THE MINISTRY OF PUBLIC HEALTH OF UKRAINE
ZAPOROZHYE STATE MEDICAL UNIVERSITY
Department of biochemistry and laboratory diagnostics

# Carbohydrate Metabolism I:

- Aerobic oxidation of glucose
- Anaerobic Glycolysis
- Gluconeogenesis

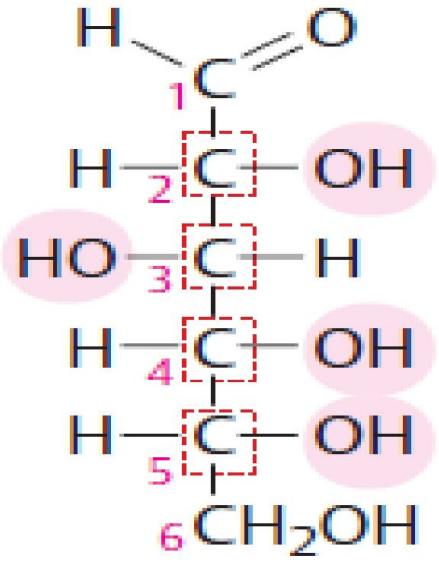
by Rudko N.P., 2011

# OBJECTIVES in Carbohydrate Metabolism

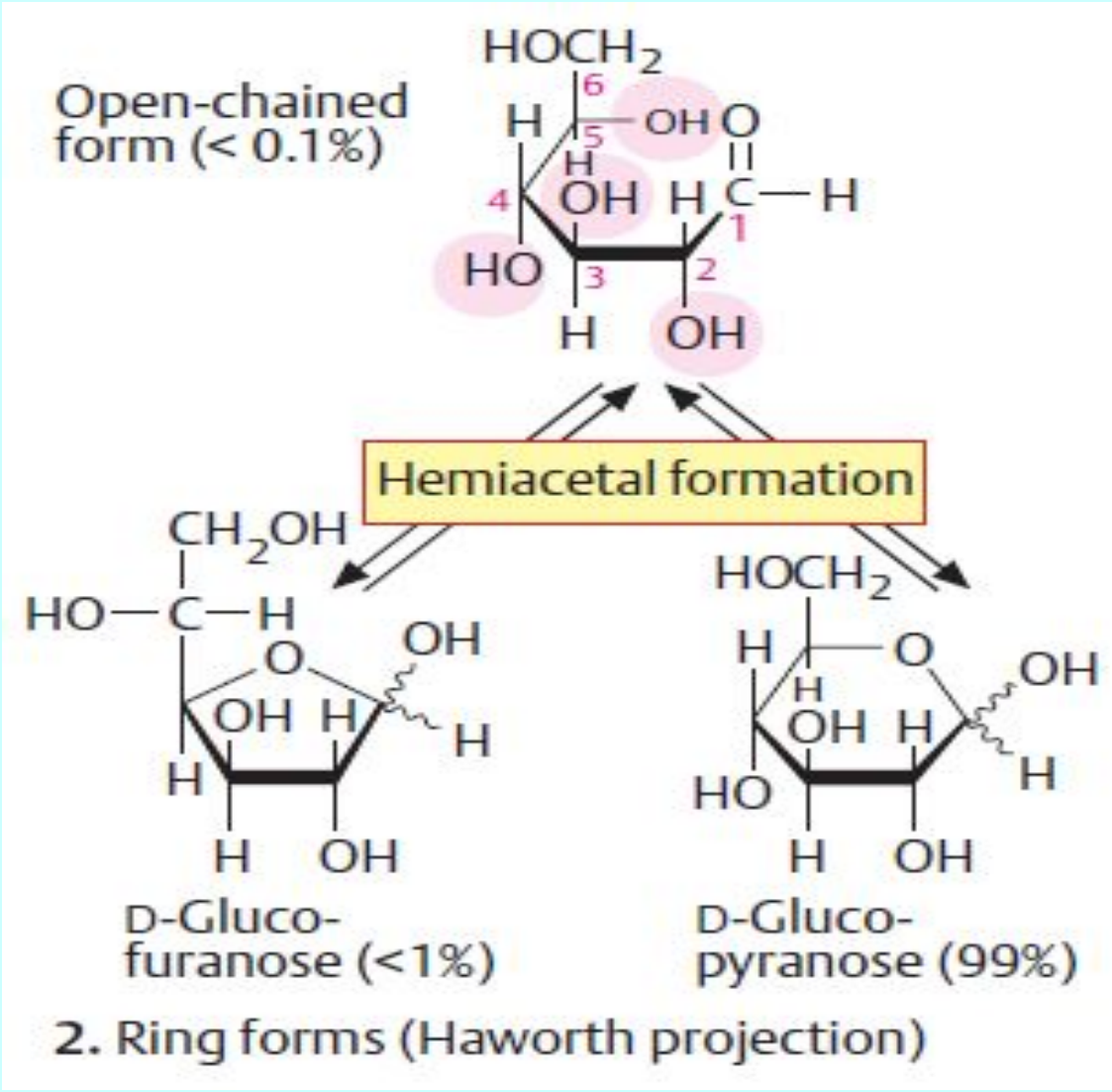
Consider the main metabolic pathways
(the intermediates, enzymes, cofactors and regulation)
for carbohydrate metabolism:

1) Aerobic oxidation of glucose (complete degradation to $CO_2$&$H_2O$)
2) Glycolysis
3) Gluconeogenesis
4) Pentose Phosphate Pathway
5) Glycogenesis
6) Glycogenolysis

# Glucose Structure




Open-chained form of glucose

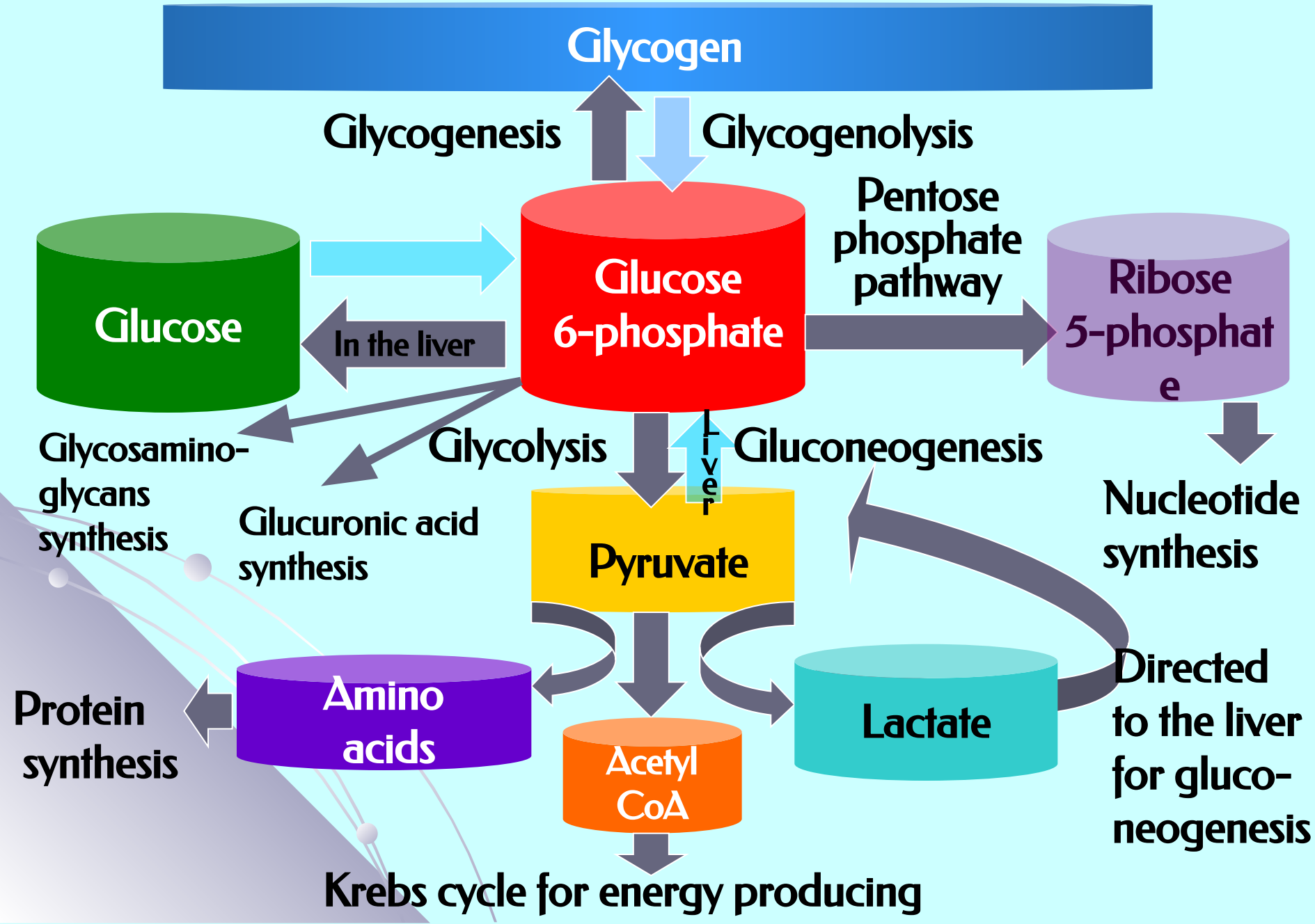
# Carbohydrate are Classified as:

**Monosaccharides:** glucose, galactose, fructose, ribose and deoxyribose

**Oligosaccharides:** sucrose (G-F), lactose (G-Gal), maltose (G-G)

**Polysaccharides:**
**homo**: glycogen, starch, cellulose
**hetero:** mucopolysaccharides such as: hyaluronic acid, heparine

# The most significant fates for glucose



# Carbohydrate Metabolism Processes that Yield Energy

1. **Tissue respiration** (**with oxygen** ): Break down 6C sugars to $CO_2$ and $H_2O$; most efficient source of energy. 70-75% of glucose are utilized through this way.
2. **Fermentation** (**without oxygen**) (in animals it is usually called **anaerobic glycolysis**): Break down 6C sugars to 3C (or 2C in yeast) compounds to derive some energy

# Tissue Respiration (Aerobic Oxidation) for Glucose Consists of 3 Main Phases:

1. Aerobic glycolysis & oxidative decarboxylation of pyruvate
2. Krebs cycle
3. Electron transport in ETC

# Aerobic Glycolysis

- **Definition:**
  Aerobic Glycolysis is the metabolic pathway in which monosaccharides (mainly glucose) are split into two molecules of pyruvate
- **Location** in the body : all type cells
- **Location** within the cell : cytosol
- **Substrates:** Glucose, galactose, fructose
- **Products:** 2 pyruvates & 2 ATP & 2 NADH

## Net reaction for aerobic Glycolysis:

$$C_6H_{12}O_6 + 2\,NAD^+ + 2ADP + 2P_i \rightarrow 2\,CH_3\text{-}CO\text{-}COOH + 2\,NADH + 2H^+ + 2ATP$$

# Functions of aerobic Glycolysis :

1) to convert glucose to pyruvate which can be:
   - burned for energy (due to PDH and TCA)
   - or converted to fatty acids, cholesterol, amino acids synthesis, etc.

2) such intermediate as dihydroxyacetone phosphate can be reduced to glycerol phosphate either
   - for use in the biosynthesis of lipids or
   - for reducing equivalents transfer from cytosolic NADH into mitochondrion (glycerol phosphate shuttle)

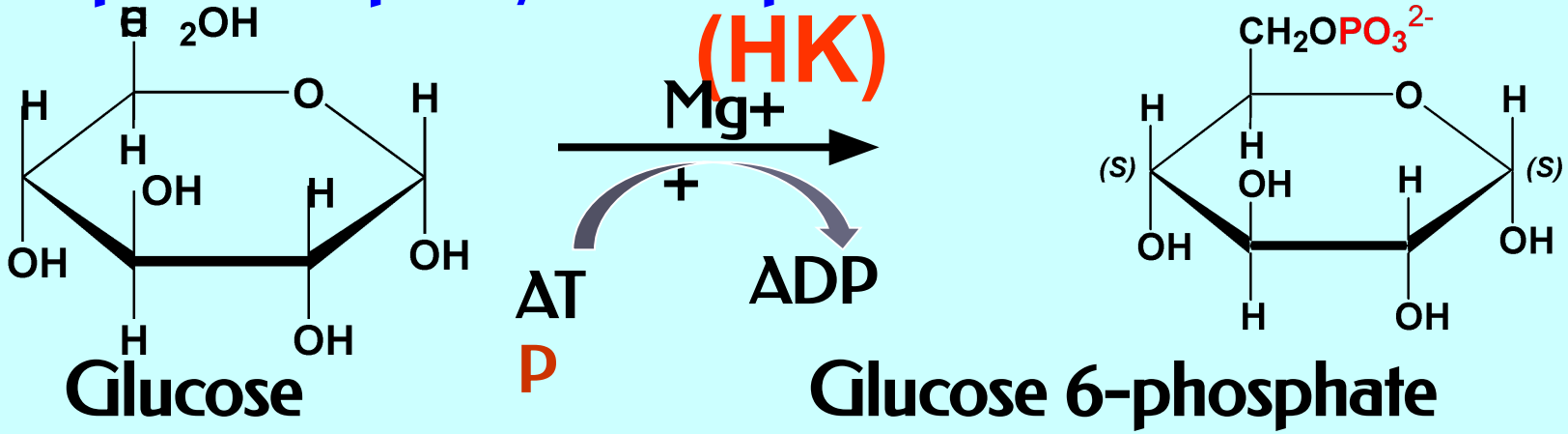
3) the reversible reactions of glycolysis in opposite direction of duration are used for gluconeogenesis

