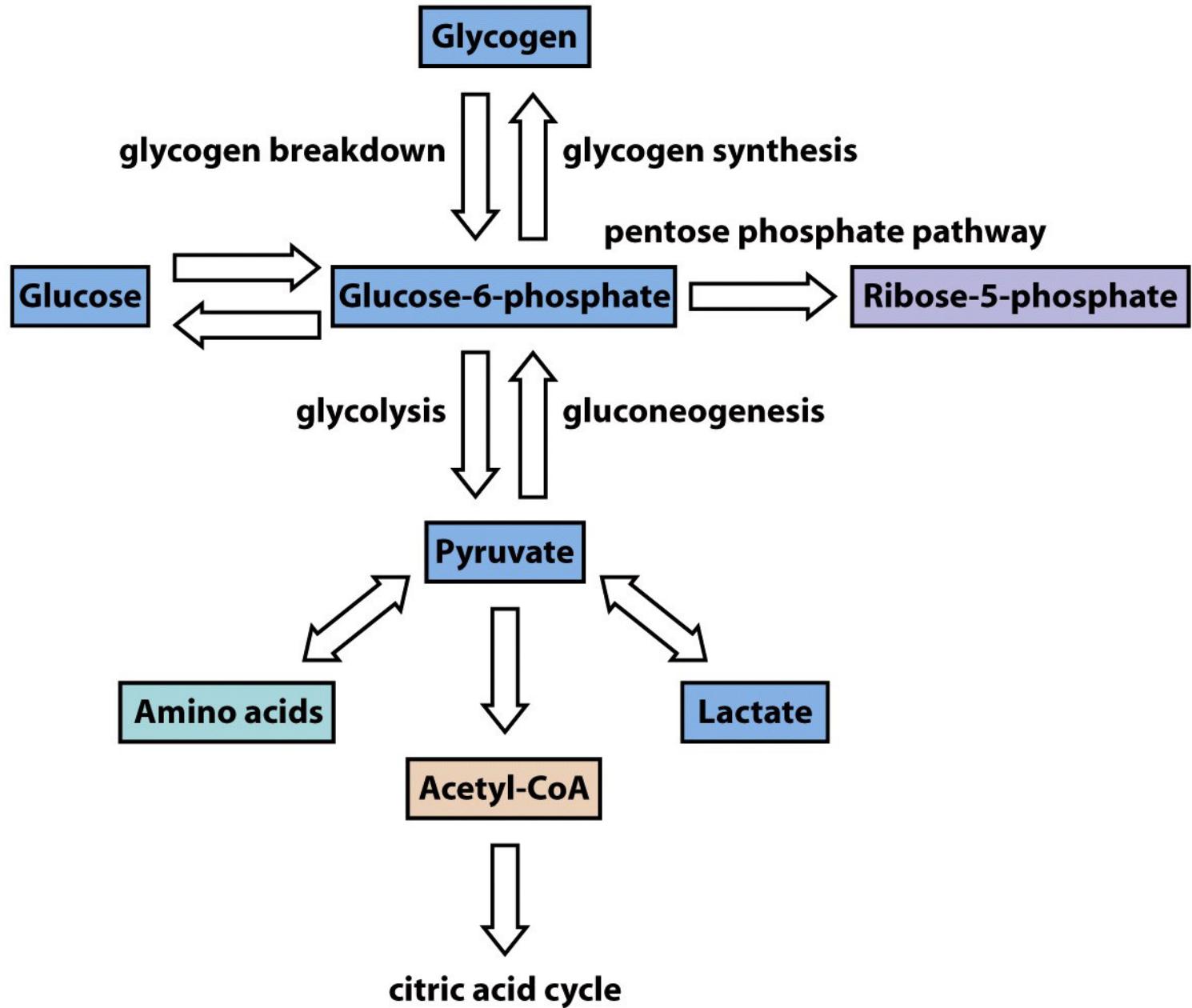
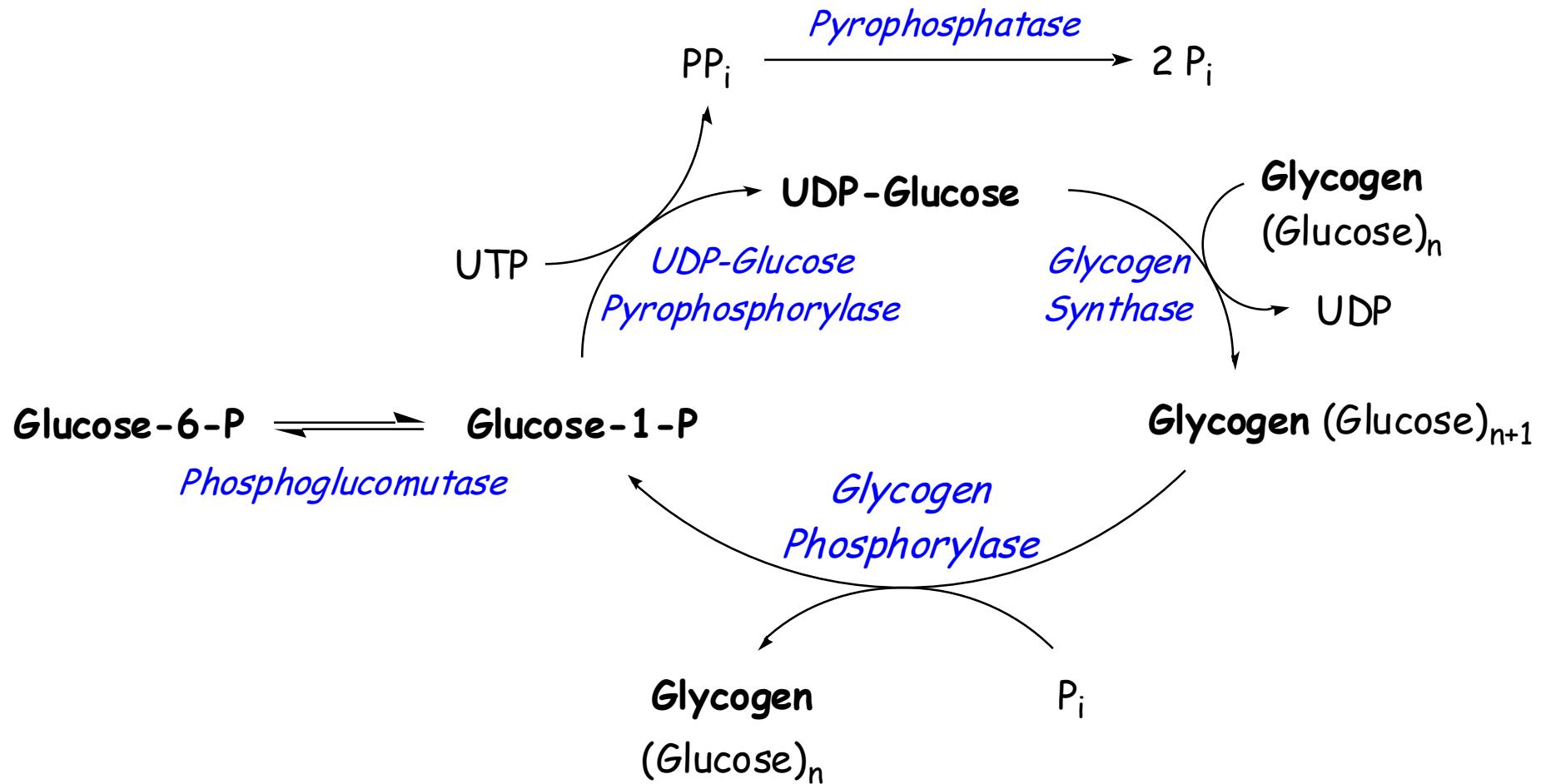


Storage Mechanisms and Control in Carbohydrate Metabolism

Chapter 18



Glycogen Metabolism

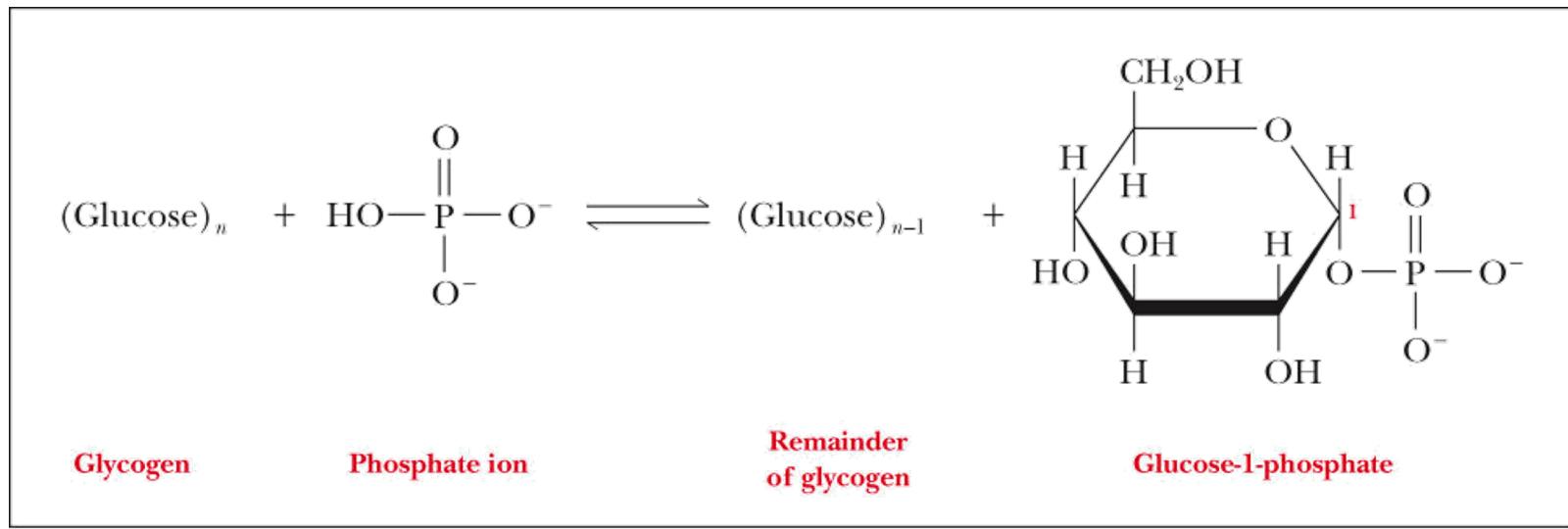


Breakdown of Glycogen

- Glycogen is primarily found in liver and muscle.
- Release of glycogen in liver is triggered by
 - ⇒ low level of glucose in blood.
- Glycogen degradation consists of 3 steps:-
 1. The release of glucose 1-phosphate from glycogen
 2. The remodeling of glycogen substrate to permit further degradation
 3. The conversion of glucose 1-phosphate into glucose 6-phosphate for further metabolism

