

# **Gluconeogenesis**

# **Glycogen metabolism**

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# Glucose in blood

**Resorption  
phase**

**Postresorption  
phase, fasting**

3,1-5,0 mmol/l

**Concentration of glucose in blood**

Saccharides from  
food

Glycogenolysis  
(liver)

Gluconeogenesis  
(liver, *kidney*)

## Main hormones in metabolism of glucose

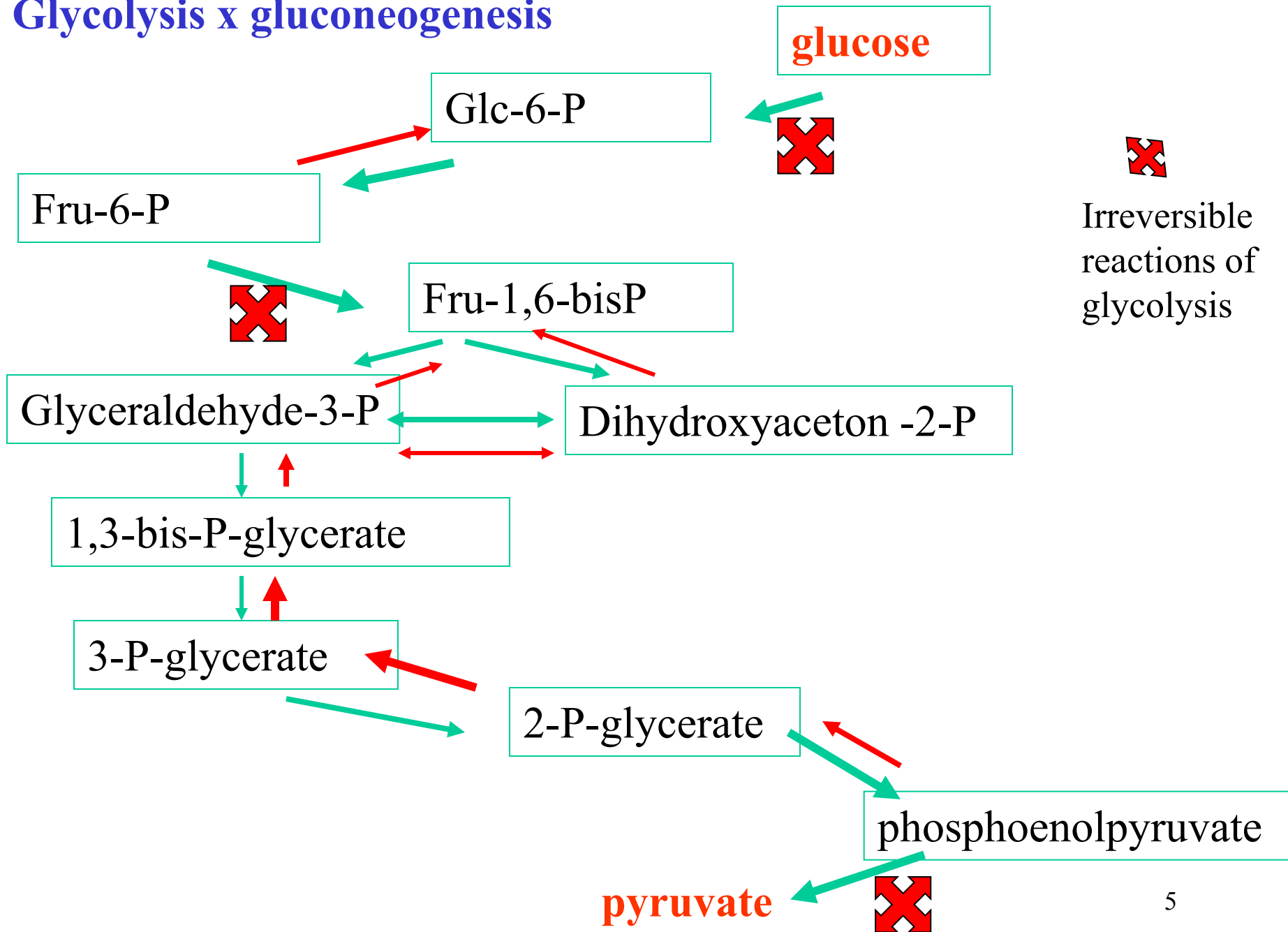
Hormone	Source	Effect on the level of glucose
<b>Insulin</b>	$\beta$ -cells of pancreas	↓
<b>Glucagon</b>	$\alpha$ -cells of pancreas	↑
<b>Adrenaline</b>	Adrenal medulla	↑
<b>Cortisol</b>	Adrenal cortex	↑

# Gluconeogenesis - synthesis of glucose *de novo*

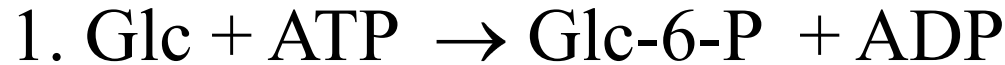
- Organ: liver (kidney)
- Location: cytoplasm
- Substrates for synthesis: non-saccharide compounds (lactate, pyruvate, gluconeogenic amino acid, glycerol)
- Reactions: enzymes of glycolysis are used for gluconeogenesis, only 3 irreversible reactions are circumvented by alternate reactions that energetically favor synthesis of glucose

**Enzymes are regulated so that either glycolysis or gluconeogenesis predominates, depending on physiologic conditions**

# Glycolysis x gluconeogenesis



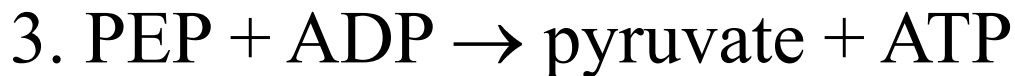
# Irreversible reactions of glycolysis (kinase reactions)



(reverse reaction is catalyzed by different enzyme)



(reverse reaction is catalyzed by different enzyme)

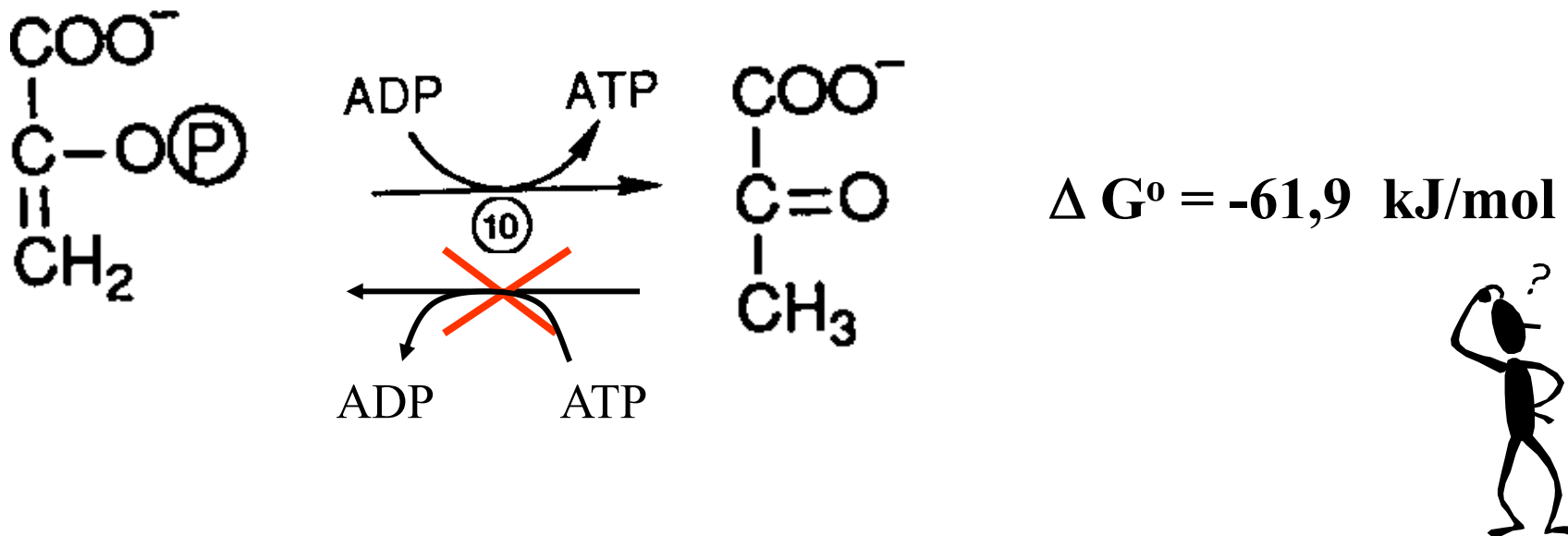


(reverse reaction is replaced by „by-pass“)

# Reactions unique to gluconeogenesis

## 1. Synthesis of phosphoenolpyruvate

Why the reverse reaction cannot proceed?



Cleavage of ATP does not provide energy sufficient for reverse reaction

# Formation of phosphoenolpyruvate occurs in two steps:

## 1. Formation of oxalacetate by carboxylation of pyruvate \*

enzyme: pyruvate carboxylase

energy: consumption of **1 ATP**

location: mitochondria

## 2. Conversion of oxalacetate to phosphoenolpyruvate

enzyme: phosphoenolpyruvate carboxykinase

energy: consumption of **1 GTP**

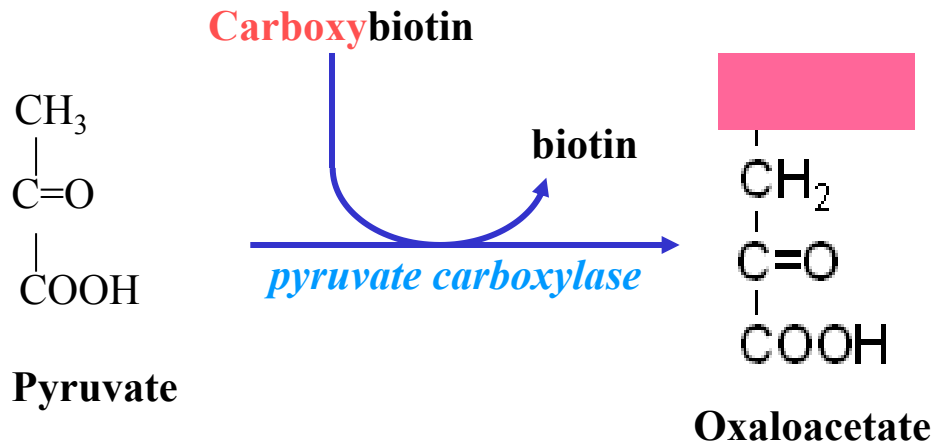
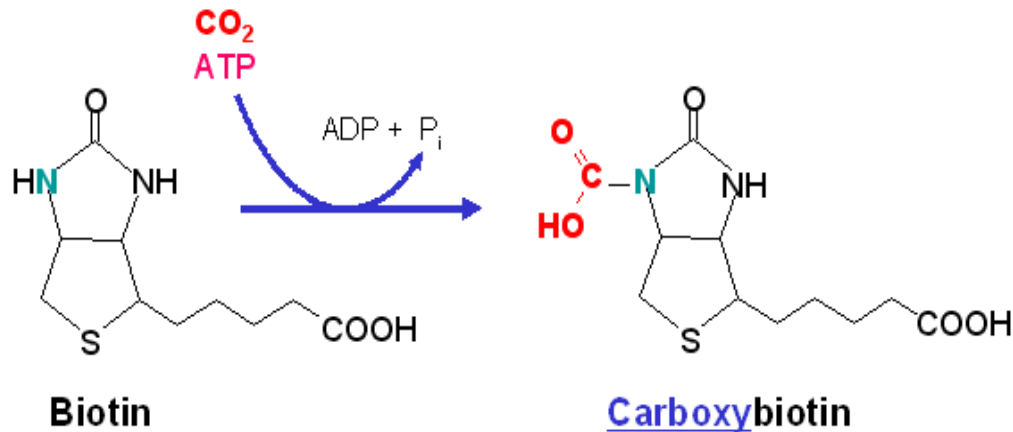
location: cytoplasm

