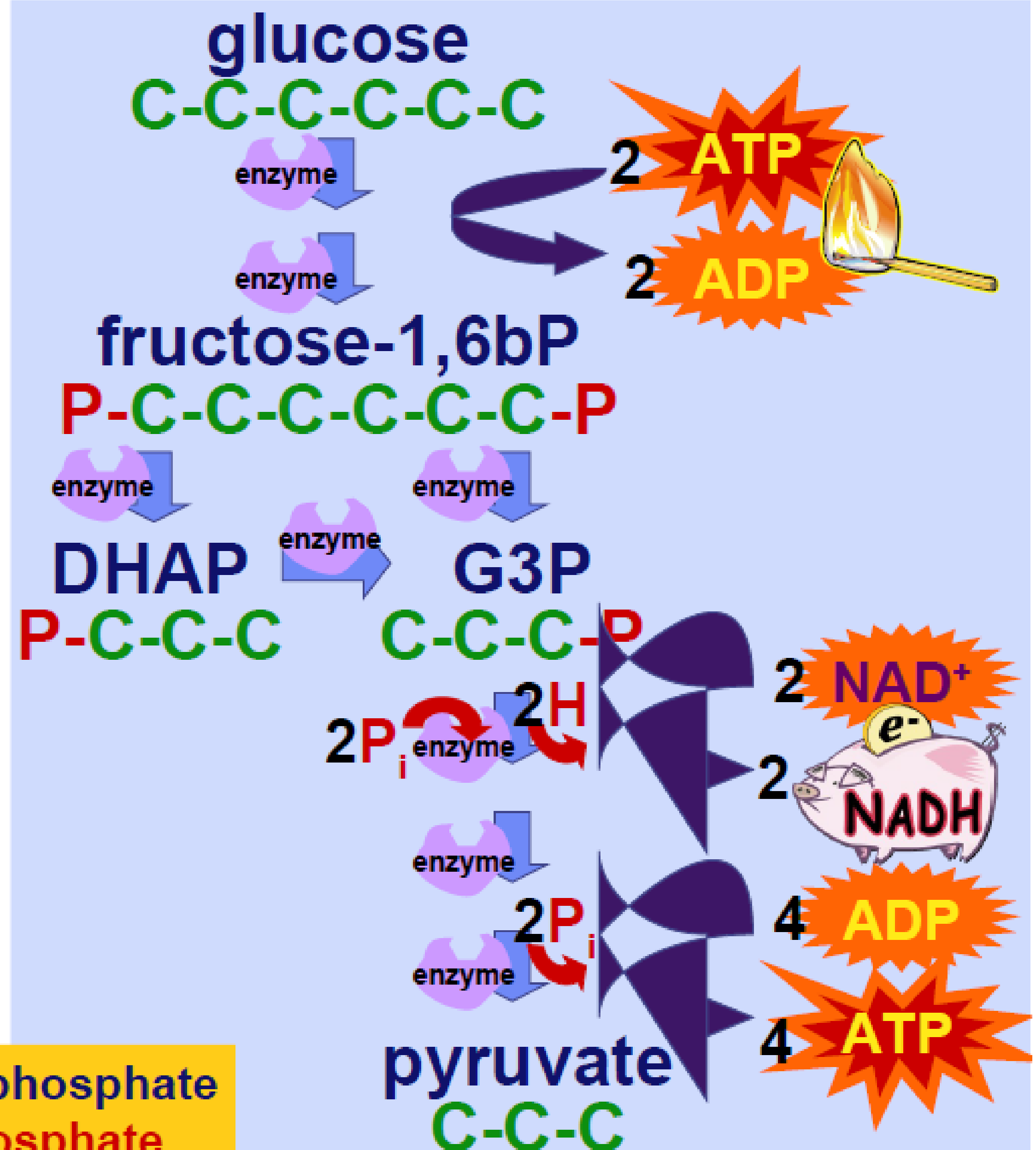
P is transferred from PEP to ADP
- ✓kinase enzyme
- ✓ADP → ATP



Overview

10 reactions

- convert
  glucose (6C) to
  2 pyruvate (3C)
- produces:
  4 ATP & 2 NADH
- consumes:
  2 ATP
- net yield:
  2 ATP & 2 NADH



DHAP = dihydroxyacetone phosphate
G3P = glyceraldehyde-3-phosphate

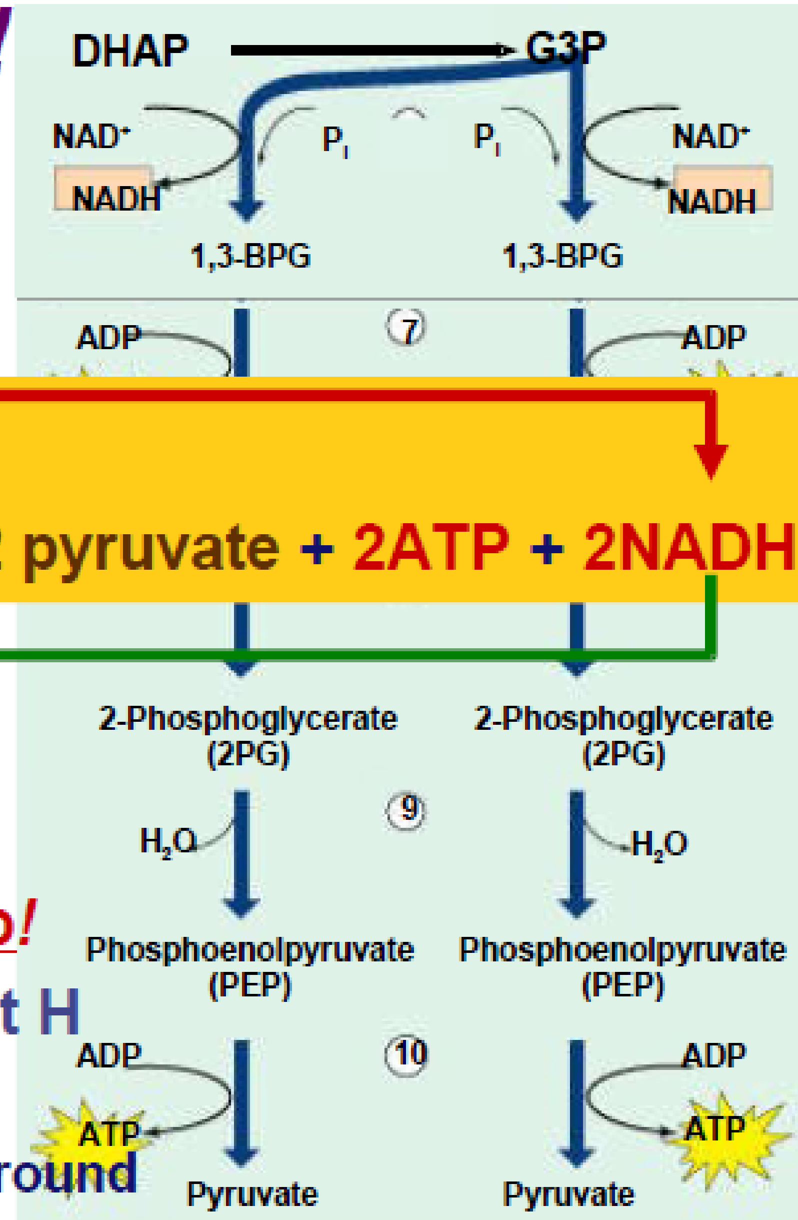
But can't stop there!

raw materials → products

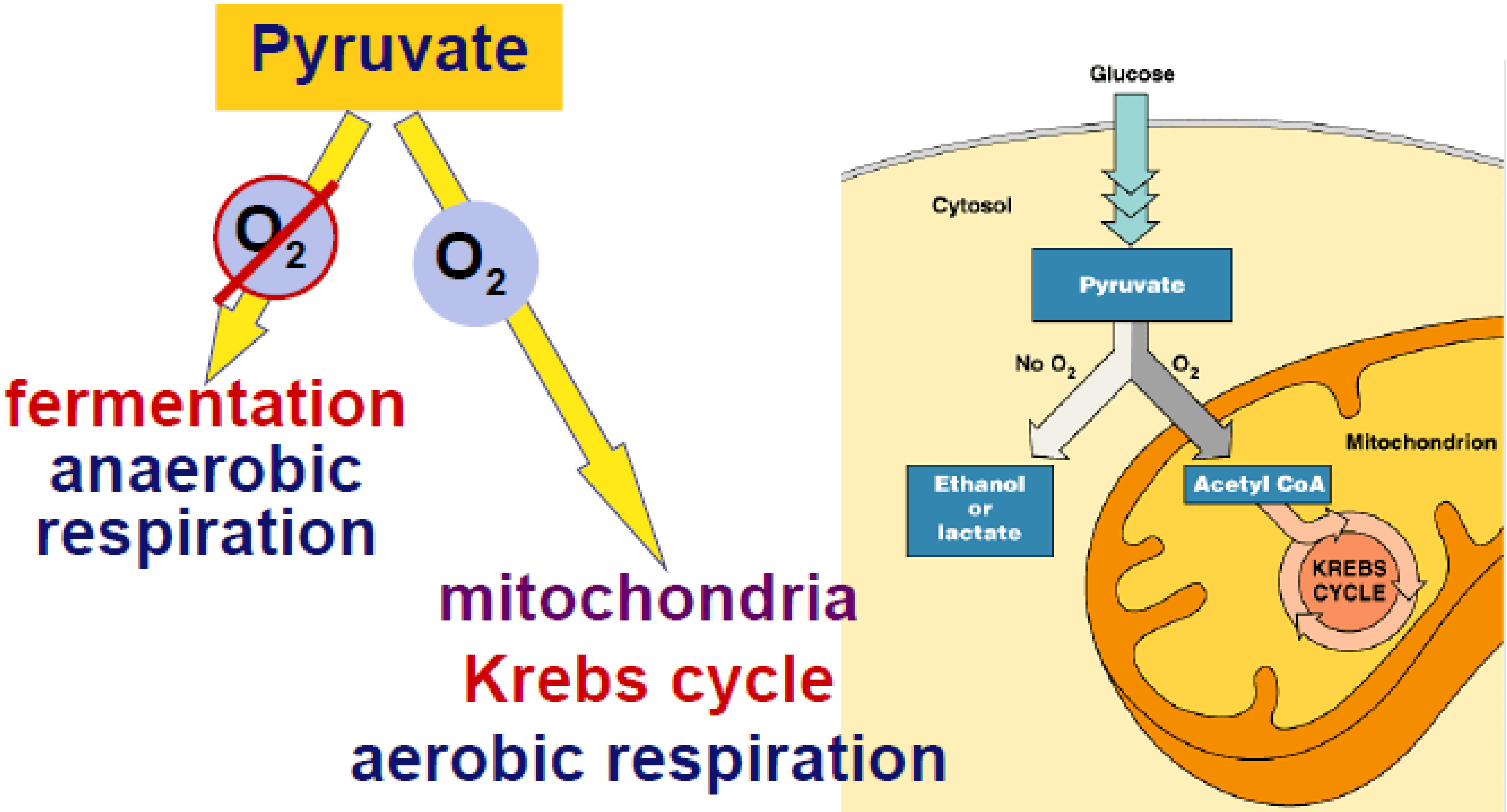
Glycolysis

glucose + 2ADP + 2P_i + 2 NAD^+ → 2 pyruvate + 2ATP + 2NADH

- Going to run out of NAD^+
  - without regenerating NAD^+, energy production would stop!
  - another molecule must accept H from NADH
    - so NAD^+ is freed up for another round