# Glycolysis reactions: overview

| 1 | 2 | 3 |
| --- | --- | --- |
| Add phosphoryl groups to activate glucose | Convert the phosphorylated intermediates into high energy phosphate compounds | Couple the transfer of the phosphate to ADP to form ATP |

# Preparatory Phase

## Step 1: Phosphorylation of Glucose Hexokinase (HK)




Glucose

Glucose 6-phosphate

Phosphorylation makes hexose unable to move or be transported out of the cell

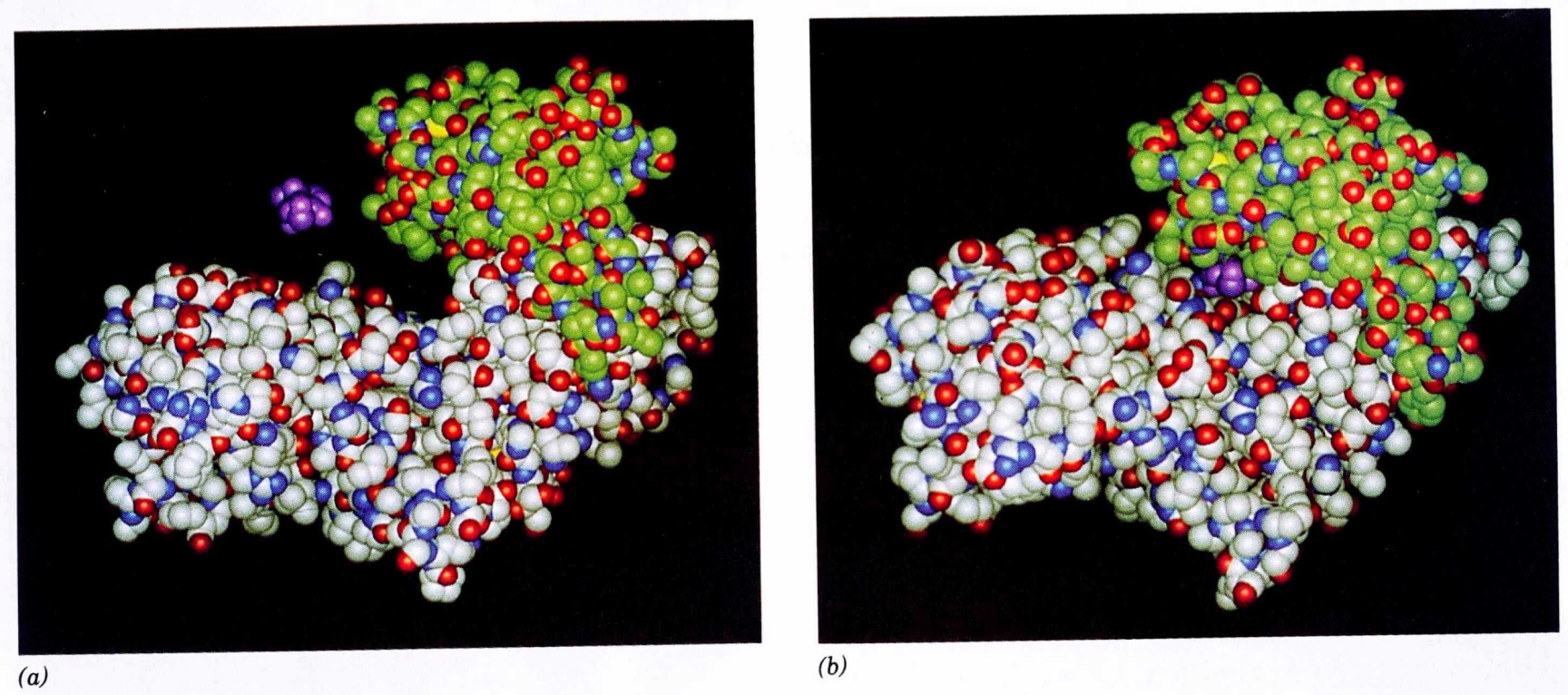
HK is a point for regulation of glycolysis

HKs are tissue specific isozymes:

Glucokinase is in the liver for control of blood glucose levels

Hexokinases are in muscles, brain and other tissues for trapping glucose from blood and its further utilization

# Yeast hexokinase




Binding of glucose (purple) causes a large conformational change

# Hexokinase characteristics

There are four important mammalian hexokinase isozymes. They are designated hexokinases I, II, III, and IV

**Hexokinases I, II, and III:**

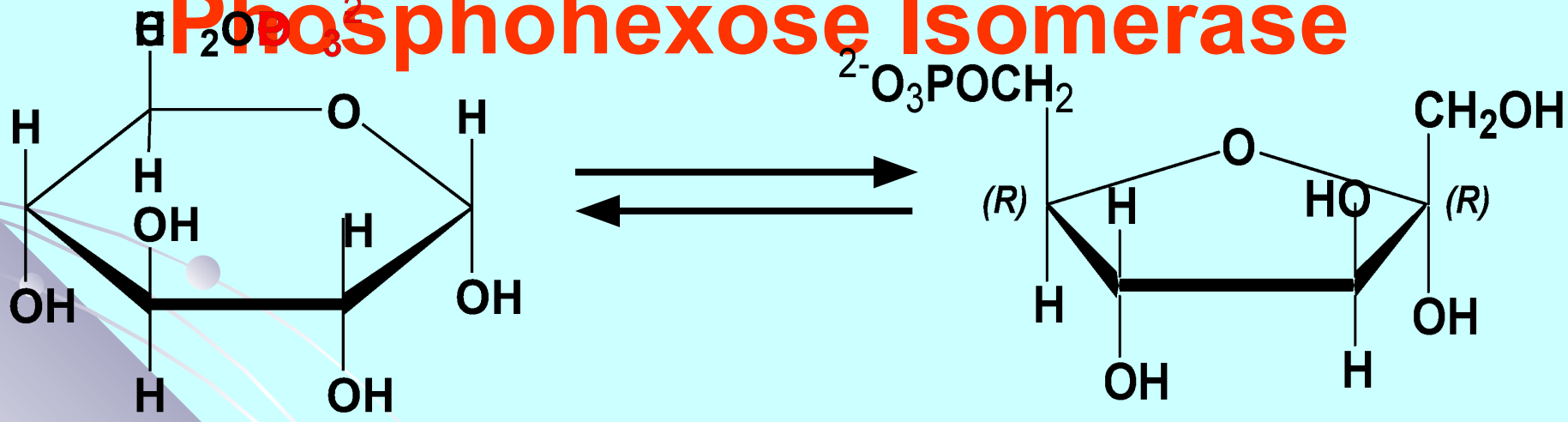
- are referred to as "low-Km" isozymes;
- also phosphorylate other hexose sugars;
- are inhibited by glucose 6-phosphate;

**Hexokinase IV**, also referred to as **glucokinase**:

- its Km for glucose is 100 times higher than that of hexokinases I, II, and III;
- phosphorylates only glucose;
- it is not allosterically inhibited by glucose-6-phosphate

# Step 2: Conversion of glucose 6-phosphate to fructose 6-phosphate

Phosphohexose Isomerase

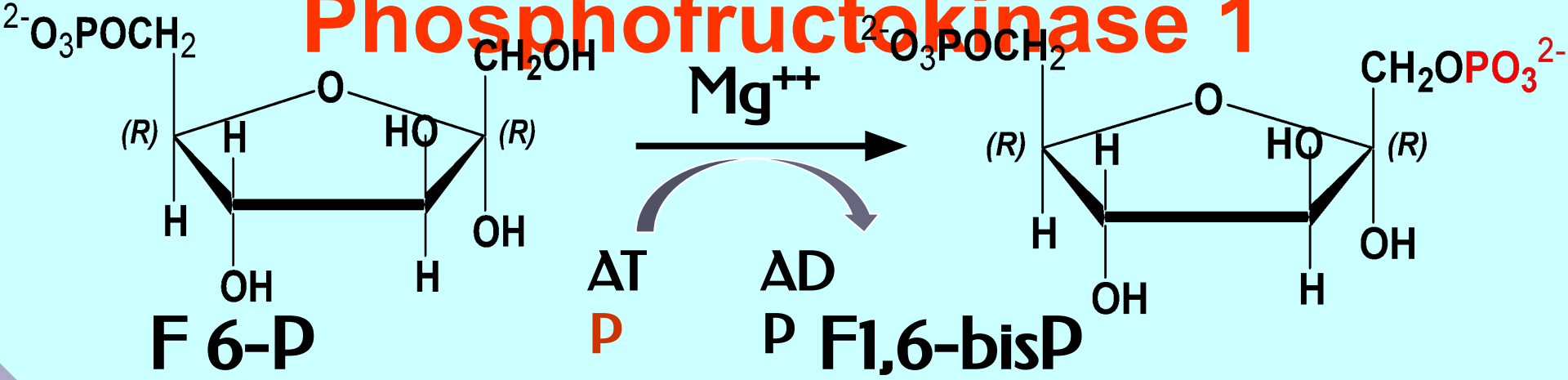



G6-P

F 6-P

# Step 3: Phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate

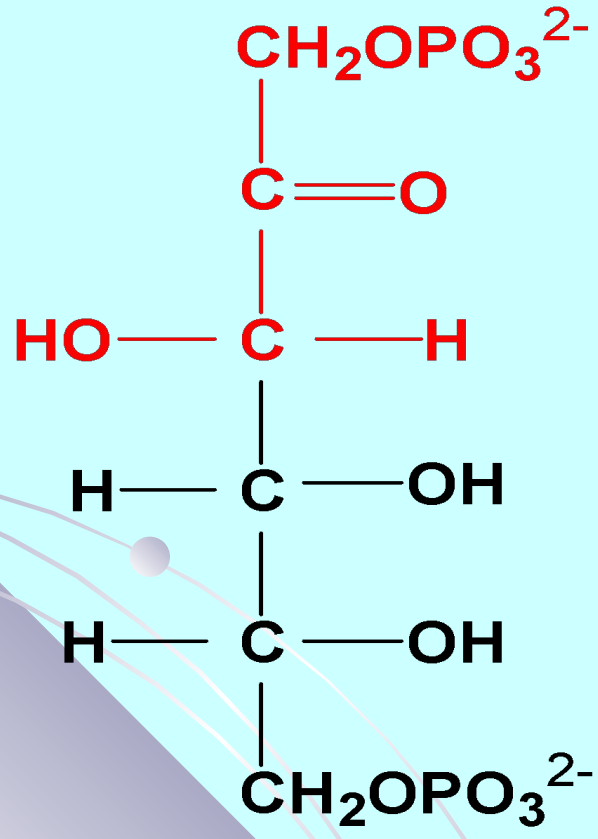
## Phosphofructokinase 1

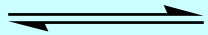


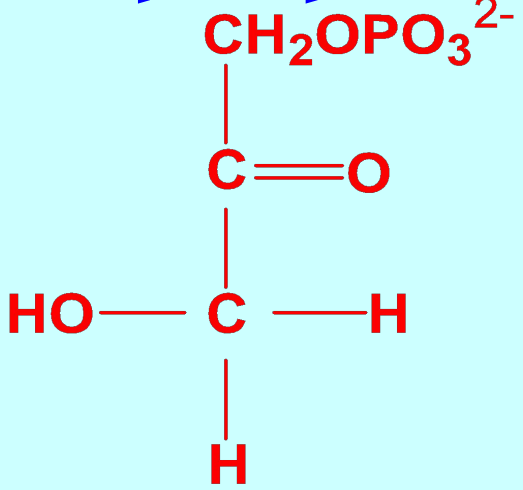

1. Rate limiting step in glycolysis
2. Irreversible step
3. The control point for regulation of glycolysis

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

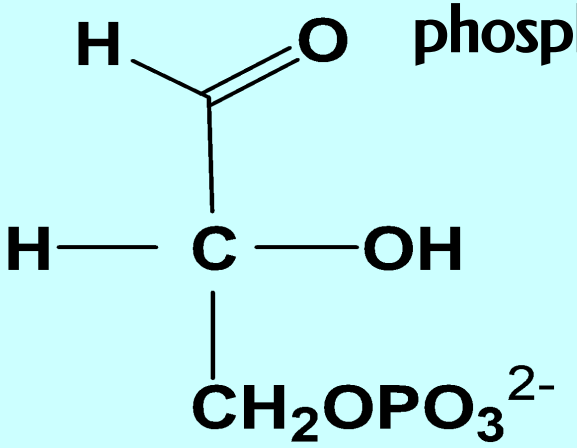
## Aldolase A





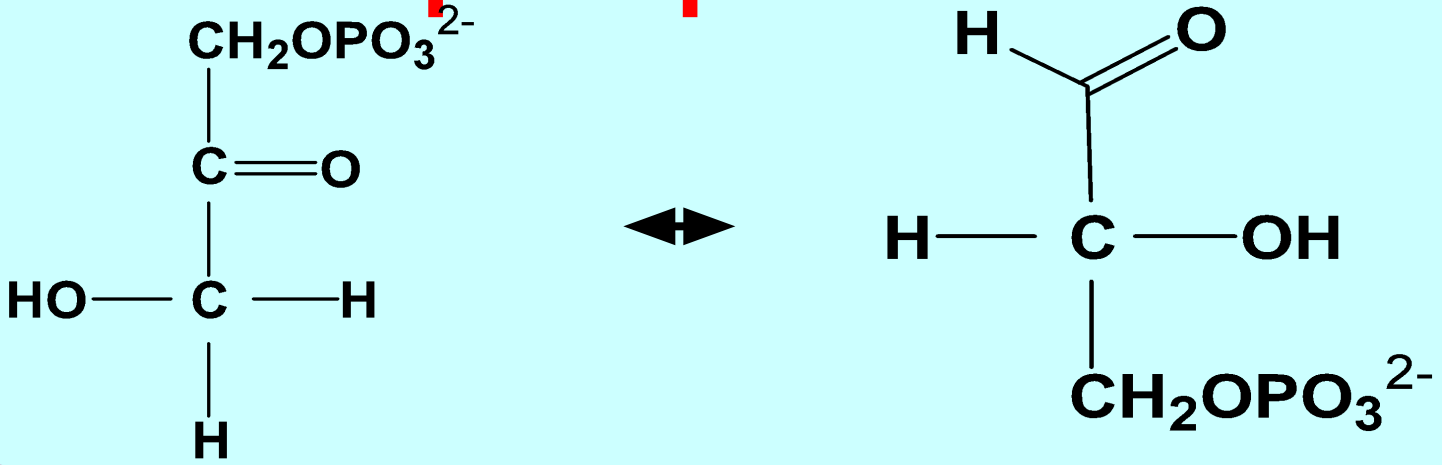

Dihydroxyacetone phosphate (DHAP)




