Glycogen Metabolism

Summary of Carbohydrate Metabolism

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Glycogen

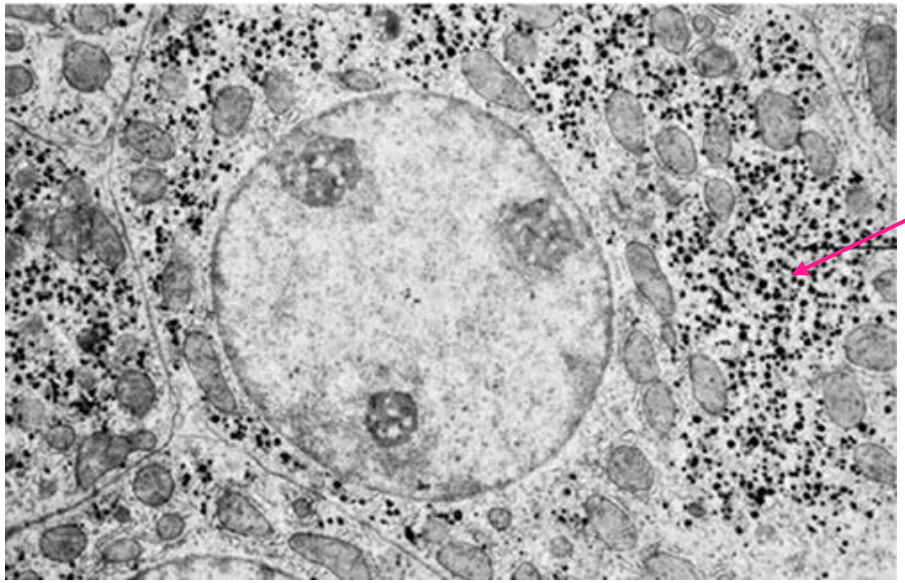
- available, **storage form of glucose** (**[Glc]**↓- degradation, **[Glc]**↑- synthesis)
- large, branched **polysaccharide**

FUNCTIONS:

LIVER (5% = 90g) → **blood glucose conc. maintenance**

MUSCLE (0,7% = 245g) → **source of ATP**

- small amount present in kidneys and intestine

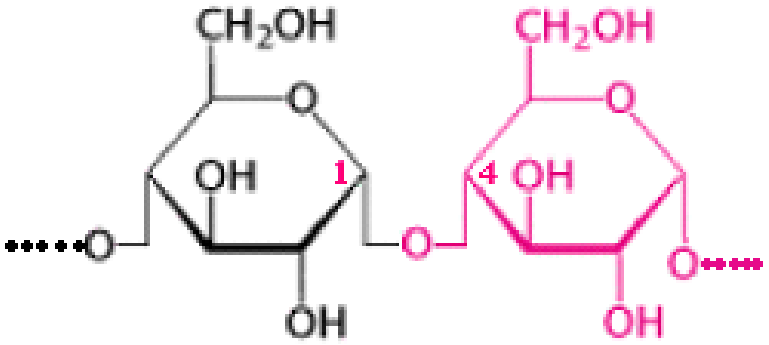


glycogen granules

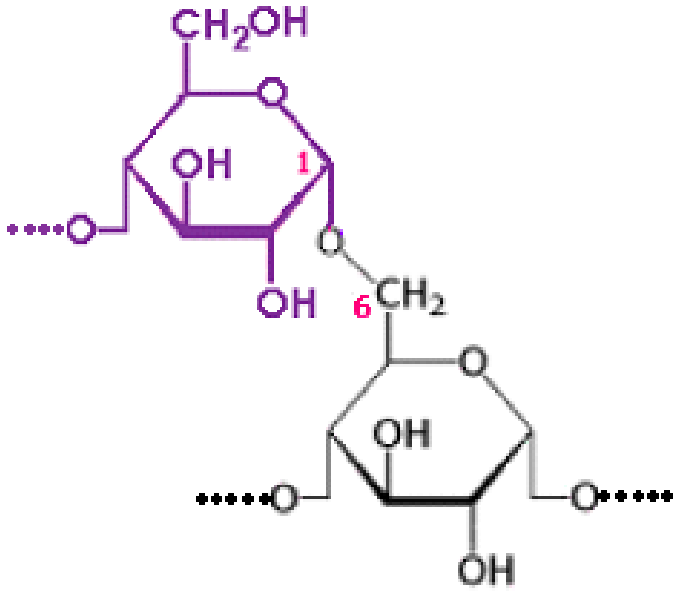
(hepatocytes cytoplasm, EM)

- **enzymes for glycogen biosynthesis and degradation** are permanently and firmly bound in glycogen granules

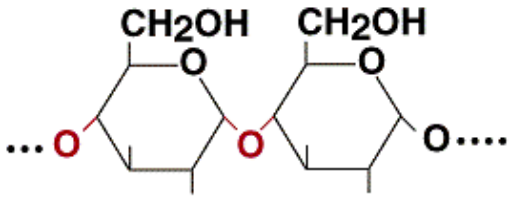
Two basic types of glycosidic bonds in glycogen



α -1,4 - glycosidic bond

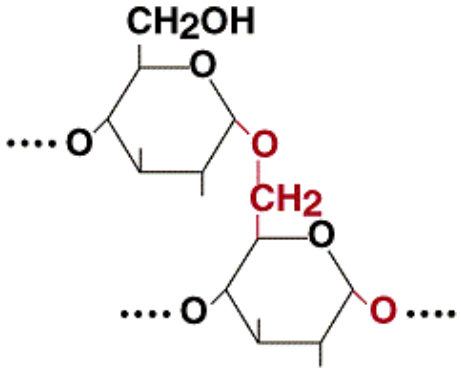


α -1,6 - glycosidic bond



α-1,4-Glycosidic linkage

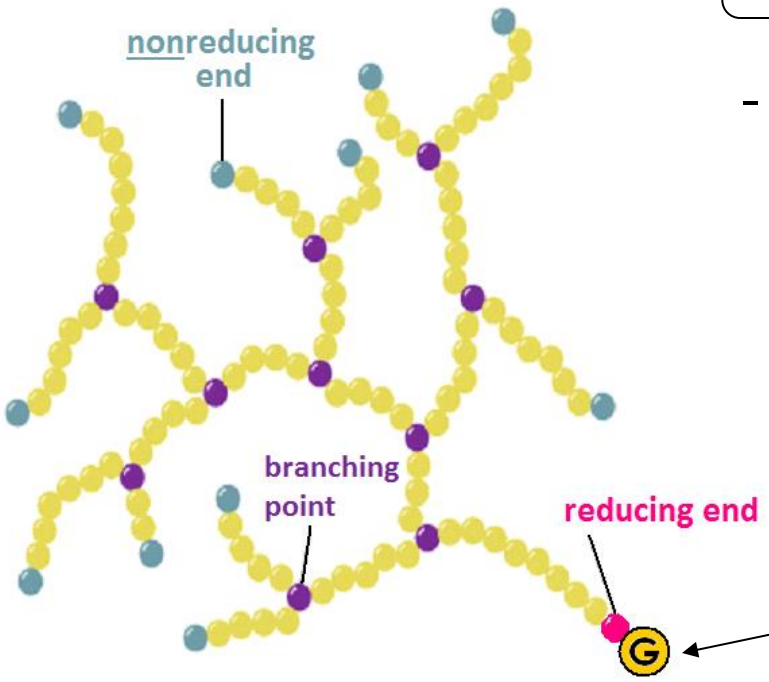
(a)



α-1,6-Glycosidic linkage

(b)

GLYCOGEN STRUCTRE

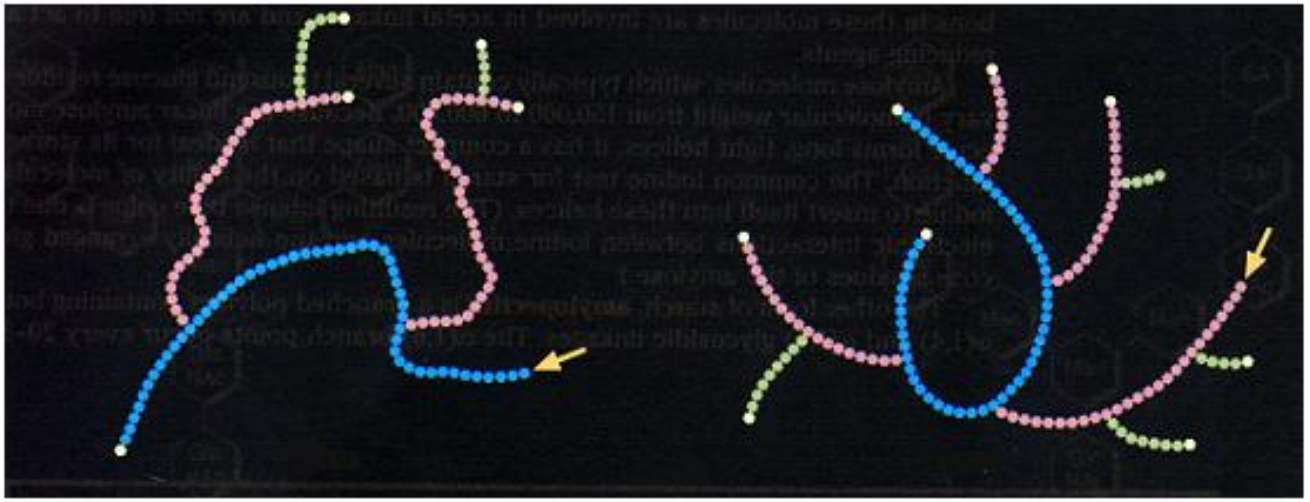


- glucose units linked by **α(1,4)** glycosidic bonds (linear molecule), while **in branching points α(1,6)** bonds (~**10:1**)

- **nonreducing ends** - DEGRADATION!!!

- ONLY **1 reducing end**, but permanently bound to **GLYCOGENIN** - self-glucosylating enzyme

Glycogen structure



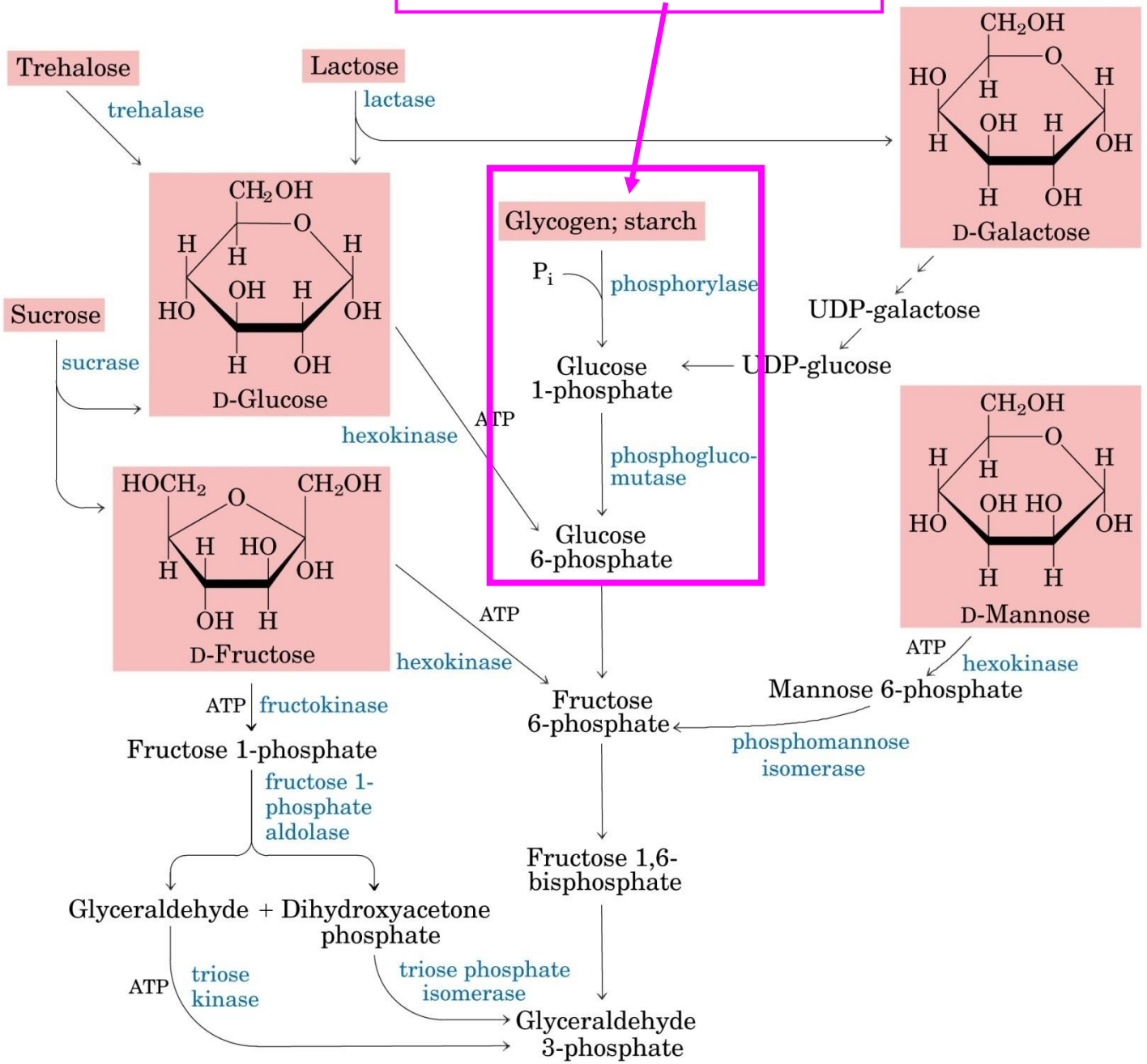
AMYLOPECTIN GLYCOGEN

- GLYCOGEN - **more branched structure** than amylopectin
  - more soluble and more easy to degrade (**non**reducing ends!!!)

- **<u>starch</u>** - **amylose** - linear molecule, **α(1,4)**-glycosidic bonds
  amylopectin - **α(1,4)** i **α(1,6)**- glycosidic bonds
- **<u>cellulose</u>** - **β(1,4)**- glycosidic bonds

humans lack ***β-glucosidase*** for cellulose degradation

GLYCOGENOLYSIS



Biosynthesis and Degradation of Glycogen - GLYCOGENESIS AND GLYCOGENOLYSIS

- different reaction pathways
- hormonal regulation
- regulate glucose blood **concentration** (liver)
- provide glucose **reserve** for muscle work

Glycogenolysis

- **3 enzymes** involved:

1. ***glycogen-phosphorylase**** hydrolyses **α(1,4)** bonds forming **glucose-1-phosphate**

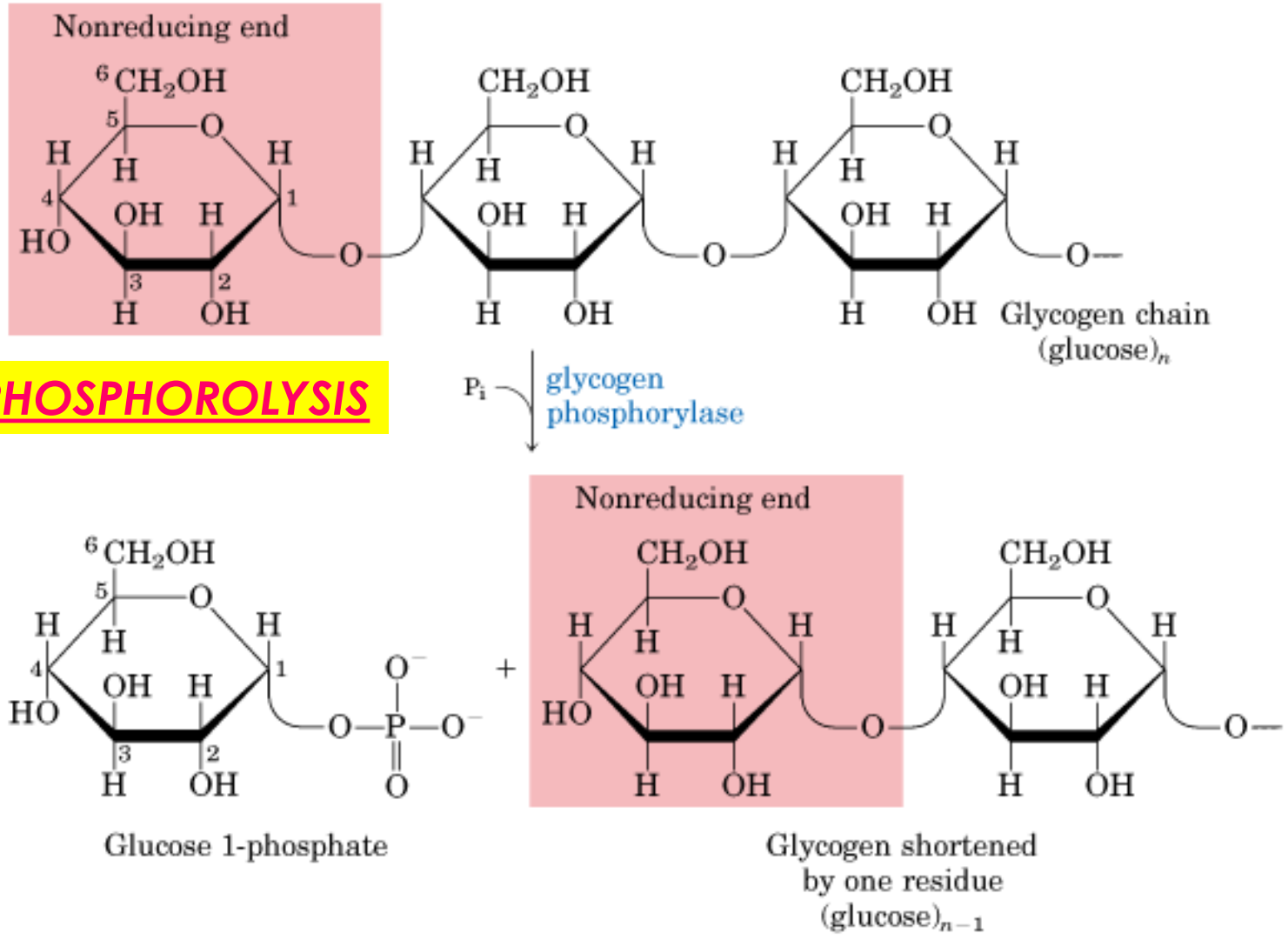
$(glucose)_n + P_i \rightarrow (glucose)_{n-1} + glucose\text{-}1\text{-}P$ ***PHOSPHOROLYSIS***

co-enzyme is **pyridoxal phosphate (PLP) – pyridoxine (vitamine B6) derivative*

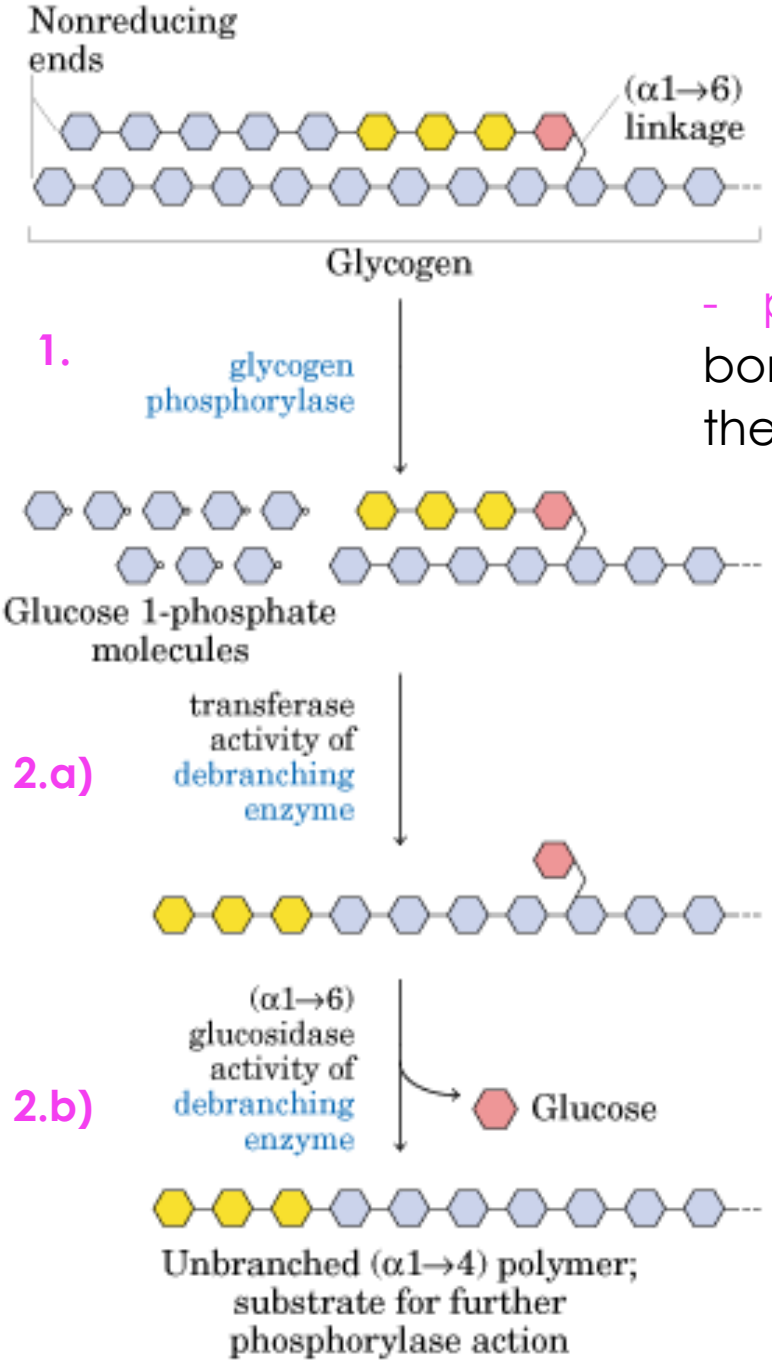
2. ***debranching enzyme***
   - *2 activities:* ***a) transferase*** - transfer of 3 glucose residues
     b) glucosidase - hydrolysis of **α(1,6)**- glycosidic bond

