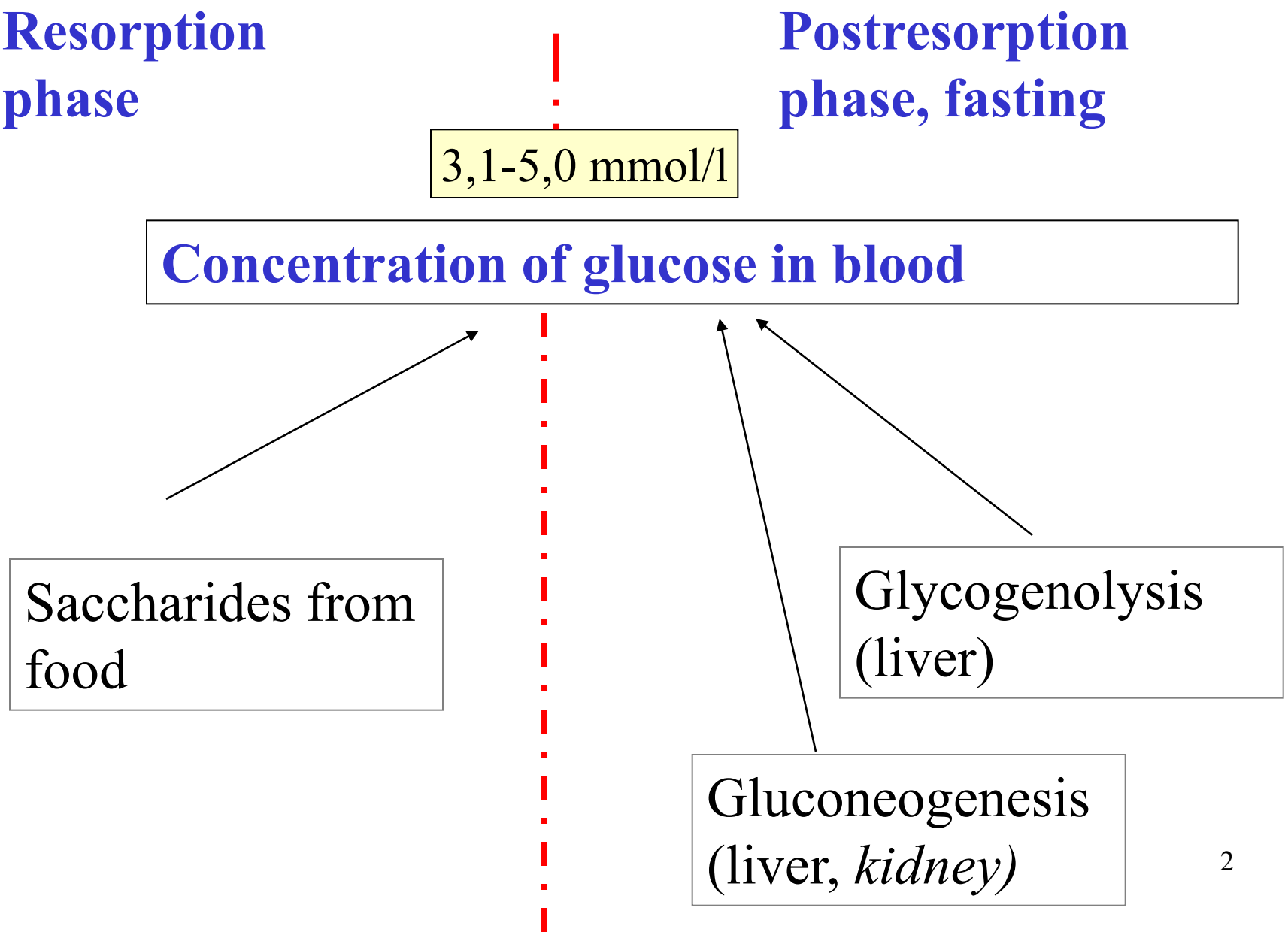
Gluconeogenesis
Glycogen metabolism

Glucose in blood



Main hormones in metabolism of glucose

Hormone	Source	Effect on the level of glucose
Insulin	β-cells of pancreas	↓
Glucagon	α-cells of pancreas	↑
Adrenaline	Adrenal medulla	↑
Cortisol	Adrenal cortex	↑

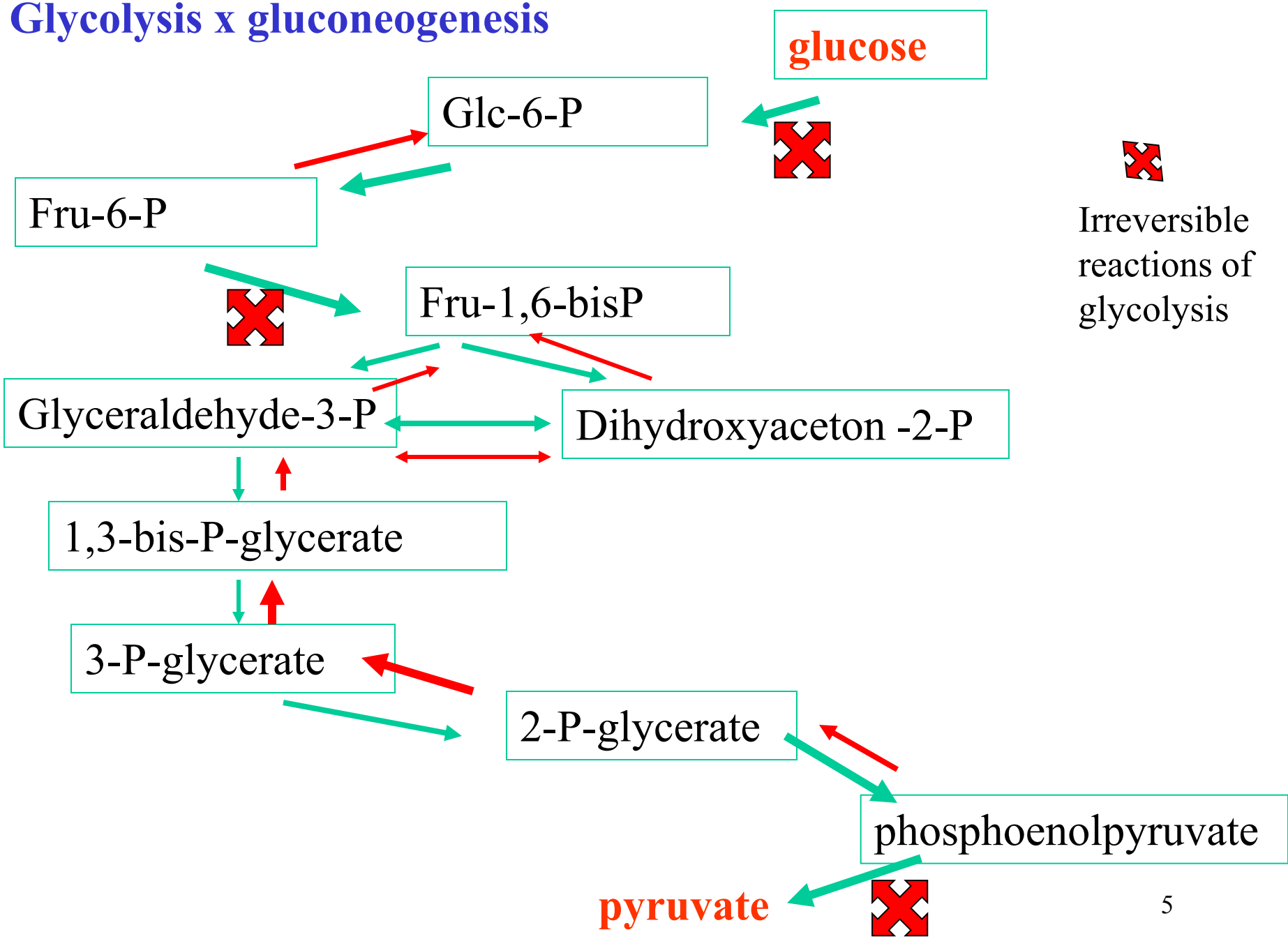
Gluconeogenesis - synthesis of glucose *de novo*

- Organ: liver (kidney)
- Location: cytoplasma
- Substrates for synthesis: non-saccharide compounds (lactate, pyruvate, glucogenic 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)

1. Glc + ATP $\rightarrow$ Glc-6-P + ADP

(reverse reaction is catalyzed by different enzyme)

2. Fru-6-P + ATP $\rightarrow$ Fru-1,6-bisP

(reverse reaction is catalyzed by different enzyme)

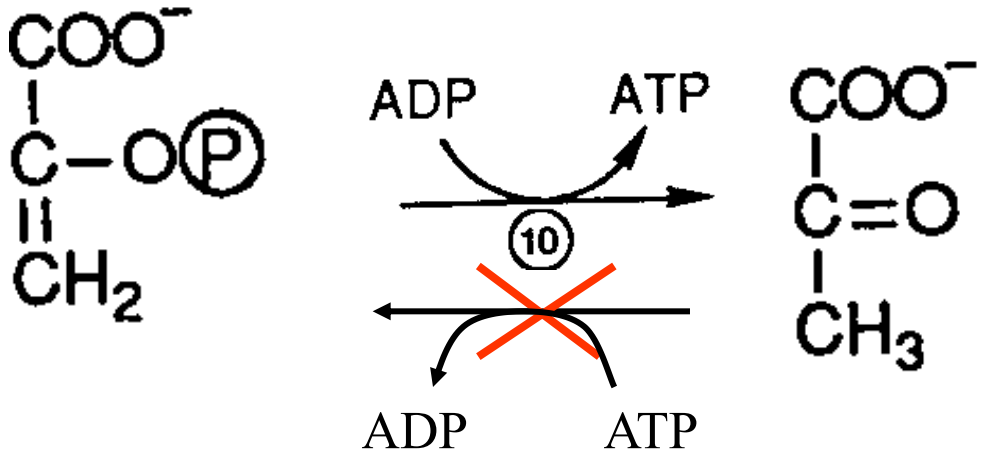
3. PEP + ADP $\rightarrow$ pyruvate + ATP

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

Reactions unique to gluconeogenesis

1. Synthesis of phosphoenolpyruvate

Why the reverse reaction cannot proceed?