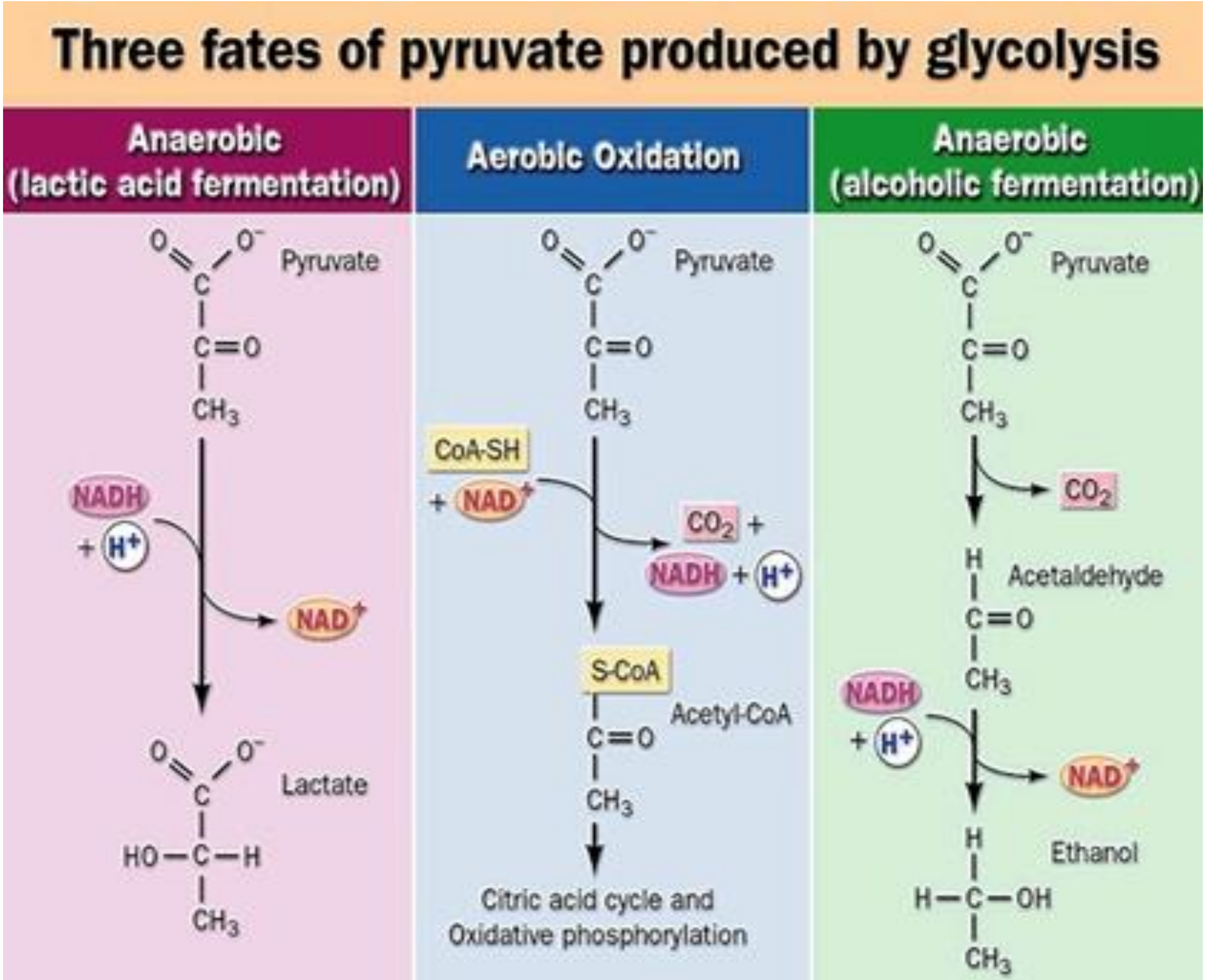


Pyruvate is a branching point



Pathways for Pyruvate

- The pyruvate produced from glucose during glycolysis can be further metabolized in three possible ways
- For **aerobic** organisms, when oxygen is plentiful the pyruvate is converted to acetyl coenzyme A (acetyl CoA)
- For aerobic organisms, when oxygen is scarce, and for some **anaerobic** organisms, the pyruvate is reduced to lactate
- For some anaerobic organisms (like yeast), the pyruvate is fermented to ethanol



Three fates of pyruvate produced by glycolysis
Anaerobic
(lactic acid fermentation)
Aerobic Oxidation
Anaerobic
(alcoholic fermentation)
Pyruvate
NADH
+ H+
NAD+
Lactate
Pyruvate
CoA-SH
+ NAD+
CO2 +
NADH + H+
S-CoA
Acetyl-CoA
Citric acid cycle and
Oxidative phosphorylation
Pyruvate
CO2
Acetaldehyde
NADH
+ H+
NAD+
Ethanol

Fermentation (anaerobic)

- Bacteria, yeast

pyruvate → ethanol + CO_2

3C   2C   1C

NADH → NAD^+

back to glycolysis→

  - beer, wine, bread

- Animals, some fungi

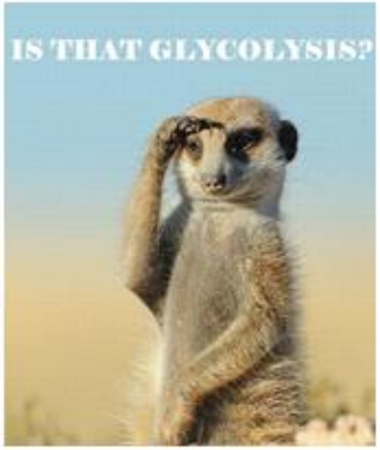
pyruvate → lactic acid

3C   3C

NADH → NAD^+

back to glycolysis→→

  - cheese, anaerobic exercise (no O_2)



Alcohol Fermentation

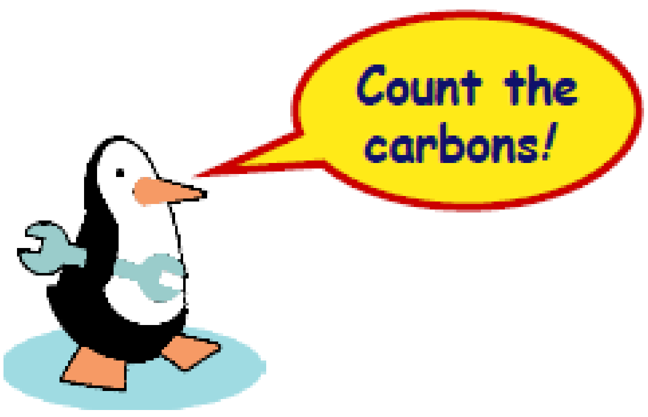
pyruvate → ethanol + CO_2

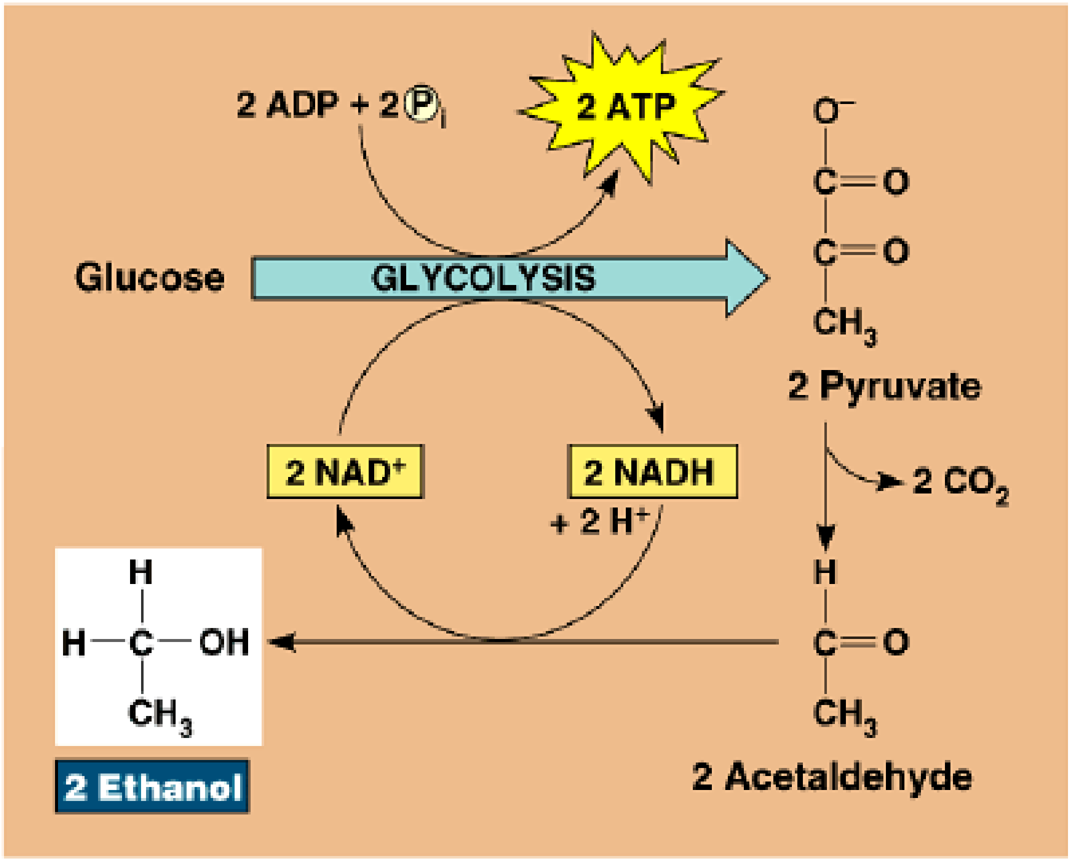
3C → 2C + 1C

NADH → NAD^+ back to glycolysis→→

bacteria
yeast

- Dead end process
  - at ~12% ethanol, kills yeast
  - can't reverse the reaction





Lactic Acid Fermentation

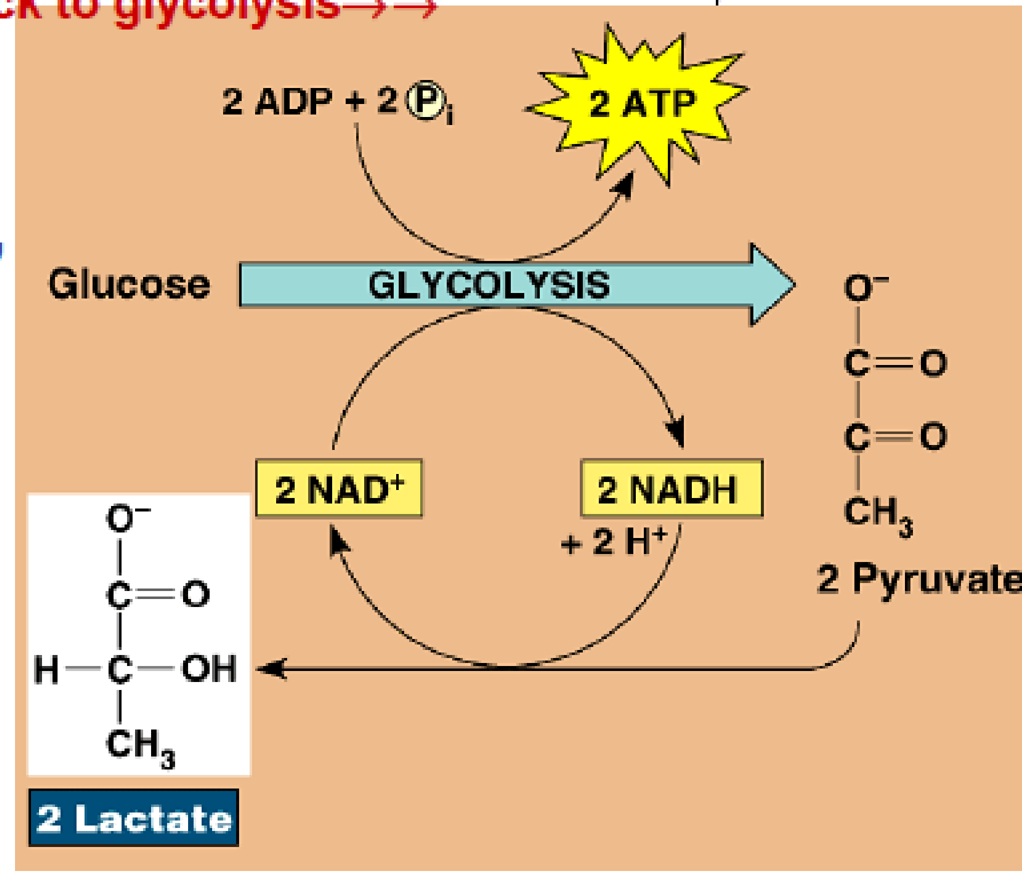
animals
some fungi

pyruvate ↔ lactic acid

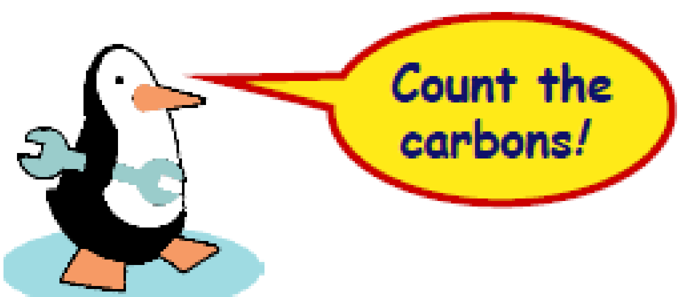
3C 3C

NADH NAD⁺ back to glycolysis→→

O_2

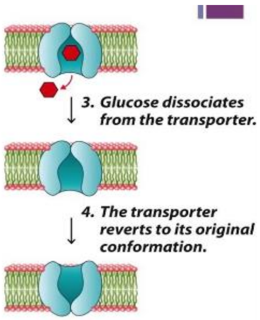


- Reversible process
  - once O_2 is available, lactate is converted back to pyruvate by the liver



Glucose Transporters

Glucose cannot diffuse directly into cells but enters by a family of 14 glucose transporters found in cell membranes (GLUTs).