3. ***phosphoglucomutase*** transfers **glucose-1-phosphate** into **glucose-6-phosphate**

Glycogenolysis



Glycogenolysis



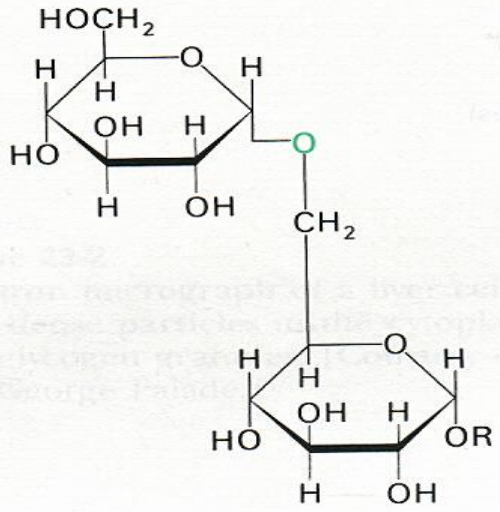
- phosphorolytic breaking of **α-(1,4)**-glycosidic bond, except for **4 glucose residues** away from the branching site

  - formation of **glucose 1-phosphate**

- transfere of **3 glucose residues** from one branch and formation of **α-(1,4)**-glycosidic bond on the other branch

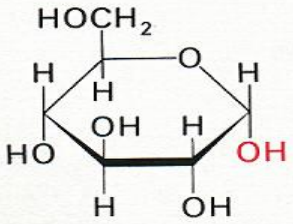
- hydrolysis of **α-(1,6)**-glycosidic bond with **glucose** formation

- products:
  glucose 1-phosphate and **glucose**
  in ratio **10 : 1**

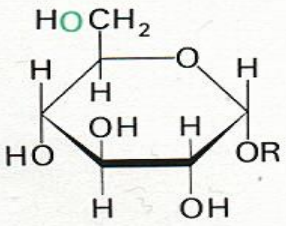


Glycogen
(*n* residues)

2.b) HYDROLYSIS



Glucose

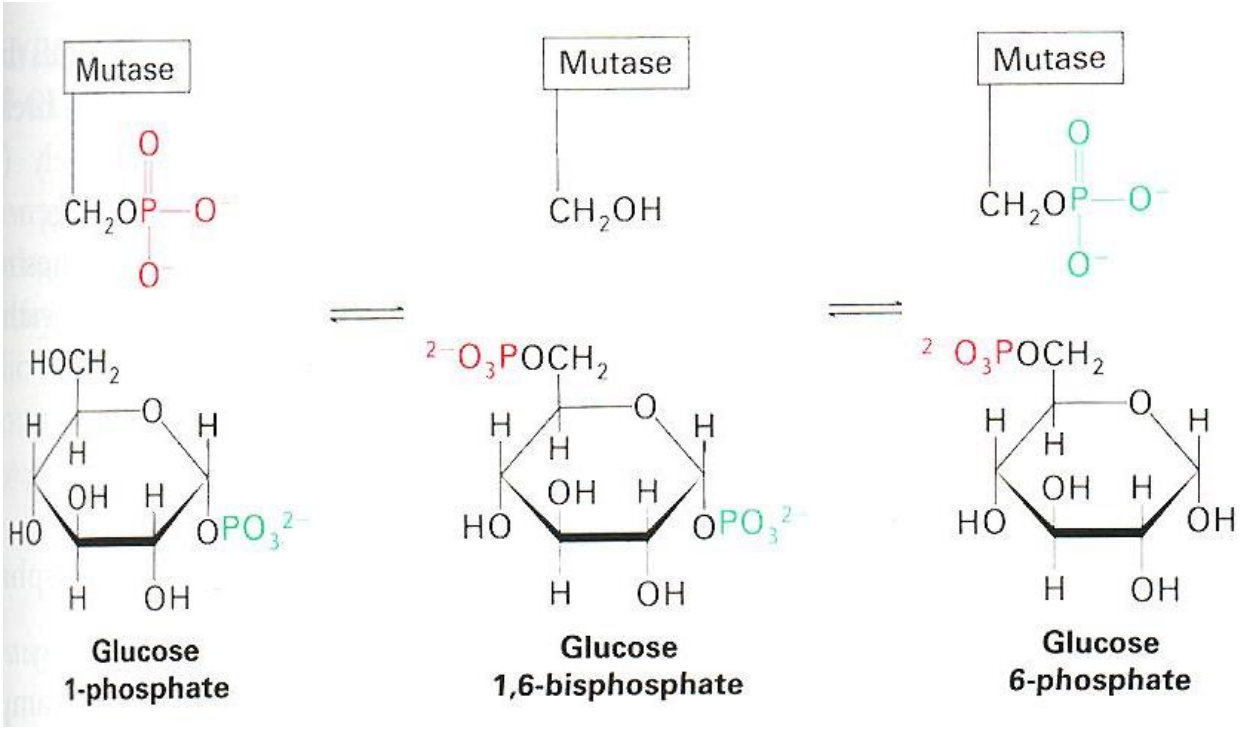


Glycogen
(*n* − 1 residues)

Glycogenolysis

3. *phosphoglucomutase*

- isomerisation of **glucose 1-P** into **glucose 6-P**

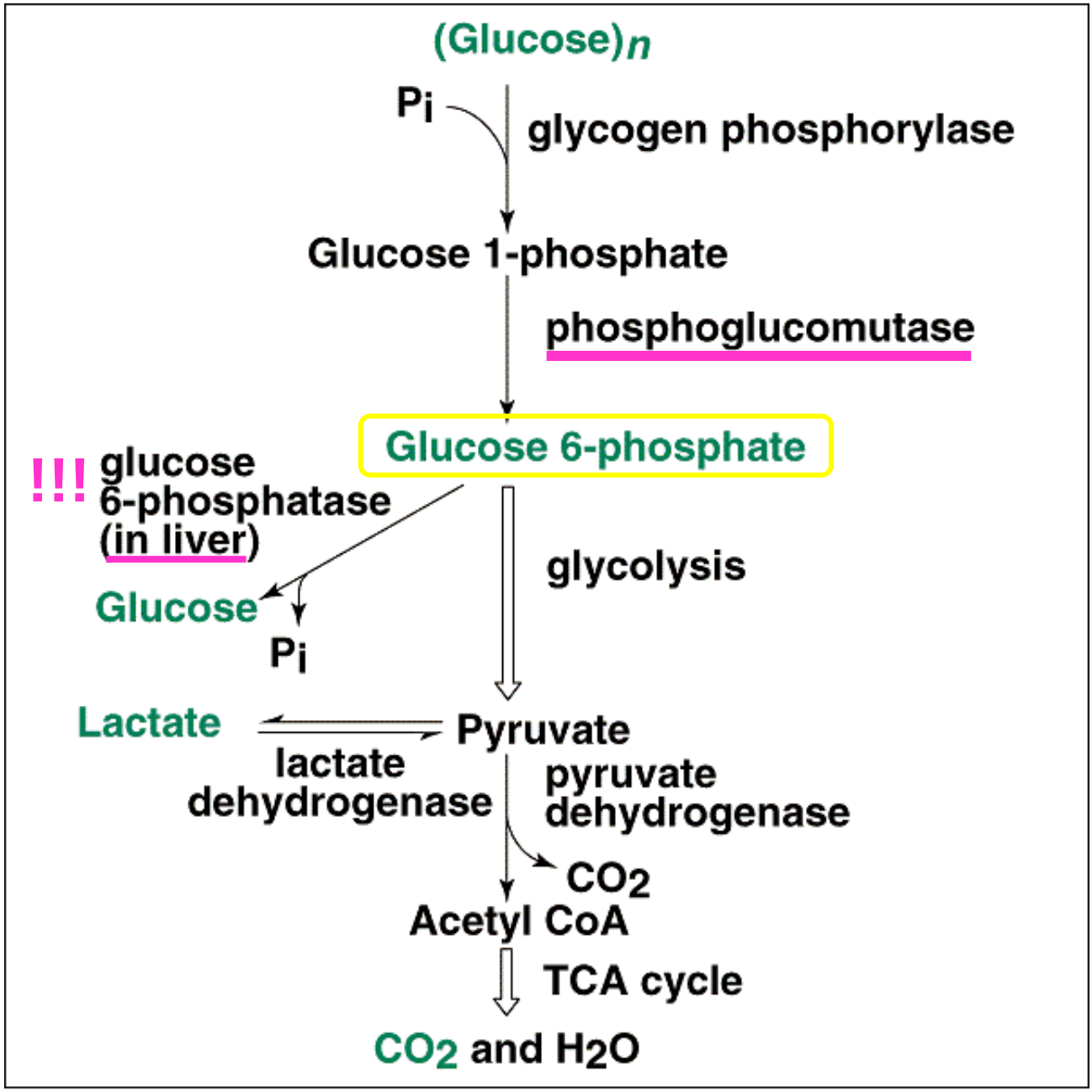


- ***phosphoglucomutase*** ***(phosphoenzyme!)*** catalyses the reaction in the direction of **glucose 6-P** formation, since the **glucose 1-P** concentration in the cell is much higher than of **glucose 6-P**

Glucose 6-phosphate
- different roles:

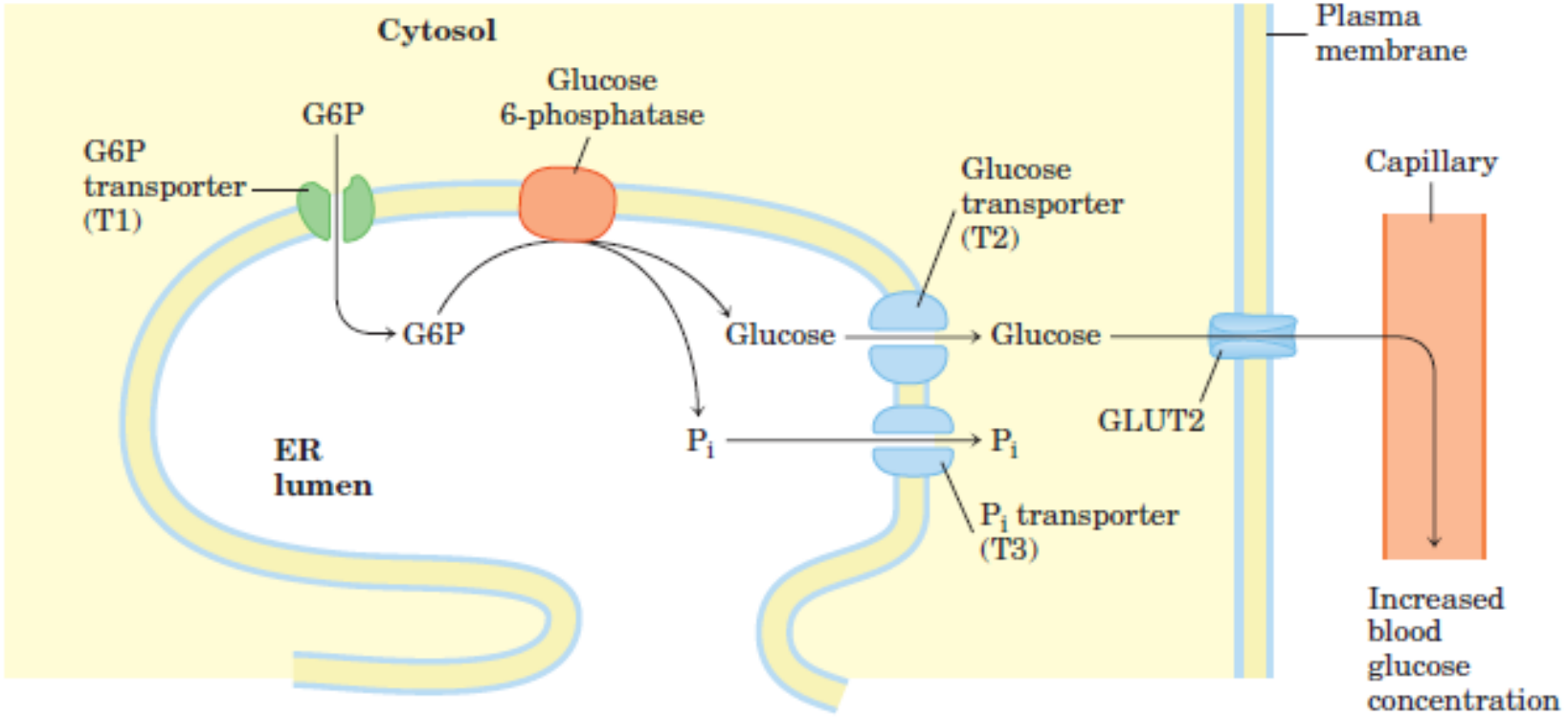
1. ***Muscle, brain***- **fuel** for aerobic and anaerobic metabolism (***pyruvate, lactate***)

2. ***Liver***, *kidneys* - tranformation of **G 6-P** into **glucose** for other tissues by **glucose 6-phosphatase** (other tissues do not have glucose 6-phosphatase!)



Glucose 6-phosphatase hydrolysis glucose 6-phosphate

- **liver, kidneys** - elevation of glucose blood concentration
- occurs in the lumen of **endoplasmatic reticulum (ER)** - separated from cytosol (glycolysis!)



- genetic defects in either **glucose 6-phosphatase** or **T1 transporter** lead to serious derangement of glycogen metabolism, resulting in **type Ia glycogen storage disease**

Glycogenolysis - Summary

- **phosphorolytic cleavage** of glycogen from the **non-reducing end**
- released glucose is **phosphorylated** and thus ready to join the metabolism without ATP cost
- glucose 1-phosphate **cannot** difuse out of the cell
- tissues which primarily use Glu as the energy source, **do not contain *glucose 6-phosphatase***, but use G-6-P as fuel for glycolysis

Glycogen Synthesis - Glycogenesis

- takes place in virtually all animal tissues, but especially prominent in the **liver** and **skeletal muscles**

- 3 enzymes:

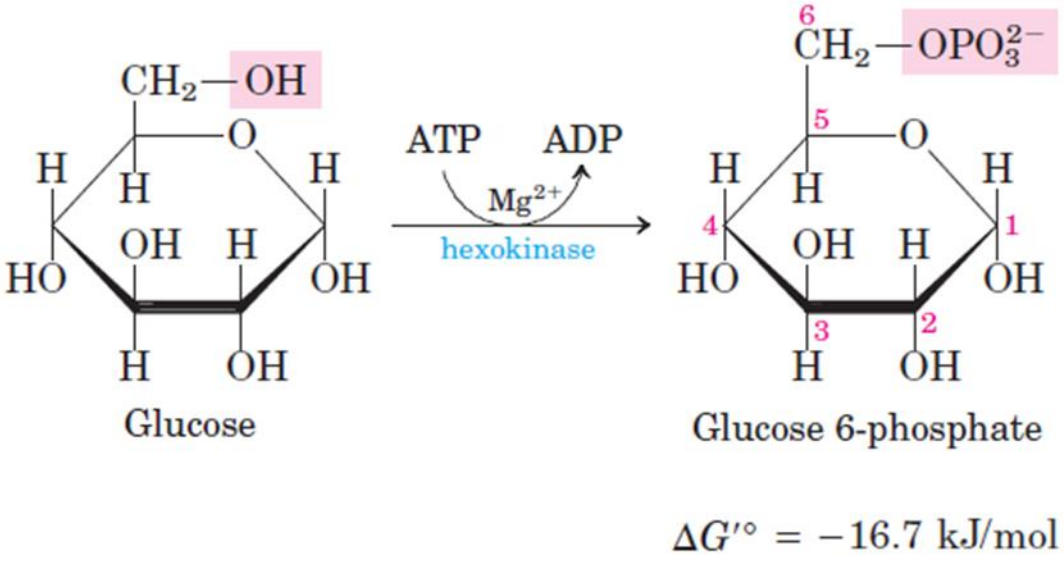
1. **GLYCOGENIN** – self-glucosylating enzyme
   - synthesis of „**primer"** - first 8 glucose molecules

2. **glycogen-synthase**
   - further extension the „primer" by adding Glc molecules
   - formation of **α(1→4)** glycosidic bonds
   - substrate for the syntesis is **UDP-glucose**

3. **branching enzyme** *(glycosyl(4→6)-transferase)*
   - formation of α(1→6) glycosidic bonds

