BMS2004 Week 9

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Questions to think about

- Why do we eat foods?
- What happens to the foods after we eat them?
- Why do we breathe oxygen all the time?
- Are other animals the same with us?

Metabolism and Energy

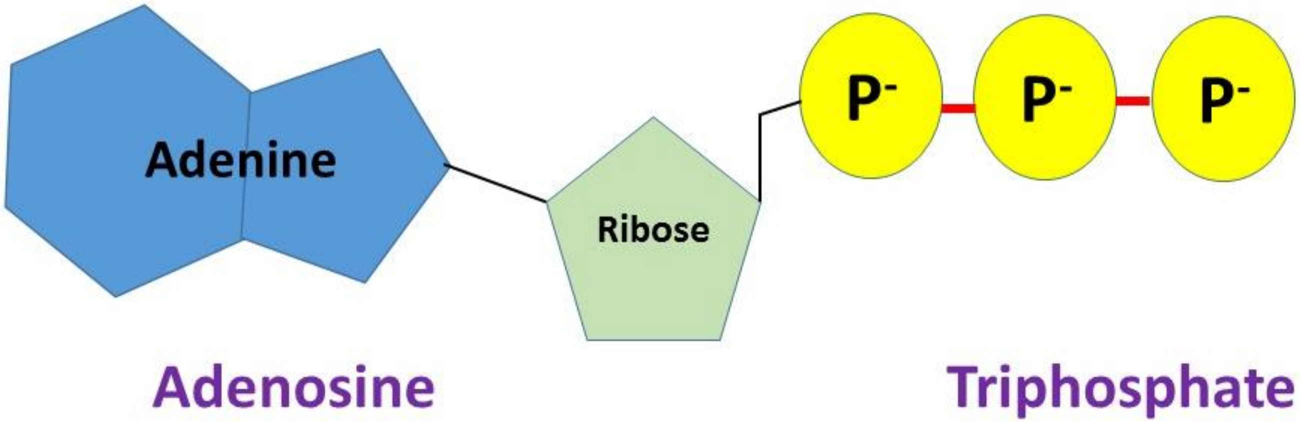
Metabolism

- The cost of living – energy.
- All the time, living organisms consumes and produces energy through chemical reactions.
- Collectively, we call these chemical reactions metabolism.
- What is true of E coli. is true of the elephant.

--- Jacques Monod

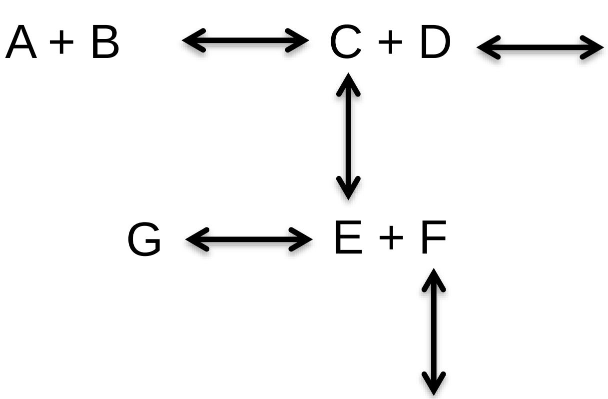
Counting the energy

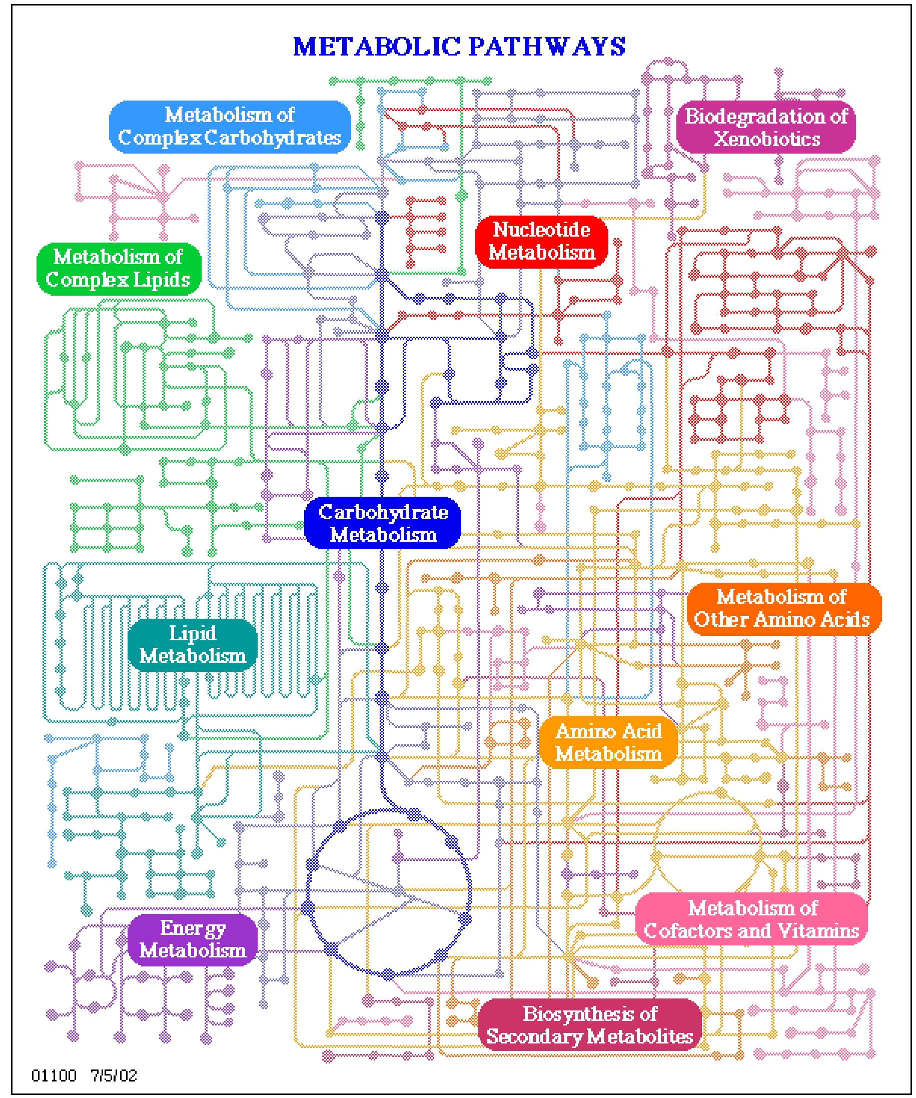
- Ribonucleoside triphosphate are the currency of energy: **ATP**, **GTP, UTP**, and **CTP**. The energy is stored in the multi-phosphate bonds.



The metabolic network

- A network of pathways.
- All inter-connected.
- Which direction to go?



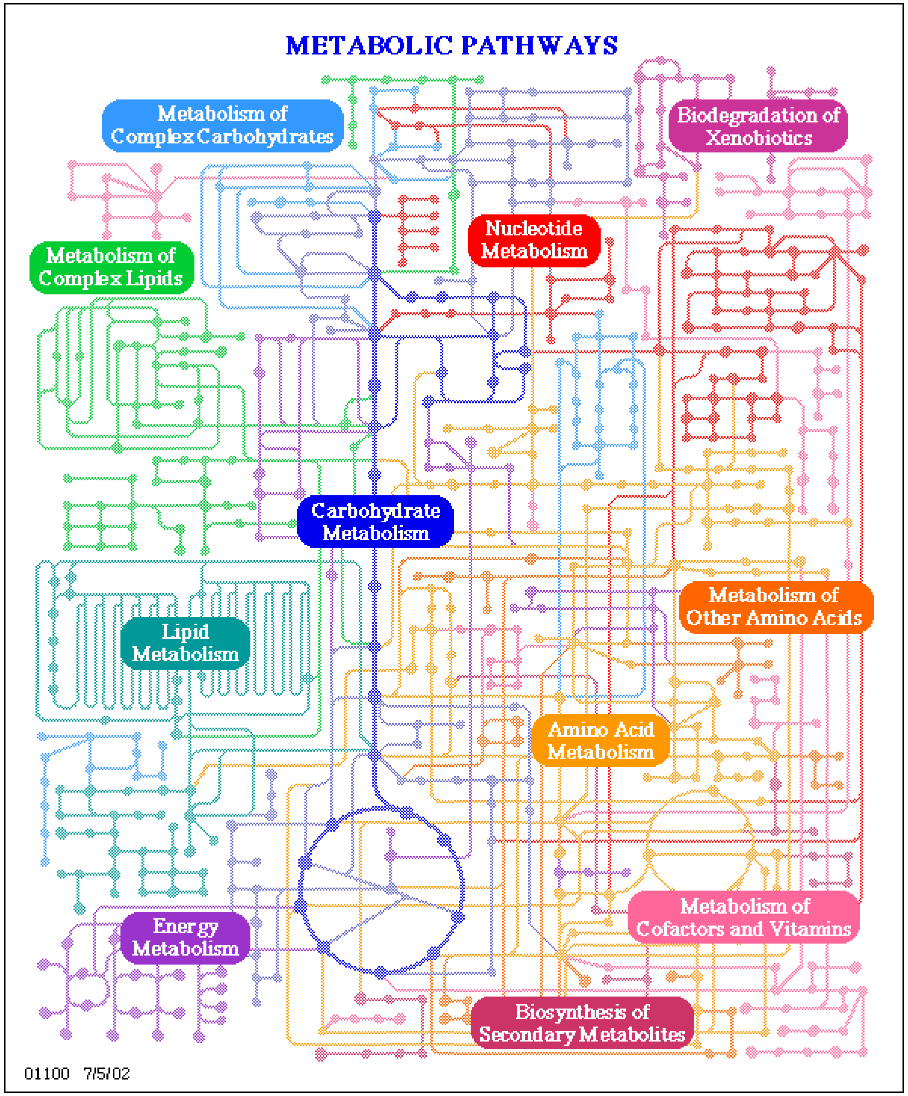


http://www.genome.jp/kegg-bin/show_pathway?map01100

The metabolic network

- A network of pathways.
- All inter-connected.
- Which direction to go?

--- Gibbs free energy and external regulation.



$$
\begin{array}{ccccc}
A + B & \longleftrightarrow & C + D & \longleftrightarrow & \\
 & & \updownarrow & & \\
G & \longleftrightarrow & E + F & & \\
 & & \updownarrow & &
\end{array}
$$

Direction of metabolic reactions – Gibbs free energy (G)

$$A + B \longleftrightarrow C + D$$

$$\Delta G = G_{(C + D)} - G_{(A + B)}$$

If $\Delta G < 0$, reaction goes left to right;

If $\Delta G = 0$, reaction is at equilibrium;

If $\Delta G > 0$, reaction goes right to left

What determines ΔG?

$$\underline{A + B} \longleftrightarrow \underline{C + D}$$

Reactants Products

$$\Delta G = \Delta G'_0 + RT\ln(\{Products\}/\{Reactants\})$$

What determines ΔG?

$$\underline{A + B} \longleftrightarrow \underline{C + D}$$

Reactants Products

$$\Delta G = \Delta G'_0 + RT\ln(\{Products\}/\{Reactants\})$$

$\Delta G'_0$: change of G at standard conditions.

For a given reaction, if $\Delta G'_0 < 0$, then that direction is <u>energetically favorable</u>, meaning the reaction tends to undergo in that direction.

If $\Delta G'_0 > 0$, then that direction is energetically unfavorable, meaning the reaction tends to undergo in the reverse direction.

What determines ΔG?

$$\underline{A + B} \longleftrightarrow \underline{C + D}$$

Reactants Products

$$\Delta G = \Delta G'_0 + RT\ln(\{Products\}/\{Reactants\})$$

But there is another part in the equation: {Products}/{Reactants}.

