



Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

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**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

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BIOCHEMISTRY

BIOKÉMIA

ANABOLISM OF CARBOHYDRATES

A SZÉNHIDRÁTOK SZINTÉZISE

TRETTÉR LÁSZLÓ



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Gluconeogenesis and its regulation

Glycogen synthesis and its regulation

Lactose synthesis



BIOCHEMISTRY: ANABOLISM OF CARBOHYDRATES

Gluconeogenesis

<http://semmelweis-egyetem.hu/>

Learning objectives

At the end of the presentation students will be able:

To demonstrate the difference between the steps of gluconeogenesis and glycolysis.

To demonstrate the reactions, specific for gluconeogenesis.

To understand the concept of gluconeogenesis i.e. that the higher multicellular organisms use many noncarbohydrate precursors for the biosynthesis of glucose, because the maintenance of blood sugar level is highly important.

To understand the principles of metabolic regulation by the comparison the regulation of gluconeogenesis and glycolysis.



Gluconeogenesis^{DEF}: biosynthesis of glucose from simpler, non-carbohydrate precursors

The most important precursors of gluconeogenesis:

lactate, pyruvate

glycerol

(glucogenic) amino acids

fatty acids with odd number of carbon atoms

Glucogenic amino acids^{DEF}: amino acids with carbon skeleton that can be used in glucose synthesis during gluconeogenesis (e.g. alanine, aspartate)

Antonym: Ketogenic amino acids^{DEF}: amino acids with carbon skeleton that can be used in ketone body synthesis during starvation (e.g. leucine, lysine)



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Gluconeogenesis

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The principles of gluconeogenesis I.

Gluconeogenesis requires reactions from glycolysis, citric acid cycle and a few special reactions

The nonequilibrium reactions during glucose catabolism obstruct the simple reversal of glycolysis

The nonequilibrium reactions:

1. phosphoenol pyruvate→pyruvate
2. fructose 6-P→fructose 1,6-P₂
3. glucose→glucose 6-P



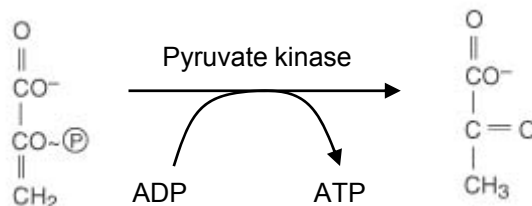
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Gluconeogenesis

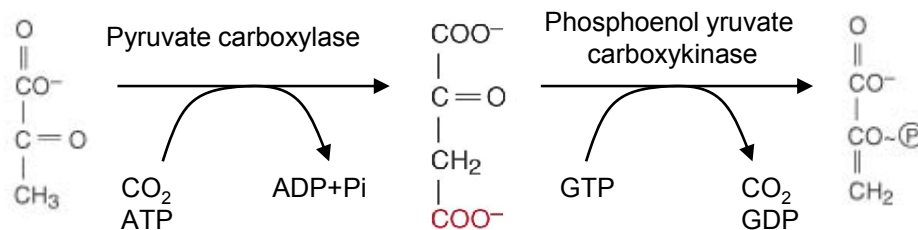
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The principles of gluconeogenesis II. The circumvention of glycolytic reactions

1. Glycolysis: phosphoenol pyruvate→pyruvate



Gluconeogenesis: pyruvate→oxaloacetate→phosphoenol pyruvate





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Gluconeogenesis

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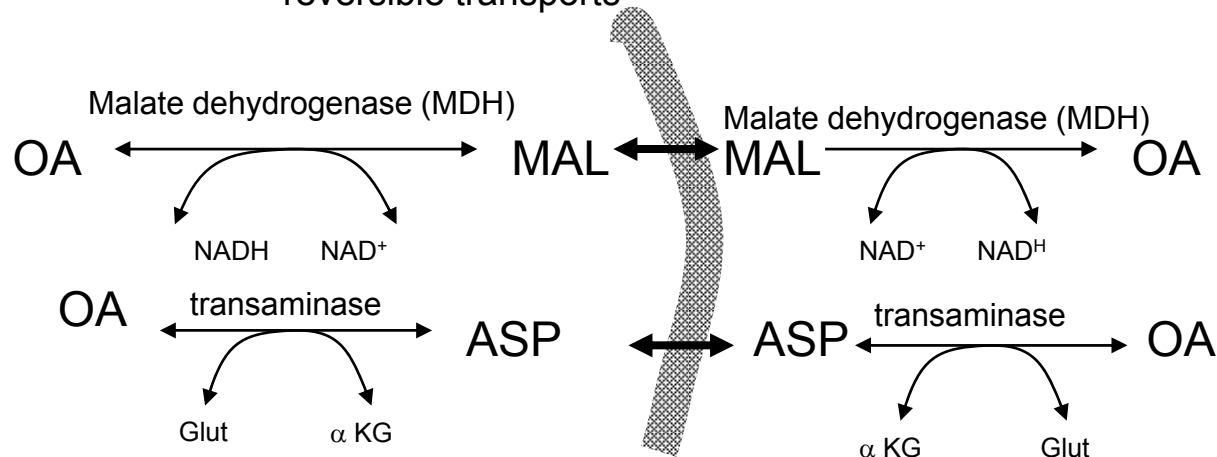
The principles of gluconeogenesis III. The circumvention of glycolytic reactions

However: mitochondria are impermeable for oxaloacetate

but oxaloacetate should be present in the cytosol
in order to
become a substrate for phosphoenol pyruvate carboxykinase (PEPCK)

Problem solved by

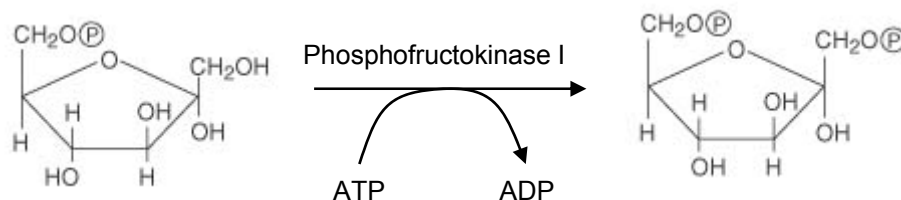
- reversible oxidoreduction and transamination
- reversible transports



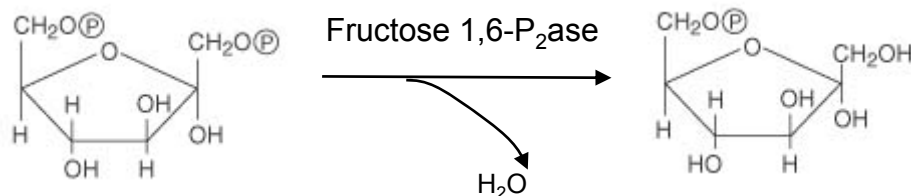
transaminases^{DEF}: enzymes catalyzing the reversible transfer of amino group
from an amino acid to an oxo acid resulting a new amino acid and a new oxo acid