$\Delta G^o = -61,9$ kJ/mol

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

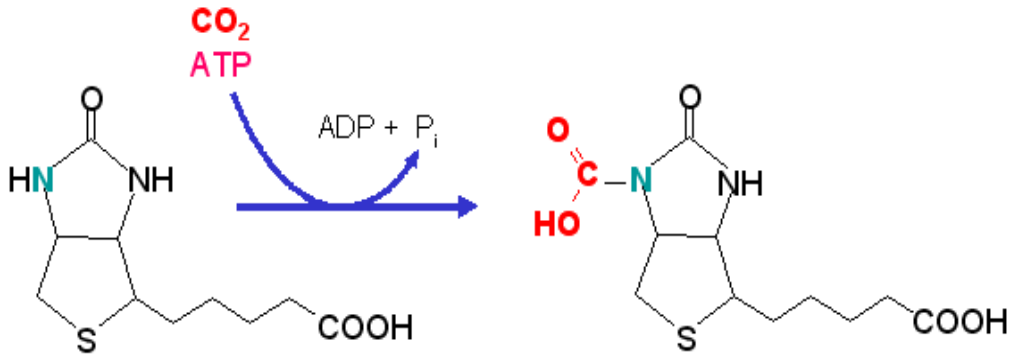
2. Conversion of oxalacetate to phosphoenolpyruvate

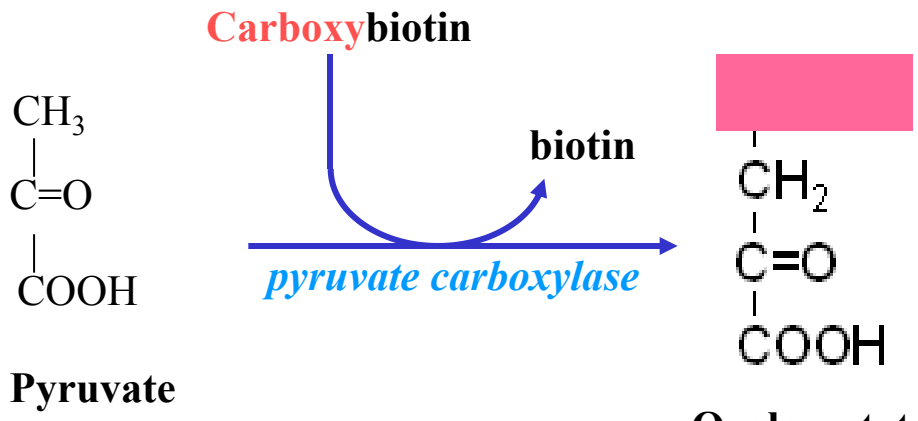
enzyme:	phosphoenolpyruvate carboxykinase
energy:	consumption of **1 GTP**
location:	cytoplasma

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

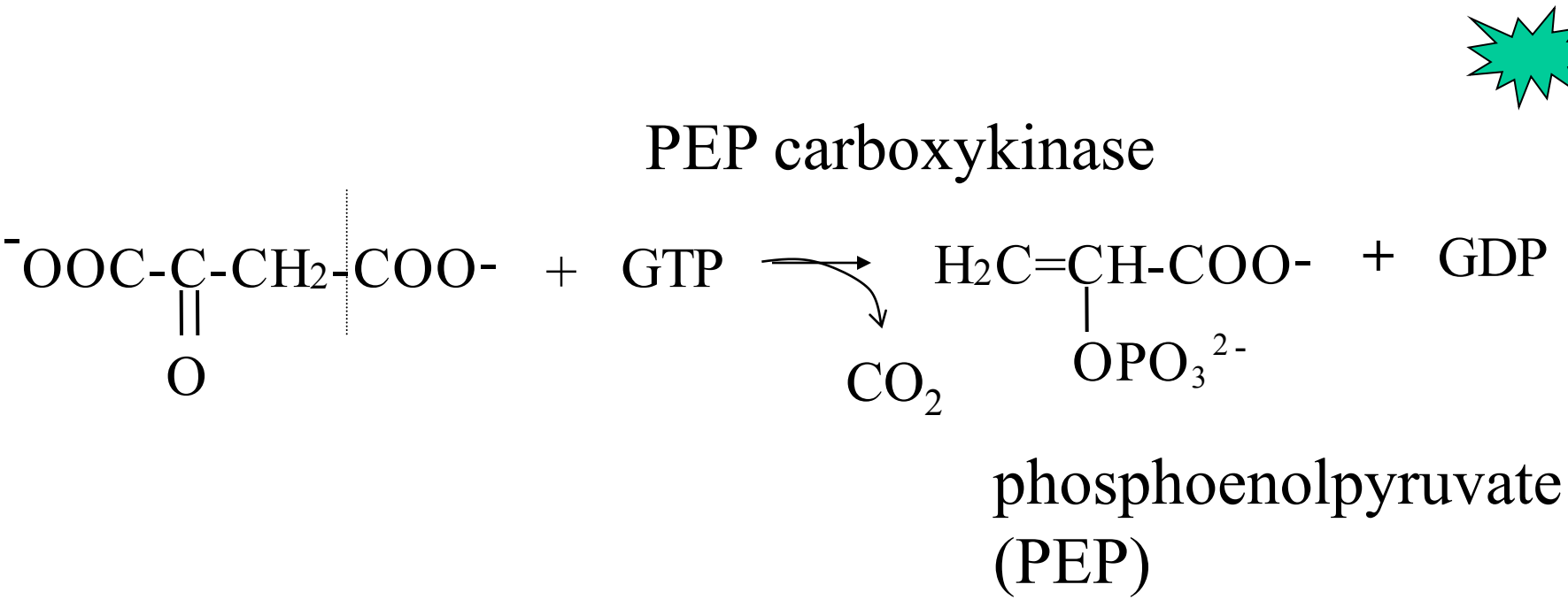
1. Conversion of pyruvate to phosphoenolpyruvate (reaction)

•carboxylation pyruvate





- **decarboxylation of oxalacetate**



PEP enters reversible reactions of glycolysis

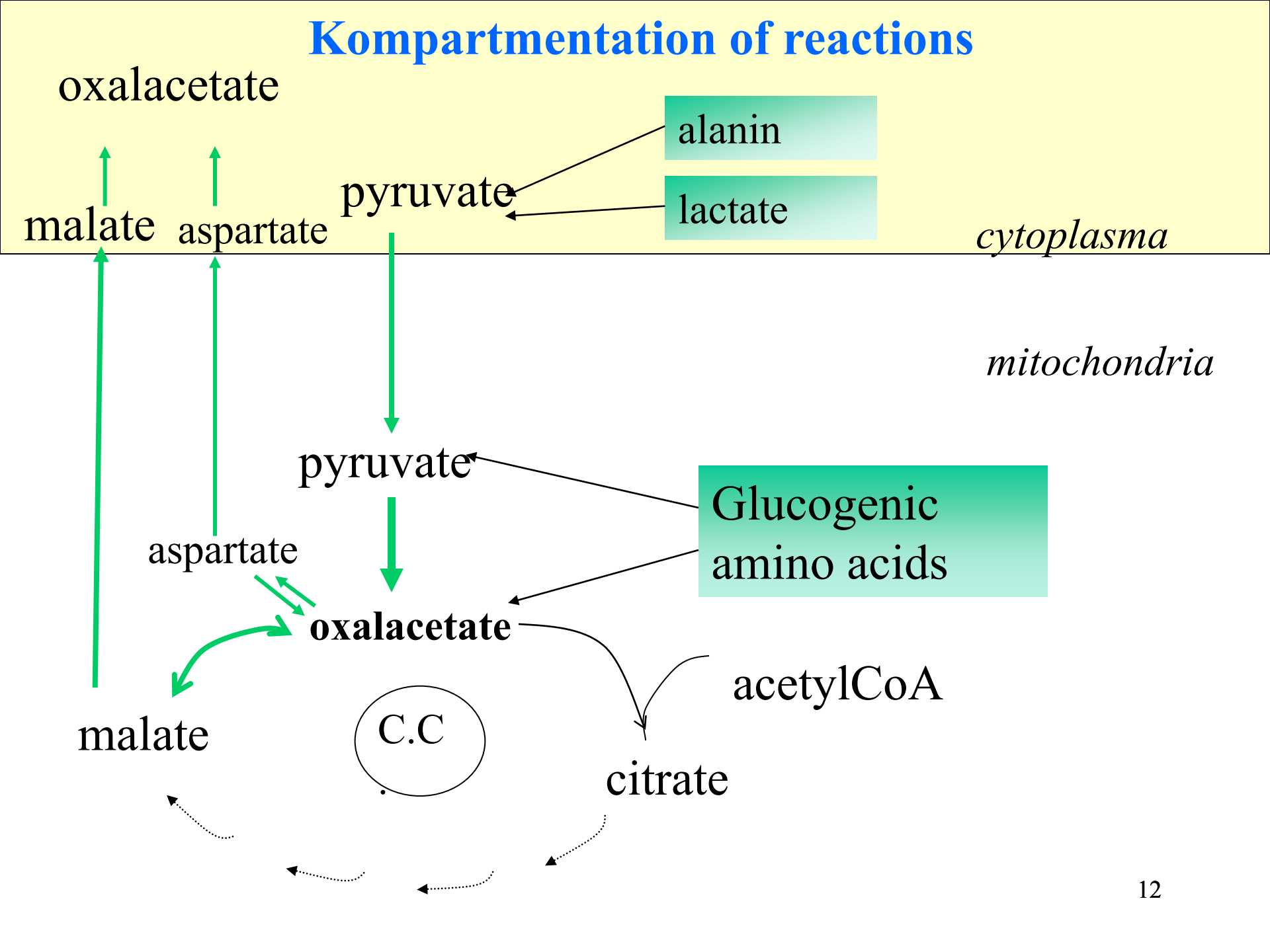
Compartmentation of reactions at phosphoenolpyruvate formation

• Carboxylation of pyruvate is located in mitochondrial matrix – at the same time it can serve as anaplerotic reaction of citric acid cycle (se lecture citric acid cycle)

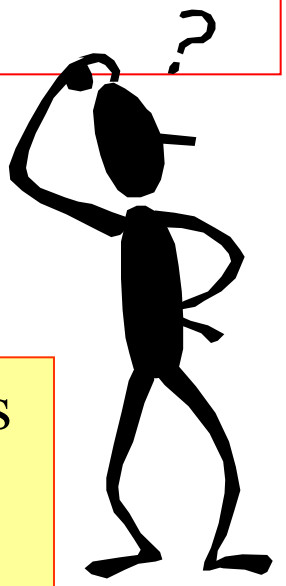
• Oxaloacetate cannot be transported across mitochondrial membrane – it must be transported in form of malate or aspartate