\*note.: carboxylation of pyruvate is also anaplerotic reaction of citric acid cycle



# 1. Conversion of pyruvate to phosphoenolpyruvate (reaction)

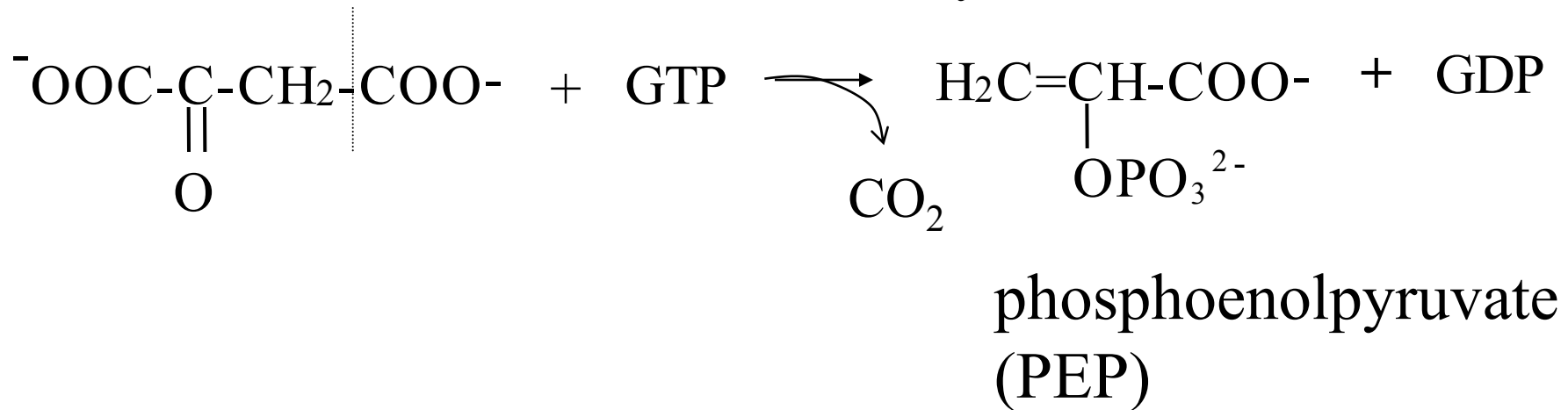
## • carboxylation pyruvate



- decarboxylation of oxalacetate



PEP carboxykinase

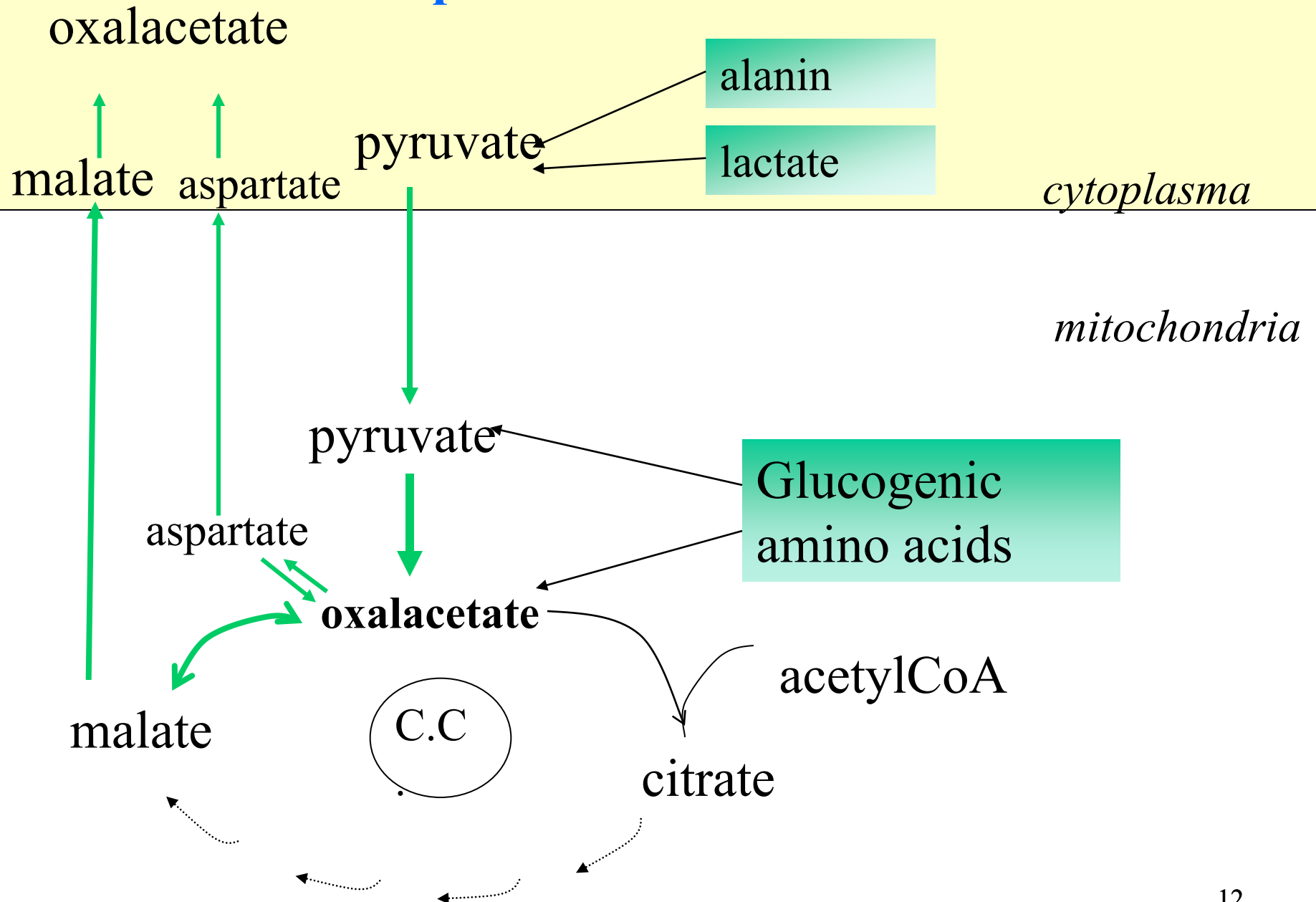


PEP enters reversible reactions of glycolysis

## Compartmentation of reactions at phosphoenolpyruvate formation

- Carboxylation of pyruvate is located in mitochondrial matrix – at the same time it can serve as anaplerotic reaction of citric acid cycle (see lecture citric acid cycle)
- Oxaloacetate cannot be transported across mitochondrial membrane – it must be transported in form of malate or aspartate
- malate and aspartate are again converted to oxaloacetate in cytoplasm

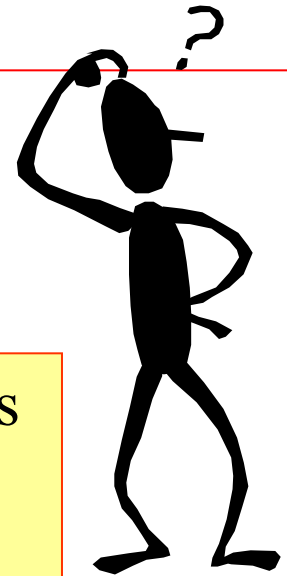
# Kompartimentation of reactions



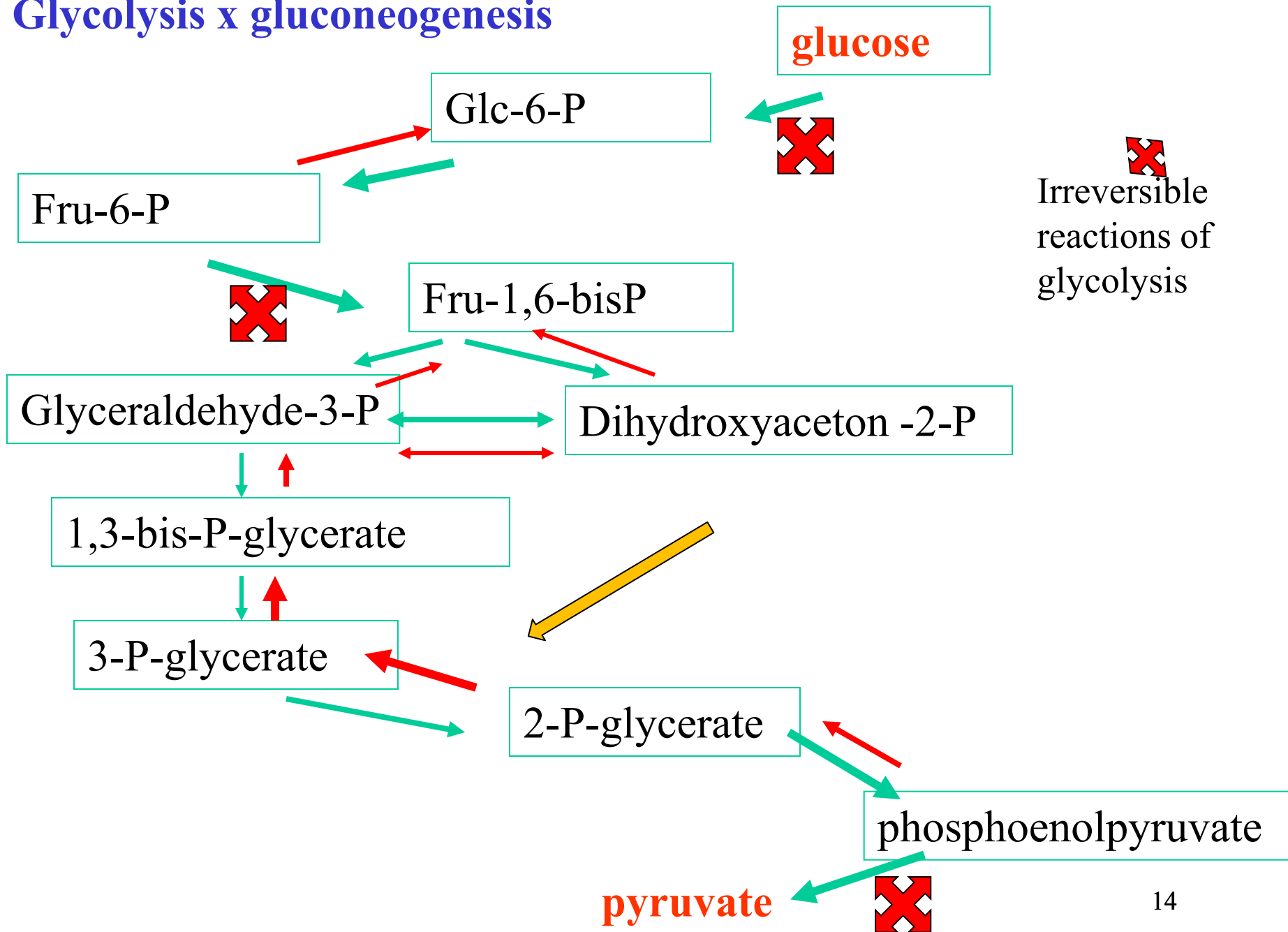
**Synthesis phosphoenolpyruvate from pyruvate or lactate requires consumption of 2 ATP**

Pairing of carboxylation and decarboxylation drives the reaction that would be otherwise energetically unfavorable.