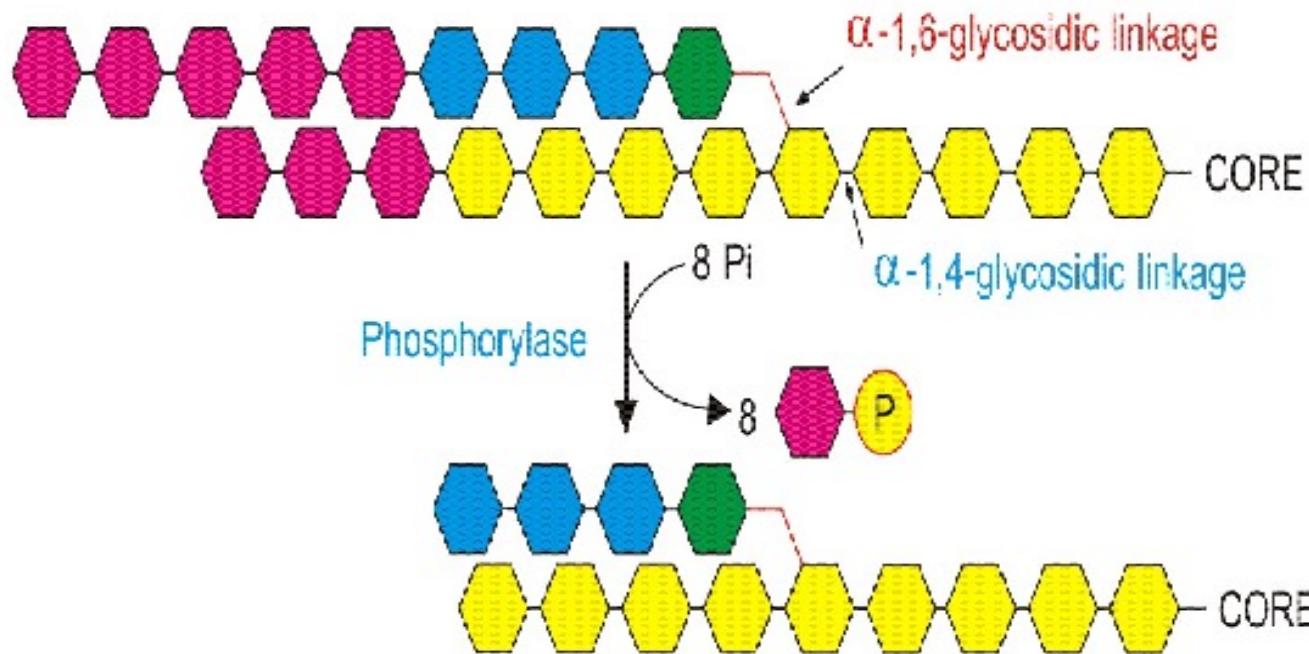
Glycogen Breakdown: Glucose-1-phosphate

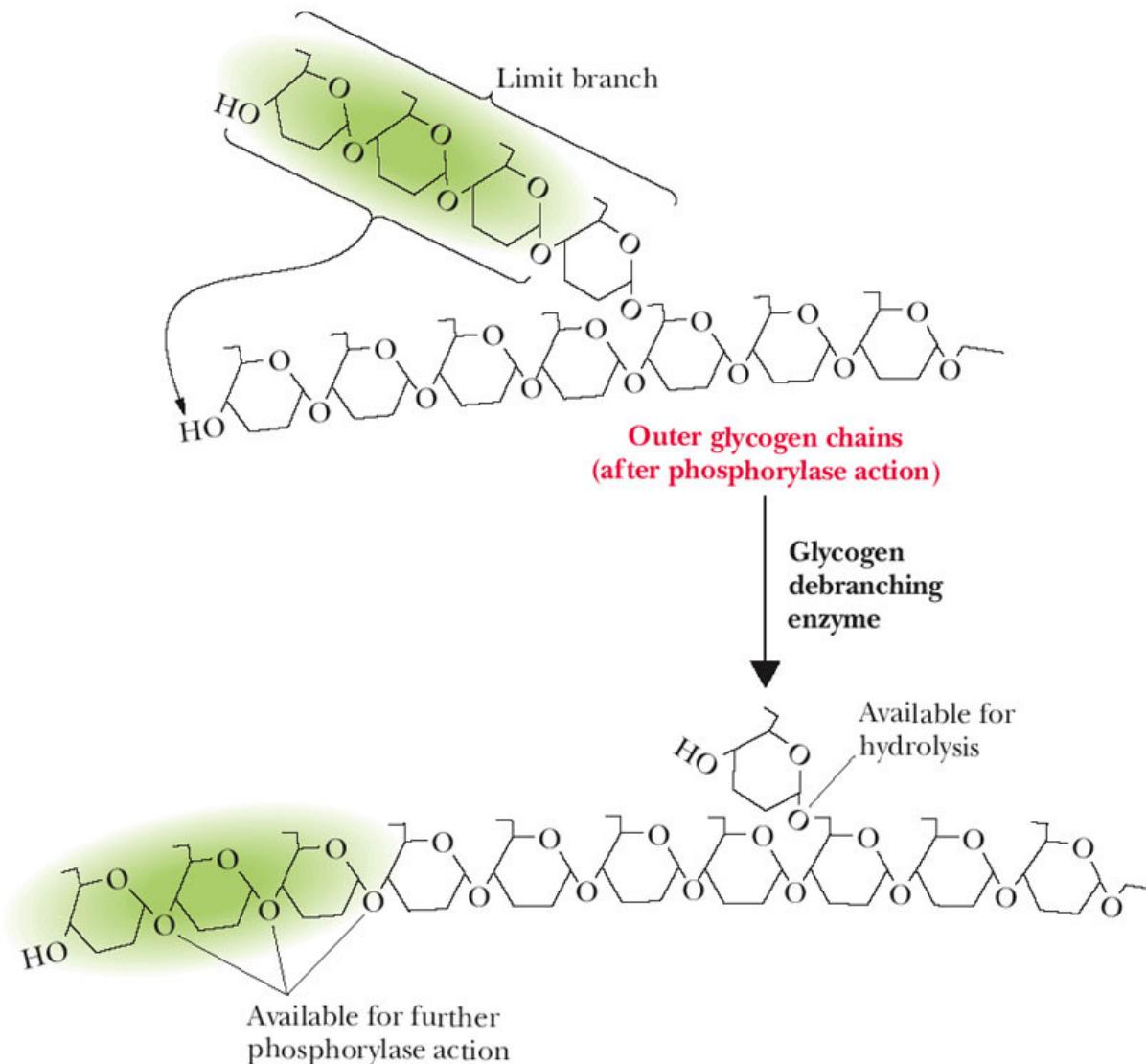
- Glycogen is cleaved by phosphate to give Glucose-1-phosphate
- Cleavage reaction is phosphorolysis not hydrolysis
 - No ATP is involved in reaction
- Reaction is catalyzed by glycogen phosphorylase



Glycogen phosphorylase

- This enzyme will only release a glucose unit that is at least 5 units from a branch point
- Leave 4 glucose attached to the branch

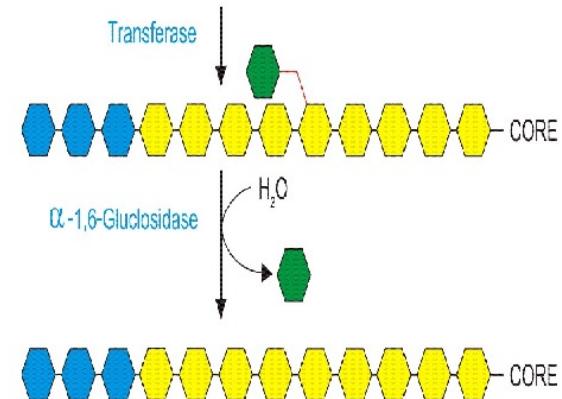




- The mode of action of the debranching enzyme in glycogen breakdown:
- The enzyme transfers three $\alpha(1 \rightarrow 4)$ -linked glucose residues from a limit branch to the end of another branch. The same enzyme also catalyzes the hydrolysis of the $\alpha(1 \rightarrow 6)$ -linked residue at the branch point.

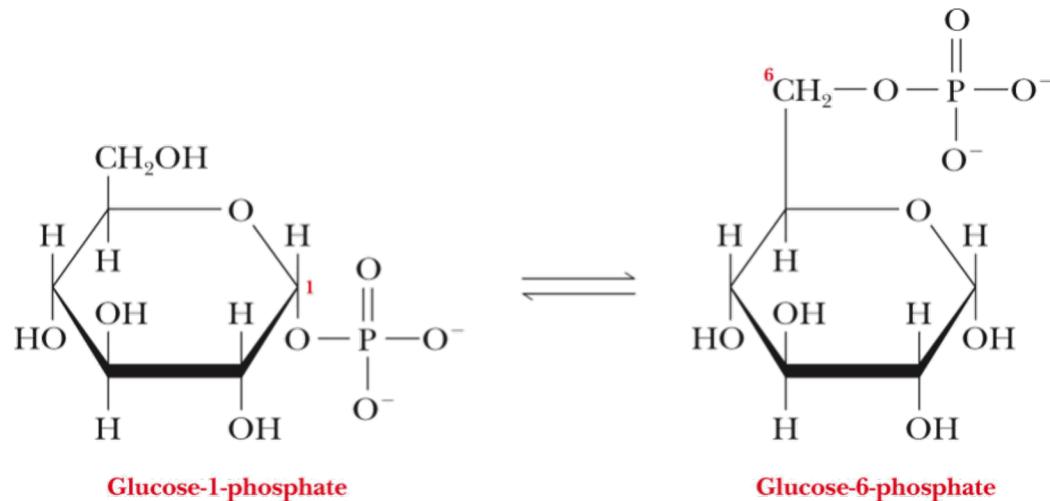
Debranching Glycogen: remodeling

- Complete breakdown requires debranching enzymes to degrade the $\alpha(1-6)$ linkage
- Glycogen debranching enzyme (transferase) shift a block of 3 residues from one outer branch to the other
- The residue remaining at the branch point is hydrolyzed by further action of debranching enzyme to yield free glucose.
 - The new elongated branched is subject to degradation by glycogen phosphorylase



Glycogen Breakdown

- In the second reaction, glucose-1-phosphate is isomerized to Glucose-6-phosphate
 - Saves ATP !
- Glucose-6-phosphate is formed in the first step of glycolysis through the action of either hexokinase or glucokinase
 - Requires ATP
- This reaction is catalyzed by phosphoglucomutase
 - G6P enter into glycolysis in muscle
 - hydrolysis to glucose in liver and transfer to blood stream



Glycogen Synthesis

- Glycogen biosynthesis and breakdown must occur by separate pathways.
- The formation of glycogen from glucose is not the exact reversal of the breakdown of glycogen to glucose.
- The enzymes catalyzing the 3 steps involved in the glycogen synthesis pathways are:-
 1. UDP-glucose pyrophosphorylase
 2. Glycogen synthase
 3. Glycogen branching enzyme

Formation of glycogen from glucose

- The synthesis of glycogen requires energy, which is provided by the hydrolysis of UTP.
 - Glucose-1-phosphate reacts with UTP to make UDPG
- UDPG is then added to a growing chain of glycogen, catalyzed by glycogen ***UDPG pyrophosphorylase***



- (by ***UDPG pyrophosphorylase***)
- Pyrophosphate is also formed

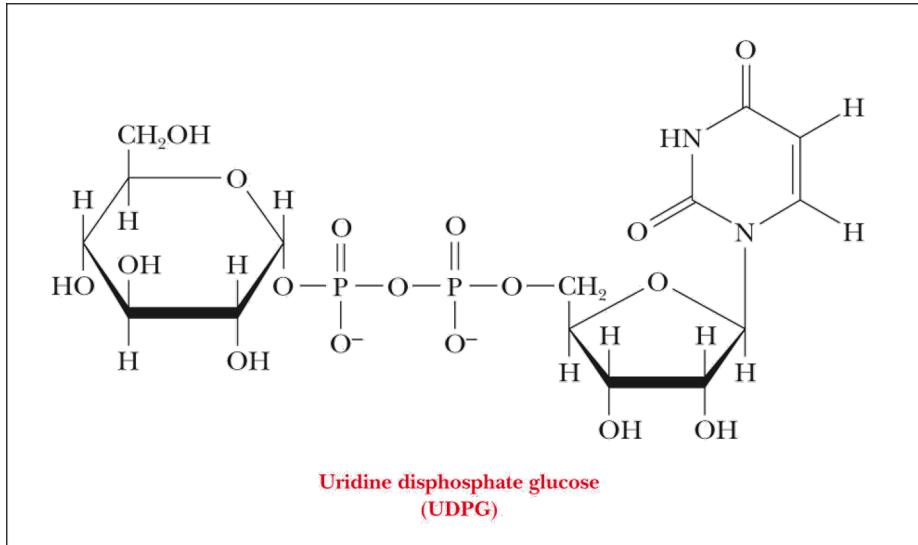


How is Glycogen formed from Glucose?