Glyceraldehyde-3-phosphate (GAP)

# Step 5: Interconversion of the triose phosphates

## Triosephosphate isomerase




DHAP

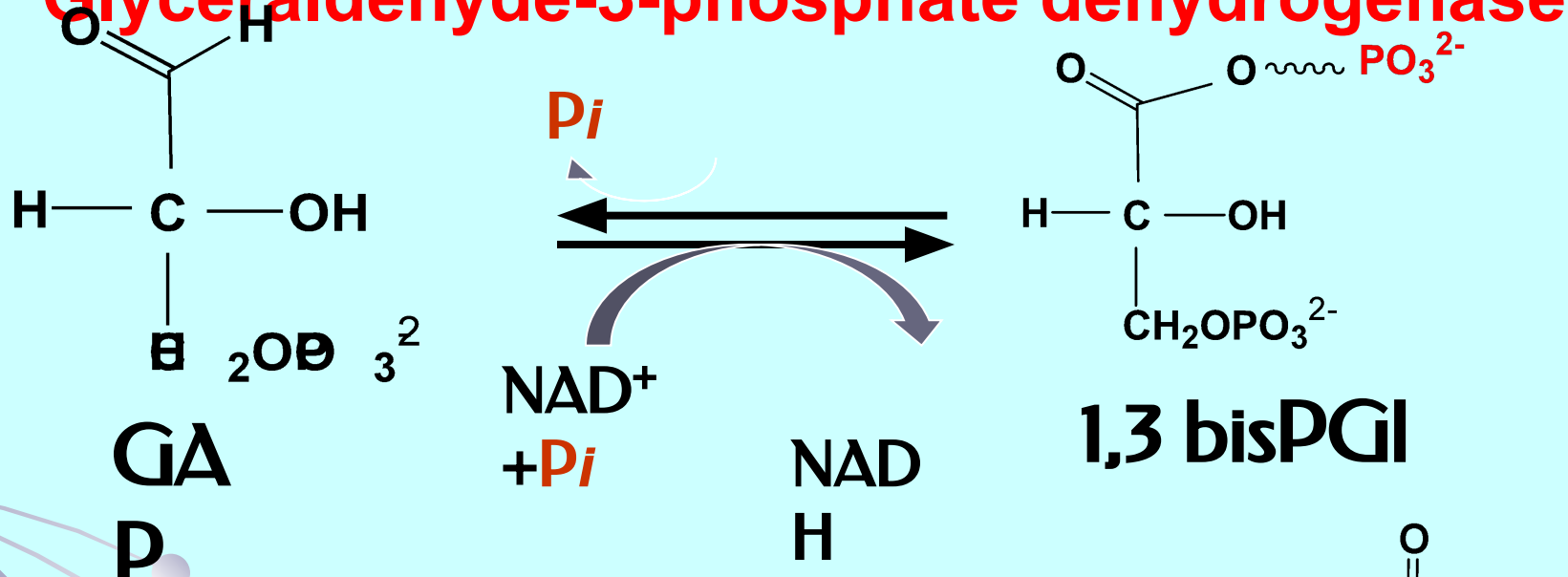
GAP

$$K_{eq} = \frac{[GAP]}{[DHAP]} = 4.7x10^{-2} = \frac{1}{96}$$

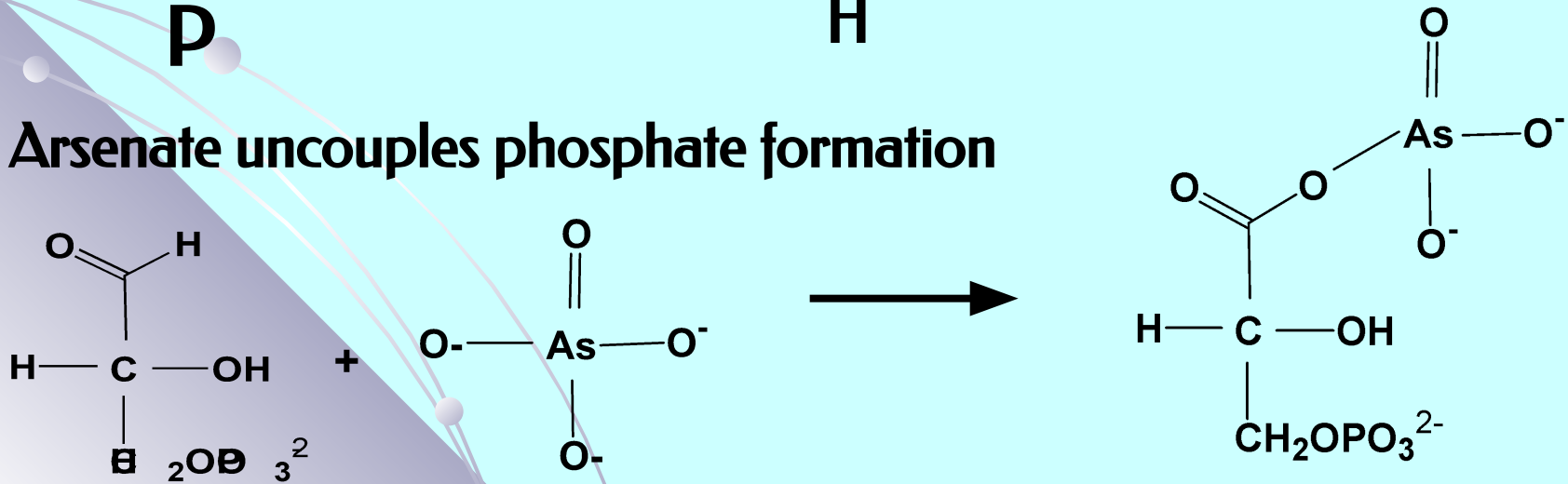
A rapid equilibrium allows GAP to be used and DHAP to replace the used GAP

# Step 6: Oxidation of glyceraldehyde 3-phosphate to 1, 3-bisphosphoglycerate

## Glyceraldehyde-3-phosphate dehydrogenase



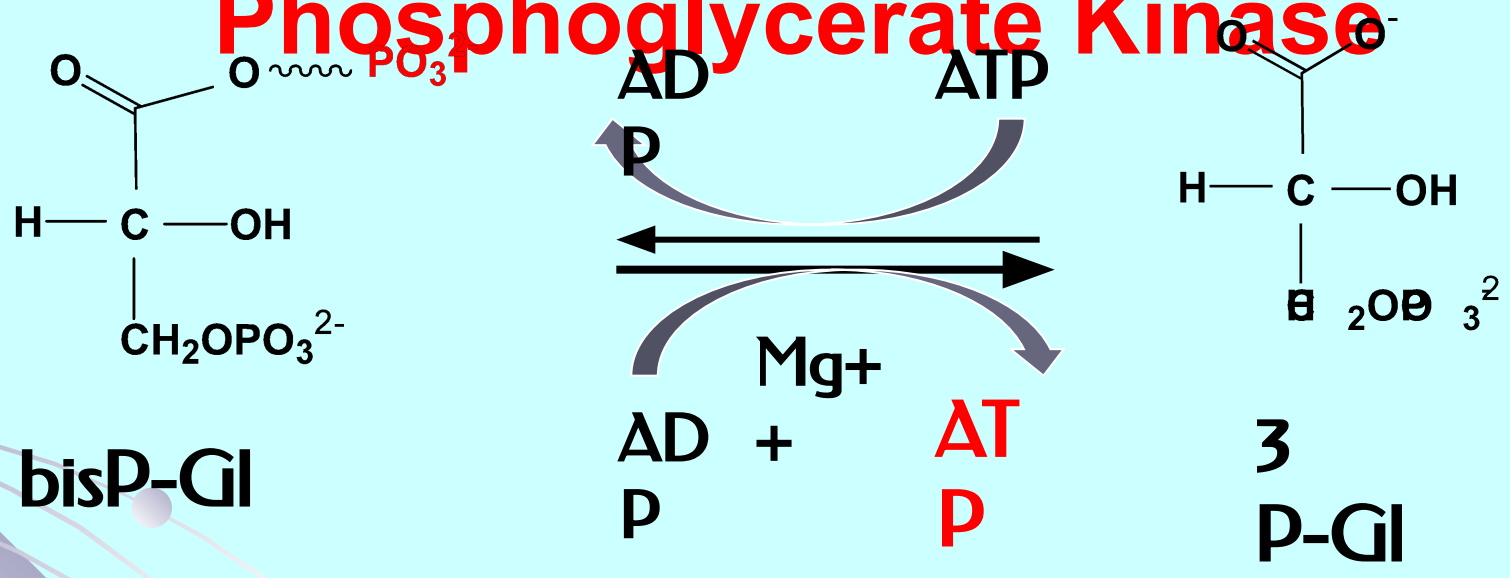

Arsenate uncouples phosphate formation



# Step 7: Phosphoryl transfer from 1,3-bisphosphoglycerate to ADP

## First ATP generation step

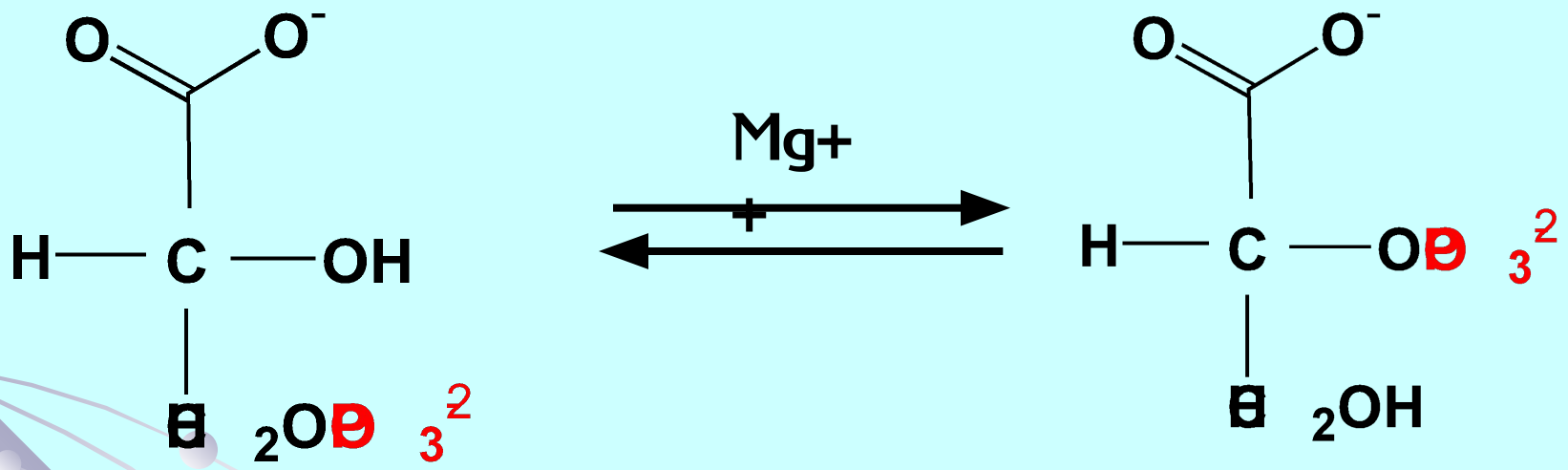
## Phosphoglycerate Kinase




Transfer of a phosphate from a substrate to ADP directly is called "substrate-level phosphorylation"