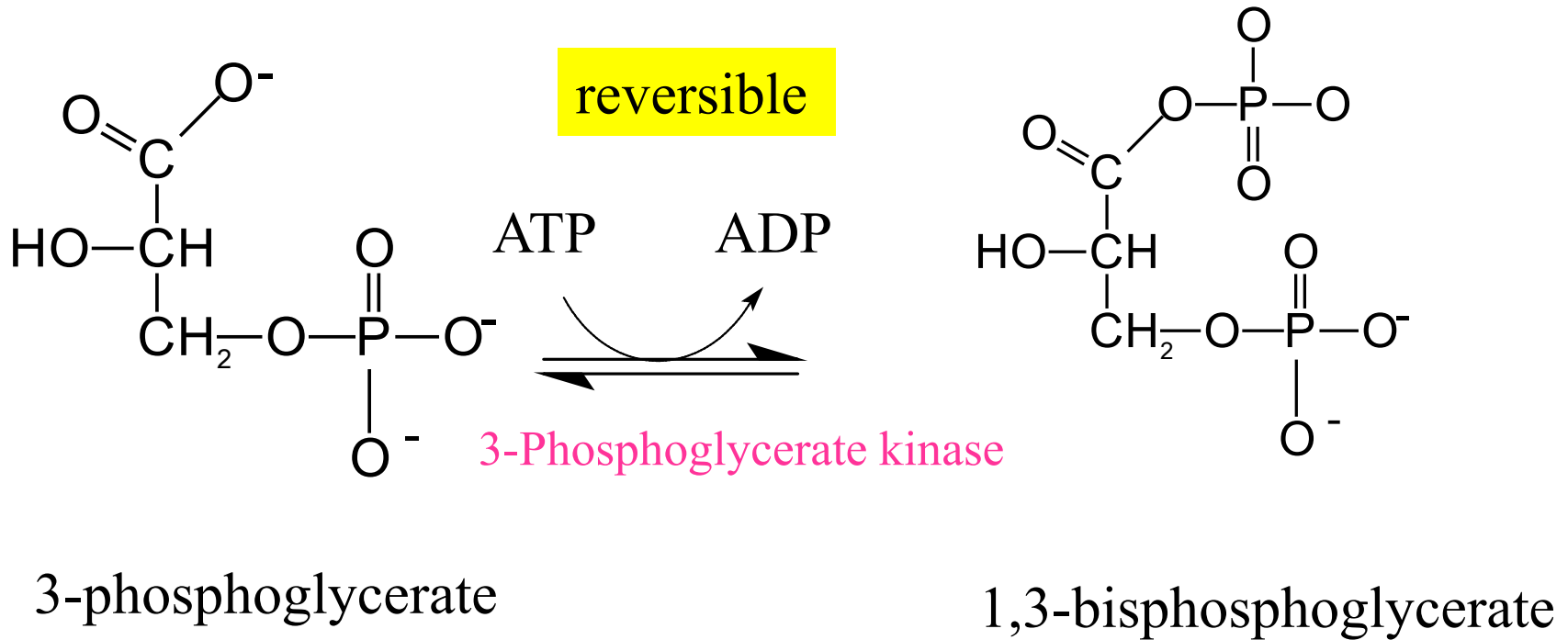
(see also the synthesis of fatty acids)



# Glycolysis x gluconeogenesis

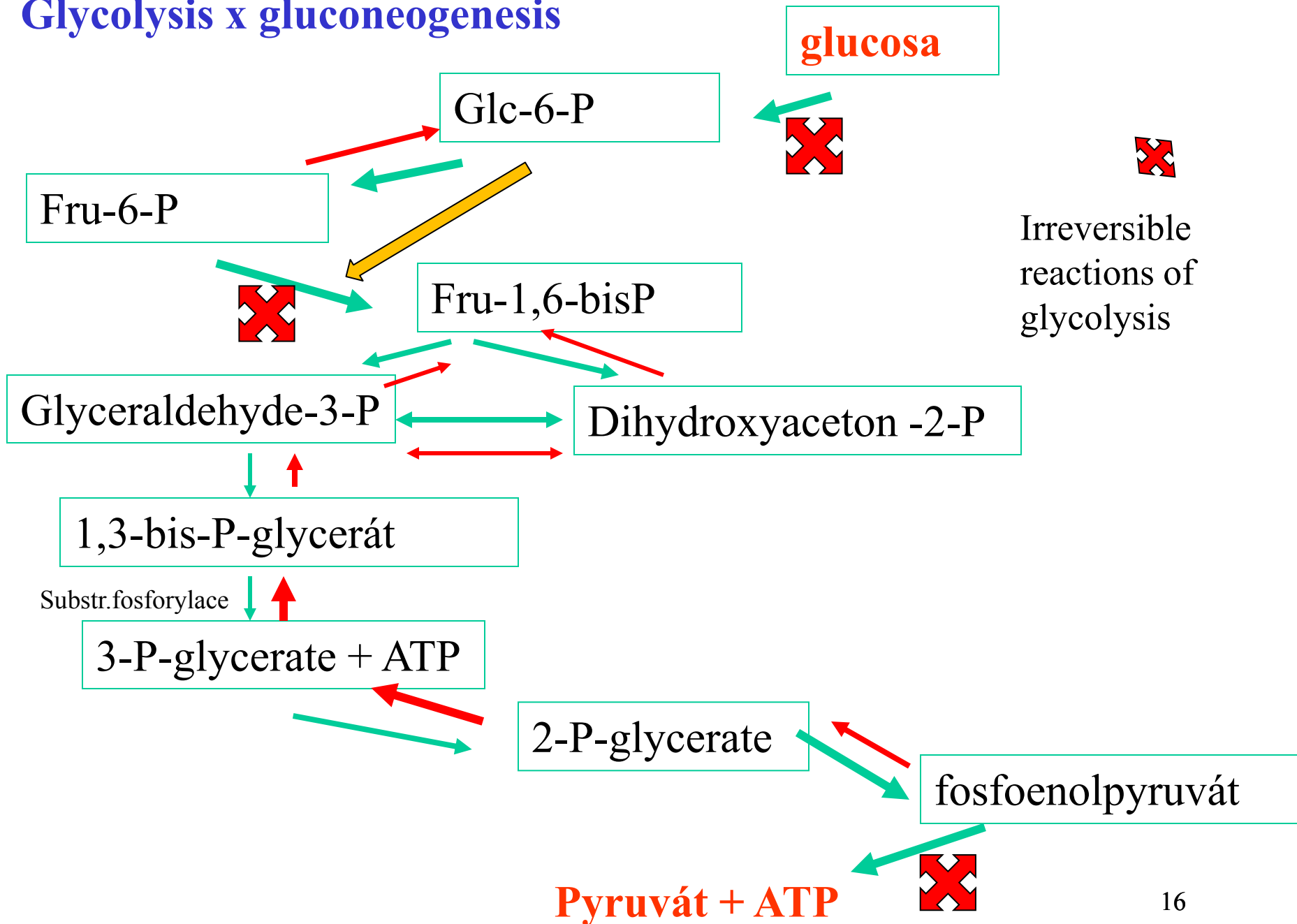


## Further consumption of ATP at gluconeogenesis



Reversal proces of substrate phosphorylation in glycolysis

# Glycolysis x gluconeogenesis

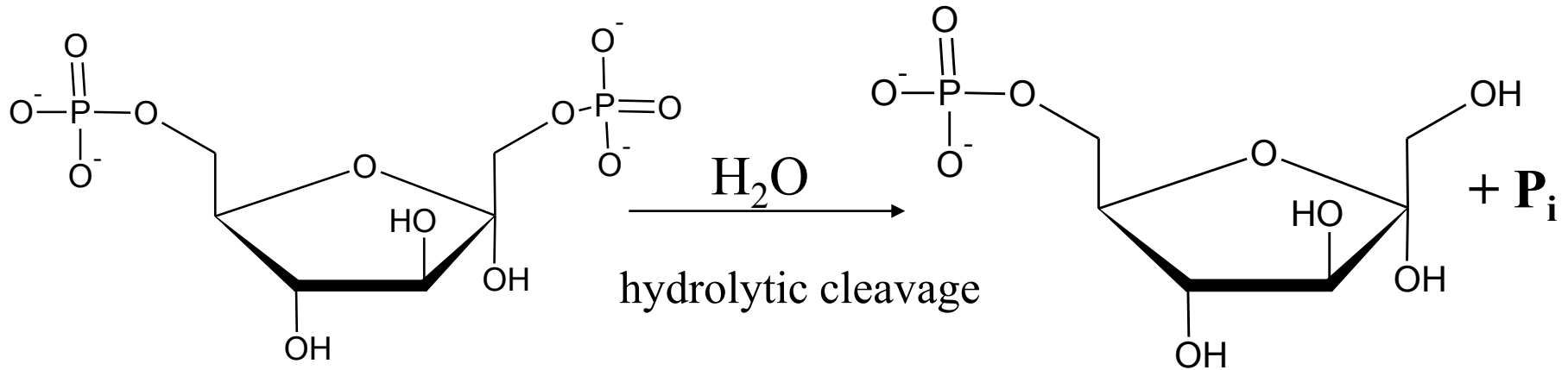


Irreversible reactions of glycolysis



The second unique reaction on gluconeogenesis

## 2. Dephosphorylation of fructose-1,6-bisphosphate



### fructose-1,6-bisphosphatase

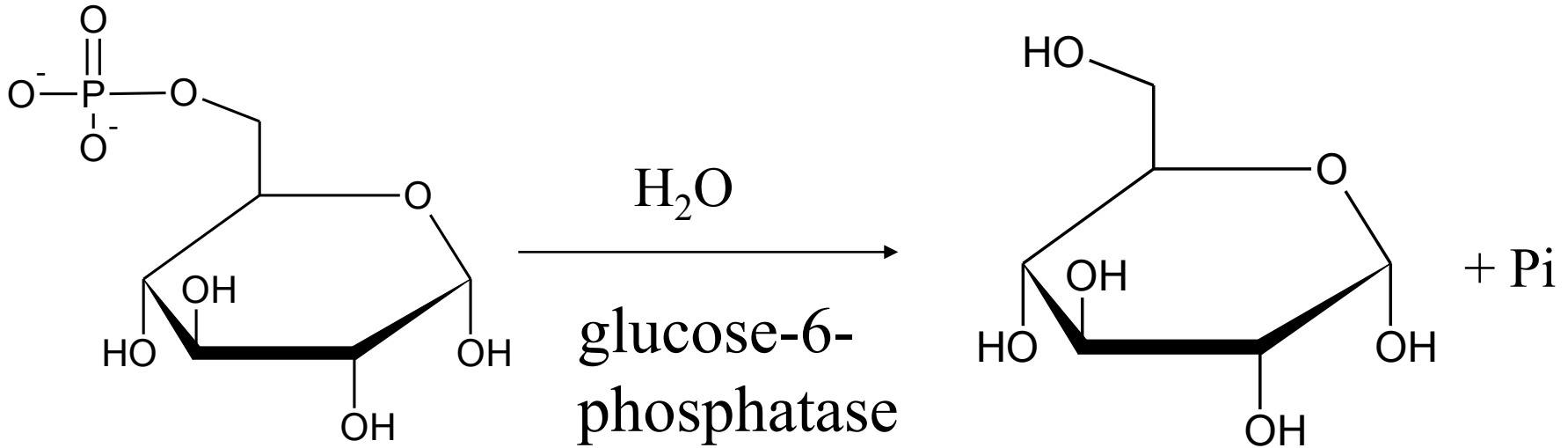
Like its glycolytic counterpart *phosphofructokinase-1*, it participates in the regulation of gluconeogenesis.

allosteric inhibition by AMP,  
activation by ATP

inhibition by fructose-2,6-  
bisphosphate (its level is  
decreased by glucagon)

The third unique reaction on gluconeogenesis

### 3. Dephosphorylation of glucose-6-P



It is present only in liver.