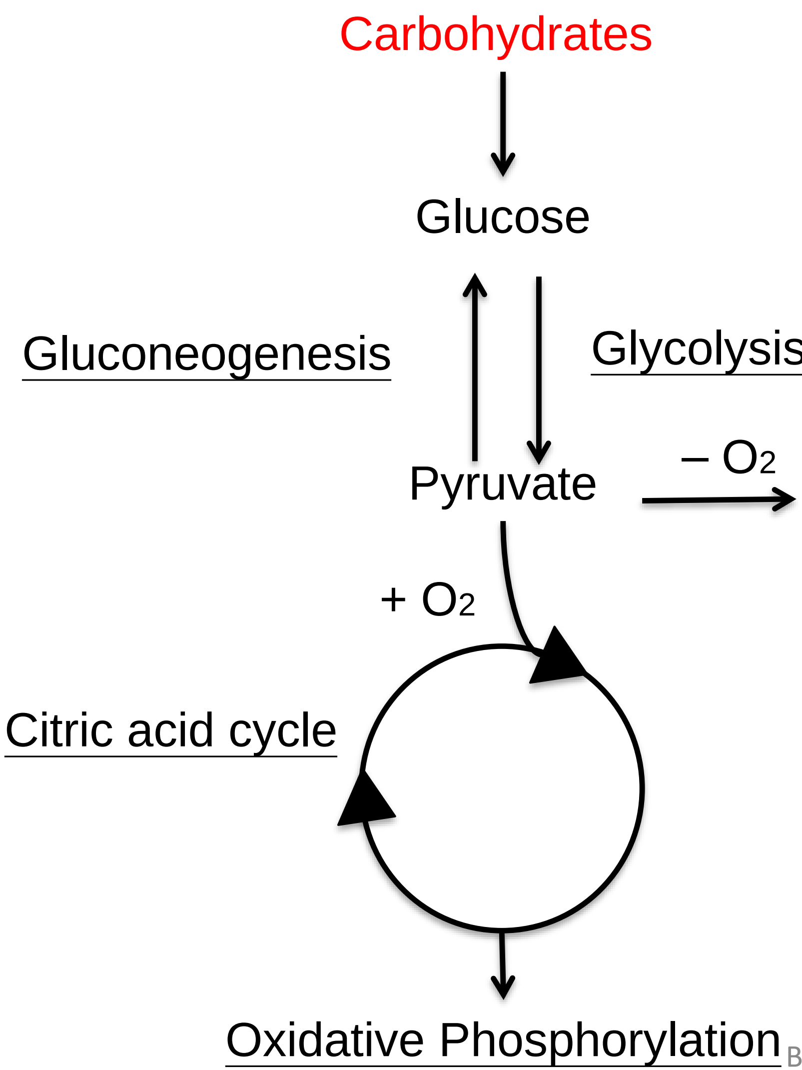
If the concentration of products is very low and the concentration of reactants is very high, even if $\Delta G'0$ is <u>positive</u> (energetically unfavorable), ΔG may be <u>negative</u> and the reaction go as written – pushing and pulling.

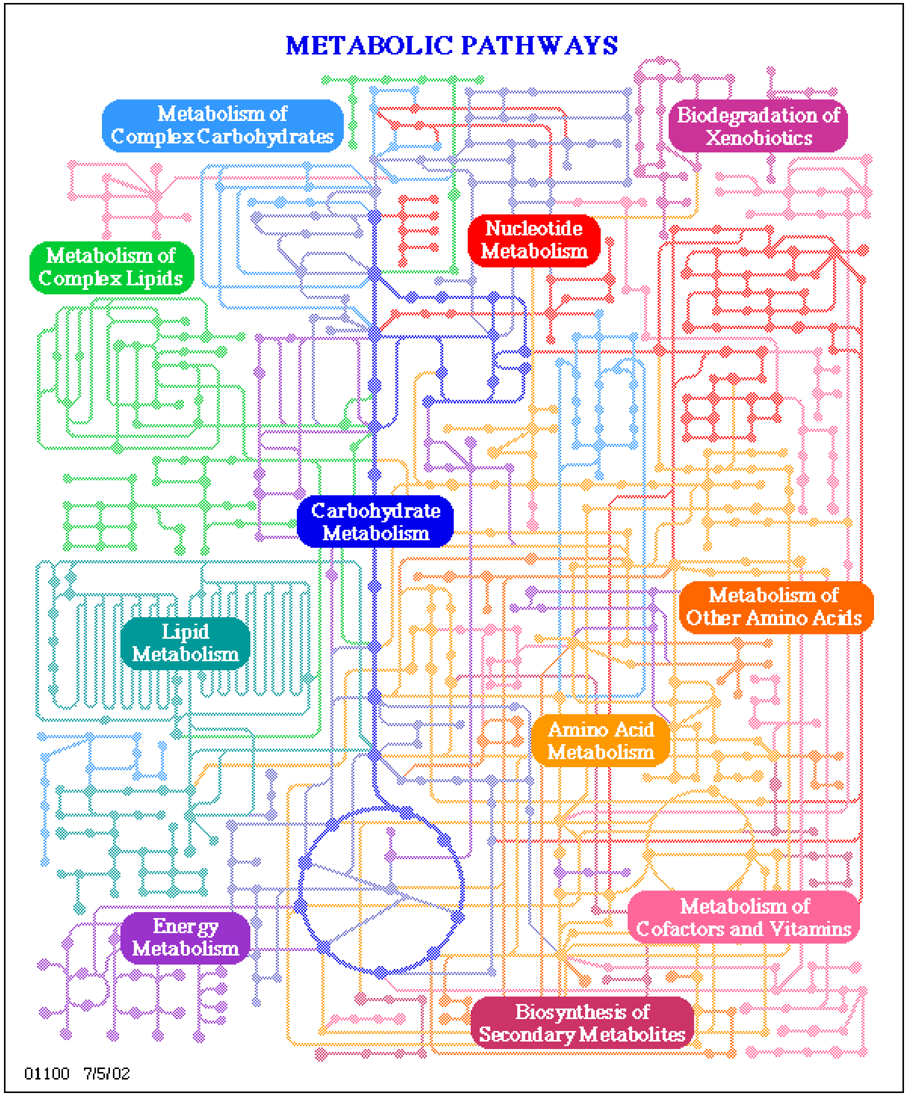
Summary of objectives

- Metabolism is collection of chemical reactions in the body
- Central theme of metabolism: energy
- Determining the direction of reactions: ΔG, $\Delta G'_0$ and concentration of reactants/products (pushing and pulling).

Glycolysis

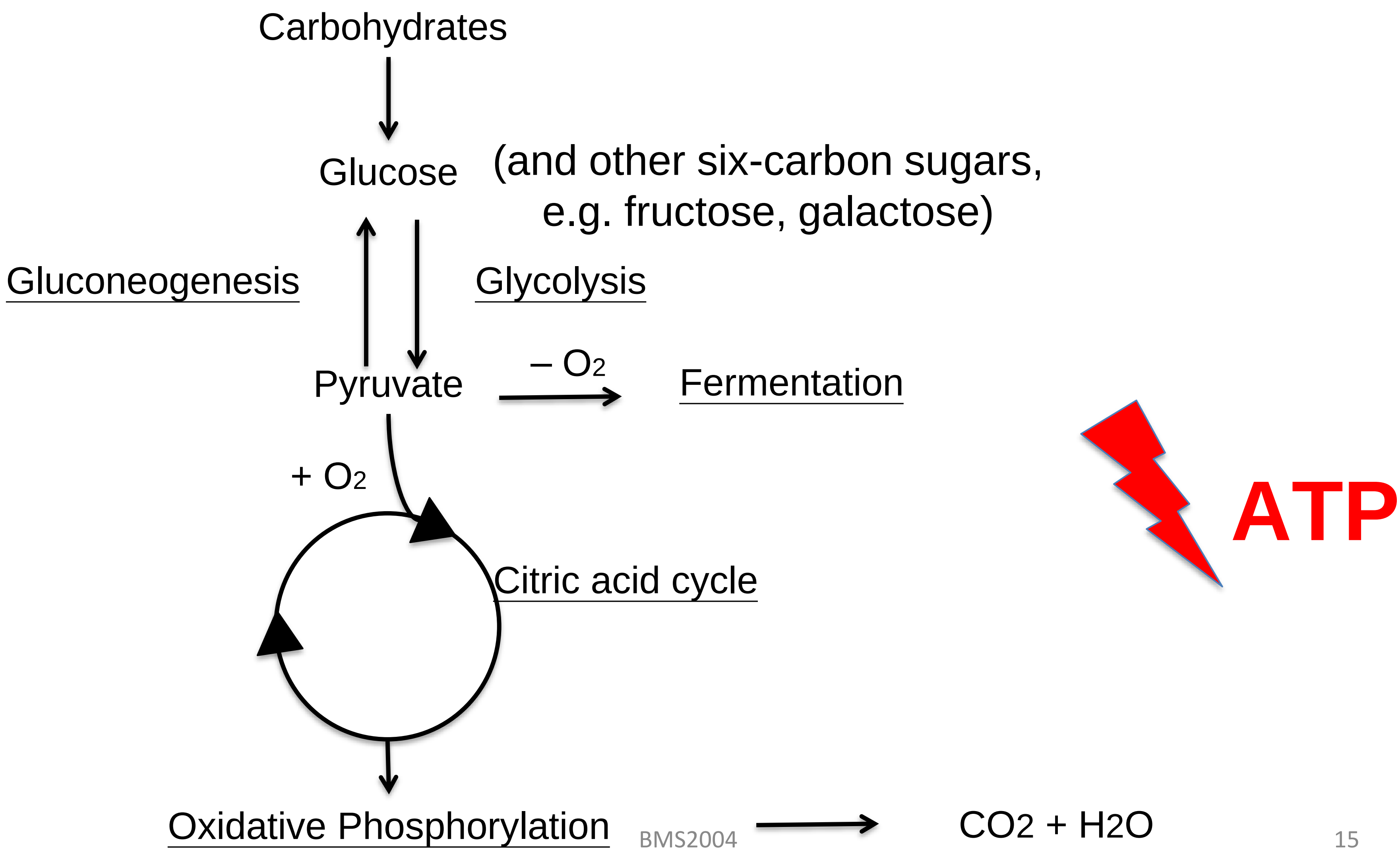
Metabolism of carbohydrates





http://www.genome.jp/kegg-bin/show_pathway?map01100

Central theme -- energy



The pathways are not isolated, and the reactions don't stop

Carbohydrates

↓

Glucose (and other six-carbon sugars, e.g. fructose, galactose)

Gluconeogenesis ↑ ↓ **Glycolysis**

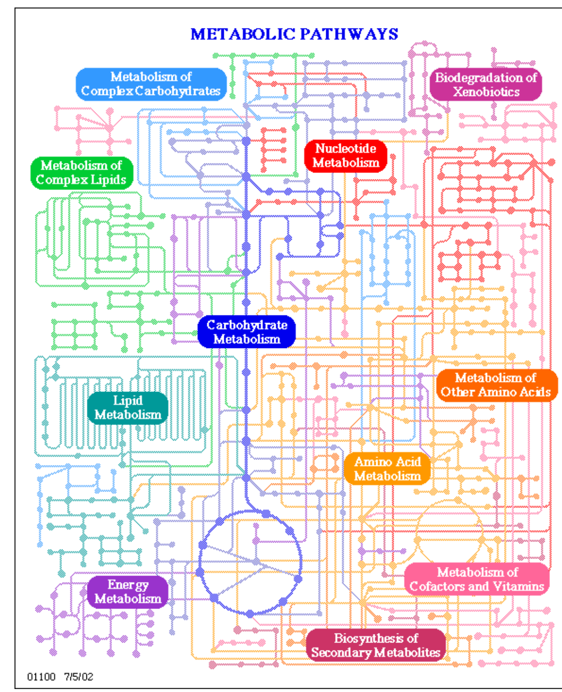
Pyruvate $\xrightarrow{-\,O_2}$ Fermentation