- Coupling of UDPG formation with hydrolysis of Pyrophosphate drives formation of UDPG to completion

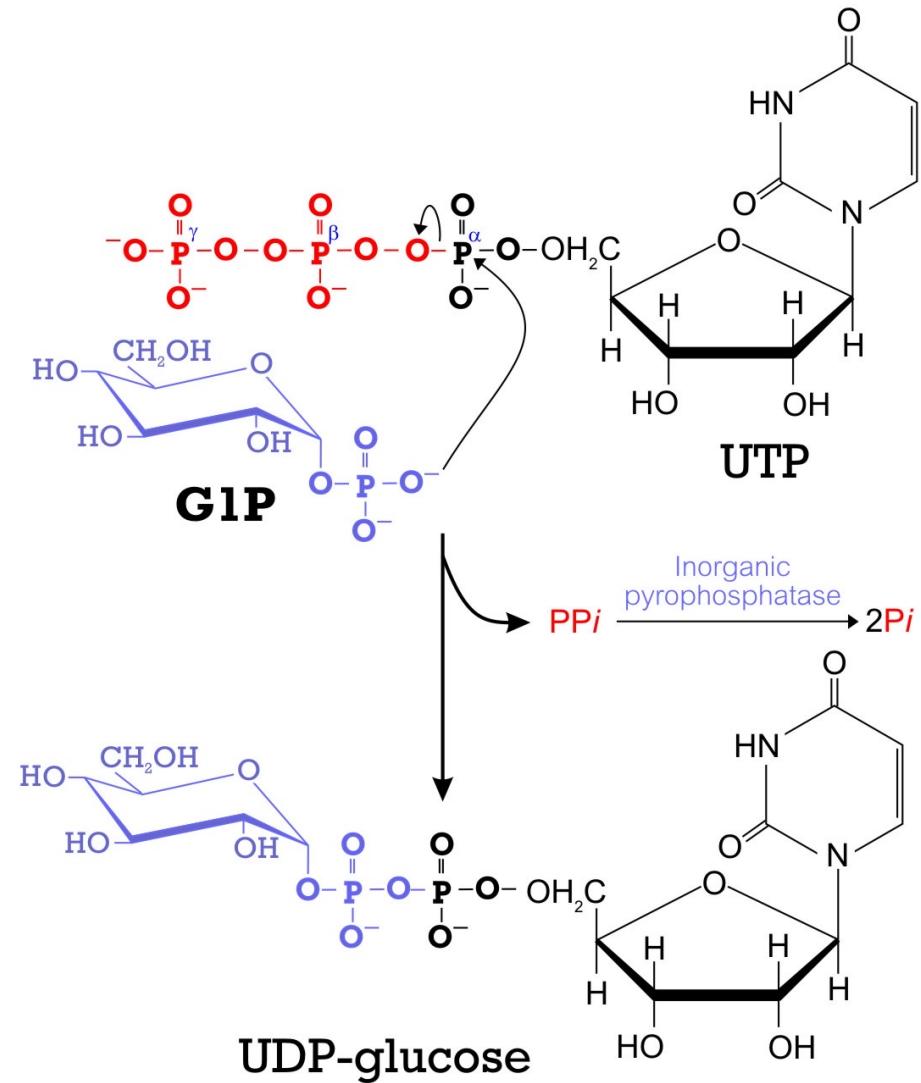
	$\Delta G^\circ'$	
	kJ mol^{-1}	kcal mol^{-1}
Glucose-1-phosphate + UTP \rightleftharpoons UDPG + PP _i	~0	~0
H ₂ O + PP _i \rightarrow 2P _i	-30.5	-7.3
Overall Glucose-1-phosphate + UTP \rightarrow UDPG + 2P _i	-30.5	-7.3

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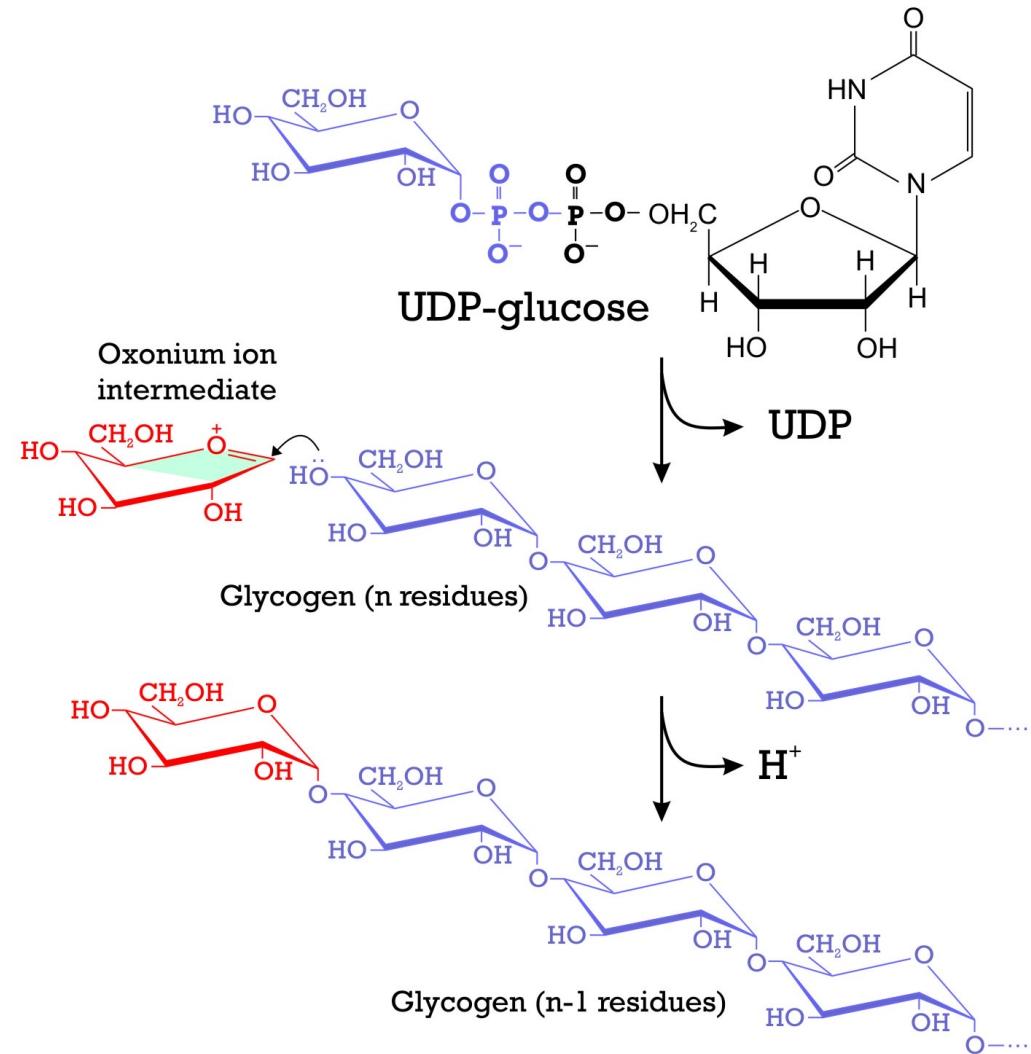
UDPG – glucose pyrophosphatase

- Phosphoryl oxygen of G1P attacks the alpha phosphorus atom of UTP to form UDPG and release PPi.



Reaction catalyzed by Glycogen Synthase

- The **key regulatory enzyme** in glycogen synthesis
- The glycosyl unit of UDPG is transferred to the **C4 – OH** group on one of glycogen's nonreducing ends to form a **alpha (1→4) glycosidic bond**
- The glycosyl synthase is thought to involve a glycosyl oxonium ion intermediate



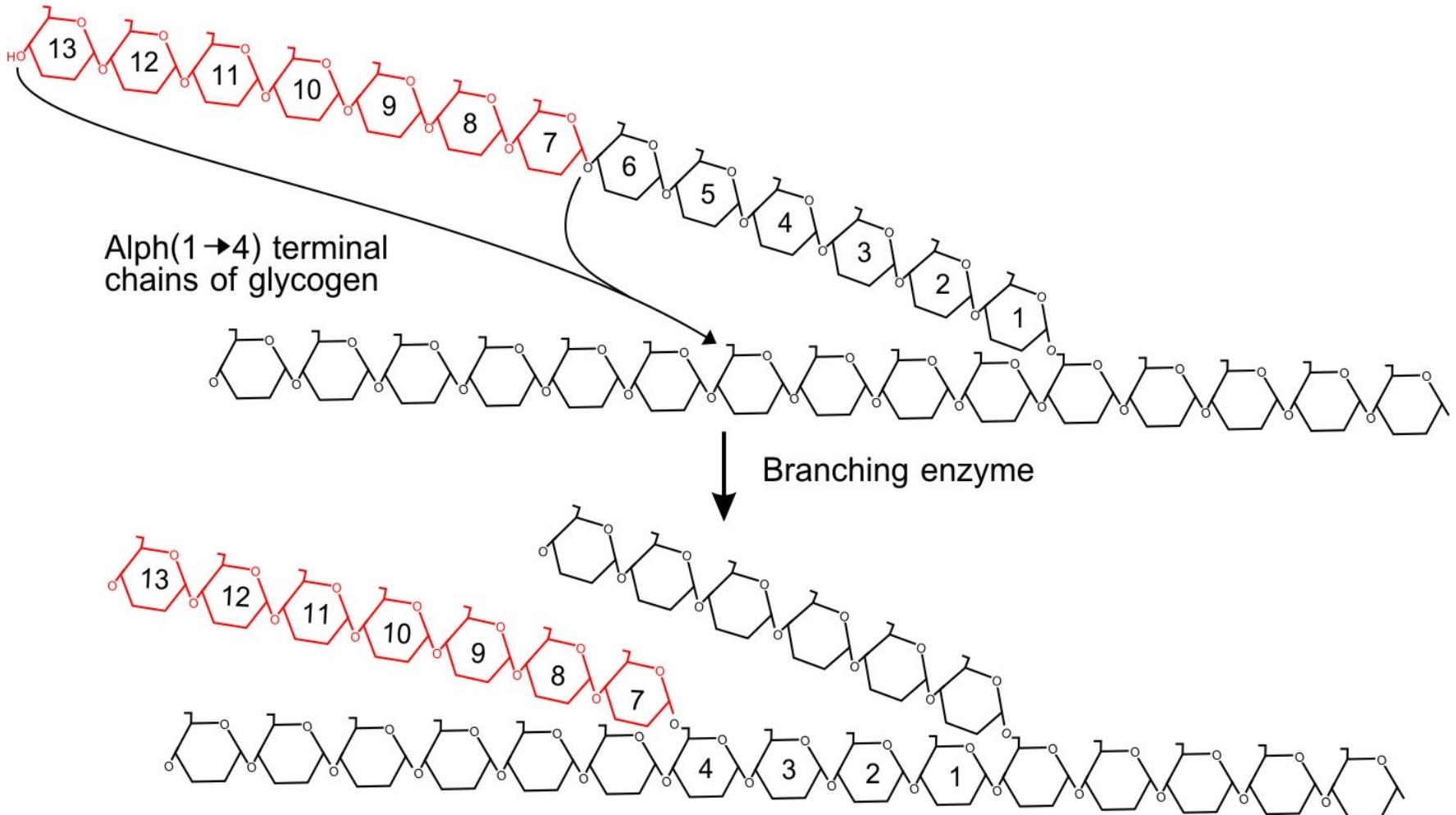
Glycogen Synthase

- Glycogen synthase can only extend an already existing alpha (1→4) glycosidic bond.
 - Glycogen synthase cannot simply link together 2 glucose residues
- So that first step in glycogen synthesis is the self-catalyzed attachment of a glucose residue to the Tyr 194 **OH group** of a protein named “**Glycogenin**” (a primer)
 - **Glycogenin** initiates glycogen synthesis.
 - Glycogenin is an enzyme that considered as glucose acceptor.
 - Act as catalyst for Glucose addition until 8 units
 - Glycogen synthase take over