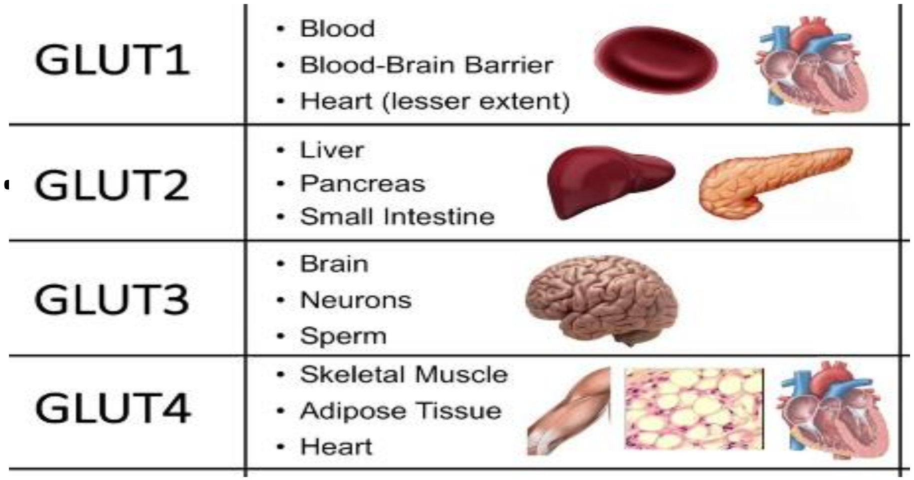
The GLUTs display a tissue-specific pattern:

GLUT-3 is the primary glucose Tr in **neurons**.

GLUT-1 is abundant in erythrocytes

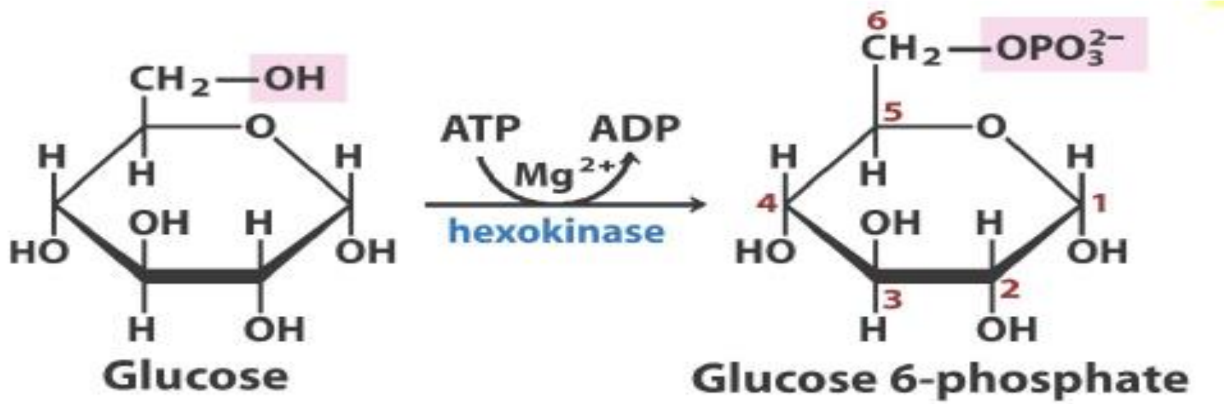
GLUT-4 is abundant in muscle and adipose tissue.

GLUT-2 is abundant in liver, kidney and β cells



GLUT1	• Blood • Blood-Brain Barrier • Heart (lesser extent)
GLUT2	• Liver • Pancreas • Small Intestine
GLUT3	• Brain • Neurons • Sperm
GLUT4	• Skeletal Muscle • Adipose Tissue • Heart

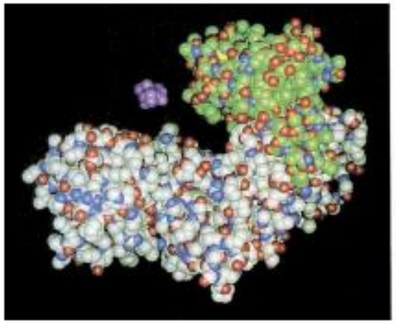
Reaction 1: Glucose Phosphorylation

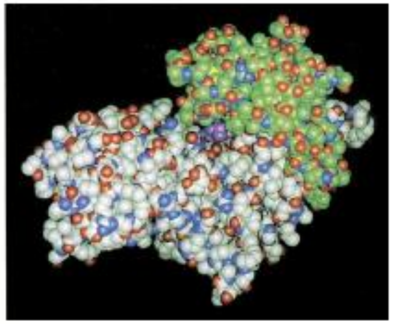


R1:Phosphorylation of Glucose by Hexokinase or Glucokinase using ATP as the phosphate donor

Phosphorylation keeps the substrate in the cell. Glucose is a neutral molecule and could diffuse across the cell membrane, but phosphorylation confers a negative charge on glucose, and the plasma membrane is essentially impermeable to glucose-6-phosphate

Thus,



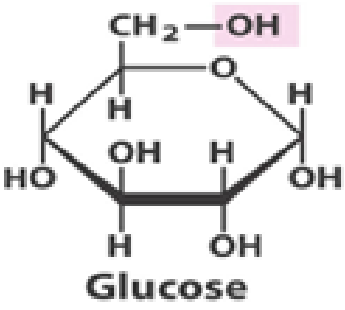


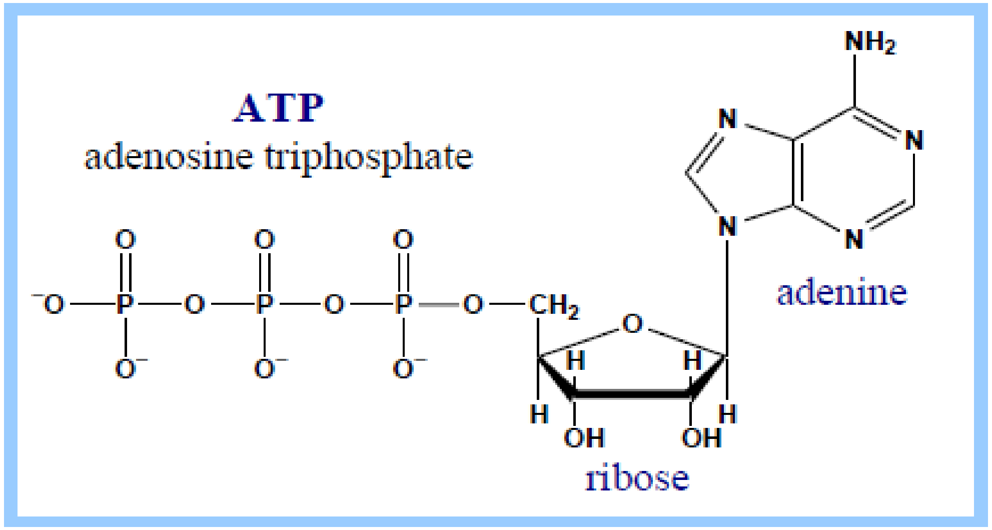
Hexokinase catalyzes:

Glucose + ATP → glucose-6-P + ADP

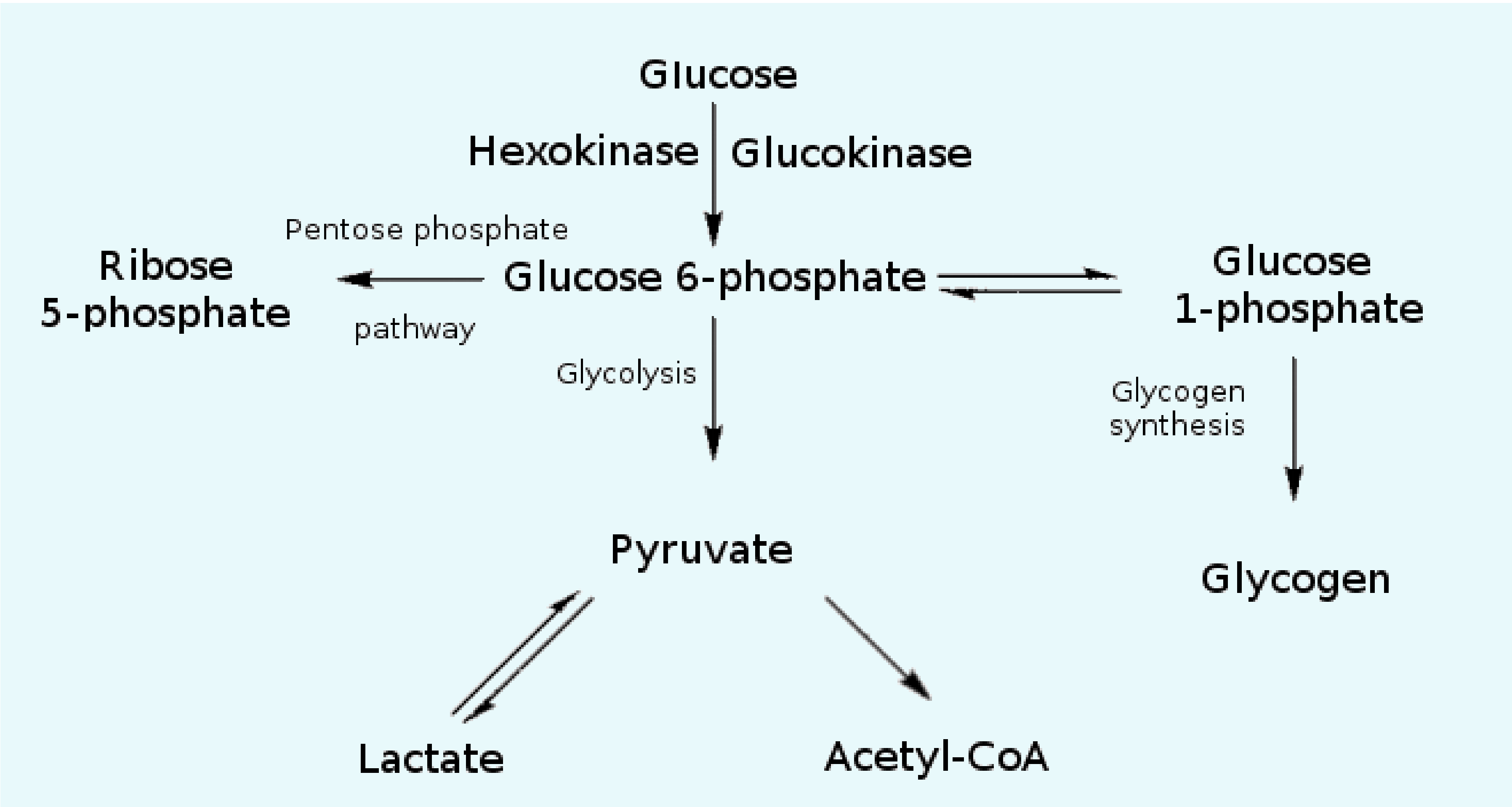
The reaction involves nucleophilic attack of the C6 hydroxyl O of glucose on P of the terminal phosphate of ATP.

ATP binds to the enzyme as a complex with **Mg**$^{++}$.