$+ O_2$

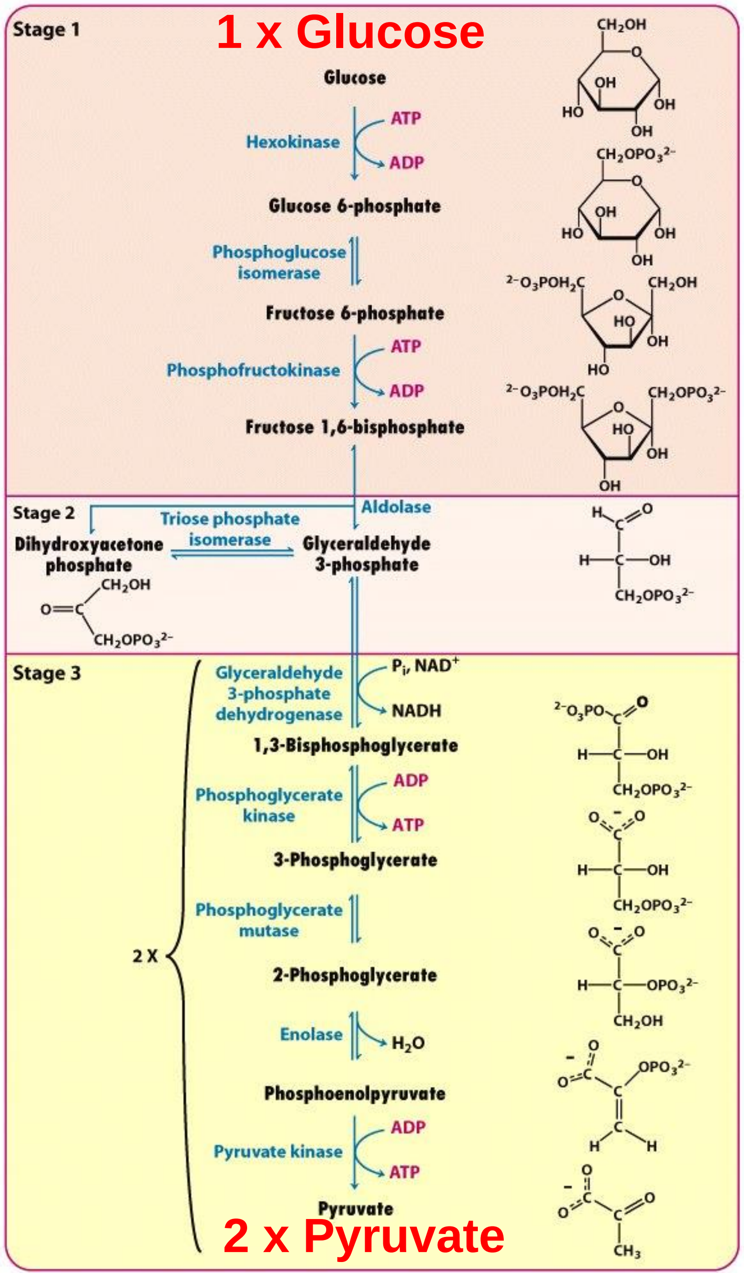
Citric acid cycle

↓

Oxidative Phosphorylation → $CO_2 + H_2O$



Glycolysis – an introduction



- Literally, glycolysis means the splitting of sugar.
- It was the first metabolic pathway to be elucidated (yet it took almost 100 years!).
- It is an almost **universal** central pathway of glucose metabolism, because it is found in mammals, plants, and microorganisms (especially anaerobic ones).

What is glycolysis?

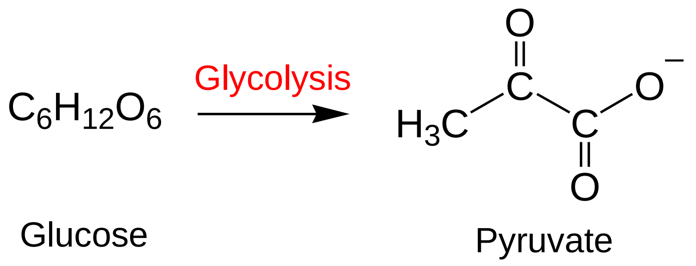
Glycolysis is the sequence of reactions that metabolizes **one molecule of glucose** to **two molecules of pyruvate** with the concomitant net production of **TWO** molecules of ATP.

The net reaction of glycolysis is:

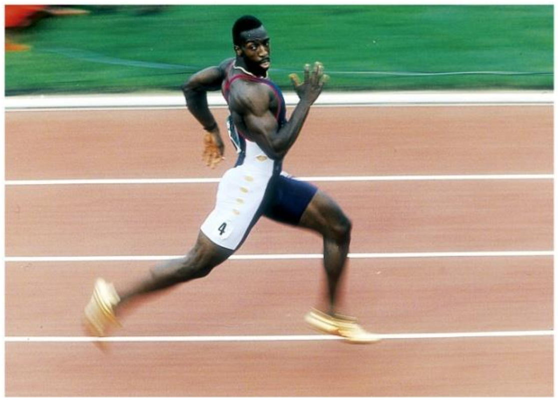
Glucose + 2ADP +2P$_i$ + 2NAD$^+$ $\rightarrow$ 2Pyruvate + 2ATP + 2NADH +2H$^+$ + 2H$_2$O

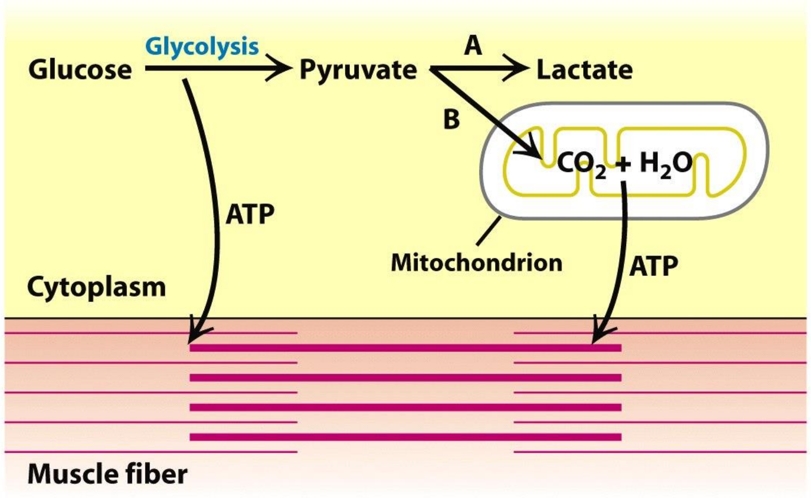
This process is ***anaerobic*** (i.e., it does not require O_2).

The reactions take place in the ***cytosol***.



Some facts about glycolysis



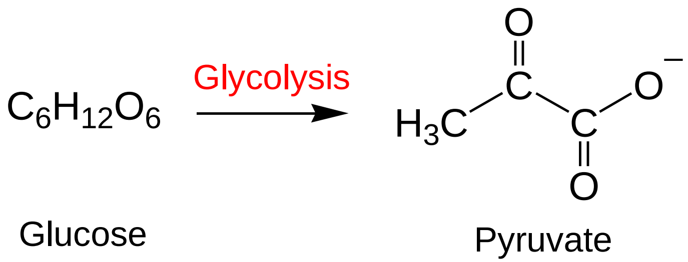


A. Low O_2 (last seconds of a sprint)

B. Normal (long slow run)

Biochemistry, 7th edition

Two ATP is generated, but is a major source of energy during intensive exercising, e.g. sprint.

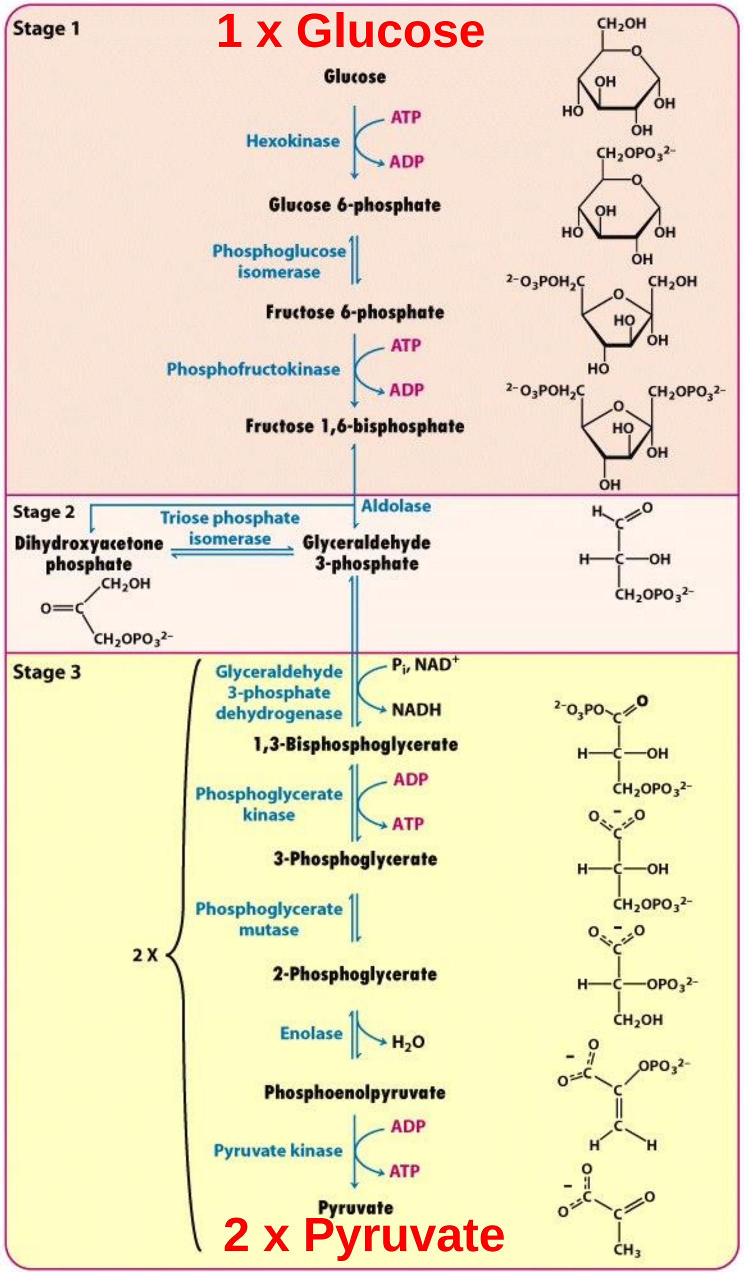


Summary of objectives

- Glycolysis is the breakdown of simple sugars.
- Starting: 1 glucose; Ending: 2 pyruvates.
- ATP generated – 2.
- Location – cytosol.