Glycogen Branching

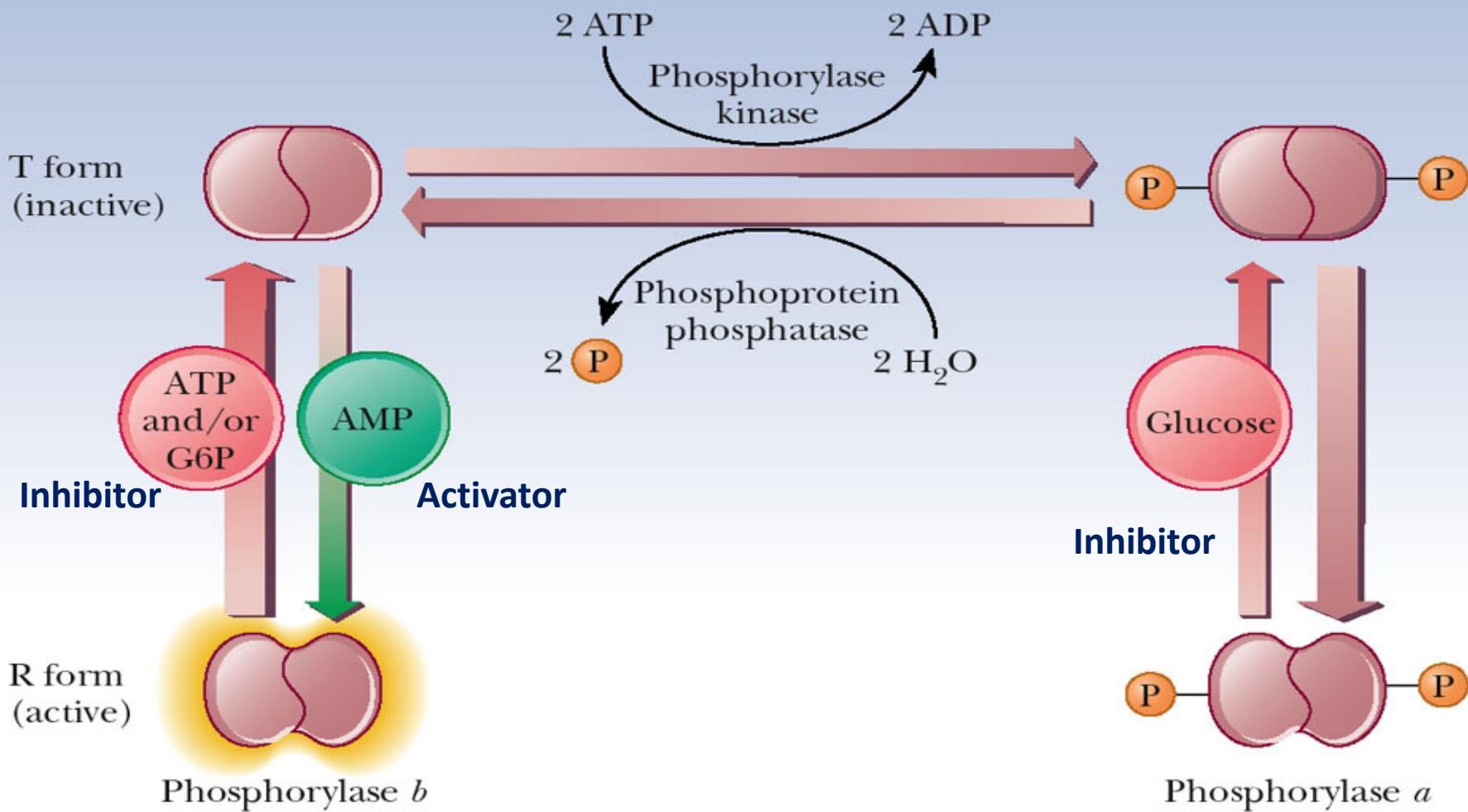
- Branches are created by the **transfer of terminal chain segments** consisting of **~7 glucosyl residues** to **C6 – OH group of glucose** residues on the same or another glycogen chain
 - Each transferred segment must come from a chain of at **least 11 residues**, and new branch point must be at **least 4 residues away** from the other branch point
- **Debranching** involves breaking alpha(1—6) and reforming alpha(1→4) glycosidic bonds
- **Branching** involves breaking alpha(1→4) glycosidic bonds and reforming alpha(1→6) linkages.

Glycogen Branching



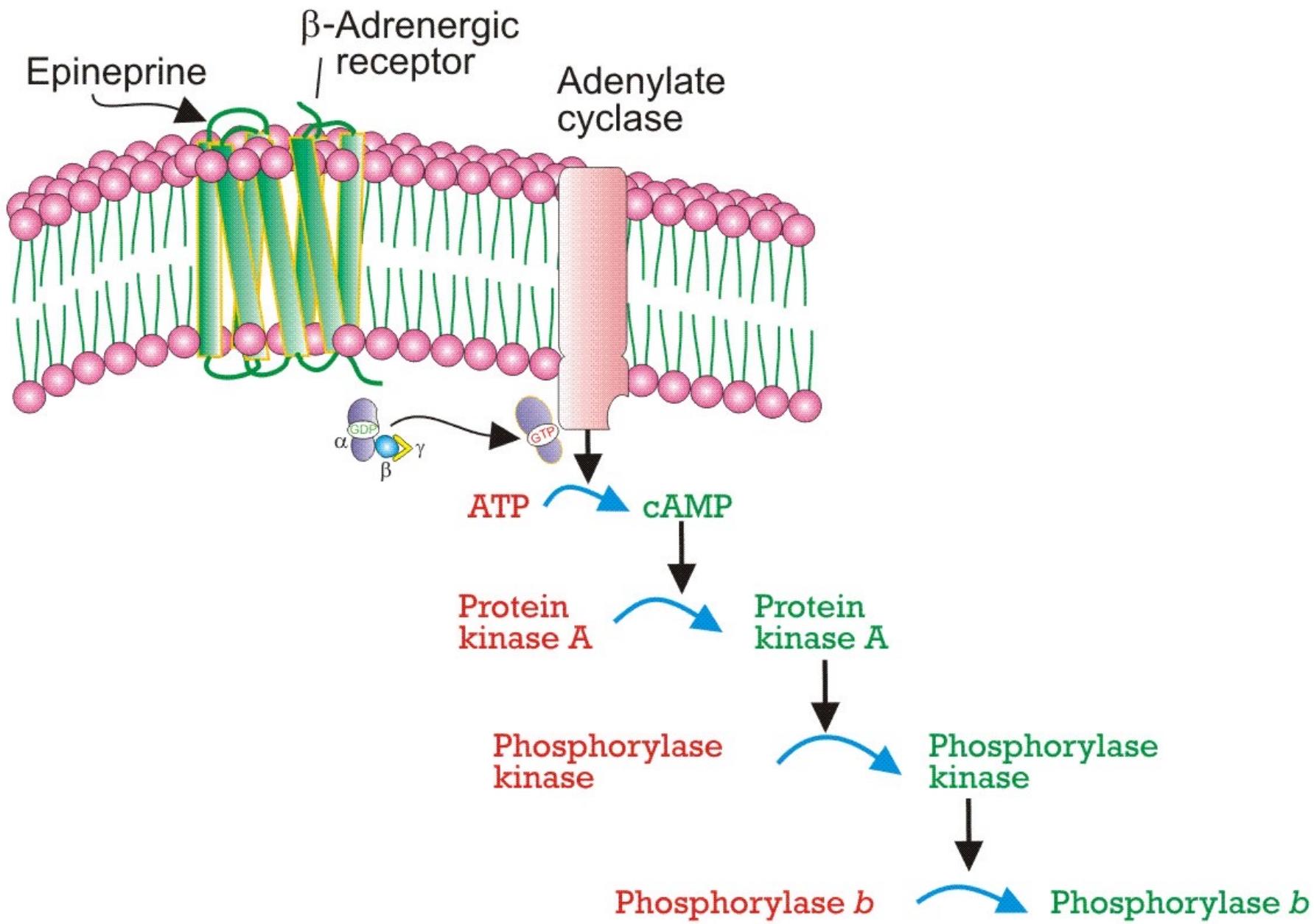
Phosphorylase is regulated by allosteric interactions and reversible phosphorylation

- Glycogen metabolism is precisely controlled by multiple interlocking mechanisms, and focus on **glycogen phosphorylase**.
- **Phosphorylase** is regulated by several **allosteric effectors** that:
 - Signal the energy state of the cell
 - Responsive to **hormones**:- **insulin**, **glucagon** and **epinephrine**



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Glycogen phosphorylase activity is subject to allosteric control and covalent modification: Phosphorylation of the **a form** of the enzyme converts it to the **b form**. Only the T form is subject to modification and de-modification. The **a** and **b** forms respond to different allosteric effectors.



Hormonal Control

Increase Glycogen Breakdown

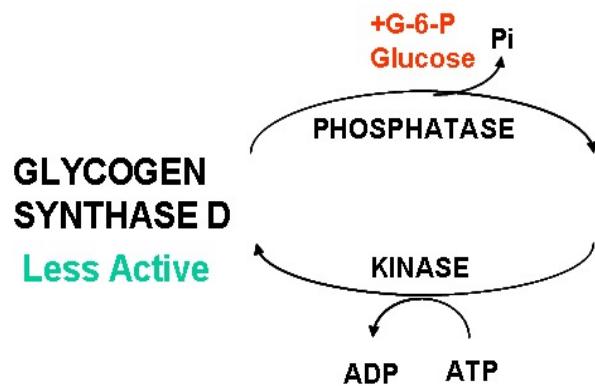
- Low Glucose
- \uparrow Glucagon
- \uparrow Epinephrine
 - B-adrenergic receptor (muscle)
 - \uparrow cAMP
 - A-adrenergic receptor (liver)
 - \uparrow Glucagon
 - \uparrow cAMP
 - \uparrow Ca^{2+}

