Gluconeogenesis
Glycogen metabolism

Biochemistry I
Lecture 4

2008 (J.S.)

In the human body, the direct glucose reserves
(about 20 g in body fluids and approximately 200 g in the form of glycogen)
are sufficient to meet glucose needs only for about a day
under basal conditions.

Gluconeogenesis

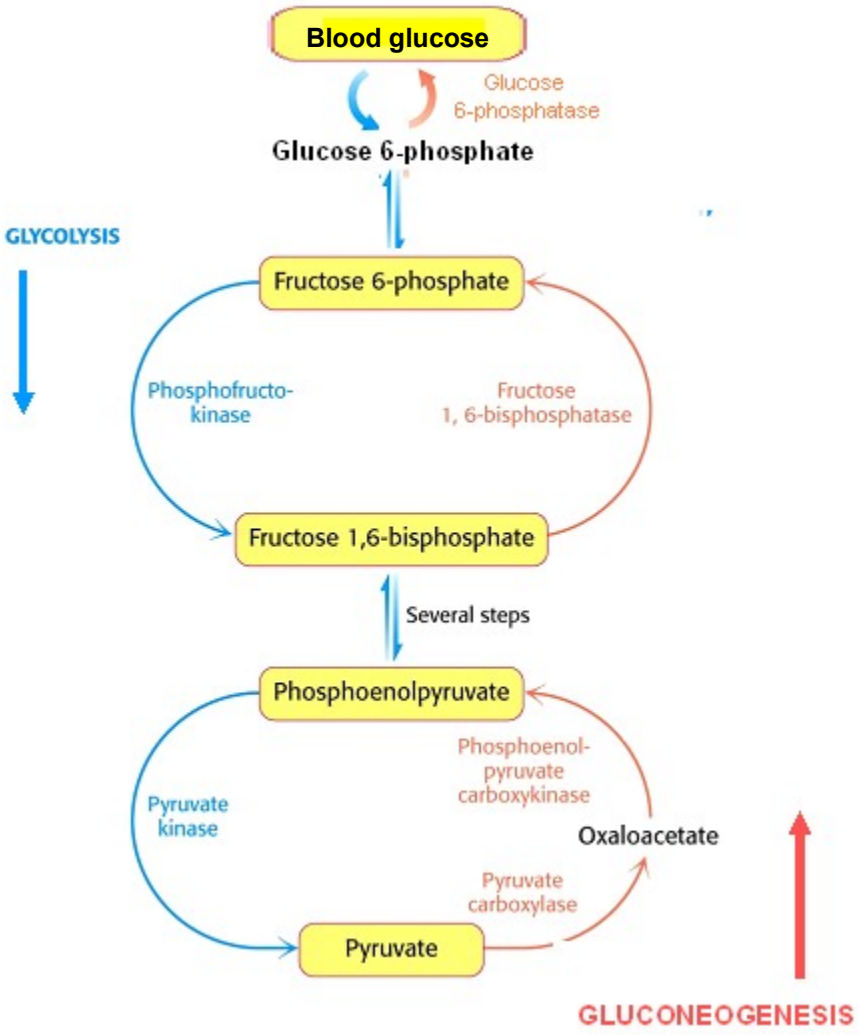
is the synthesis of glucose from nonsaccharide compounds

- **lactate,**
- **glycerol,** and
- **some amino acids** (called glucogenic amino acids).

Gluconeogenesis occurs in the **liver** (approximately 90 %)
and in the **kidney** (about 10 %),
only those two tissues can provide blood glucose by gluconeogenesis.

Gluconeogenesis is not a reversal of glycolysis,

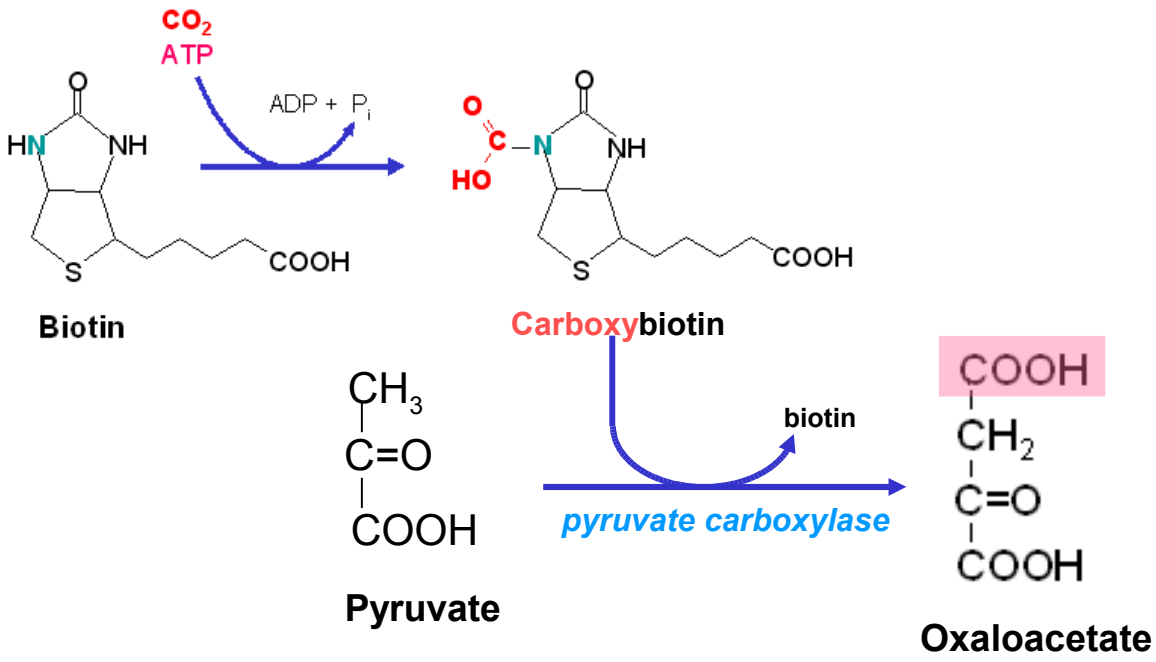
because there are
three irreversible steps in glycolysis.

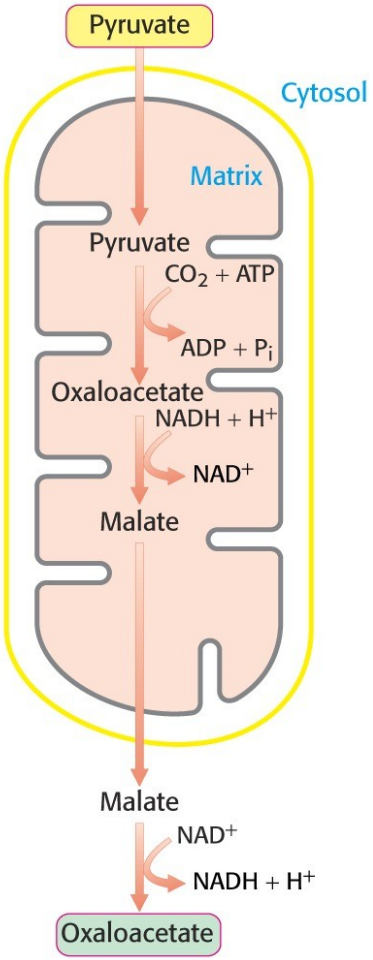


In gluconeogenesis,
four alternate reactions bypass
these irreversible steps of glycolysis.

1 Carboxylation of pyruvate to oxaloacetate

In the **mitochondria** of liver and kidney cells, pyruvate is carboxylated. Carboxybiotin is the donor of carboxyl group:



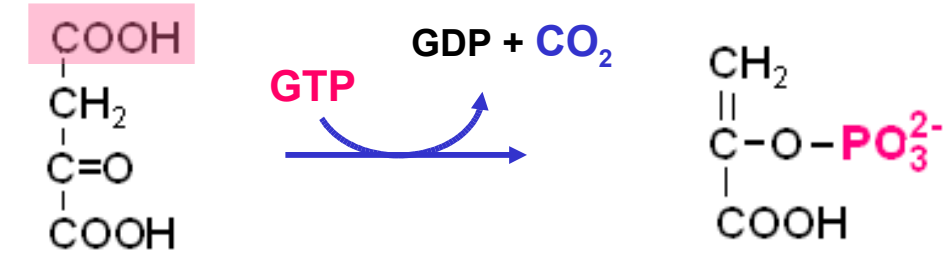


The activity of pyruvate carboxylase depends on the presence of an allosteric activator - acetyl-CoA.

Oxaloacetate is transported **into the cytosol in the form of malate**, which is then reoxidized to oxaloacetate.

2 Conversion of oxaloacetate to phosphoenolpyruvate (PEP)

Oxaloacetate is simultaneously **decarboxylated** and **phosphorylated** by ***phosphoenolpyruvate carboxykinase*** in the cytosol:



Oxaloacetate **Phosphoenolpyruvate**

The two-step pathway for the formation of phosphoenolpyruvate (the sum of reactions 1 and 2)

$$\text{Pyruvate} + \text{ATP} + \text{GTP} + H_2O \rightleftharpoons \text{Phosphoenolpyruvate} + \text{ADP} + \text{GDP} + P_i + 2\,H^+$$

is much more favourable than the reaction catalyzed by pyruvate kinase, because of the use of a molecule of ATP in the carboxylation reaction 1. The added molecule of CO_2 is then removed to power the endergonic formation of PEP in the decarboxylation step.

3 Dephosphorylation of fructose 1,6-bisphosphate

The hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate is catalyzed by ***fructose 1,6-bisphosphatase***.

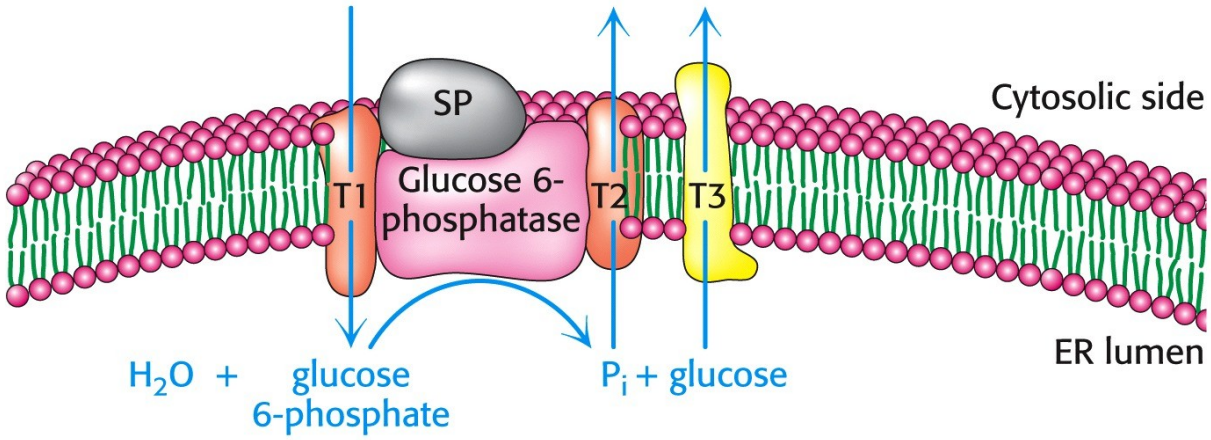
Fructose 1,6-bisphosphatase is an allosteric enzyme. Like its glycolytic counterpart ***phosphofructokinase*-1**, it participates in the regulation of gluconeogenesis.

Both enzymes are reciprocally controlled by **fructose 2,6-bisphosphate** in the liver. Fructose 2,6-bisphosphate strongly stimulates phosphofructokinase-1 and inhibits fructose 1,6-bisphosphatase.

In most tissues, gluconeogenesis (if there is any) ends at glucose 6-phosphate, free glucose is not generated.