The reactions

The reactions



10 steps in total

Can be divided into 2 phases

Biochemistry, 7th edition
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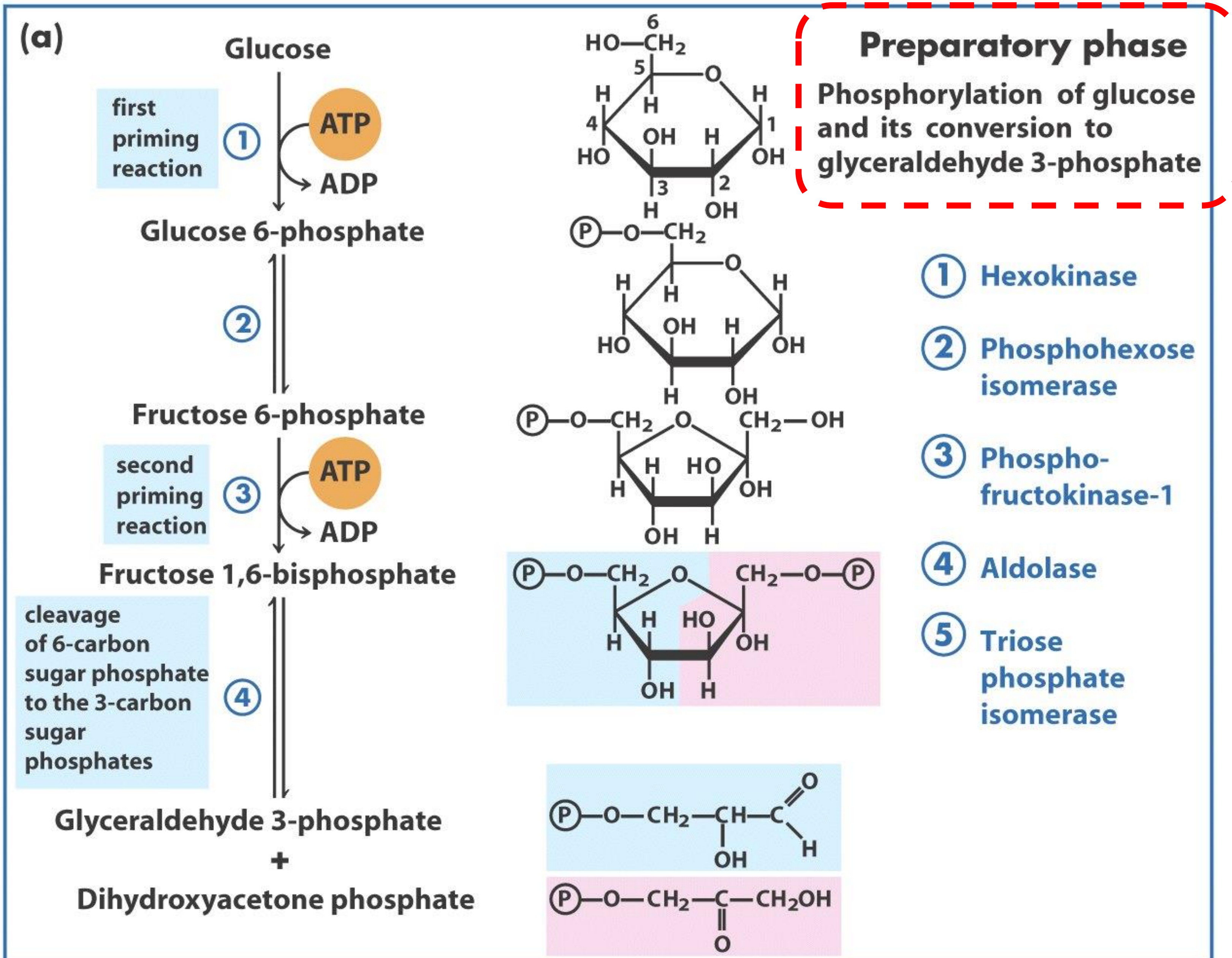
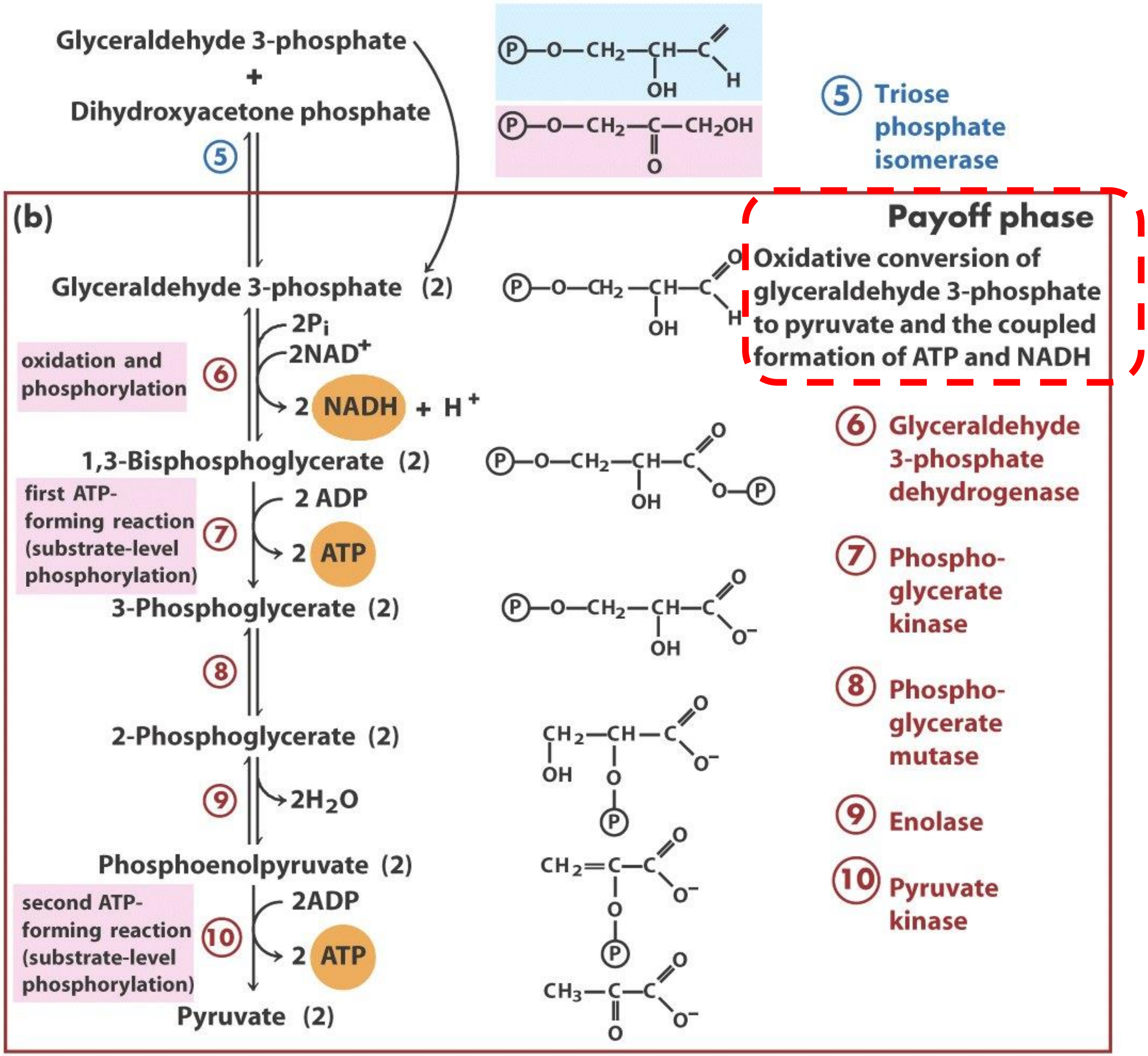
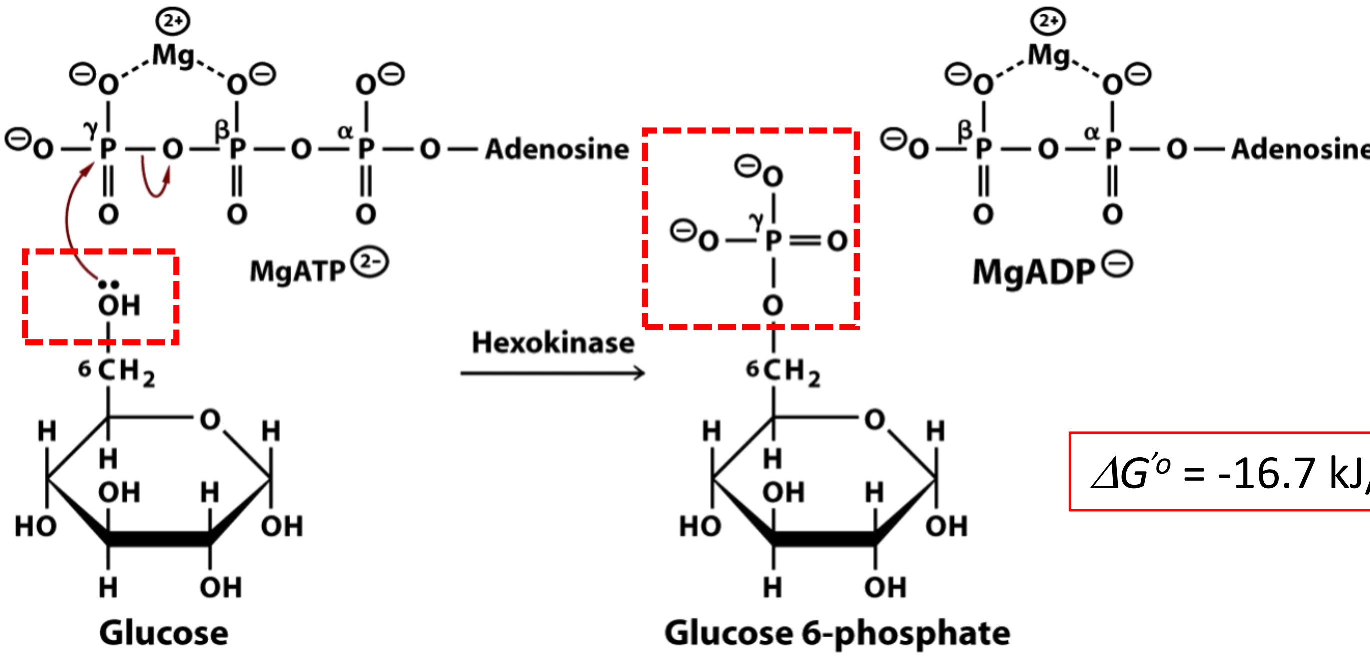


(a)
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to the 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase



Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Triose phosphate isomerase
(b)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
Glyceraldehyde 3-phosphate (2)
$2P_i$
$2NAD^+$
2 NADH + H^+
oxidation and phosphorylation
6
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2 ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
$2H_2O$
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phosphoglycerate kinase
8 Phosphoglycerate mutase
9 Enolase
10 Pyruvate kinase

Step (1): Formation of glucose 6-phosphate



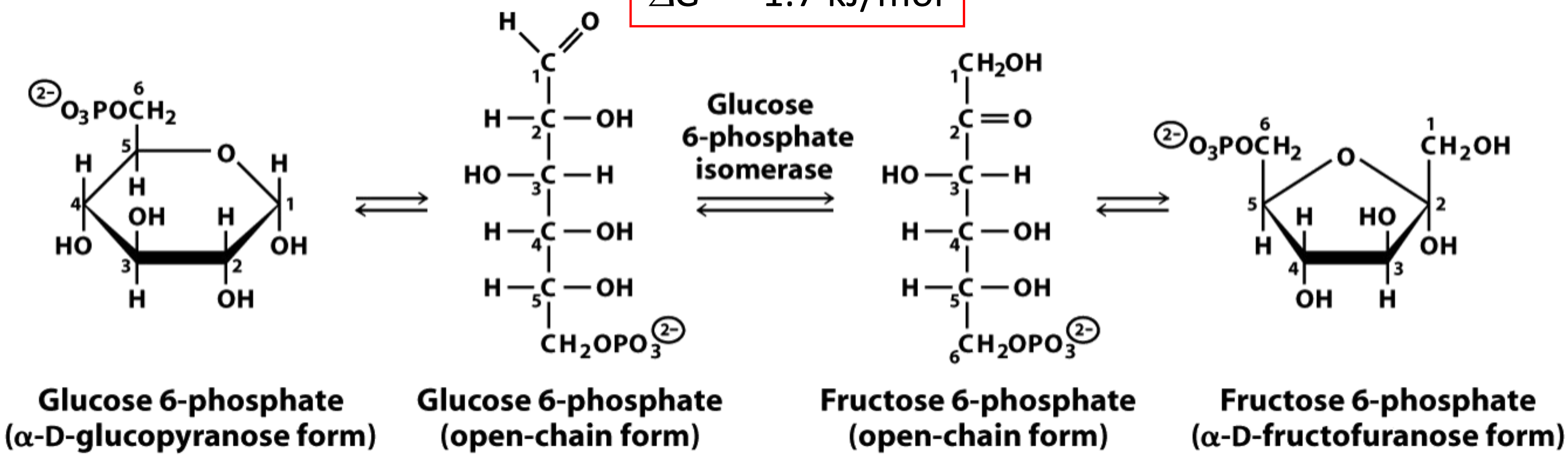
$\Delta G'^{o}$ = -16.7 kJ/mol

(1) **Hexokinase** traps glucose in the cell because glucose 6-phosphate cannot diffuse through the membrane (as it is negatively charged).

(2) The addition of the phosphoryl group begins to <u>destabilize</u> glucose, facilitating its further metabolism.

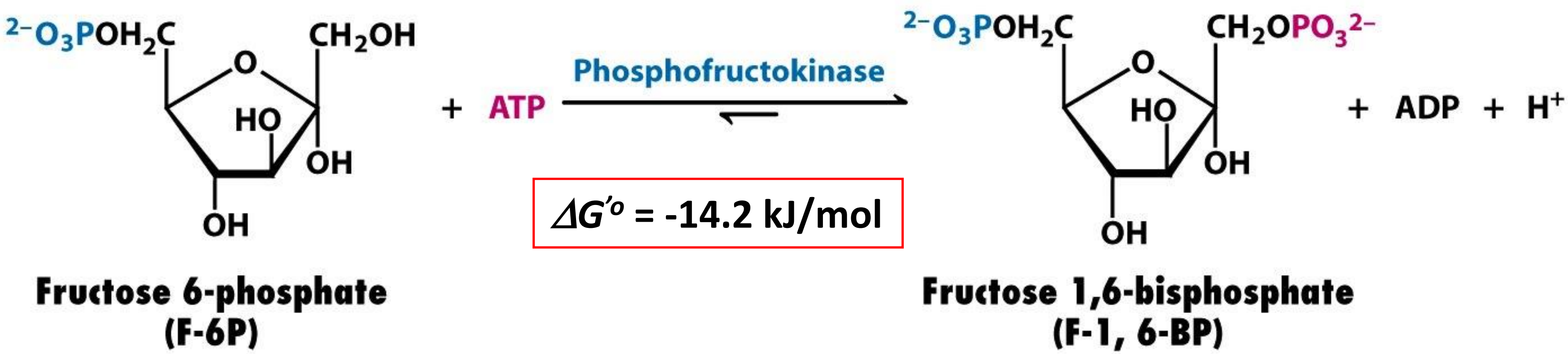
Step (2): Isomerization of glucose 6-phosphate to fructose 6-phosphate

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



In the open chain form, glucose has an aldehyde group on C1, whereas the fructose has a keto group on C2. Thus the isomerization of glucose 6-phosphate to fructose 6-phosphate is a conversion of an aldose to ketose.
The purpose of this step is to make the C1 carrying a OH group for the next step.