Glycogen Synthesis

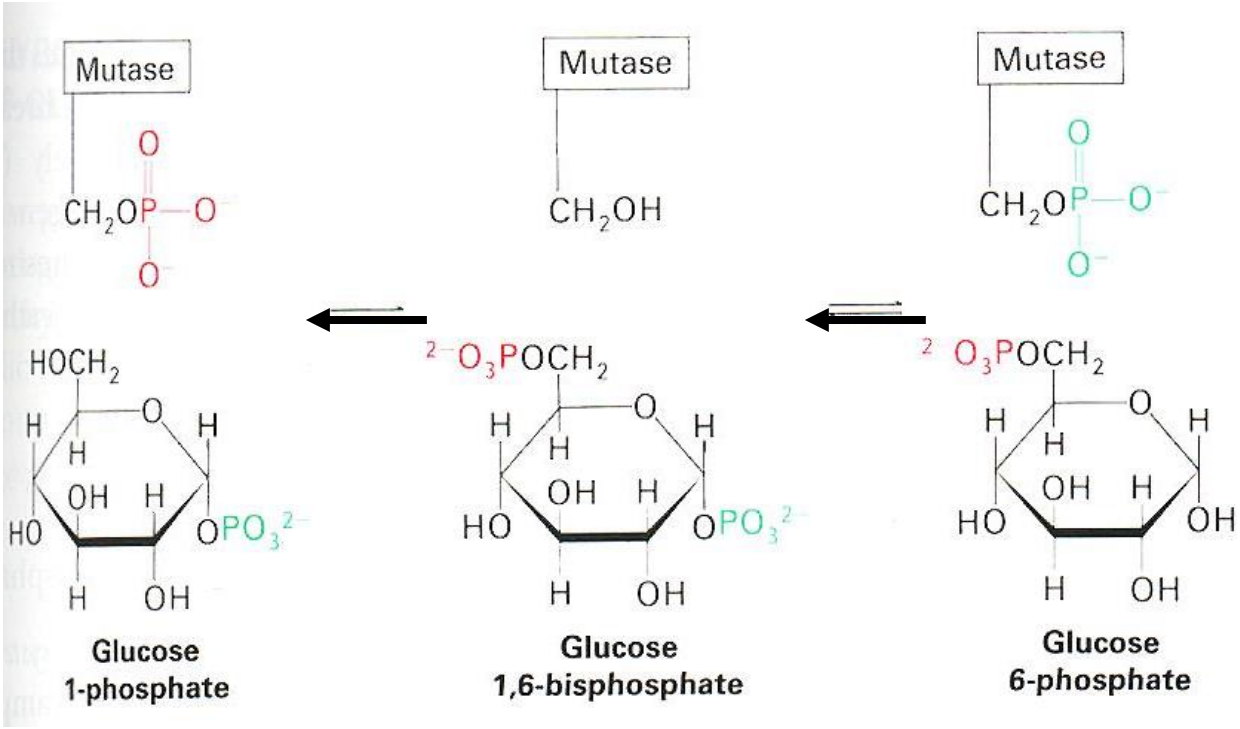
- after entering the cell, **glucose** is phosphorylated by the activity of ***hexokinase**** forming **glucose 6-phosphate**



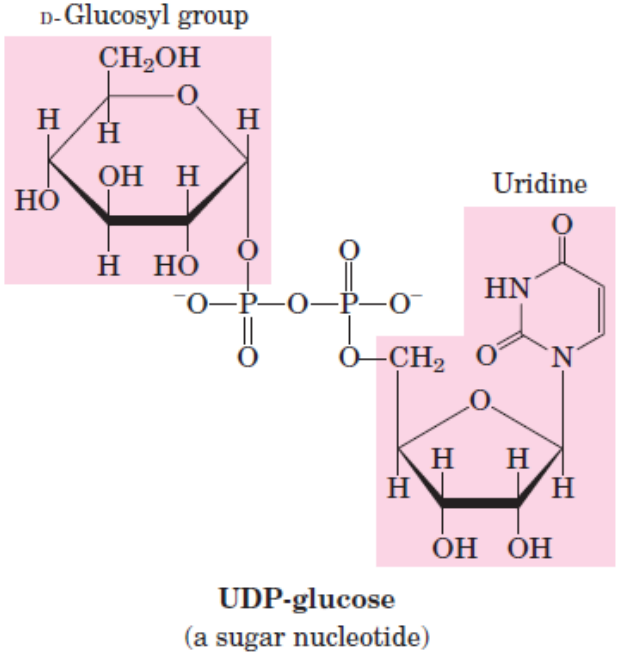
* ***hexokinase I*** *and* ***II*** - muscle; ***hexokinase IV (glucokinase)*** - liver

Glycogen Synthesis

- **glucose 6-phosphate** isomerisation (reversible reaction) into **glucose 1-P** by the activity of ***phosphoglucomutase***

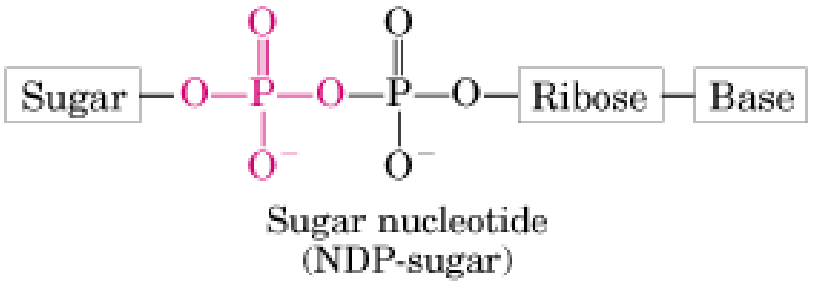


- when higher amount of **glucose 6-phosphate** is present in the cell, the equilibrium of the reactions is shifted to the left, towards the formation of **glucose 1-phosphate**



→ **UDP-glucose is activated form of glucose** - anomeric carbon of a sugar is activated by attachment to a nucleotide through a phosphate ester linkage

- **sugar nucleotides** are the **substrates** for polymerization of monosaccharides into disaccharides, glycogen, starch, cellulose, and more complex extracellular polysaccharides



Sucrose: *UDP-Glc i Fru-6-P*

Lactose: *UDP-Glc i UDP-Gal*

Glucuronides: *UDP-Glc → UDP-GA (UDP-glucuronate)*

The mechanism of NDP-sugar formation

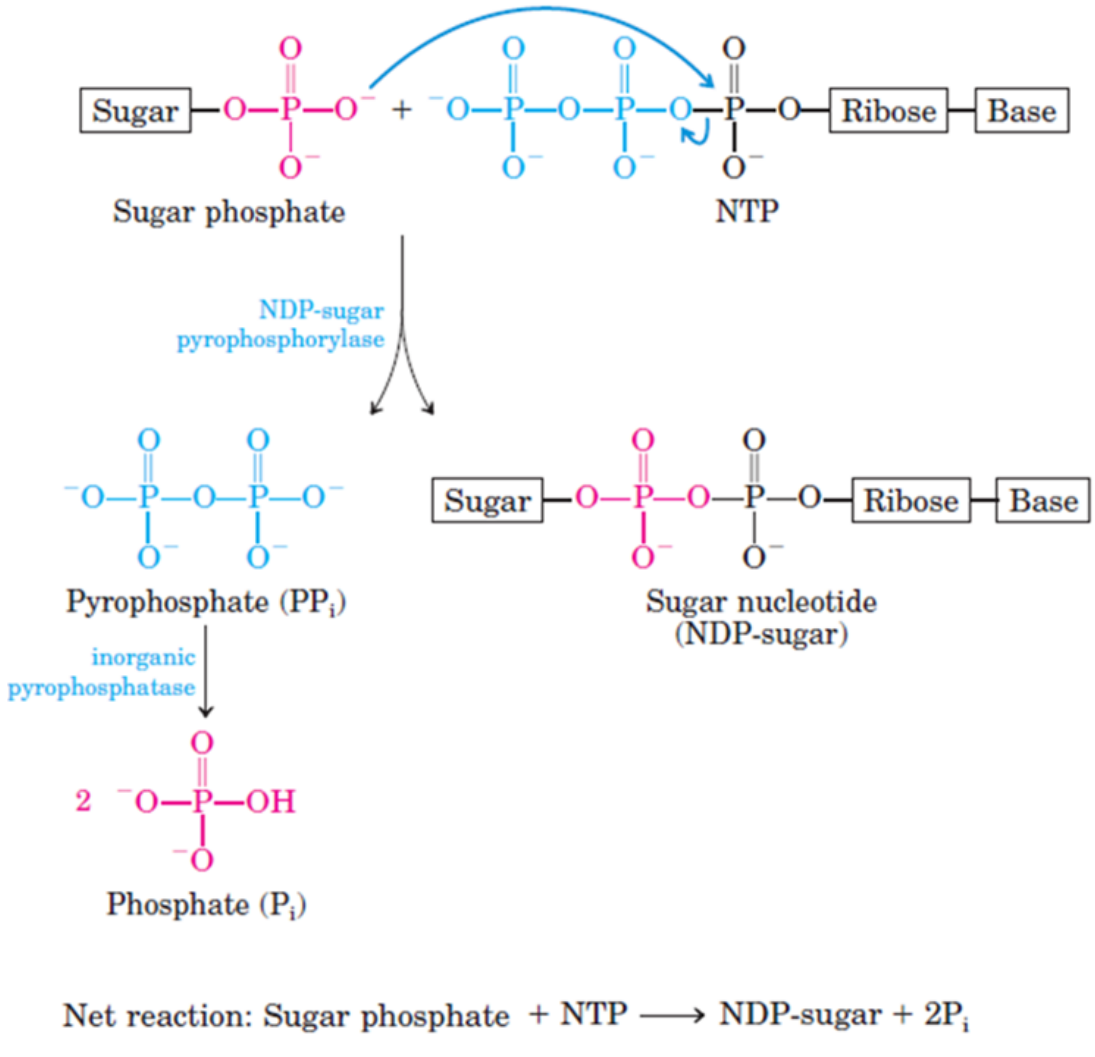
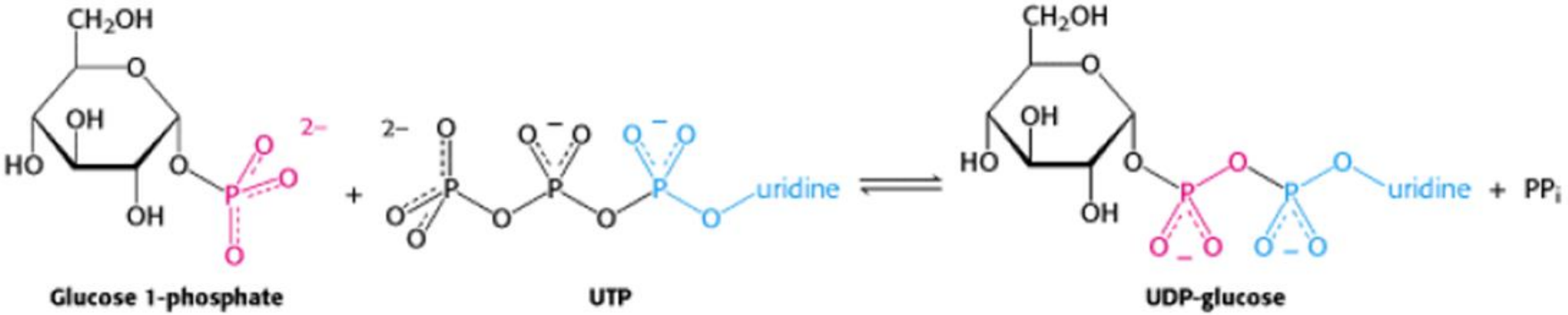


FIGURE 15–7 Formation of a sugar nucleotide. A condensation reaction occurs between a nucleoside triphosphate (NTP) and a sugar phosphate. The negatively charged oxygen on the sugar phosphate serves as a nucleophile, attacking the α phosphate of the nucleoside triphosphate and displacing pyrophosphate. The reaction is pulled in the forward direction by the hydrolysis of PP_i by inorganic pyrophosphatase.

Glycogen Synthesis

UDP glucose formation

- **UDP-glucose** (uridine diphosphate-glucose) formed by the activity of ***UDP-glucose pyrophosphorylase***



- the synthesis of UDP-glucose is driven by the essentially irreversible **hydrolysis of pyrophosphate** catalyzed by **pyrophosphatase**

$$\text{Glucose 1-phosphate} + \text{UTP} \rightleftharpoons \text{UDP-glucose} + \text{PP}_i$$

$$\text{PP}_i + \text{H}_2\text{O} \longrightarrow 2\,\text{P}_i$$

$$\text{Glucose 1-phosphate} + \text{UTP} + \text{H}_2\text{O} \longrightarrow \text{UDP-glucose} + 2\,\text{P}_i$$

$\Delta G^{\circ\prime}$ = ~ -19 kJmol^{-1}

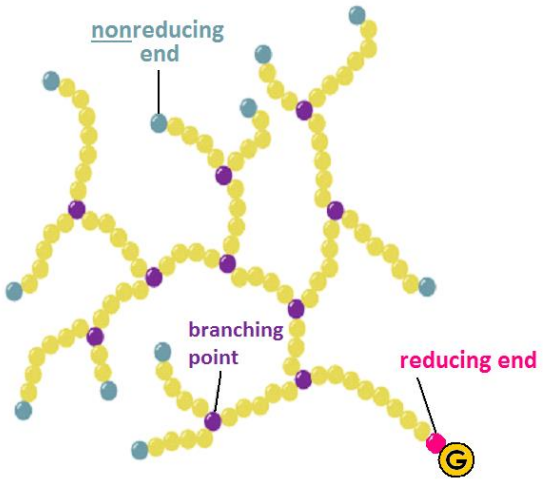
The synthesis of UDP-glucose exemplifies another recurring theme in biochemistry: ***many biosynthetic reactions are driven by the hydrolysis of pyrophosphate***.

Glycogen Synthesis

- ***glycogen synthase*** **cannot** **synthetise glycogen *de novo***
- **GLYCOGENIN** - self-glucosylating enzyme - **starts glycogen synthesis**

  - functions: **bonding** of **1st molecule UDP-glucose** (with UDP release), and **oligomerisation** of following **7 molecules od glucose**

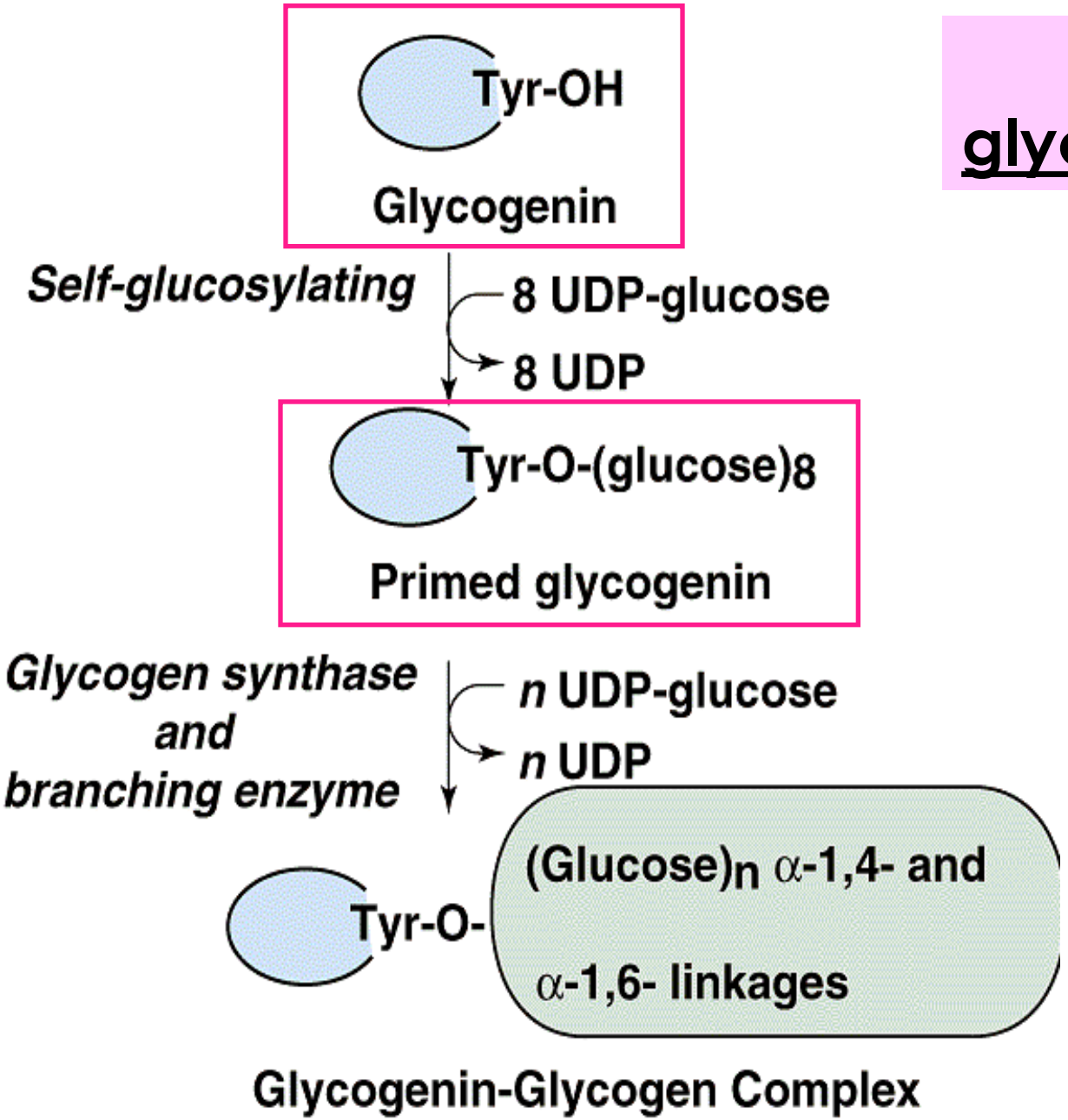
"*PRIMER*" (starter)
for ***glycogen synthase*** activity



- ***glycogen synthase*** can act (by adding Glu units) **only** upon existing oligosaccharride chain containing **at least 8 glucose residues**

! **reducing end of glycogen is permanently bond to glycogenin**

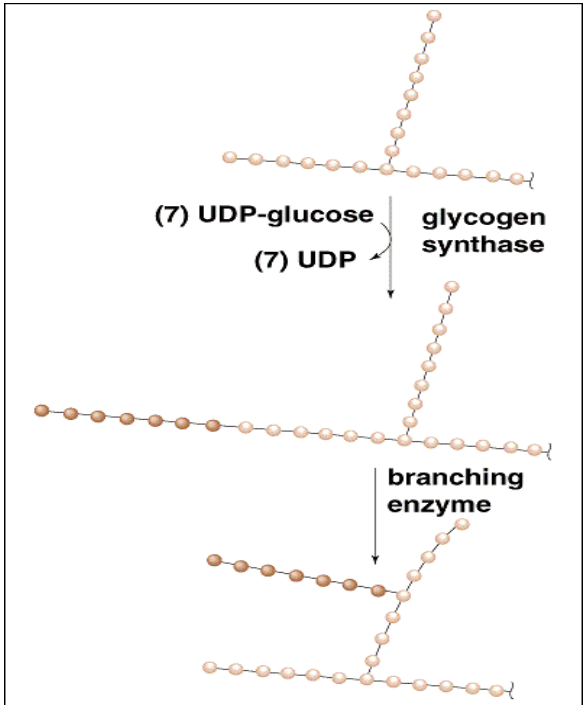
De novo glycogen synthesis

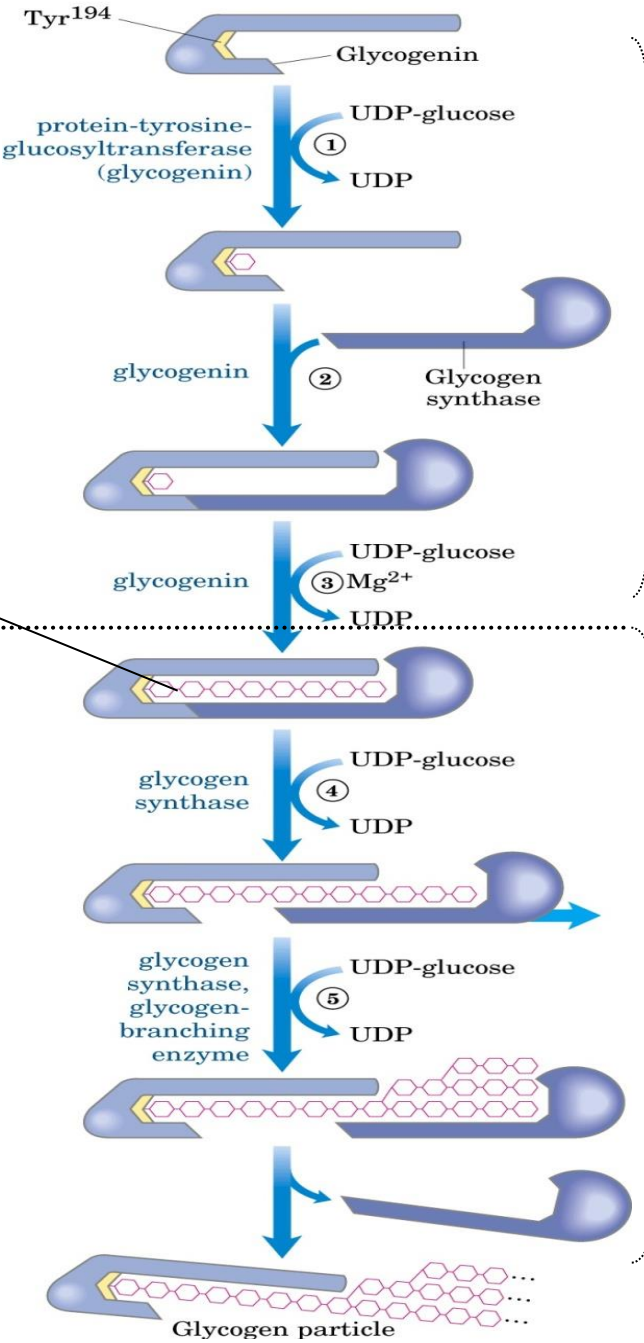


Glycogenin

- self-glucosylating enzyme!