**Not present in muscle!!!**

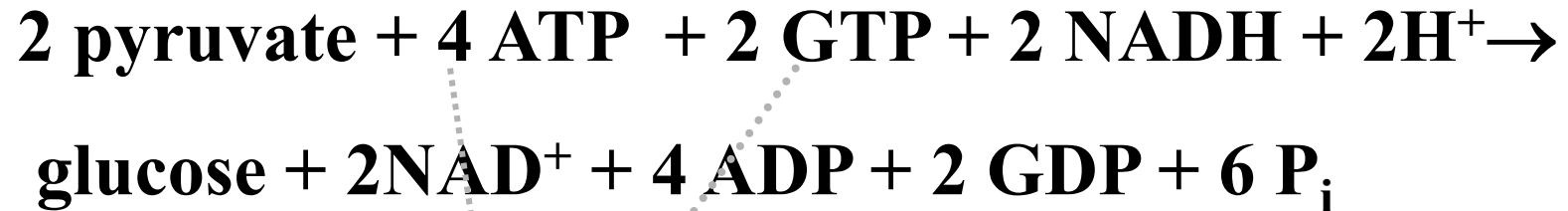
Enzyme is  
located in  
lumen of  
ER

# Energetic requirements for gluconeogenesis

reaction	ATP/glucose
2 pyruvate $\rightarrow$ 2 oxalacetate	-2
2 oxalacetate $\rightarrow$ 2 phosphoenolpyruvate	-2 (GTP)
2 3-phosphoglycerate $\rightarrow$ 2 1,3-bisphosphoglycerate	-2
	<hr/>
	<b>-6 ATP/glucose</b>

Source of energy is mainly  $\beta$ -oxidation of fatty acids

## Summary equation of gluconeogenesis



Consumption: -6 ATP

**Gluconeogenesis is energy demanding process**

# Origin of substrates for gluconeogenesis

## Pyruvate

E.g. from transamination of alanine, dehydrogenation of lactate

## Lactate

formation in tissues, transport by blood to the liver



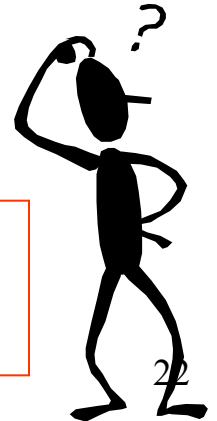
(Cori cycle)

## Glycerol

- formation in adipocytes at cleavage of triacylglycerols
- transport by blood to the liver
- in liver (cytoplasm):



What is the energy requirement for synthesis of 1 mol of glucose from glycerol?



## Glucogenic amino acids

They provide pyruvate or intermediates of citric acid cycle, that can be converted to oxalacetate

Acetyl CoA – is not the substrate for gluconeogenesis !!!

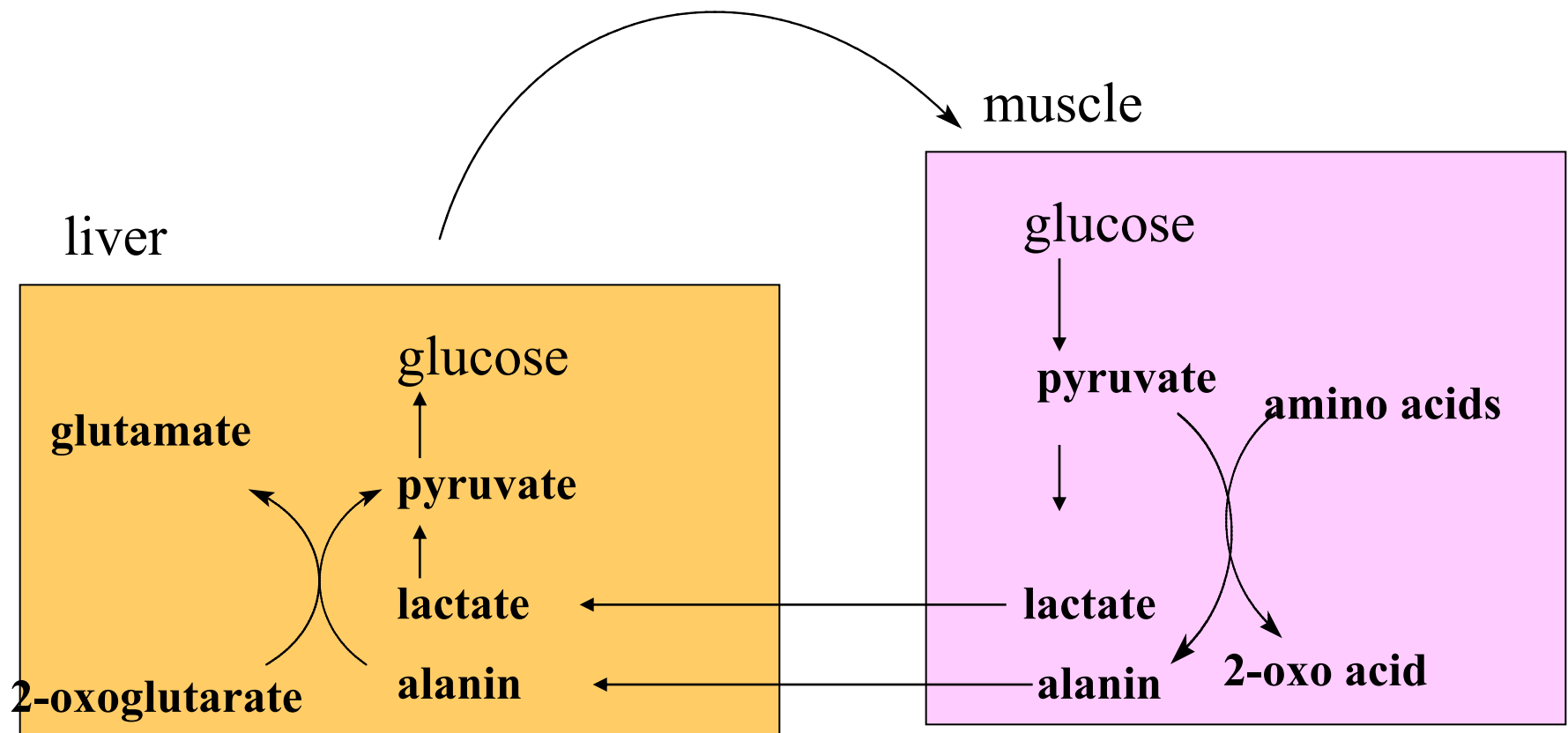
It is metabolised to  $\text{CO}_2$  in citric acid cycle.

**Fatty acid cannot be converted to glucose in animals!**

# The most important amino acid for gluconeogenesis is alanin

It is formed mainly in muscle by transamination of pyruvate and is transported by blood to the liver.

Here is again converted to pyruvate by reverse transamination





## Gluconeogenesis from lactate and glycerol requires $\text{NAD}^+$

The ratio  $\text{NADH}/\text{NAD}^+$  may be high at some metabolic conditions – gluconeogenesis can not occur

The ratio  $\text{NADH}/\text{NAD}^+$  is increased e.g. at ethanol metabolism (alcohol dehydrogenase).

Therefore intake of alcohol can decrease gluconeogenesis  $\Rightarrow$  hypoglycemia at alcoholics

# The main features of gluconeogenesis regulation

Availability of substrates.

Allosteric and hormonal regulation of irreversible reactions.

Allosteric effects are rapid (they affect the reaction immediately)

Hormons can act through

- direct inhibition or activation by a second messenger (rapid effect)
- induction or repression of enzyme synthesis (slow effect – hours - days)

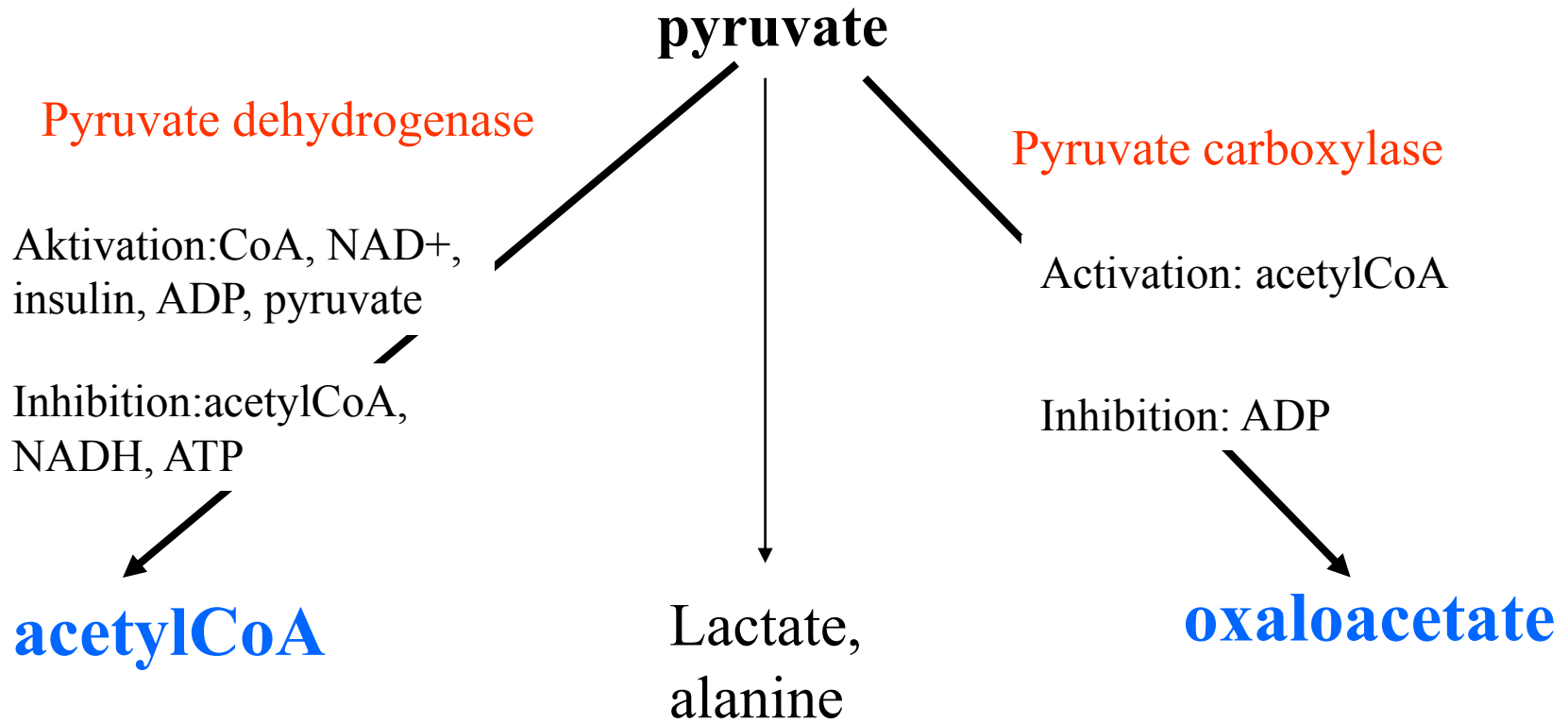
## Activation and inhibition of enzymes involved in glycolysis and gluconeogenesis

Enzyme	Activator	Inhibitor
<b>Hexokinase</b>		glucose-6-phosphate
<b>Phosphofructo kinase</b>	5'AMP, fructose-6-phosphate, fructose-2,6-bisphosphate	Citrate, ATP, glucagon
<b>Pyruvate kinase</b>	fructose-1,6-bisphosphate,	ATP, alanin
<b>Pyruvate dehydrogenase</b>	CoA, NAD <sup>+</sup> , ADP, pyruvate	acetylCoA, NADH, ATP
<b>Pyruvate carboxylase</b>	acetylCoA	ADP