Step (3): Formation of fructose 1,6-biphosphate



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

This reaction is catalyzed by *phosphofructokinase* (**PFK**), an **allosteric enzyme** that sets the pace of glycolysis.

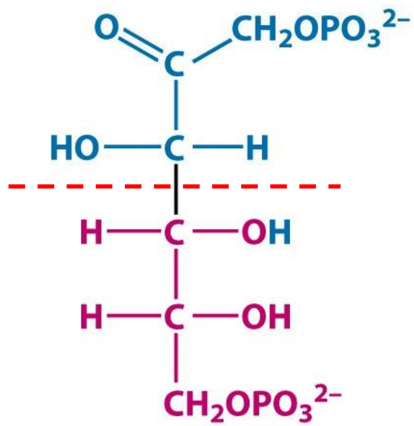
The reaction is almost irreversible under cellular condition, and it is the ***first committed step*** in the glycolytic pathway.

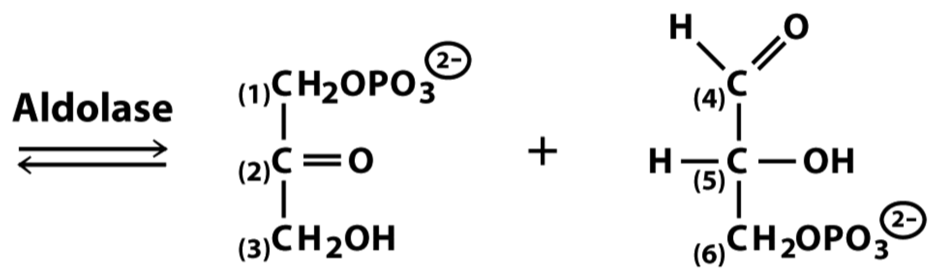
Thus fructose 1,6-bisphosphate is only targeted for glycolysis.

Step (4): Splitting of fructose 1,6-biphosphate

$\boxed{\Delta G'^{o} = 23.8 \text{ kJ/mol (so how the cells drive this reaction?)}}$

A reversible reaction

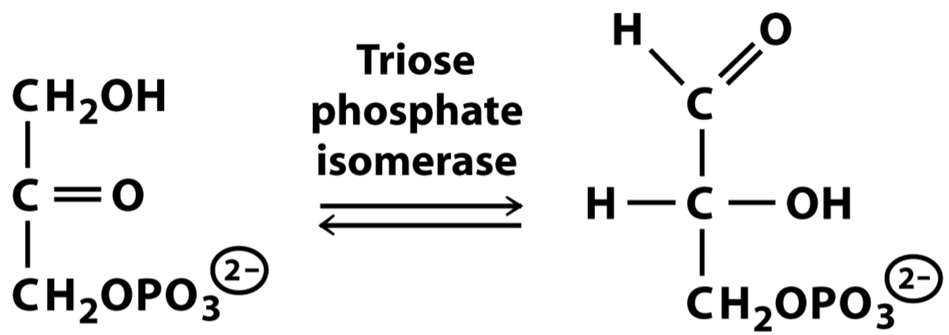




Fructose 1,6-*bis*phosphate	Dihydroxyacetone phosphate	Glyceraldehyde 3-phosphate
6C unit	3C unit	3C unit

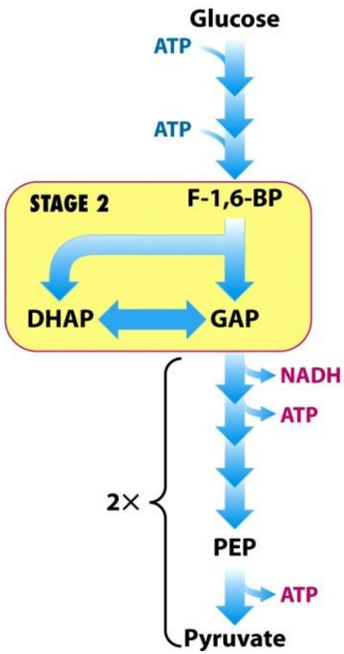
Glyceraldehyde 3-phosphate is on the direct pathway of glycolysis, while dihydroxyacetone phosphate is not (see next step)!

Step (5): Isomerization of dihydroxyacetone phosphate



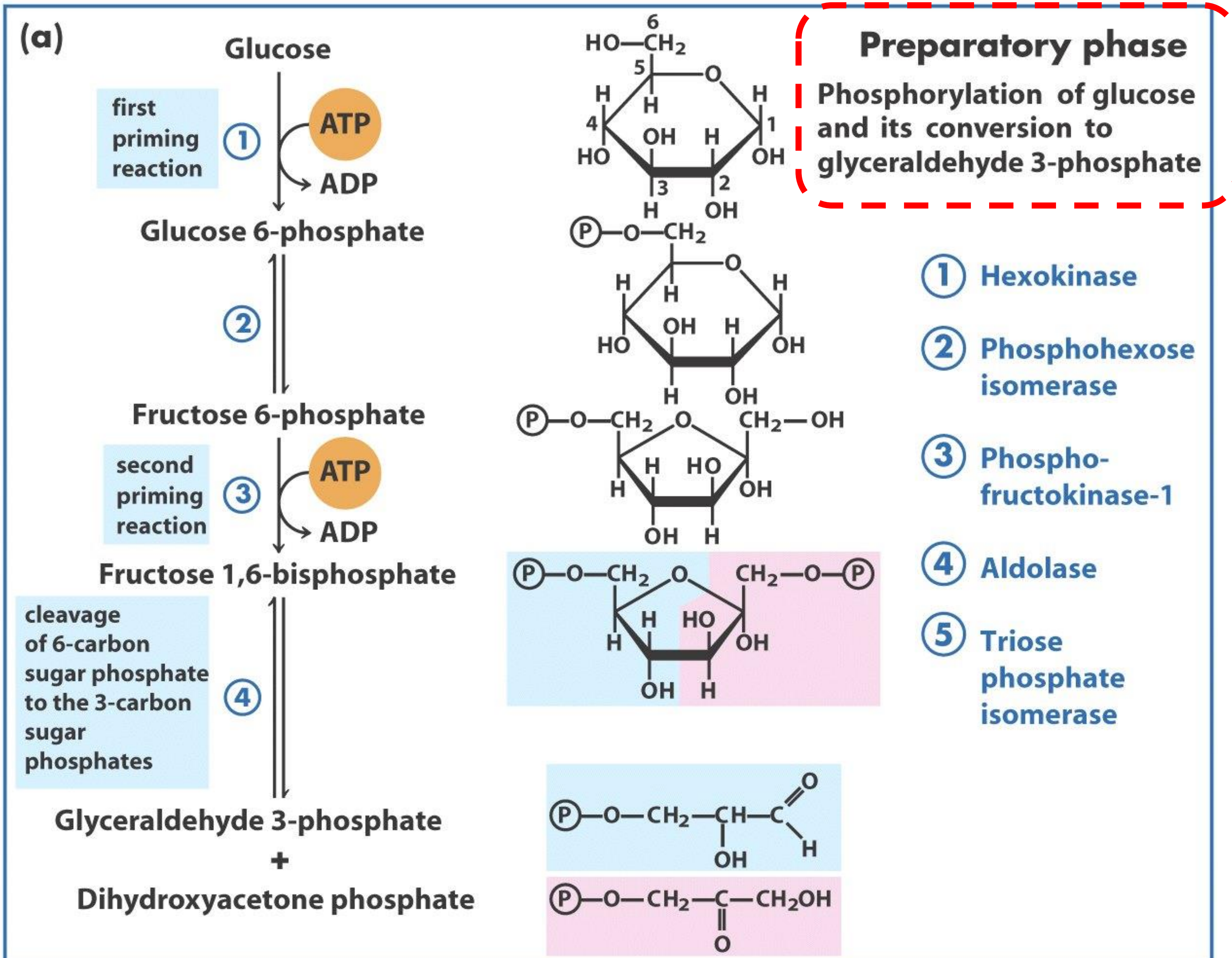
Dihydroxyacetone phosphate — **Glyceraldehyde 3-phosphate**

ketose — $\boxed{\Delta G'^{o} = 7.5 \text{ kJ/mol}}$ — aldose



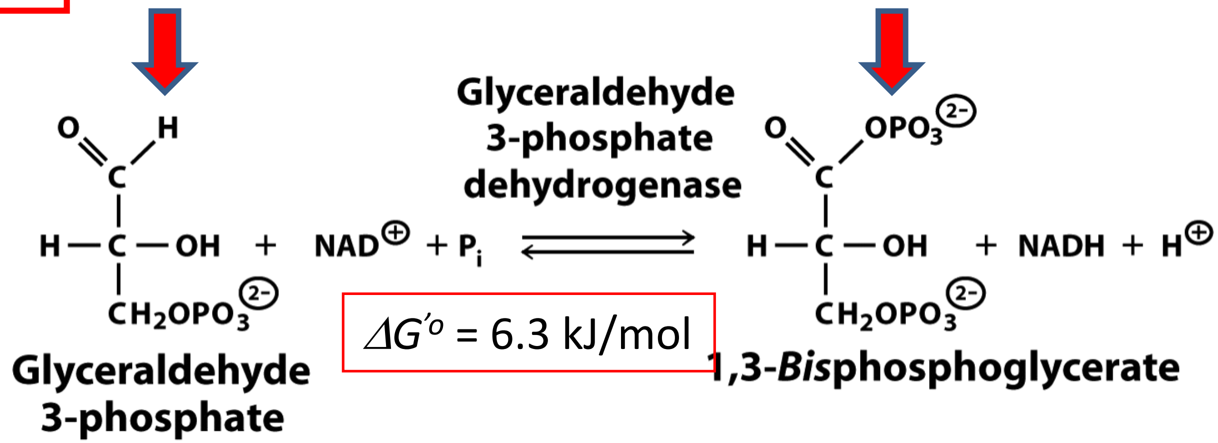
N.B. At equilibrium, 96% of the triose phosphate is dihydroxyacetone phosphate. However, the reaction proceeds readily from dihydroxyacetone phosphate to glyceraldehyde 3-phosphate because of the efficient removal of this product by subsequent reactions.

At this point, *2 molecules of glyceraldehyde 3-phosphate* are formed from *1 glucose* molecule! *2 ATP* have been invested so far!


(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to the 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase

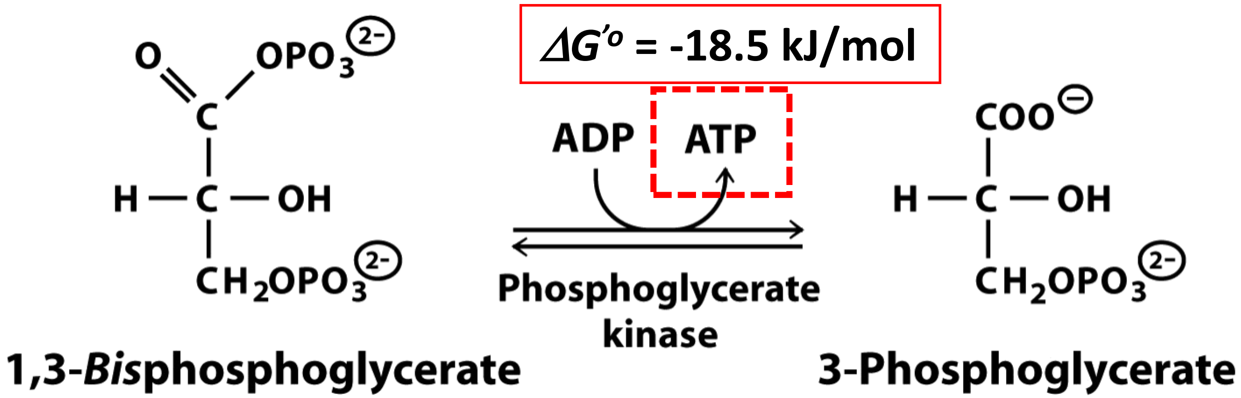
Step (6): Conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate

Start of payoff stage



1,3-Bisphosphoglycerate is an acyl phosphate, and one of its phosphoryl groups is transferred to ADP in the next step in glycolysis.