Glycogen Synthesis





"PRIMER"

glycogenin

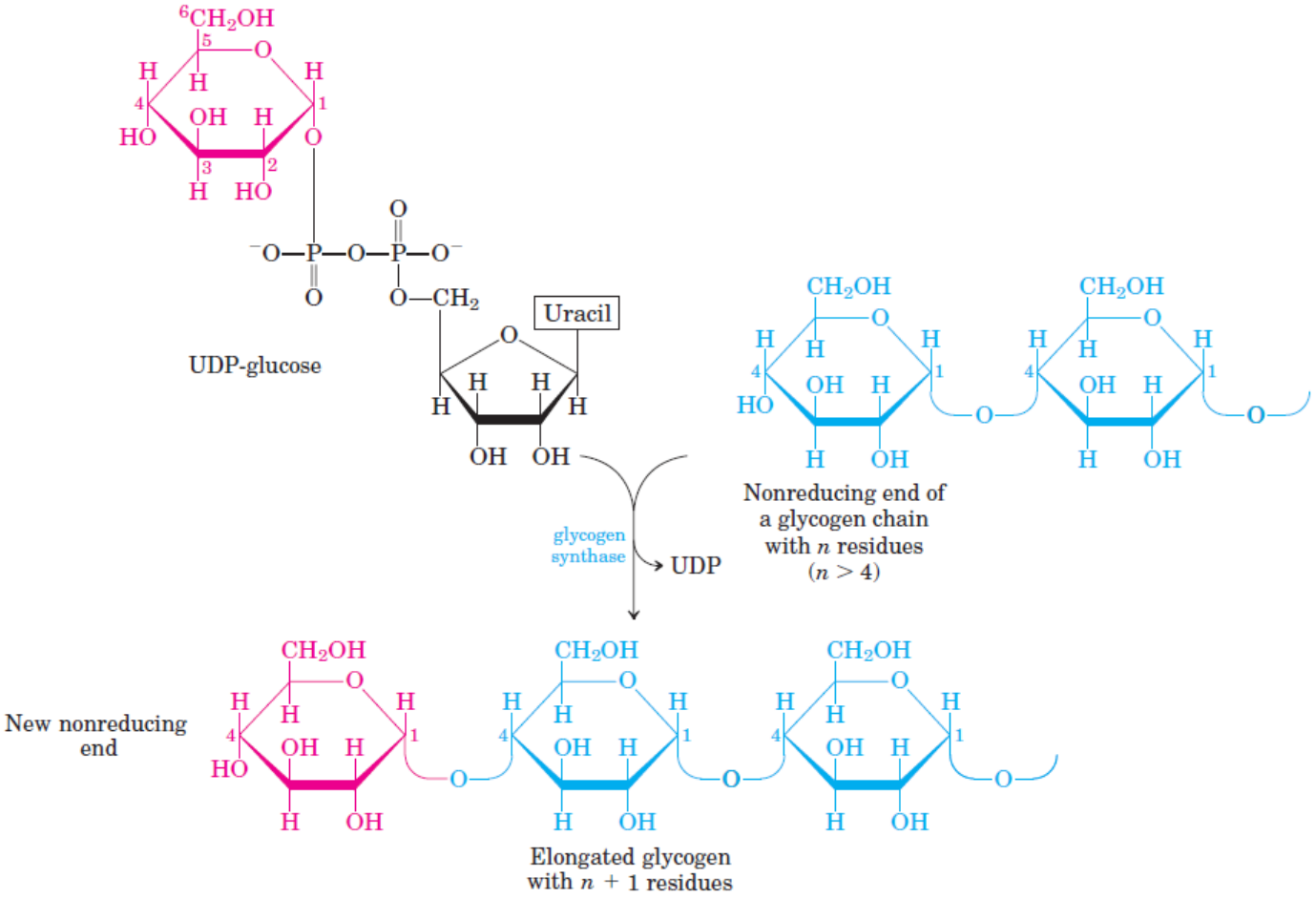
glycogen synthase (α1,4)

branching enzyme (α1,6) or

glucano-1,6 transferase

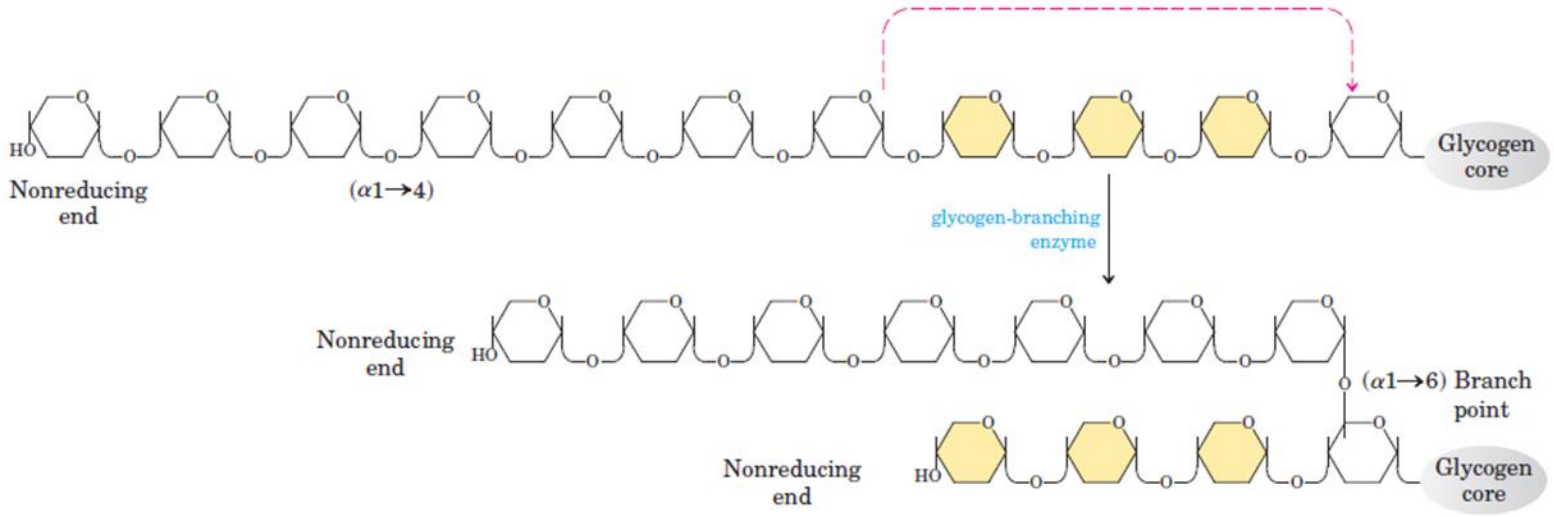
Glycogen Synthesis

- **glycogen synthase** transfers the glucose residue of **UDP-glucose** to the glycogen **nonreducing end** to make a new **(α1,4) linkage**



GLYCOGEN BRANCHING - formation of α(1→ 6) bond

- **branching enzyme** *(glycosyl-(4→6) transferase)*
  - ***transfer*** of an oligosaccharide chain and ***formation*** of a new **α(1→6) glycosidic bond**, forming a new branch point



- some athletes consume large amounts of carbohydrates after training *(carbohydrate loading)*→ rapid glycogen synthesis and faster recovery

- „*The consumption of high-glycemic carbohydrates soon after exercise can maximize and sustain the rate of glycogen synthesis to help speed glycogen restoration.*" (Murray et al, Nutr Rew, 2018)

What is the "price" of glucose storage?

glucose + ATP —(hexokinase)→ glucose 6-P + ADP

glucose 6-P —(phosphoglucomutase)→ glucose 1-P

glucose 1-P + UTP —(UDP-Glu pyrophosphorylase)→ UDP-glucose + PP_i

PP_i + H_2O —(pyrophosphatase)→ 2 P_i

UDP-glucose + glycogen $_n$ —(glycogen synthase)→ glycogen $_{n+1}$ + UDP

UDP + ATP —(nucleoside diphosphokinase)→ UTP + ADP

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glucose + 2ATP + glycogen $_n$ + H_2O ⟶ glycogen $_{n+1}$ + 2 ADP + 2 P_i

- if the starting substrate is **Glu 6-P**, **1 ATP** is spent to store **1 Glu molecule** (for UTP regeneration)

- if the starting substrate is **glucose**, **2 ATPs** are needed (for Glu phosphorylation and UTP regeneration)

REGULATION OF GLYCOGEN METABOLISM:

GLYCOGEN-PHOSPHORYLASE

GLYCOGEN-SYNTHASE

* ALLOSTERIC EFFECTORS
* COVALENT MODIFICATION

Regulation of Glycogen Metabolism

- synthesis and breakdown of glycogen - **different pathways**
- ***glycogen synthase*** and ***glycogen phosphorylase*** are **regulated by:**

1. **covalent modification** (enzyme interconversion) *(seconds, min)*

- **hormonal regulation** - **the same enzymes** are regulated by cells respond to **hormone activity:**

epinephrine - muscle , liver
glucagon - liver
→ induce glycogen **breakdown**

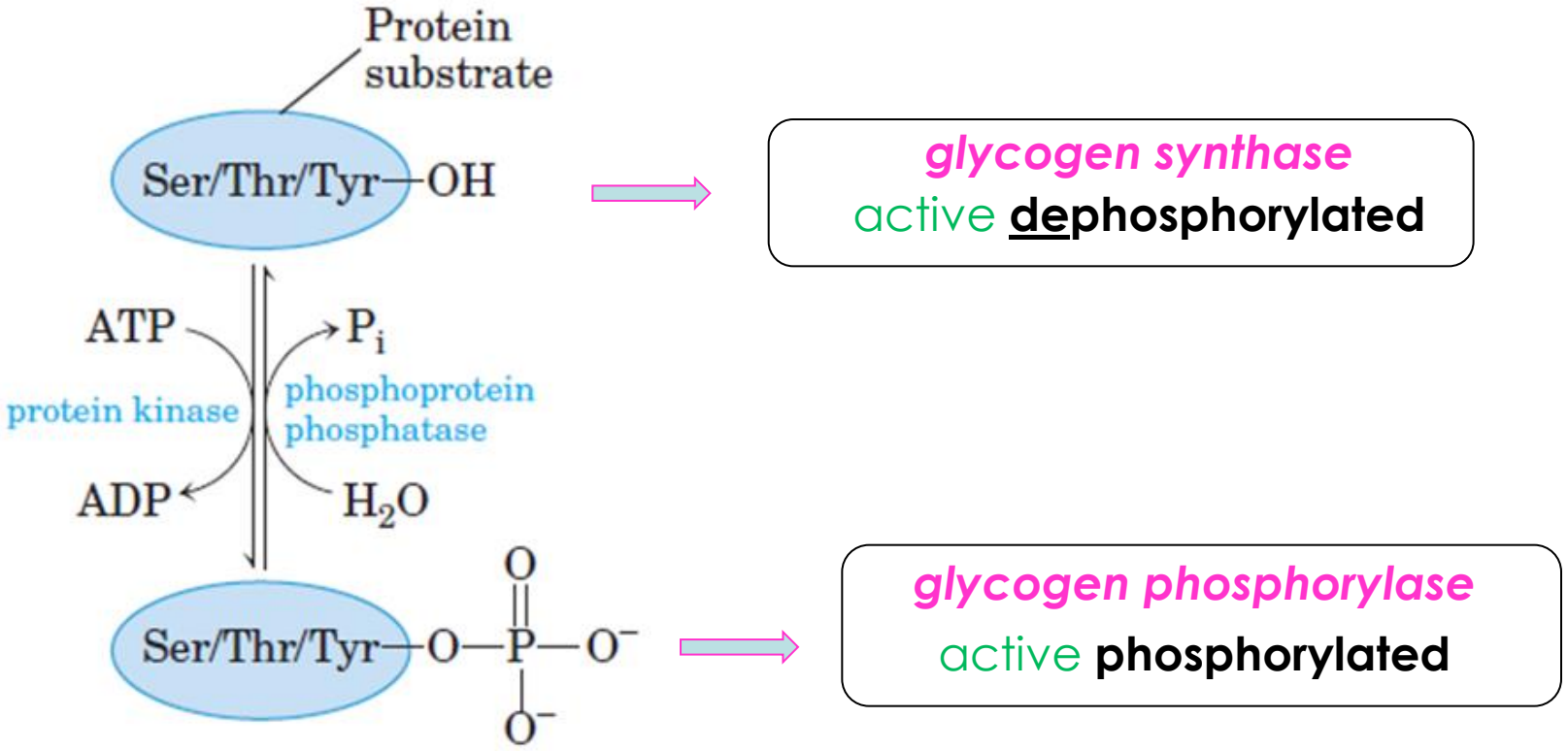
insuline → induce glycogen **synthesis**

2. **allosteric control** *(miliseconds)*

- **ATP**, **AMP**, **Glu 6-P** and **Glu** are allosteric effectors of *glycogen synthase* and *glycogen phosphorylase*

Covalent modification

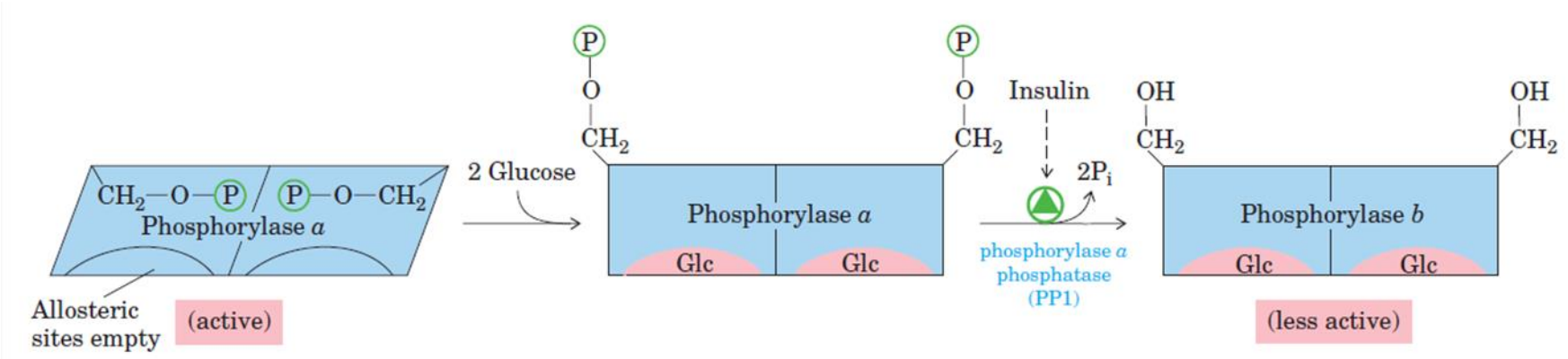
- **enzyme interconversion**
- one of the most often type is **phosphorylation/dephosphorylation**



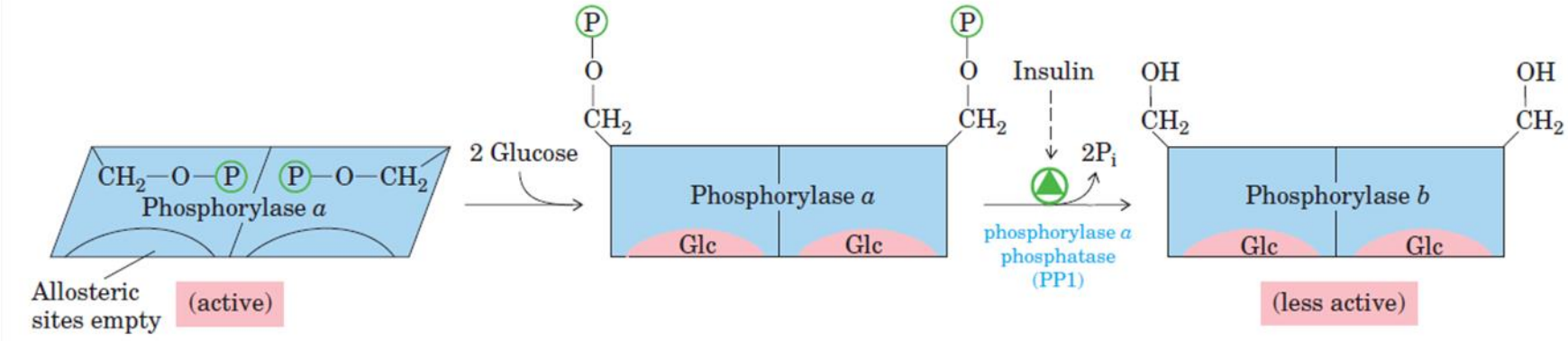
Glycogen phosphorylase

- major regulatory enzyme of glycogen breakdown
- active in phosphorylated form – **phosphorylase a**

phosphorylase *a* - **active** = phosphorylation - **glycogen breakdown**
phosphorylase *b* - **inactive/less active** = **breakdown does not occur**



Glycogen phosphorylase (liver) - *allosteric control*



- glycogen phosphorylase in the liver acts as a glucose sensor

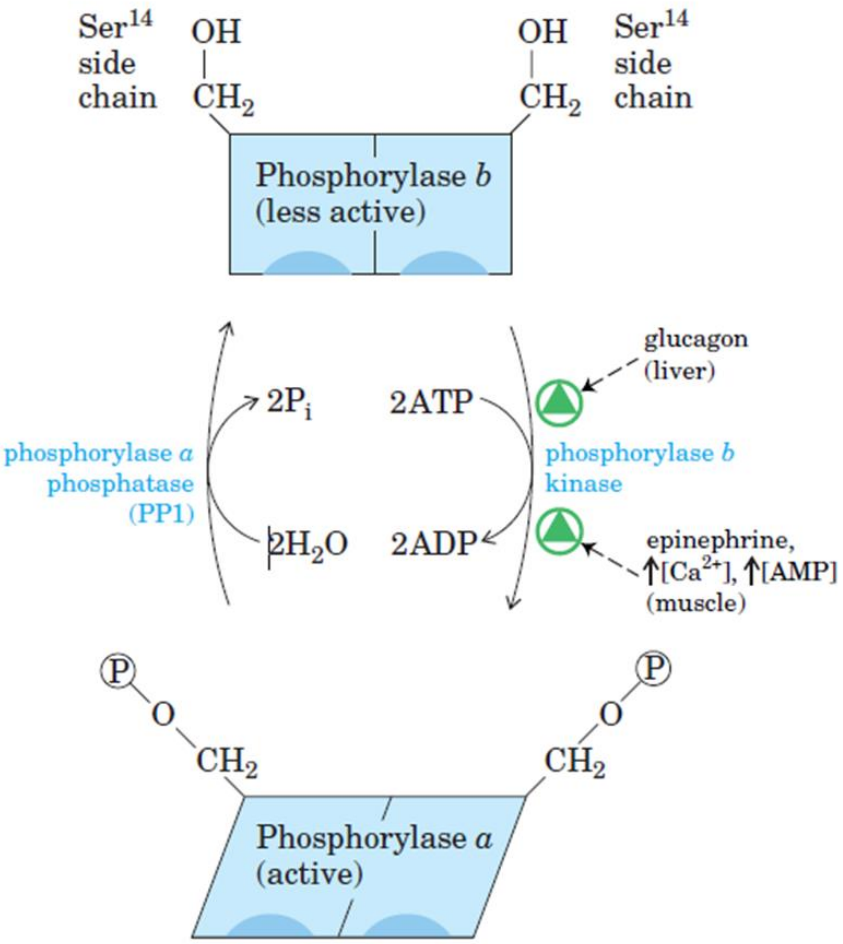
- when blood glucose concentration is high, **glucose** binds to an ***inhibitory*** allosteric site of the **phosphorylase *a*** isozyme
- glucose binding induces a conformational change that exposes its phosphorylated residues to **phosphorylase *a* phosphatase 1 (PP1)**
- phosphatase converts **phosphorylase *a*** to **phosphorylase *b***, sharply reducing the activity of phosphorylase and **slowing glycogen breakdown**
- insulin also acts indirectly to stimulate PP1 and slow glycogen breakdown