• malate ans aspartate are again converted to oxaloacetate in cytoplasma

Kompartmentation of reactions



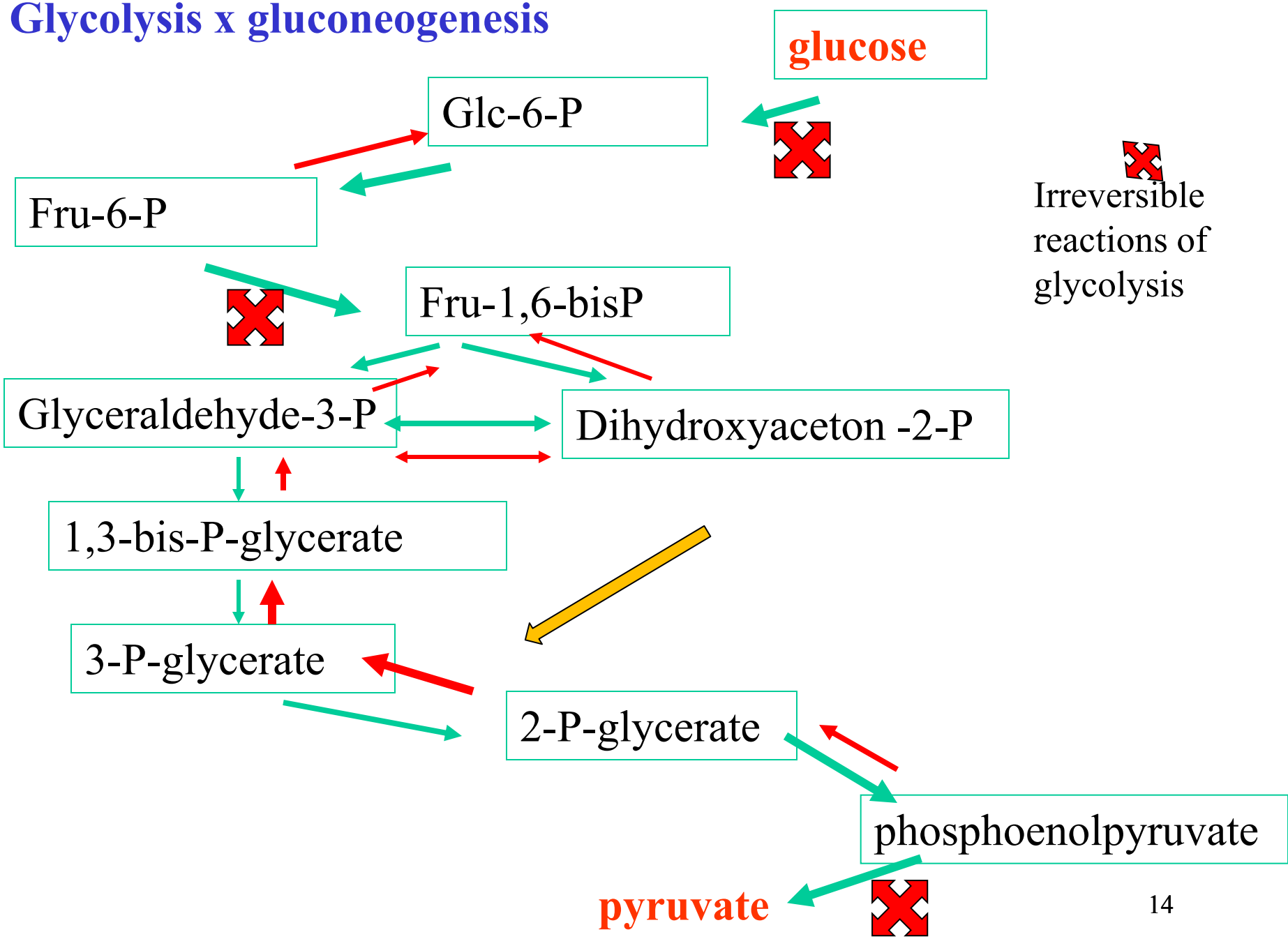
Synthesis phosphoenolpyruvate from pyruvate or lactate requires consumption of 2 ATP



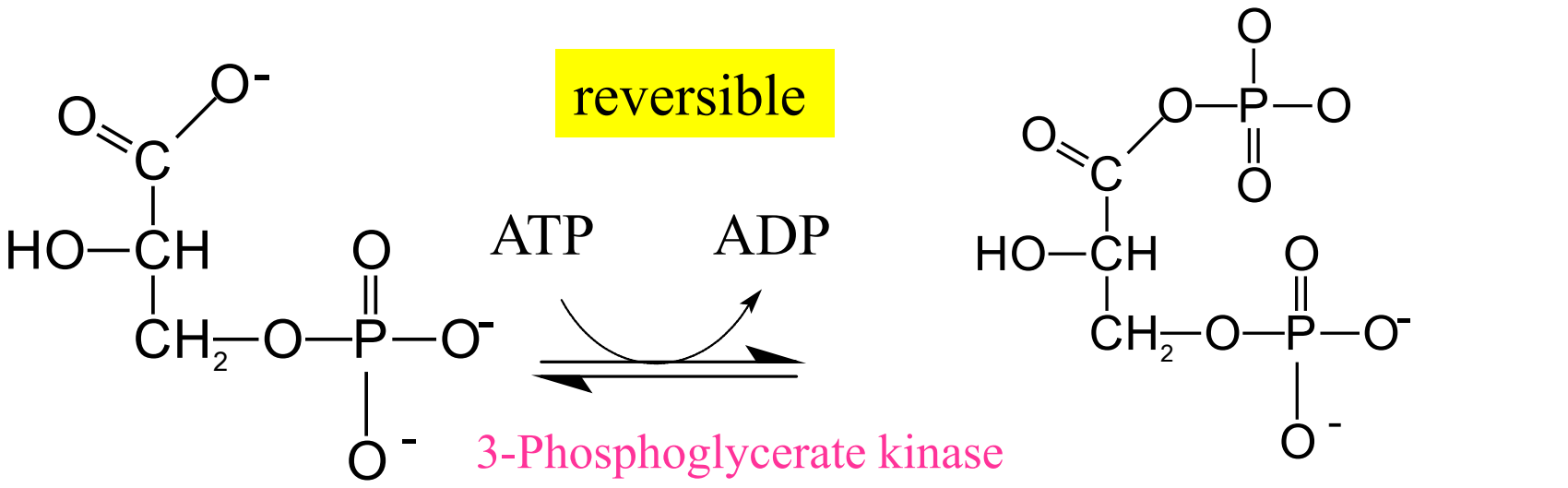
Pairing of carboxylation and decarboxylation drives the reaction that would be otherwise energetically unfavorable.

(see also the synthesis of fatty acids)

Glycolysis x gluconeogenesis



Further consumption of ATP at gluconeogesis

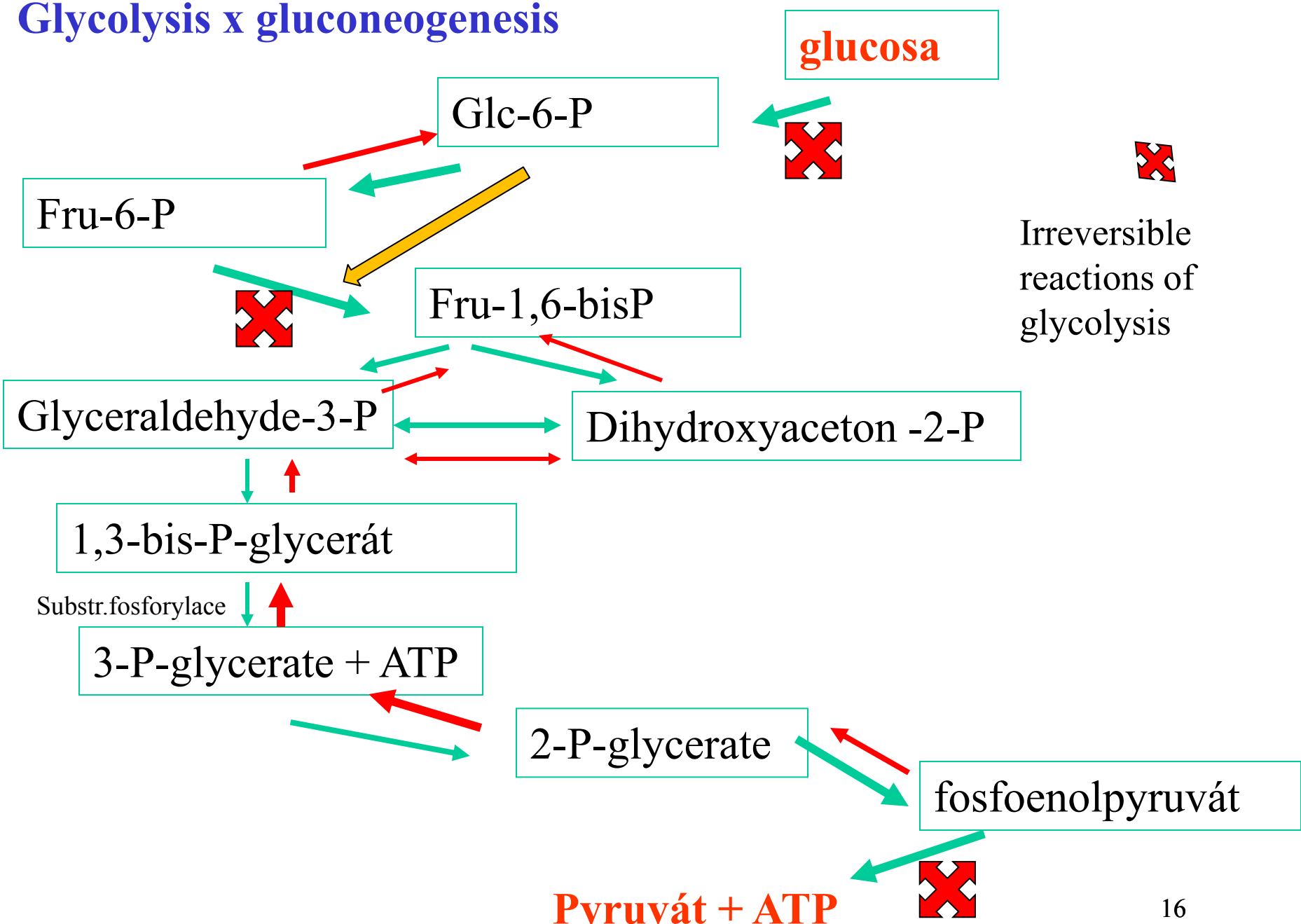


3-phosphoglycerate

1,3-bisphosphoglycerate

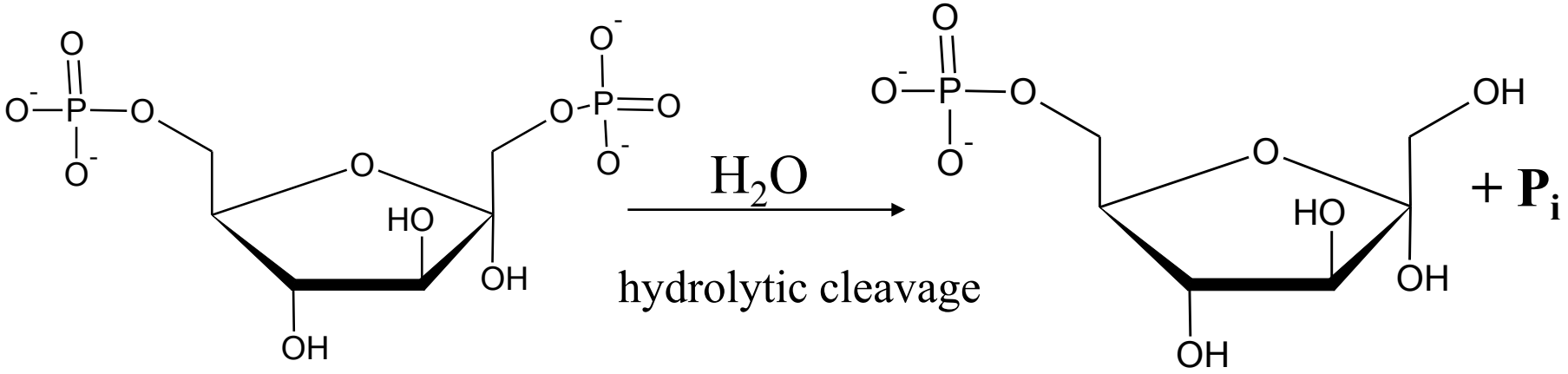
Reversal proces of substrate phosphorylation in glycolysis