The principles of gluconeogenesis IV. The circumvention of glycolytic reactions

2. Glycolysis: fructose 6-P → fructose 1,6-P₂

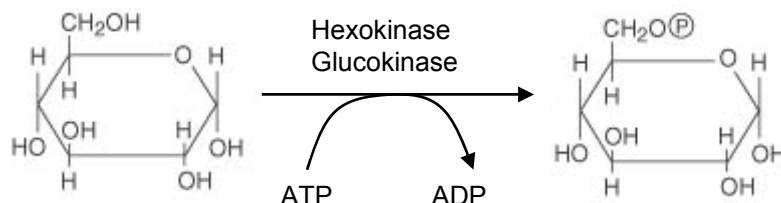


Gluconeogenesis: Fructose 1,6-P₂ → Fructose 6-P

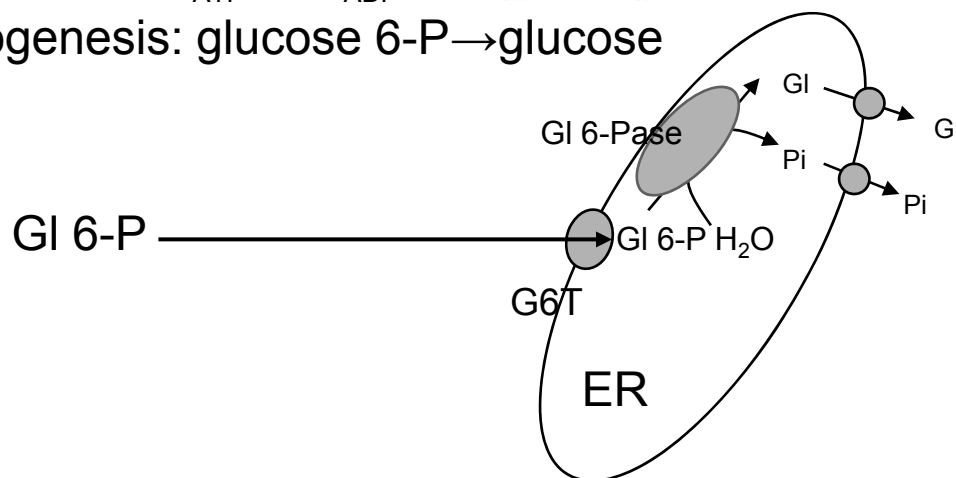


The principles of gluconeogenesis V. The circumvention of glycolytic reactions

3. Glycolysis: glucose → glucose 6-P



Gluconeogenesis: glucose 6-P → glucose



Glucose 6-P is transported by glucose 6P transporter (G6T) then hydrolyzed in the ER by glucose 6Pase



Gluconeogenesis in the liver from lactate and pyruvate



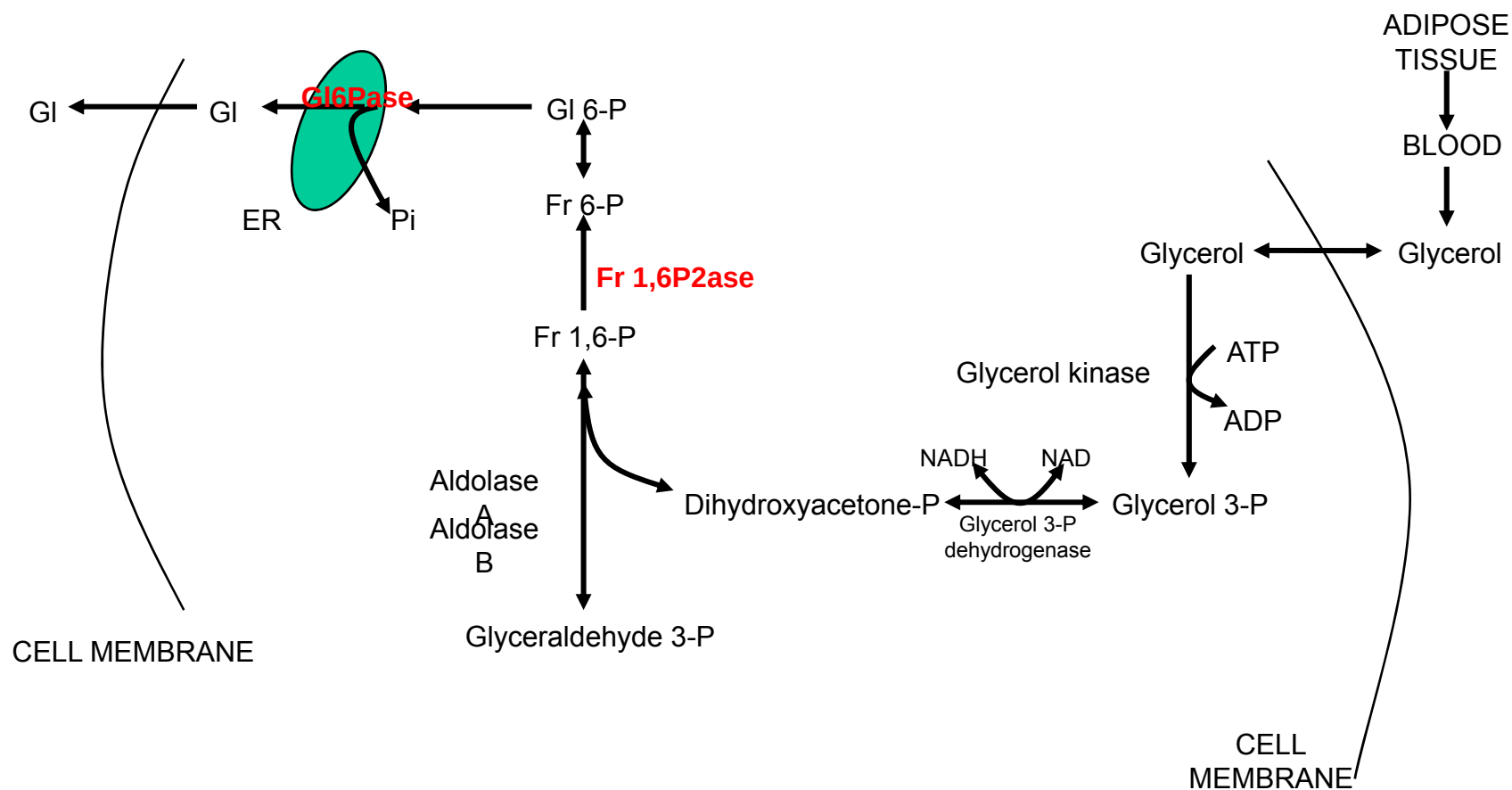


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Gluconeogenesis

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Entry of glycerol into the gluconeogenesis (Liver)