# Step 8: Conversion of 3-phosphoglycerate to 2-phosphoglycerate

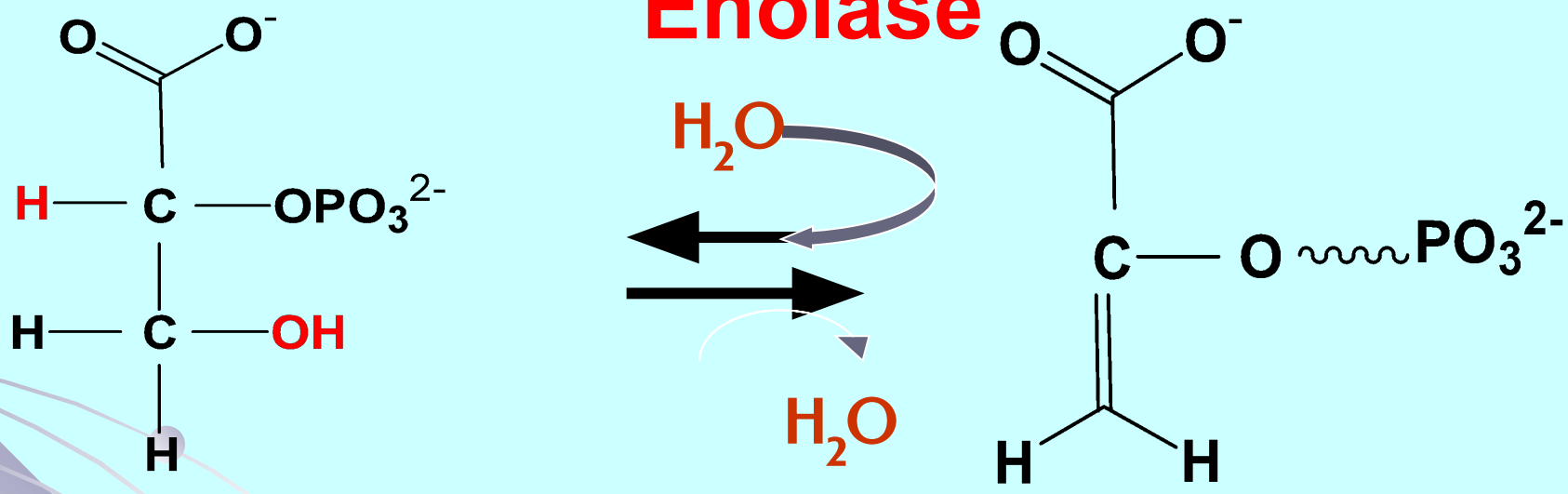
## Phosphoglycerate mutase




3 P-Gl

2 P-Gl

# Step 9: Dehydration of 2-phosphoglycerate to phosphoenolpyruvate



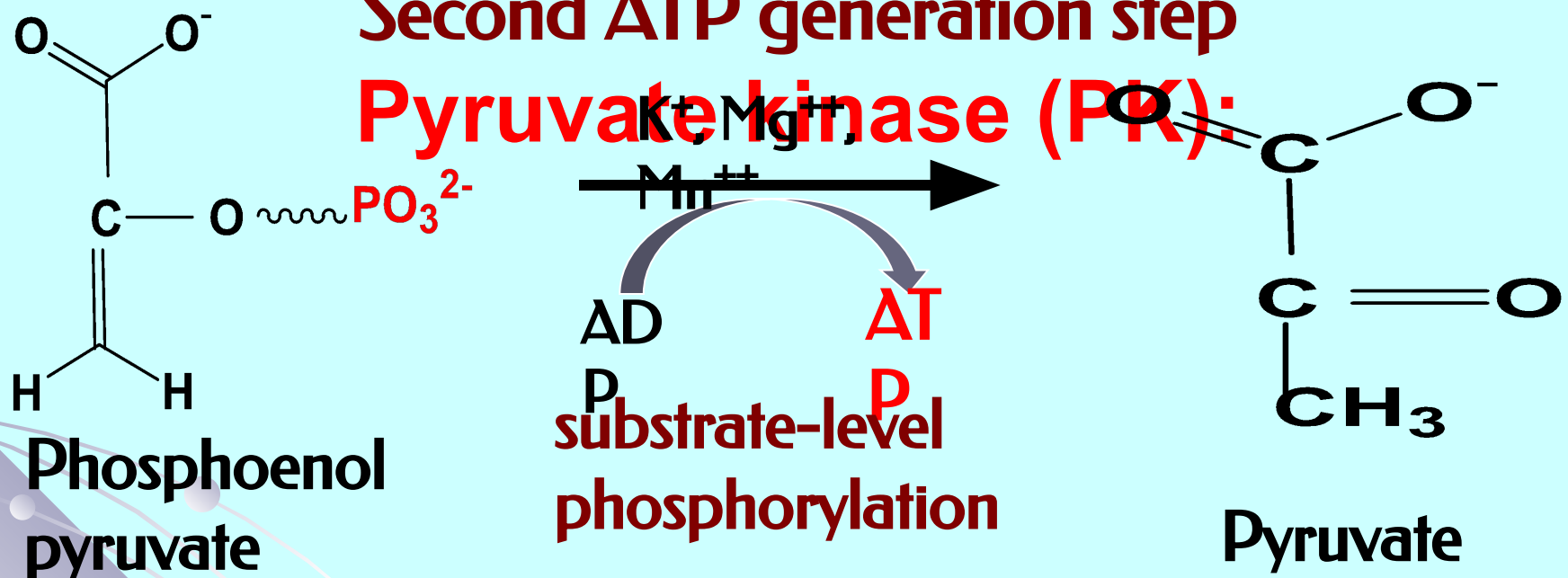

2 P-Gl

Phosphoenol pyruvate

# Step 10: Transfer of the phosphoryl group from phosphoenolpyruvate to ADP

## Second ATP generation step

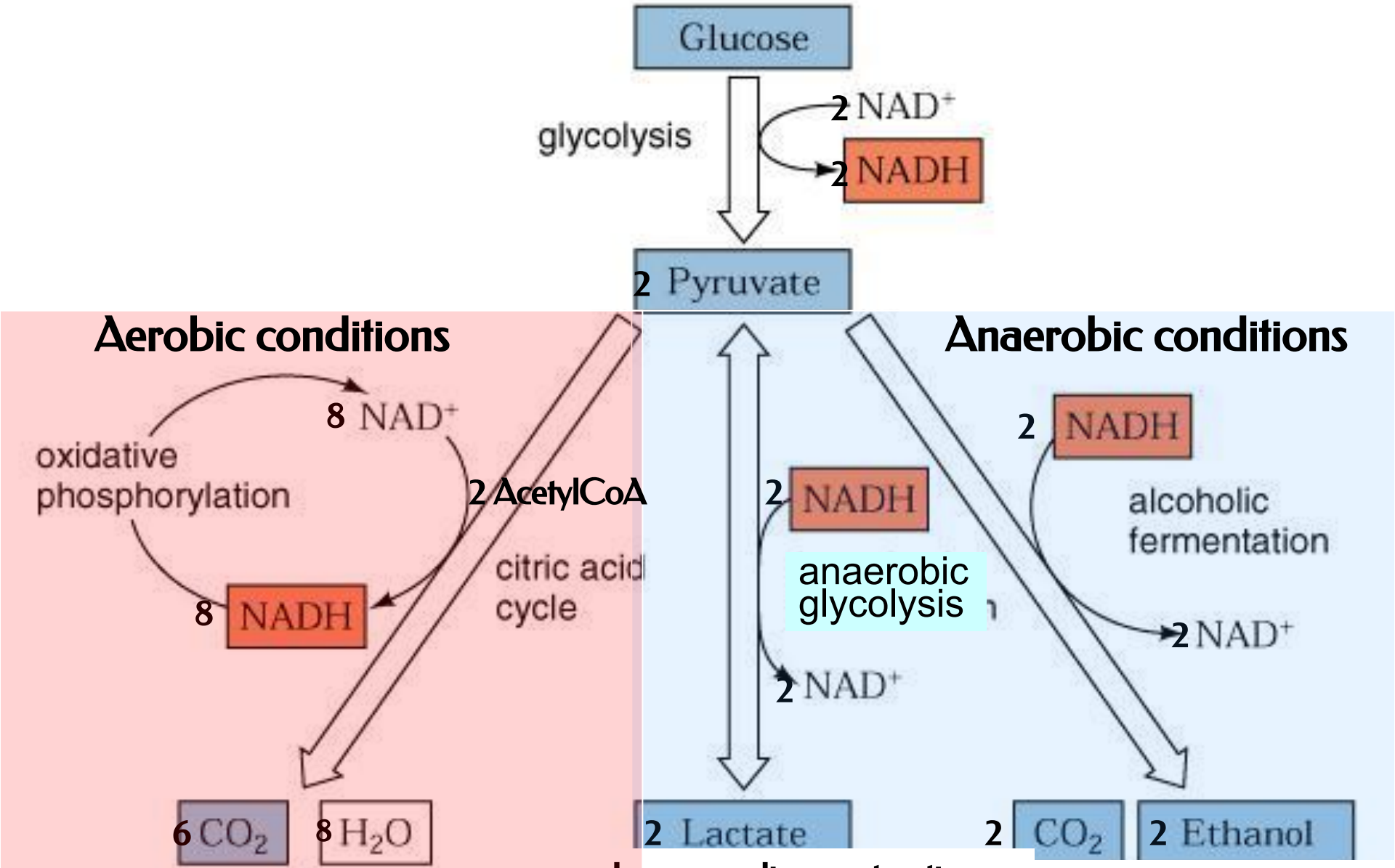
## Pyruvate kinase (PK):




PK is a point for regulation of glycolysis

There are two isozymes of PK: L (liver, kidneys) & M (muscle and other tissues) which are distinct in regulation

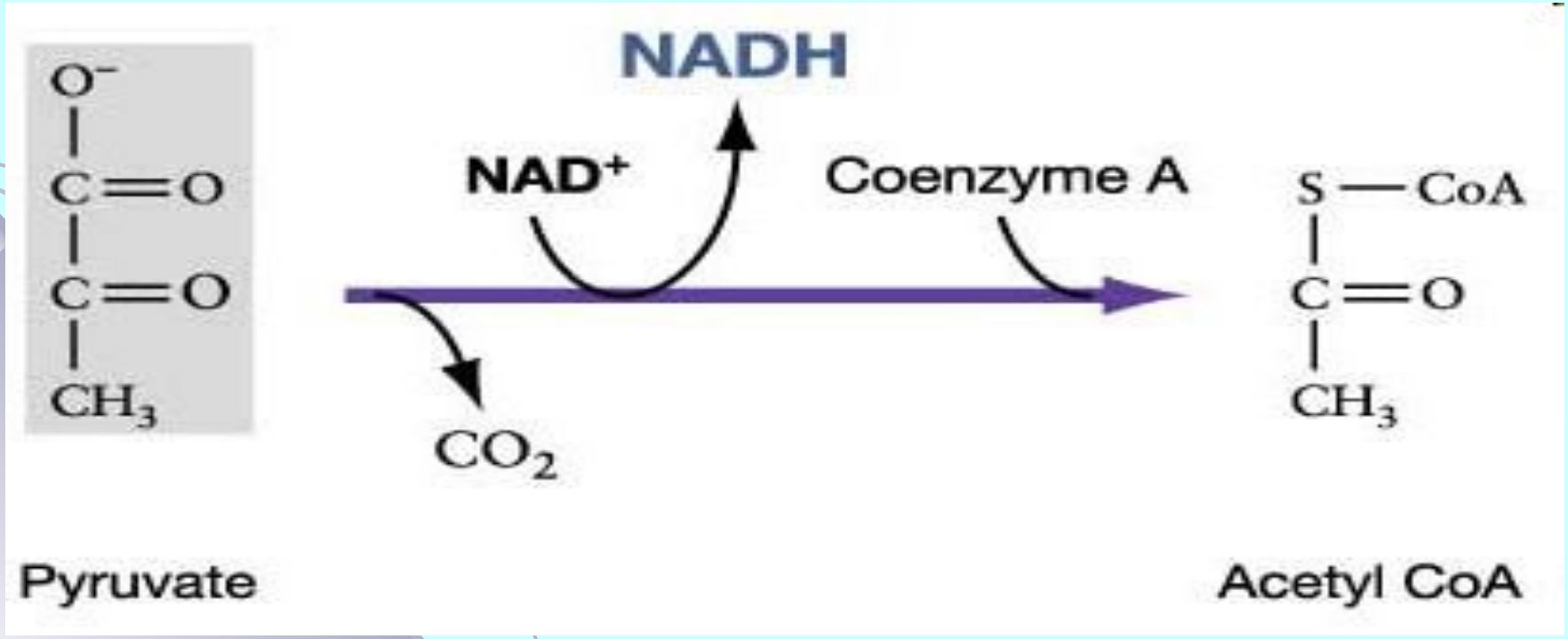
# Oxidizing power of $NAD^+$ must be recycled