Glycolysis x gluconeogenesis



The second unique reaction on gluconeogesis

2. Dephosphorylation of fructose-1,6-bisphosphate



fructose-1,6-bisphosphatase

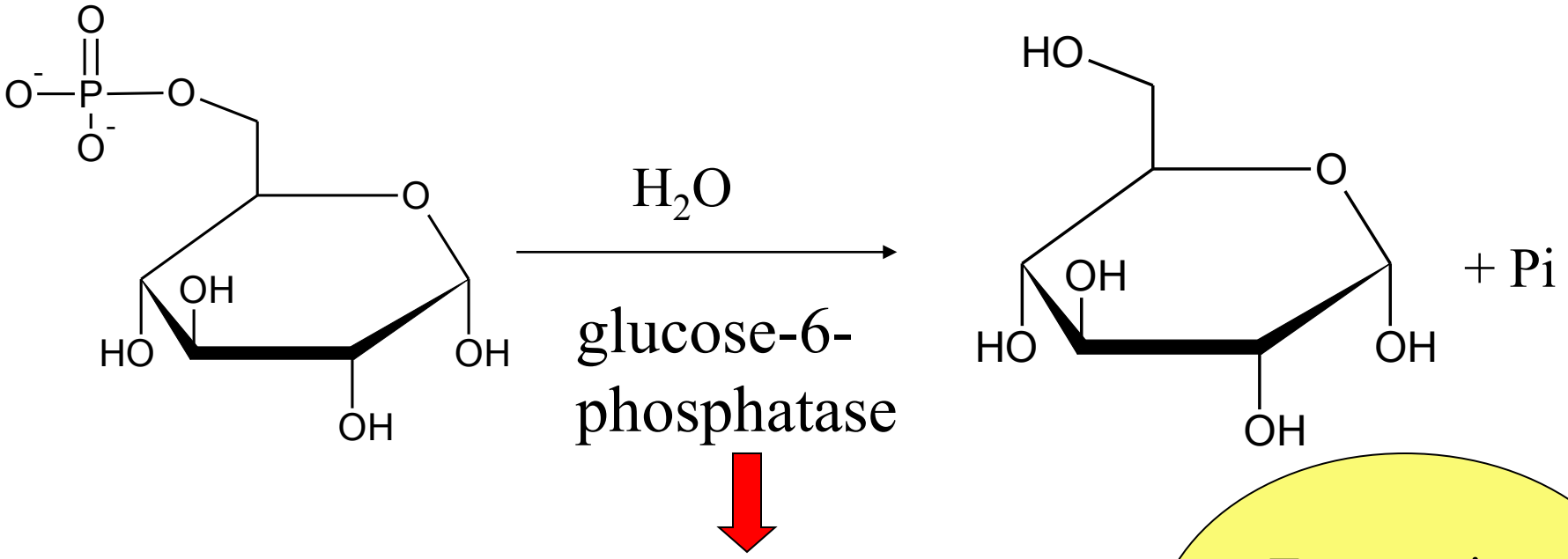
Like its glycolytic counterpart ***phosphofructokinase*-1**, it participates in the regulation of gluconeogenesis.

allosteric inhibition by AMP, activation by ATP

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

The third unique reaction on gluconeogesis

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 gluconeogenis

reaction	ATP/glucose
2 pyruvate → 2 oxalacetate	-2
2 oxalacetate → 2 phosphoenolpyruvate	-2 (GTP)
2 3-phosphoglycerate → 2 1,3-bisphosphoglycerate	-2

-6 ATP/glucose

Source of energy is mainly β-oxidation of fatty acids

Sumary equation of gluconeogenesis

2 pyruvate + 4 ATP + 2 GTP + 2 NADH + $2H^+ \rightarrow$

glucose + $2NAD^+$ + 4 ADP + 2 GDP + 6 P_i

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

lactate + $NAD^+ \rightarrow$ pyruvate + NADH + H^+ (cytoplasma)

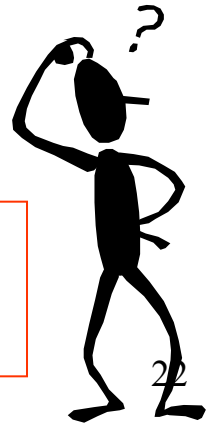
(Cori cycle)

Glycerol

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

glycerol + ATP $\rightarrow$ glycerol-3-P + ADP

glycerol-3-P + NAD^+ $\leftrightarrows$ dihydroxyaceton-P + NADH + H^+



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 !!!

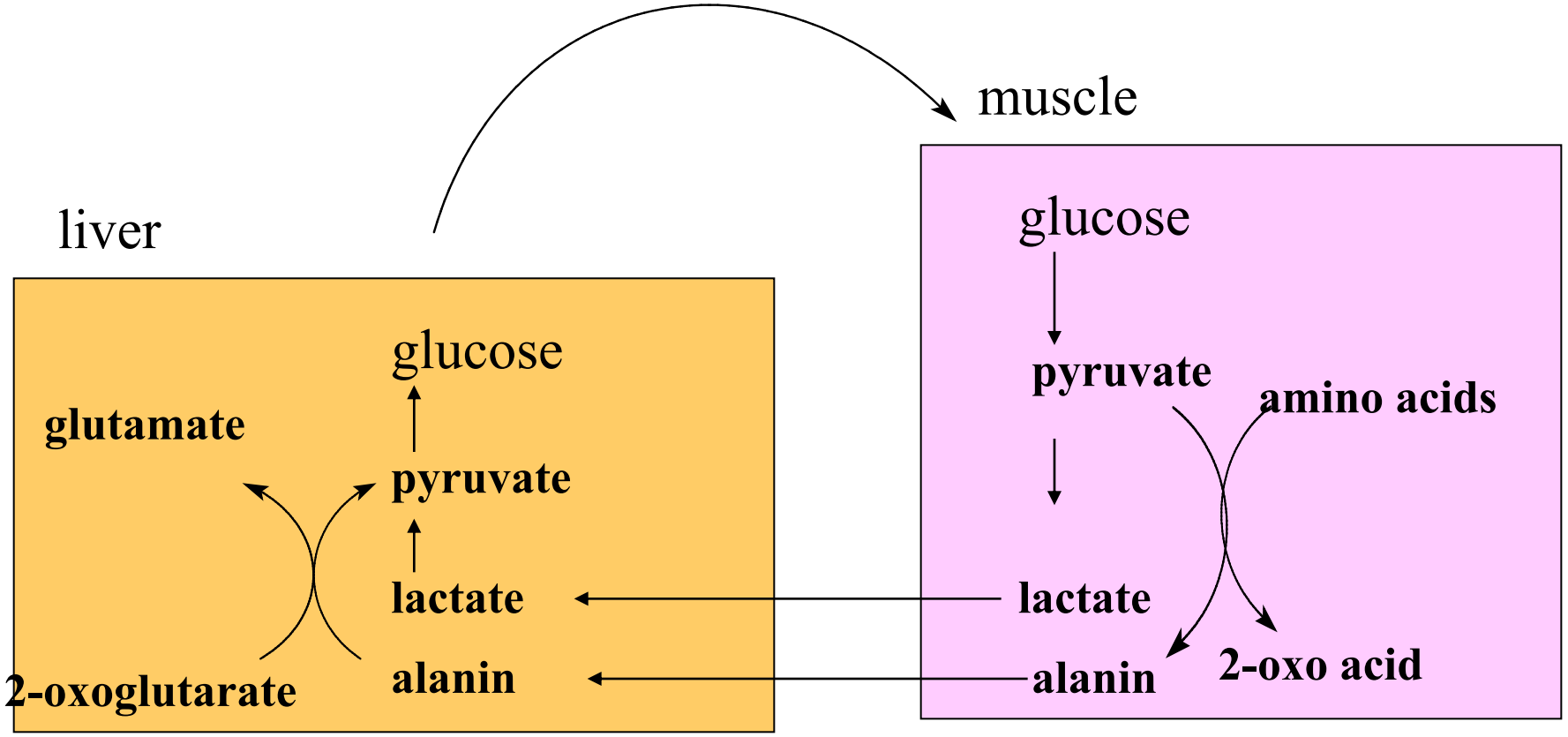
It is metabolised to CO_2 in citric acid cycle.

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 NAD^+

The ratio $NADH/NAD^+$ may by high at some metabolic conditions – gluconeogenesis can not occur

The ratio $NADH/NAD^+$ 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)

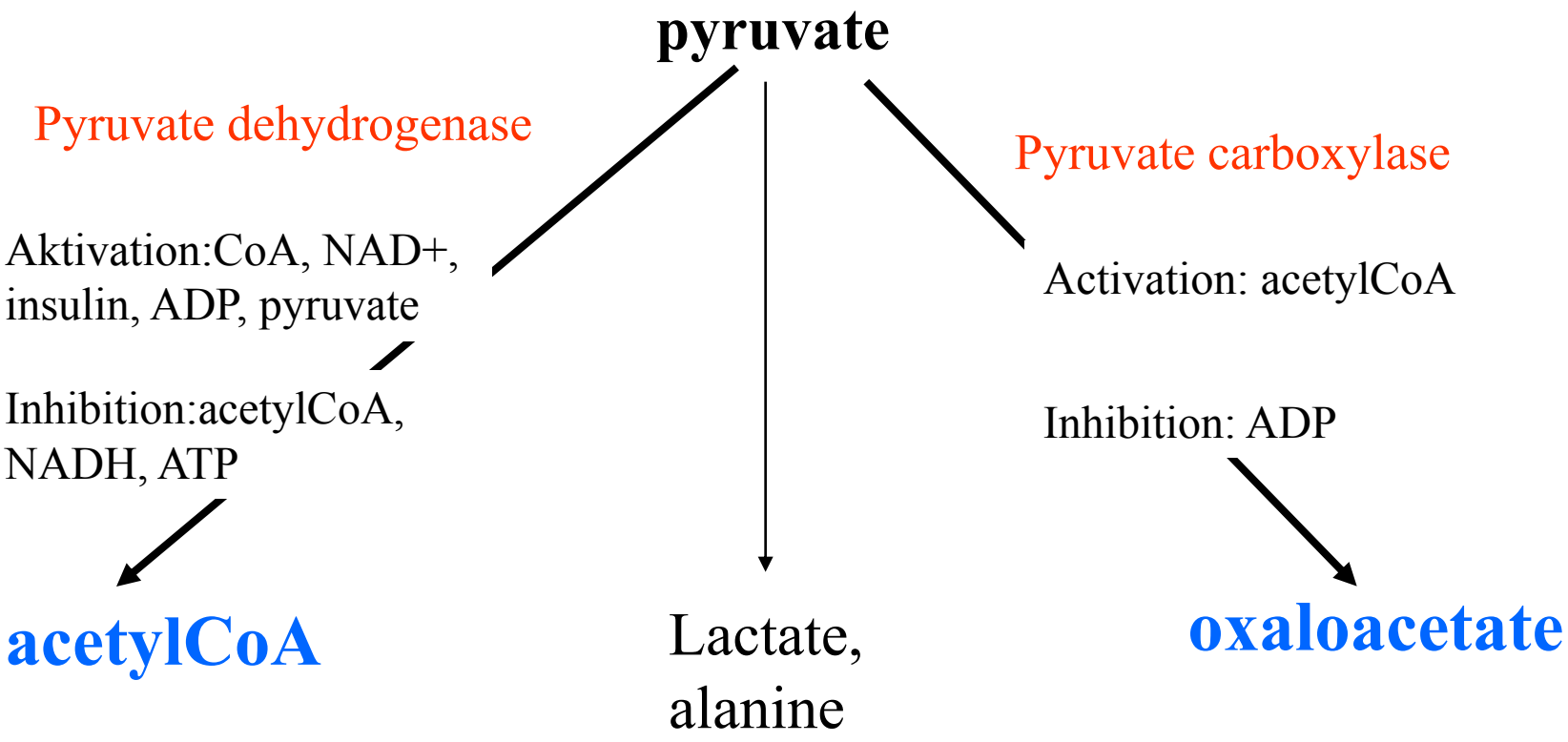
Activation and inhibition of enzymes involved in glycolysis and gluconeogenesis