## Effects of hormones on enzyme expression

Enzyme	Inductor	Represor
<b>glucokinase</b>	insulin	glucagon
<b>phosphofructokinase</b>	insulin	glucagon
<b>Pyruvate kinase</b>	insulin	glucagon
<b>Pyruvate carboxylase</b>	glucokortikoids glucagon Adrenalin	insulin
<b>phosphoenolpyruvate carboxykinase</b>	glucocorticoids glucagon adrenalin	insulin
<b>glucose-6-phosphatase</b>	glucocorticoids glucagon adrenalin	insulin

# Conversions of pyruvate at different conditions



# Gluconeogenesis in kidneys

Substrates: mainly lactate, glycerol and glutamin

Glucose can be released from kidneys – in post-resorptive state or during starvation, at acidosis

# Glycogen

- synthesis and degradation

## Glycogen storage

- synthesis and degradation of glycogen occurs in most types of cells, the largest stores are in liver and skeletal muscle.
- glycogen is a storage form of glucose in cells, that is rapidly released
- Muscle – the mass of glycogen is about 1-2% of muscle mass, glycogen is degraded during intensive muscle work or stress
- Liver: about 5-10 % of liver mass (after the meal)

Glycogen is degraded when glucose level in blood drops



## Storage of glucose in human (70 kg)

<b>Tissue</b>	<b>% tissue mass</b>	<b>Tissue mass (kg)</b>	<b>Mass of glucose (g)</b>
<b>Liver</b>	5,0	1,8	90 (glycogen)
<b>Muscle</b>	0,7	35	245 (glycogen)
<b>Extracellular glucose</b>	0,1	10	10

# Location of synthesis and degradation of glycogen

Glycogen is deposited cytoplasm of cells in form of glycogen particles (10-40 nm)

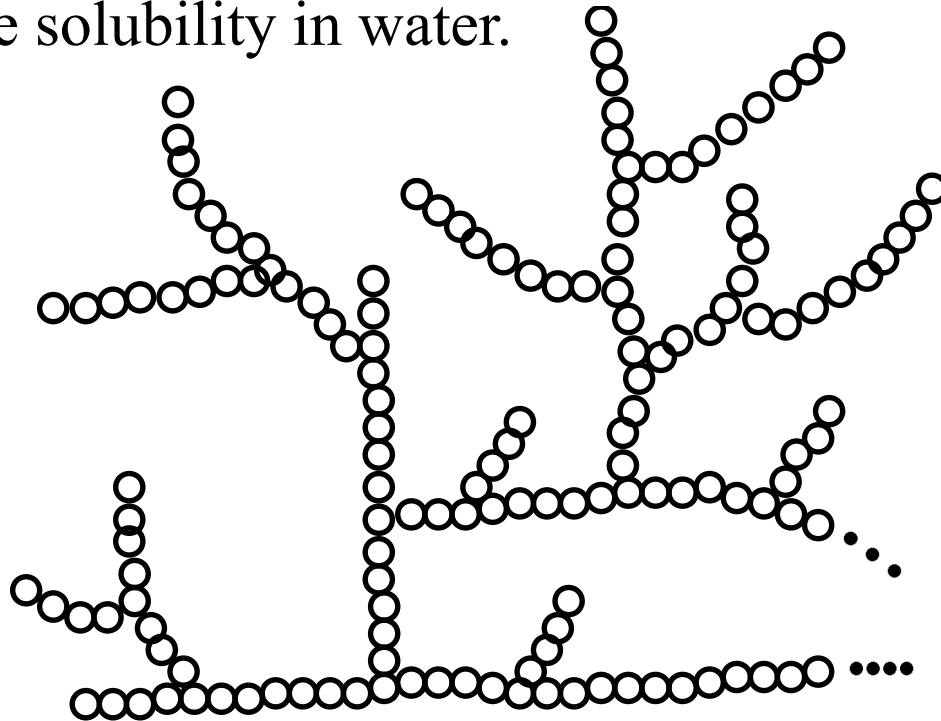
Enzymes of degradation and synthesis are on the surface of particles

Glycogenolysis is not a reversal process of synthesis.

## Molecules of glycogen have $M_r \sim 10^8$

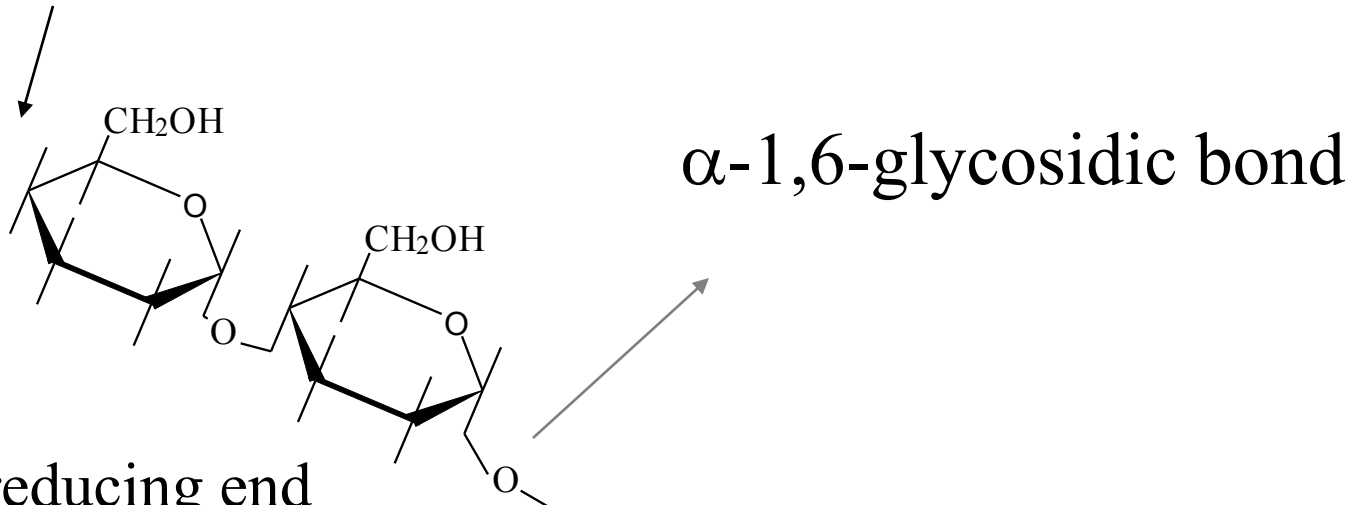
The branched structure permits rapid degradation and rapid synthesis, because enzymes can work on several chains simultaneously.

It also increases the solubility in water.

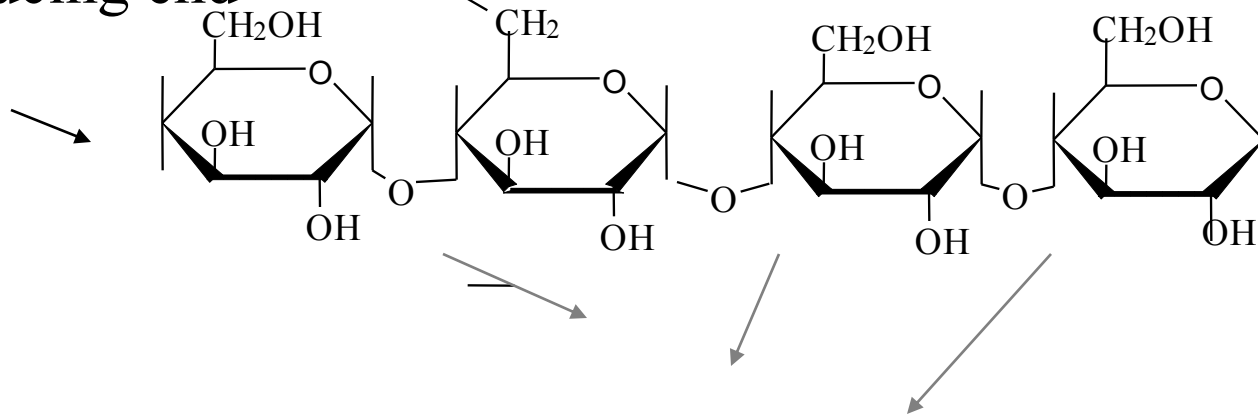


# Types of bonds in glycogen

Non-reducing end



Non-reducing end



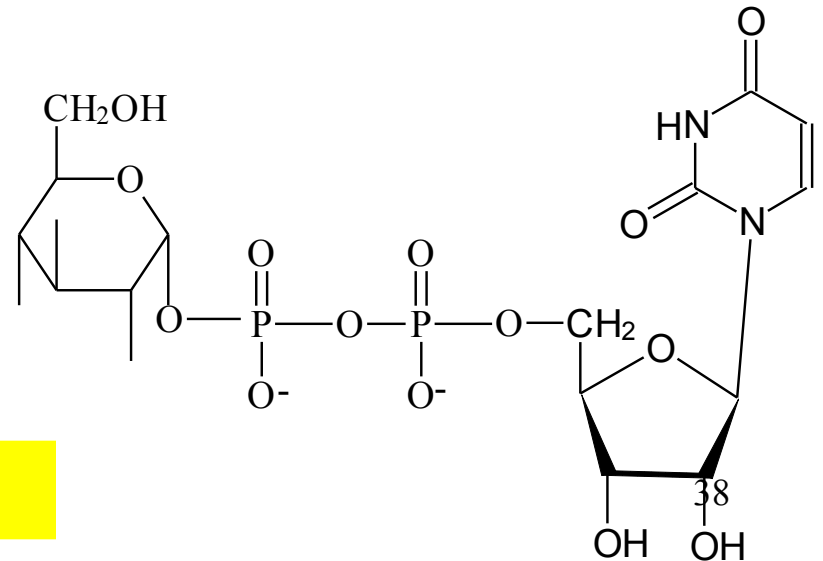
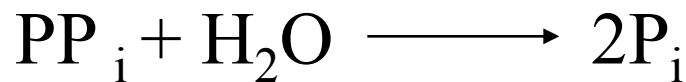
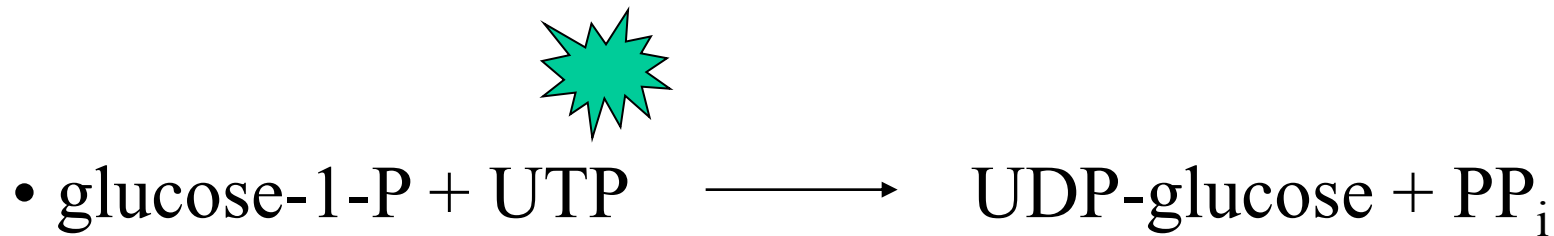
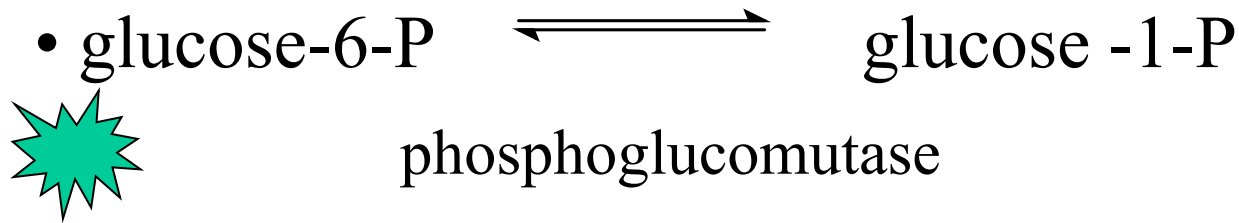
$\alpha$ -1,4-glycosidic bond