# I. The metabolic fate of pyruvate in aerobic conditions

## Pyruvate dehydrogenase complex (PDC)

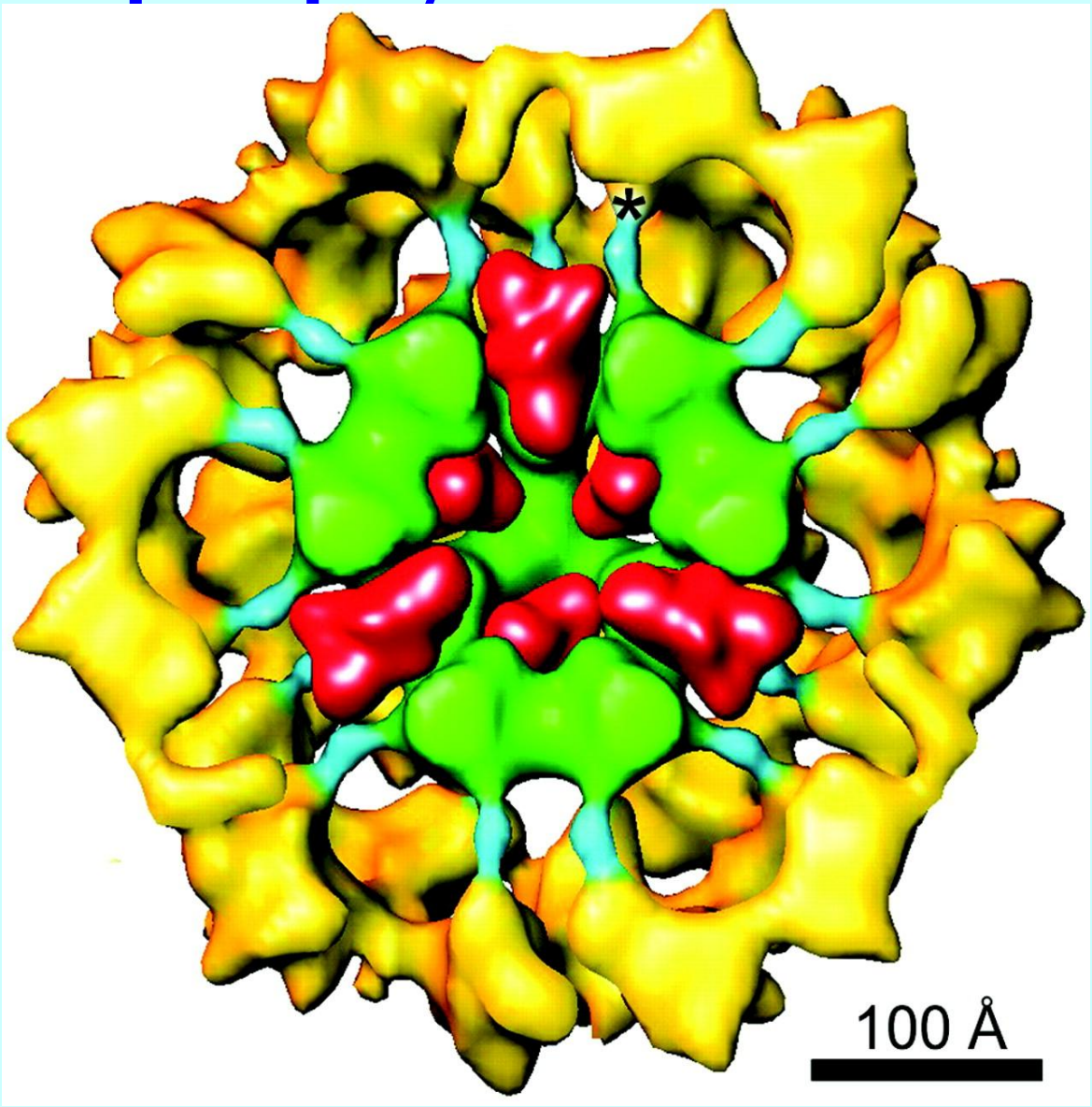
transforms pyruvate into acetyl CoA & thereby links the glycolysis to the citric acid cycle



# Cut-away model of the fully assembled PDC

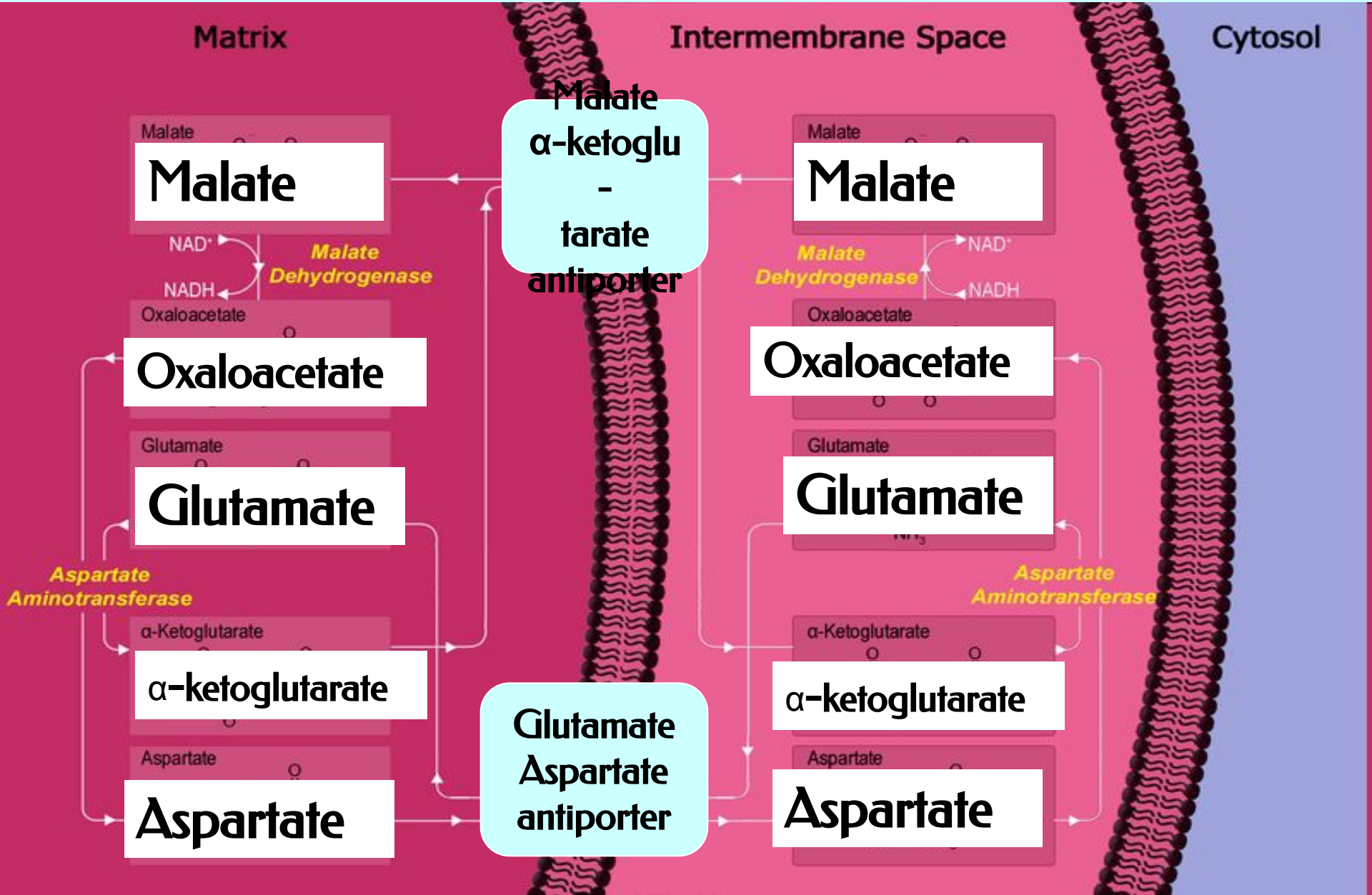
It consists of a total of 96 subunits, organized into three functional enzyme

and conteins 5 kinds of coenzymes: TPP, $NAD^+$, FAD, CoA, lipoamide



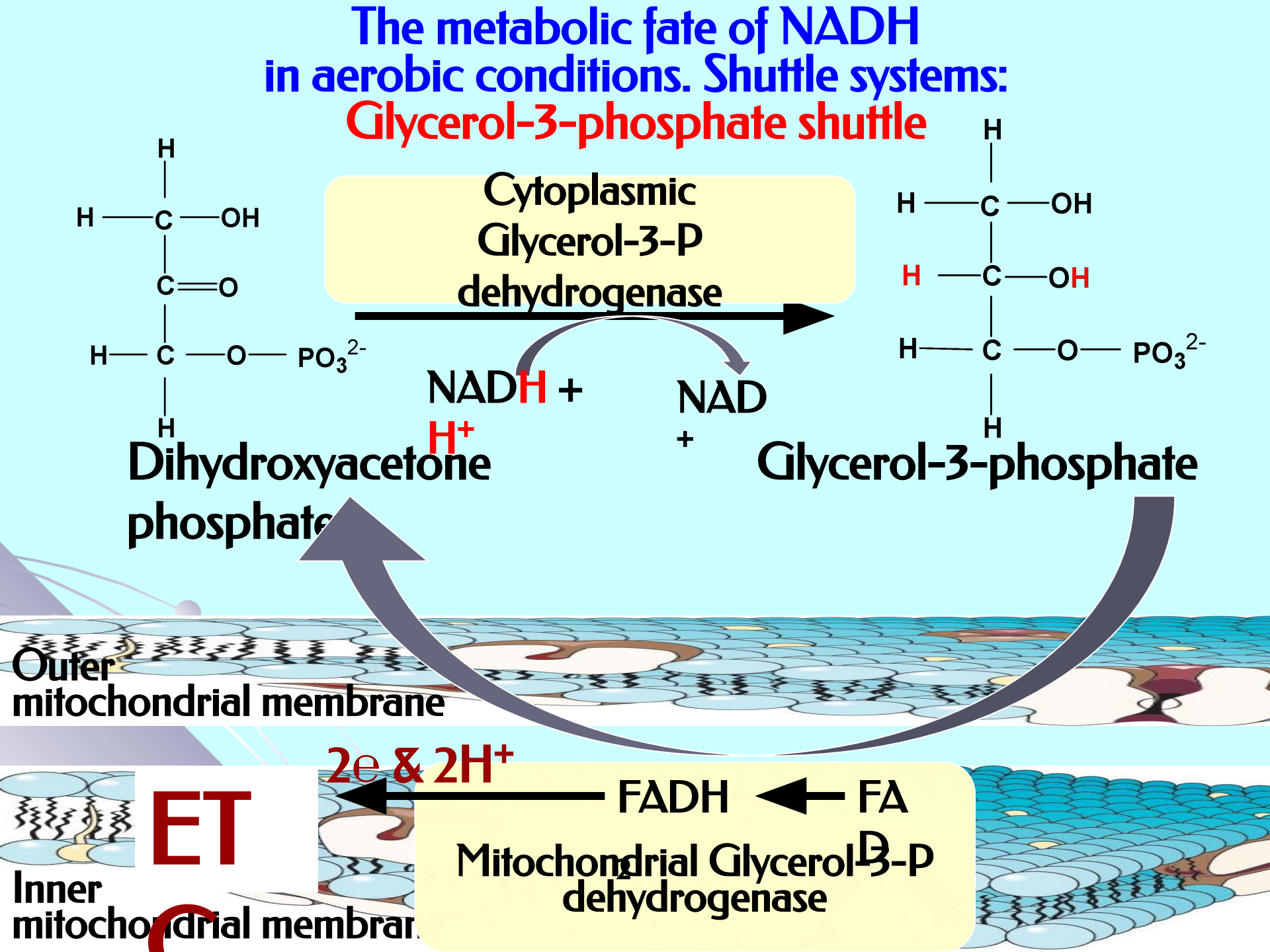
# Mechanism of PDC action (see in a text-book)

# The metabolic fate of NADH in aerobic conditions.
# Shuttle systems: Malate-aspartate shuttle (liver, heart)



# The metabolic fate of NADH in aerobic conditions. Shuttle systems:

## Glycerol-3-phosphate shuttle



# II. The metabolic fate of pyruvate in anaerobic conditions.

## Anaerobic glycolysis

- **Definition:** Anaerobic Glycolysis is the metabolic pathway in which monosaccharides (mainly glucose) are split into two molecules of lactate
- **Location** in the body : takes place in erythrocytes, cornea, lens, skeletal muscle tissue (significant at first 40-50 sec of continuous muscle work)
- **Location** within the cell : cytosol
- **Substrates:** Glucose
- **Products:** 2 lactates & 2 ATP

- **Functions of anaerobic Glycolysis :**

- ATP production

- 2,3 bisphosphoglycerate as powerful effector of $O_2$ binding with haemoglobin in RBC is formed from 1,3 bisphosphoglycerate (glycolysis intermediate)

- **Anaerobic Glycolysis reactions:**

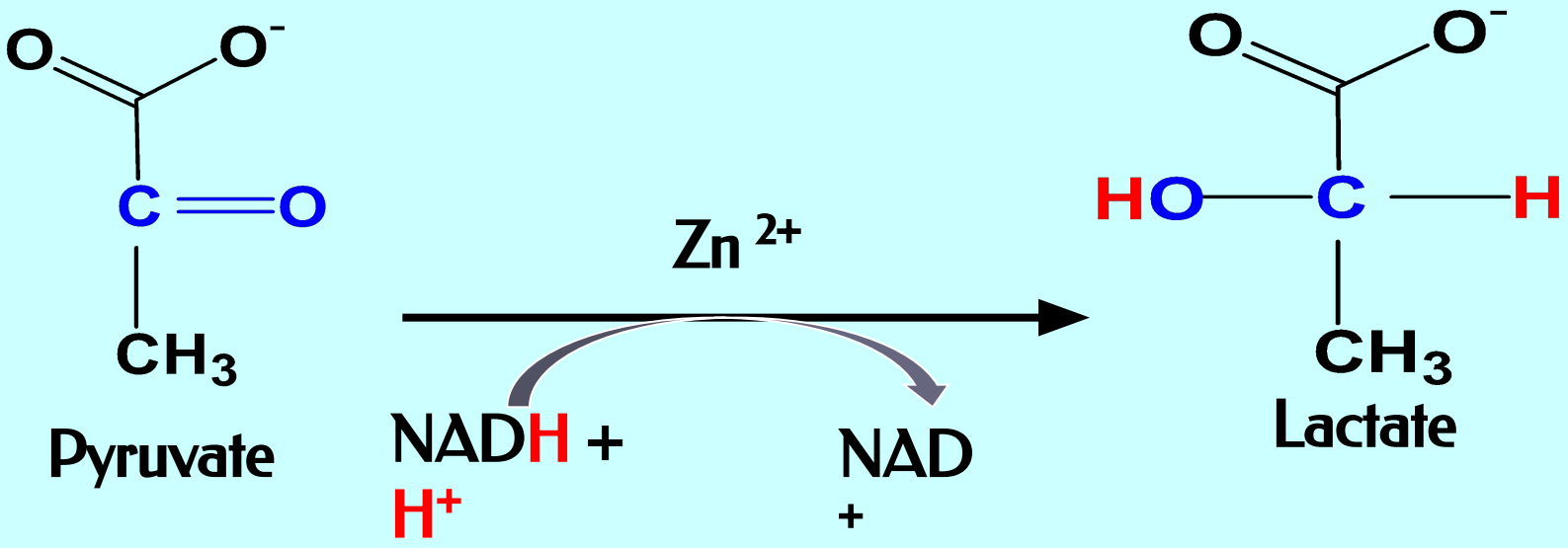
  All reactions of anaerobic glycolysis to pyruvate are the same as they are in aerobic glycolysis but one reaction is added else : Pyruvate is reduced by NADH to lactate