Increase Glycogen Synthesis

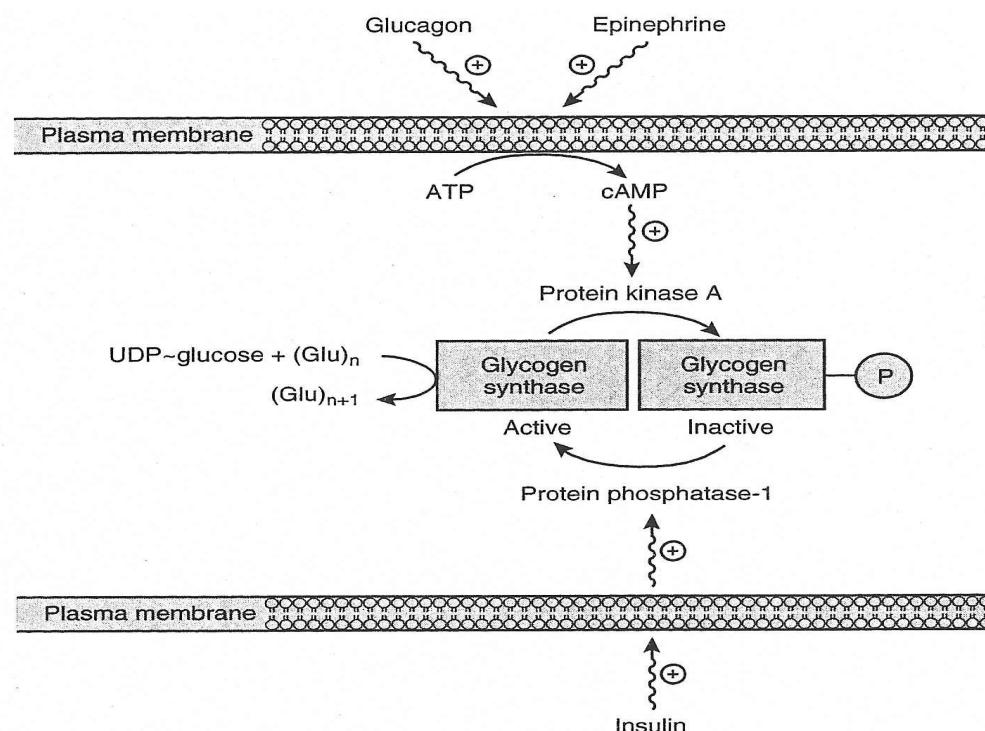
- High Glucose
- Insulin
 - GLUT4
 - Muscle, fat
 - \downarrow cAMP

Control of Glycogen Metabolism

- When cAMP decrease, phosphorylation rate decrease, dephosphorylation increase and the fraction of enzymes in their dephosphorylated form increase
- The resultant activation of glycogen synthase, and the inhibition of glycogen phosphorylase cause a change in the flux direction toward net glycogen synthesis

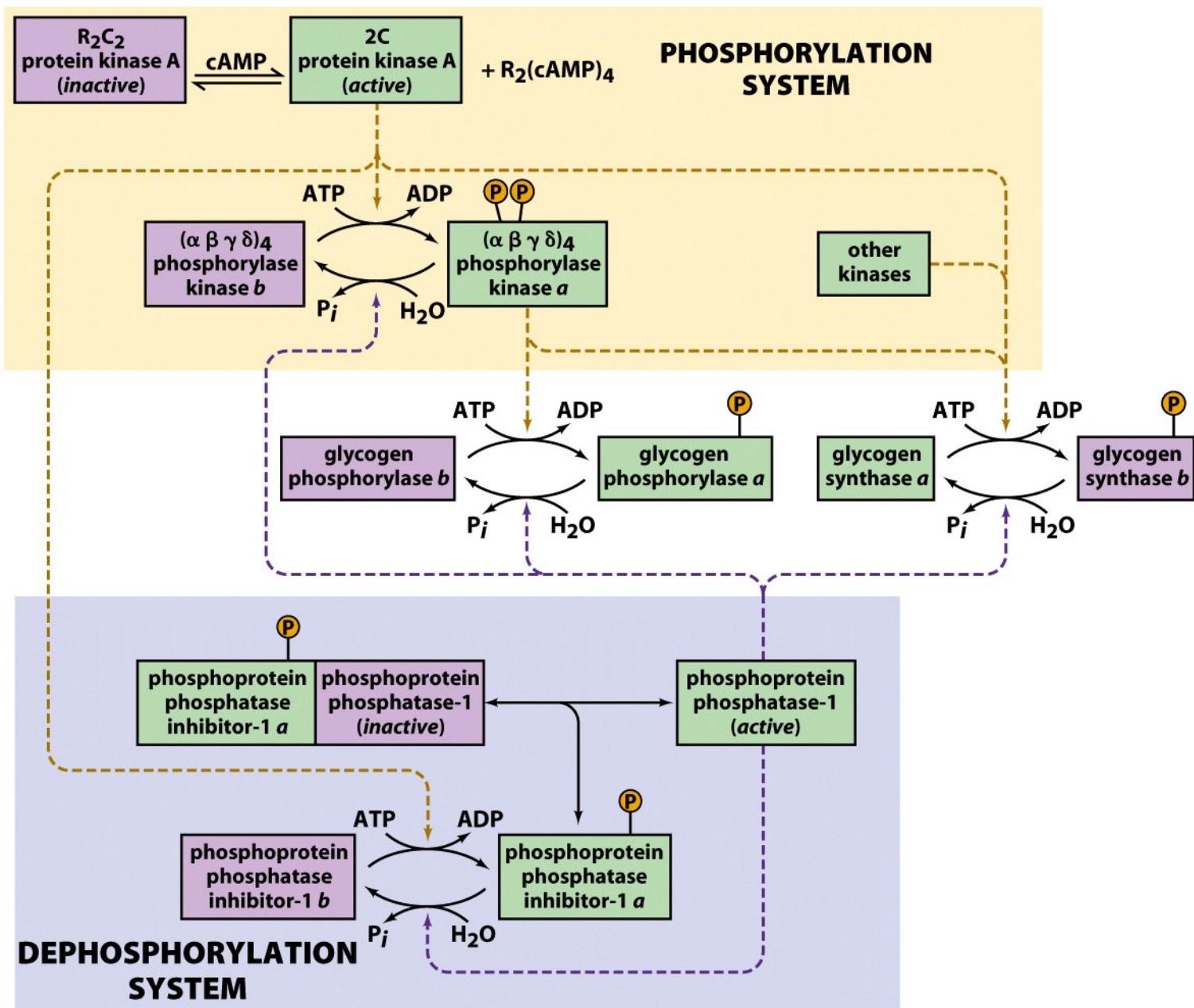


Activated by G-6-P



Control of Glycogen Metabolism

- The covalent modification is under hormonal control
 - Hormonal signals (glucagon or epinephrine) stimulate glycogen synthase phosphorylation
 - After phosphorylation, glycogen synthase becomes inactive at the same time the same hormonal signal is activating phosphorylase
 - Glycogen synthase can be phosphorylated by several other enzymes including phosphorylase kinase
 - Dephosphorylation is by phosphoprotein phosphatase