Step (7): Formation of ATP from 1,3-bisphosphoglycerate

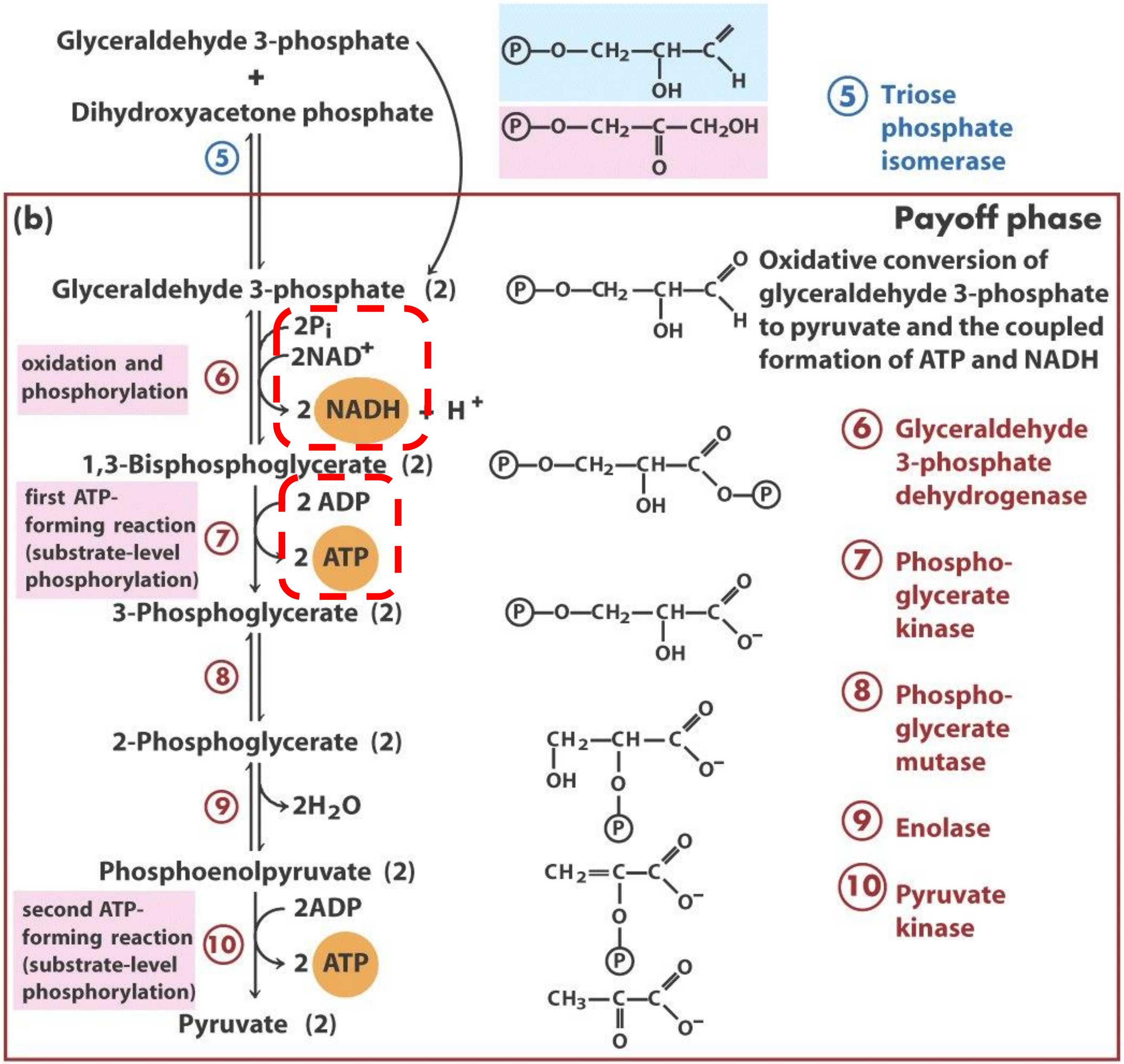


Substrate-level phosphorylation

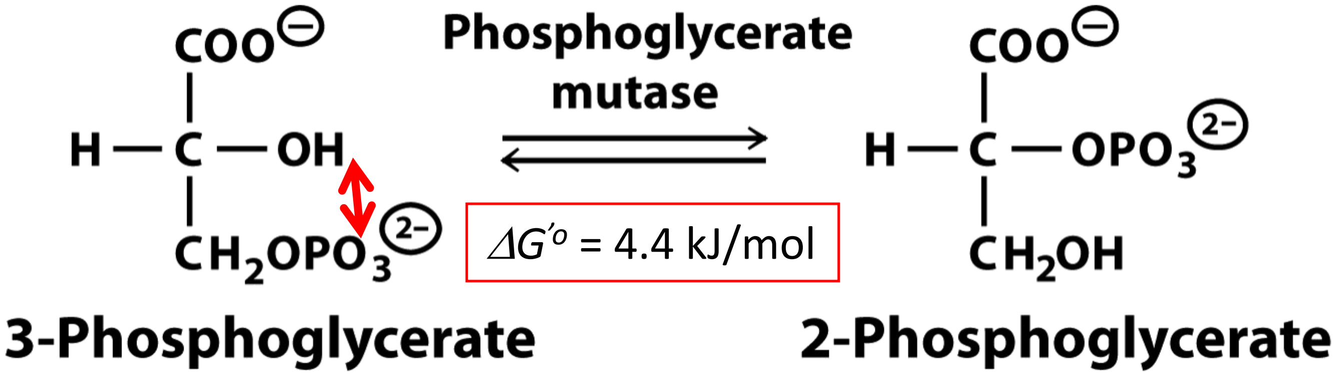
ATP is formed!!!

The formation of ATP by phosphoryl group transfer from 1,3-bisphosphoglycerate is referred to as a **substrate-level phosphorylation**. It occurs in the cytoplasm and involves soluble enzymes and chemical intermediates.

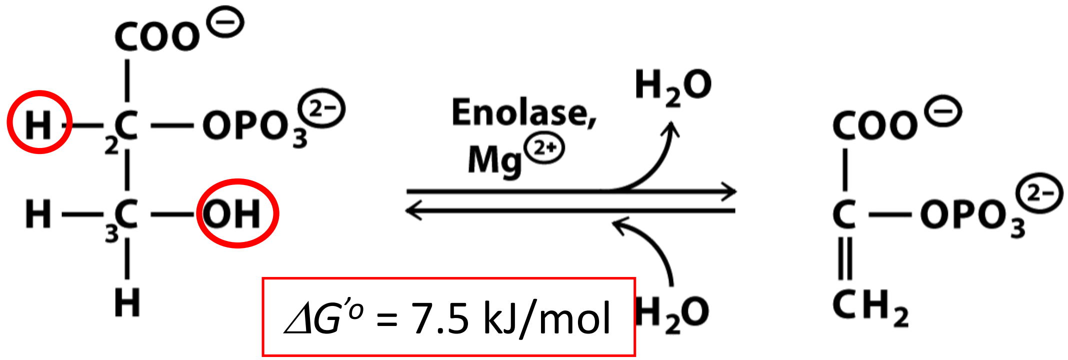
Oxidative phosphorylation in mitochondria.
Photophosphorylation in plants.


Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Triose phosphate isomerase
(b)
Payoff phase
Glyceraldehyde 3-phosphate (2)
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
$2P_i$
$2NAD^+$
2 NADH + H^+
oxidation and phosphorylation
6
Glyceraldehyde 3-phosphate dehydrogenase
1,3-Bisphosphoglycerate (2)
2 ADP
2 ATP
first ATP-forming reaction (substrate-level phosphorylation)
7
Phosphoglycerate kinase
3-Phosphoglycerate (2)
8
Phosphoglycerate mutase
2-Phosphoglycerate (2)
9
$2H_2O$
Enolase
Phosphoenolpyruvate (2)
2ADP
2 ATP
second ATP-forming reaction (substrate-level phosphorylation)
10
Pyruvate kinase
Pyruvate (2)

Step (8): Formation of 2-phosphoglycerate



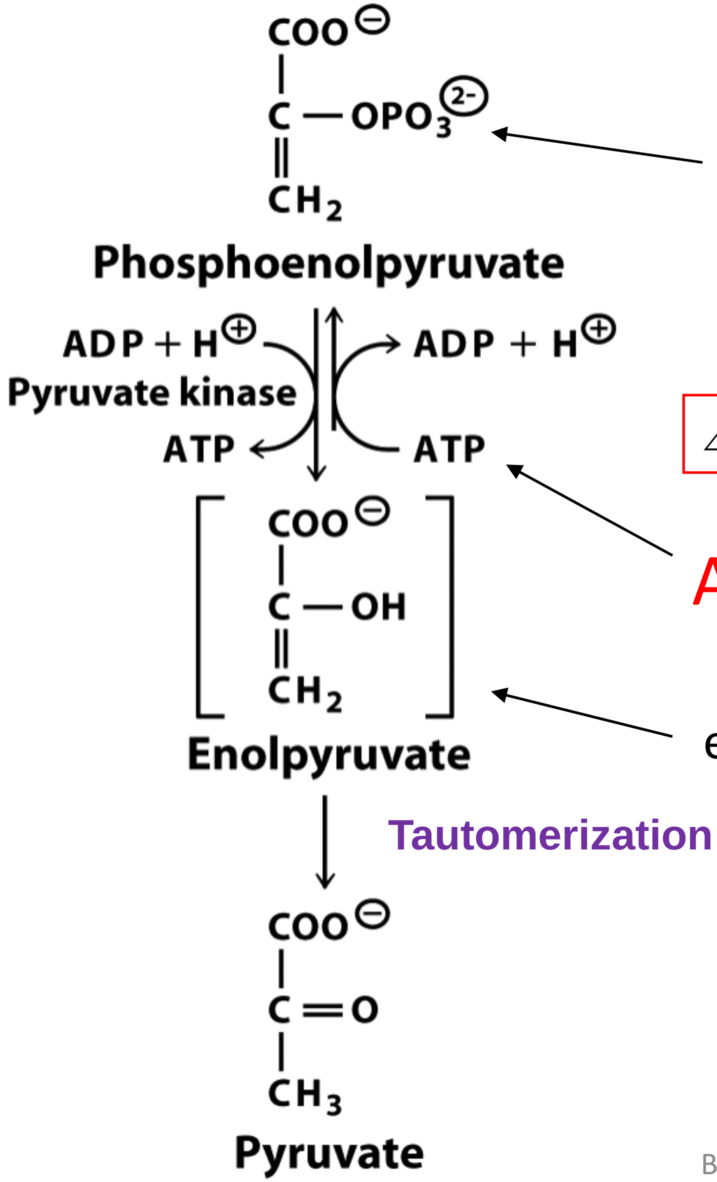
Step (9): Formation of phosphoenolpyruvate



2-Phosphoglycerate   Phosphoenolpyruvate PEP

PEP: one of the highest energy in the cells.

Step (10): Formation of Pyruvate



The phosphoryl group traps the molecule in its unstable enol form. When the phosphoryl group has been donated to ATP, the enol molecule undergoes a conversion into the more stable ketone namely, pyruvate.