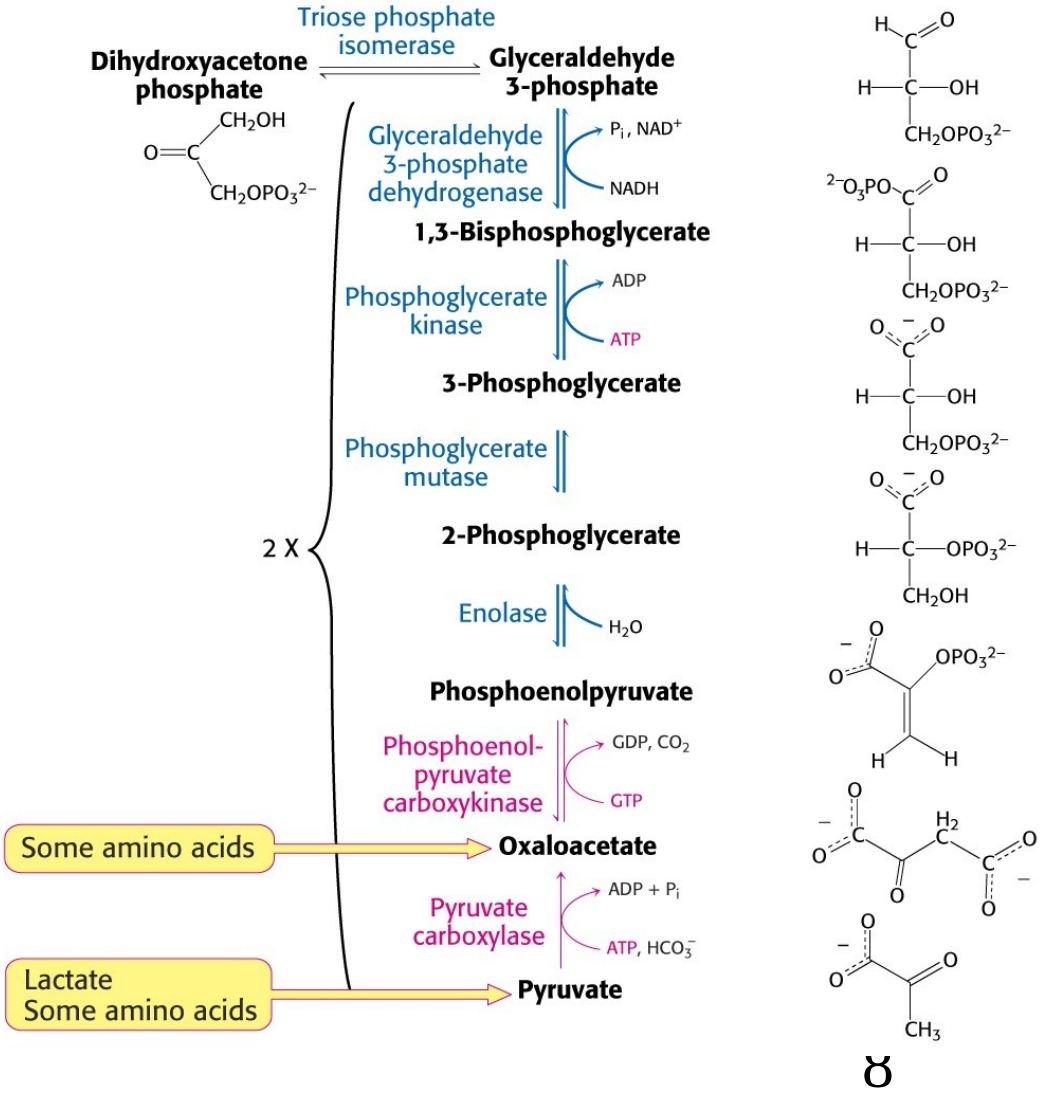
4 Glucose 6-phosphatase is present only in the liver cells and to a lesser extent **in the kidney**, only these tissues can release free glucose into the blood.

The dephosphorylation of glucose 6-phosphate takes place within the **lumen of endoplasmic reticulum**.

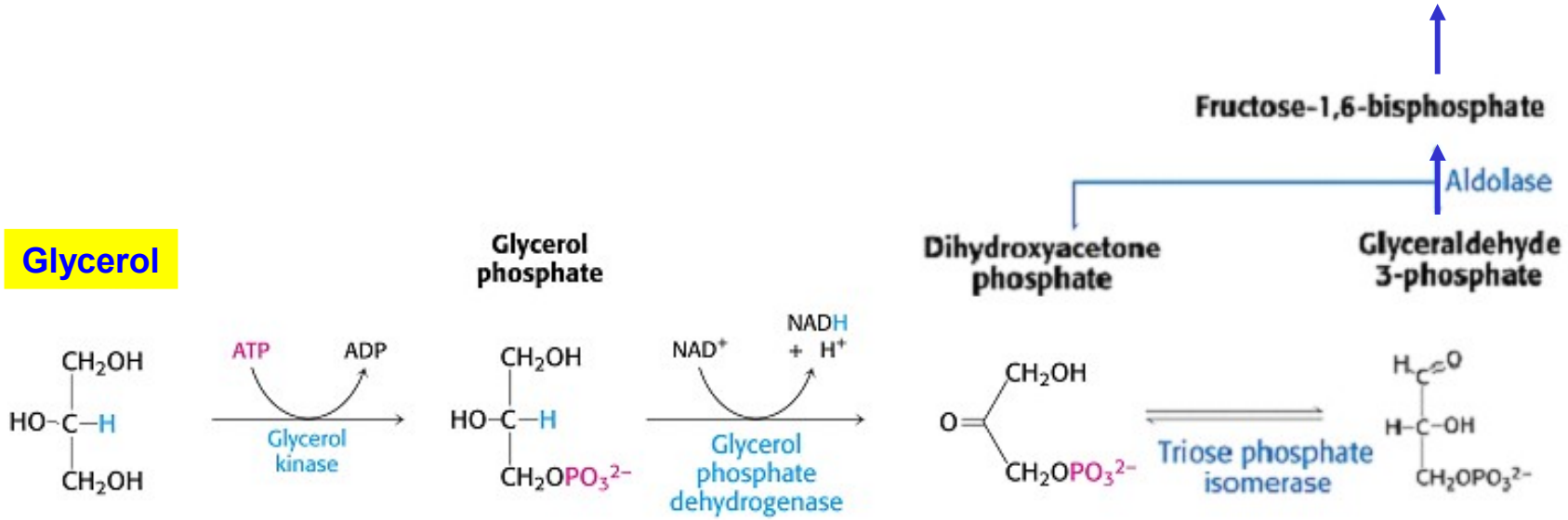


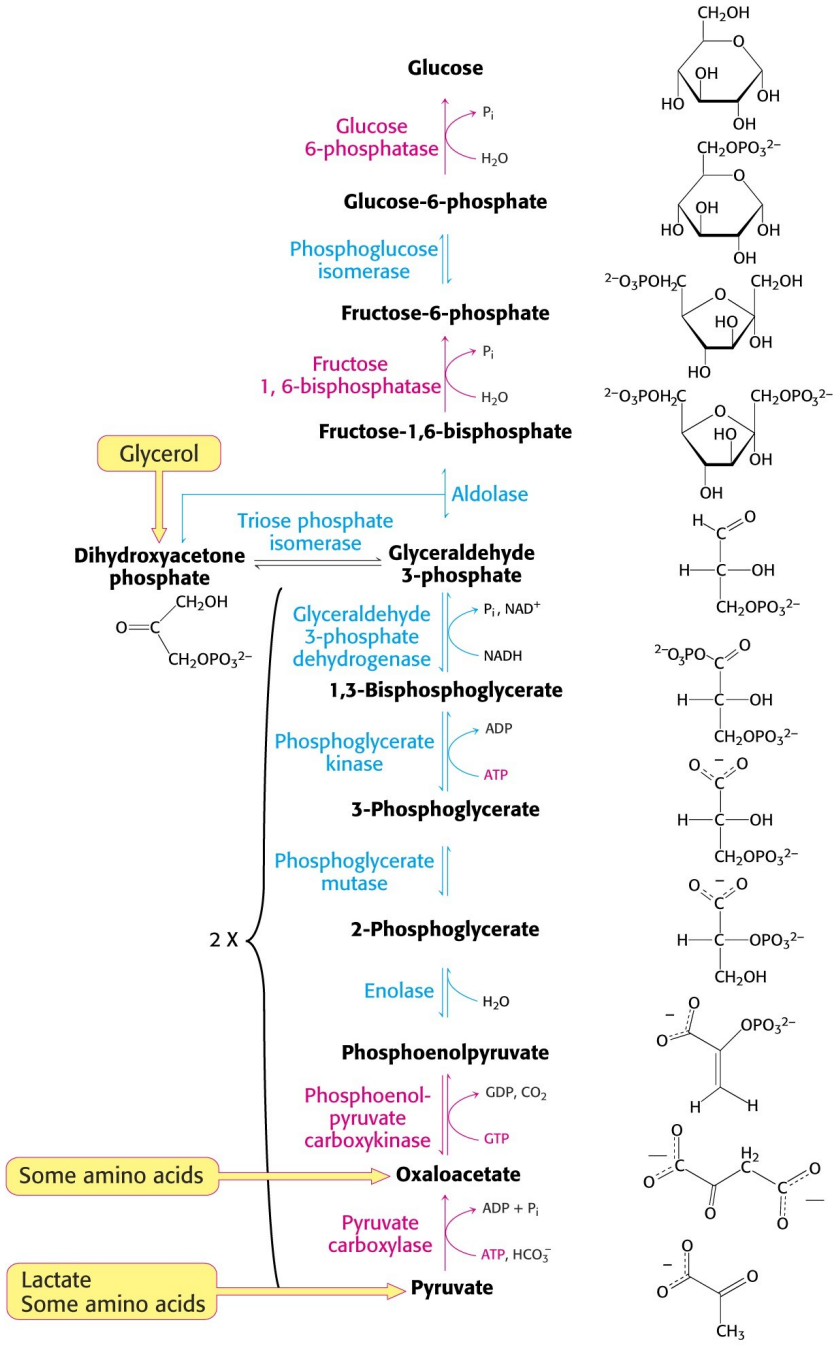
SP – Ca^{2+}-binding stabilizing protein is essential for Glu-6-phosphatase activity

Nonsaccharide precursors **lactate** and some **glucogenic amino acids** are first converted to **pyruvate**,
other glucogenic amino acids enter the gluconeogenic pathway as **oxaloacetate**:



Glycerol (from mobilized reserve fat) enters the gluconeogenesis as **dihydroxyacetone phosphate**:



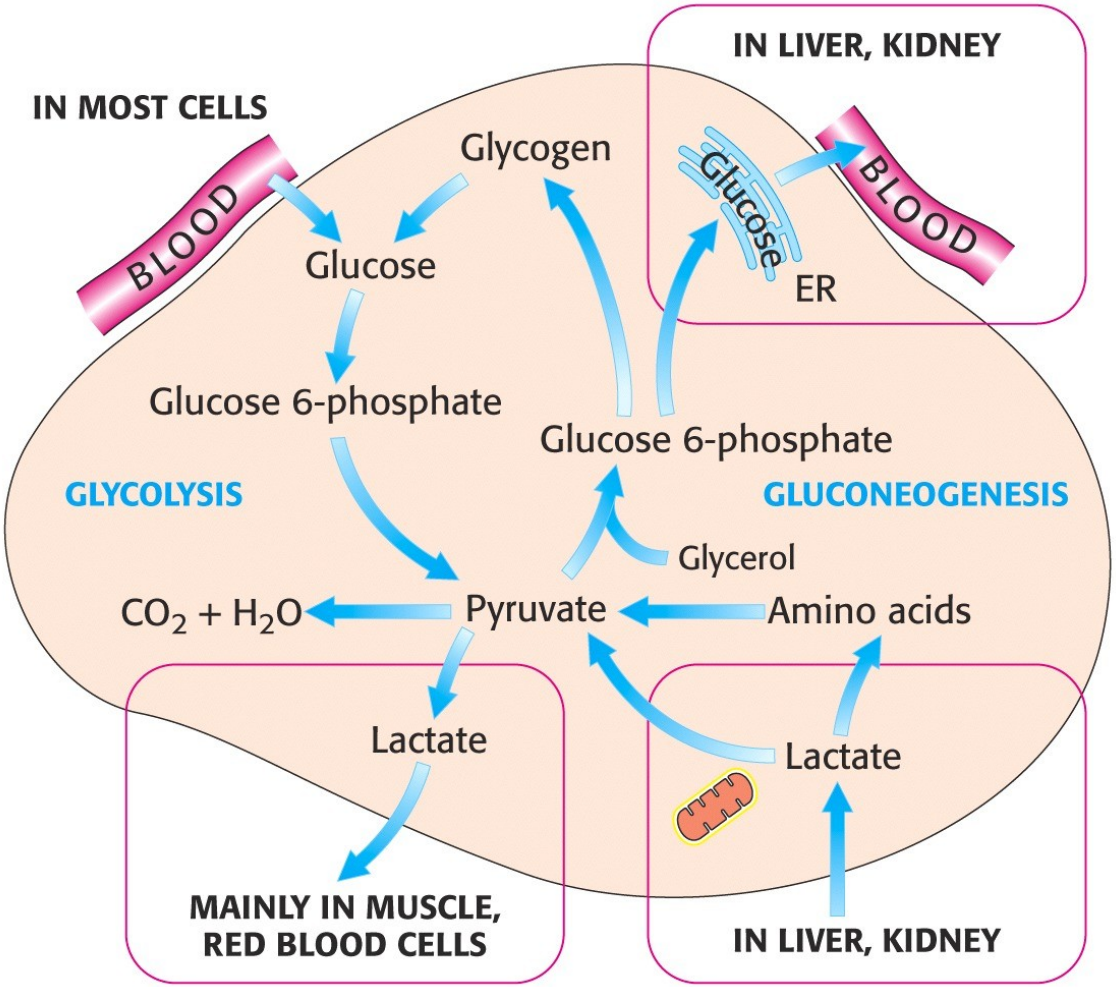


In the gluconeogenesis from pyruvate, six high-energy phosphate bonds are spent.

Only two molecules of ATP are generated in glycolysis in the conversion of glucose into pyruvate.

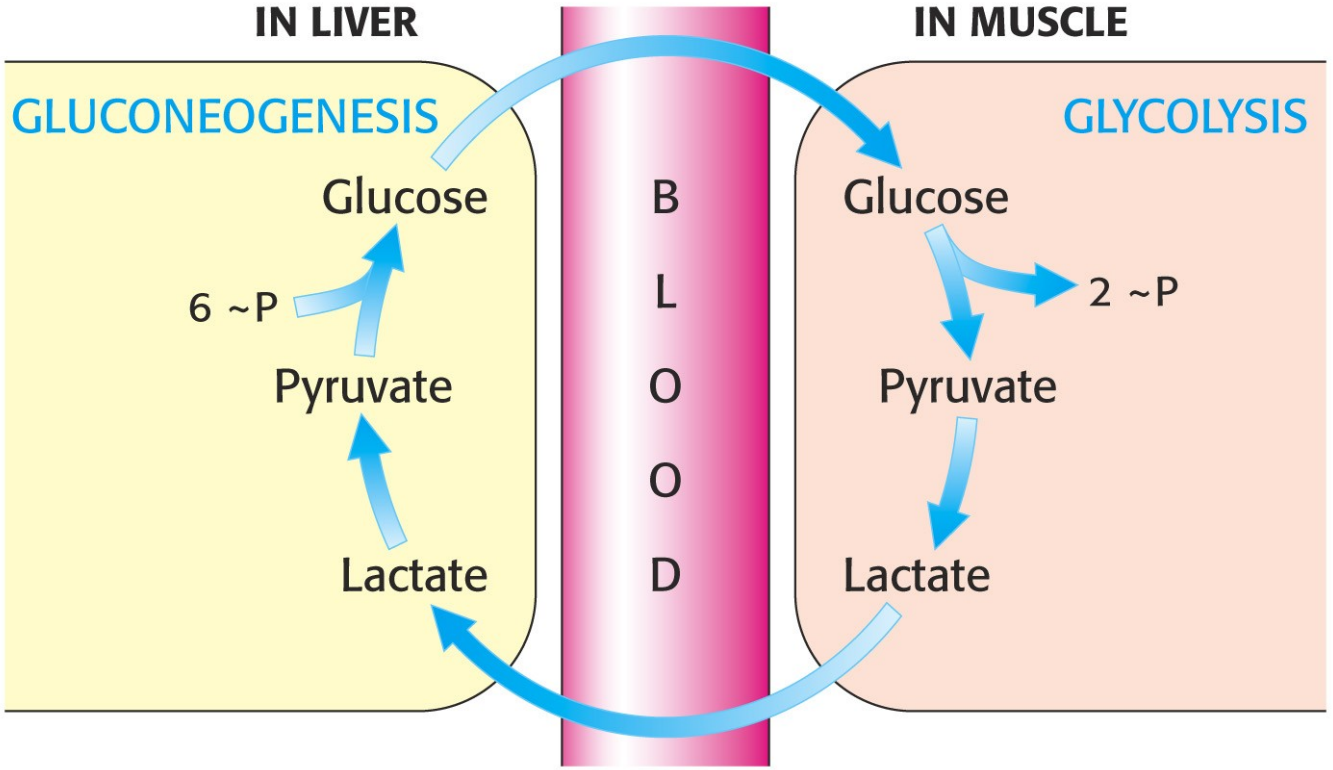
Cooperation between glycolysis and gluconeogenesis

in a tissue-specific fashion

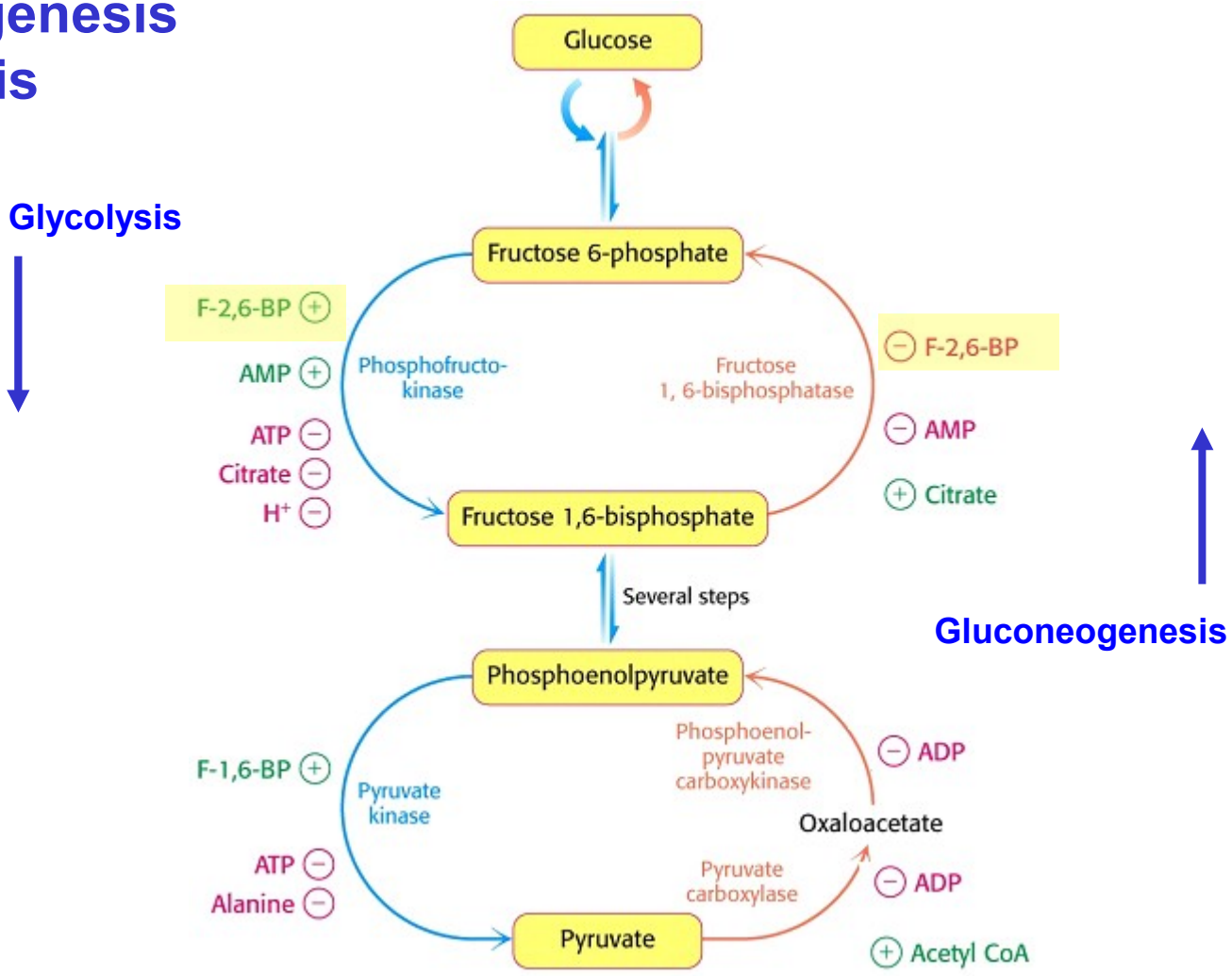


The Cori cycle

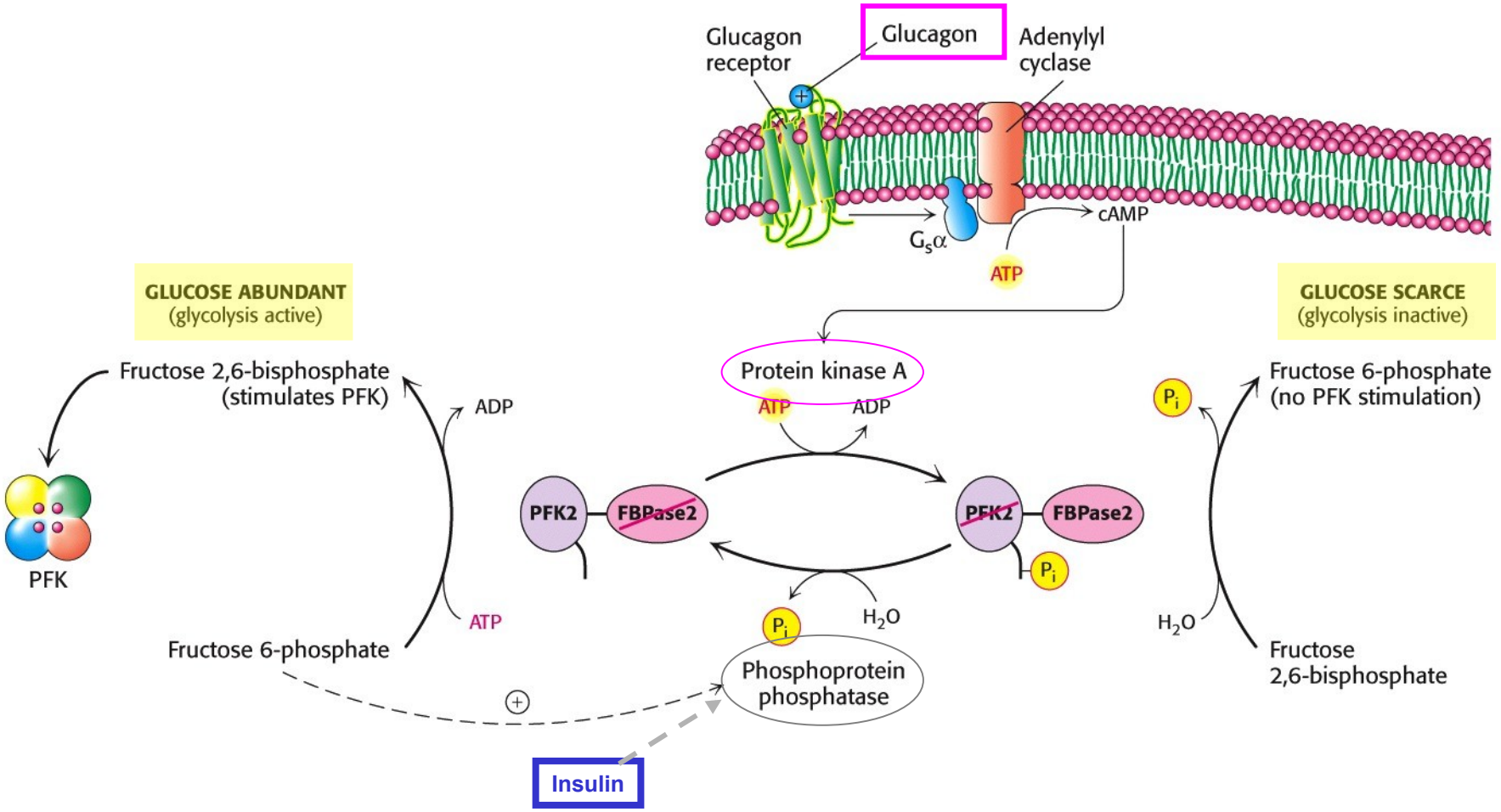
Gluconeogenesis in the liver transforms part of the **lactate** formed by active skeletal muscle to **glucose:**