Enzyme	Activator	Inhibitor
Hexokinase		glucose-6-phosphate
Phosphofructo kinase	5´AMP, fructose-6-phosphate, fructose-2,6-bisphosphate	Citrate, ATP, glucagon
Pyruvate kinase	fructose-1,6-bisphosphate,	ATP, alanin
Pyruvate dehydrogenase	CoA, NAD^+, ADP, pyruvate	acetylCoA, NADH, ATP
Pyruvate carboxylase	acetylCoA	ADP

Effects of hormones on enzyme expression

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

Tisue	% tissue mass	Tissue mass (kg)	Mass of glucose (g)
Liver	5,0	1,8	90 (glycogen)
Muscle	0,7	35	245 (glycogen)
Extracelular glucose	0,1	10	10

Location of synthesis and degradation of glycogen

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

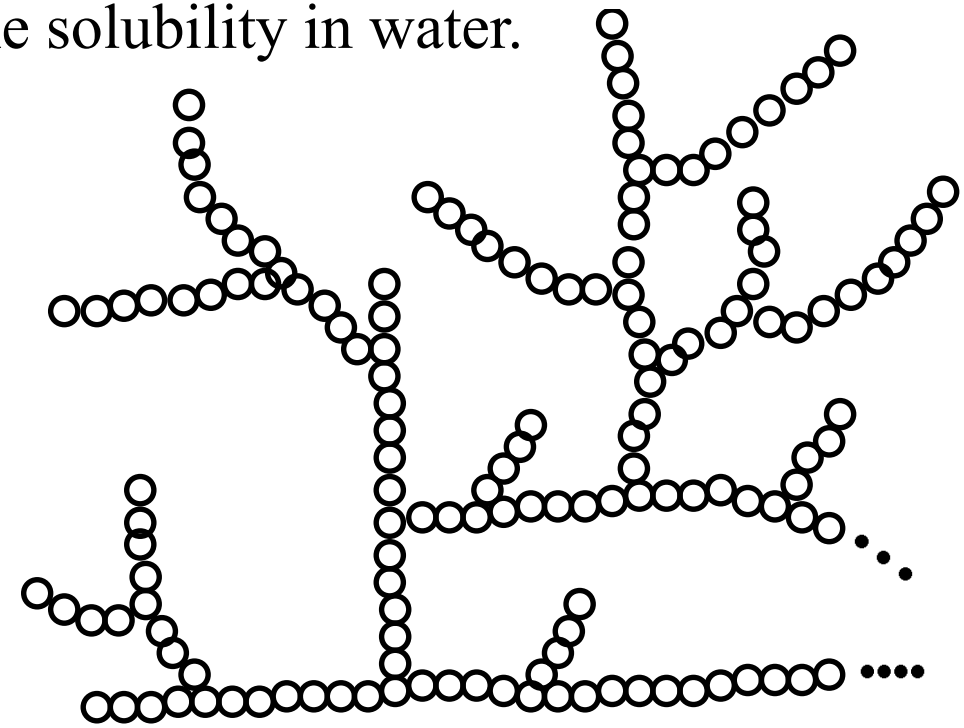
Enzymes od degradation and synthesis are on the surface of particles

Glycogenolysis is not a reversal proces of synthesis.

Molecules of glycogen have M_r ~10^8

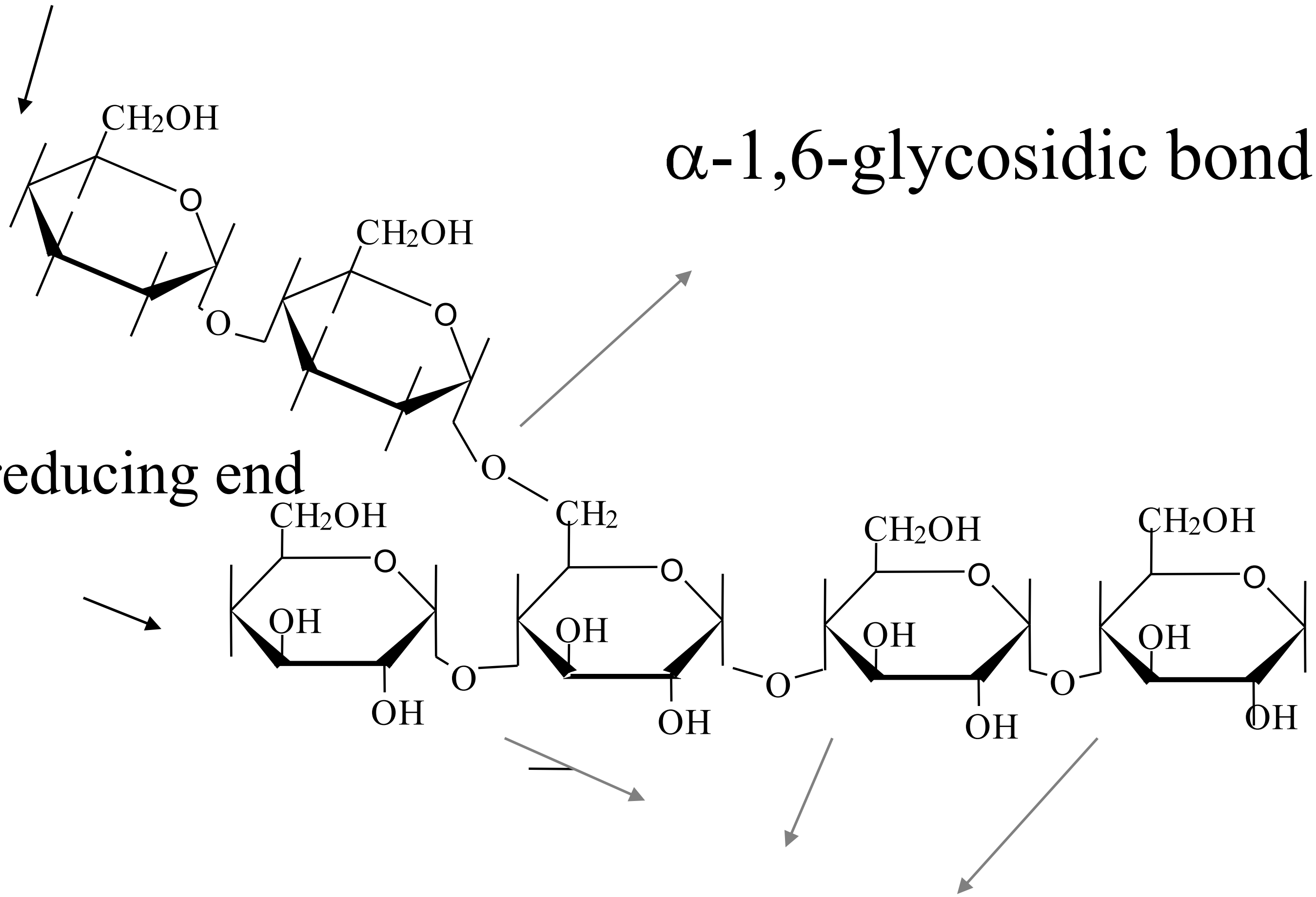
The branched structure permits rapid degradation and rapid synthesis, because enzymes can work on several chains simultaneously.

It also increases the solubility in water.



Types of bonds in glycogen

Non-reducing end



Non-reducing end

α-1,6-glycosidic bond

α-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 α -1,4 glycosidic bond
4. Branching

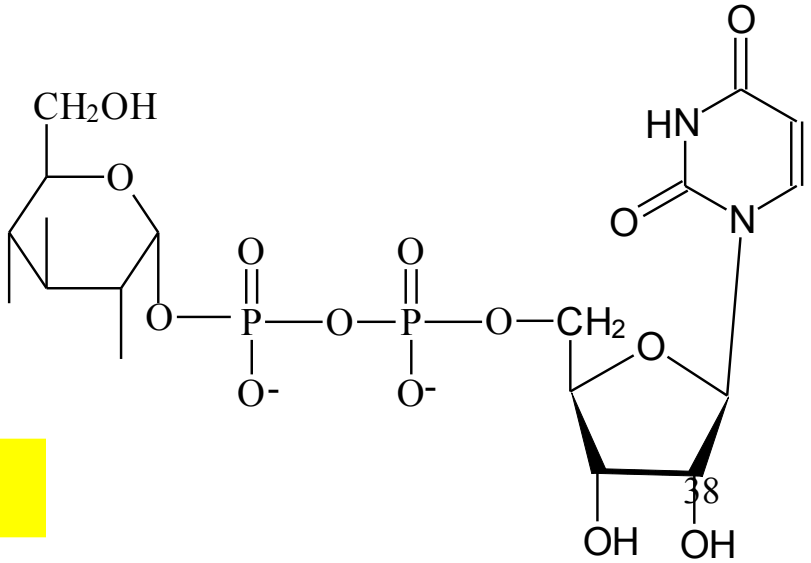
1. Synthesis of UDP-glucose

- glucose-6-P ⇌ glucose -1-P

phosphoglucomutase

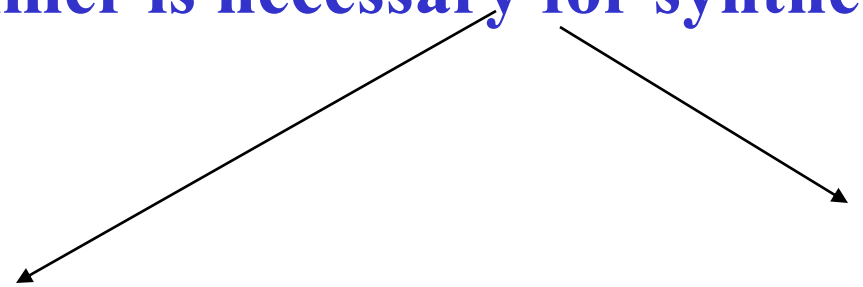
- glucose-1-P + UTP ⟶ UDP-glucose + PP_i

$PP_i + H_2O \longrightarrow 2P_i$



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 α -1,4 glycosidic bonds glycogensynthase

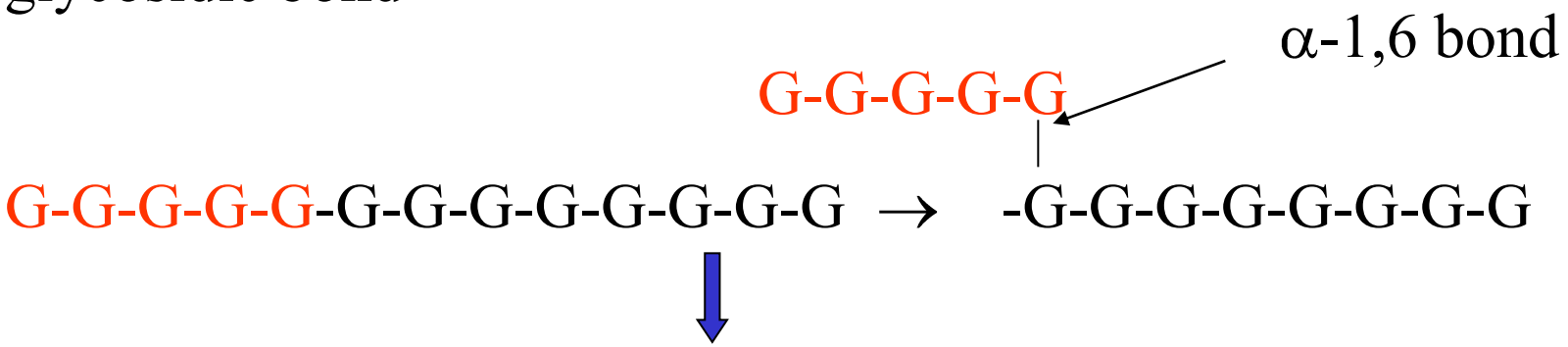
- Iniciation – glucosyl residue is added from UDP-glucose to the non-reducing terminal of the primer by glycogen synthase

- Elongation by glycogensynthase - formation of linear chains with α-1,4 glycosidic bond UDP-glucose + $[glucose]_n \rightarrow [glucose]_{n+1}$ + 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).