## Net reaction for anaerobic Glycolysis:

$$C_6H_{12}O_6 + 2ADP + 2P_i \rightarrow 2\,CH_3\text{-}CHOH\text{-}COOH + 2ATP$$

# Anaerobic glycolysis last step

## Lactate dehydrogenase (LDH)




**Functional LDH are homo or hetero tetramers composed of M and H protein subunits:**

**LDH-1 (4H) – in the heart (at hypoxia), renal cortex & RBCs**
**LDH-2 (3H1M) – in the reticuloendothelial system**
**LDH-3 (2H2M) – in the lungs**
**LDH-4 (1H3M) – in the kidneysLDH-4 (1H3M) – in the kidneys, placentaLDH-4 (1H3M) – in the kidneys, placenta and pancreas**

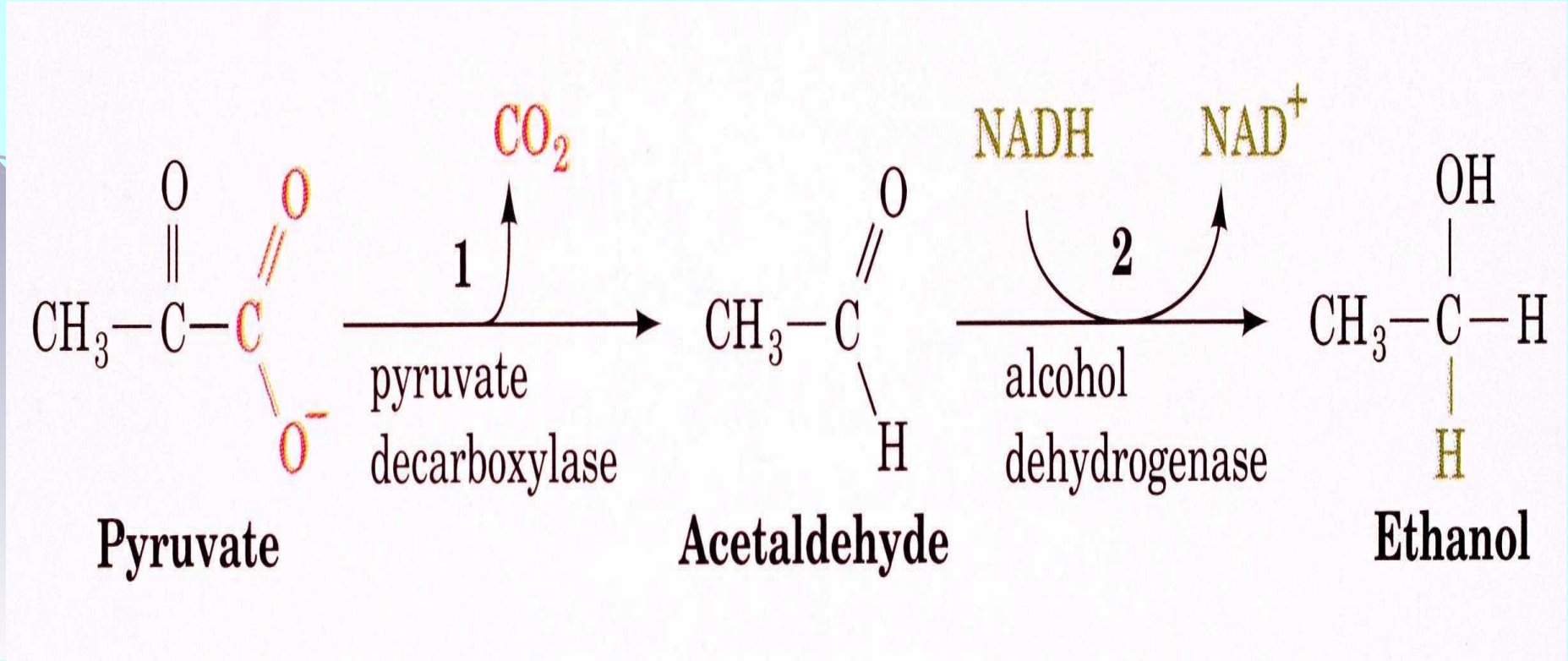
# II. The metabolic fate of pyruvate in anaerobic conditions in yeast

Alcoholic fermentation: Glucose → 2 pyruvates → 2 ethanol & $CO_2$

Double-step conversion of Pyruvate to Ethanol:

1) Pyruvate decarboxylase requires TPP (thiamine pyrophosphate) as a coenzyme.

2) Alcohol dehydrogenase requires $Zn^{2+}$ as a cofactor



# Comparative characteristics of aerobic oxidation of glucose (to $CO_2$&$H_2O$) and anaerobic glycolysis energy balance

## Aerobic oxidation of glucose (to $CO_2$&$H_2O$)

I. Glycolysis stage:

- 2 ATP (used for phosphorylation of glucose & fructose 6-P)

+ 4 ATP (produced by 1,3 bis P-glycerate and pyruvate kinases)

+ 6 ATP (if malate-aspartate shuttle translocates electrons from 2 NADH for oxidative phosphorylation (OP))

or + 4 ATP (if glycerol-3-phosphate shuttle translocates electrons from 2 NADH for OP)

= 8 (or 6)

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II. Oxidative decarboxylation of pyruvate stage (2 pyruvates enter) :

+ 6 ATP (due to utilization of 2 NADH: OP)

III. Krebs cycle (2 acetyl CoA enter) stage:

+ 18 ATP (due to utilization of 6 NADH for OP)
+ 4 ATP (due to utilization of 2 $FADH_2$ for OP)
+ 2 ATP (due to 2 GTP conversion)
= 24

In all = 38 (or 36) ATP

## Anaerobic glycolysis:

- 2 ATP (used for phosphorylation of glucose & fructose 6-P)
+ 4 ATP (produced by 1,3 bis-P-glycerate kinase and pyruvate kinase)

2 NADH are not used for oxidative phosphorylation but are consumed in LDH reaction

In all = 2 ATP

Glycolysis is normally faster than the TCA cycle capacity, and lactate is the usual product of glycolysis even in resting muscle.

The lactate/pyruvate ratio is about 10 in resting muscle, but in working muscle this ratio may hit 200

# Regulation

# Glycolysis is regulated at 3 steps involving non equilibrium reactions

- Step 1: Hexokinase
- Step 3: Phosphofructokinase 1
- Step 10: Pyruvate kinase

These three enzymes are key enzymes for Glycolysis

# Specific effectors of Glycolysis

| Enzyme | Activator | Inhibitor |
|---|---|---|
| Hexokinase (muscle) Glucokinase (liver) | | G 6-P |
| PFK1 | ADP, AMP (muscle), Pi, $NH_4^+$ ↑ F-2,6-biP (in the liver due to insulin) | ATP Citrate, PEP $H^+$ (low pH) ↓ F-2,6-biP (in the liver due to glucagon) |
| Pyruvate kinase | F-1,6-biP | ATP Acetyl-CoA Fatty acids Alanine -c-AMP dependent PK (in the liver due to glucagon) |

# Regulation of PDC

- PDC is inhibited when one or more of the three following ratios are increased: ATPPDC is inhibited when one or more of the three following ratios are increased: ATP/ADP, NADH/$NAD^+$ and acetyl-CoA and acetyl-CoA/CoA.
- In eukaryotes PDC is tightly regulated by its own specific pyruvate dehydrogenase kinaseIn eukaryotes PDC is tightly regulated by its own specific pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP), deactivating and activating it respectively.
  Products of the reaction (acetyl-CoA, NADH, ATP) act as allosteric activators of the PDK, therefore PDC is also inhibited.
  Substrates ($HAD^+$,CoA) in turn are inhibitors of the PDK, therefore PDC is also activated
- Calcium ion has a role in regulation of PDC in muscle tissue, because it activates PDP

# Gluconeogenesis

- **Definition:** Gluconeogenesis is an anabolic pathway whereby non-carbohydrate precursors are converted to glucose

**Functions:**

- It is one of the two main mechanisms humans and many other animals use to keep blood glucose levels from hypoglycemia (dropping too low)
- This process occurs during periods of fasting, starvation, low-carbohydtrate diets, or intense exercise
- acidic components of the blood can be utilized due to gluconeogenesis (mainly in kidney) at metabolic acidosis state and as result the pH of the blood is normalized

# Gluconeogenesis

- **Location** in the body :

Glucose is synthesized between almost nil and perhaps 200 g/day in adults

- Liver ( 90% )
- Kidney cortical layer (10%)
- Small intestine (0,1%)

- **Location** within the cell (if pyruvate is the substrate):

- It is started in mitochondrion &
- is continued in cytoplasm &
- is finished in the lumen of the endoplasmic reticulum

# Gluconeogenesis

**Substrates:**

- Lactate ( produced in RBC, muscles)
- Glycerol (produced in adipocytes due to lipolysis)
- Glucogenic amino acids (all except Leu, Lys)
- Propionyl CoA (due to oxidation of odd carbon chain fatty acids from vegetable foodstuff mainly)

Most precursors must enter the Krebs cycle at some point to be converted to oxaloacetate

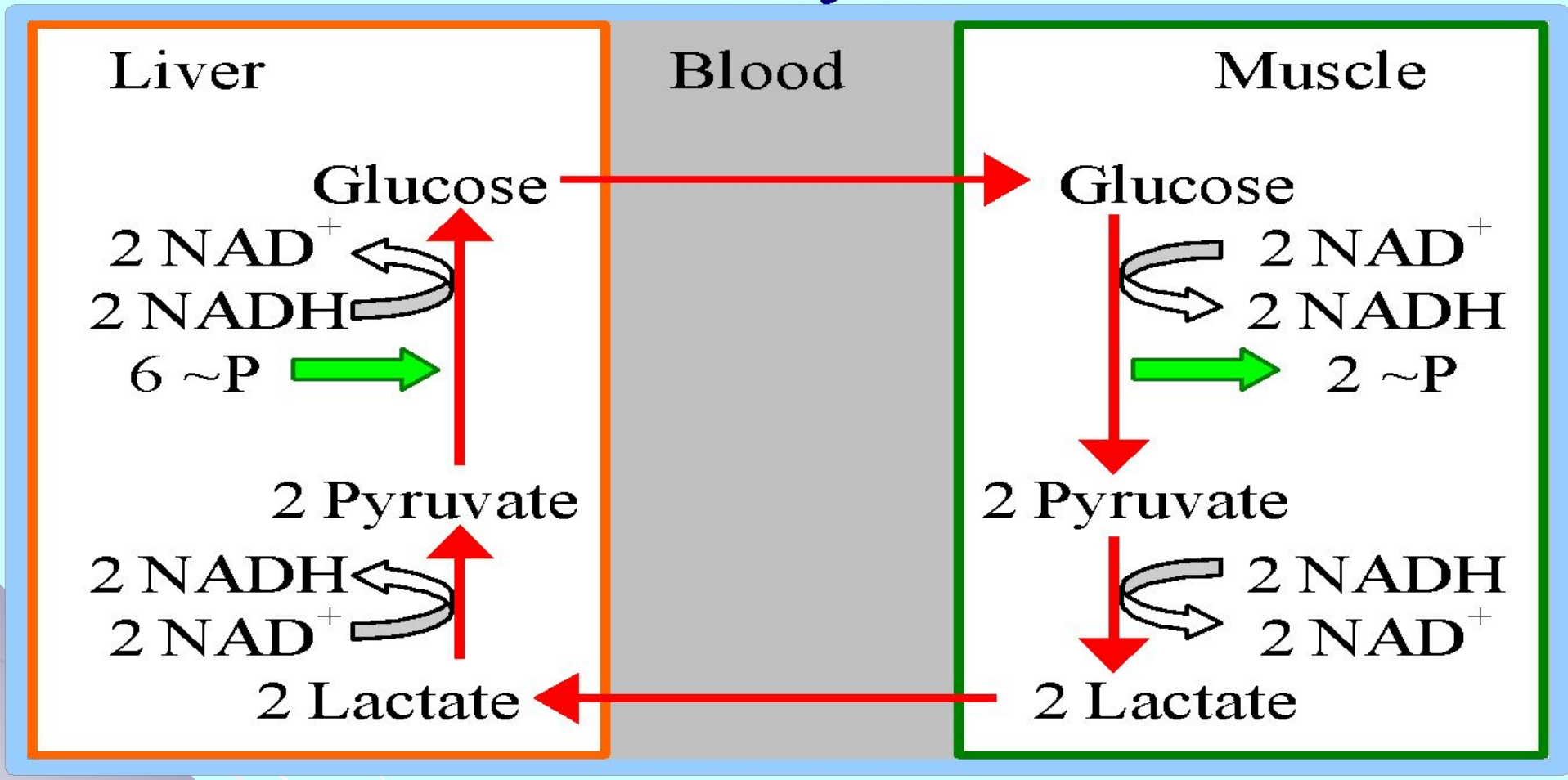
**Product:**

- Glucose

**Net reaction for Gluconeogenesis:**

$$2\,CH_3\text{-}CO\text{-}COOH + 4ATP + 2GTP + 2NADH + 2H^+ + 6H_2O \longrightarrow C_6H_{12}O_6 + 2\,NAD^+ + 4ADP + 2GDP + 6P_i$$

# Cori Cycle




The major metabolic product produced under normal circumstances by erythrocytes and by muscle cells during intense exercise lactate is recycled to glucose through the liver in the Cori cycle