Reciprocal allosteric regulation of gluconeogenesis and glycolysis in the liver

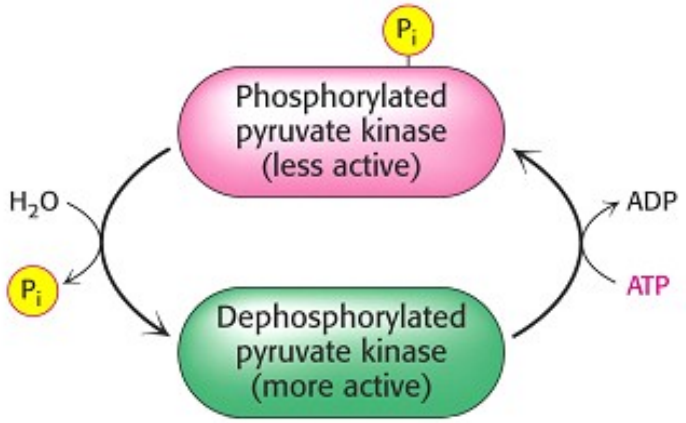


Control of phosphofructokinase-2 / fructose 2,6-bisphosphatase (a bifunctional enzyme) by phosphorylation and dephosphorylation



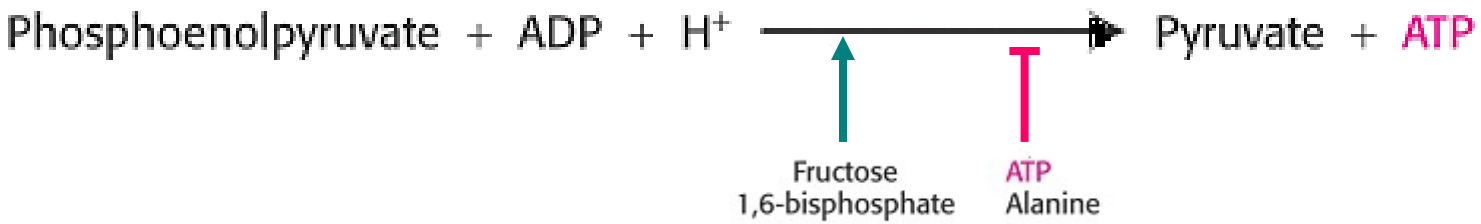
Control of pyruvate kinase activity

- by phosphorylation and dephosphorylation, and
- by allosteric effectors



LOW BLOOD GLUCOSE LEVEL

During starvation, pyruvate kinase is inhibited by phosphorylation



Allosteric effects

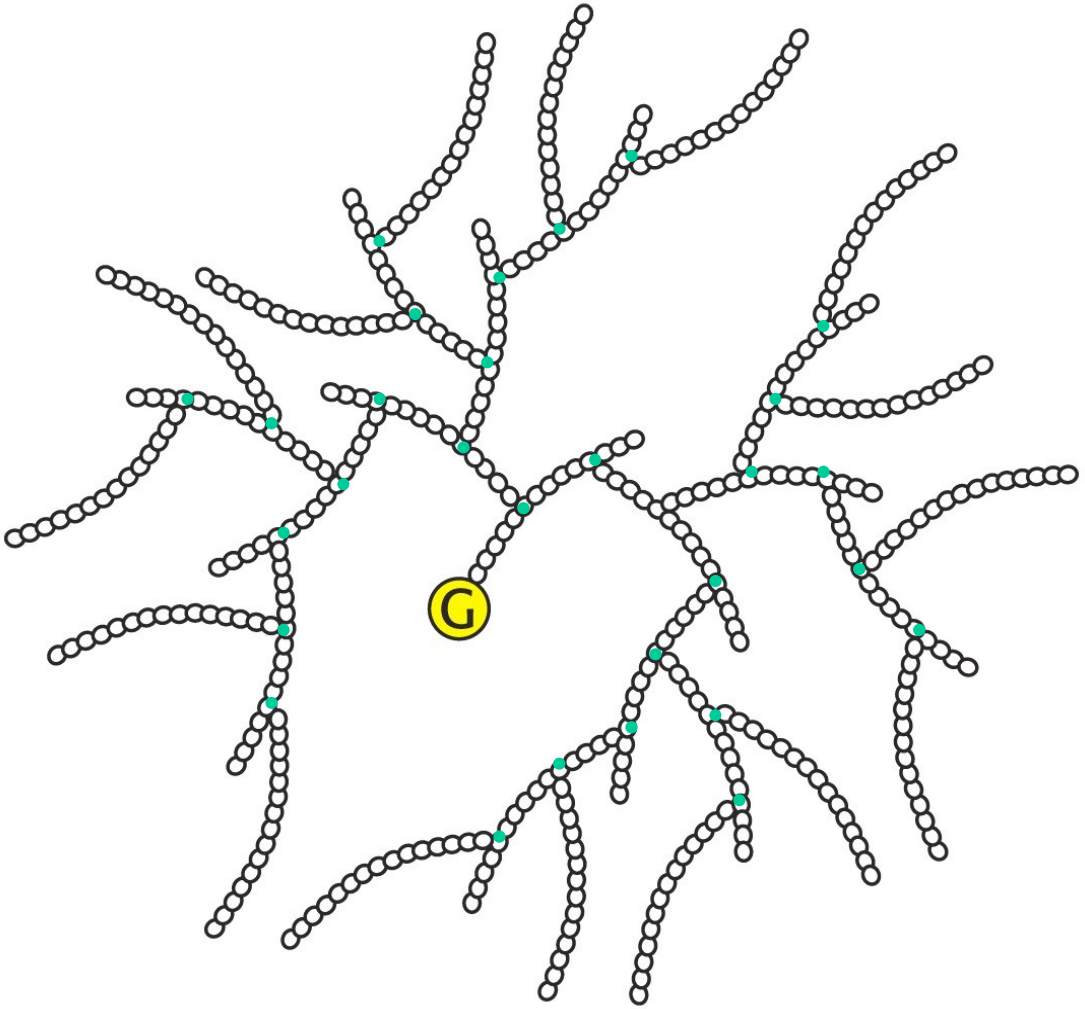
Glycogen metabolism

Structure of glycogen

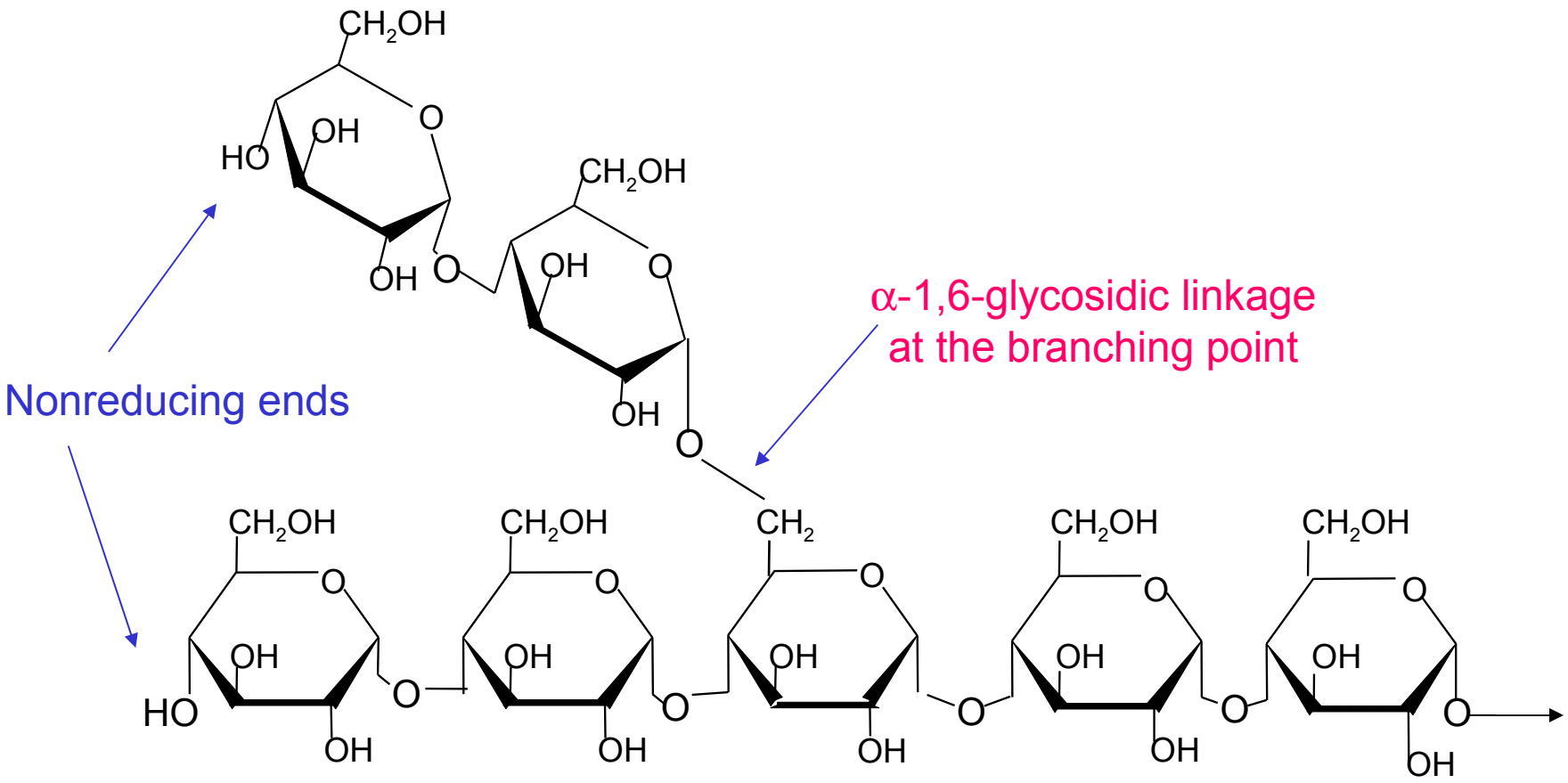
A very large **branched polymer** of glucose residues, M_r about 10^7 (≈ 50 000 glucose units).

Glycogen is present in the cytosol of animal cells in the form of **granules** ranging in diameter from 10 to 40 nm. The two major sites of glycogen storage are **the liver and skeletal muscle**.

The core of the glycogen particle is a protein (glycogenin, G).



Structure of glycogen – **two types of α-glucosidic bonds**



Glycogen digestion in the gastrointestinal tract

is essentially the same as the digestion of amylopectin.

Both saliva and pancreatic secretion contain ***α-amylase***, which catalyses **hydrolytic splitting of α-1,4-glucosidic bonds** at random, unless they are near chain ends or branch points.

The products are then **maltose**, **maltotriose** and a mixture of small branched fragments (with 5 - 9 glucose residues) called **α-dextrins**.

Those products are **hydrolysed** to **free glucose** by the action of both ***maltase*** and ***saccharase-isomaltase***, bound in the plasma membrane of mucosal cells of the duodenum and jejunum.