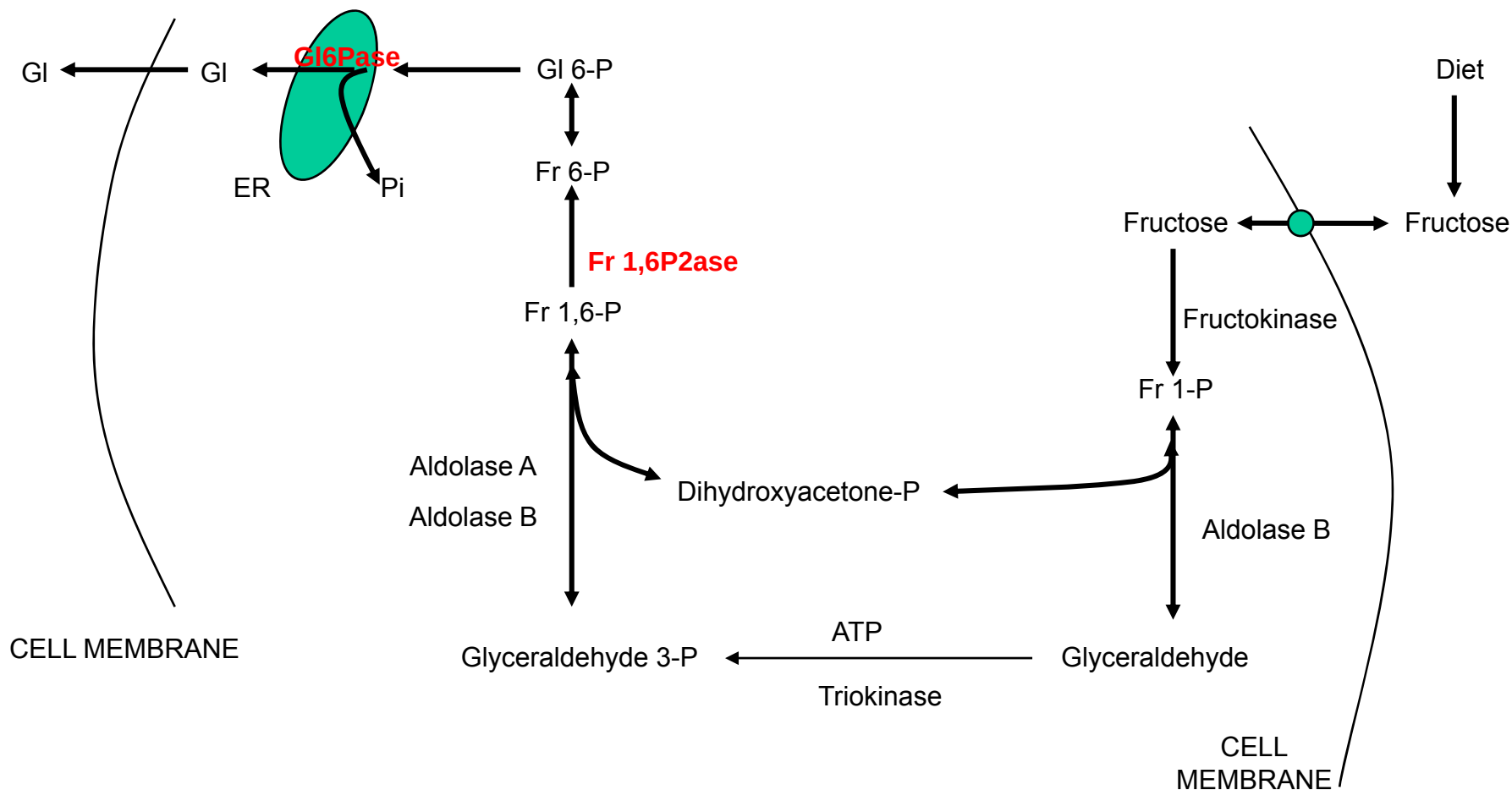


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Gluconeogenesis

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Entry of fructose into the gluconeogenesis (Liver)





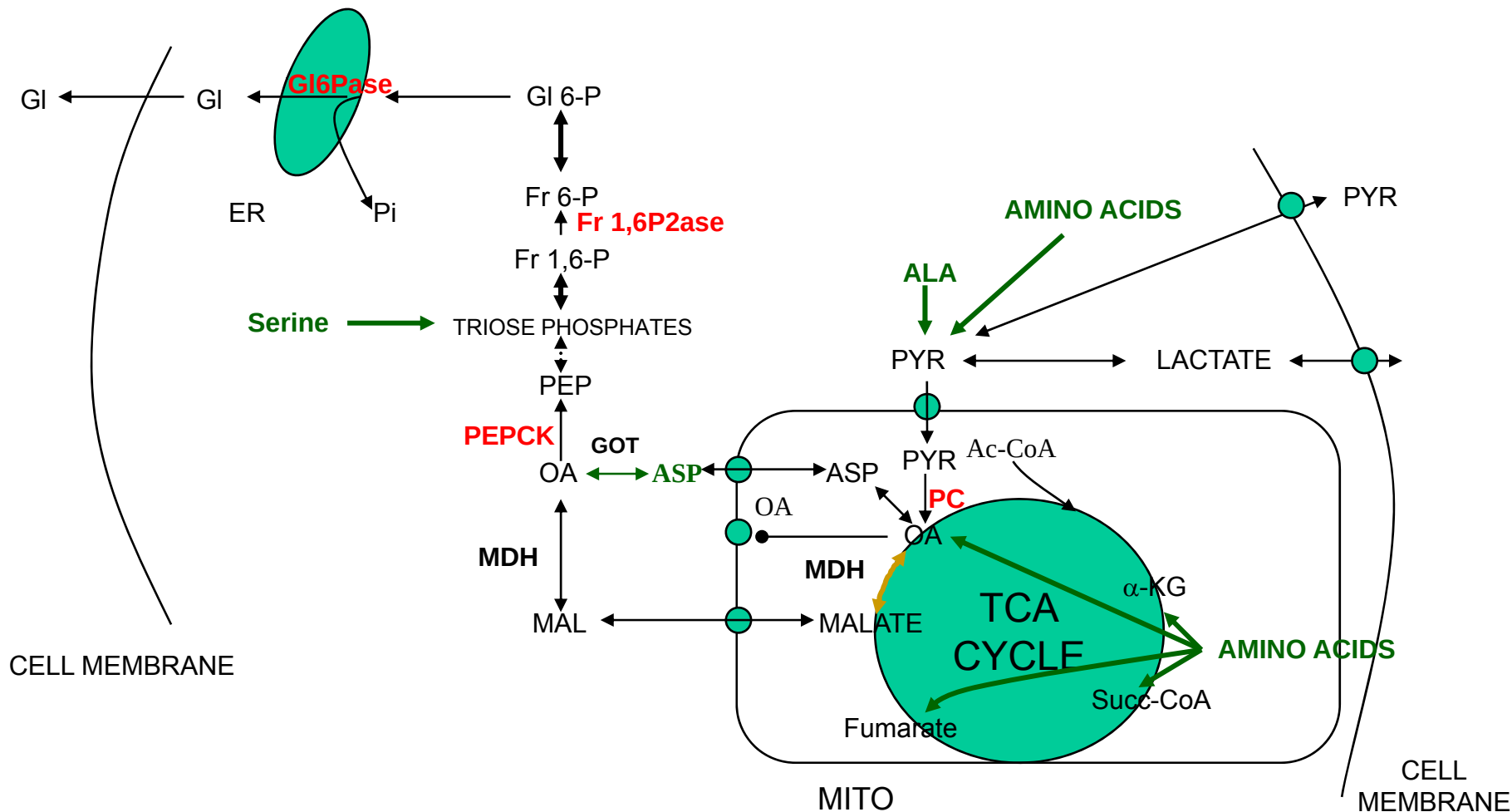
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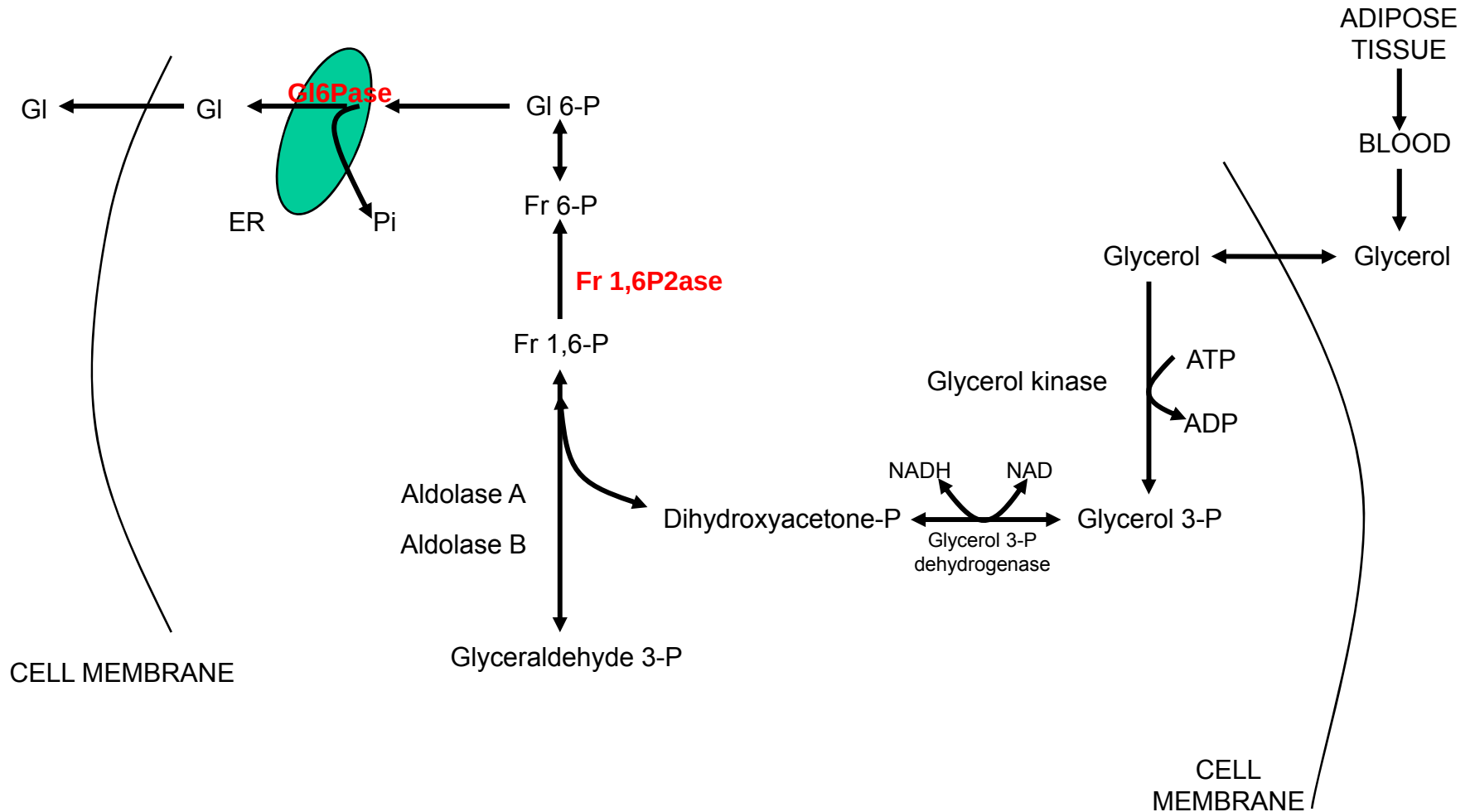
Entry of amino acids into the gluconeogenic pathway