# Gluconeogenesis reactions

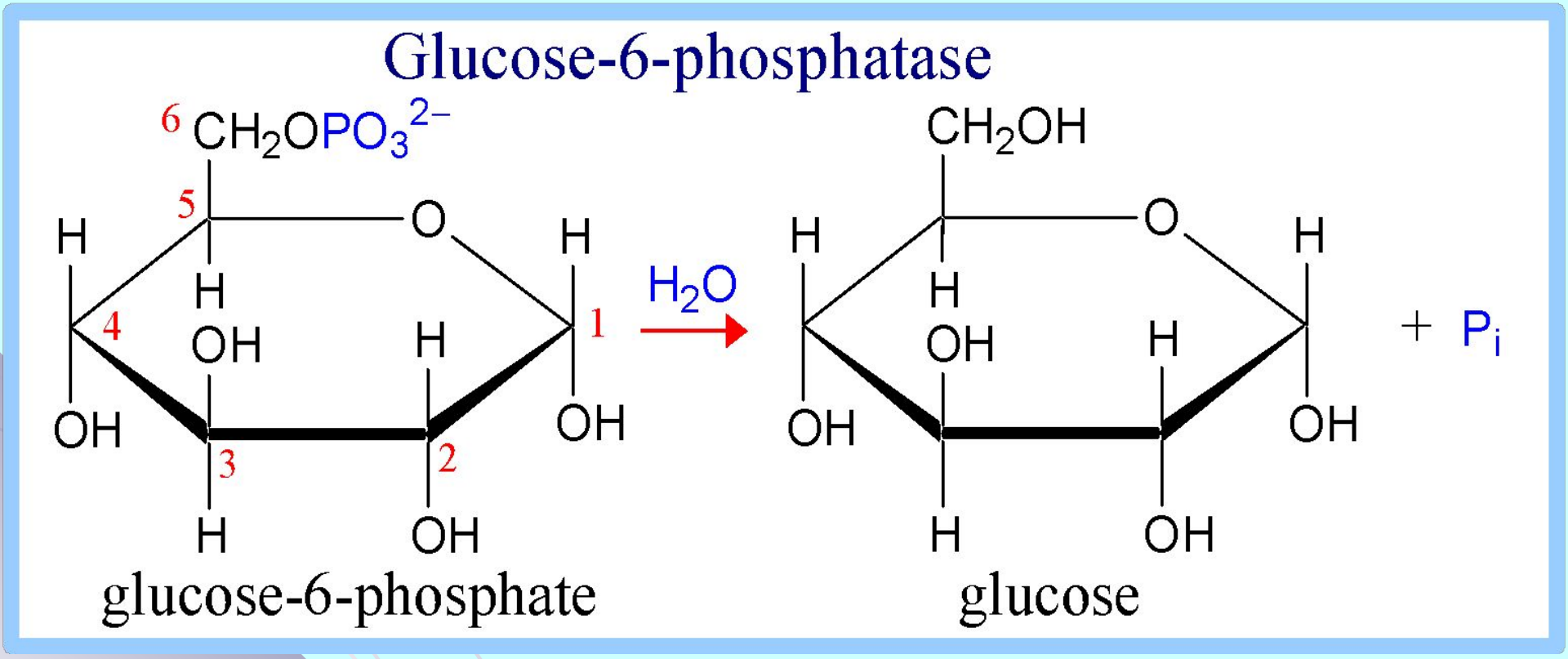
Synthesis of glucose from pyruvate utilizes many of the same enzymes as Glycolysis.
Gluconeogenesis is not just the reverse of glycolysis.
Three Glycolysis reactions are essentially irreversible:
Hexokinase (or Glucokinase);
PFK1;
Pyruvate kinase
These steps must be bypassed in gluconeogenesis

Two of the bypass reactions involve simple hydrolysis reactions:

# Bypass of Hexokinase reaction

**Hexokinase (or Glucokinase) (Glycolysis)**

**G 6-Pase (Gluconeogenesis) catalyzes:**

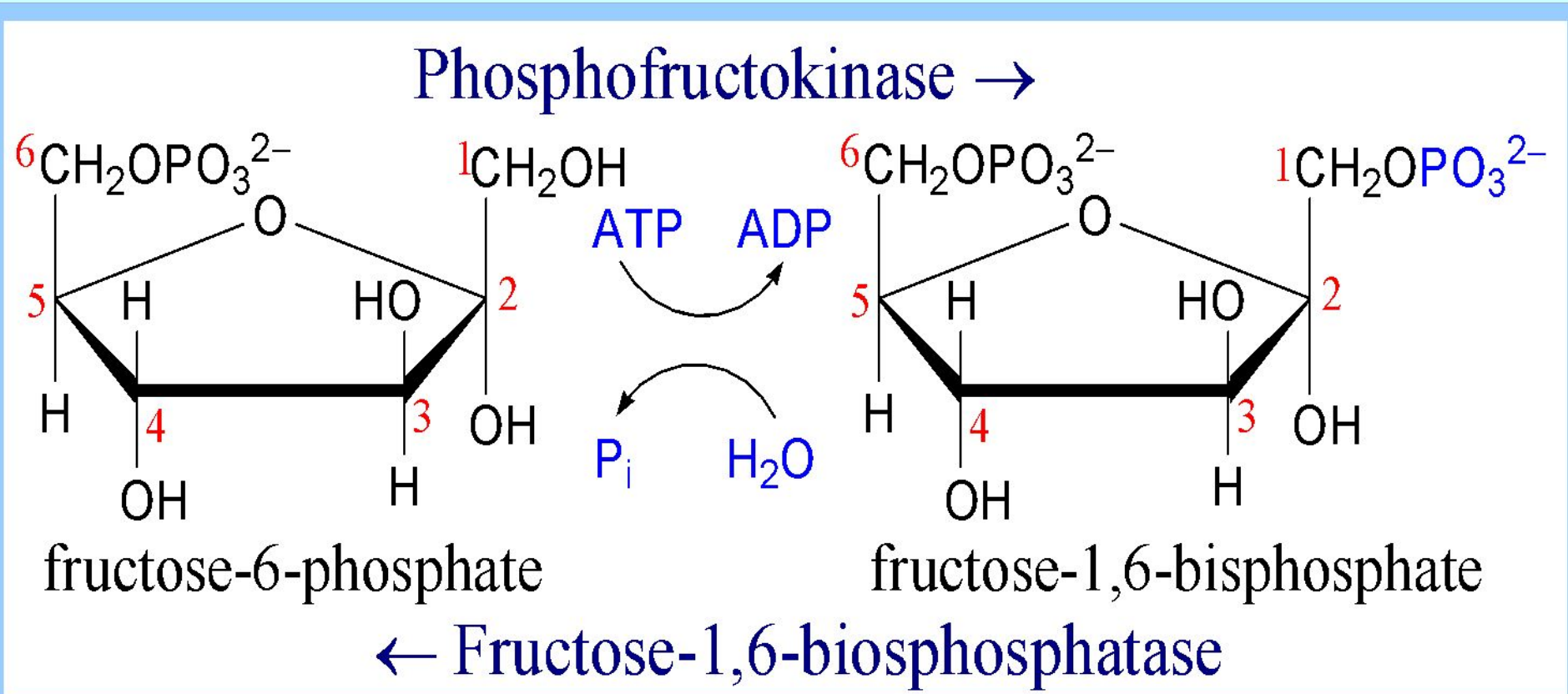



**G 6-Pase enzyme is embedded in the endoplasmic reticulum (ER) membrane in liver cells**

# Bypass of PFK 1 reaction

PFKase 1 (Glycolysis)

F 1,6-bisPase (Gluconeogenesis) catalyzes:

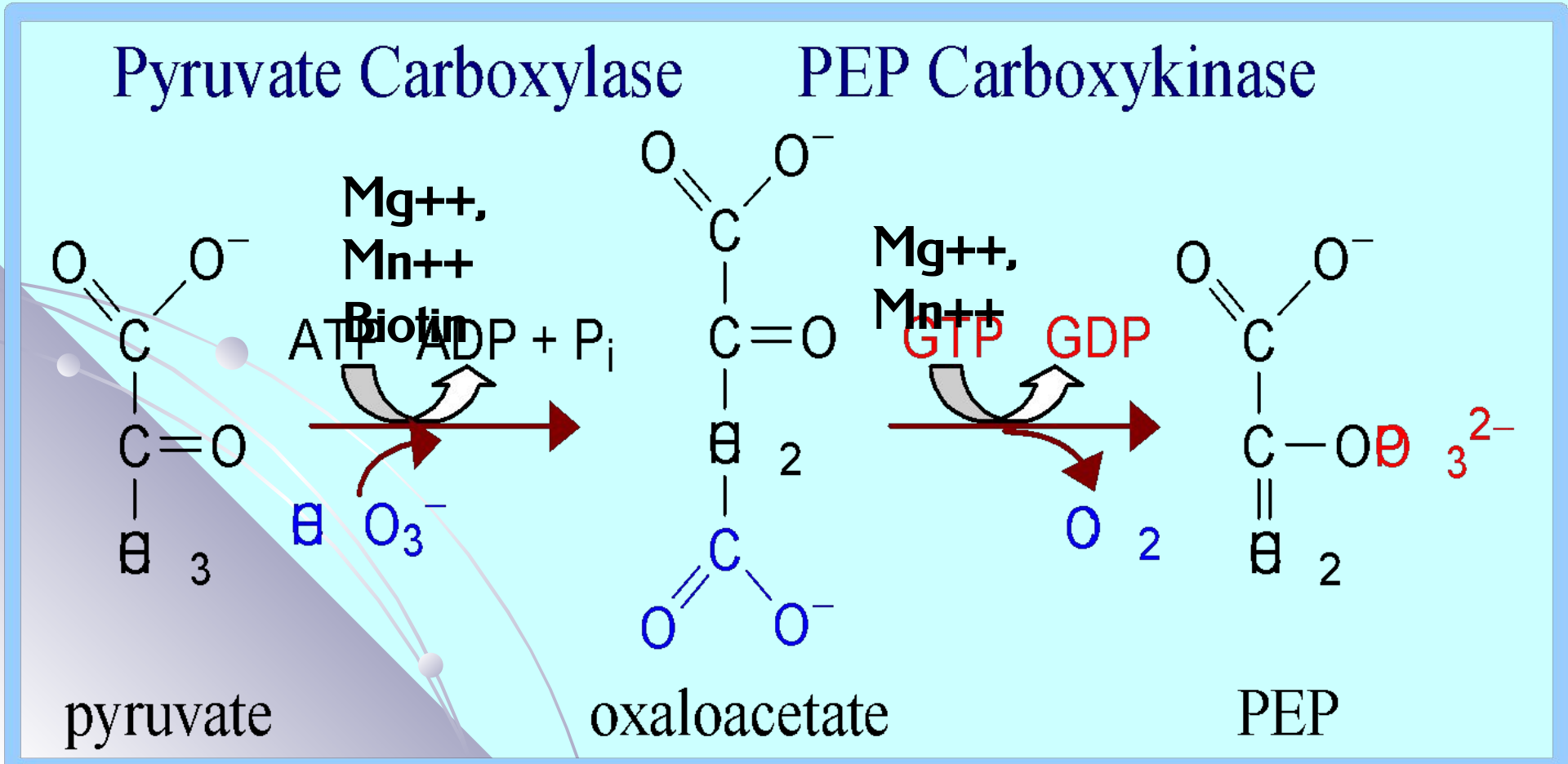


# Bypass of Pyruvate Kinase reaction

Pyruvate Kinase (last step of Glycolysis)

Pyruvate Carboxylase (PC)

Phosphoenolpyruvate Carboxykinase (PEPCK)



# Energy balance for 1 mole of glucose synthesis from 2 moles of pyruvate

- PC reaction – 2ATP;
- PEPCK reaction – 2 GTP;
- 1,3-bisPGl kinase reaction – 2 ATP;