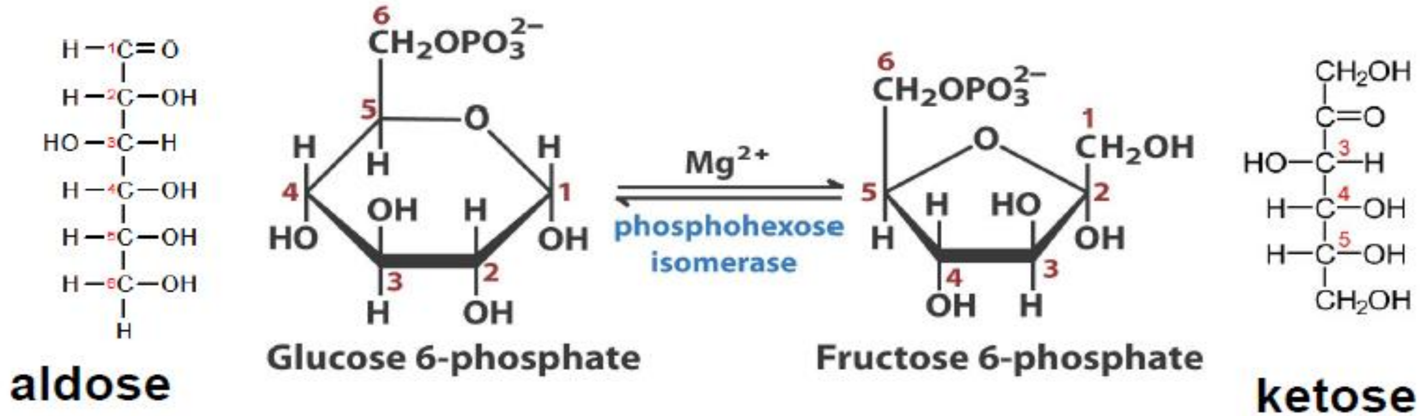


Don’t forget:



Glucose
Hexokinase
Glucokinase
Pentose phosphate
pathway
Ribose
5-phosphate
Glucose 6-phosphate
Glucose
1-phosphate
Glycolysis
Glycogen
synthesis
Pyruvate
Glycogen
Lactate
Acetyl-CoA

Reaction 2: Isomerization

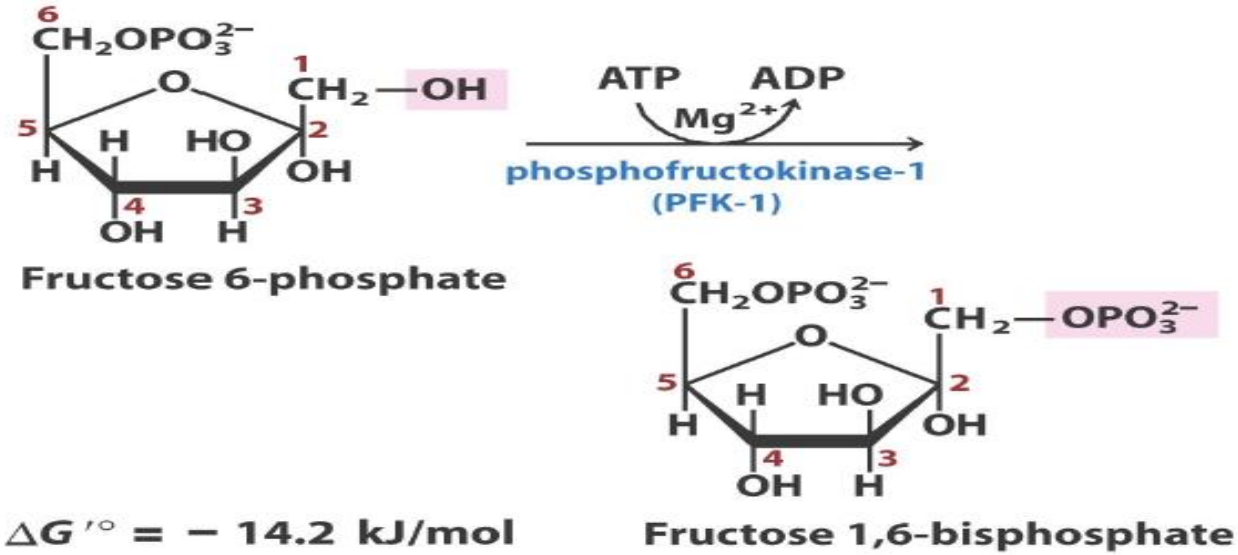


R 2: Isomerization of Glucose-6-Phosphate to Fructose-6-Phosphate catalyzed by Phospho- glucose isomerase

- This is an isomerization of an aldose (glucose-6-phosphate) to a ketose—fructose-6-phosphate.

This reaction is freely reversible under normal cell conditions. However, it is often driven forward because of low F6P concentration. Under conditions of high F6P concentration, this reaction readily runs in reverse.

Reaction 3: Phosphorylation



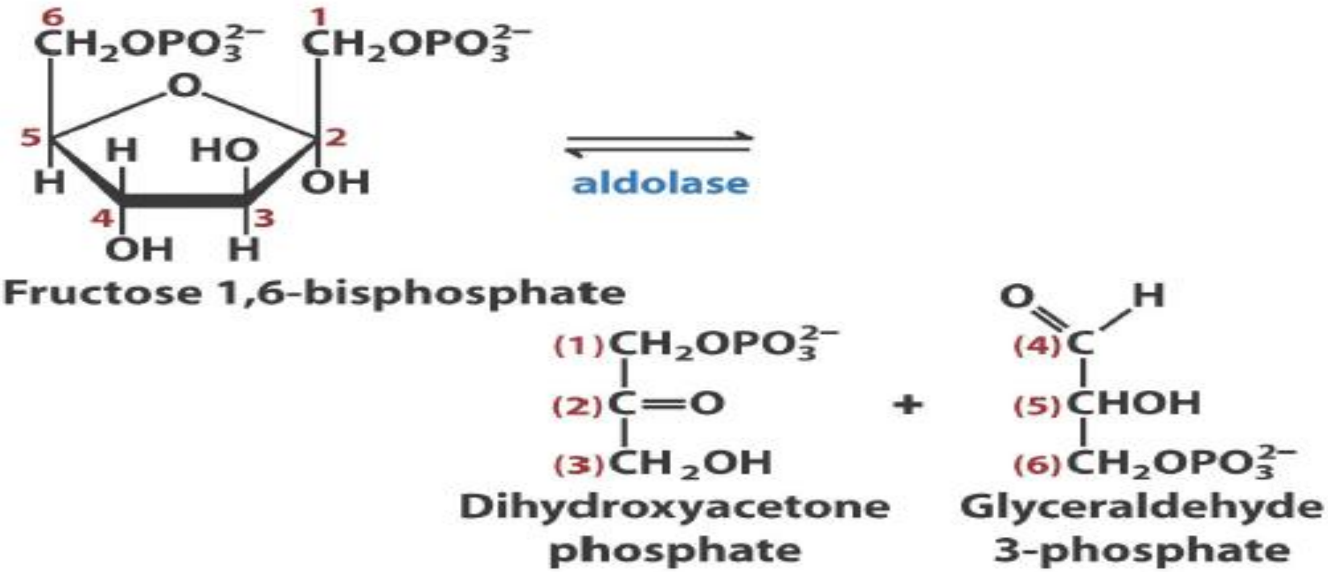
R3: The next step in the glycolytic pathway is the phosphorylation of this group by phosphofructo-kinase.

Phosphofructokinase catalyzes:

fructose-6-P + ATP → fructose-1,6-bisP + ADP

- This highly **spontaneous** reaction has a mechanism similar to that of Hexokinase. The Phosphofructokinase reaction is the **rate-limiting step** of Glycolysis

Reaction 4: Cleavage of Fructose-1,6-Bisphosphate

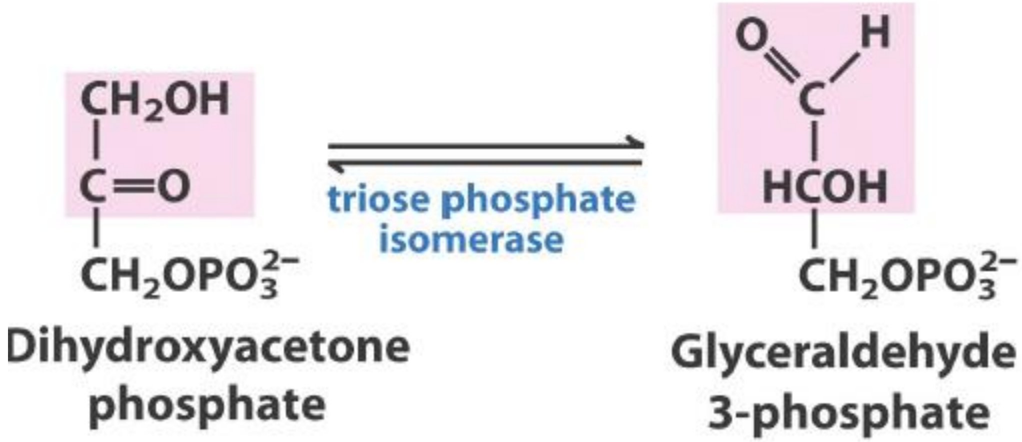


Fructose-1,6-bisphosphate splits to form two three-carbon sugars: dihydroxyacetone phosphate {DHAP}, and glyceraldehyde-3-phosphate.

Aldolase catalyzes: **fructose-1,6-bisphosphate dihydroxyacetone-P + glyceraldehyde-3-P**

They are isomers of each other, but only one—glyceraldehyde-3-phosphate—can directly continue through the next steps of glycolysis.

Reaction 5: Triose Phosphate Isomerase



Triose Phosphate Isomerase (TIM) catalyzes:

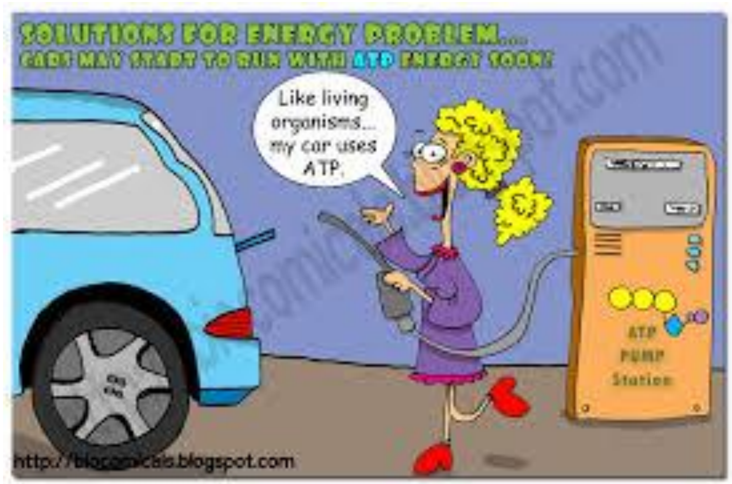
dihydroxyacetone-P ⟷ glyceraldehyde-3-P

This reaction thus permits both products of the aldolase reaction to continue in the glycolytic pathway

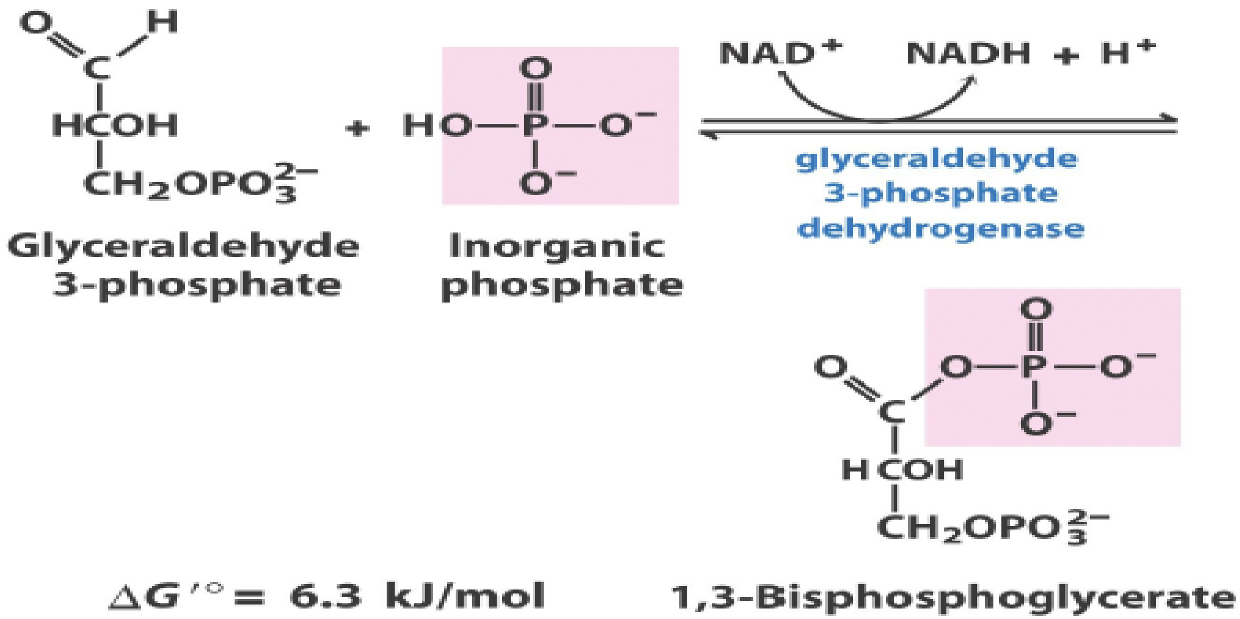
- The triose phosphate Isomerase reaction completes the first phase of glycolysis, each glucose molecule that passes through being converted to two molecules of glyceraldehyde-3-phosphate.