> The importance of glycogen in food is not very large, because the glycogen content of meat products is usually negligible due to post-mortem glycogenolysis.

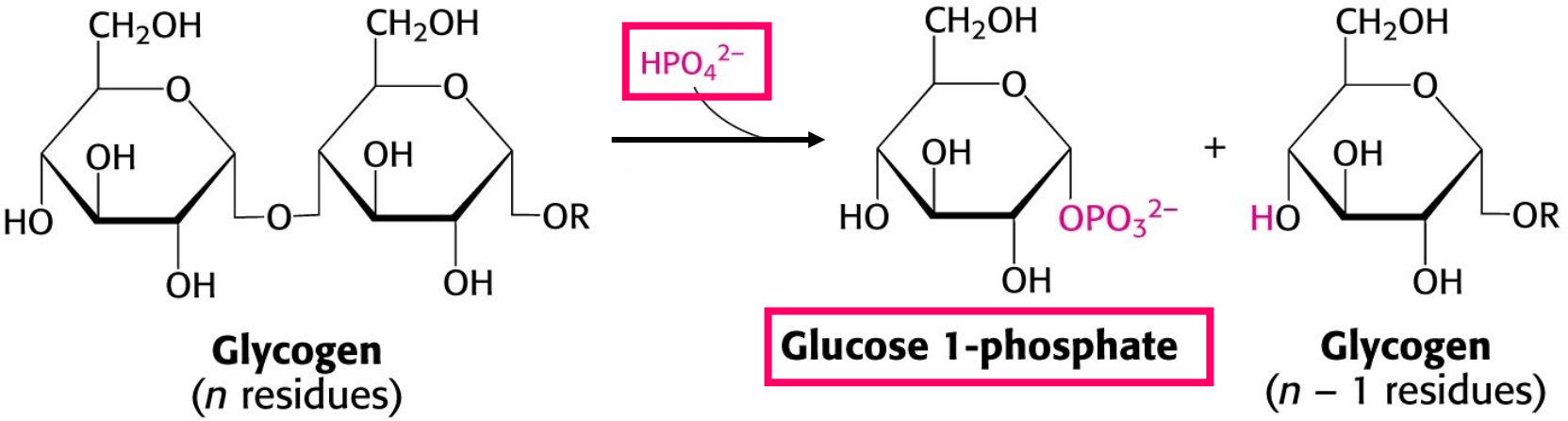
Glycogen breakdown in cells

requires the cooperation of two enzymes – glycogen phosphorylase and
– a debranching enzyme.

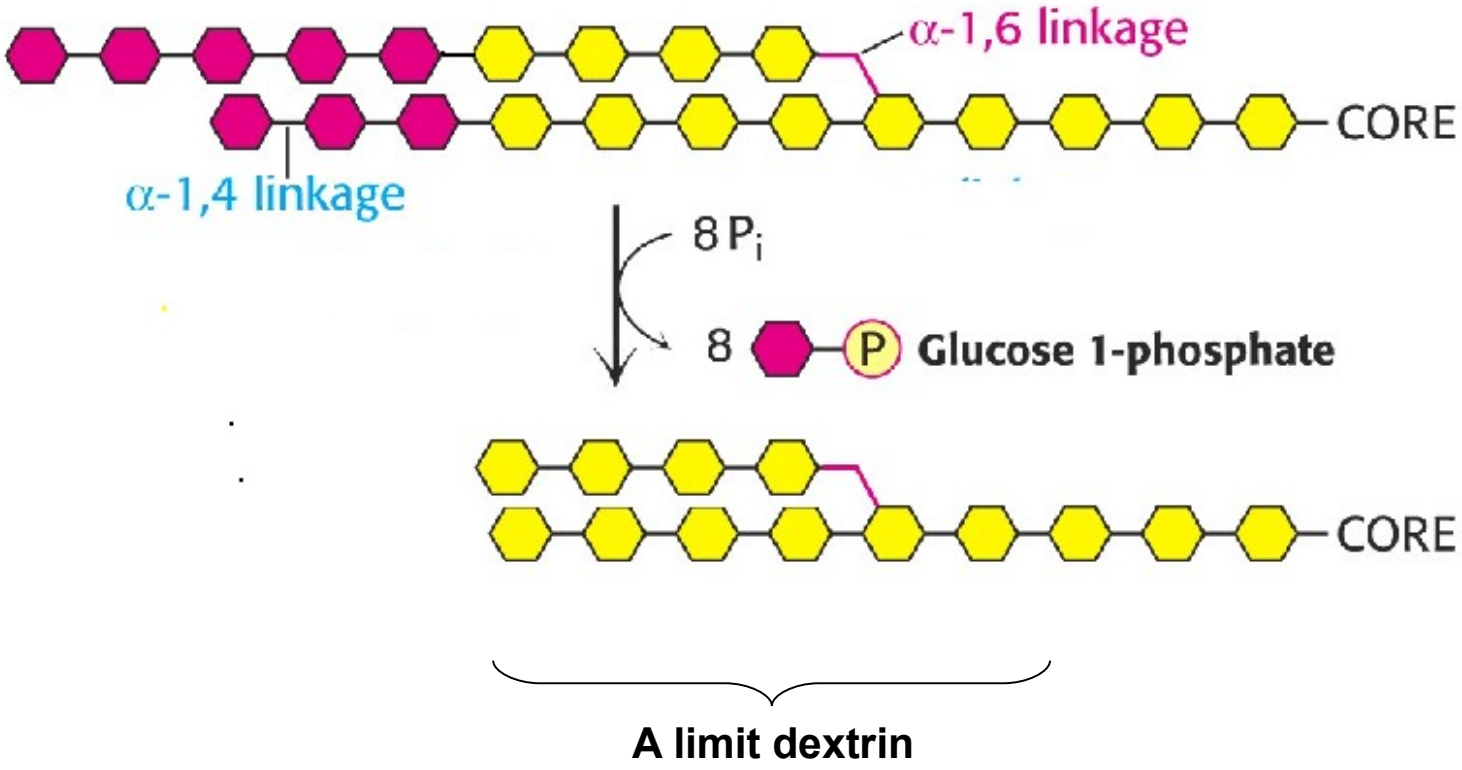
Glycogen phosphorylase (phosphorylase)

- the key regulatory enzyme in glycogenolysis

catalyses the sequential **phosphorolysis** (not hydrolytic splitting!) of α-1,4-glycosidic bonds of glycosyl residues from the non-reducing ends, and these only if they are more distant than four residues from a branch point. So its action ends with a production of several molecules of **glucose 1-phosphate** and a **limit dextrin**.



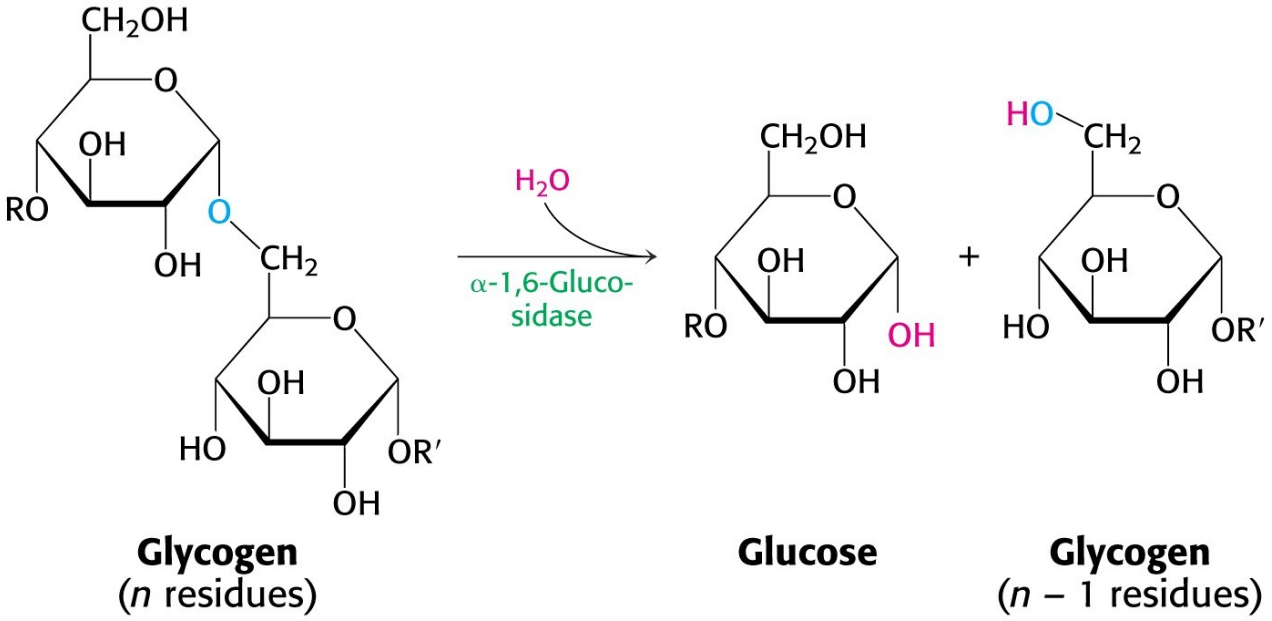
Phosphorylase can split α-1,4-links,
its action ends with the production of limit dextrin:



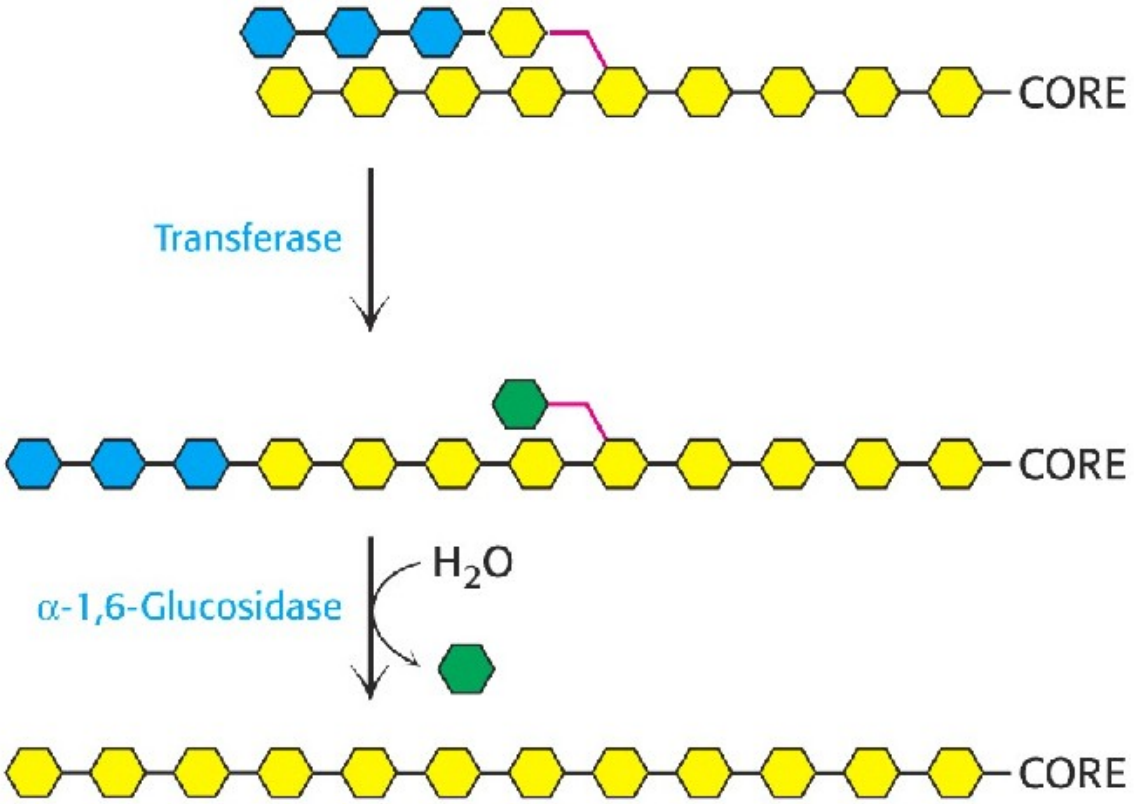
Glycogen debranching enzyme exhibits two catalytic activities, it is a bifunctional enzyme:

The ***transferase*** activity shifts a block of three glucosyl residues from one outer branch to the other, and

α-1,6-glucosidase activity **hydrolysis** the α-1,6-glycosidic bond resulting in the release of a **free glucose molecule.**

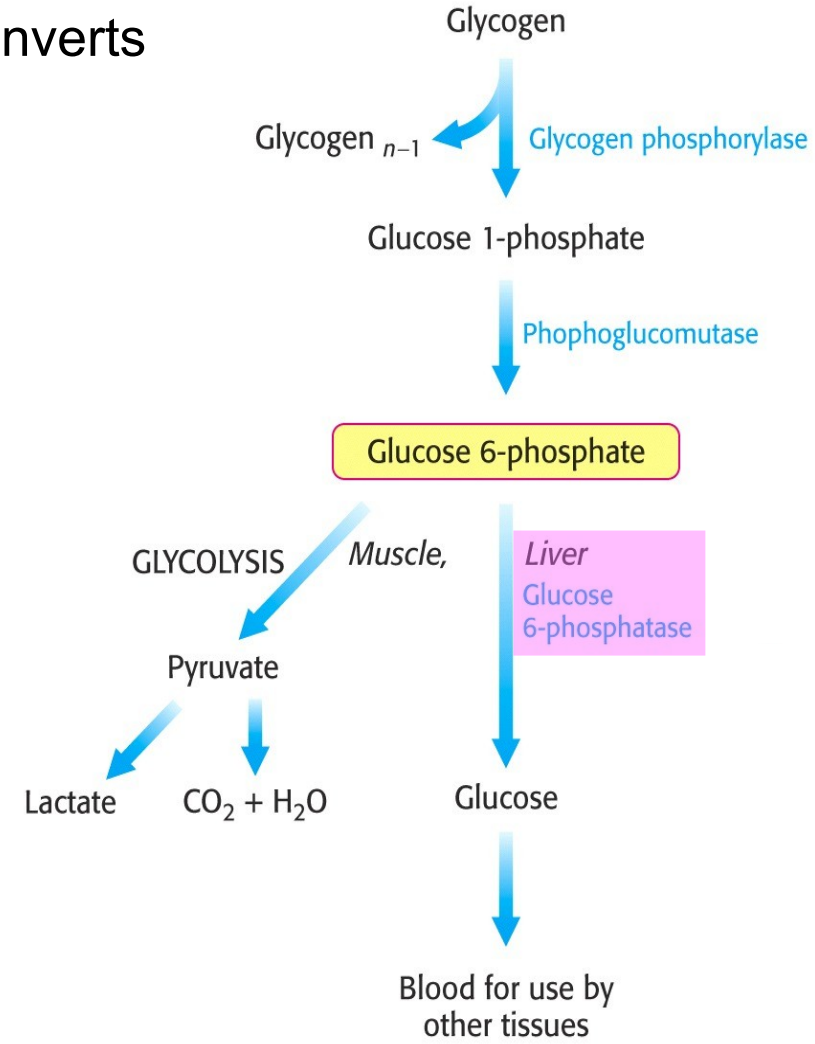


The debranching enzyme converts the branched structure of a limit dextrin into a linear one:



Phosphorylase can now attack the remaining α-1,4-linked chain.

Phosphoglucomutase converts glucose 1-phosphate into **glucose 6-phosphate** – the intermediate of glycolysis.



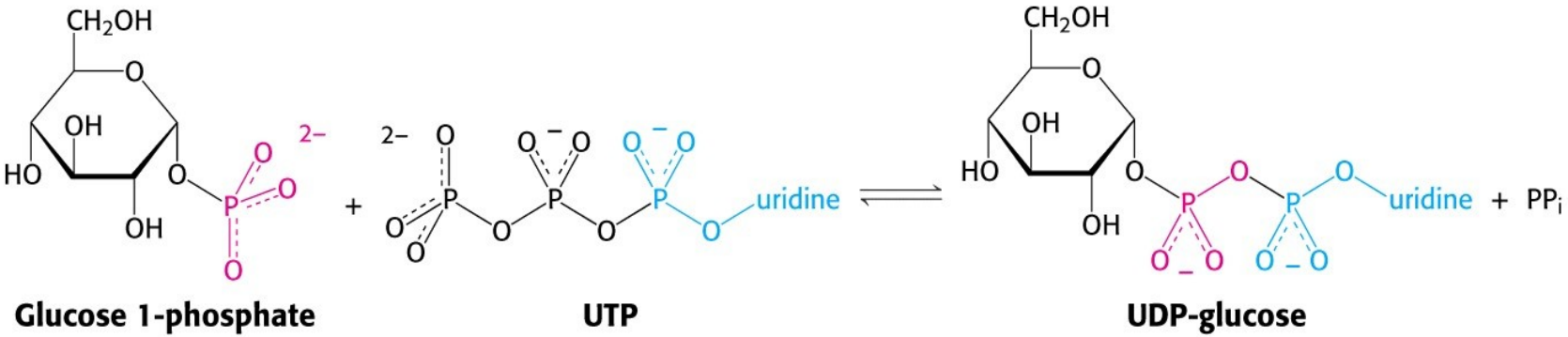
Glycogen synthesis (glycogenesis)

A distinct system of enzymes exists for endergonic glycogen synthesis, coupled ultimately to the hydrolysis of ATP.

Glucose 6-phosphate isomerizes to **glucose 1-phosphate** by the action of phosphoglucomutase.

Synthesis of an activated form of glucose – UDP-glucose

from glucose 1-phosphate and UTP (uridine triphosphate) in a reaction catalyzed by ***UDP-glucose pyrophosphorylase***:



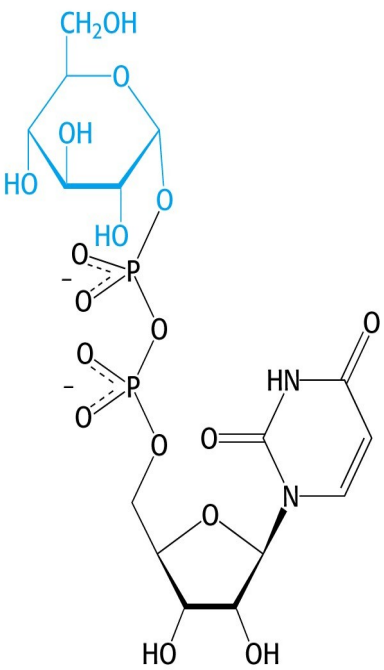
Glucose 1-phosphate **UTP** **UDP-glucose**

This reaction is reversible, but it is driven by the essentially irreversible and rapid hydrolysis of diphosphate catalysed by inorganic pyrophosphatase:

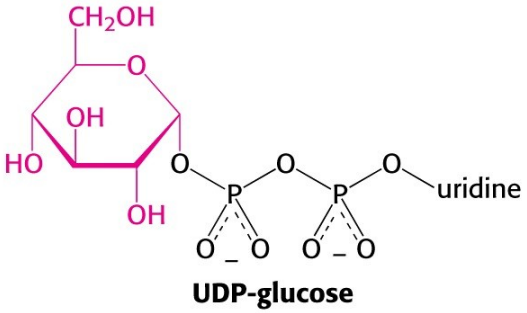
$$PP_i + H_2O \longrightarrow 2\,P_i$$

Glycogen synthase – the key regulatory enzyme in glycogenesis

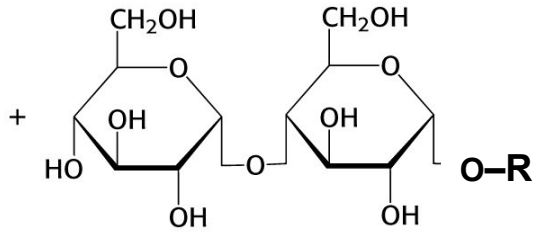
catalyses formation of an α-1,4-glycosidic bonds by the transfer of glucosyl from UDP-glucose to an existing chain (a primer):



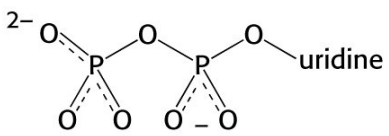
Uridine diphosphate glucose (UDP-glucose)



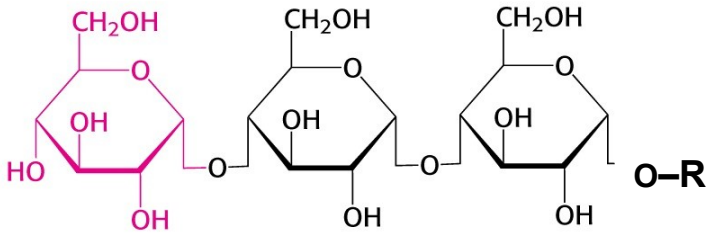
UDP-glucose



A primer (an existing chain of glycogen or autoglucosylated glycogenin)



UDP



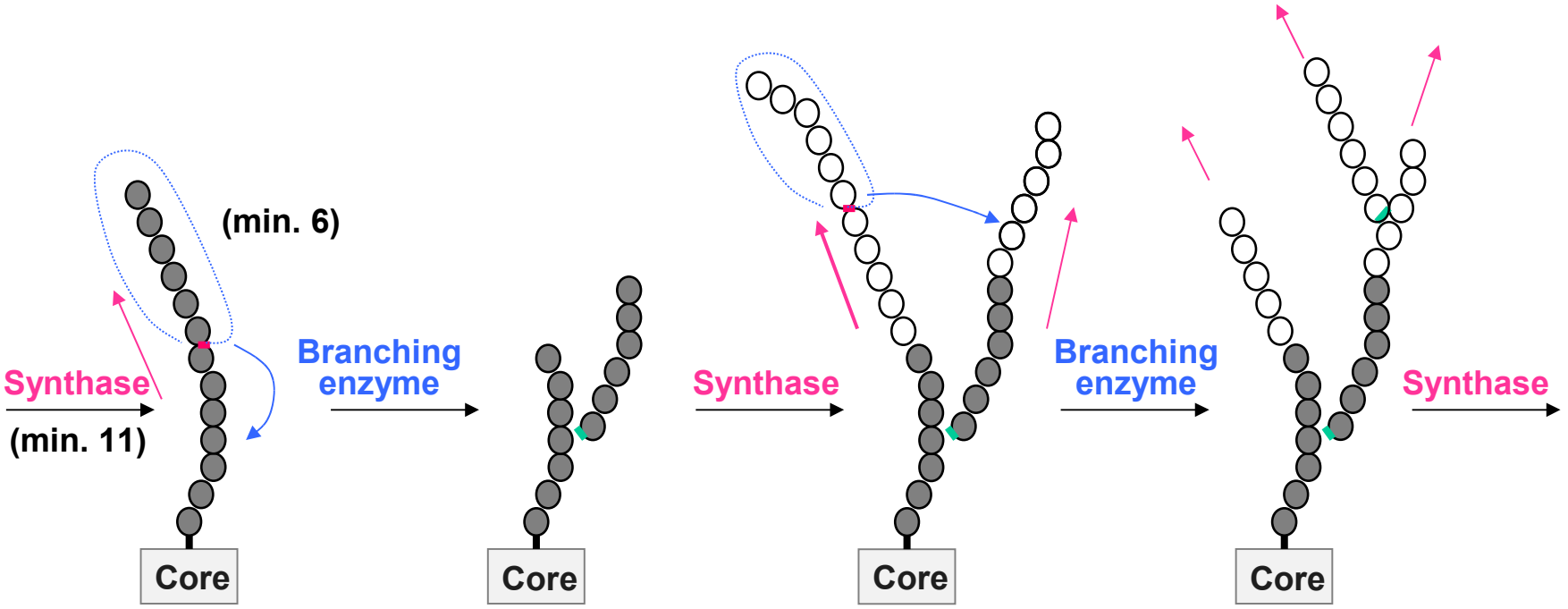
Glycogen (n + 1 residues)

*The **branching enzyme***

forms α-1,6-linkages that make glycogen a branched polymer.