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Entry of glycerol into the gluconeogenesis (Liver)

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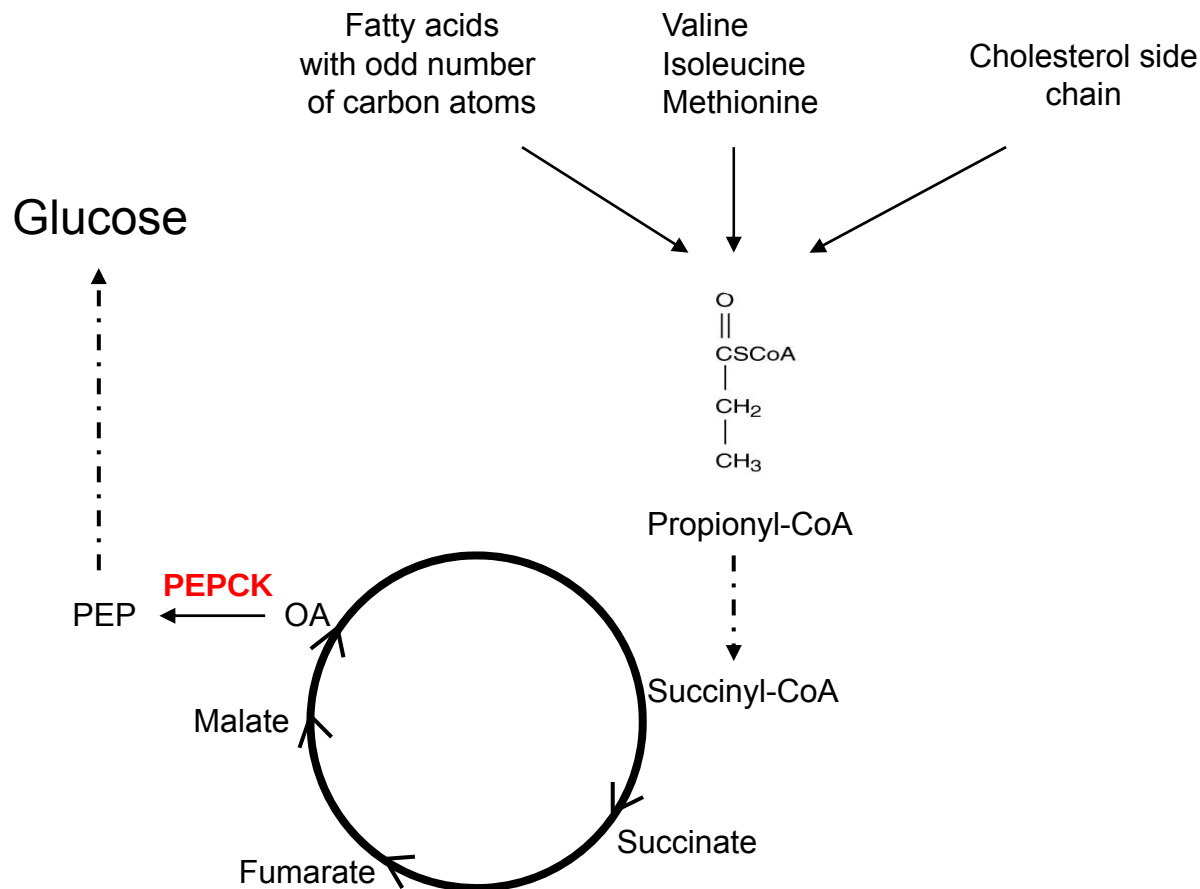


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Gluconeogenesis

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Entry of propionyl-CoA into the gluconeogenesis (Liver)





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Gluconeogenesis

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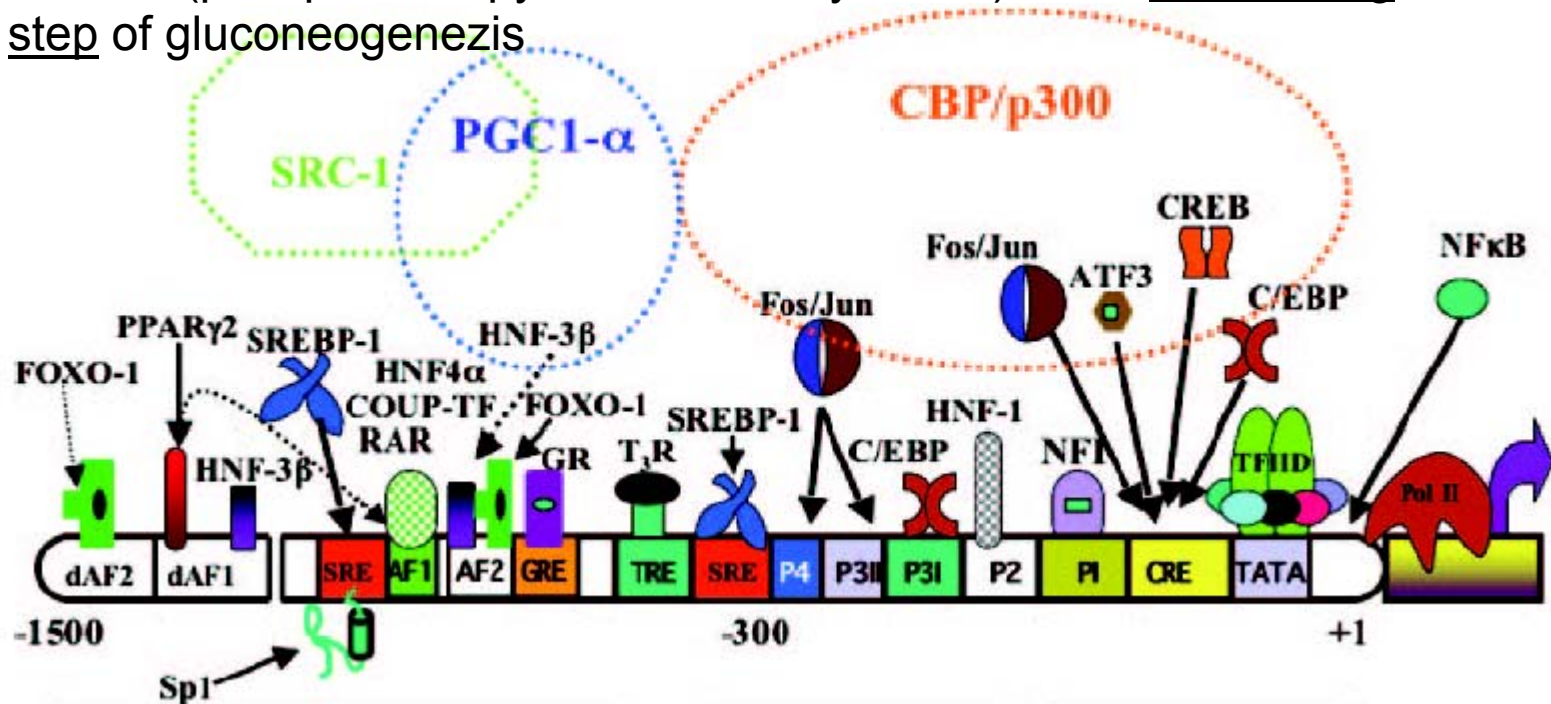
Regulation of gluconeogenesis