The Second Phase of Glycolysis

The second half of the glycolytic pathway involves the reactions that convert the metabolic energy in the glucose molecule into ATP.



Reaction 6: Oxidation

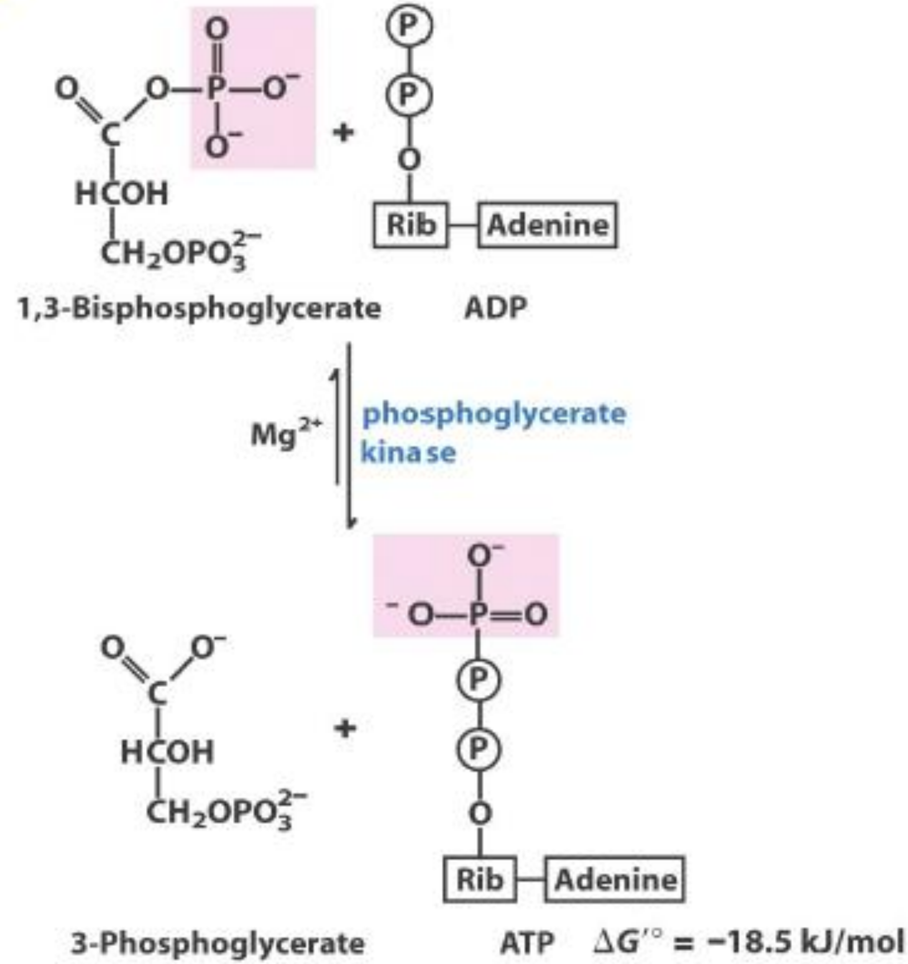


GAP is dehydrogenated by the enzyme glyceraldehyde **3-phosphate dehydrogenase (GAPDH)**. In the process, NAD^+ is reduced to NADH + H^+ from NAD.

Oxidation is coupled to the phosphorylation of the C1 carbon. The product is **1,3-bisphosphoglycerate**

Glyceraldehyde-3-P dehydrogenase catalyzes the formation of a high energy compound. This is the first step in the payoff phase

Reaction 7: Phosphorylation

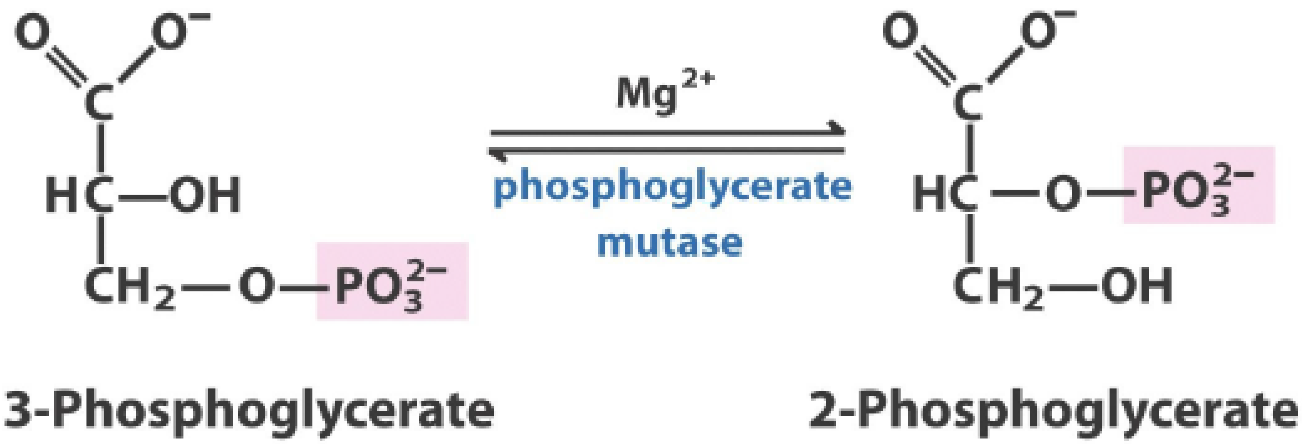


Phosphoglycerate Kinase catalyzes:

1,3-bisphosphoglycerate + ADP ←→ 3-phosphoglycerate + ATP

This phosphate transfer is reversible.

Reaction 8: Shift of Phosphoryl Group



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

Phosphoglycerate Mutase catalyzes:

3-phosphoglycerate ←→ 2-phosphoglycerate

Mutase: An enzyme that catalyzes the transposition of functional groups, such as phosphates, sulfates, etc.

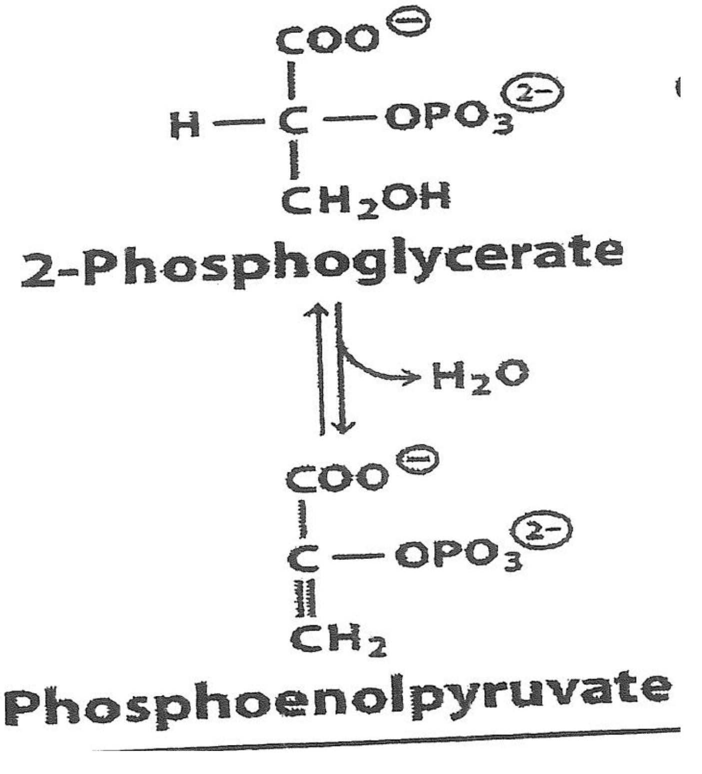
Reaction 9: Dehydration

Dehydration catalyzed by **enolase** (a lyase). A water molecule is removed to form phosphoenolpyruvate

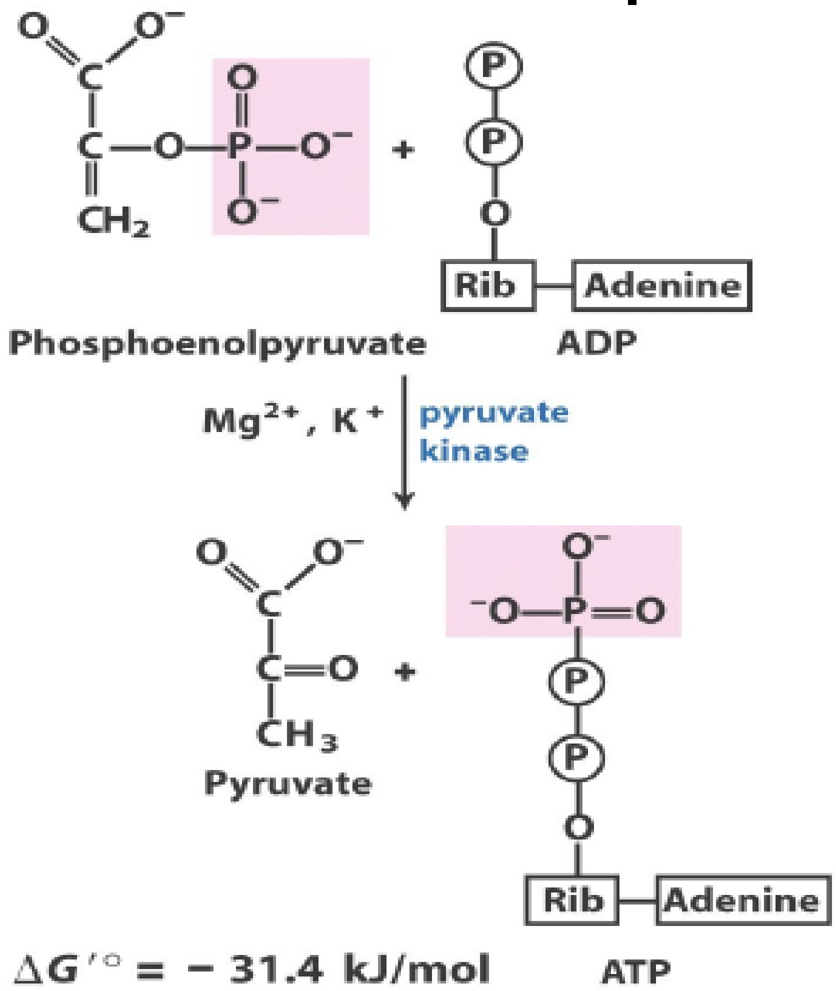
which has a double

bond between

C2 and C3.