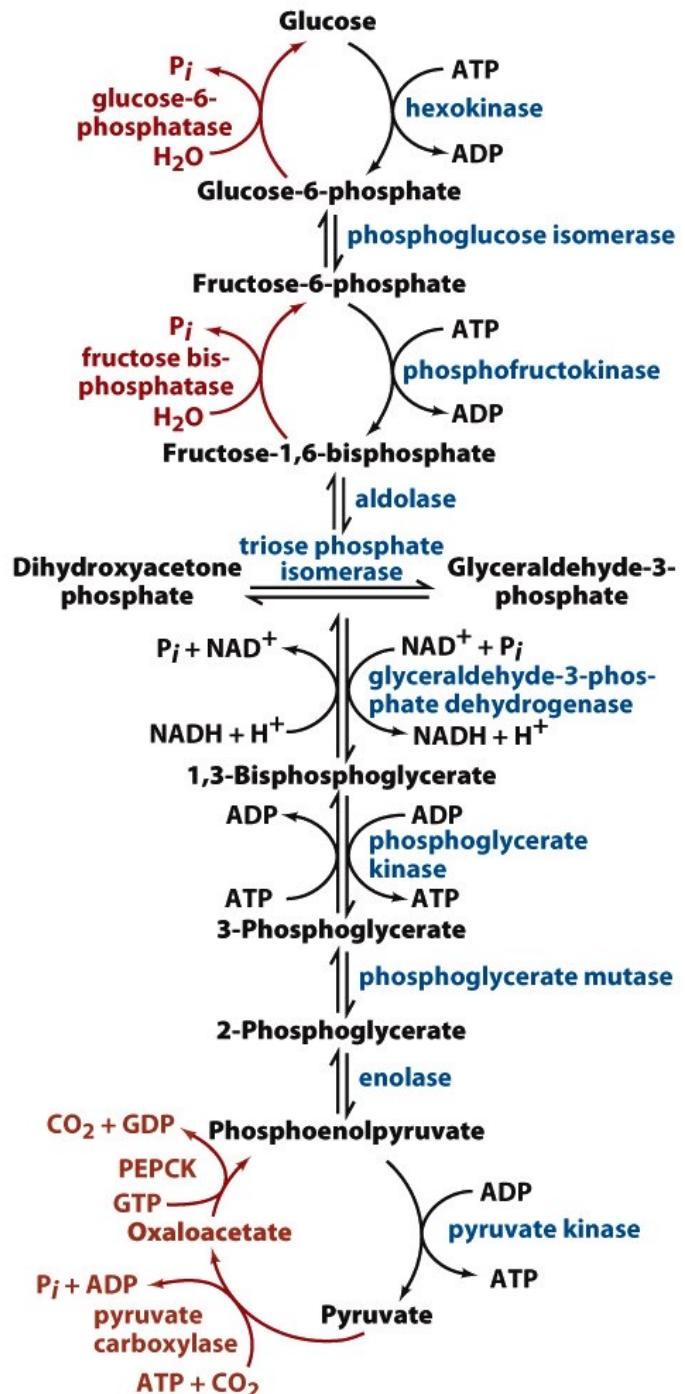


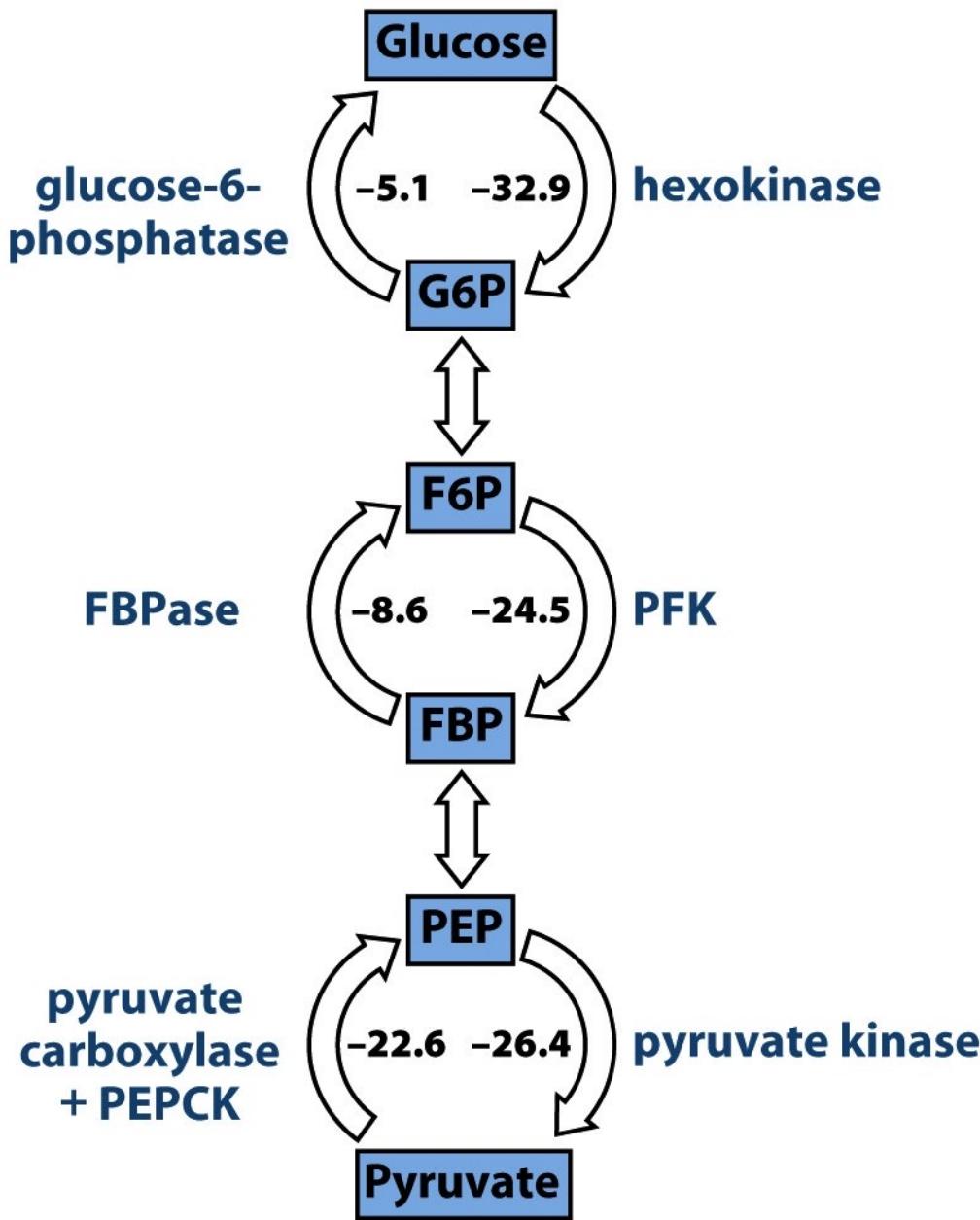
Gluconeogenesis

- Gluconeogenesis: pyruvate → glucose
 - Gluconeogenesis is not exact reversal of glycolysis; that is, pyruvate to glucose does not occur by reversing the steps of glucose to pyruvate
 - Three Glycolysis reactions have such a large negative DG that they are essentially irreversible.
 1. **Hexokinase** (Glucose to glucose-6-phosphate)
 2. **Phosphofructokinase** (Fructose-6-phosphate to fructose-1,6-bisphosphate)
 3. **Pyruvate Kinase** (Phosphoenolpyruvate to pyruvate + ATP)
 - Net result of gluconeogenesis is reversal of these three steps, but by different pathways and using different enzymes

Gluconeogenesis

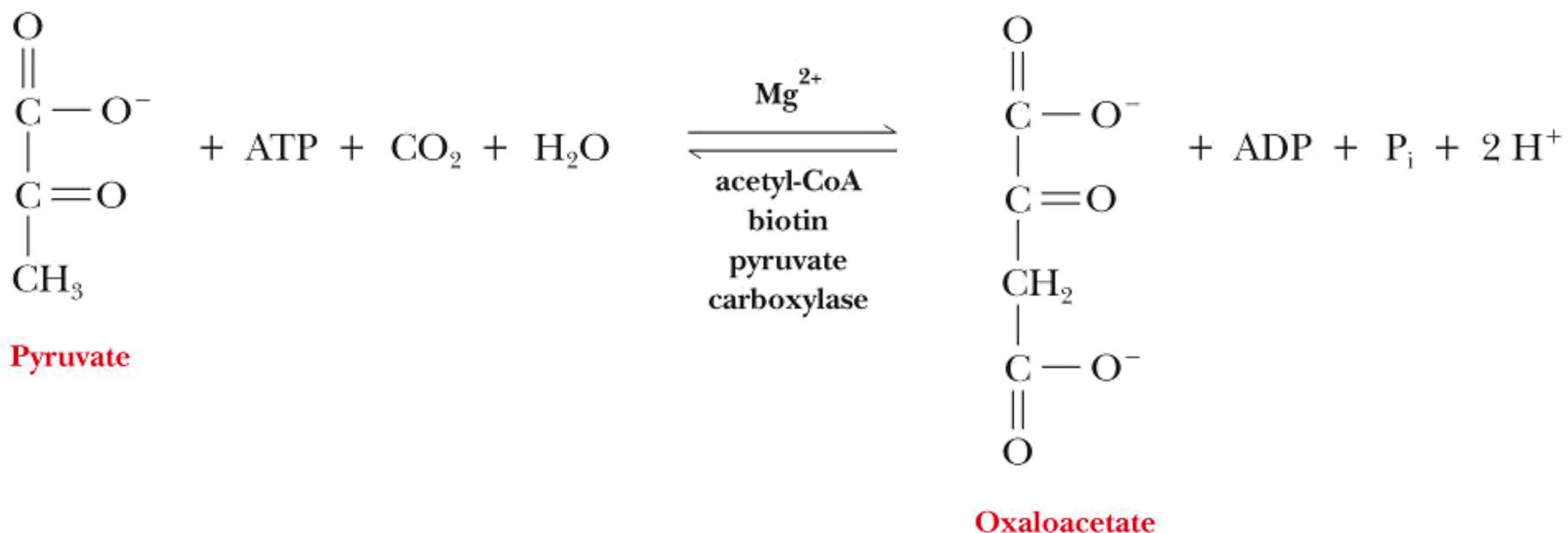
- Most of Gluconeogenesis is Glycolysis in reverse.
- Only irreversible steps must be different
- Oxaloacetate is the required molecule to start gluconeogenesis





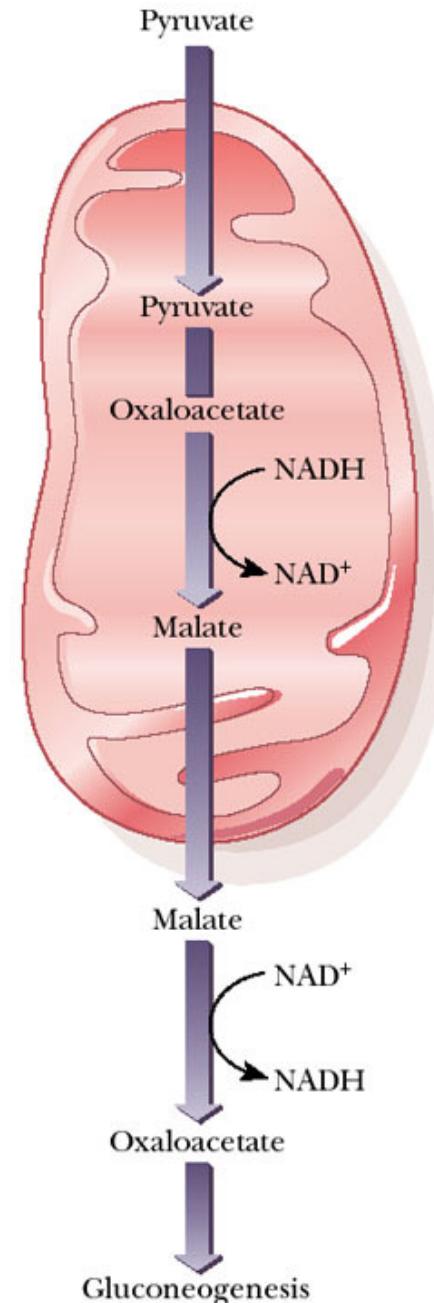
Oxaloacetate is an Intermediate

- In first step, pyruvate is carboxylated to oxaloacetate
 - Requires Biotin (**CO₂ carrier**)
 - Requires ATP
 - Pyruvate carboxylase (mitochondria) is subject to allosteric control; it is activated by Acetyl CoA



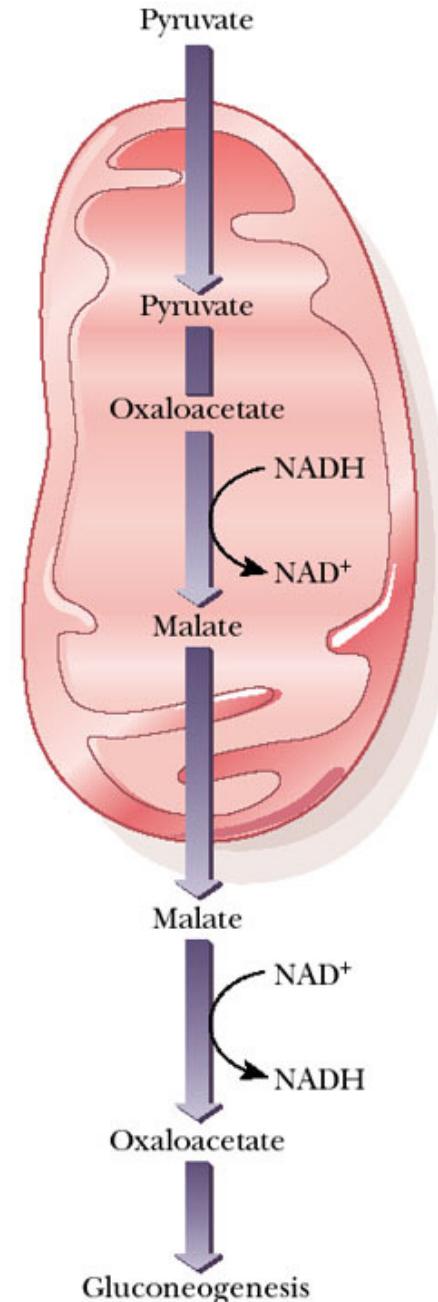
Fate of Oxaloacetate

- Oxaloacetate formed in mitochondria (trapped)
 - Must leave because all the rest of the gluconeogenesis enzymes are in the cytosol
- Malate dehydrogenase a mitochondrial enzyme change Oxaloacetate to malate
 - $\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{malate} (\text{mitochondria}) + \text{NAD}^+$
 - Malate can be transported through the mitochondrial membrane
- Malate dehydrogenase cytosolic enzyme



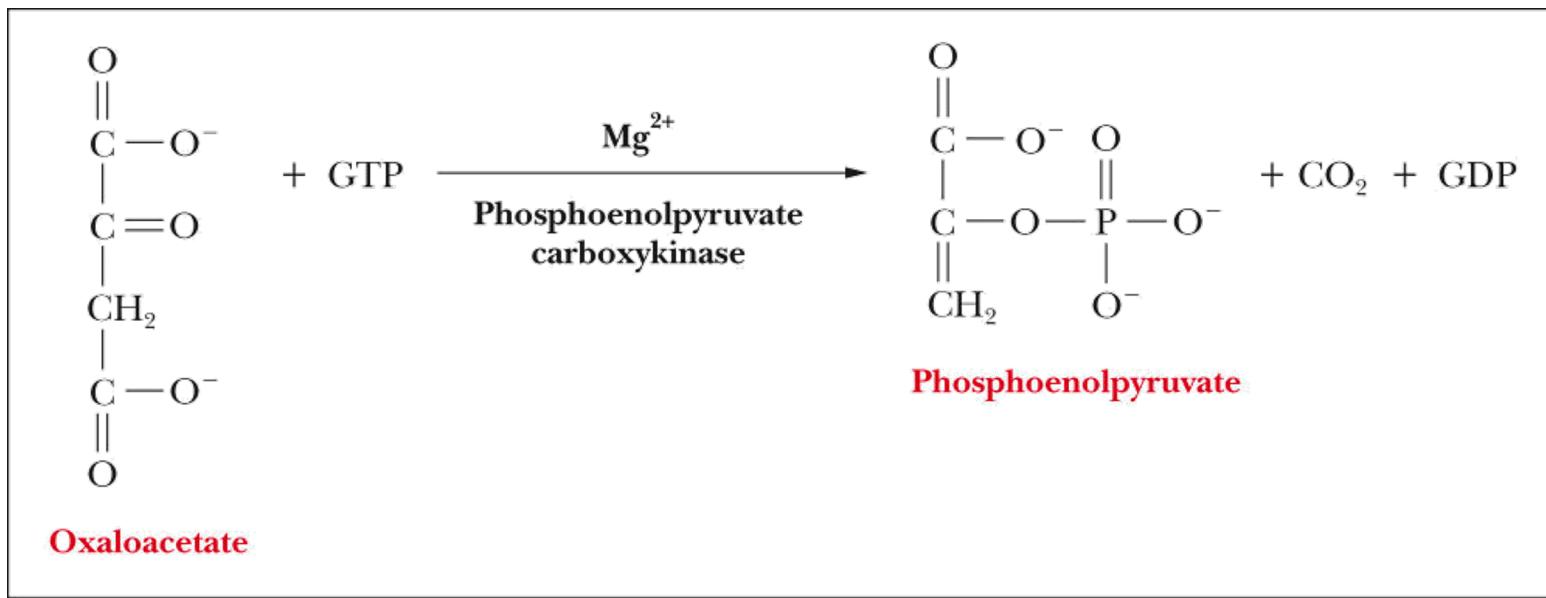
Fate of Oxaloacetate

- The GTP-dependent decarboxylation of oxaloacetate
- Enzyme = **PEP carboxykinase** is a *cytosolic and mitochondria* enzyme
- Oxaloacetate continues to form PEP (in cytoplasm or mitochondria)
- PEP leaves mitochondria to continue the Gluconeogenesis in cytoplasm