Enzyme	Gene expression		Covalent modification		Allosteric	
	Inducer	repressor	phosphorylation	dephosphorylation	activator	inhibitor
Pyruvate carboxylase	Glucocort. Glucagon epinephrine (cAMP)	insulin	-	-	-	-
Phosphoenolpyruvate carboxykinase (PEPCK)	Glucocort. Glucagon epinephrine (cAMP)	insulin	-	-	-	-
Fr 1,6-P ₂ ase	Glucocort. Glucagon epinephrine (cAMP)	insulin	-	-	-	Fructose 2,6-P ₂ AMP
Pyruvate kinase	Glucocort. Glucagon epinephrine (cAMP)	insulin	-	-	Fructose 1,6-P ₂	ATP, alanine

Regulation at the PEPCK level

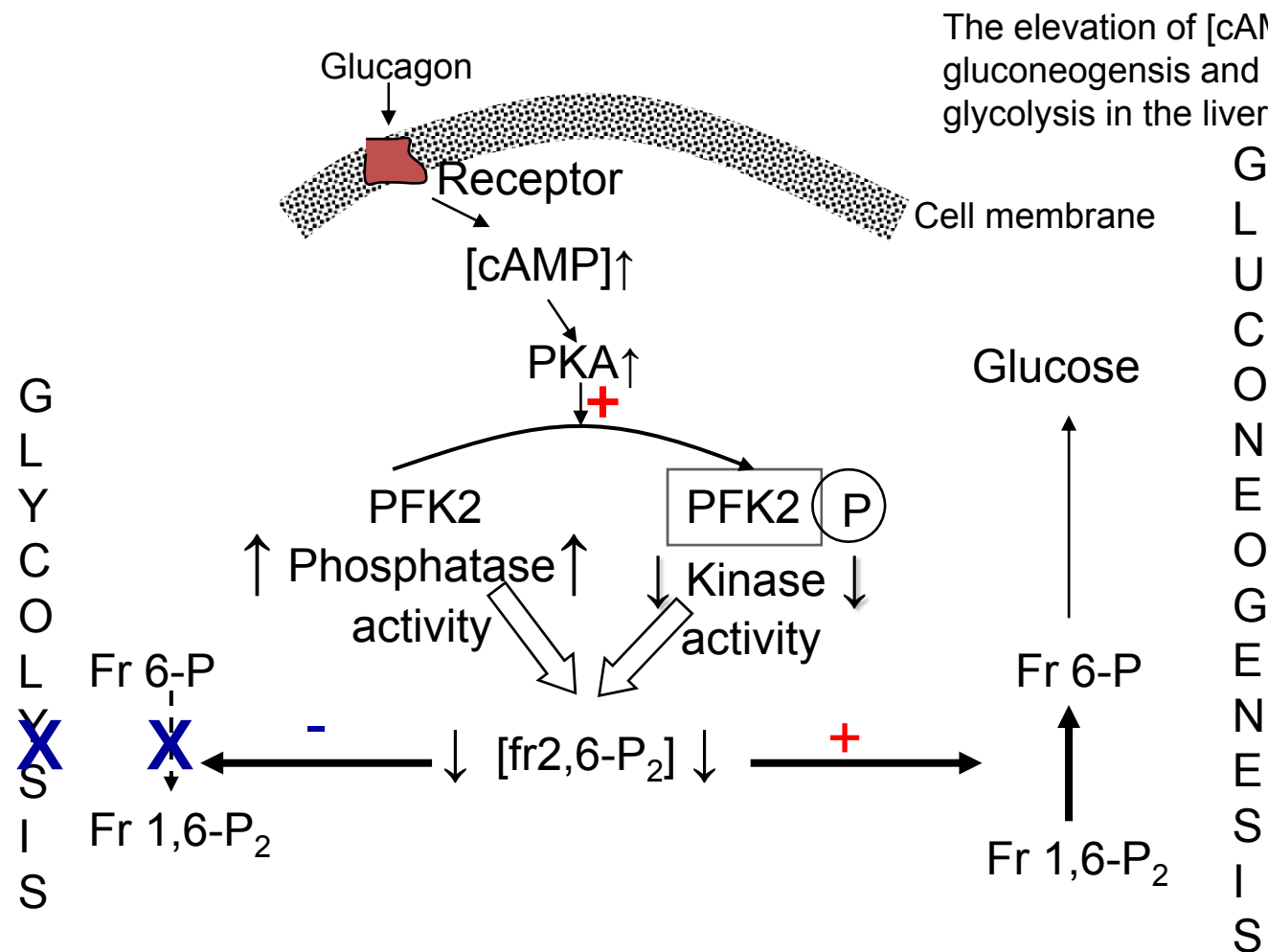
The promoter region of PEPCK gene

PEPCK (phosphoenol pyruvate carboxykinase) is the rate limiting step of gluconeogenesis



All of the important hormones in the metabolism can regulate the PEPCK expression

Regulation at the fr 2,6-P₂ase level (liver)





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Gluconeogenesis

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Regulation of gluconeogenesis I

Regulated steps: pyruvate carboxylase
 PEPCK
 fr 2,6-P₂ase
 Gl 6-Pase

Pyruvate carboxylase is regulated step, it catalyses an irreversible reaction, it is at the beginning of the pathway, but it is not entirely committed to the gluconeogenesis. Pyruvate carboxylase is also an anaplerotic enzyme of the citric acid cycle. Anaplerotic reactions can not be entirely shut off.

Anaplerotic^{DEF}: a reaction which can replenish the supply of intermediates in a metabolic pathway, e.g. in the citric acid cycle.

PEPCK is a rate limiting, irreversible, heavily regulated step at the initial part of the pathway. It is important to note that neither allosteric nor phosphorylation type of regulation has not been detected on the enzyme. It is regulated exclusively on the gene expression level.

Rate limiting step^{DEF}: The slowest step in a metabolic pathway. Usually it is heavily regulated. E.g. The rate limiting step of glycolysis is the fr 6-P fr 1,6-P₂ transformation.



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Gluconeogenesis

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Regulation of gluconeogenesis II.

Fr 1,6-P₂ase is also heavily regulated. This enzyme however, not the main regulatory site of the gluconeogenesis. It is far from the beginning of the pathway. It is particularly interesting that the regulation of PFK1 and the fr 1,6-P₂ase is very much coordinated. Those conditions and effectors which stimulate Fr 1,6-P₂ase activity inhibit PFK1 activity and vice versa. It is very tempting to say that because PFK1 is the rate-limiting enzyme of the glycolysis, Fr 1,6-P₂ase should be the rate-limiting one of the gluconeogenesis, however it is not true.

Gl6-Pase is also regulated, but not the rate limiting step either. The reasons for that

1. It is at the very end of the pathway,
2. It is not entirely committed to the gluconeogenesis but playing a role in the glycogenolysis as well. Glycogenolysis is activated earlier than gluconeogenesis, so the enzyme is not suitable for the fine tuning of the gluconeogenesis.