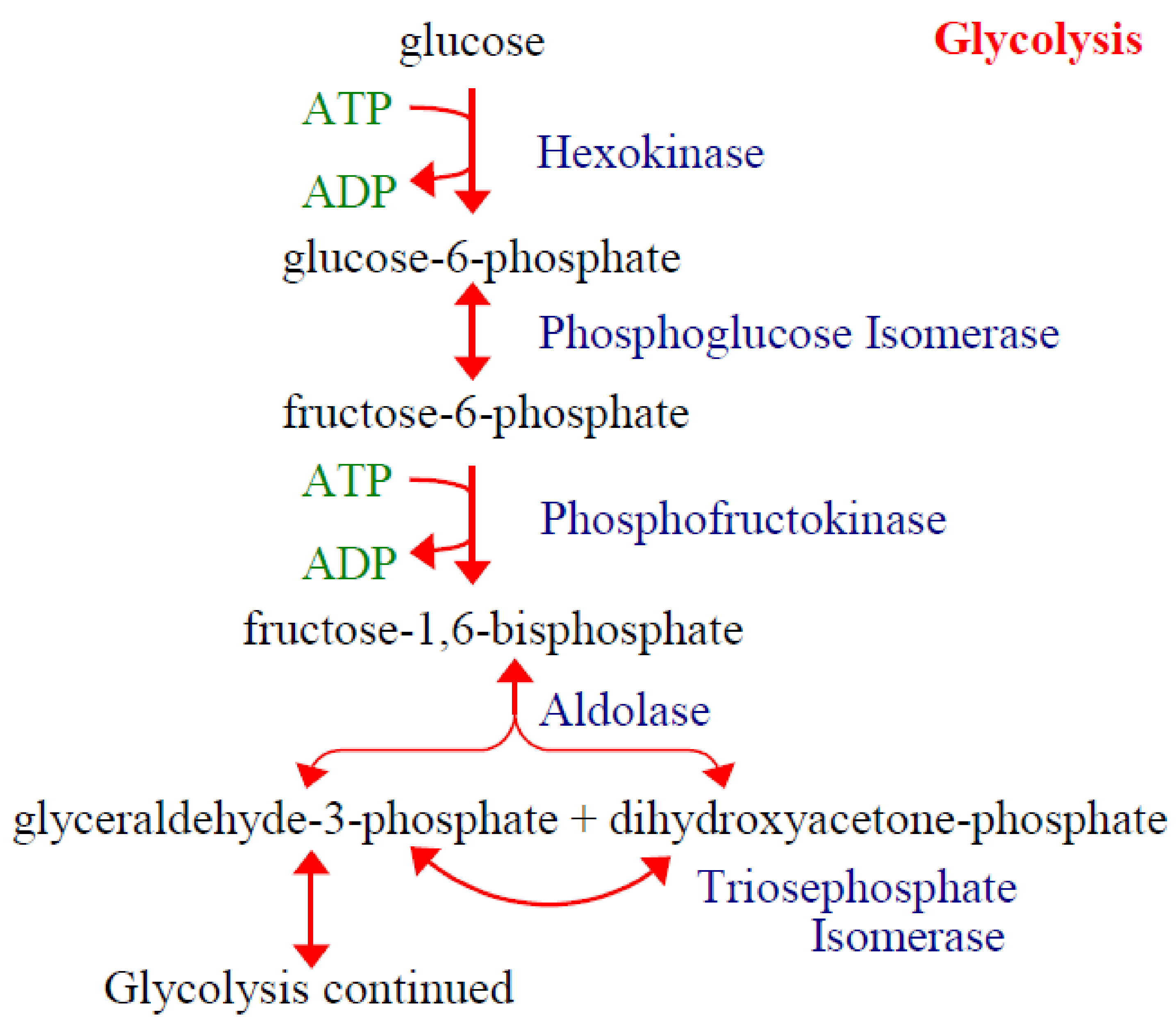


Reaction 10: Substrate Phosphorylation



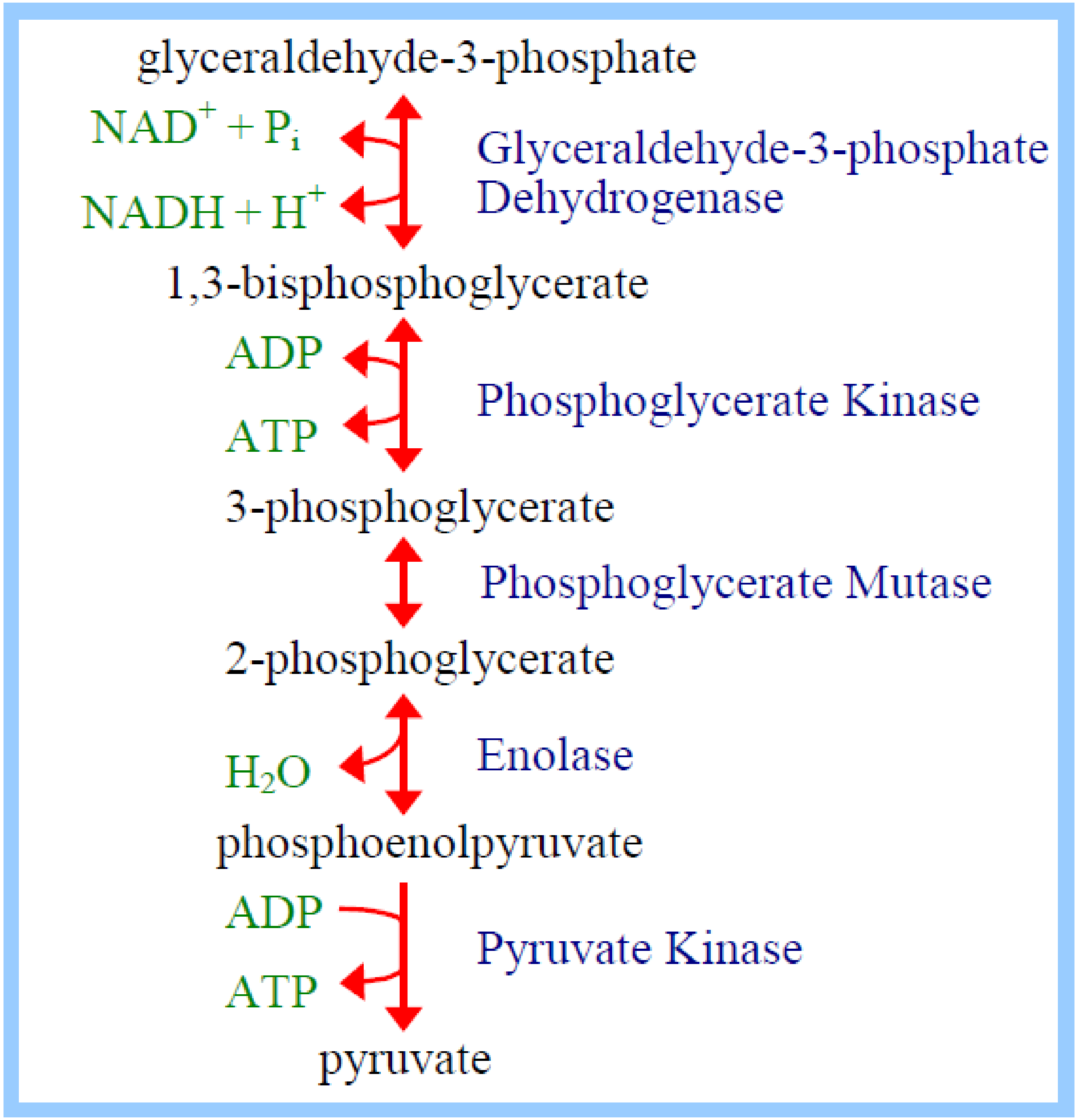
Pyruvate Kinase catalyzes:

phosphoenolpyruvate + ADP → pyruvate + ATP



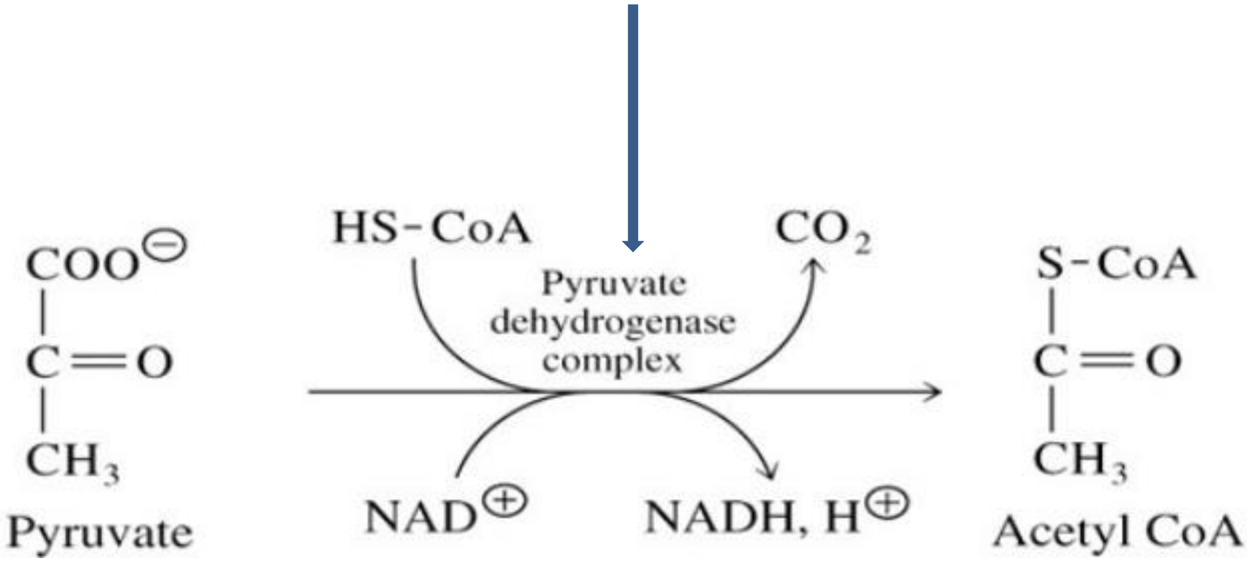
Glycolysis
glucose
ATP
ADP
Hexokinase
glucose-6-phosphate
Phosphoglucose Isomerase
fructose-6-phosphate
ATP
ADP
Phosphofructokinase
fructose-1,6-bisphosphate
Aldolase
glyceraldehyde-3-phosphate + dihydroxyacetone-phosphate
Triosephosphate Isomerase
Glycolysis continued

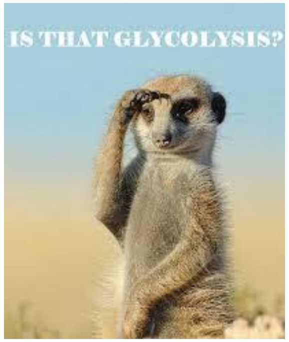
Glycolysis continued.



Fate of Pyruvate and NADH

1- Pyruvate: If oxygen is available, the pyruvate can be broken down (oxidized) to carbon dioxide in cellular respiration + **Acetyl CoA (**which enters TCA cycle and gets completely oxidized to CO2.)