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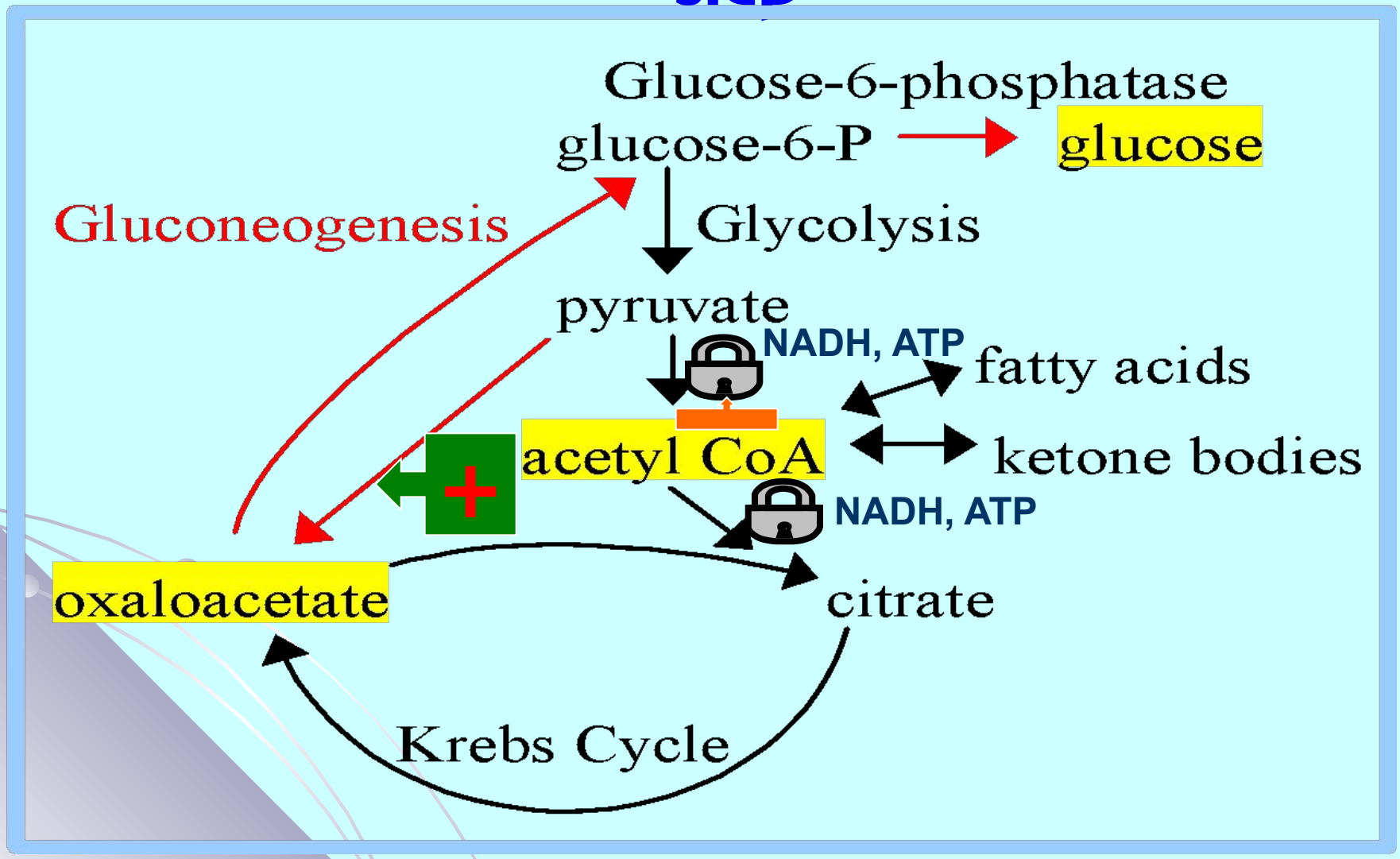
**In all** : The use of **6 ATP** for 1 mole of glucose synthesis from pyruvate or lactate

# Gluconeogenesis regulation: mitochondrial step




Acetyl CoA is allosteric activator of Pyruvate Carboxylase

# Gluconeogenesis regulation: cytosol stage

To prevent the waste of a futile cycle, Glycolysis (producing 2 ATP) & Gluconeogenesis (consuming 4 ATP and 2 GTP) are reciprocally regulated:

## Local Control

It includes reciprocal allosteric regulation by adenine nucleotides:

- Phosphofructokinase 1 (Glycolysis) is inhibited by ATP and activated by AMP, ADP
- Fructose-1,6-bisphosphatase (Gluconeogenesis) is inhibited by AMP

# Global Control in liver cells

It includes reciprocal effects of a cyclic AMP cascade, triggered by the hormone glucagon when blood glucose is low and epinephrine during stress

Phosphorylation of enzymes & regulatory proteins in liver by Protein Kinase A (cAMP Dependent Protein Kinase) results in

- inhibition of glycolysis
- stimulation of gluconeogenesis,

making glucose available for release to the blood

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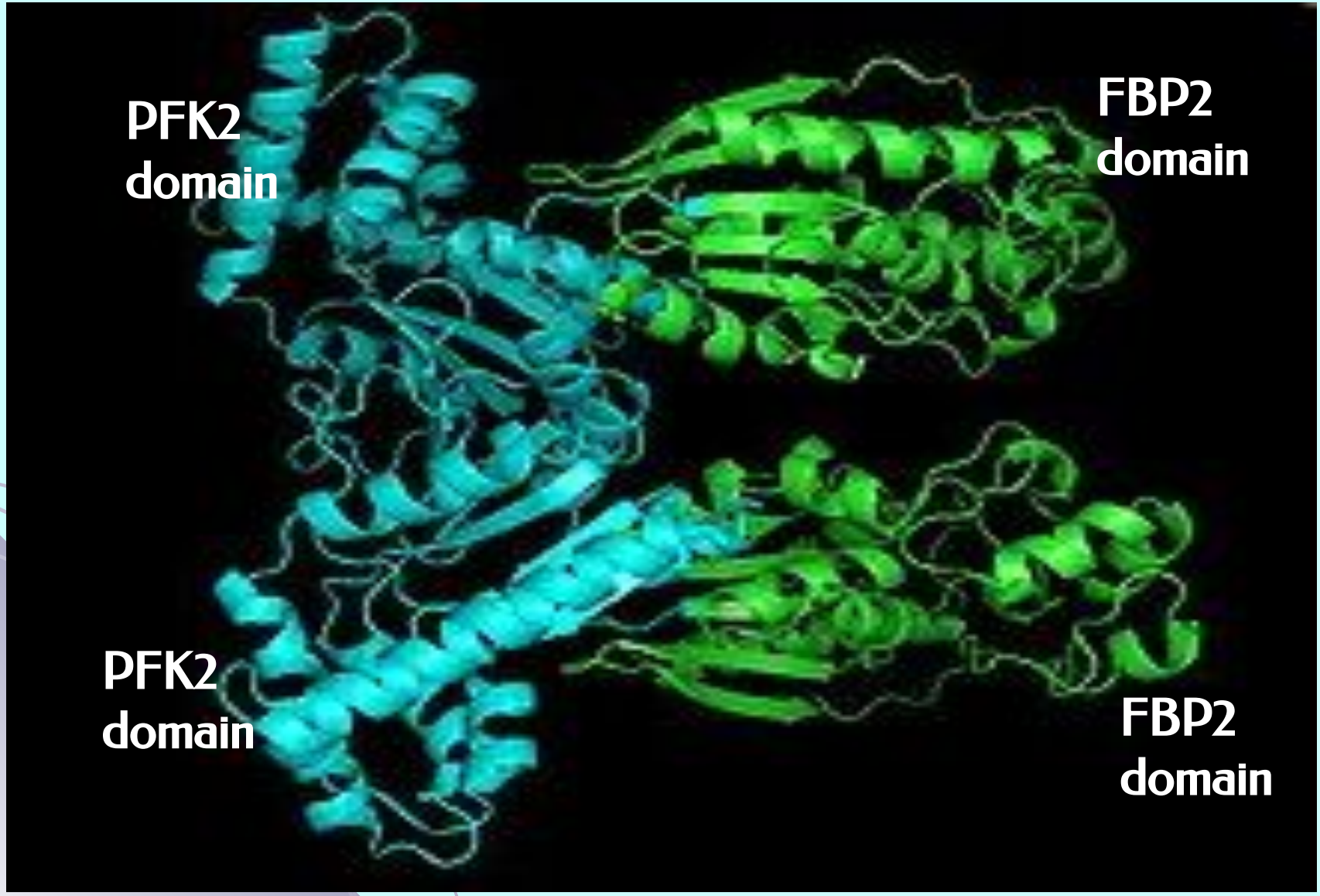
Enzymes relevant to these pathways that are phosphorylated by Protein Kinase A include:

- Pyruvate Kinase, a glycolysis enzyme that is inhibited when phosphorylated.
- CREB (cAMP response element binding protein) which activates, through other factors, transcription of the gene for PEP Carboxykinase, leading to increased gluconeogenesis.
- A bi-functional enzyme that makes and degrades an allosteric regulator, fructose-2,6-bisphosphate


PFK2
domain
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PFK2
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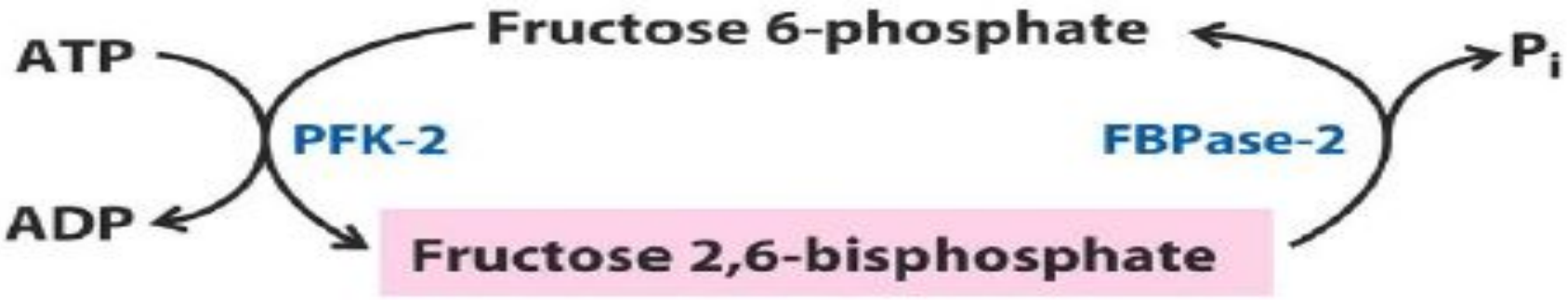
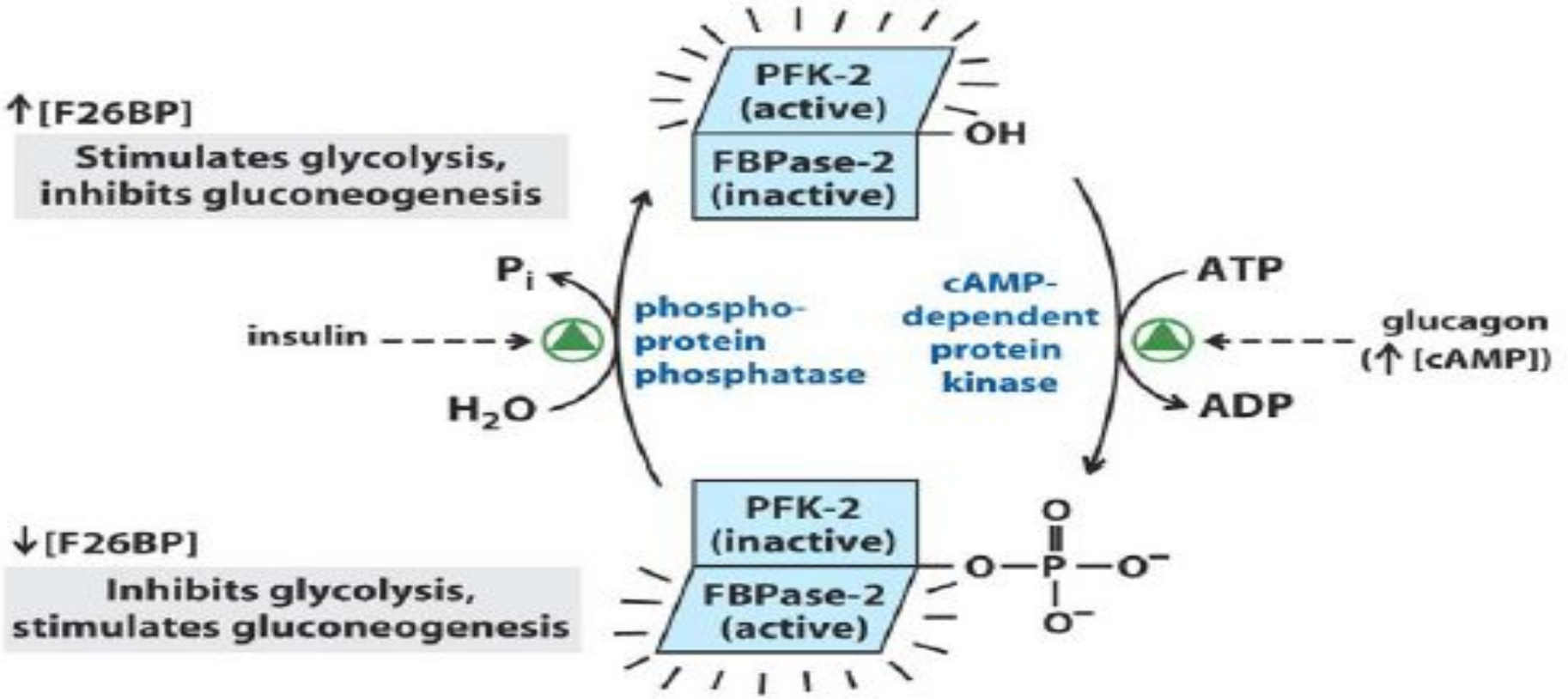
# Reciprocal hormonal regulation through F-2,6-bisP



# Phosphofructokinase (PFK) characteristics

There are two types of the enzyme:

- Mammalian PFK1:

- catalyzes the irreversible transformation of F6P to F1,6bisP;
- is enzyme out of glycolysis;
- the main way of PFK1 activity regulation is allosteric;

- Mammalian PFK2 or FBPase2 (fructose bisphosphatase2):

- catalyzes the reversible transformation of F6P to F2,6bisP
- is enzyme for regulation of glycolysis in the liver;
- the main regulation of its activity is realized through phosphorylation-dephosphorylation (cAMP-dependent);
- each polypeptide chain consisting of independent kinase and phosphatase domains

# Specific and common effectors for Glycolysis & Gluconeogenesis (in liver)

| Enzyme | Allosteric Inhibitors | Allosteric Activators | Enzyme Phosphorylation | Synthesis of protein part of E |
|---|---|---|---|---|
| GK | | | | Insulin |
| G6Pase | | | | Glucagon |
| PFK1 | ATP, citrate etc | AMP, F2,6P etc | | Insulin |
| FBPase 1 | AMP, F2,6P | | | Glucagon |
| PK | Alanine etc | F1,6P etc | Inactivates | Insulin |
| PC | | AcetylCoA | | |
| PEPCK | | | | Glucagon Cortisol |
| PFK-2 | Citrate | AMP, F6P, Pi | Inactivates | |
| FBPase-2 | F6P | Glycerol-3-P | Activates | |

# Thank You !