# Synthesis of glycogen (glycogenesis)

It occurs after the meal, activation by insulin

1. Activation of glucose to UDP-glucose
2. Transfer of glucosyl units from UDP-glucose to the 4' ends of glycogen chains or primers
3. Formation  $\alpha$ -1,4 glycosidic bond
4. Branching

# 1. Synthesis of UDP-glucose



2 ATP are consumed

## 2. Primer is necessary for synthesis of glycogen



Pre-existing  
fragment of  
glycogen

When glycogen stores are  
totally depleted, specific  
protein glycogenin serves an  
acceptor of first glucose  
residue

Autoglycosylation on serine  
residues

### 3. Formation of $\alpha$ -1,4 glycosidic bonds glycogensynthase

- Initiation – glucosyl residue is added from UDP-glucose to the non-reducing terminal of the primer by glycogen synthase
- Elongation by glycogensynthase - formation of linear chains with  $\alpha$ -1,4 glycosidic bond  
$$\text{UDP-glucose} + [\text{glucose}]_n \rightarrow [\text{glucose}]_{n+1} + \text{UDP}$$



## 4. Branching

(branching enzyme)

5-8 glucosyl residues are transferred from non-reducing end to another residue of the chain and attached by 1,6-glycosidic bond



↓

Elongation of both non-reducing ends by glycogensynthase

↓

New branching by branching enzyme

# Degradation of glycogen (phosphorolysis)

Proceeds during fasting (liver), muscle work (muscle) or stress (liver and muscle).

1. **phosphorolytic** cleavage of  $\alpha$ -1,4 glycosidic bonds by phosphorylase
2. Removal of  $\alpha$ -1,6 branching (debranching enzyme)

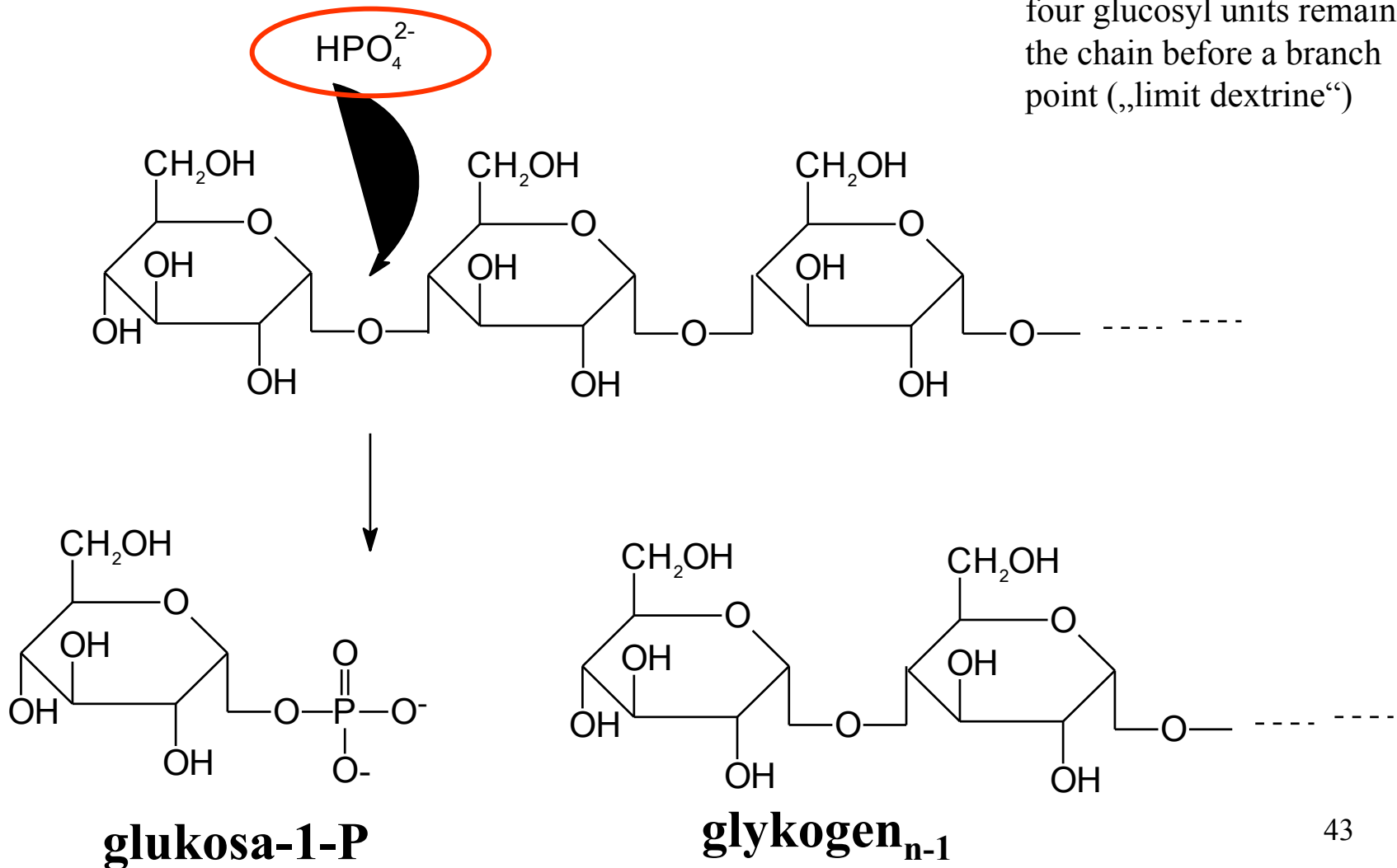
Compare:

**Hydrolysis x phosphorolysis**



# 1. Phosphorylase - phosphorolytic cleavage of $\alpha$ -1,4 glycosidic bonds at the non-reducing ends

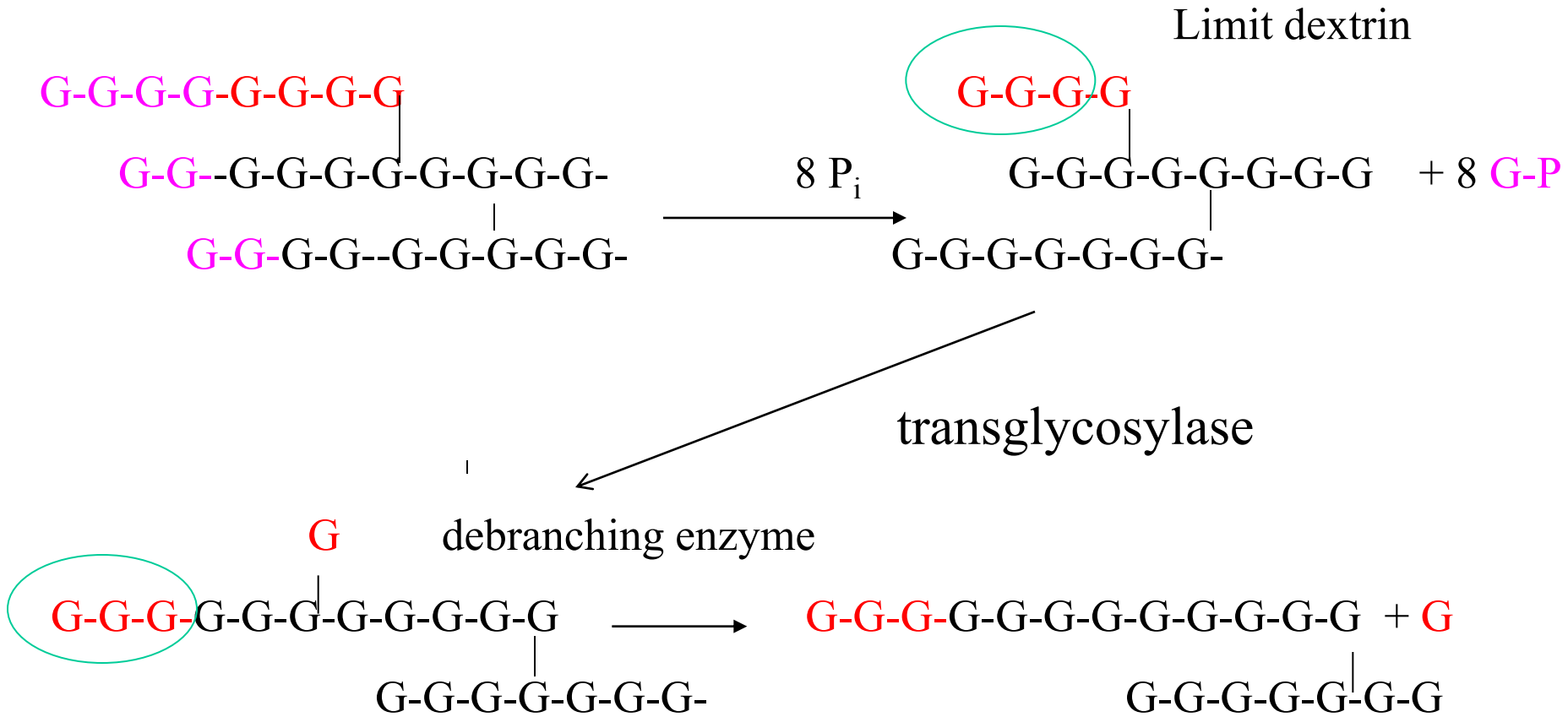
The cleavage continues until four glucosyl units remain on the chain before a branch point („limit dextrine“)



# Degradation of glycogen

*Phosphorylase* can split  $\alpha$ -1,4-links,  
its action ends with the production of **limit dextrins**

:



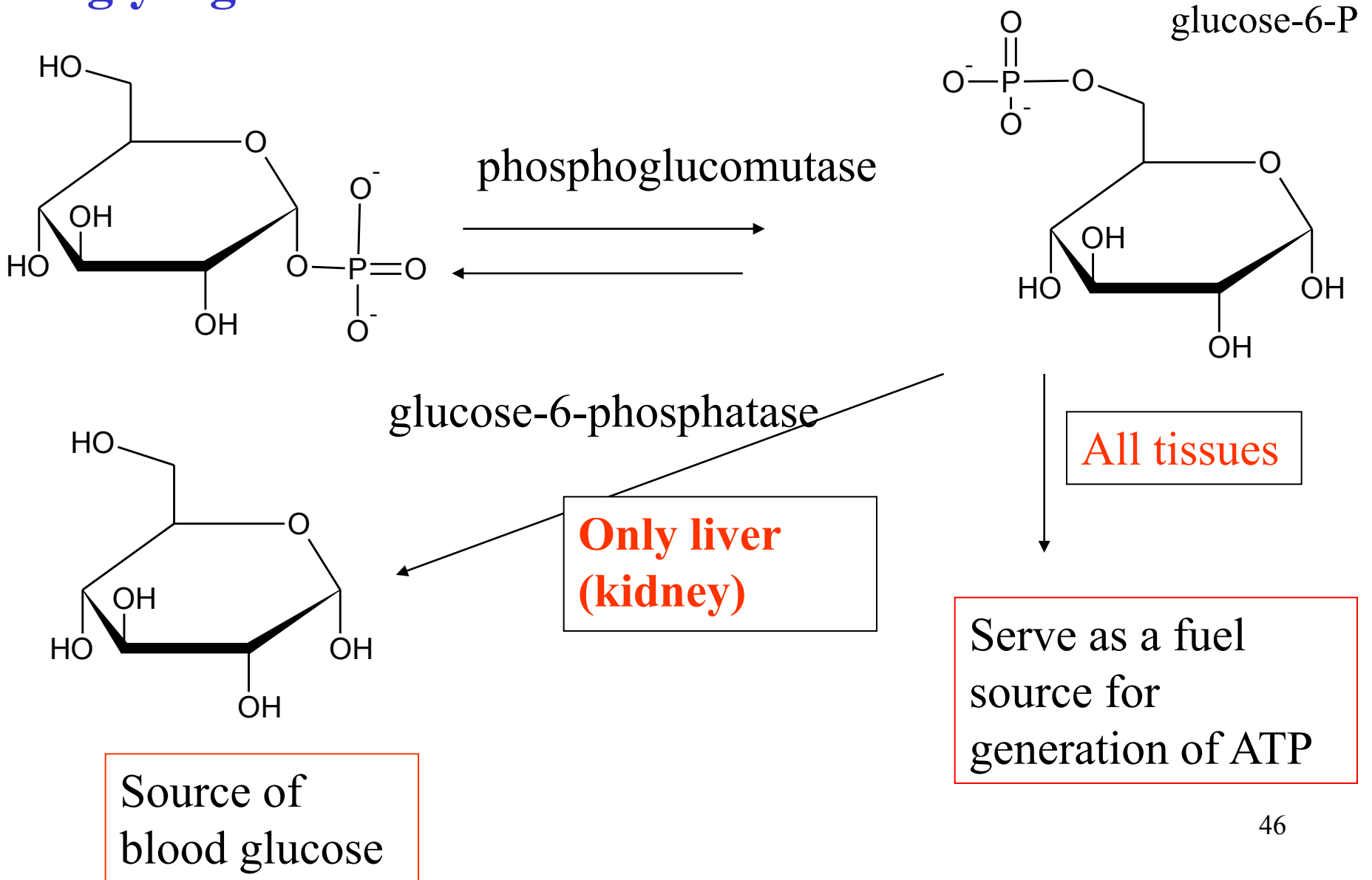
## 2. Debranching enzyme

**transferase activity:** enzyme transfers unit containing 3 from 4 glucose molecules remaining on the 1,6-branch and adds it to the end of a longer chain by  $\alpha$ -1,4 glycosidic bond

**glucosidase activity:** the one glucosyl residue remaining at the end of  $\alpha$ -1,6 branch is hydrolyzed by the 1,6 – glucosidase activity of debranching enzyme

Free **glucose** is released ! Not Glc-1-P

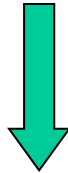
# Further fates of glucose-1-phosphate formed from glycogen



## Significance of glucose-6-phosphatase

glucose-6-P cannot permeate across the cellular membrane, only free glucose can diffuse

Enzyme glucose-6-phosphatase is only in liver and kidneys – it is not present in muscle.



Blood glucose can be maintained only by cleavage of liver glycogen but not by cleavage of muscle glycogen

Cleavage of glycogen in muscle and other cells provides glucose-6-P that can be metabolized only within the given cell (by glycolysis)

# Lysosomal degradation of glycogen

Lysosomal acidic glucosidase (pH optimum 4)

Degradation of about 1-3% of cellular glycogen (glycogen particles are surrounded by membranes that then fuse with the lysosomal membrane)

- enzyme degrades  $\alpha$ -1,4-bonds from non-reducing end

- glucose is released

(see also Pompe disease)



# Regulation glycogen metabolism

Allosteric regulation



**Glycogen synthase**

**X**

**glycogen phosphorylase**



Hormonal control

## Hormons affecting synthesis and degradation of glycogen

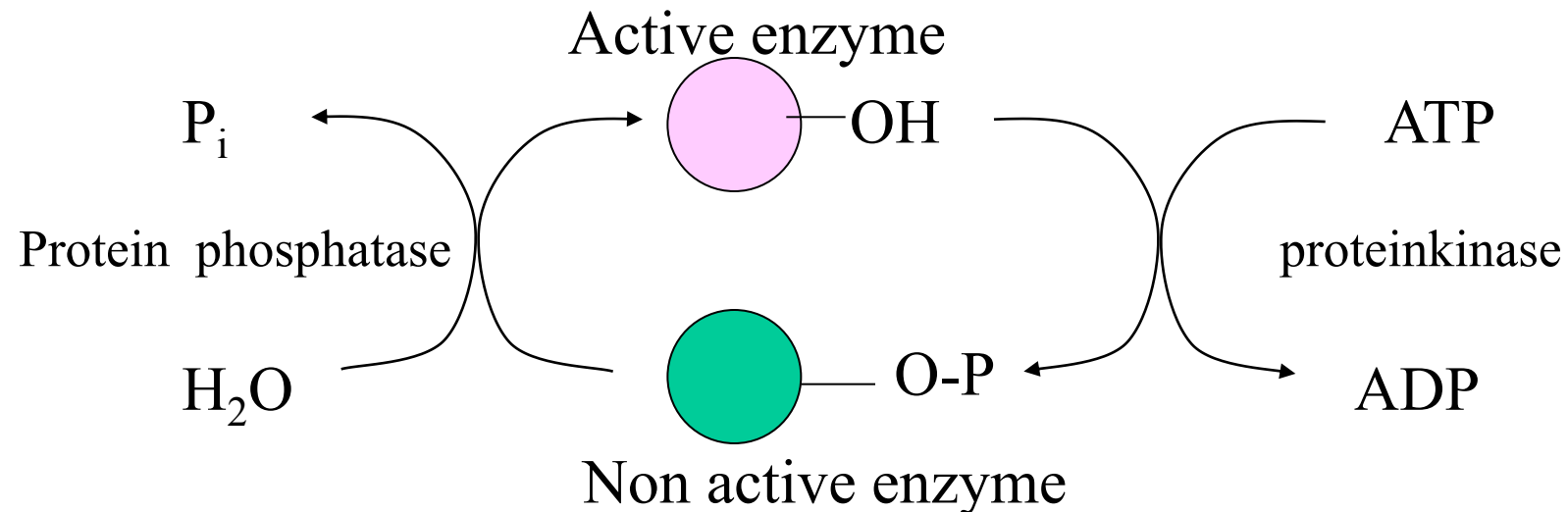
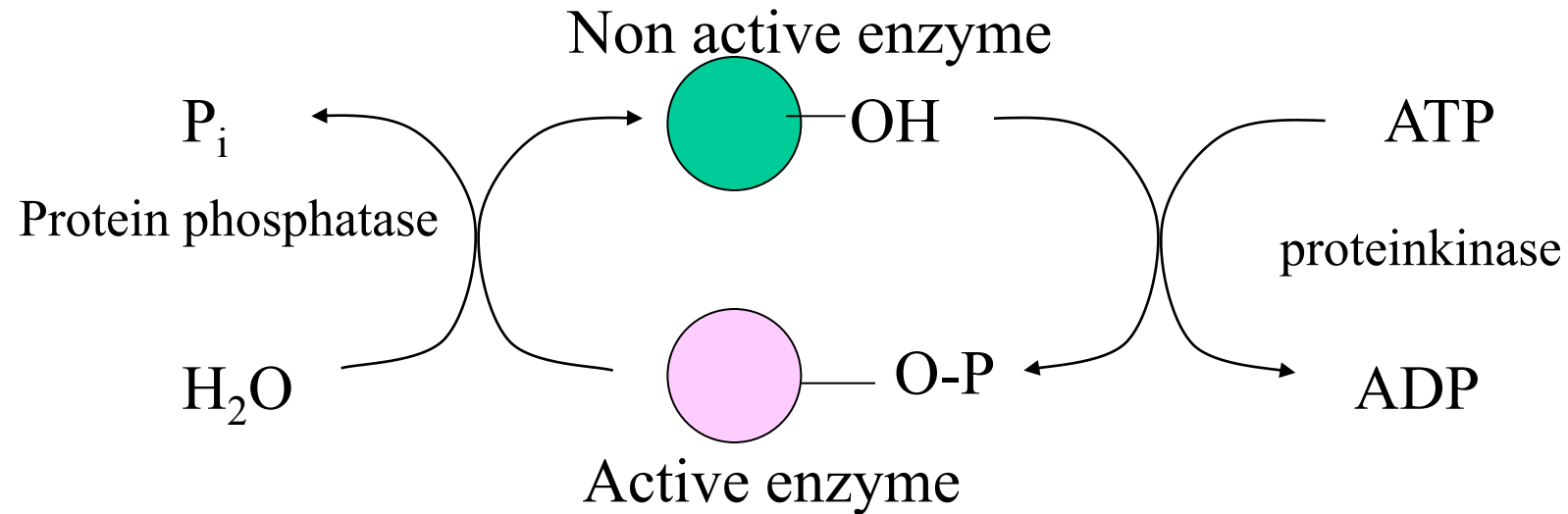
Hormon	synthesis	degradation
Insulin	↑	↓
Glucagon	↓	↑
Adrenalin	↓	↑

Hormons action is mediated by their second messengers.

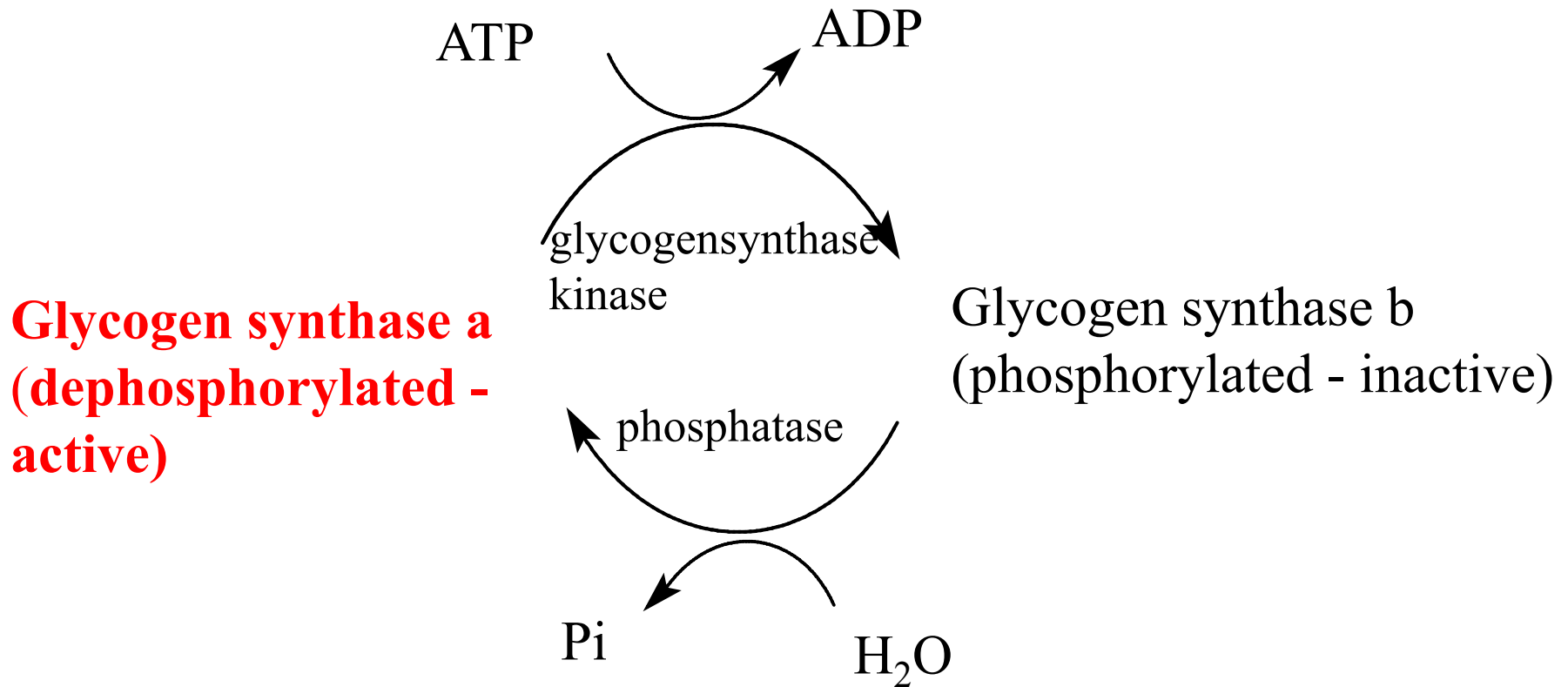
## **Phosphorylation and dephosphorylation plays important role at regulation of glycogen metabolism**