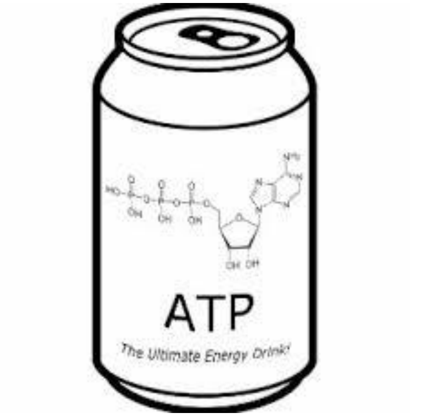




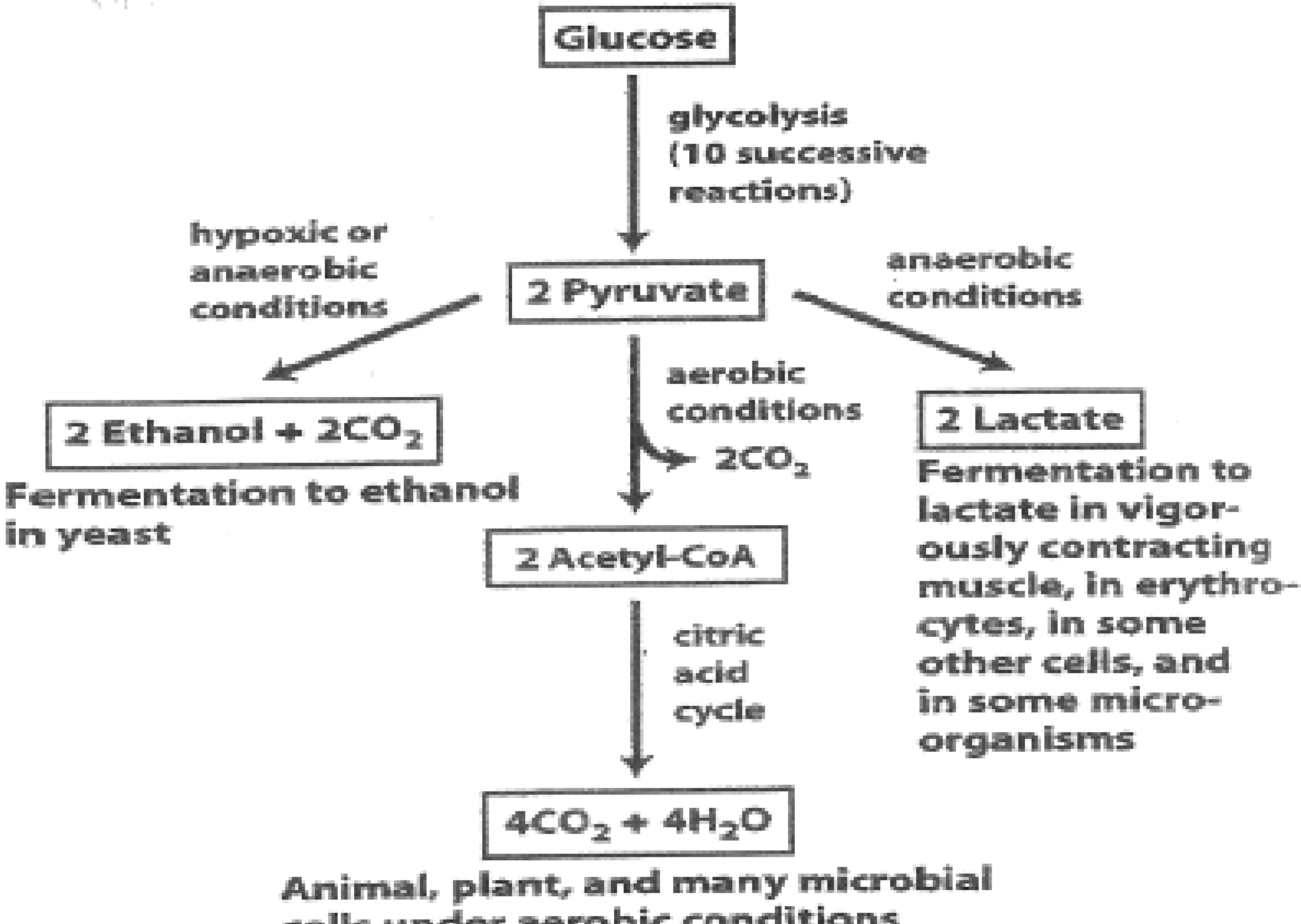
2- NADH

Aerobic: NADH is re-oxidized in the electron transport pathway, making ATP in oxidative phosphorylation

Anaerobic: NADH is re-oxidized by lactate dehydrogenase (LDH), providing additional NAD+ for more glycolysis



Fate of the products, pyruvate and NADH



Ethanol Fermentation

Formation of ethanol is catalyzed by 2 enzymes:

- Pyruvate decarboxylase catalyzes the first irreversible reaction to form acetaldehyde:

CH_3-CO-COOH → CH_3-CHO + CO_2

- Acetaldehyde is reduced by Alcohol dehydogenase (reversible reaction):

CH_3-CHO + NADH + H^+ ⇌ ... OH + NAD^+

- Ethanol fermentation is used

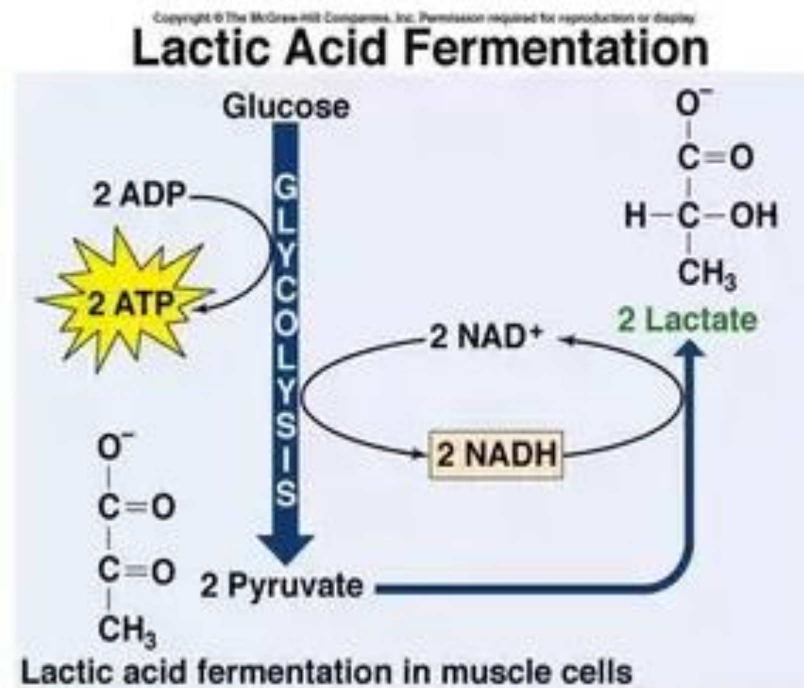
Lactate Formation

Lactate formation is catalyzed by lactate dehydrogenase:

CH3-CO-COOH+NADH+H+ ⟷ CH3-CHOH-COOH + NAD+

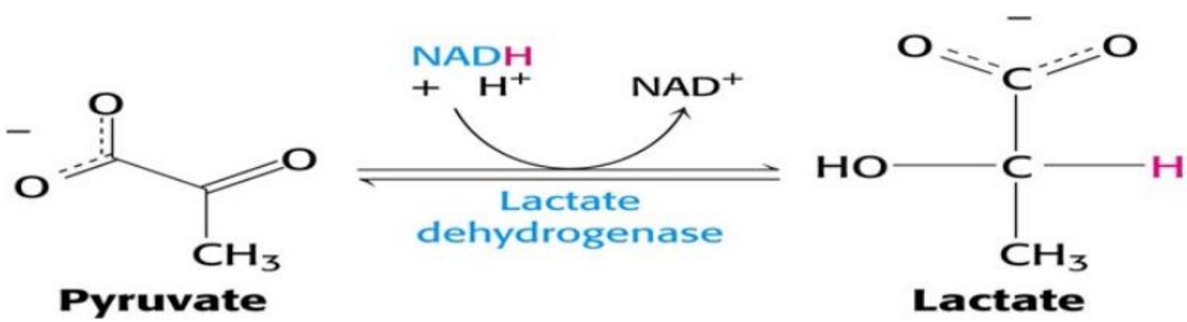
Lactate is formed from pyruvate in animals in anaerobic conditions.

The conversion of glucose into lactate is called lactic acid fermentation التخمر اللبني



Enzyme - lactate dehydrogenase.

Coenzyme – NADH.



- **Muscles** of higher organisms and humans **lack pyruvate decarboxylase** and cannot produce ethanol from pyruvate
- Muscle contain *lactate dehydrogenase* . During intense activity when the amount of oxygen is limiting the lactic acid can be accumulated in muscles **(lactic acidosis).**
- Lactate formed in skeletal muscles during exercise is transported to the liver.
- Liver lactate dehydrogenase can reconvert lactate to pyruvate.

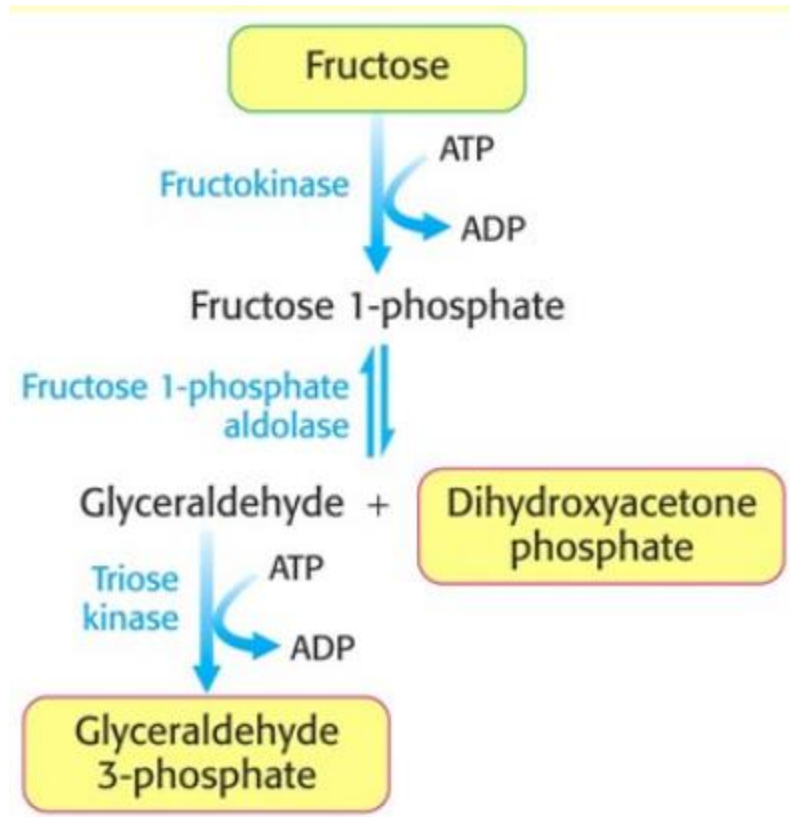
Other Sugars Can Enter Glycolysis as well

Glucose is the main metabolic fuel …

Other sugars *convert* to glycolytic intermediates:

Fructose:

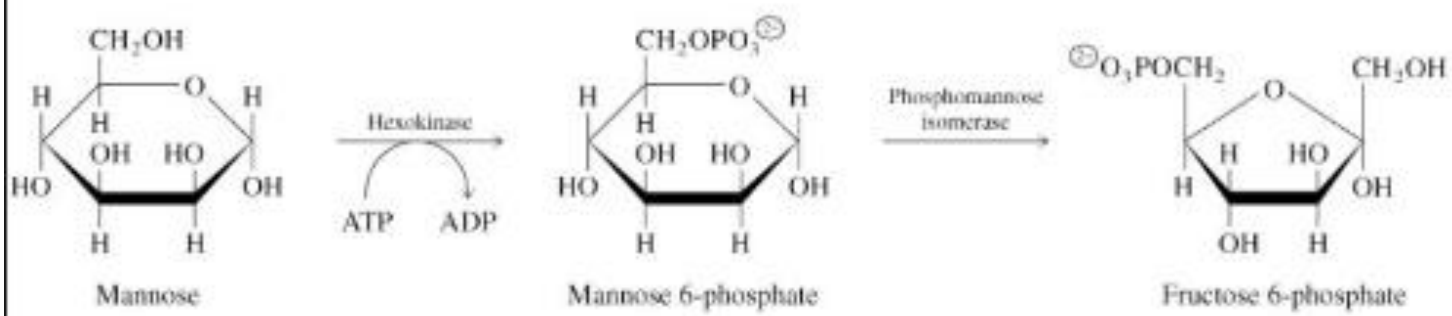
The first step is the phosphorylation of fructose to fructose 1-phosphate by fructokinase. Fructose 1-phosphate is then split into glyceraldehyde and dihydroxyacetone phosphate



Mannose:

Hexokinase catalyzes the convertion of **mannose** into **mannose 6-phosphate** .

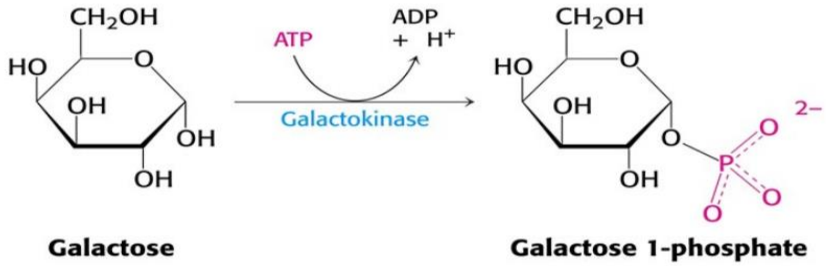
Isomerase converts **mannose 6-phosphate** into **fructose 6-phosphate** (metabolite of glycolysis).