Gluconeogenesis

- Next, decarboxylation of oxaloacetate is coupled with phosphorylation by Carboxikinase to give PEP



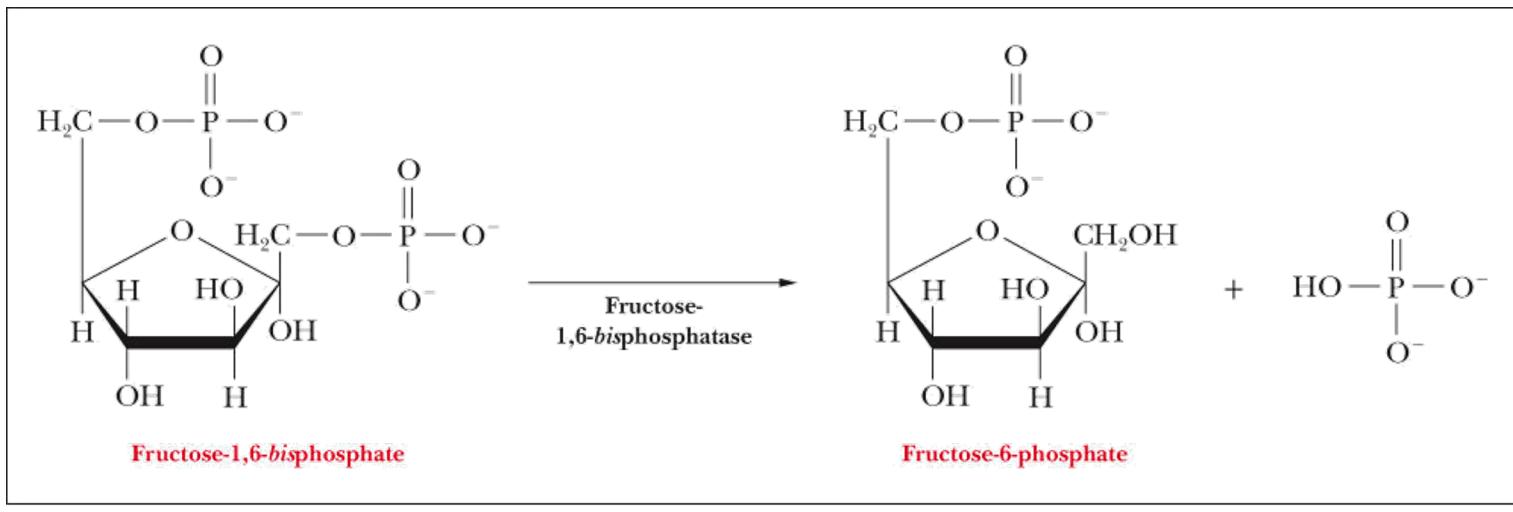
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- The net reaction of carboxylation/decarboxylation is at equilibrium as small amount accumulated will drive the reaction
 - Law of mass action (relates concentration of R and P in equ

Pyruvate + ATP +GTP → Phosphoenolpyruvate + ADP + GDP + Pi

Role of Sugar Phosphates

- Other different reactions in gluconeogenesis relative to glycolysis involve phosphate-ester bonds bound to sugar-hydroxyl groups being hydrolyzed

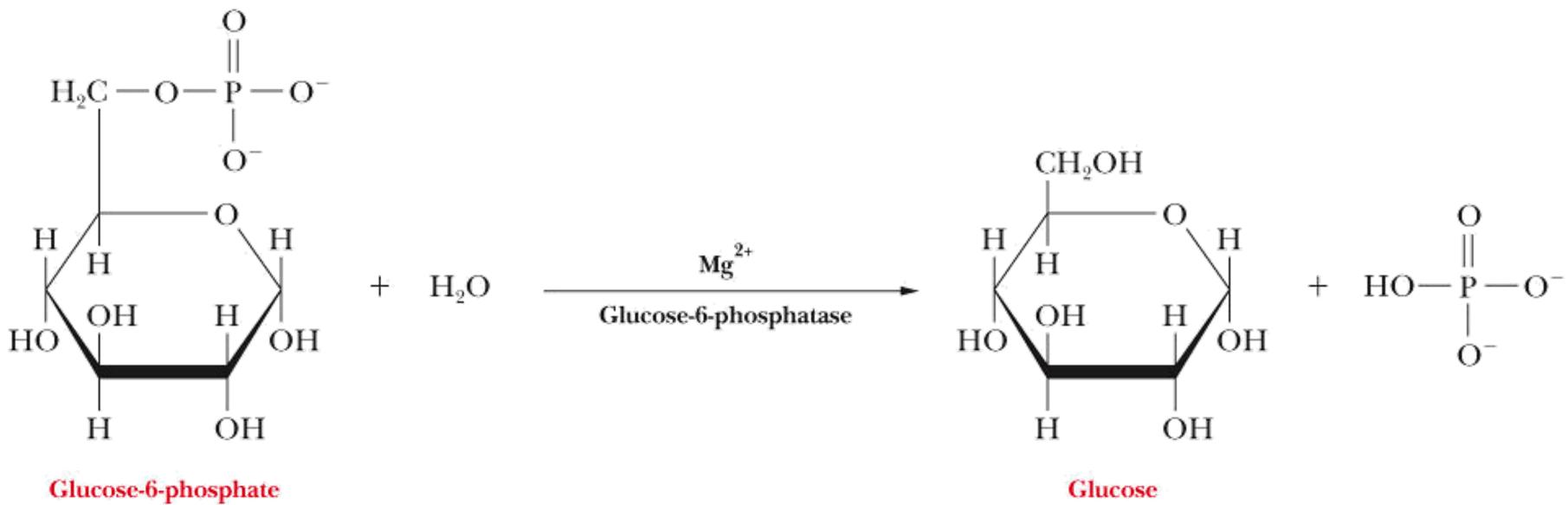


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- $\Delta G^\circ = -16.7 \text{ kJ mol}^{-1}$
- Fructose-1,6-bisphosphatase is an allosteric enzyme, inhibited by AMP and activated by ATP

Role of Sugar Phosphates (Cont'd)

- Another reaction is the hydrolysis of glucose-6-phosphate to glucose and phosphate ion
 - Takes place in ER



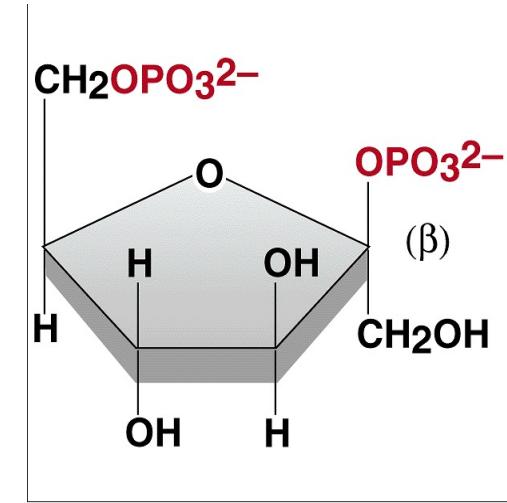
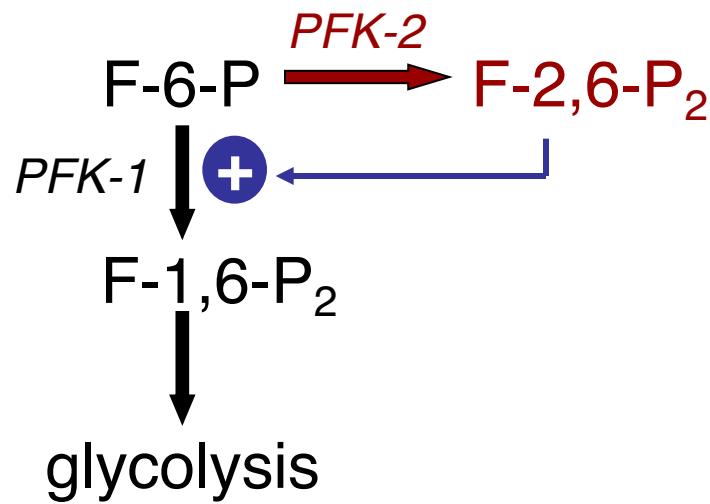
- Reaction also spontaneous ($\Delta G^\circ = -13.8 \text{ kJ mol}^{-1}$)
- Reaction catalyzed by glucose-6-phosphatase

Why is phosphofructokinase, rather than hexokinase, the key control point of glycolysis?

- Because glucose-6-phosphate is not only an intermediate in glycolysis. It is also involved in glycogen synthesis and the pentose phosphate pathway.
- PFK catalyzes the first unique and irreversible reaction in glycolysis.

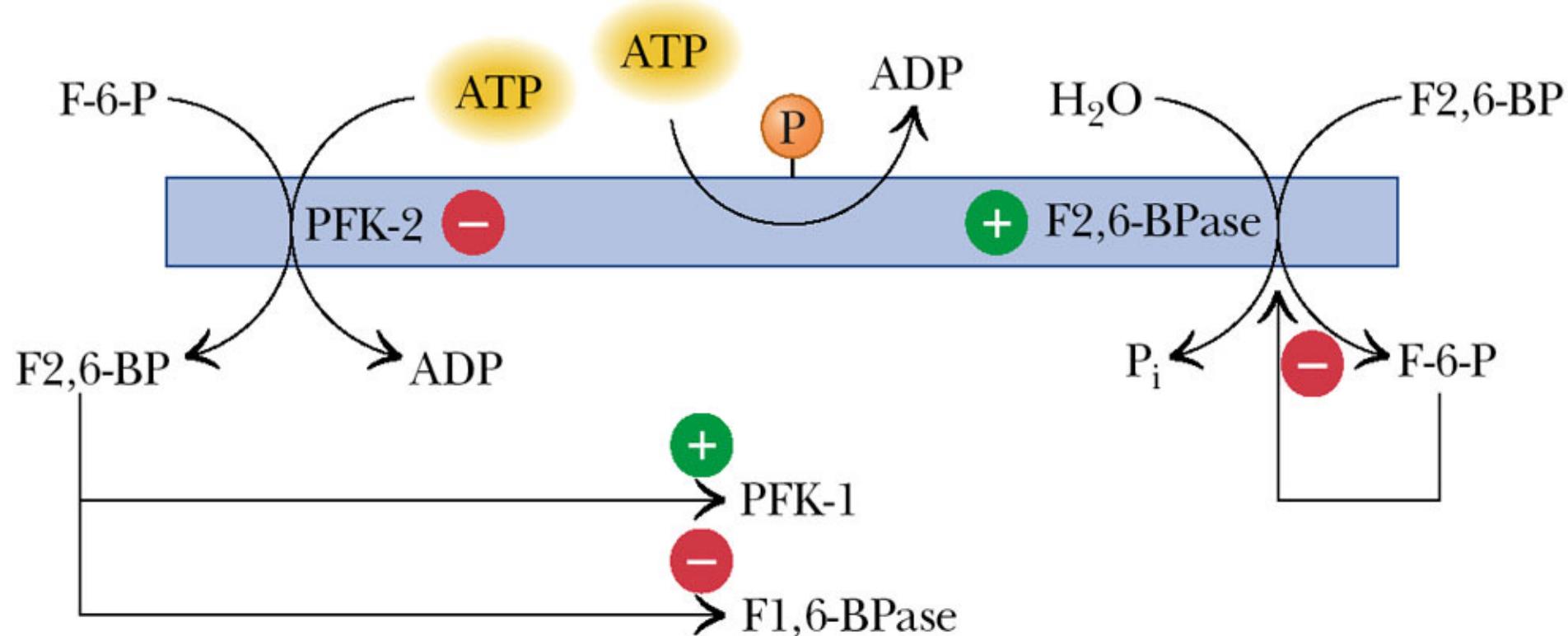
Phosphofructokinase (PFK-1) as a regulator of glycolysis