Glycogen synthase and *glycogen phosphorylase* allosteric regulation

Effector	*Glycogen synthase*	*Glycogen phosphorylase*
ATP	activator	_
AMP	_	activator
G 6-P	activator	inhibitor
Glucose	_	inhibitor

Glycogen phosphorylase covalent modification

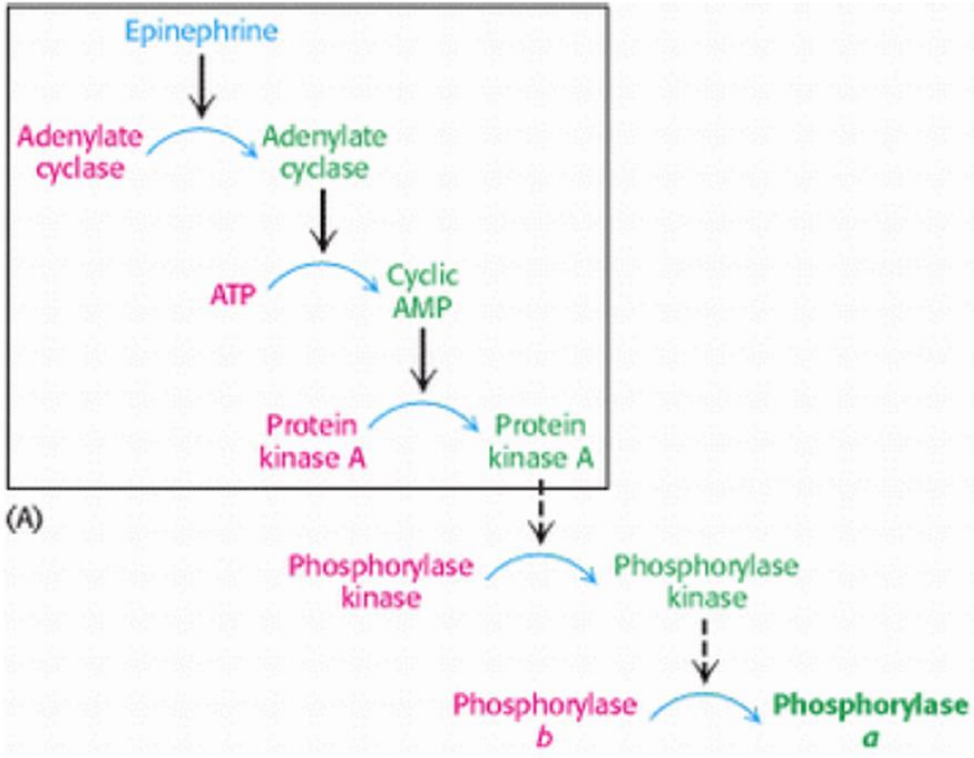
rest

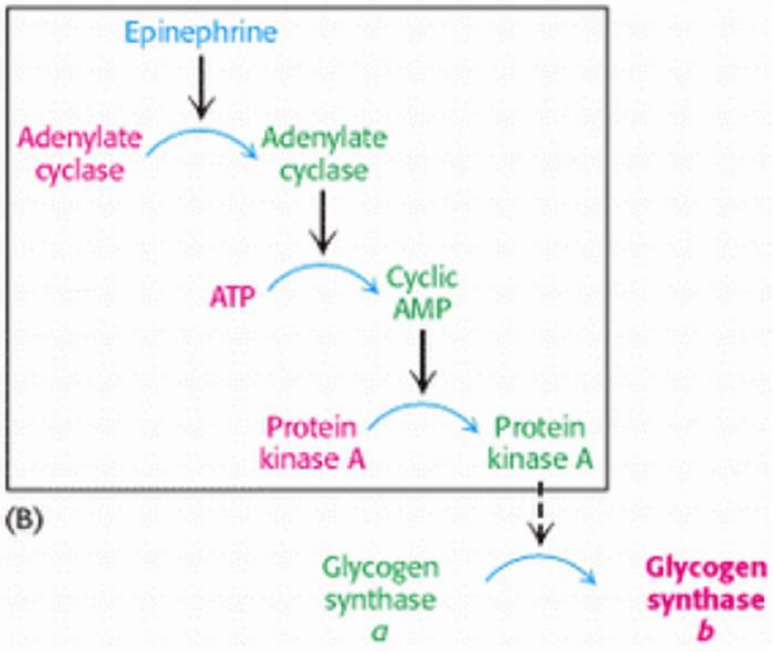


muscle activity

FIGURE 15–24 Regulation of muscle glycogen phosphorylase by covalent modification. In the more active form of the enzyme, phosphorylase *a*, Ser^{14} residues, one on each subunit, are phosphorylated. Phosphorylase *a* is converted to the less active form, phosphorylase *b*, by enzymatic loss of these phosphoryl groups, catalyzed by phosphorylase *a* phosphatase (PP1). Phosphorylase *b* can be reconverted (reactivated) to phosphorylase *a* by the action of phosphorylase *b* kinase.

* muscle and liver phosphorylase are **isoenzymes**, coded by different genes and differently regulated





(A) glycogen degradation

(B) glycogen synthesis

- the sequence of reactions leading to the activation of **protein kinase A** is the same in the regulation of glycogen degradation and synthesis:
  - **phosphorylase kinase** activates **glycogen phosphorylase** and inactivates **glycogen synthase**

inactive forms, **active forms**

Phosphorylase b kinase

- double control:

  - activated by **phosphorylation** (protein kinase A)

  - activated by **Ca^{2+}** binding to **calmodulin** (δ subunit), which acts like a sensor for Ca^{2+}

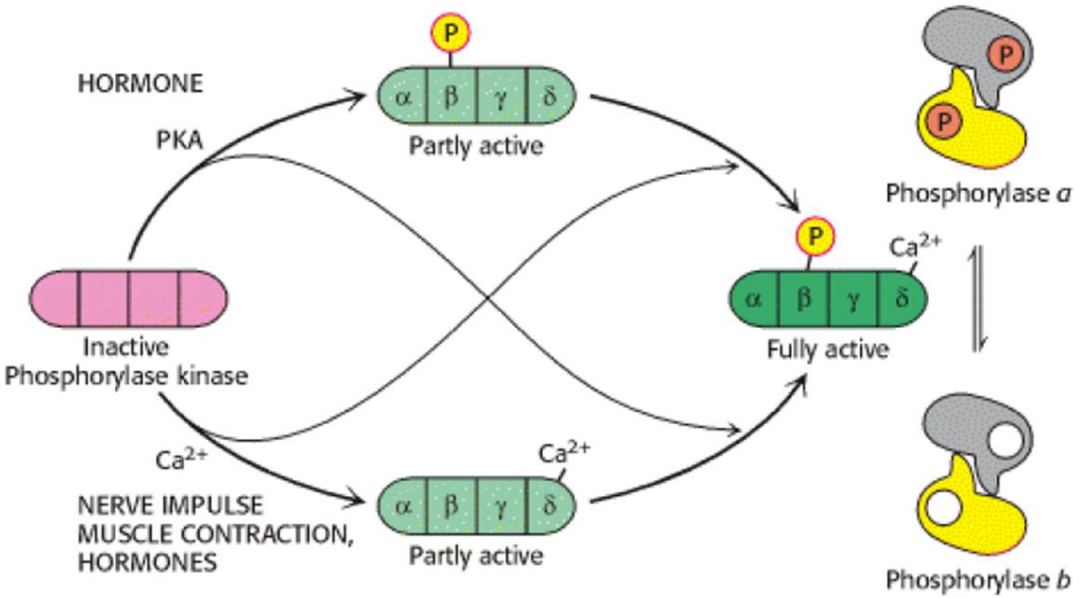


Figure 21.13. Activation of Phosphorylase Kinase. Phosphorylase kinase is activated by hormones that lead to the phosphorylation of the β subunit and by Ca^{2+} binding of the δ subunit. Both types of stimulation are required for maximal enzyme activity.

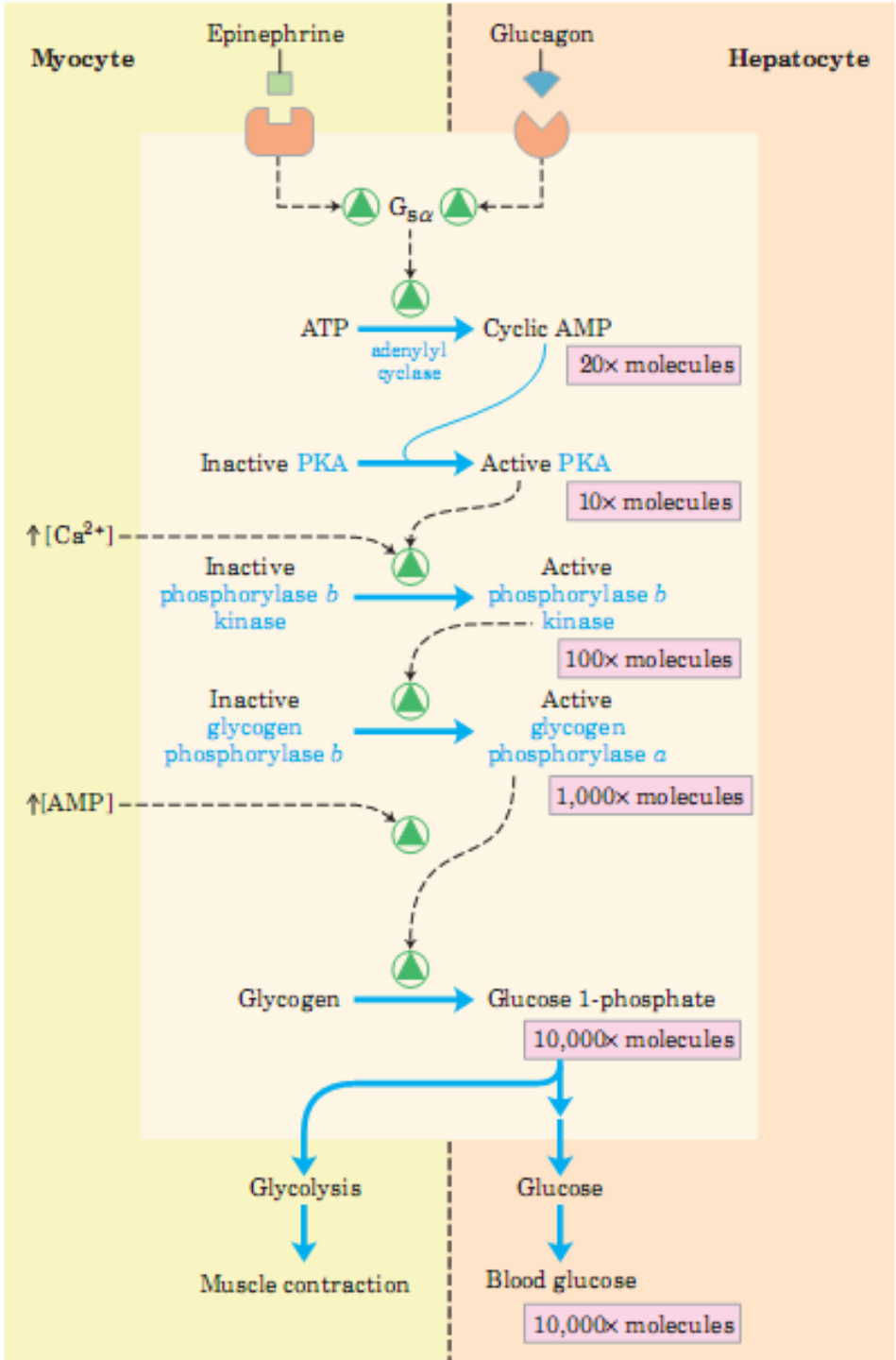
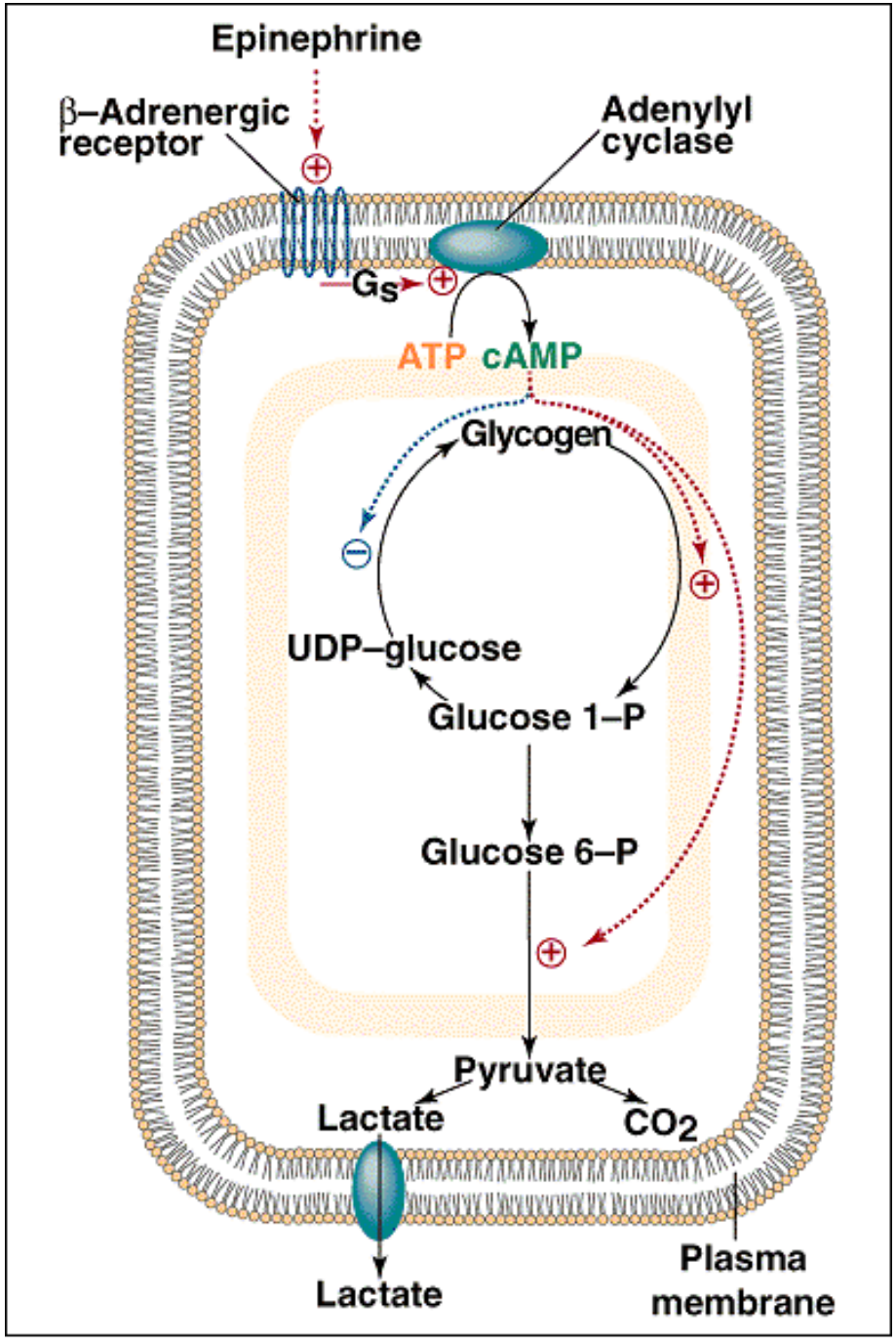
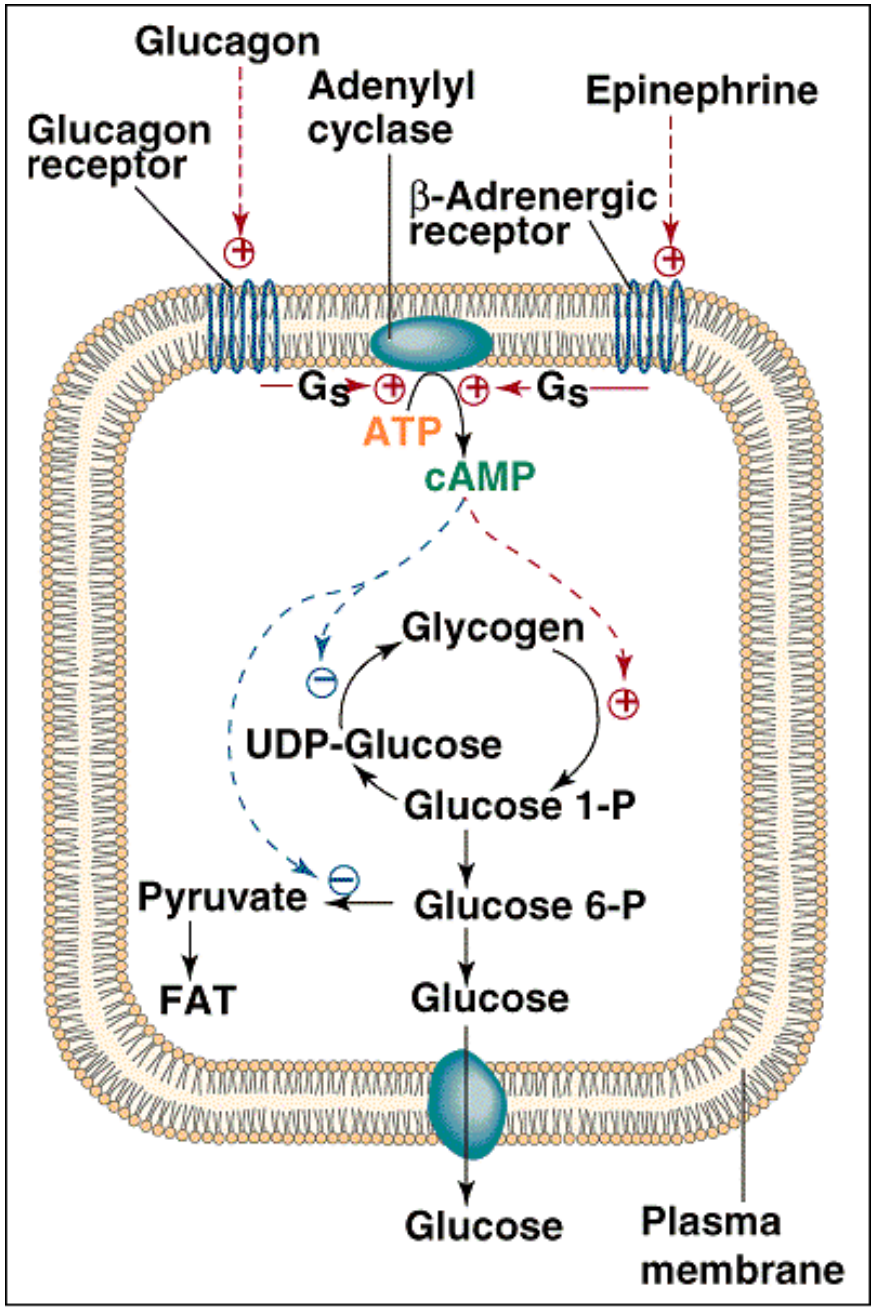