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Gluconeogenesis

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Summary

Because of thermodynamic reasons gluconeogenesis is not a simple reversal of glycolysis

The irreversible steps of glycolysis should be bypassed

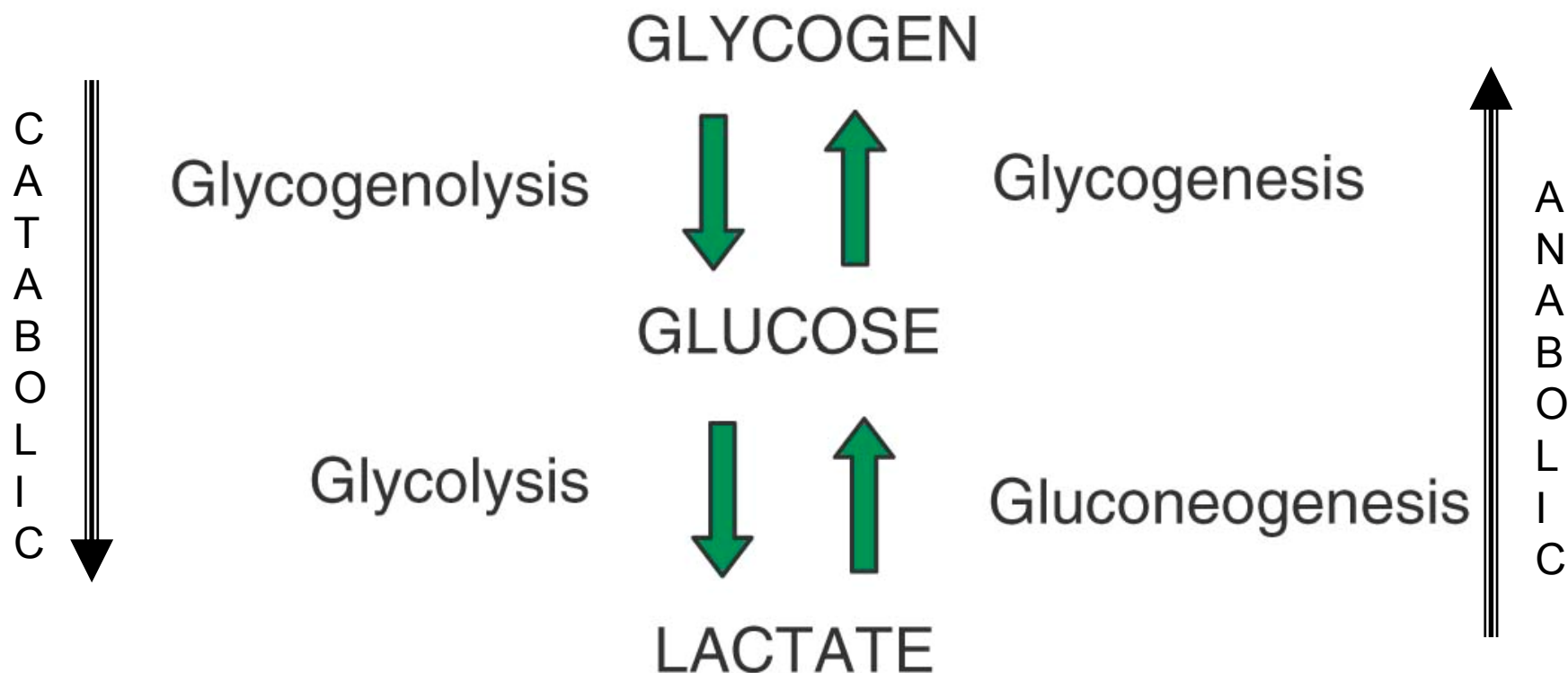
The most important gluconeogenic precursors join to the pathway at different levels

The regulation of gluconeogenesis occurs at the irreversible reactions.

The most important regulatory site of gluconeogenesis is the PEPCK enzyme



Major anabolic and catabolic pathways in glucose metabolism





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Glycogen synthesis

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Learning objectives

At the end of the presentation students will be able:

To demonstrate the difference between the steps of glycogenesis and glycogenolysis.

To demonstrate the reactions, specific for glycogenesis.

To understand the principles of simultaneous and opposite regulation of glycogenolysis and glycogenesis.

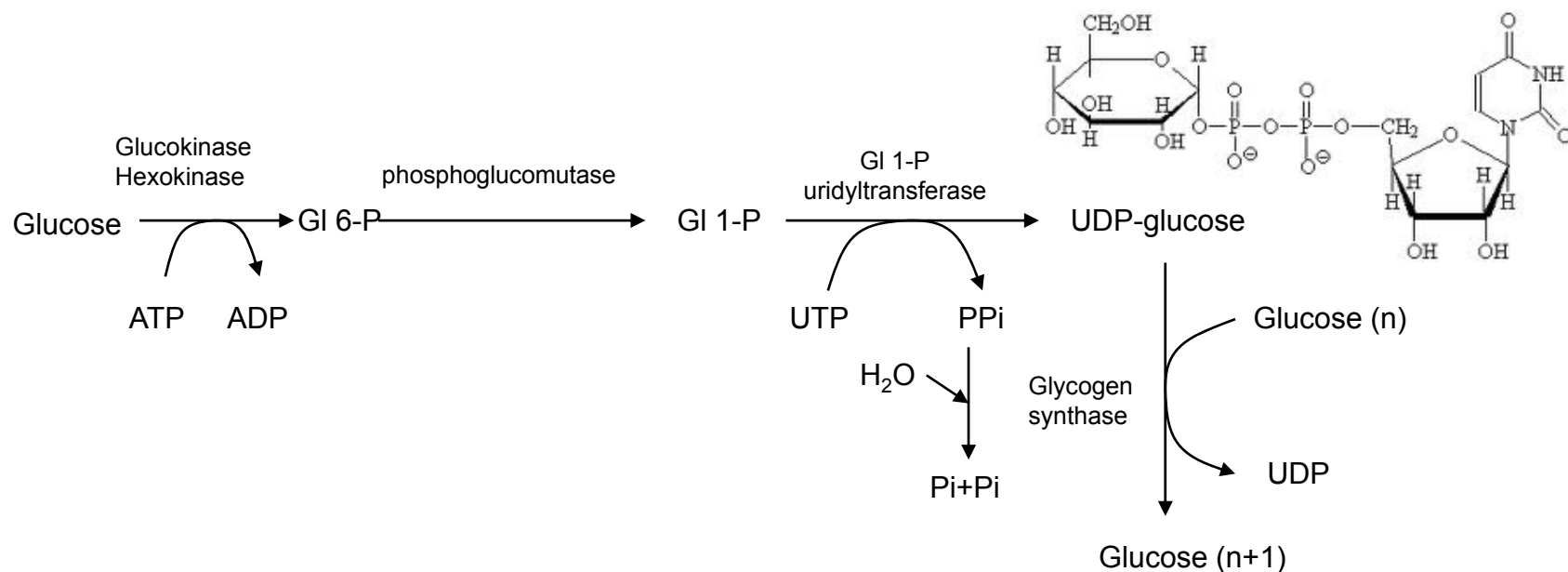


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Glycogen synthesis

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Overview of glycogen synthesis



Cost of glycogen synthesis: gluco-, hexokinase
uridylyltransferase

Total cost

1 ATP
1 UTP
2 ATP equivalent

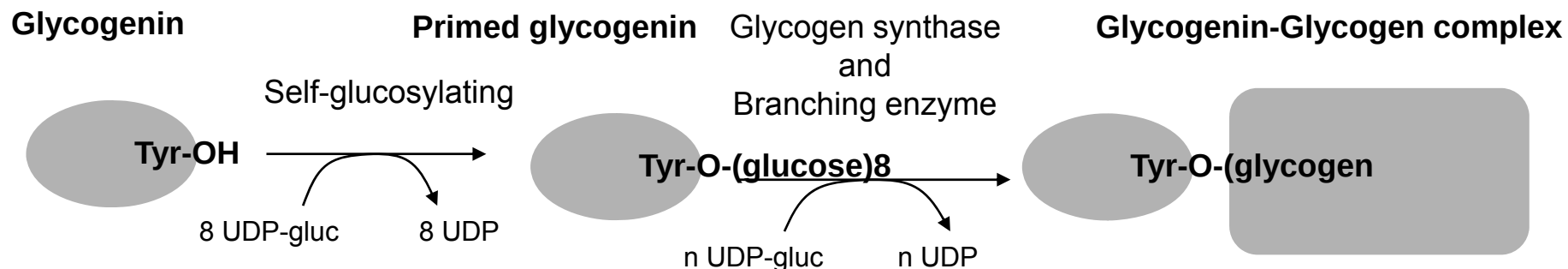


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Glycogen synthesis

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Initiation of glycogen synthesis,
The role of glycogenin