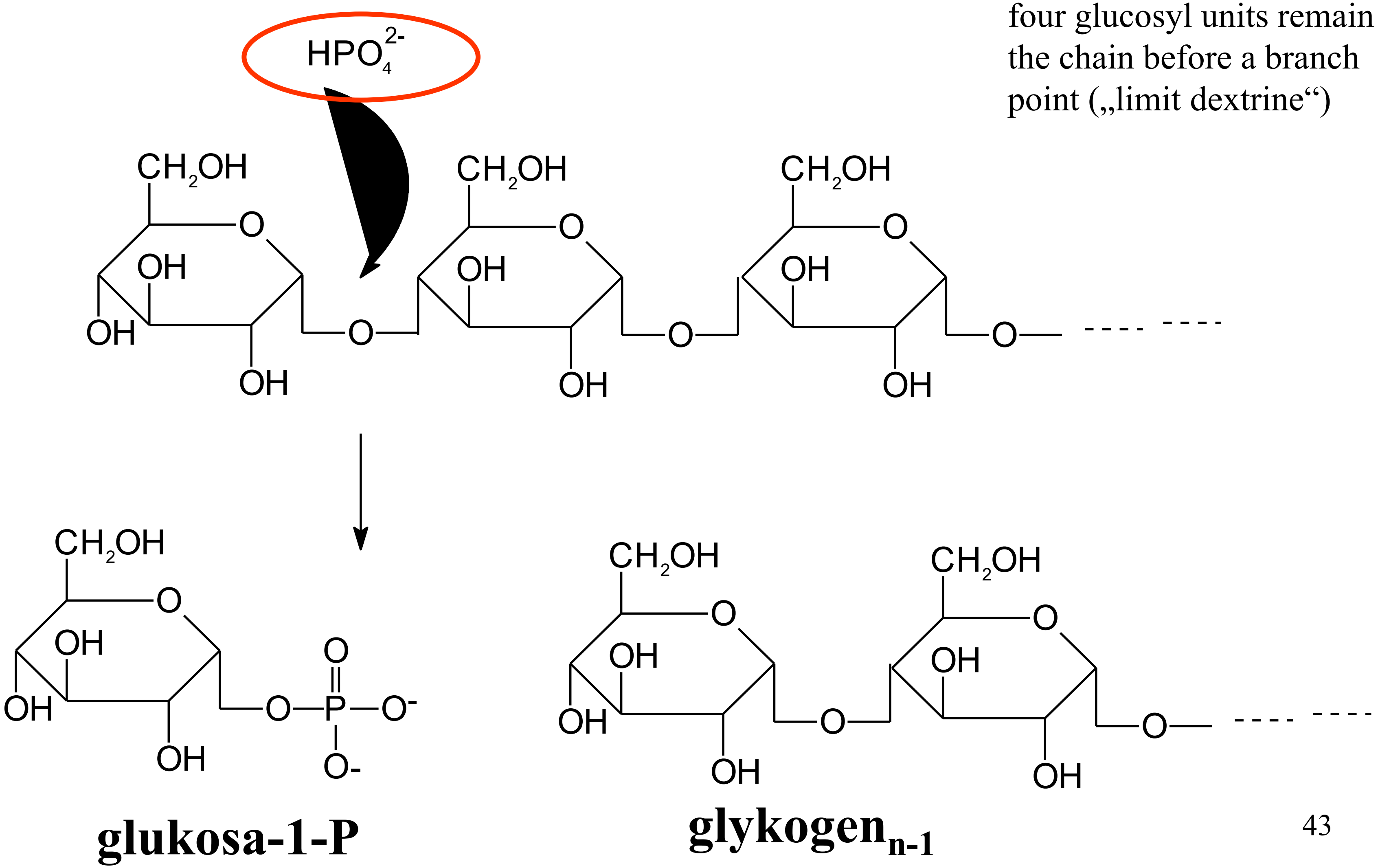
1. phosphorolytic cleavage of α-1,4 glycosidic bonds by phosphorylase
2. Removal of α-1,6 branching (debranching enzyme)

Compare:

Hydrolysis x phosphorolysis

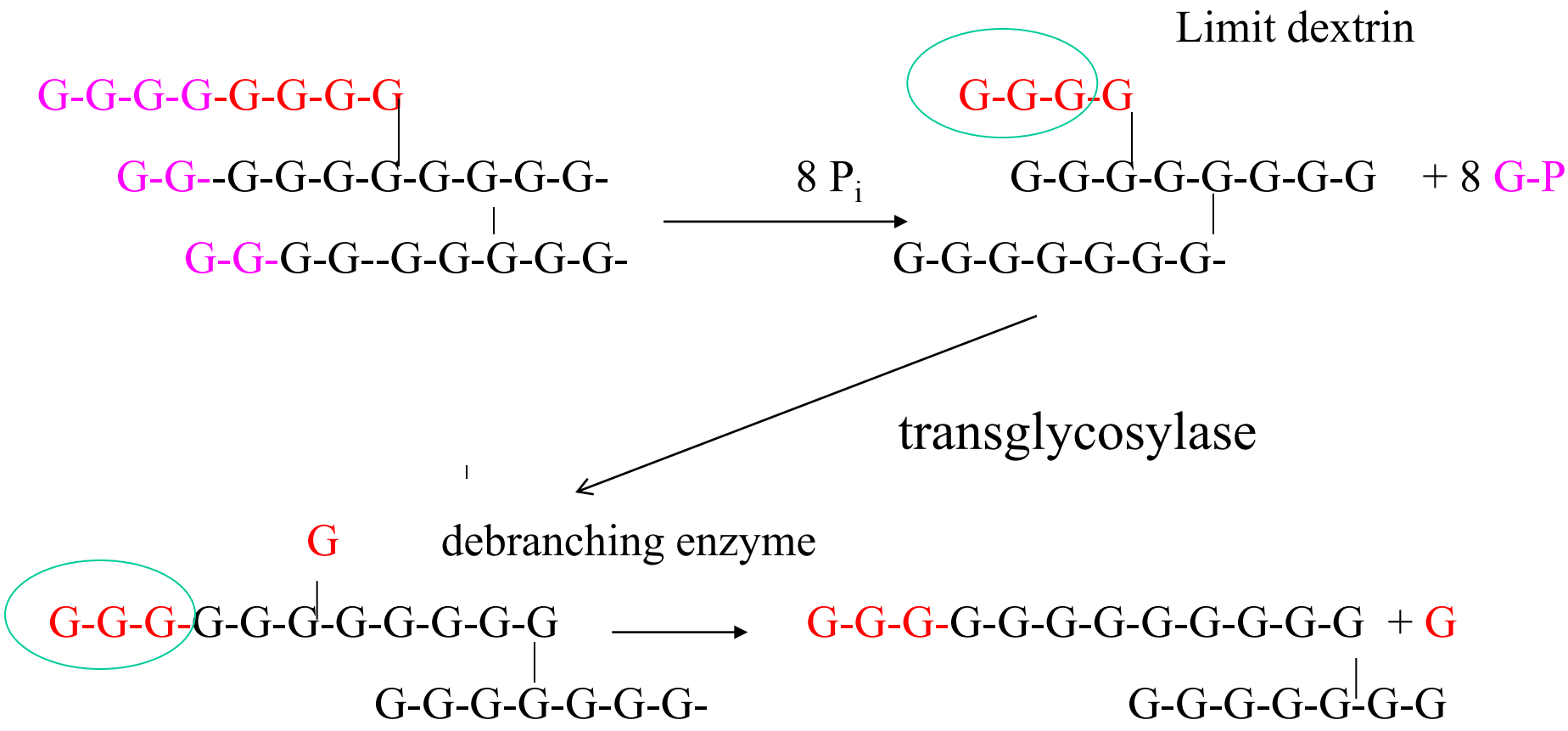
1. Phosphorylase - phosphorolytic cleavage of α-1,4 glycosidic bonds at the non-reducing ends

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



Degradation of glycogen

Phosphorylase can split α-1,4-links,
its action ends with the production of **limit dextrins**
:



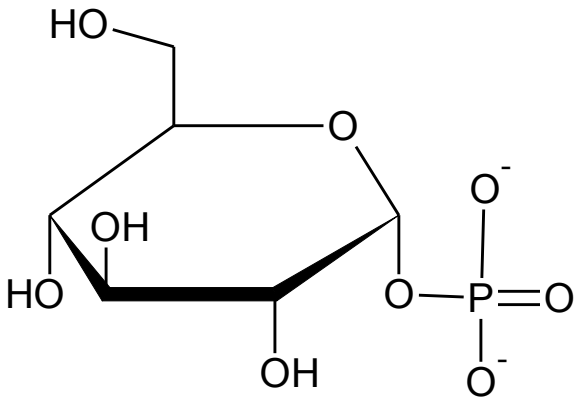
2. Debranching enzyme

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 α-1,4 glycosidic bond

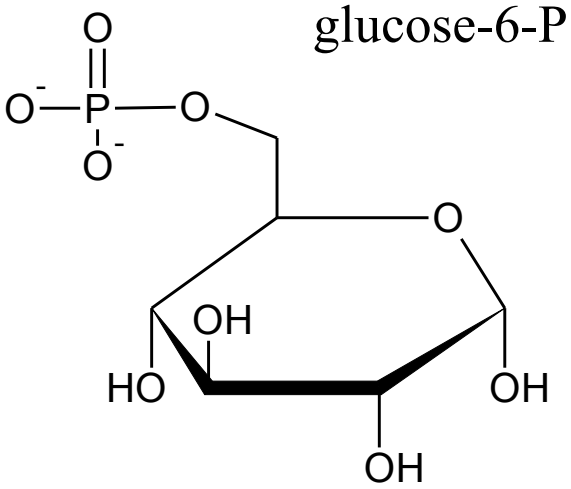
glucosidase activity: the one glucosyl residue remaining at the end of α-1,6 branch is hydrolyzed by the 1,6 – glucosidase activity of debranching enzyme