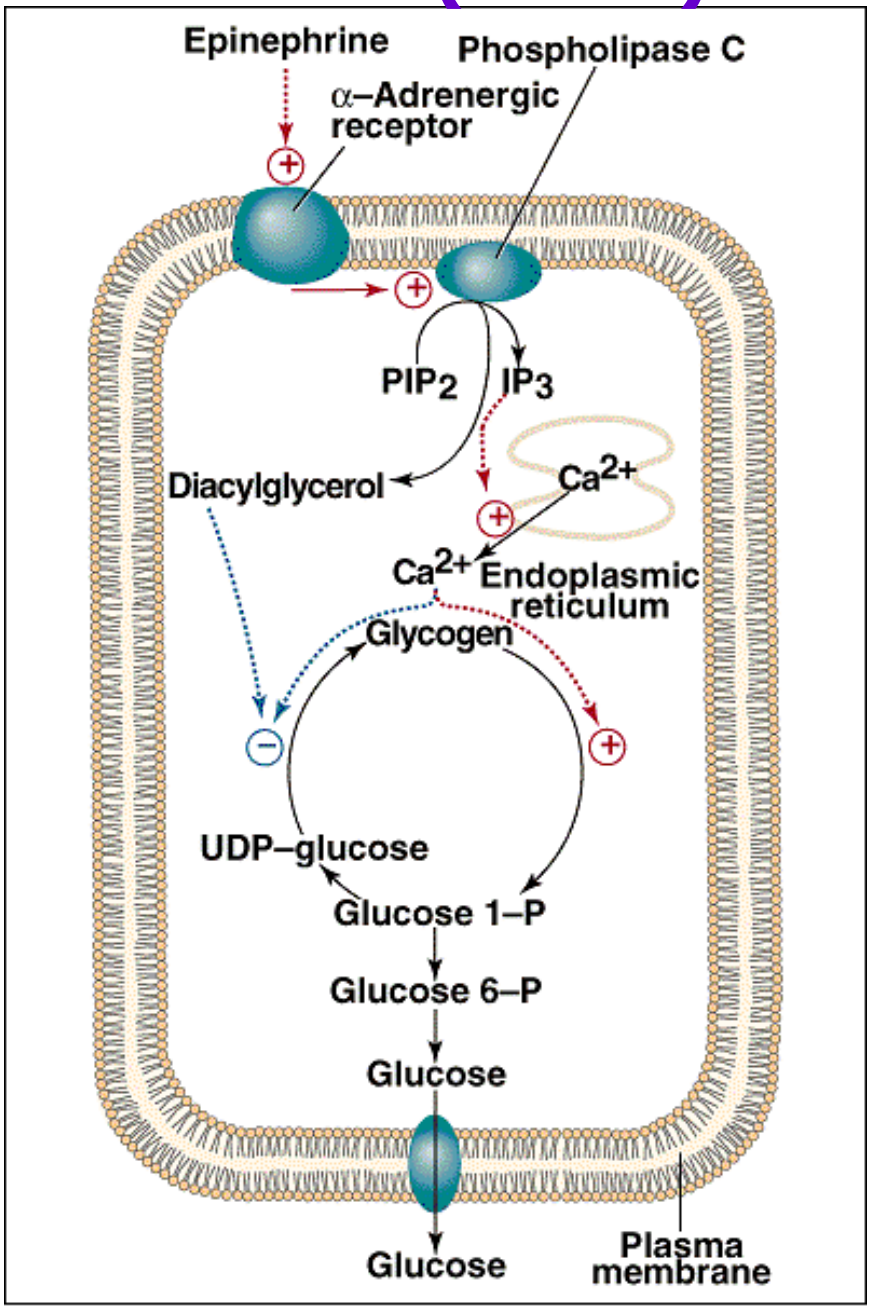
FIGURE 15–25 Cascade mechanism of epinephrine and glucagon action. By binding to specific surface receptors, either epinephrine acting on a myocyte (left) or glucagon acting on a hepatocyte (right) activates a GTP-binding protein $G_{s\alpha}$ (see Fig. 12–12). Active $G_{s\alpha}$ triggers a rise in [cAMP], activating PKA. This sets off a cascade of phosphorylations; PKA activates phosphorylase *b* kinase, which then activates glycogen phosphorylase. Such cascades effect a large amplification of the initial signal; the figures in pink boxes are probably low estimates of the actual increase in number of molecules at each stage of the cascade. The resulting breakdown of glycogen provides glucose, which in the myocyte can supply ATP (via glycolysis) for muscle contraction and in the hepatocyte is released into the blood to counter the low blood glucose.

EPINEPHRINE (MUSCLES)



GLUCAGON, EPINEPHRINE (LIVER)



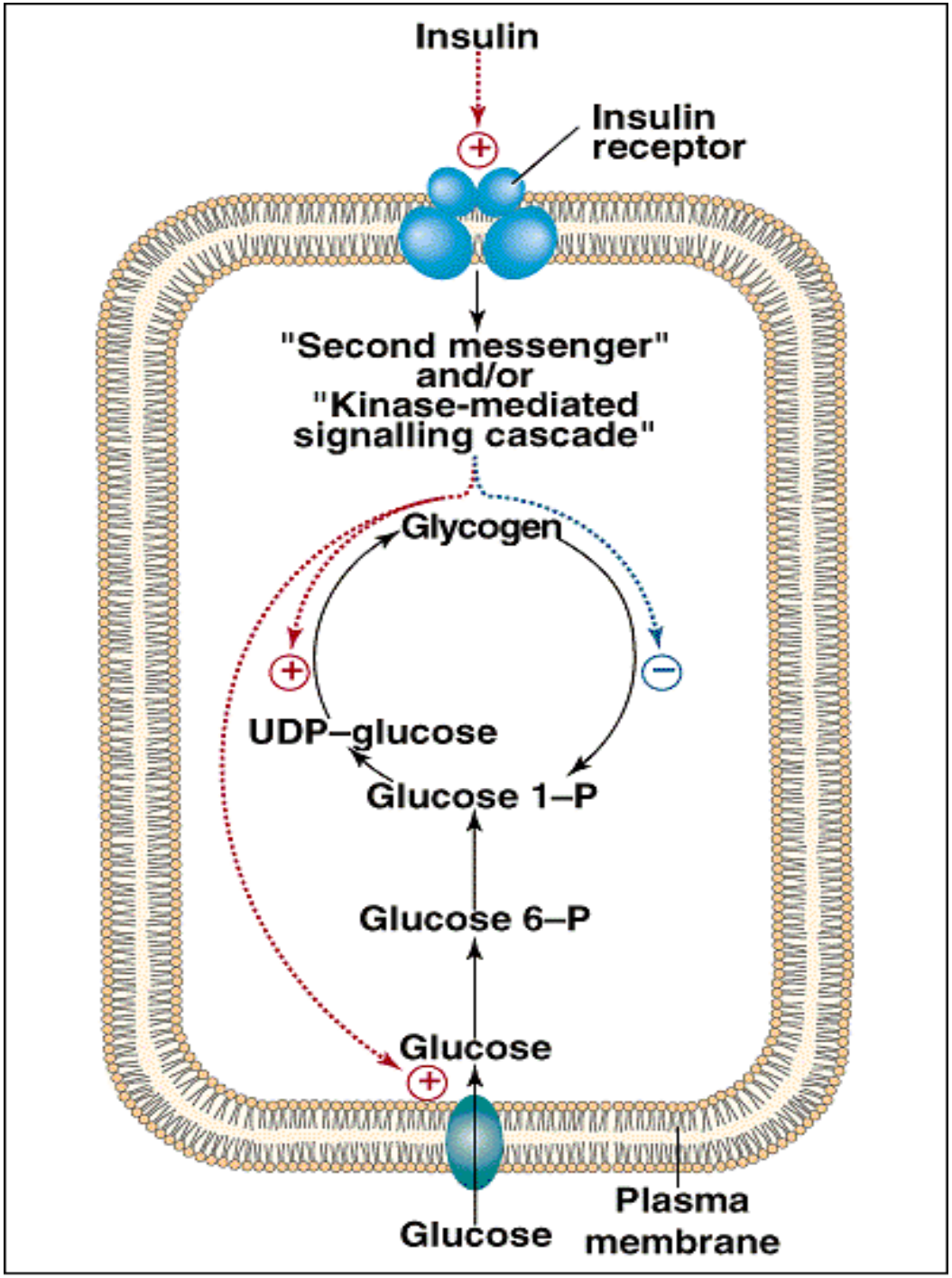


INSULIN:

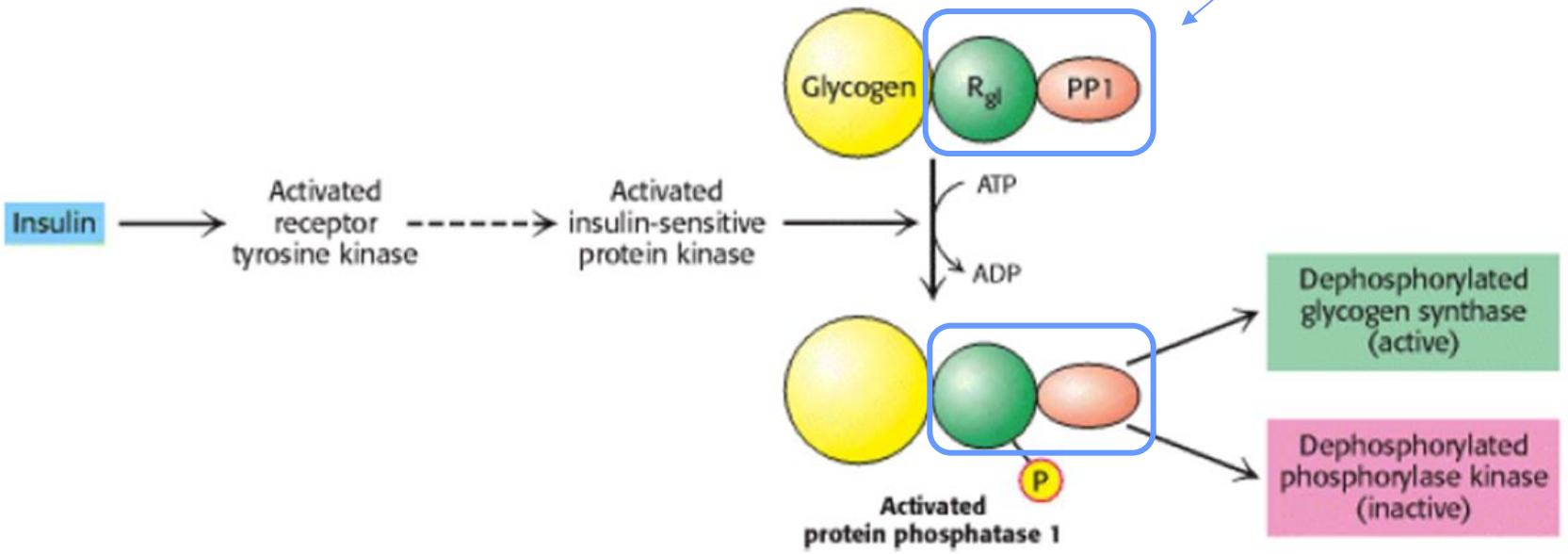
+ +

GLYCOGENESIS

- **activates** glycogen **synthesis** and **inactivates** glycogen degradation



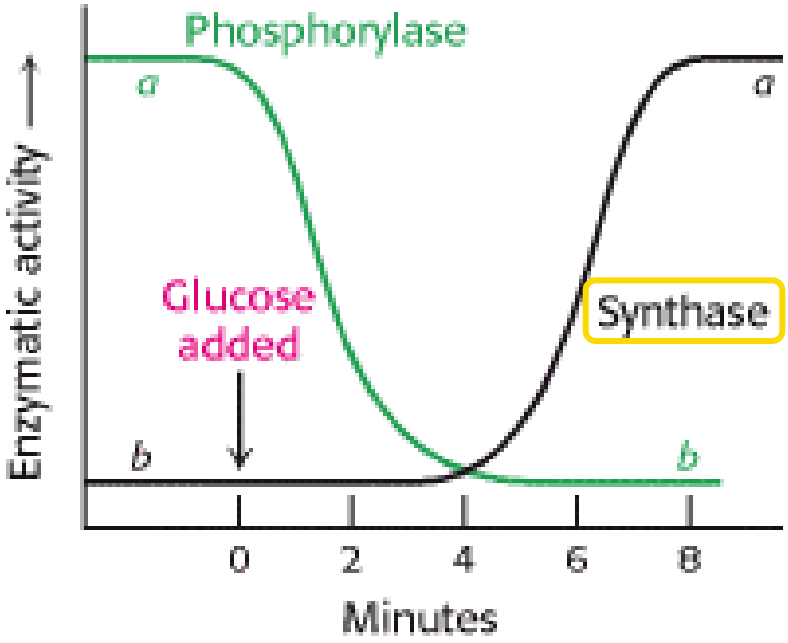
Insulin activates protein phosphatase 1 (PP1) and stimulates glycogen synthesis



- when blood Glu level is **high**, insulin stimulates Glu entrance into tissues and glycogen synthesis - **PP1 activation**

- phosphorylation of R subunit → **de**phosphorylation of **synthase** and **phosphorylase** → **synthesis activation** and **inactivation od glycogen degradation**

Glycogen metabolism in the liver regulates glucose blood concentration

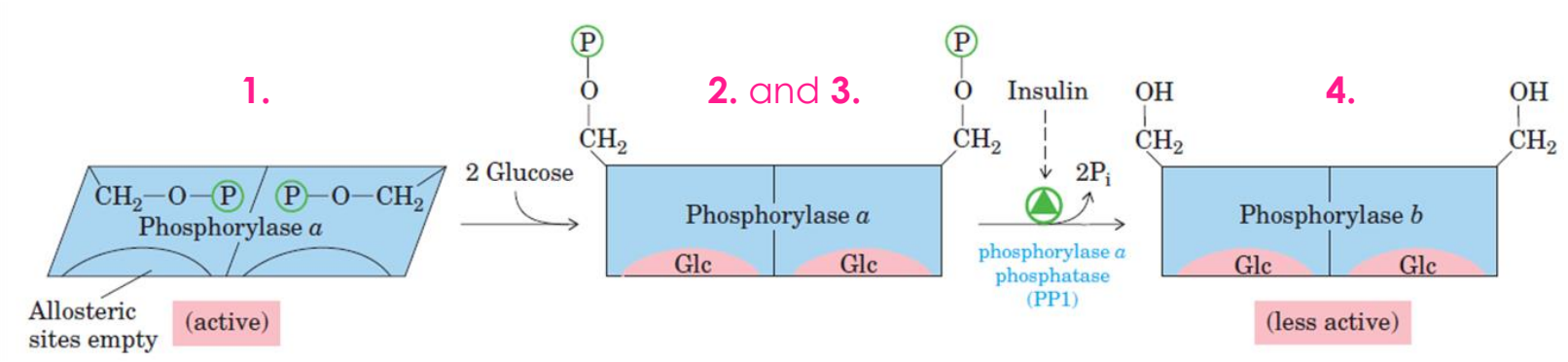


1. **glucose** sensor in the liver is **phosphorylase *a***

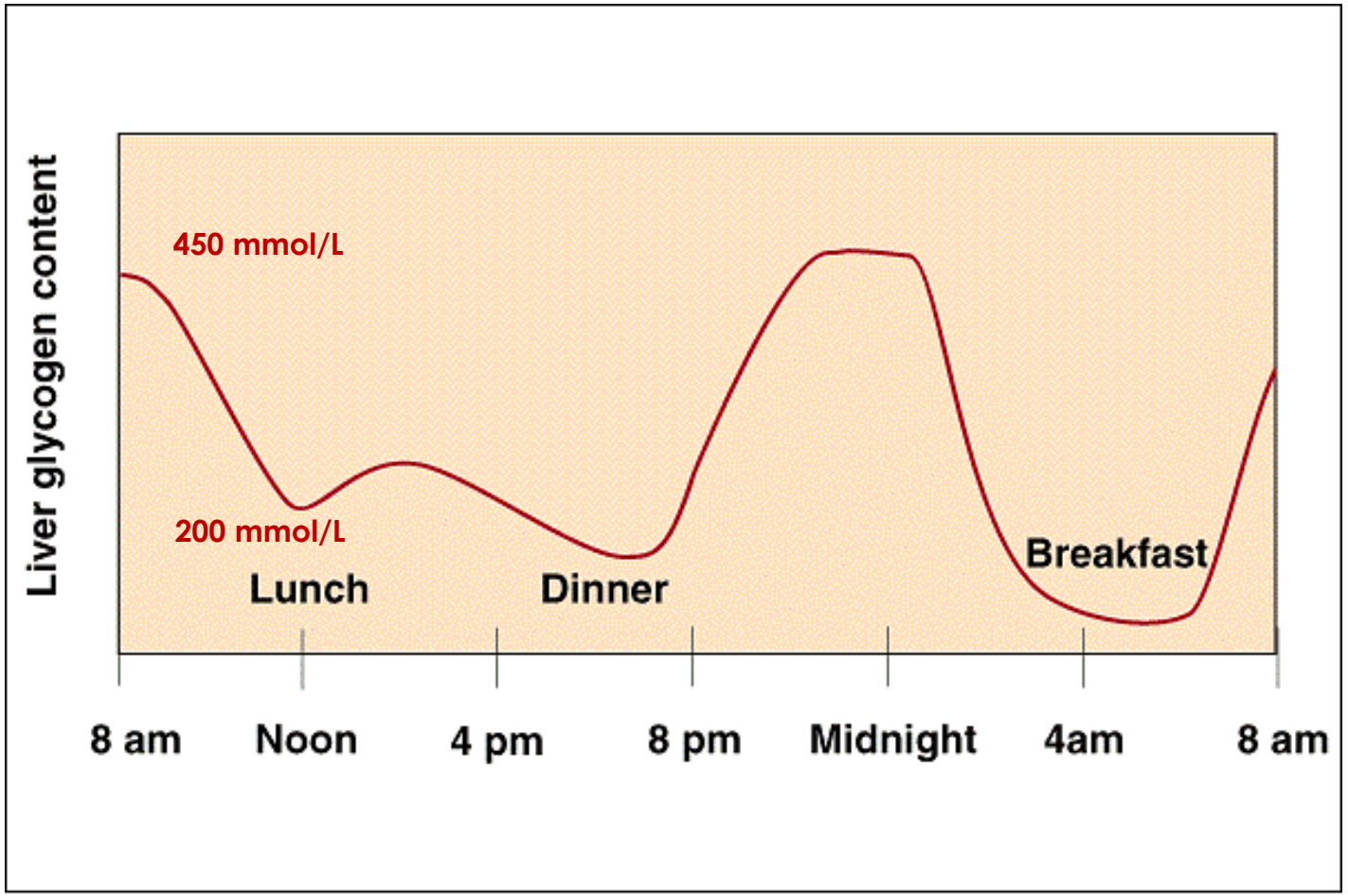
2.,3. **glucose** binds to allosteric sites on **phosphorylase *a*** → conformational change - phosphorylated residues are more exposed for action of *PP1*