Glycogen synthase cannot initiate synthesis without having a primer

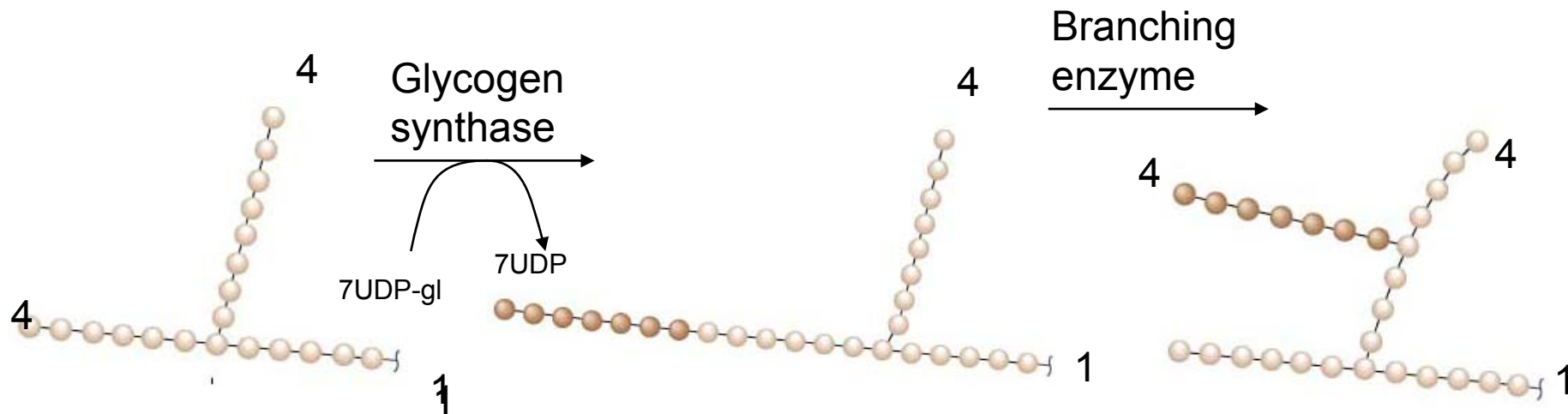


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Glycogen synthesis

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The role of branching enzyme in the synthesis





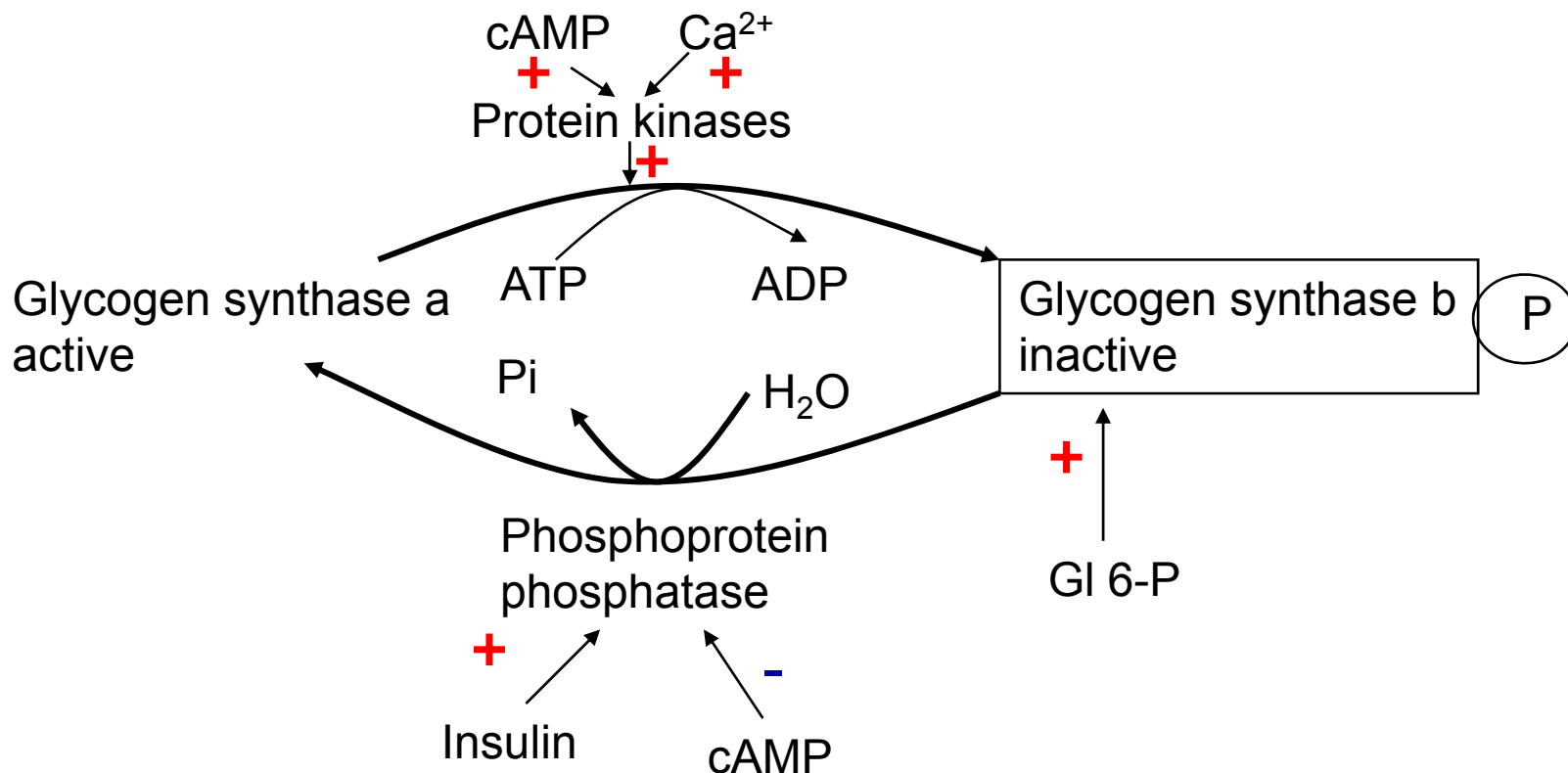
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Glycogen synthesis

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Regulation of glycogen synthesis I

Main regulator: reversible phosphorylation/dephosphorylation





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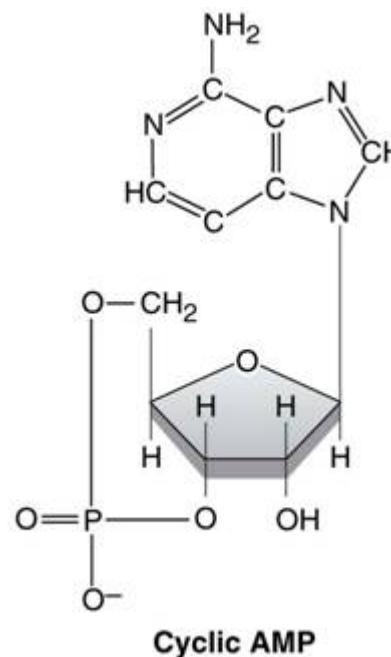
Glycogen synthesis

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Cyclic AMP

Secondary messenger^{DEF}: An effector molecule synthesized within the cell in response to an external signal (first messenger) such as a hormone

Cyclic AMP (cAMP) is formed from ATP by the adenylate cycle enzyme. First messengers, which could elevate cAMP level are glucagon, adrenalin on beta 1,2 receptors, ACTH etc. cAMP can activate protein kinase-A which can initiate a cascade of phosphorylation





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Glycogen synthesis

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Regulation of glycogen synthesis II

Elevation of [cAMP], or $[Ca^{2+}]$ in the liver indicates the presence of glucagon and adrenaline.

Meaning of glucagon: hypoglycemia, glycogen should be mobilized
Glycogenesis should be inactivated.

Meaning of adrenaline “fight or fly” stress, glycogen should be mobilized,
glycogenesis should be inactivated

cAMP, Ca^{2+} , activate protein kinases which phosphorylate glycogen synthase
Phosphorylated glycogen synthase is inactive – synthesis stopped

Phosphatases could remove the phosphorylation (inhibition) of the enzyme.
cAMP inhibits the phosphatase, maintains phosphorylation of glycogen synthase (GS), maintains inhibition of the synthesis
Insulin stimulates the phosphatase, relieves GS from inhibition, stimulates synthesis

GI 6-P precursor of the glycogen synthesis stimulates the inactive GS.



Glycogen synthesis

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Glucose stimulates glycogen synthesis in the liver

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Increased blood glucose stimulates glycogen synthesis in the liver in an insulin independent manner as well.

Glucose binds to phosphorylase a, and glucose-bound phosphorylase is a better substrate for phosphoprotein phosphatase.