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

Further fates of glucose-1-phosphate formed from glycogen

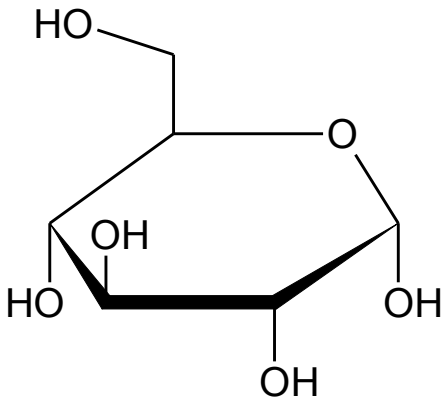


phosphoglucomutase



glucose-6-P

glucose-6-phosphatase



Only liver (kidney)

All tissues

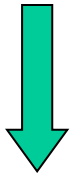
Source of blood glucose

Serve as a fuel source for generation of ATP

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 maintened 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)

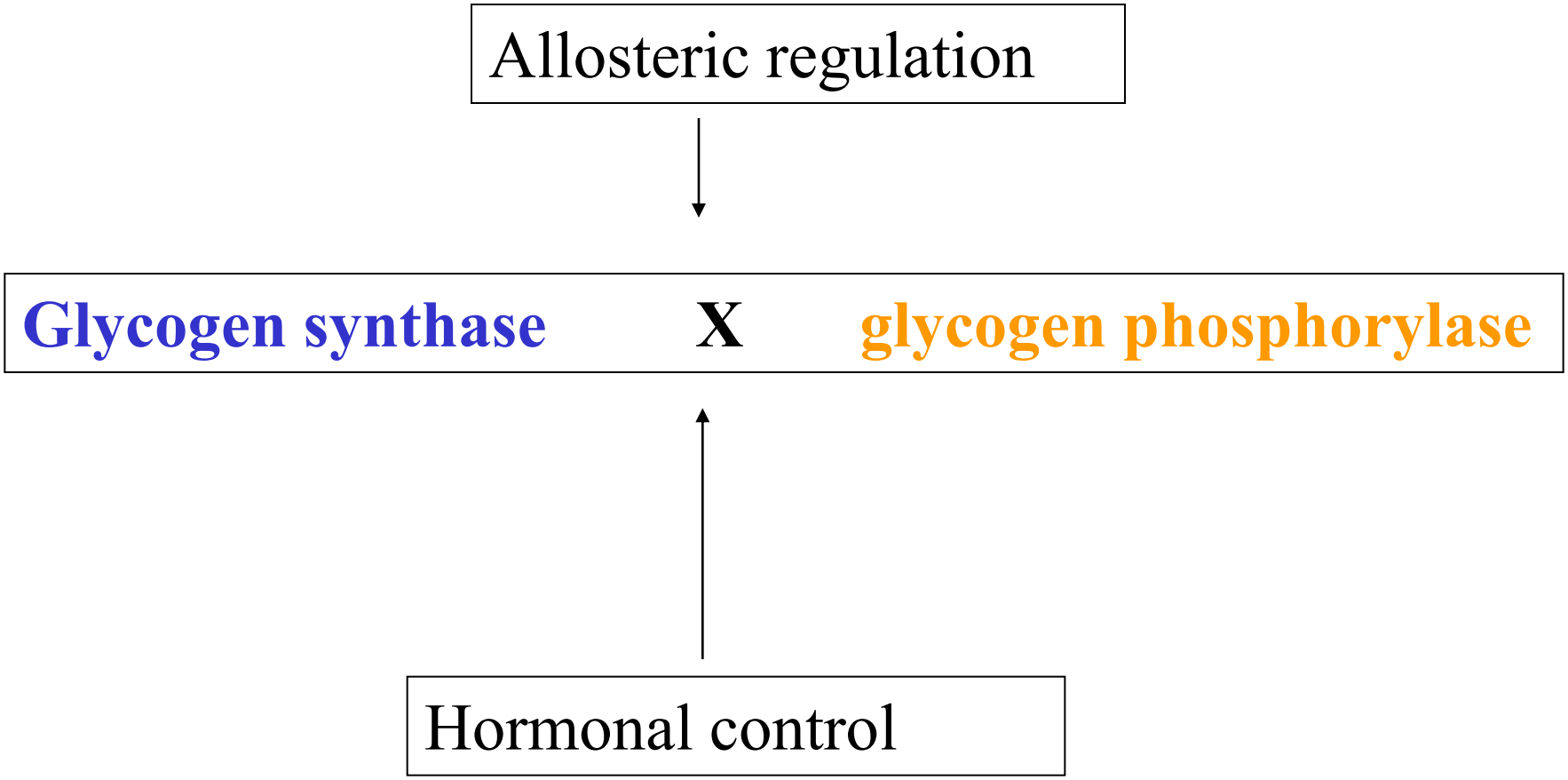
Degradation of about 1-3% of cellular glycogen (glycogen particles are surrounded by membranes that then fuse with the lysosomal membrane

-enzyme degrades α-1,4-bonds from non-reducing end

- glucose is released

(see also Pompe disease)

Regulation glycogen metabolism



Hormons affecting synthesis and degradation of glycogen

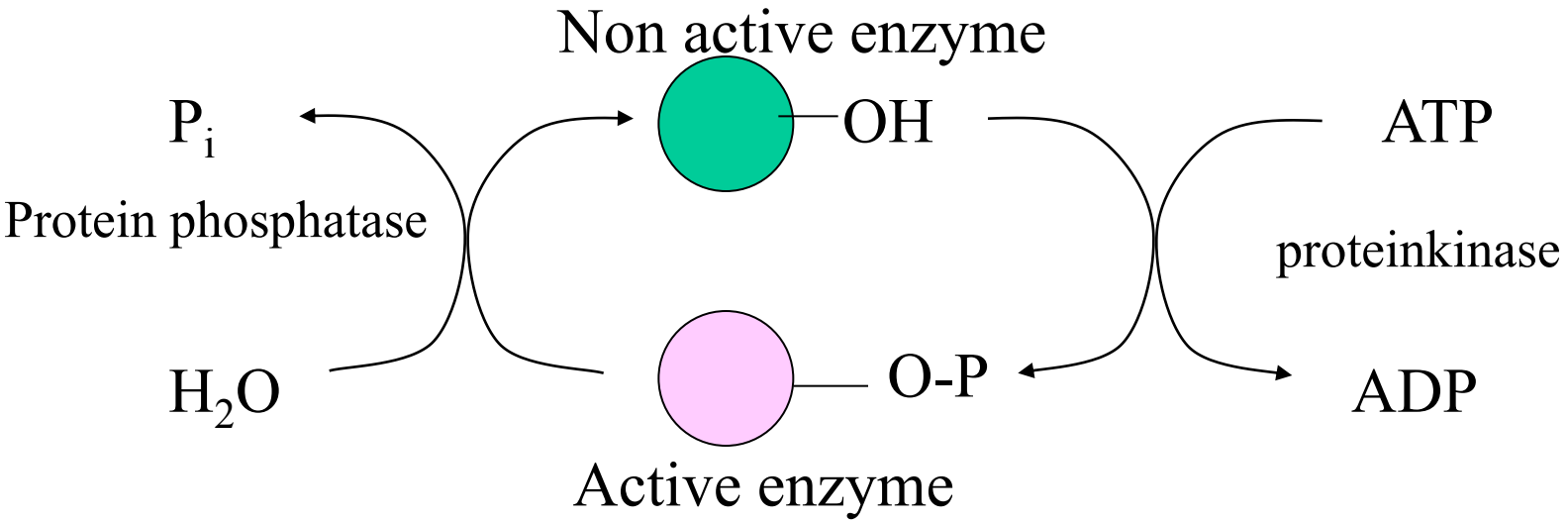
Hormon	synthesis	degradation
Insulin	↑	↓
Glucagon	↓	↑
Adrenalin	↓	↑

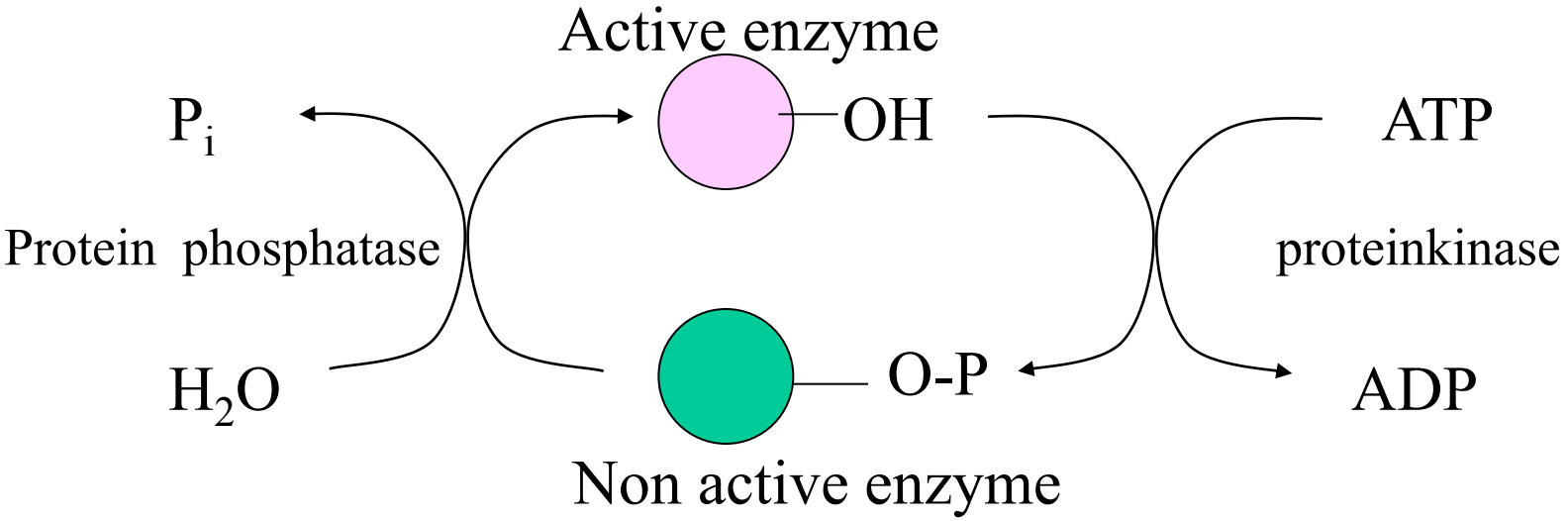
Hormons action is mediated by their second messengers.