- phosphorylation by kinases and ATP
- dephosphorylation by phosphatases

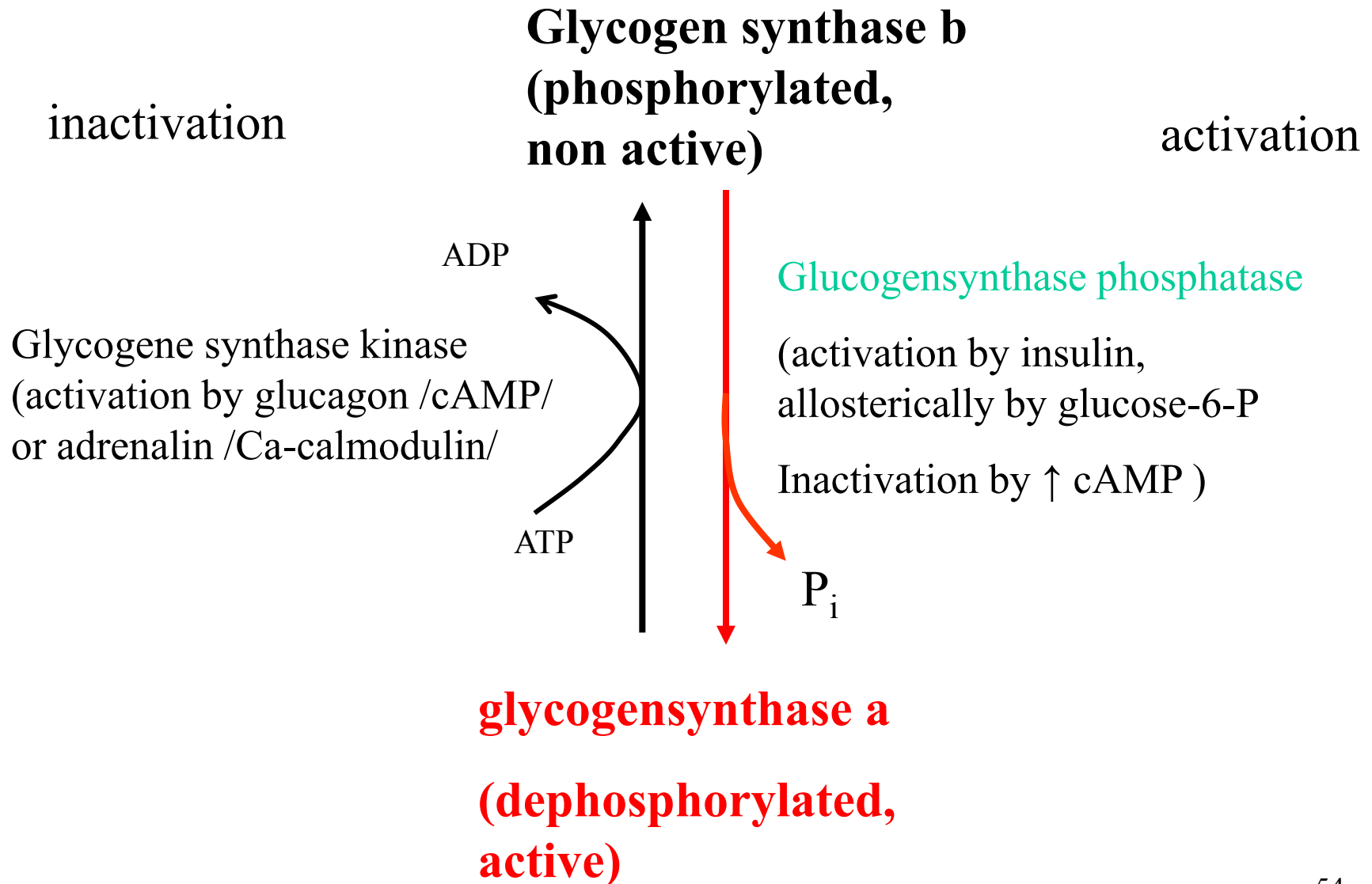
# Common examples of enzyme activity regulation by phosphorylation and dephosphorylation



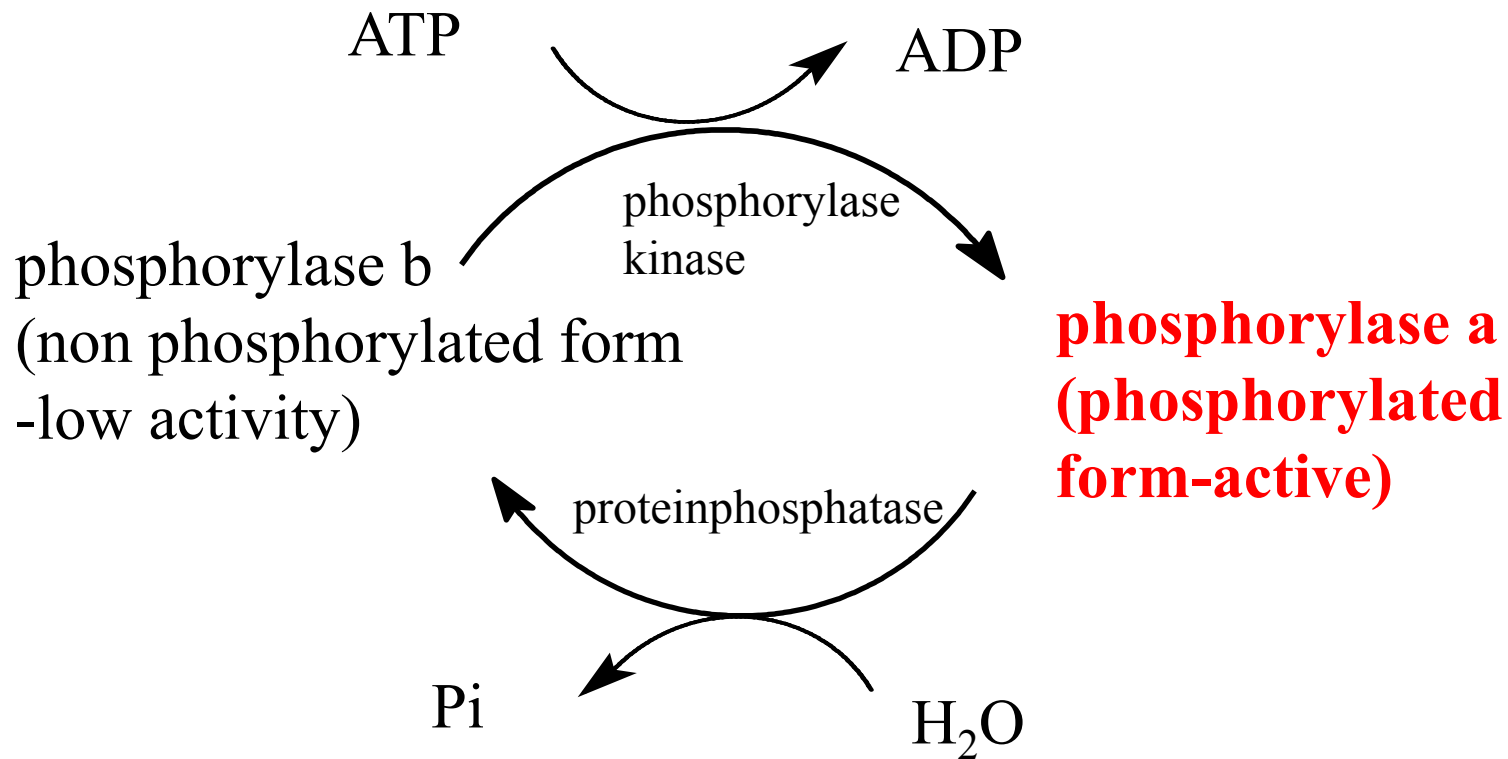
# Activation and inactivation of glycogen synthase



# Activation and inactivation of glycogen synthase in liver



# Activation and inactivation of glycogen phosphorylase



**Phosphorylases in liver and muscles are different**

# Degradation of glycogen

## Effect of **hormons**:

### **Liver:**

glucagon (cAMP),

adrenalin (cAMP,  $\text{Ca}^{2+}$ calmodulin)

### **Muscle:**

adrenalin (cAMP) at the stress

No effect of glucagon !

## **allosteric regulation**

Glucose, ATP, Glc-6P: allosteric inhibition

$\uparrow \text{Ca}^{2+}$  during muscle contraction

AMP



# Glycogen storage diseases - enzyme defects

Inherited enzyme deficiencies. They can be tissue specific, as in various tissues can be various isoenzymes.

Typ	Enzyme defect	Organ	Characteristics
0	Glycogen synthase	Liver	Hypoglycemia
I	Glc-6-phosphatase	Liver, kidney	Enlarged liver, kidney. Hypoglykemia. Celly are overloaded by glycogen
II	Lysosome $\alpha$ -glucosidase	All organs	Accumulation of glycogen in lyzosomes
III	Debranching enzyme	Liver, muscle, heart	Accumulation of branched polysaccharide.
IV	Branching enzyme	Liver	Accumulation of unbranched polysaccharide
V	Muscle phosphorylase	Muscle	High content of glycogen in muscle, exercise induced muscular pain
VI	Liver phosphorylase	Liver	High content of glycogen in liver, mild hypoglycemia
VII	Phosphofructokinase	Muscle, ercs	As in tyne V

Enlarged liver, increased  
glycogen store

## Von Gierke disease (type I)



Most common

Deficit of glucose-6-phosphatase or transporter for glucose-6-P

Consequences:

Inability to provide glucose during fasting state

- hypoglycemia at fasting
- lactacidemia
- (hyperlipidemia, hyperurikemia)

Growth reatardation, delayed puberty

## Pompe disease (type II)

Absence of  $\alpha$ -1,4-glucosidase in lysosomes

Accumulation of glycogen in lysosomes

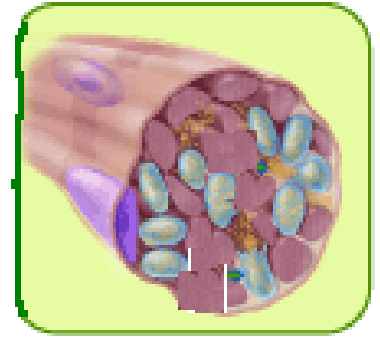
Loss of lysosomal function

Damage of muscles  $\rightarrow$  muscle weakness

Infantile form: death before age 2 years

Juvenile form: later –onset myopathy with variable cardiac involvement

Adult form: limb-girdle muscular dystrophy-like features.



# McArdle disease (type V)

Absence of muscle phosphorylase

Stores of glycogen are not available for production of energy

Muscle is not able to perform exercise or work