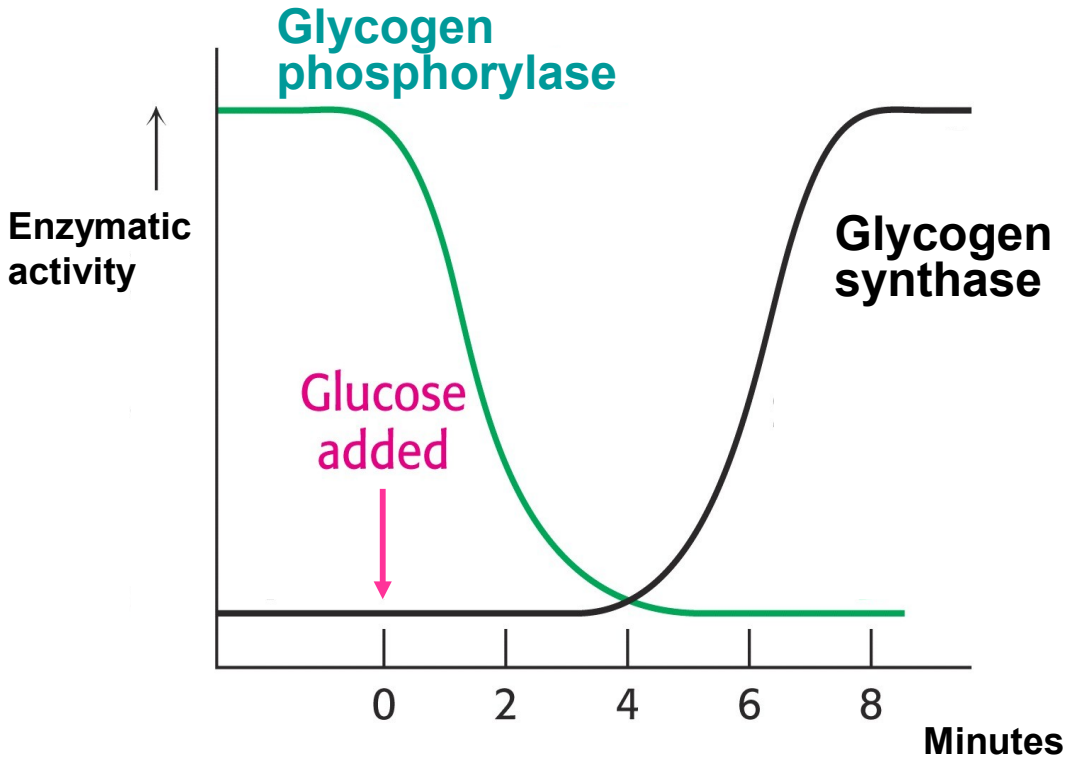
Branching is important because it increases the solubility of glycogen and increases the velocity of glycogen synthesis and breakdown (creating a large number of non-reducing ends).

The branching enzyme is the **amylo-(α-1,4→α-1,6)-transglucosylase:**



Glycogen breakdown and synthesis are regulated reciprocally, under hormonal control

Example: If the blood-glucose concentration increases after a glucose load, there is a very rapid change in glycogen metabolism **in the liver,** a switch from glycogen catabolism to glycogen synthesis.



The liver is the organ that serves as a supplier of glucose **for the whole body** (having glycogen as a reservoir of glucose), and the liver therefore responds to changes in the blood glucose level by degrading or synthesizing glycogen, as required. The response is mediated mostly by hormones –
by the action of **insulin**, or by the opposed action of **glucagon** and **adrenaline**.

Control of glycogen metabolism **in muscle** is slightly different – glycogen is an energy store only **for the tissue**.

The control acts through phosphorylation and dephosphorylation

of the key enzymes (glycogen phosphorylase and glycogen synthase)

and some regulatory proteins.

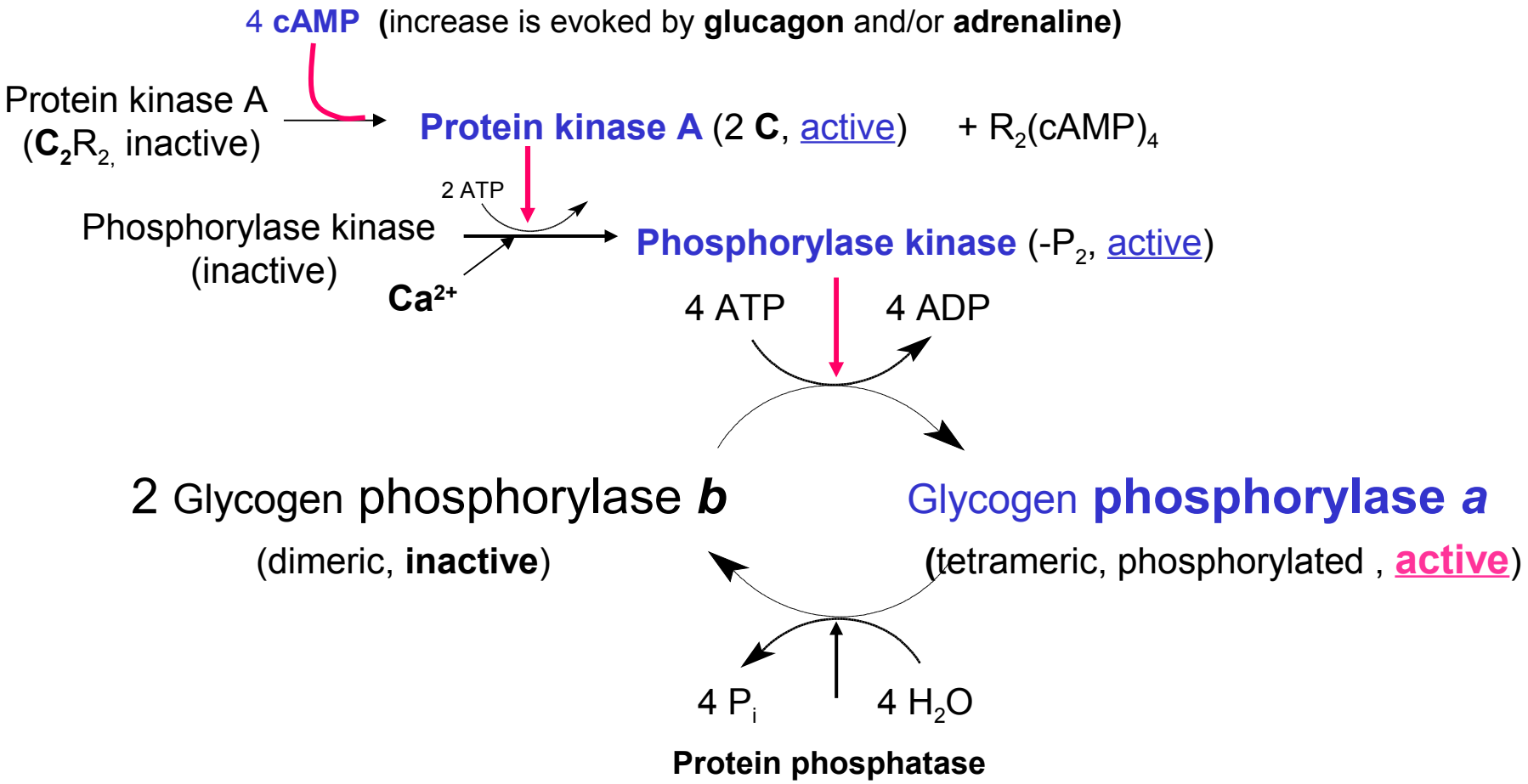
These phosphorylations are catalysed by the action of **protein kinases**,

dephosphorylations by the action of **phosphoprotein phosphatases.**

Phosphorylated glycogen phosphorylase is the **active** form,

phosphorylated glycogen synthase is **inactive**.