Phosphorylation and dephosphorylation plays important role at regulation of glycogen metabolism

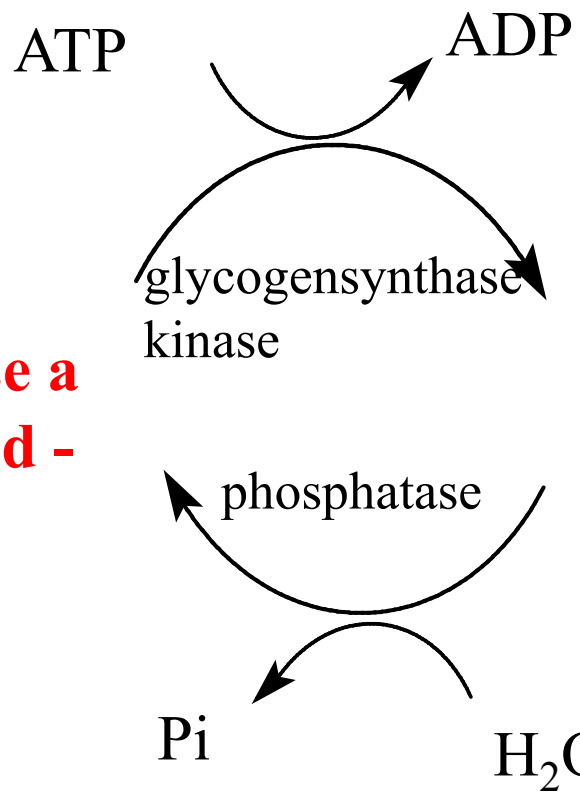
- phosphorylation by kinases and ATP
- dephosphorylation by phosphatases

Common examples of enzyme activity regulation by phosphorylation and dephosphorylation





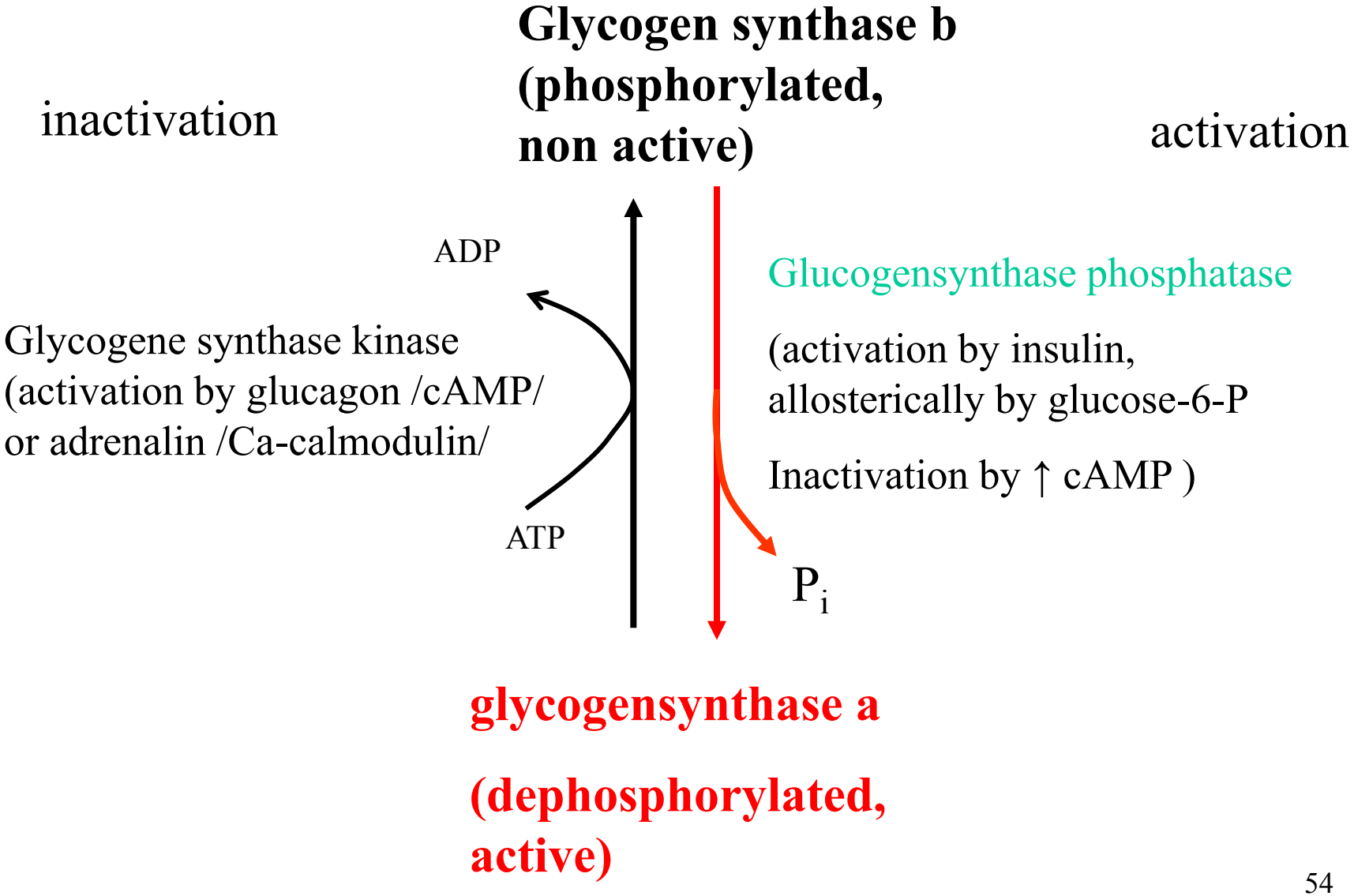
Activation and inactivation of glycogen synthase



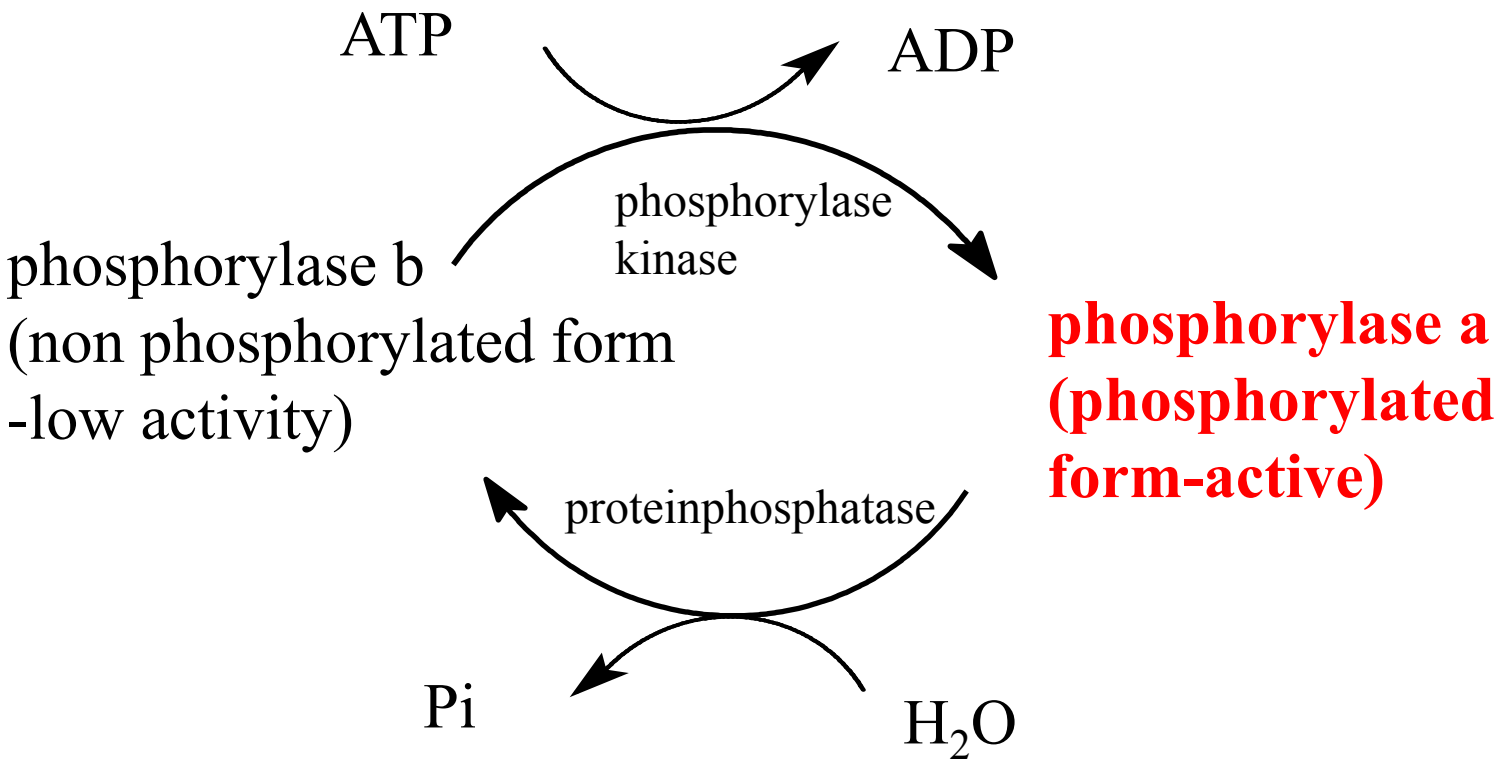
Glycogen synthase a (dephosphorylated - active)

Glycogen synthase b (phosphorylated - inactive)

Activation and inactivation of glycogensynthase in liver



Activation and inactivation of glycogen phosphorylase



Phosphorylases in liver and muscles are different

Degradation of glycogen

allosteric regulation

Effect of **hormons:**

Liver:

glucagon (cAMP),

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

Glucose, ATP, Glc-6P: allosteric inhibition

Muscle:

adrenalin (cAMP) at the stress

↑ Ca^{2+} during muscle contraction

AMP

No effect of glucagon !

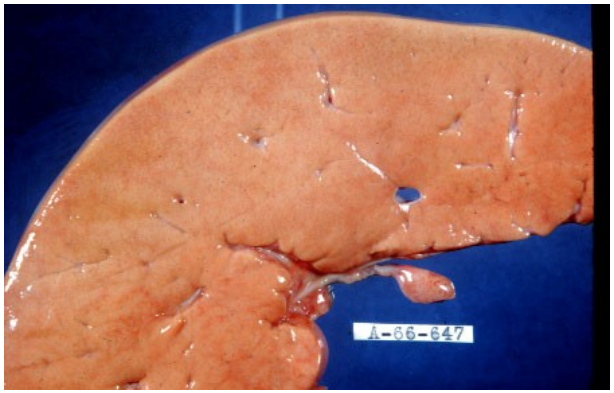
Glycogen storage diseases - enzyme deffects

Inherited enzyme deficiences. 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 α-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 type V

Von Gierke disease (type I)

Enlarged liver, increased glycogen store



Most common

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

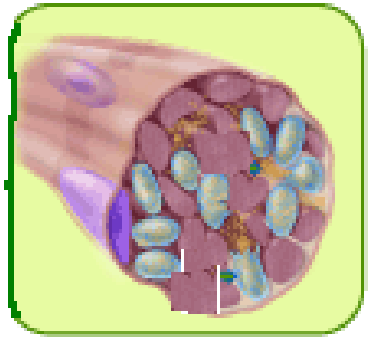
Concequences:

Inability to provide glucose during fasting state

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

Growth reatardation, delayed puberty

Pompe disease (type II)



Absence of α-1,4-glucosidase in lysosomes

Acummulation of glycogen in lysosomes

Loss of lysosomal function

Damage of muscles→muscle weakness

Infantile form: death before age 2 years

Juvenile form: later –onset myopathy with variable cardiac involvment

Adult form: limb-girdle muscular distrophy-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