$\Delta G'^{o}$ = -31.4 kJ/mol

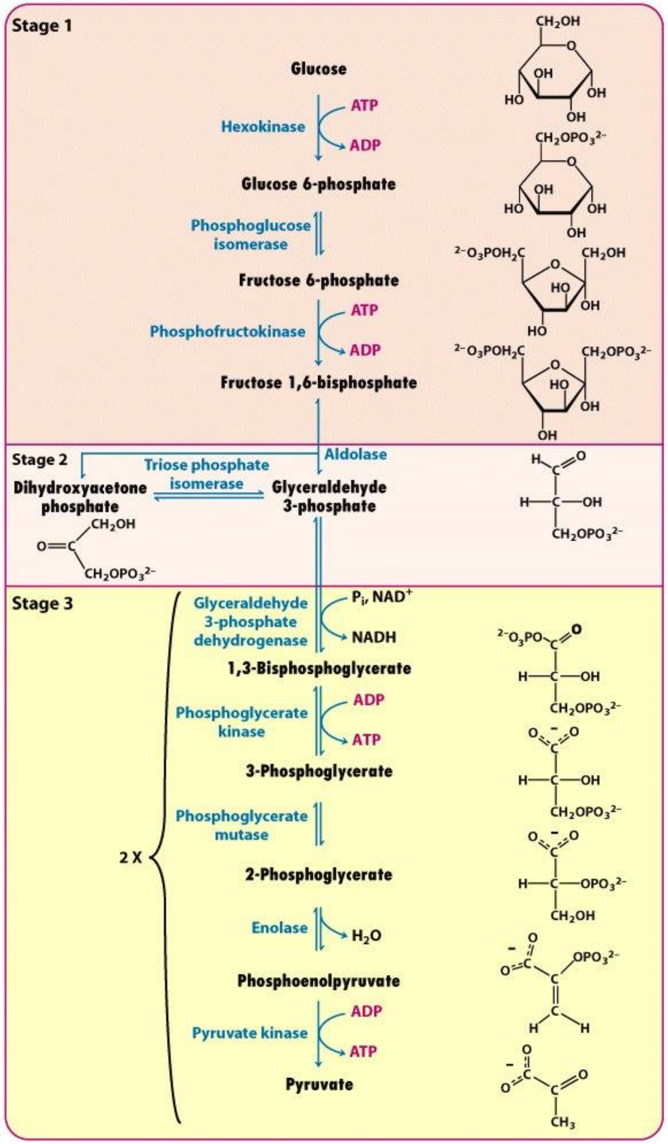
ATP is formed!!!

enol form

The second substrate-level phosphorylation in glycolysis.

"Big bang" – almost enough energy to form a second ATP, but gets lost as heat.

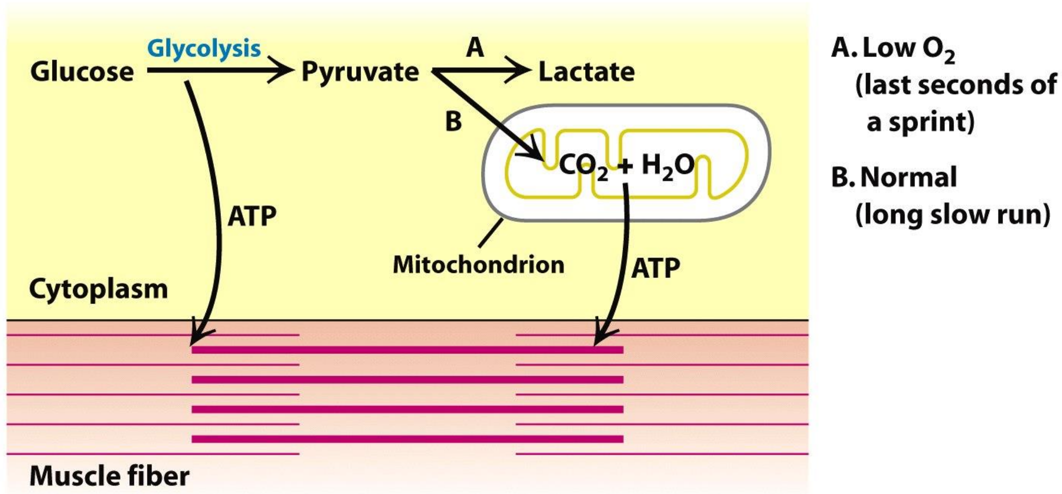
Summary of objectives

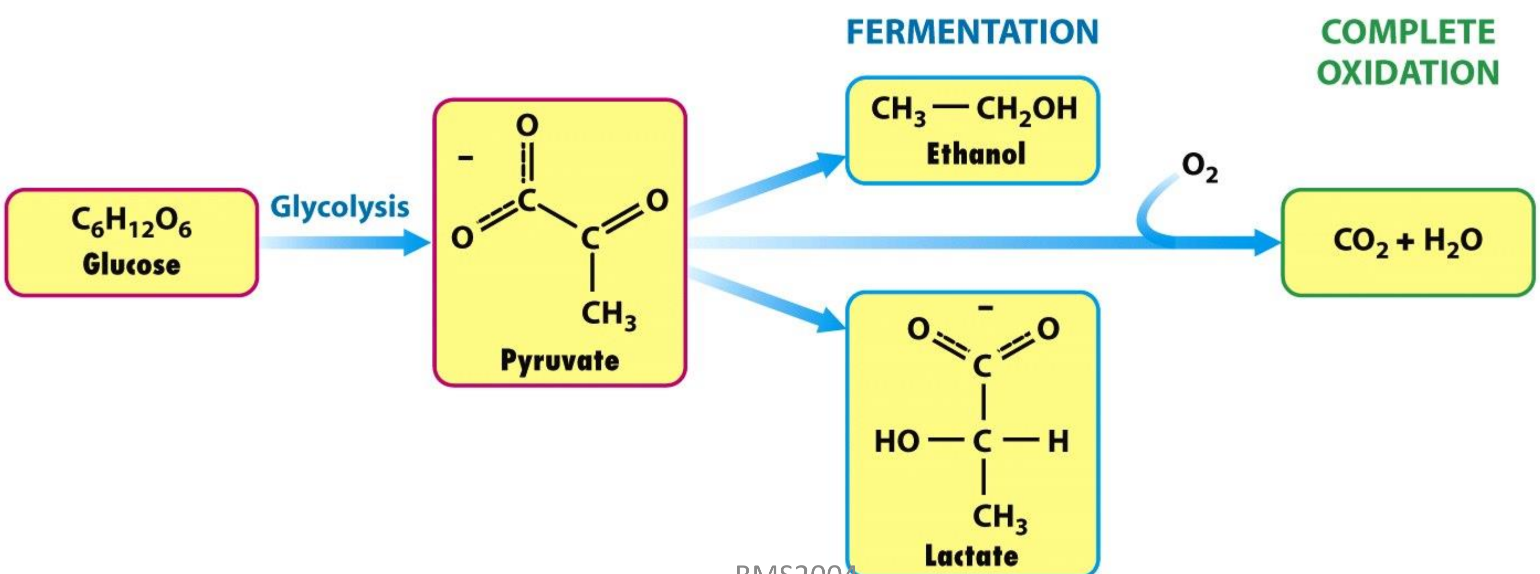


1. Two phases: preparatory and payoff.
2. Not all reactions are energetically favorable.
3. 1 glucose molecule is converted into 2 molecules of pyruvate.
4. Investment of two ATP molecules generates 4 molecules of ATP (per glucose) and 2 molecules of NADH.

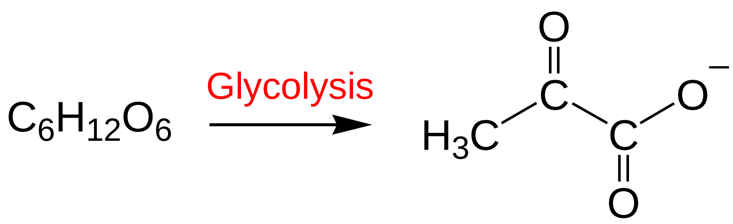
The fates of pyruvate

What happens to pyruvate?





The fate of pyruvate – aerobic



Glucose

Pyruvate

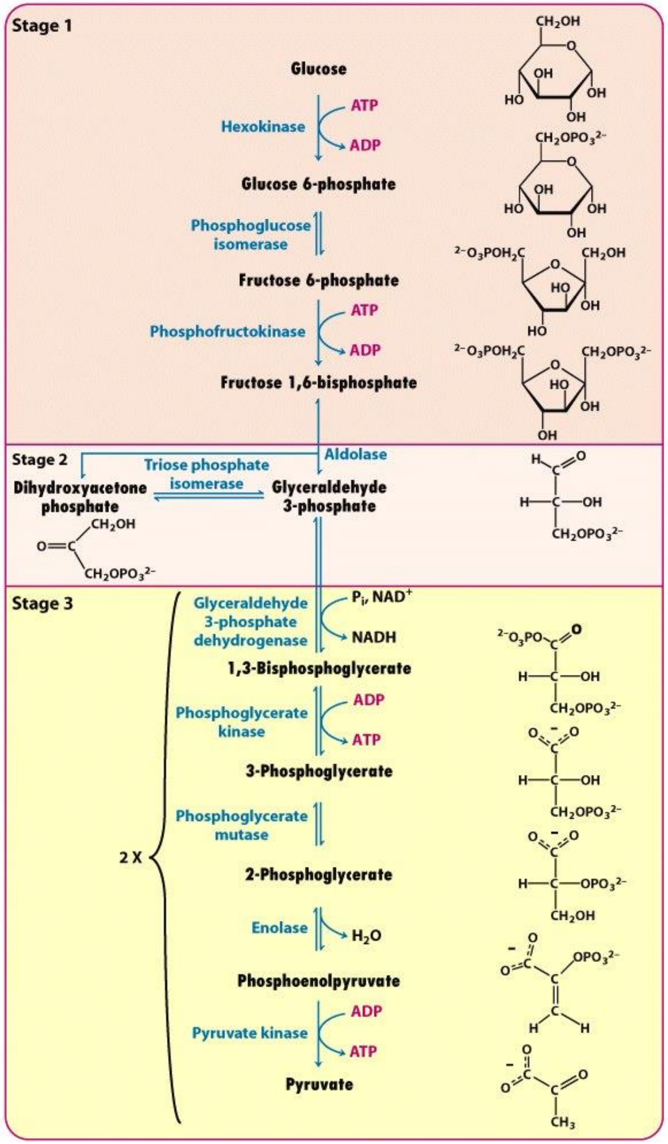
$\longrightarrow$ CO_2 and H_2O + ATP

+O2, citric acid cycle,
oxidative phosphoryation

$$2\ NADH + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O + \text{energy}$$

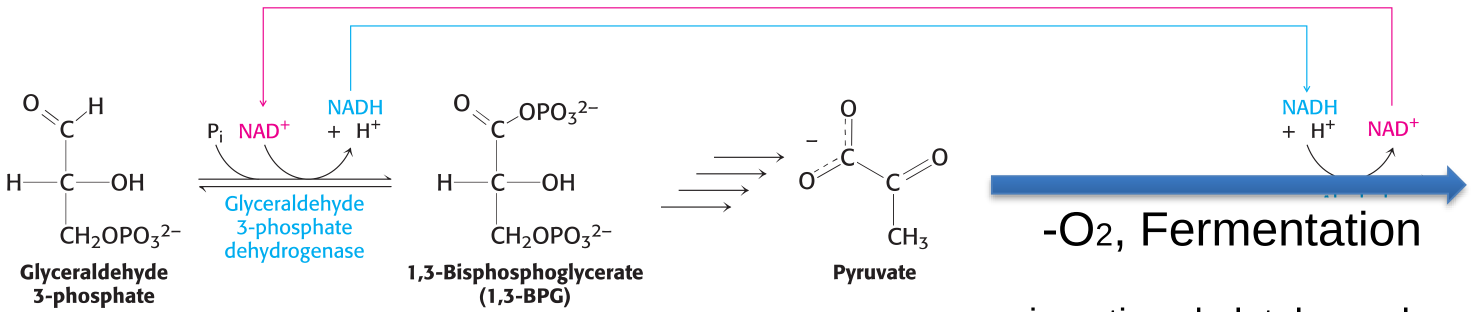
Under aerobic conditions ***pyruvate is oxidized to acetate*** (or acetyl-CoA), which enters the citric acid cycle and is oxidized to CO_2 and H_2O, and NADH formed in glycolysis is reoxidized to NAD^+ by passage of its electrons to O_2 in mitochondria respiration, and this provides more energy for ATP synthesis (see coming sections).