4. PP1 → dephosphorylation and inactivation of **phosphorylase *(a → b)***

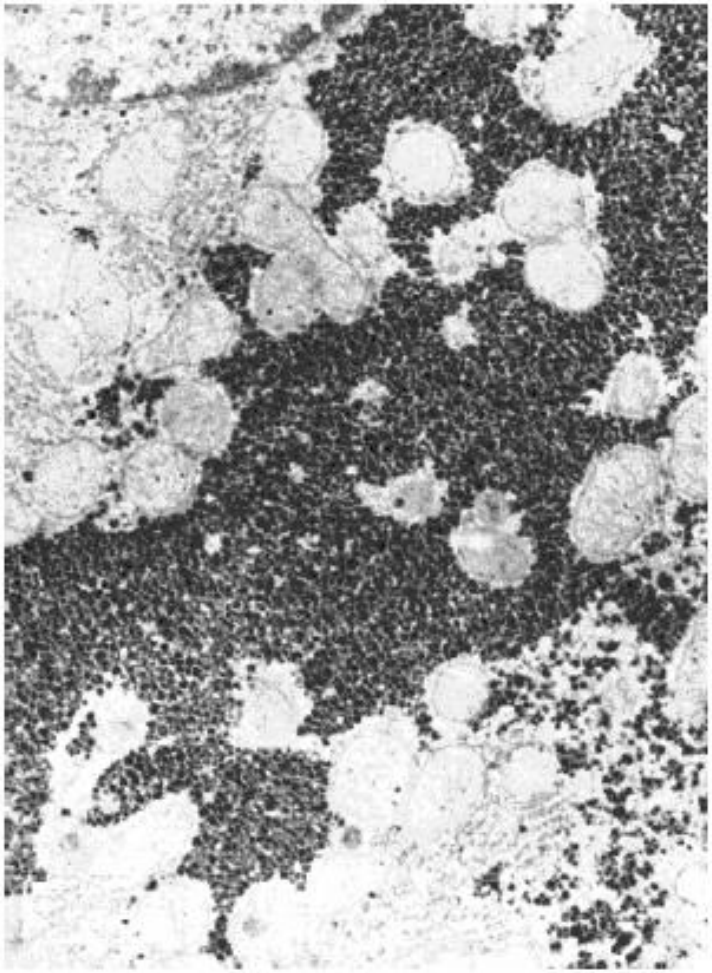
5. PP1 dephosphorylates and activates ***glycogen synthase***



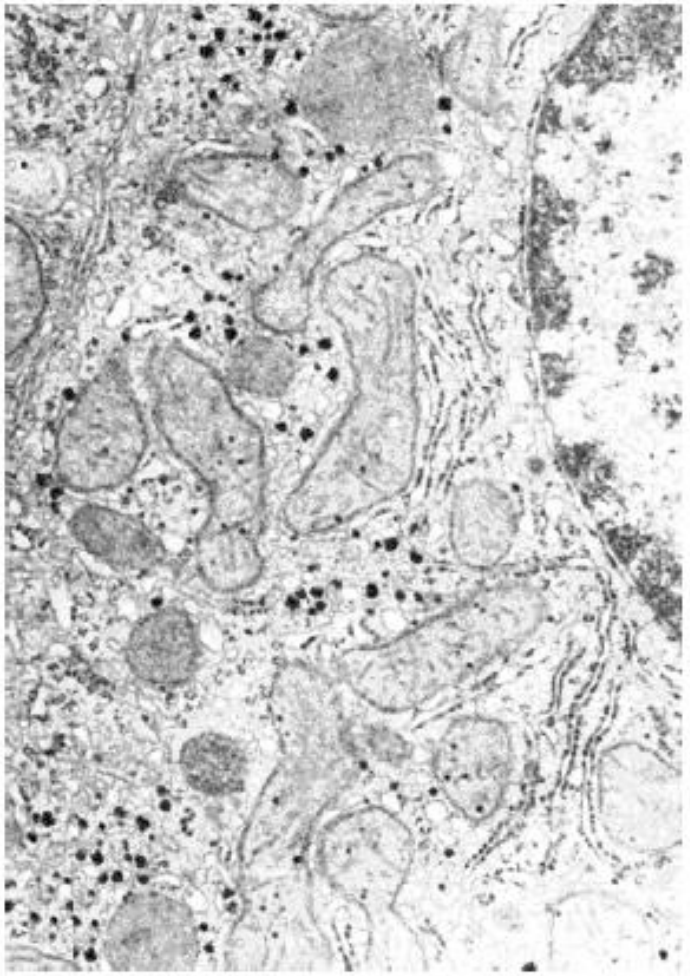
Liver glycogen concentration changes



Glycocen granules in rat liver

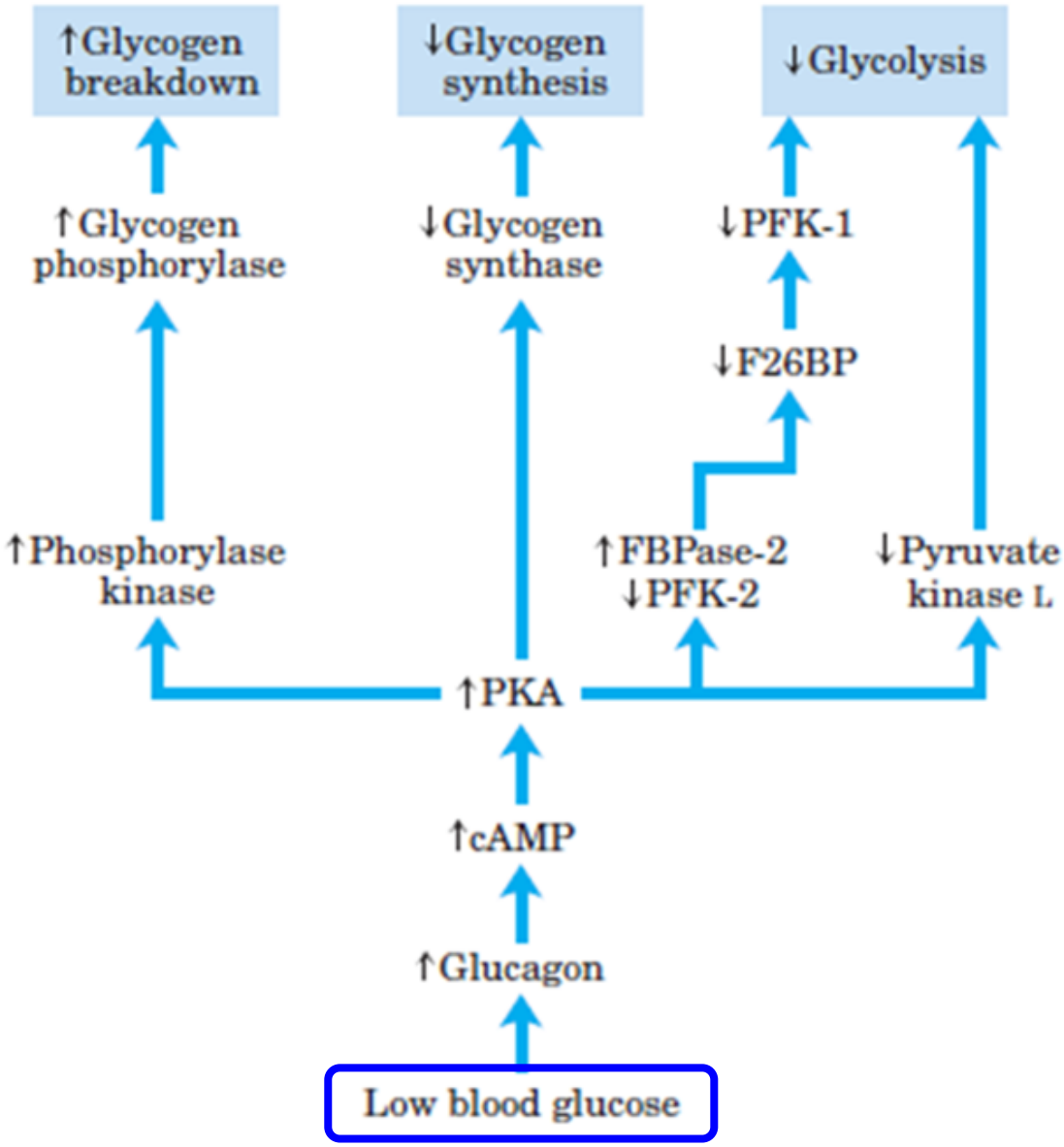


After heavy meal

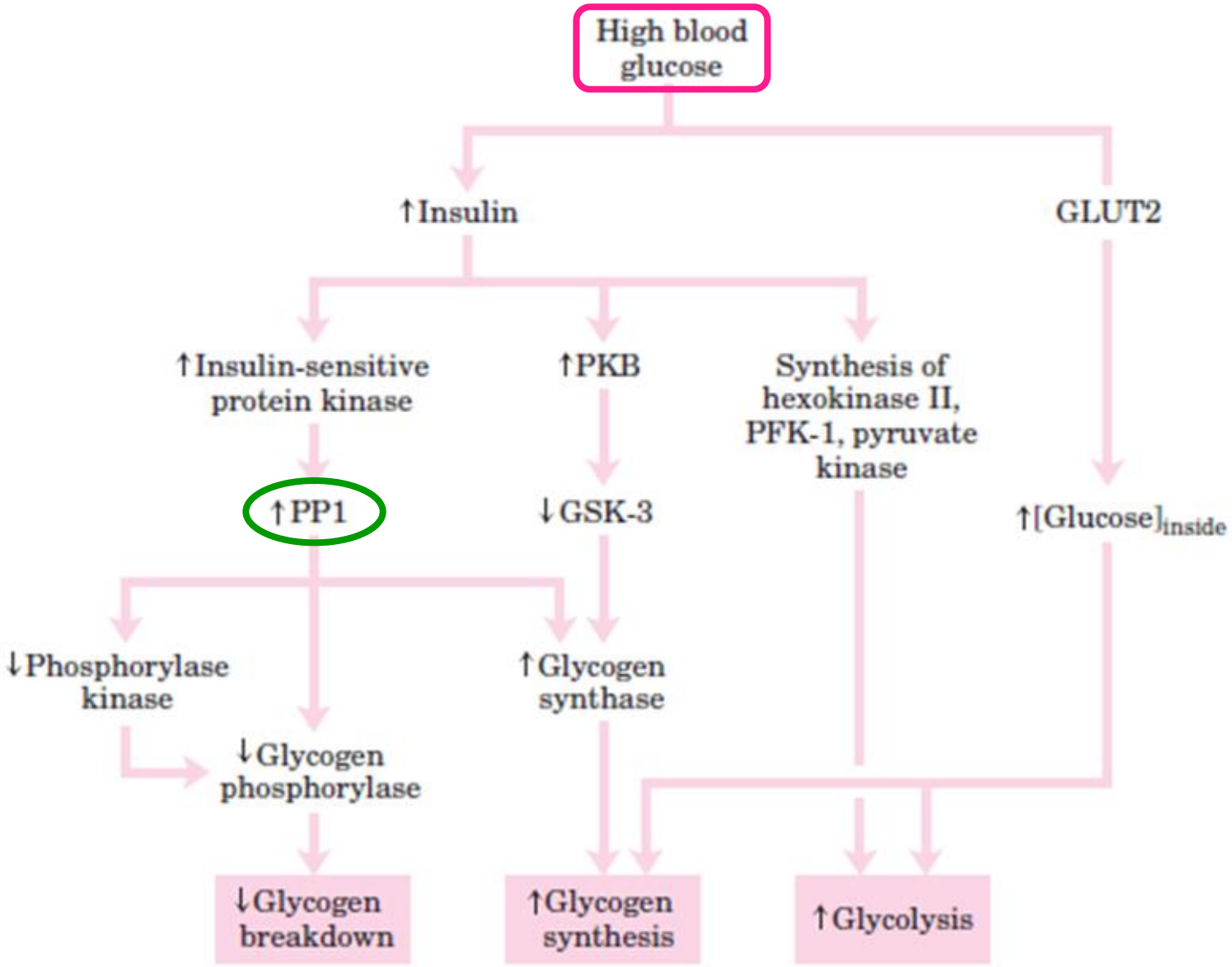


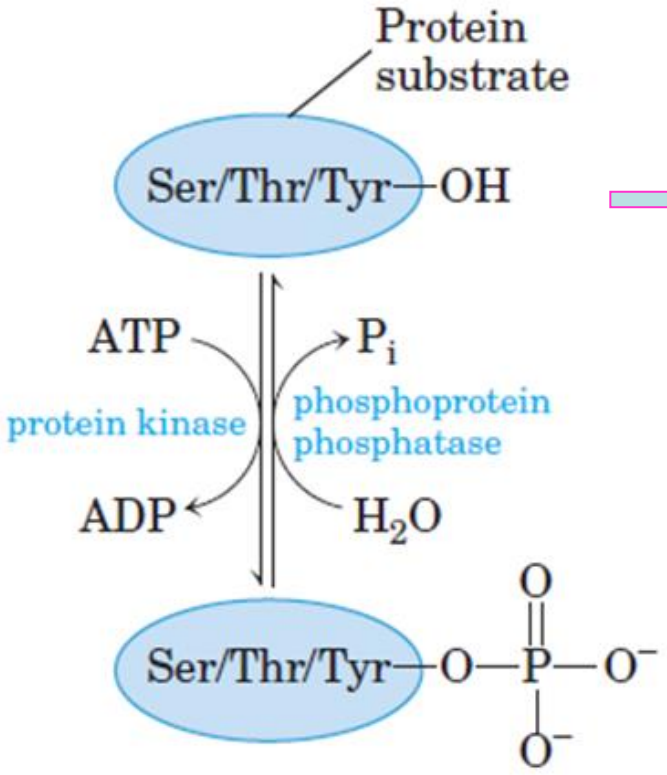
After 24h fasting

In hepatocytes



In hepatocytes





glycogen synthase
active **dephosphorylated**

glycogen phosphorylase
active **phosphorylated**

Edgar von Gierke

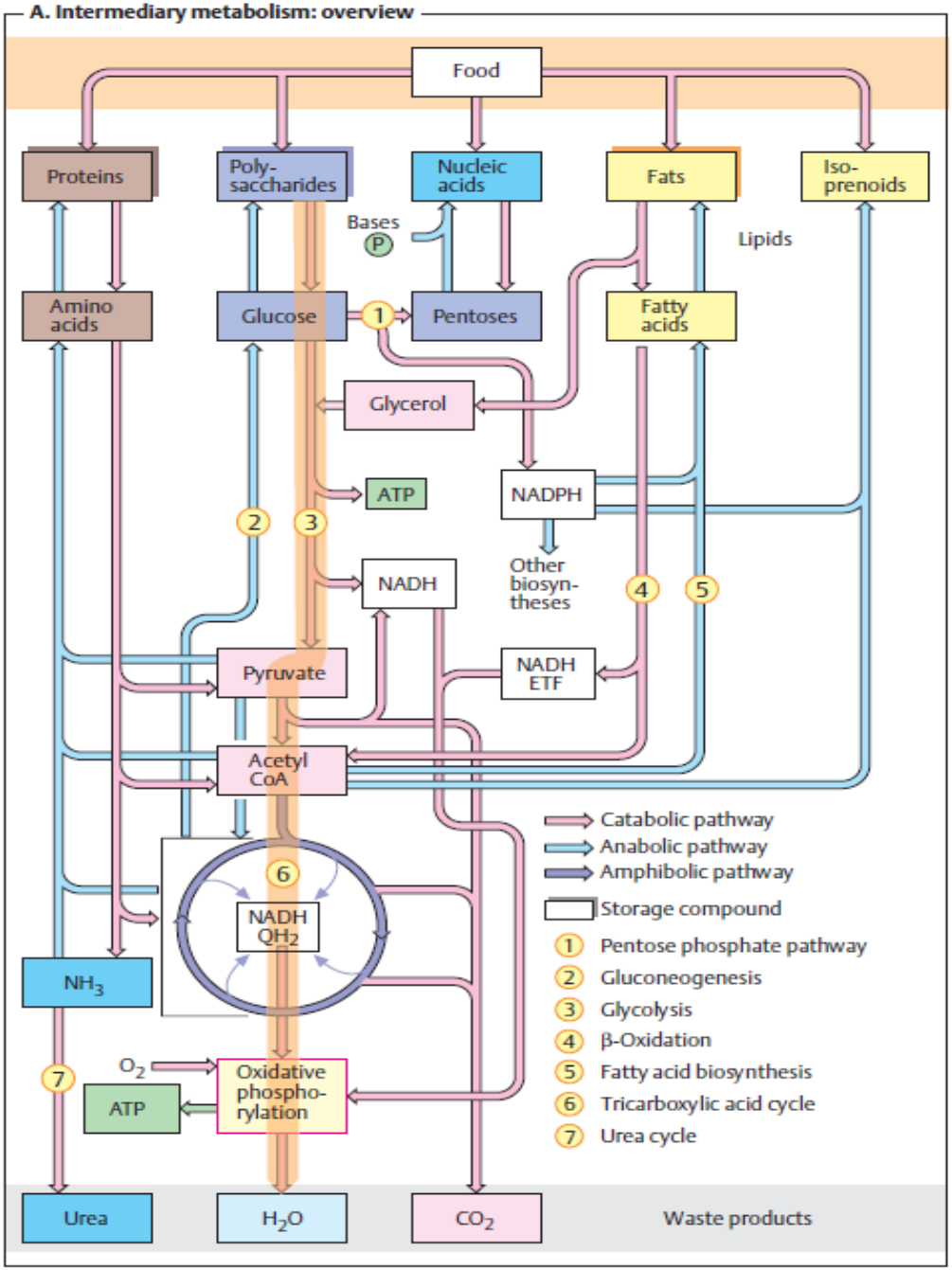
- 1929. described the first glycogen storage disease - type I (von Gierke's disease)

Gerty i Carl Cori

- 1947. Nobel prize for glycogen metabolism
- 1952. explained enzymatic defect in von Gierke's disease

TABLE 1 Glycogen Storage Diseases of Humans

Type (name)	*Enzyme affected*	*Primary organ affected*	*Symptoms*
Type 0	Glycogen synthase	Liver	Low blood glucose, high ketone bodies, early death
Type Ia (von Gierke's)	Glucose 6-phosphatase	Liver	Enlarged liver, kidney failure
Type Ib	Microsomal glucose 6-phosphate translocase	Liver	As in Ia; also high susceptibility to bacterial infections
Type Ic	Microsomal P_i transporter	Liver	As in Ia
Type II (Pompe's)	Lysosomal glucosidase	Skeletal and cardiac muscle	Infantile form: death by age 2; juvenile form: muscle defects (myopathy); adult form: as in muscular dystrophy
Type IIIa (Cori's or Forbes's)	Debranching enzyme	Liver, skeletal and cardiac muscle	Enlarged liver in infants; myopathy
Type IIIb	Liver debranching enzyme (muscle enzyme normal)	Liver	Enlarged liver in infants
Type IV (Andersen's)	Branching enzyme	Liver, skeletal muscle	Enlarged liver and spleen, myoglobin in urine
Type V (McArdle's)	Muscle phosphorylase	Skeletal muscle	Exercise-induced cramps and pain; myoglobin in urine
Type VI (Hers's)	Liver phosphorylase	Liver	Enlarged liver
Type VII (Tarui's)	Muscle PFK-1	Muscle, erythrocytes	As in V; also hemolytic anemia
Type VIb, VIII, or IX	Phosphorylase kinase	Liver, leukocytes, muscle	Enlarged liver
Type XI (Fanconi-Bickel)	Glucose transporter (GLUT2)	Liver	Failure to thrive, enlarged liver, rickets, kidney dysfunction