Phosphorylase acts as a glucose receptor. Glucose promotes inactivation of phosphorylase thus inhibits glycogenolysis.

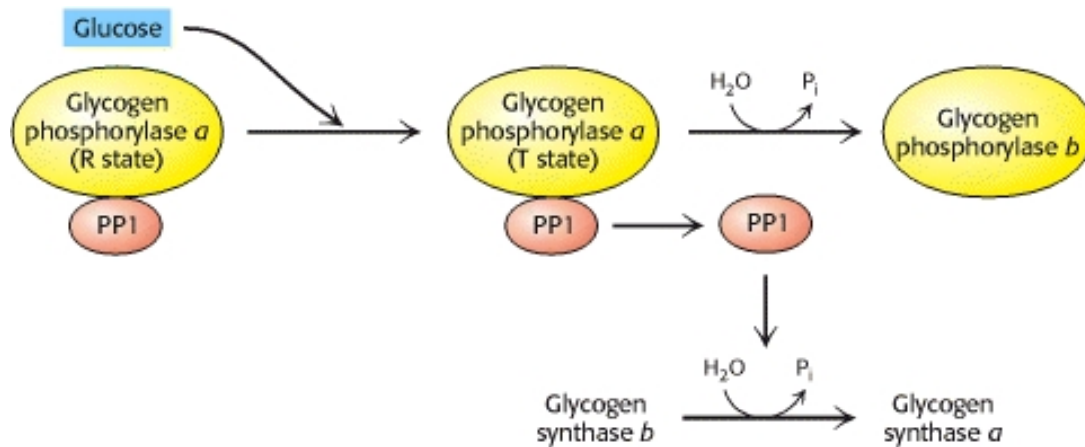
However glycogen synthesis should also be stimulated. Active phosphorylase inhibits dephosphorylation of GS by protein phosphatase. But only phosphorylase a can inhibit this process. Conversion of phosphorylase a to b relieves the inhibition, GS will be dephosphorylated, active, glycogen synthesis could start.

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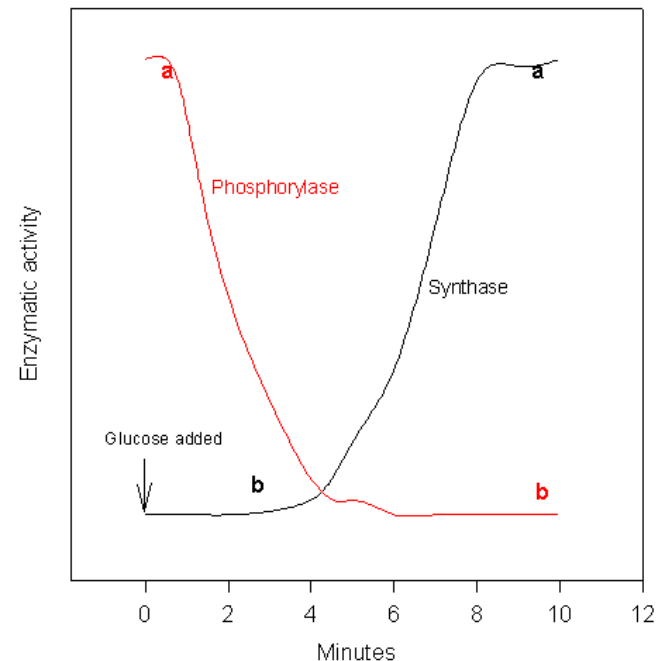
Glycogen synthesis

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Glucose stimulates glycogen synthesis in the liver



PP=phosphoprotein phosphatase





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Glycogen synthesis

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Summary

As gluconeogenesis is not the reversal of glycolysis, glycogen synthesis is also not the reversal of glycogenolysis.

Glycogen synthesis needs energy, 2 high energy phosphate required/glucose incorporated into glycogen.

Glycogen synthase is unable to prime “de novo” glycogen synthesis, In order to create a new glycogen molecule, a primer is needed, This primer is the self-catalytic glycogenin protein.

The glycogen synthesis is regulated both by reversible phosphorylation/dephosphorylation, both by allosteric effectors. Among the allosteric effectors in the liver, the glucose is the most important. Glucose is able to stimulate glycogen synthesis in the liver in the absence of insulin as well.



Lactose synthesis

Lactose^{DEF}: The disaccharide of the milk, containing glucose and galactose

Lactose synthesis: in lactating mammary gland

The enzyme: **galactosyltransferase** catalyzed reaction

UDP-galactose + N-acetyl-glucosamine → Dgalactosyl-N-acetyl-glucosamine
protein attached

This enzyme has a role in the glycoprotein synthesis

The enzyme does not accept glucose as a galactose acceptor.

However, after delivery the specificity of the enzyme changes

UDP-galactose + glucose → lactose + UDP
lactose synthase

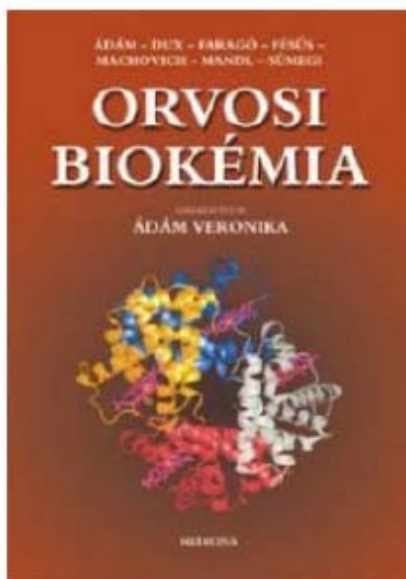
Reason for change in the specificity: lactalbumin: produced by the mammary gland upon hormonal influence. Lactalbumin changes substrate specificity of galactosyltransferase.

Lactalbumin-galactosyltransferase complex = lactose synthase

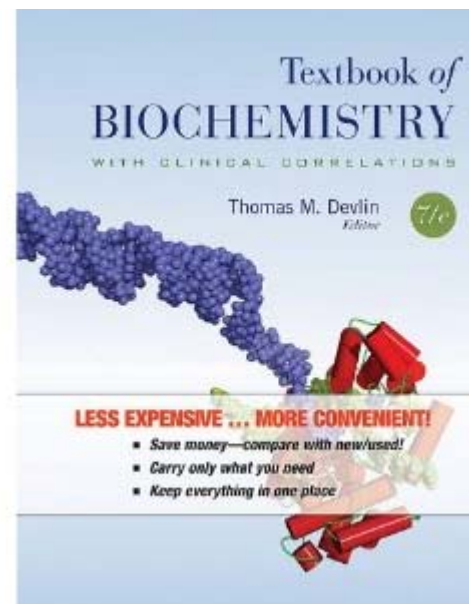
Glycoprotein^{DEF}: Proteins containing carbohydrate groups

Recommended literature

Orvosi Biokémia (Ed. Ádám Veronika)



Textbook of Biochemistry
Ed. Thomas Devlin 5th-7th edition





Questions:

1. Which allosteric regulators can regulate both the glycolysis and the gluconeogenesis?
2. How would influence the elevation of cAMP the gluconeogenesis
3. Which reactions require ATP in gluconeogenesis starting from lactate
4. Which enzymes are active in phosphorylated form in the glycogen metabolism.
5. What is the effect of insulin on the gluconeogenesis?



Questions:

Which statements are true for the synthesis of glycogen?

1. Glycogen synthase reaction connects free glucose molecules
2. Glycogen synthase requires ATP for the catalysis
3. During synthesis inorganic phosphate will be incorporated into the glycogen
4. The source of incorporated glucose is UDP-glucose
5. Branches are formed as postsynthetic modifications

A:1,2,3,

B:2,4

C:1,5,

D:4,5

E:4

Which statements are true for the regulation of glycogen metabolism?

1. Phosphorylation stimulates glycogen phosphorylase activity
2. Phosphorylation decreases the activity of glycogen synthase
3. Phosphorylation increases the activity of glycogen synthase
4. Calcium increases the activity of phosphorylase kinase
5. Glucose decreases the activity of glycogen synthase in liver

A:1,2,5,

B:1,2,4,

C:2,3,5,

D:3,4,5