Recycling NAD^+ is important !



$$2\ NADH + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O + \text{energy}$$

The fate of pyruvate in hypoxia



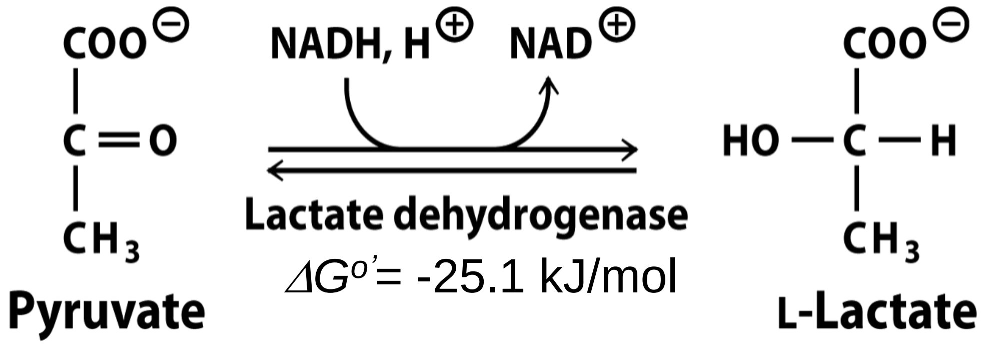
-O_2, Fermentation

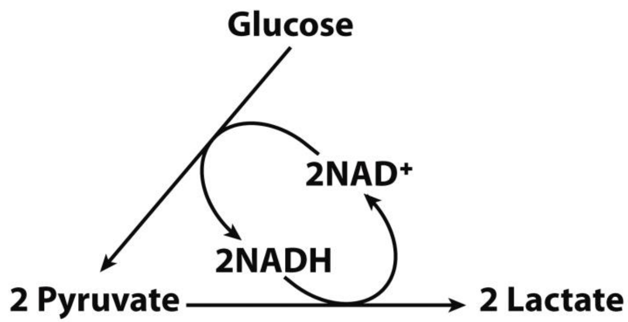
in active skeletal muscles, solid tumor, submerged plant tissues, and lactic acid bacteria.

Under hypoxic conditions, NADH generated by glycolysis cannot be reoxidized by O_2. Failure to regenerate NAD^+ would stop glycolysis and no energy can be produced. Most organisms have the ability to continually regenerate NAD^+ during anaerobic glycolysis by transferring electrons from NADH to form lactate or ethanol.

Fermentation:

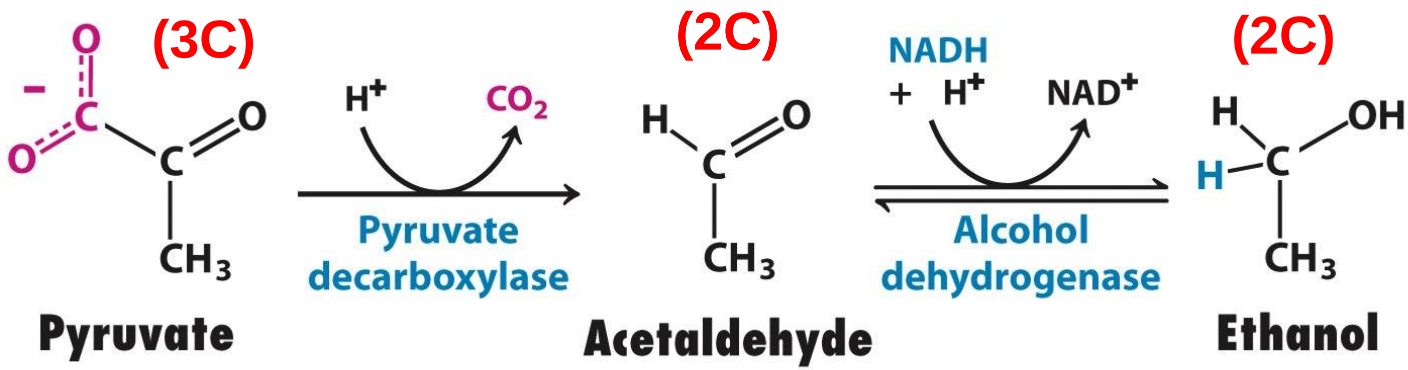
Formation of lactic acid





- The **lactate** formed by active skeletal muscles can be recycled: it is carried in the blood to the liver where it is **converted to glucose** during the recovery from strenuous muscular activity (see later sections).
- When lactate is produced in large amount, the acidification that results from ionization of lactic acid in muscles and blood causes pain and limits the period of vigorous activity.
- Erythrocytes have no mitochondria and thus cannot reoxidize pyruvate to carbon dioxide. These cells produce lactate even in the presence of oxygen.
- Thus through glycolysis and fermentation, the conversion of one molecule of glucose yields two lactate ions and two molecules of ATP.

Alcoholic Fermentation



- Yeast and other microorganisms ferment glucose to ethanol and CO_2. Pyruvate is converted to ethanol and CO_2 in two steps.
- One molecule of glucose produces 2 molecules of ethanol, 2 molecules of carbon dioxide and 2 molecules of ATP.

1 glucose → 2 ethanol + 2 CO_2 + 2 ATP

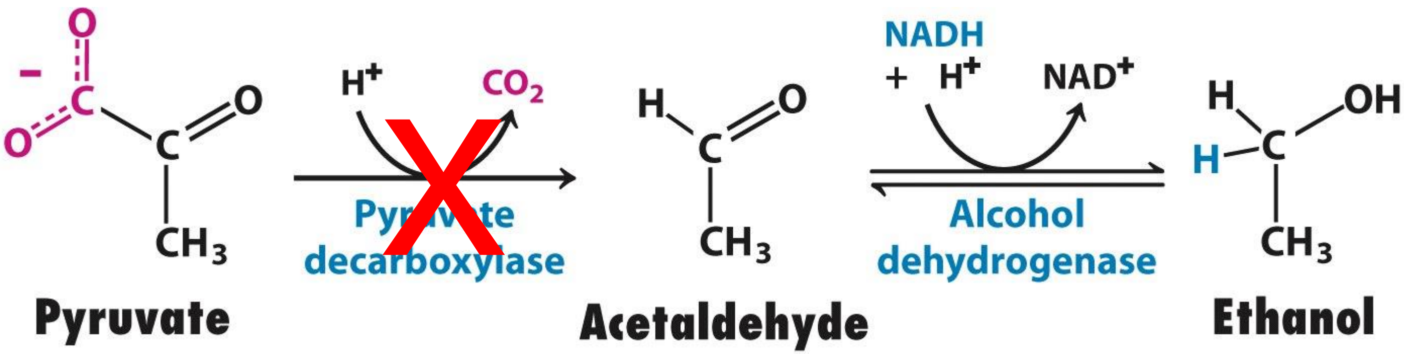
Carbon dioxide release in fermentation

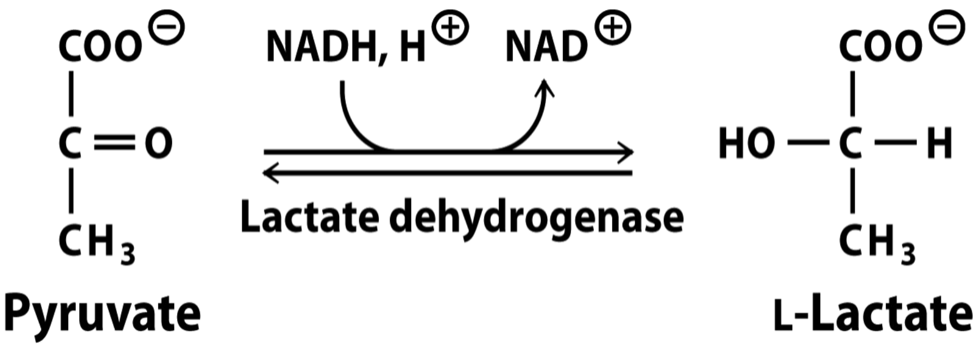
- Pyruvate decarboxylase is present in brewer's and **baker's yeast** (*Saccharromyces cerevisiae*), plants, and other microorganisms.

dough

- Carbon dioxide released in the reaction can be seen in the bottles of champagne and in the dough of bread.

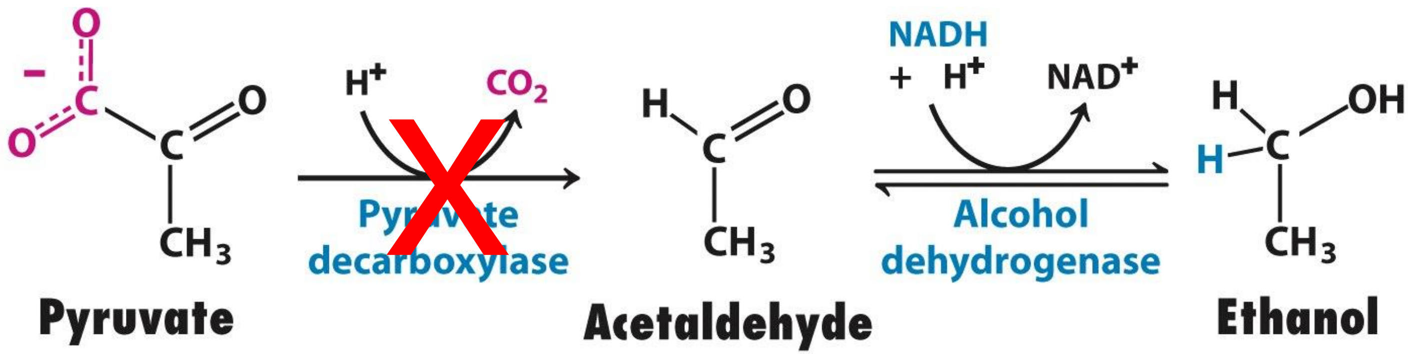
What about animals?





- ❑ In animals including human, pyruvate decarboxylase is not present.
- ❑ Therefore, pyruvate needs to go down the lactate pathway.

But we have the other half to consume ethanol

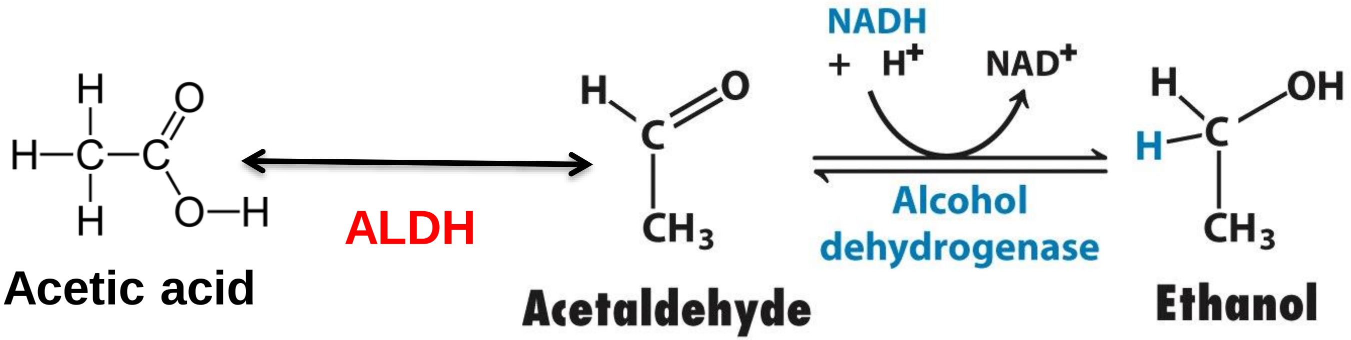


So it is not always right:

What is true of E coli. is true of the elephant.

--- Jacques Monod

What is the product of ethanol consumption?



- ❑ In many organisms including human, alcohol dehydrogenase is present to convert ethanol back to acetaldehyde.

- ❑ Ethanol in the blood of human is converted back into acetaldehyde by **alcohol dehydrogenase (ADH)**, which is further oxidized to acetic acid by **aldehyde dehydrogenase (ALDH)**. High level of acetaldehyde (in alcoholics) exerts its toxic effect by inhibiting mitochondrial functions.

- ❑ Half of the east Asians have a genetic variant of the **aldehyde dehydrogenase (ALDH)**, which is less efficient at converting acetaldehyde into acetate. When acetaldehyde builds up, one would have flushing of their face, hot sensations, nausea, and palpitation.

Some common foods made by fermentation

Microorganism	Starting material	Fermentation product	Food Product
Lactobacillus bulgaricus (lactic acid bacteria)	milk	lactic acid	yogurt
Propionibacterium freudenreichii	milk	Propionic acid and CO_2	Swiss cheese

The acids produced in fermentation can lower the pH to denature and precipitate the proteins in the process and make the food sour in taste.

Other foods made by fermentation: pickles, sauerkraut, sausage, soy sauce, kimchi.

Summary of objectives

1. The fate of pyruvate depends on the availability of oxygen.
2. With oxygen – citric acid cycle.
3. Low oxygen – fermentation.
4. Reason – recycle of NAD^+.