1. REGULATION OF GLYCOLISIS AND GLUCONEOGENESIS

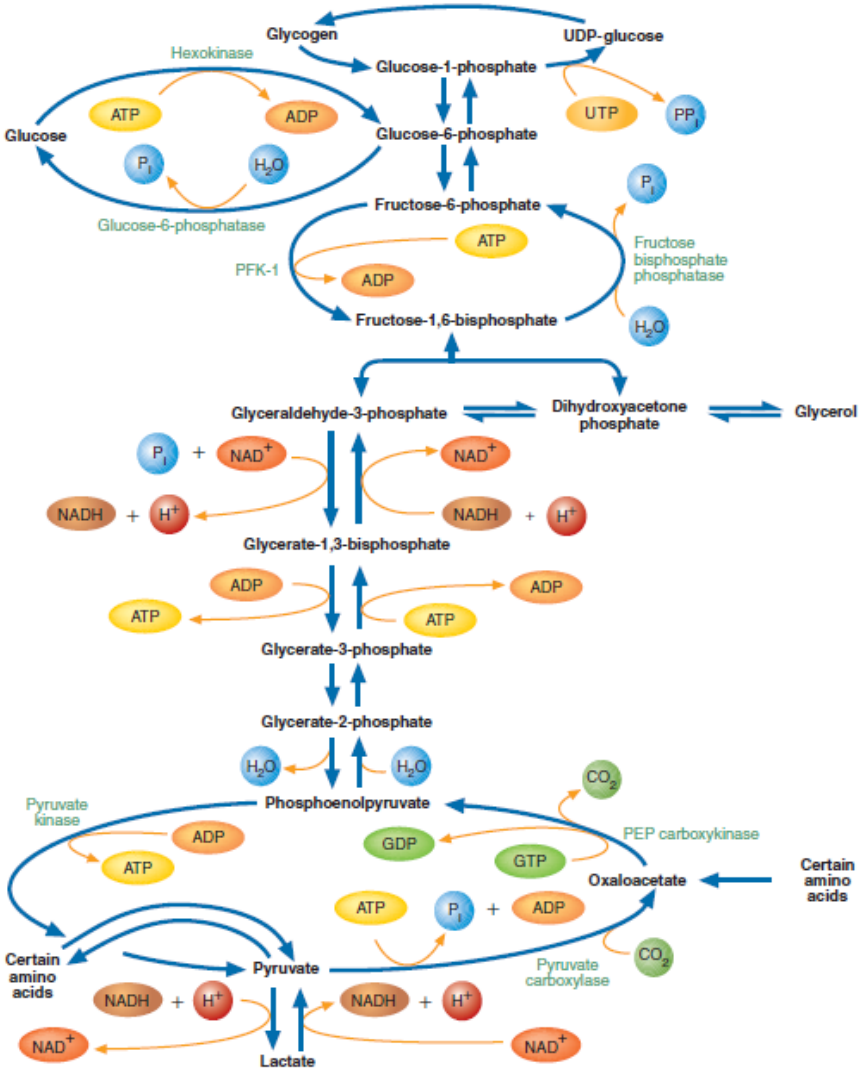


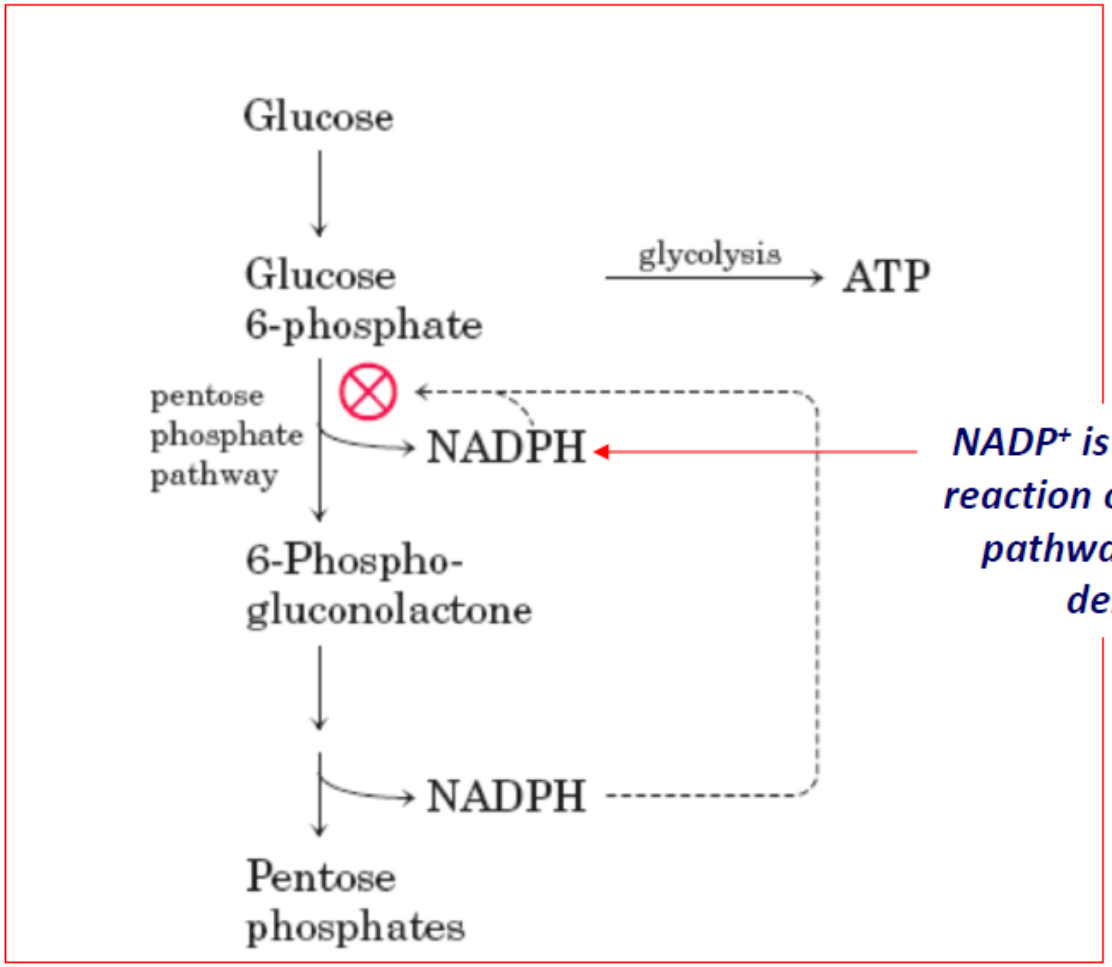
FIGURE 8.10

Carbohydrate Metabolism: Gluconeogenesis and Glycolysis

In gluconeogenesis, which occurs when blood sugar levels are low and liver glycogen is depleted, 7 of the 10 reactions of glycolysis are reversed. Three irreversible glycolytic reactions are bypassed by alternative reactions. The major substrates for gluconeogenesis are certain amino acids (derived from muscle), lactate (formed in muscle and red blood cells), and glycerol (produced from the degradation of triacylglycerols). In contrast to the reactions of glycolysis, which occur only in cytoplasm, the gluconeogenesis reactions catalyzed by pyruvate carboxylase and, in some species, PEP carboxykinase occur within the mitochondria. The reaction catalyzed by glucose-6-phosphatase takes place in the endoplasmic reticulum. Note that gluconeogenesis and glycolysis do not occur simultaneously. In glycolysis, pyruvate is converted either to acetyl-CoA (not shown) or to lactate.

2. REGULATION OF PENTOSE PHOSPHATE PATHWAY:

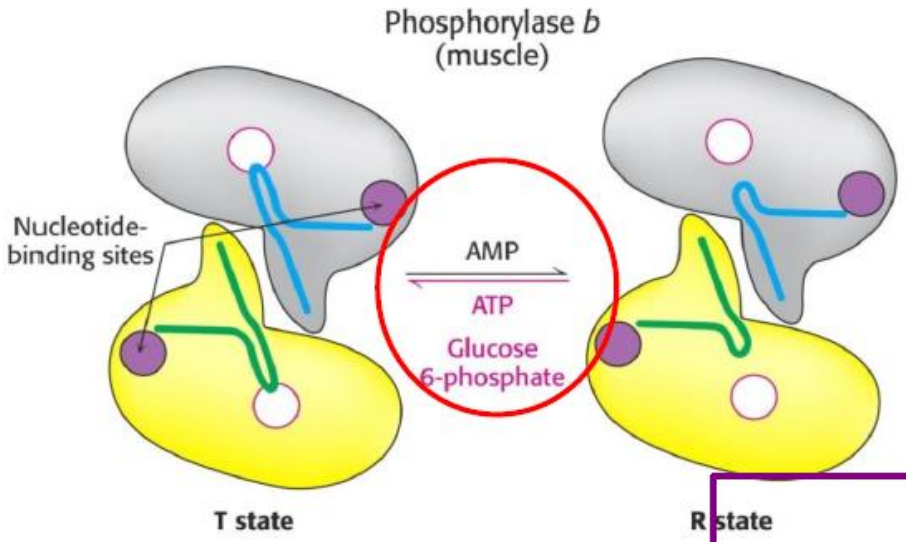
NADP⁺/NADPH ratio and oxidized/reduced glutathione



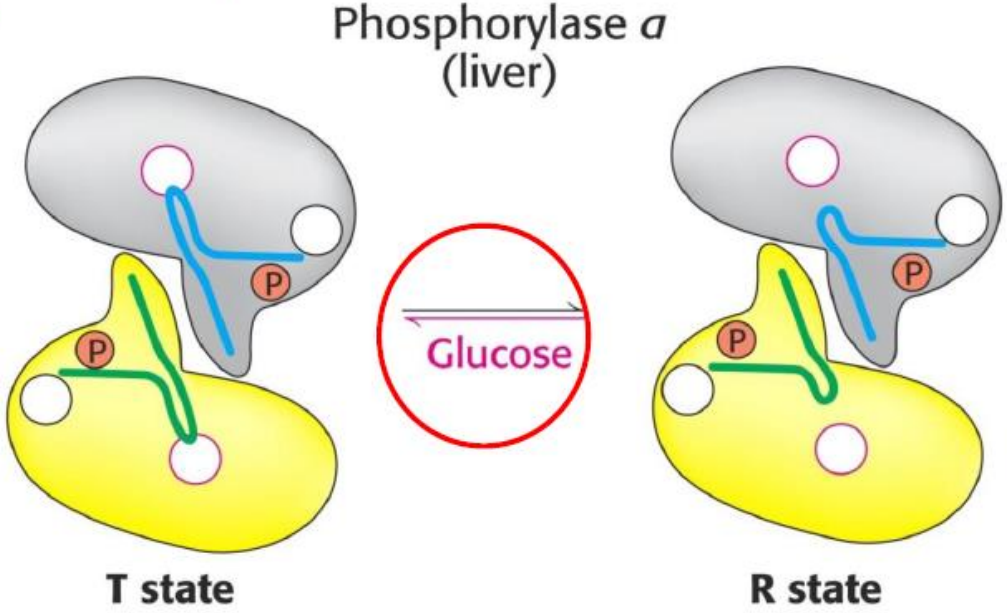
NADP⁺ is required for the first reaction of pentose phosphate pathway (enzyme Glc-6-P-dehydrogenase)!

When NADPH is forming faster than it is being used for biosynthesis and glutathione reduction, [NADPH] rises and inhibits the first enzyme in the pentose phosphate pathway. As a result, more glucose 6-phosphate is available for glycolysis.

3. REGULATION OF GLYCOGEN METABOLISM – ALLOSTERIC CONTROL



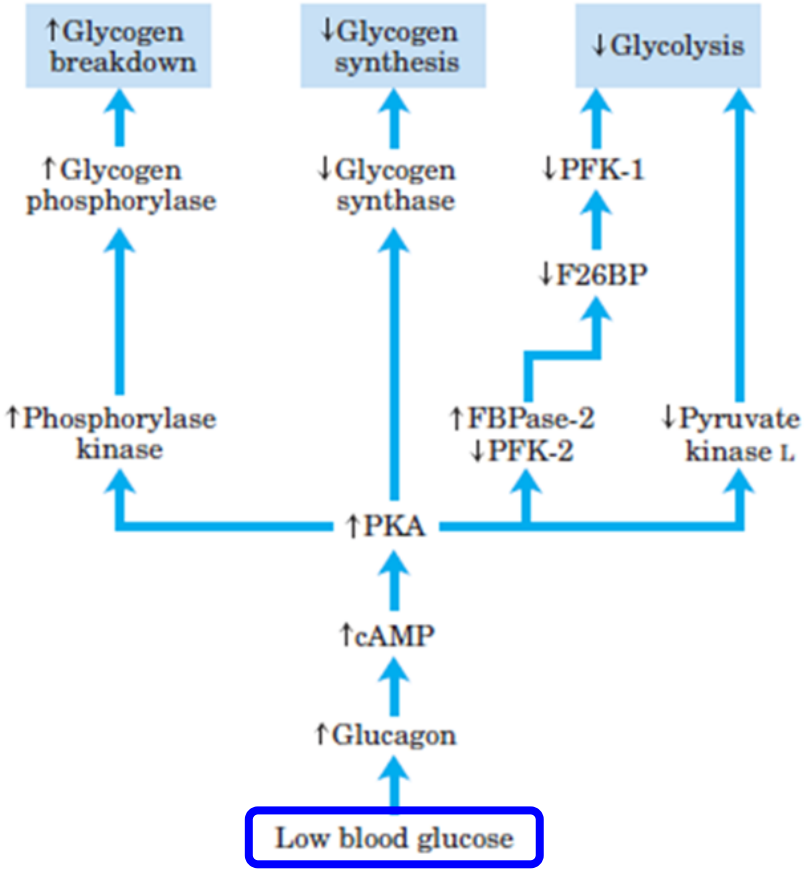
musle
↑ AMP
↓ ATP, Glc-6-P

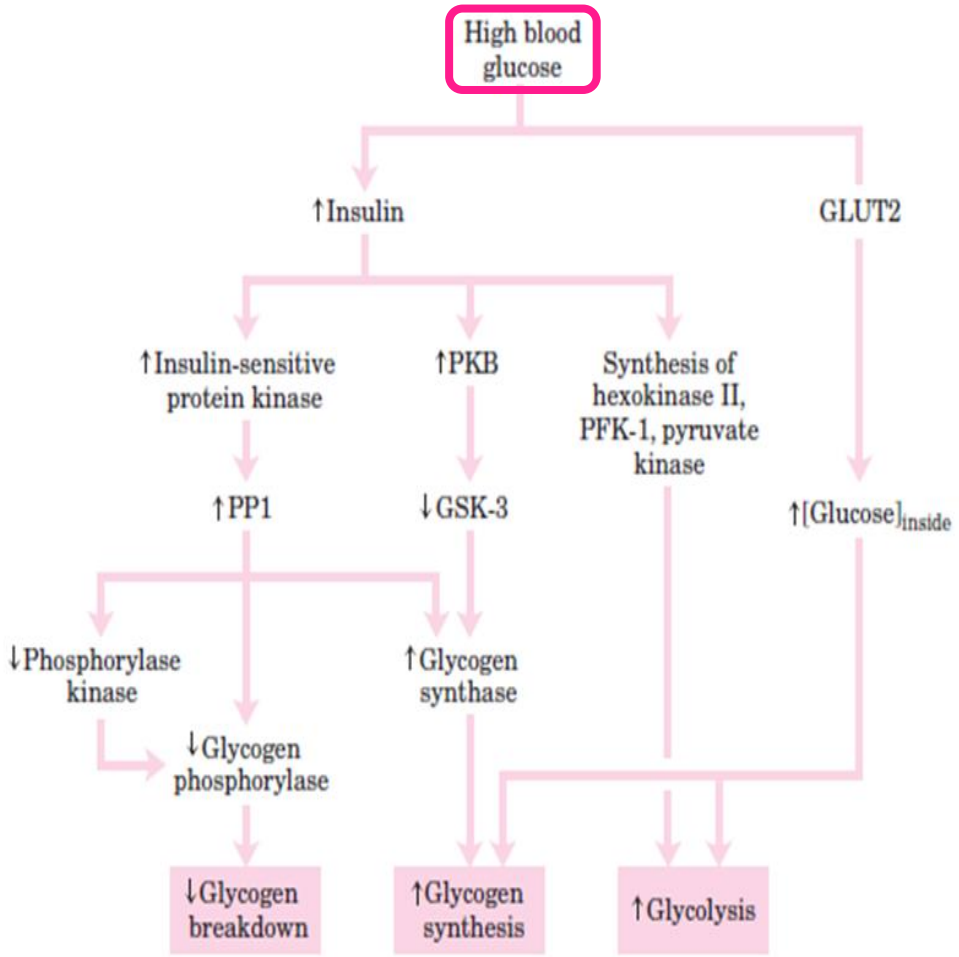


liver
↓ Glc

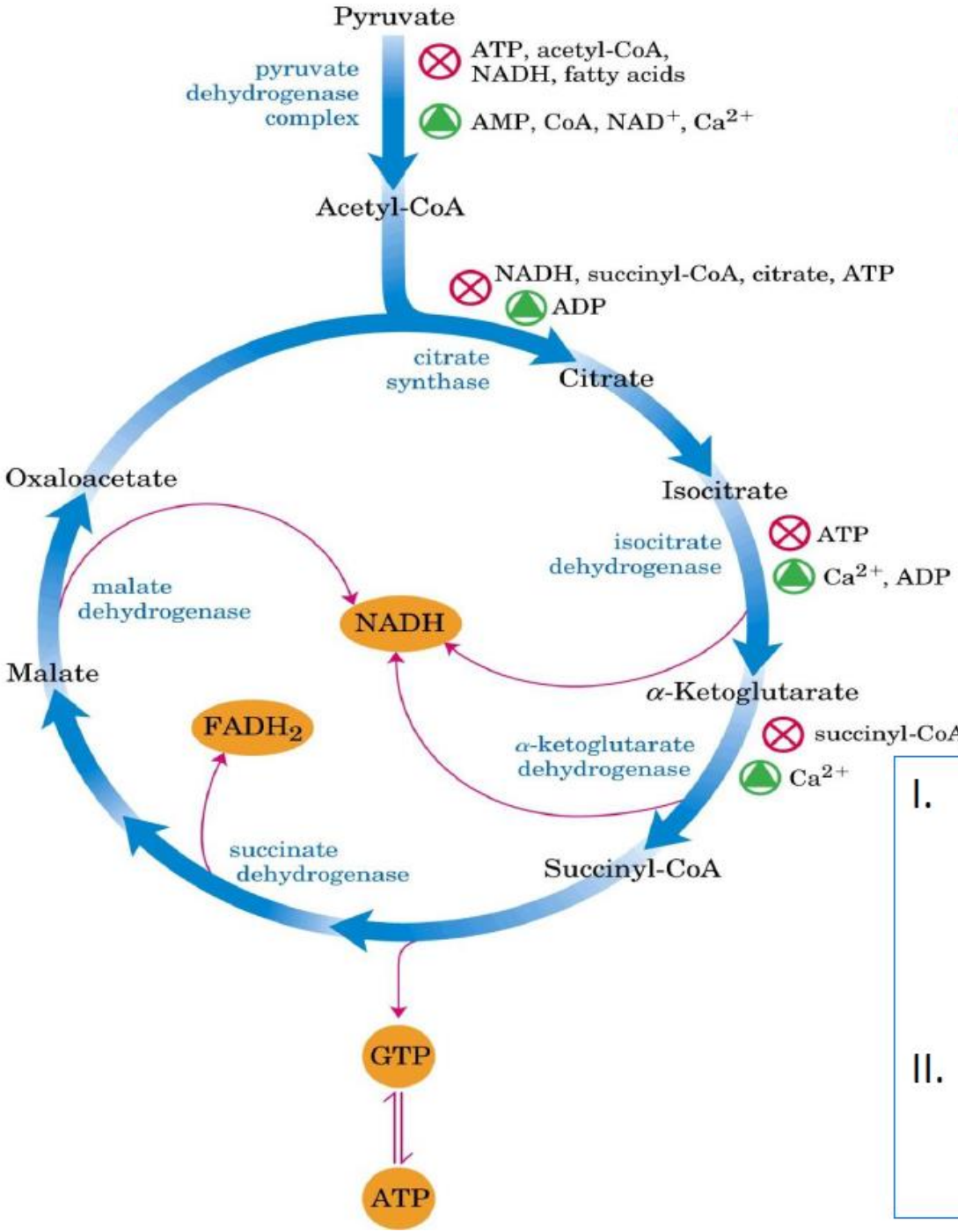
HORMONE REGULATION OF GLYCOGEN METABOLISM AND GLYCOLYSIS

- hepatocytes





4. CITRIC ACID CYCLE REGULATION



I. Formation of acetyl-CoA: activity of pyruvate dehydrogenase complex is regulated by allosteric effectors and covalent modification
II. Regulation of 3 exergonic reactions of the cycle by allosteric effectors

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Ppt presentations: Prof. Vukelić, Prof. Kalanj Bognar, Prof. Karmelić, Prof. Flögel (PMF, FBF)