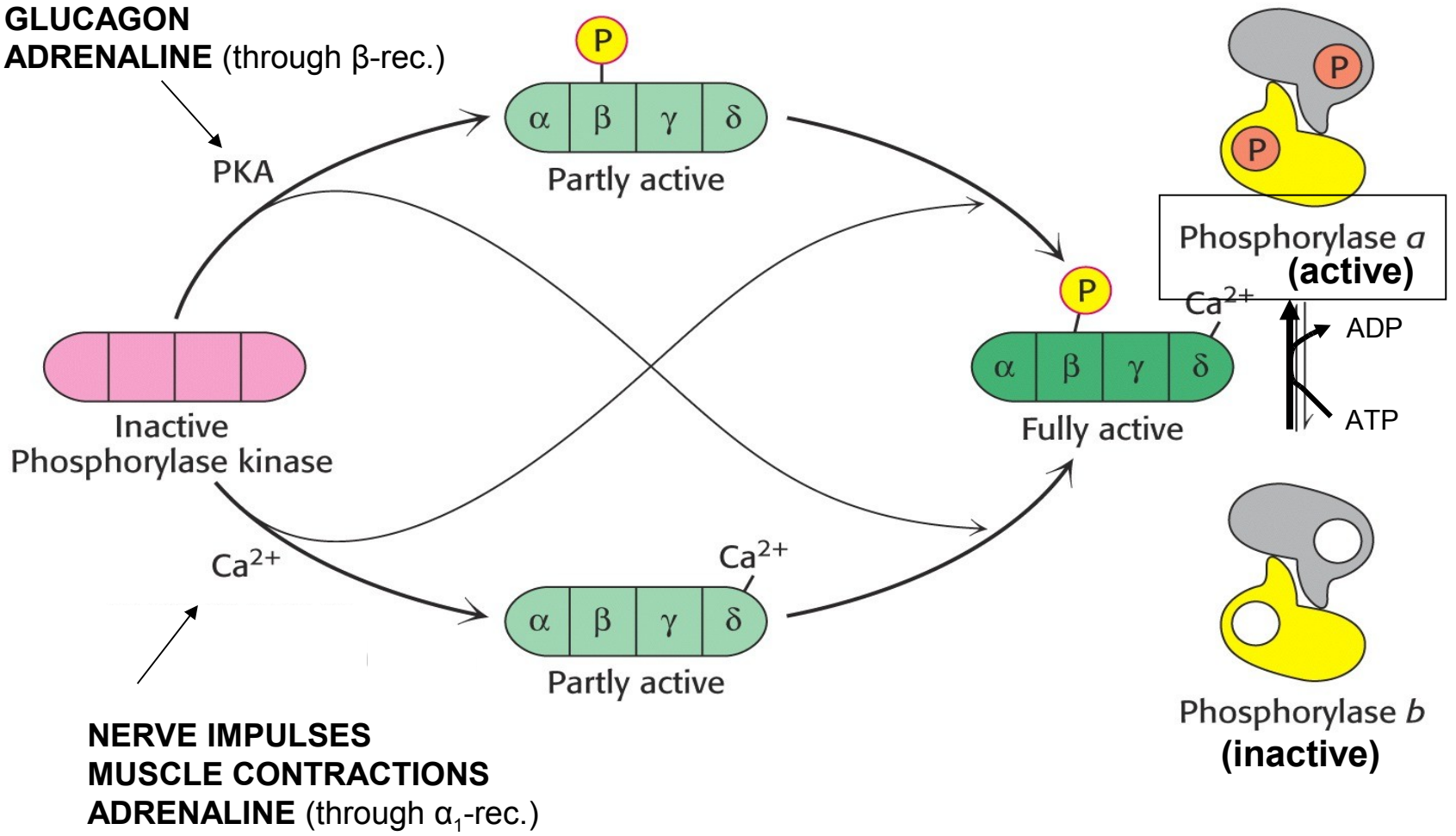
Control of glycogen degradation



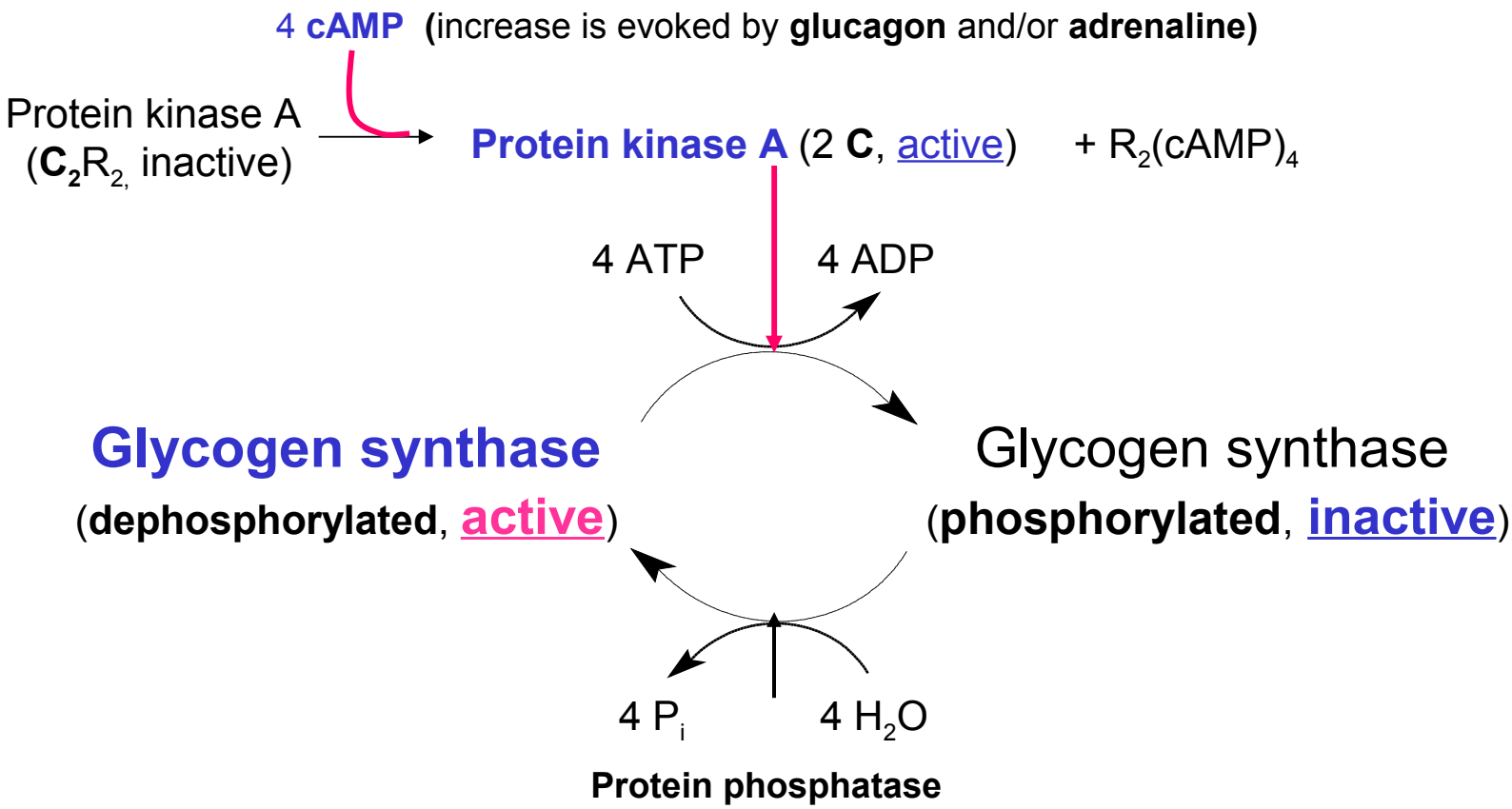
Phosphorylase ***a*** is also **allosterically activated** by **AMP** and **inorg. phosphate**.

In muscle, the phosphorylase ***b*** (relatively inactive) is activated allosterically by **AMP, even being not phosphorylated.**

Phosphorylase kinase – activation by phosphorylation and Ca^{2+}



Control of glycogen synthesis

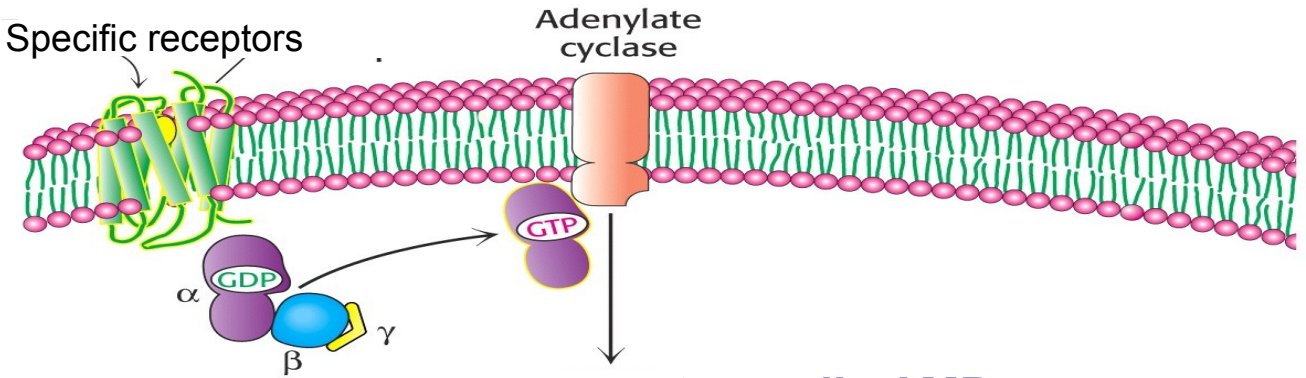


On the contrary to glycogen breakdown, the key enzyme of glycogen synthesis **glycogen synthase is inactivated by phosphorylation**.

The phosphorylation of both key enzymes depends primarily on the intracellular **concentration of cAMP**.

Intracellular concentration of cAMP is regulated by extracellular signals (hormones and neurotransmitters)

Glucagon is secreted by the pancreas (A cells of the Langerhans islets) if there is a low blood-glucose concentration, **adrenaline** is secreted from the adrenal medulla as a consequence of stress.
Both hormones are bound to specific receptors in cytoplasmic membranes and activate adenylate cyclase that catalyses the formation of cAMP, a second messenger.



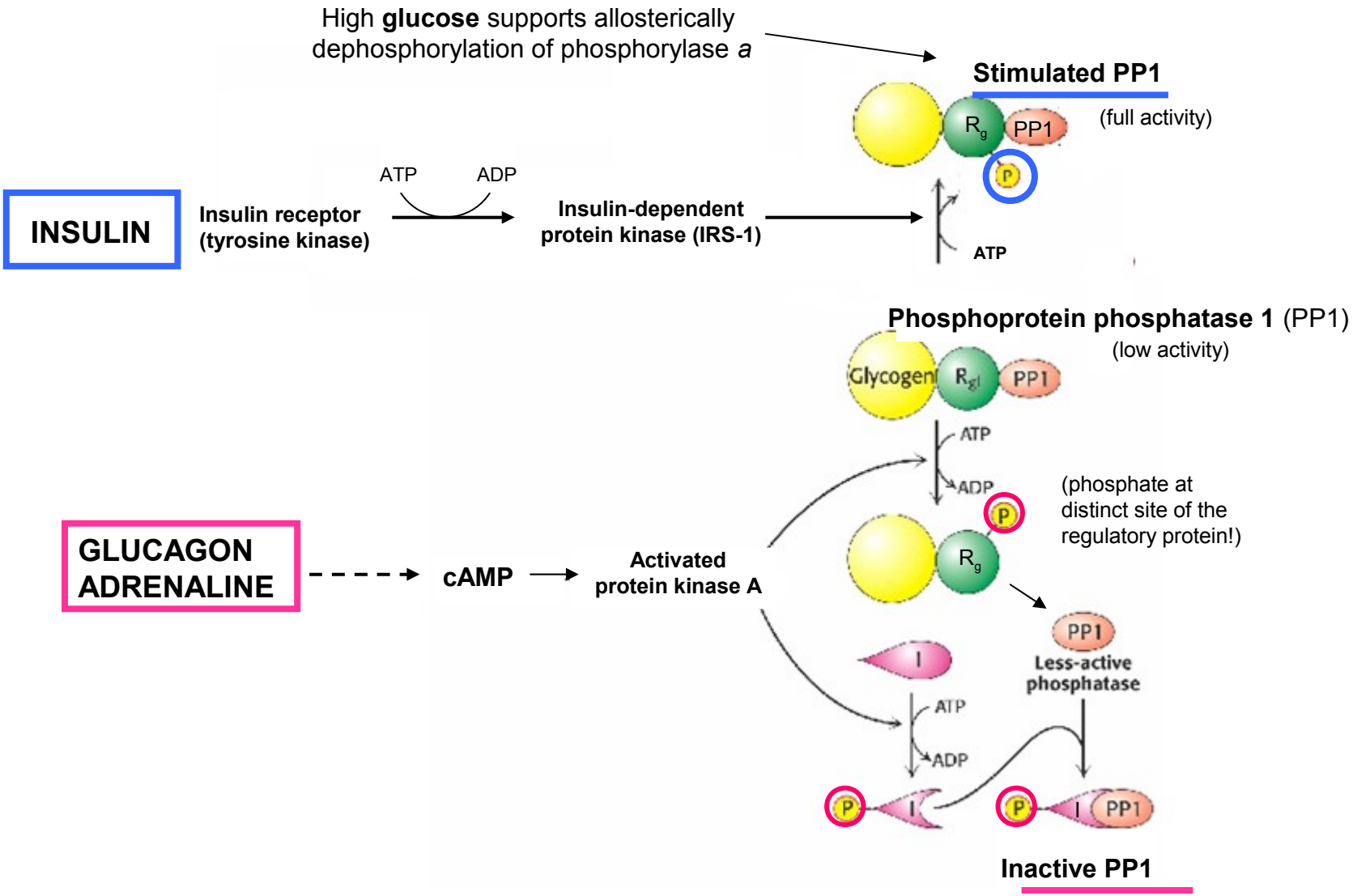
ATP ⟶ **cyclic AMP** (cAMP) + diphosphate

4 cAMP binds onto the regulatory subunits of the **inactive form of protein kinase A** (heterotetramer C_2R_2). The tetramer decomposes to the dimer $R_2(cAMP)_4$ and two catalytically **active** subunits of **protein kinase A.**

The active form of protein kinase A catalyses phosphorylation of phosphorylase kinase, glycogen synthase and two regulatory proteins that inhibit phosphoprotein phosphatase (able to reverse the phosphorylating effect of both kinases).

So glucagon or adrenaline, through the action of cAMP, stop glycogen synthesis and evoke glycogen breakdown.

Regulation of phosphoprotein dephosphorylation:



Glycogen storage diseases (glycogenoses)

are (not very common) inborn errors of metabolism:

Type	Defective enzyme	Organ affected	Glycogen in the affected organ	Clinical features
I Von Gierke disease	Glucose 6-phosphatase or transport system	Liver and kidney	Increased amount; normal structure.	Massive enlargement of the liver. Failure to thrive. Severe hypoglycemia, ketosis, hyperuricemia, hyperlipemia.
II Pompe disease	α-1,4-Glucosidase (lysosomal)	All organs	Massive increase in amount; normal structure.	Cardiorespiratory failure causes death, usually before age 2.
III Cori disease	Amylo-1,6-glucosidase (debranching enzyme)	Muscle and liver	Increased amount; short outer branches.	Like type I, but milder course.
IV Andersen disease	Branching enzyme (α-1,4 ⟶ α-1,6)	Liver and spleen	Normal amount; very long outer branches.	Progressive cirrhosis of the liver. Liver failure causes death, usually before age 2.
V McArdle disease	Phosphorylase	Muscle	Moderately increased amount; normal structure.	Limited ability to perform strenuous exercise because of painful muscle cramps. Otherwise patient is normal and well developed.
VI Hers disease	Phosphorylase	Liver	Increased amount.	Like type I, but milder course.
VII	Phosphofructokinase	Muscle	Increased amount; normal structure.	Like type V.
VIII	Phosphorylase kinase	Liver	Increased amount; normal structure.	Mild liver enlargement. Mild hypoglycemia.

Note: Types I through VII are inherited as autosomal recessives. Type VIII is sex linked.