Galactose:

Galactose is converted into glucose 6-p:

The first reaction is the phosphorylation of galactose to galactose 1-phosphate by galactokinase.



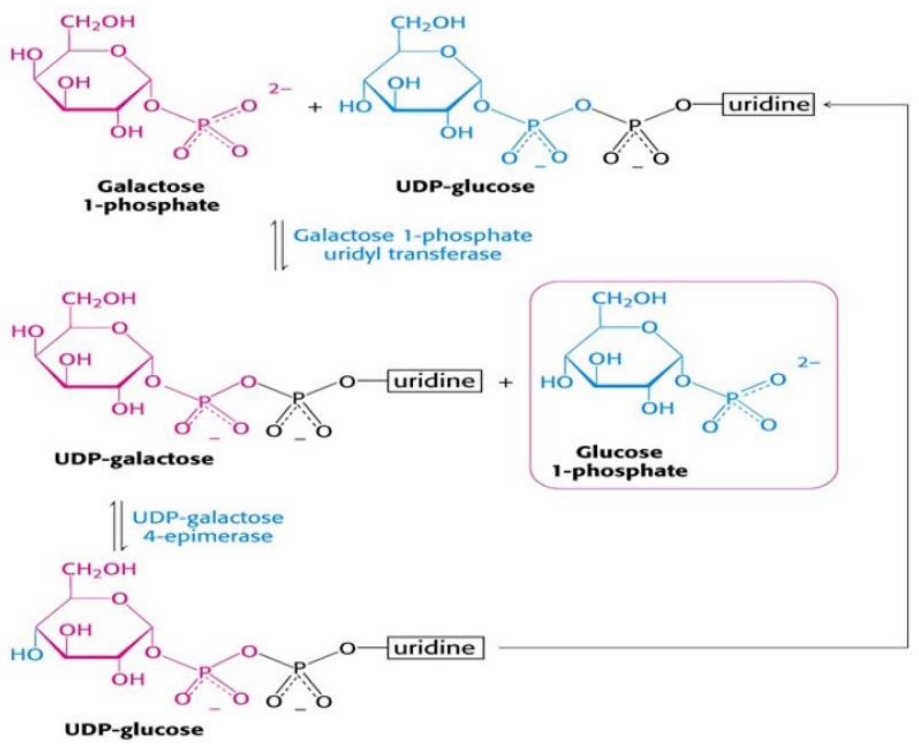
Galactose 1-phosphate react with uridine diphosphate glucose (UDP-glucose).

UDP-galactose and glucose 1-phosphate are formed.

Enzyme - *galactose 1-phosphate uridyl transferase.*

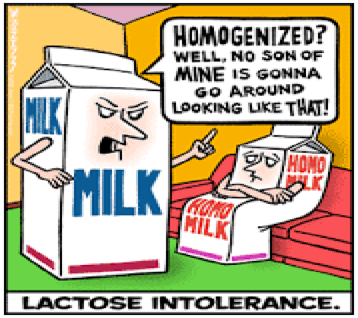
The galactose moiety of UDP-galactose is then epimerized to glucose.

Glucose 1-phosphate , formed from galactose, is isomerized to glucose 6-phosphate by *phosphoglucomutase.*



Milk Intolerance

Many people are unable to metabolize the milk sugar **lactose** and experience gastro-intestinal disturbances if they drink milk:



Lactose intolerance , or hypolactasia , **is caused by a deficiency of the enzyme** *lactase*, **which cleaves** lactose **into glucose and** galactose.

Microorganisms in the colon ferment undigested lactose to **lactic acid** generating **methane (CH_4)** and **hydrogen gas (H_2).** The gas produced creates the uncomfortable feeling of **gut distention** and the annoying problem of **flatulence**.

The lactic acid is osmotically active and draws water into the intestine, as does any undigested lactose, resulting in **diarrhea**.

Regulation of Glycolysis

The rate glycolysis is regulated to meet two major cellular needs:
(1) the production of ATP, and
(2) the provision of building blocks for synthetic reactions.

There are three control sites in glycolysis - the reactions catalyzed by

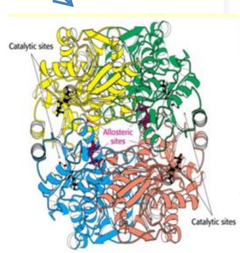
- ***hexokinase***,
- ***phosphofructokinase 1***, and
- ***pyruvate kinase***

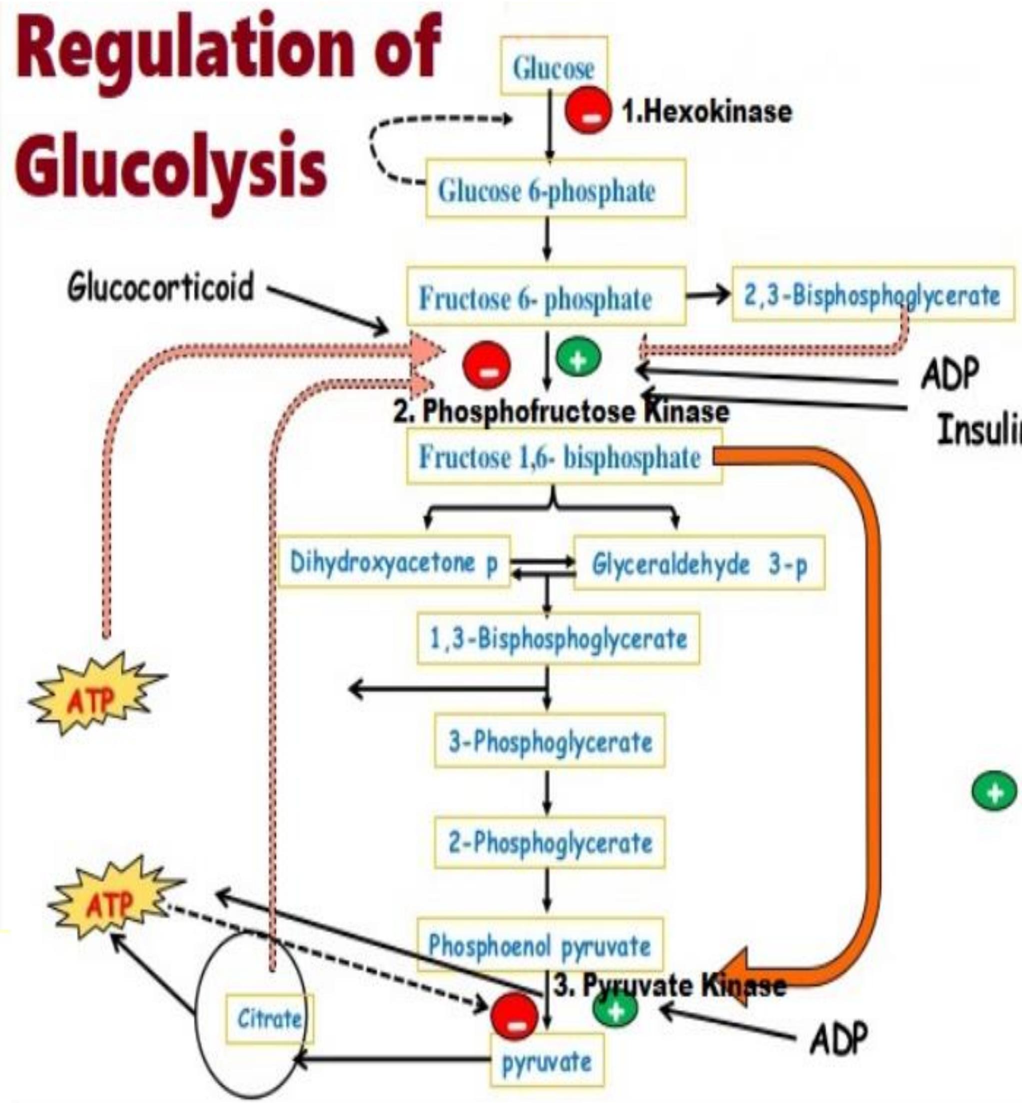
These reactions are **irreversible**.

Hexokinase is inhibited by its product, glucose 6-phosphate (G-6-P).High concentrations of G-6-P signal that the cell no longer requires glucose for energy, for glycogen, or as a source of biosynthetic precursors.

Phosphofructokinase 1 Is the Key Enzyme in the Control of Glycolysis

Phosphofructokinase-1 (tetramer of four subunits).

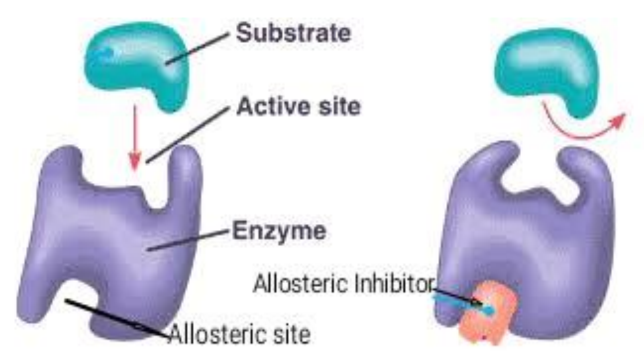




High levels of **ATP** allosterically inhibit the phosphofructokinase 1 in the liver lowering its affinity for fructose 6-phosphate.
The activity of the enzyme increases when the ATP/AMP ratio is lowered (glycolysis is stimulated as the energy charge falls).
A fall in **pH** also inhibits phosphofructokinase 1 activity. Phosphofructokinase 1 is inhibited by **citrate**, an early intermediate in the citric acid cycle. A high level of citrate means that biosynthetic precursors are abundant and additional glucose should not be degraded for this purpose.

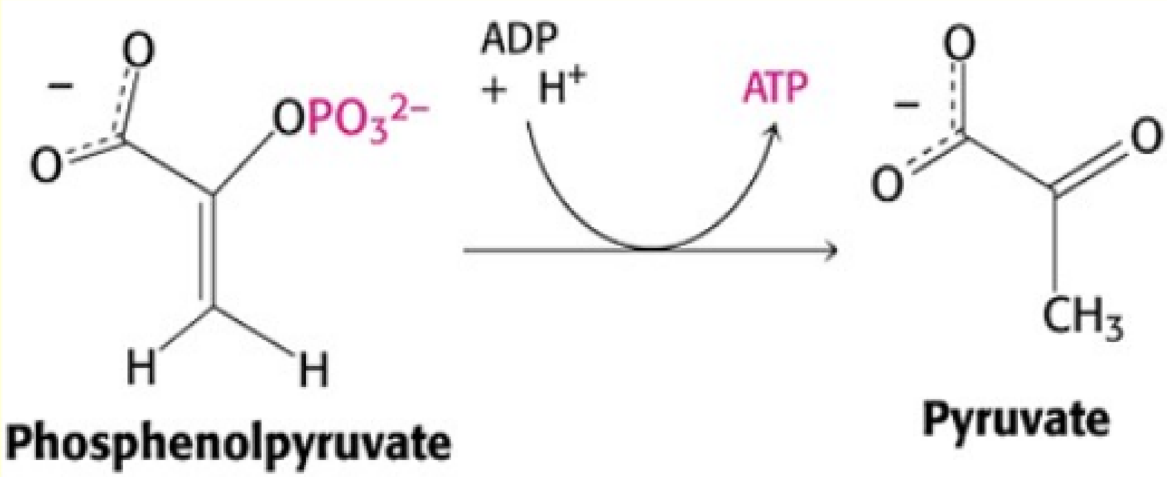
Fructose 2,6-bisphosphate (F-2,6-BP) is a potent activator of phosphofructokinase 1.

F-2,6-BP activates phosphofructokinase by increasing its affinity for fructose 6-P



Pyruvate Kinase

Several isozymic forms of ***pyruvate kinase*** are present in mammals (the **L type** predominates in liver, and the **M type** in muscle and brain).



Fructose 1,6-bisphosphate allosterically **activates *pyruvate kinase***.

ATP allosterically **inhibits *pyruvate kinase*** to slow glycolysis when the energy charge is high.

Finally, **alanine** (synthesized in one step from pyruvate) also allosterically **inhibits the *pyruvate kinases*** (signal that building blocks are abundant).

Where do the intermediates of Glycolysis go?