- PFK-1 activated by:
- Fructose-2,6-bisphosphate (F-2,6-P₂)



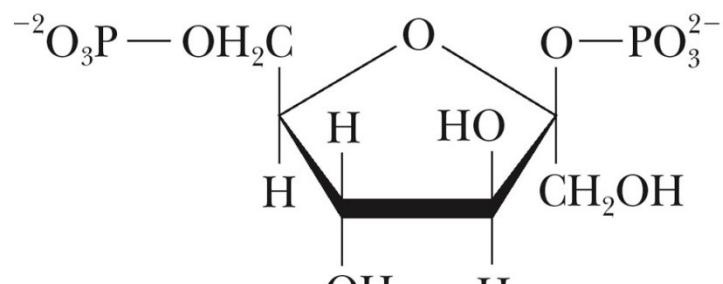
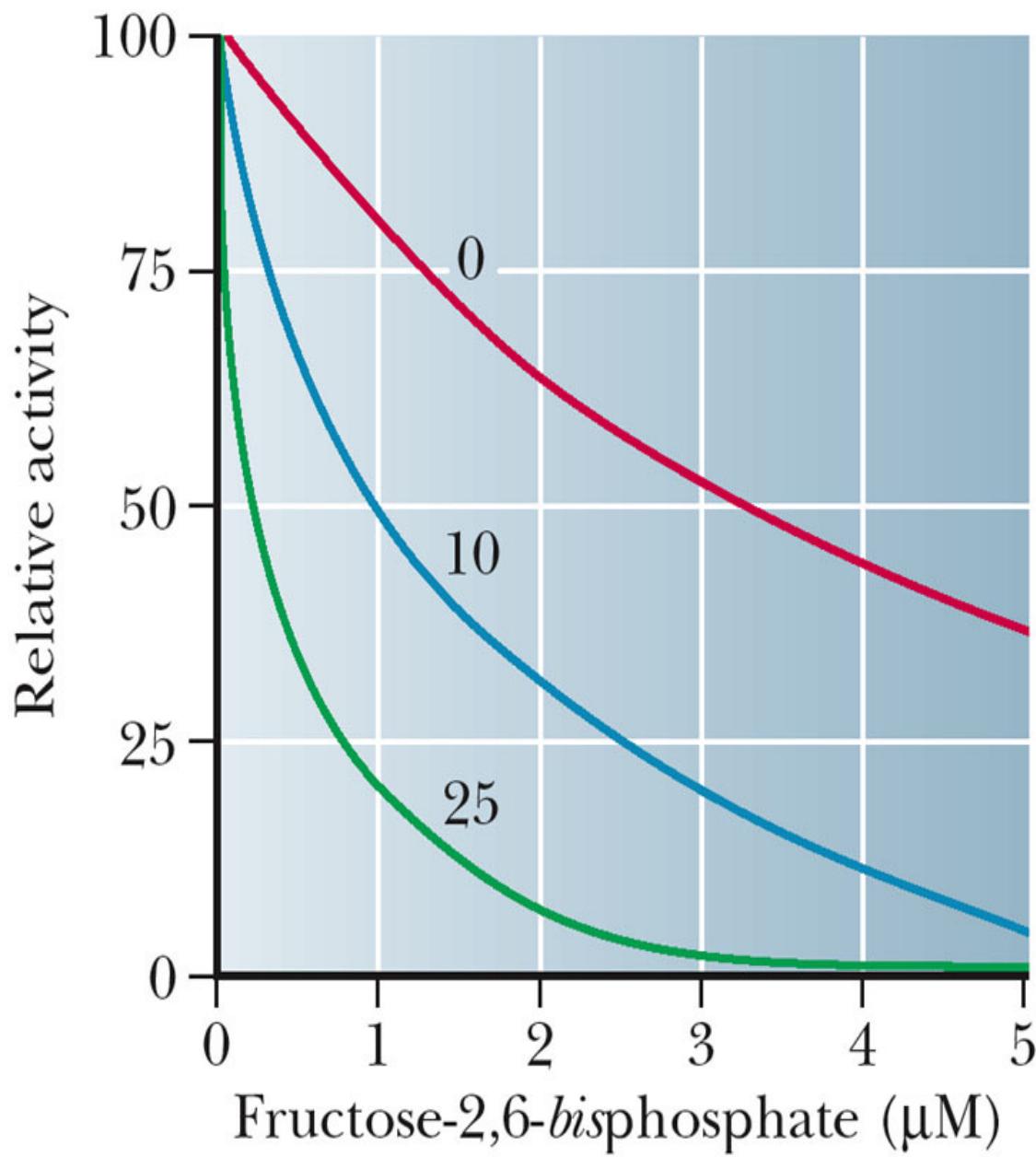
- Activates PFK-1 by increasing its affinity for fructose-6-phosphate and **diminishing the inhibitory effect of ATP**.

Protein kinase



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- The formation and breakdown of fructose-2,6-bisphosphate (F2,6P) are catalyzed by two enzyme activities on the same protein:
- These two enzyme activities are controlled by a phosphorylation/ dephosphorylation mechanism. Phosphorylation activates the enzyme that degrades F2,6P whereas dephosphorylation activates the enzyme that produces it.



**Fructose-2,6-bisphosphate
(F2,6P)**

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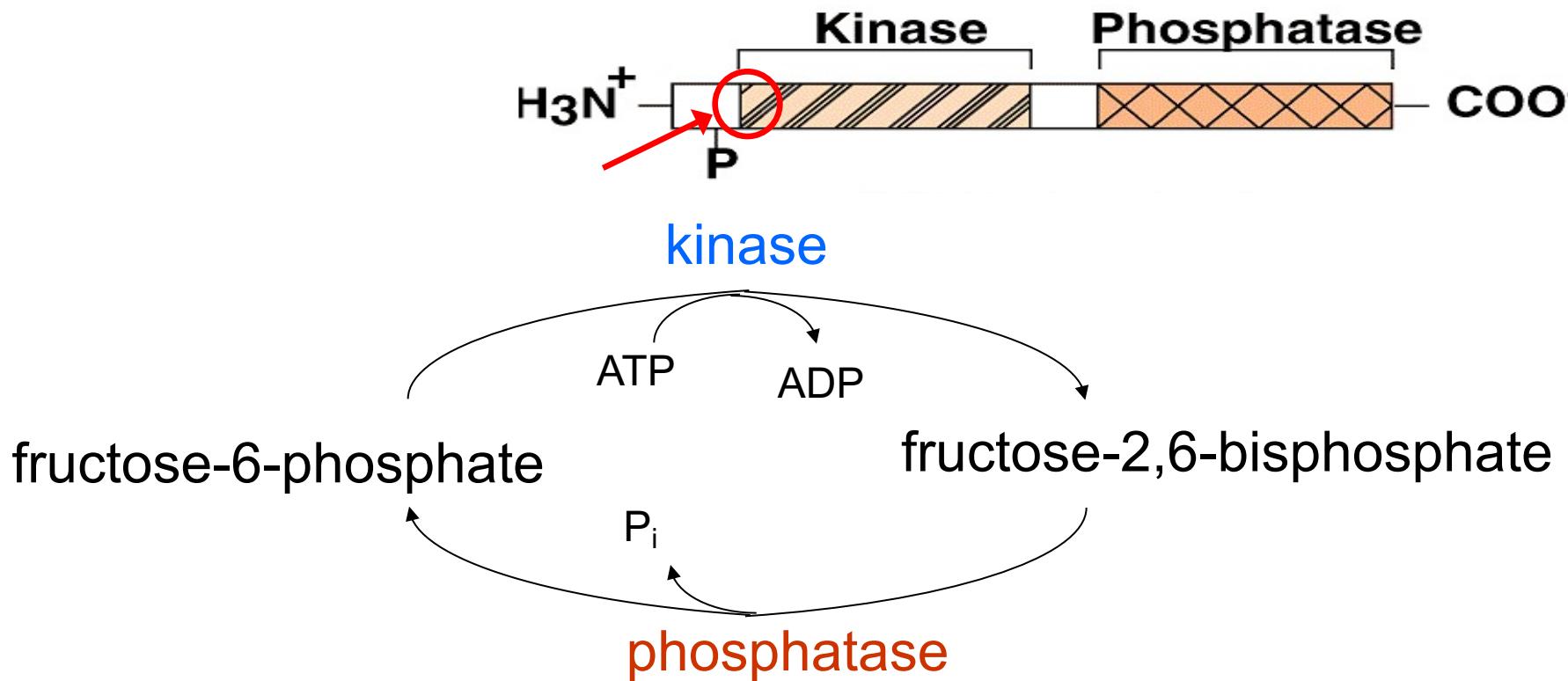
The effect of AMP (0, 10, and 25 μM [micromolar]) on the inhibition of fructose-1,6-bisphosphatase by fructose-2,6-bisphosphate.

Activity was measured in the presence of 10 μM fructose-1,6-bisphosphate.

AMP: allosteric Inhibitor to F-1,6-BisP.

Phosphofructokinase-2 (PFK-2) is also a phosphatase (bifunctional enzyme)

- Bifunctional enzyme has two activities:
 - Phosphofructokinase-2 (PFK2) activity**
 - decreased by phosphorylation
 - 2,6-Fructose bisphosphatase (FBPase) activity**
 - increased by phosphorylation



Control of Carbohydrate Metabolism

- High concentration of F2,6P stimulates glycolysis
- Low concentration stimulates Gluconeogenesis
 - Concentration of F2,6P in a cell depends on the balance between
 1. Synthesis (catalyzed by phosphofructokinase-2)
 2. Breakdown (catalyzed by fructose bisphosphatase-2)

Mechanisms of Metabolic Control

Table 18.1

Mechanisms of Metabolic Control

Type of Control	Mode of Operation	Examples
Allosteric	Effectors (substrates, products, or coenzymes) of a pathway inhibit or activate an enzyme. (Responds rapidly to external stimuli.)	ATCase (Section 7.2); Phosphofructokinase (Section 17.2)
Covalent modification	Inhibition or activation of enzyme depends on formation or breaking of a bond, frequently by phosphorylation or dephosphorylation. (Responds rapidly to external stimuli.)	Sodium–potassium pump (Section 8.6); Glycogen phosphorylase, glycogen synthase (Section 18.1)
Substrate cycles	Two opposing reactions, such as formation and breakdown of a given substance, are catalyzed by different enzymes, which can be activated or inhibited separately. (Responds rapidly to external stimuli.)	Glycolysis (Chapter 17) and gluconeogenesis (Section 18.2)
Genetic control	The amount of enzyme present is increased by protein synthesis. (Longer-term control than the other mechanisms listed here.)	Induction of β -galactosidase (Section 11.2)

Substrate cycling

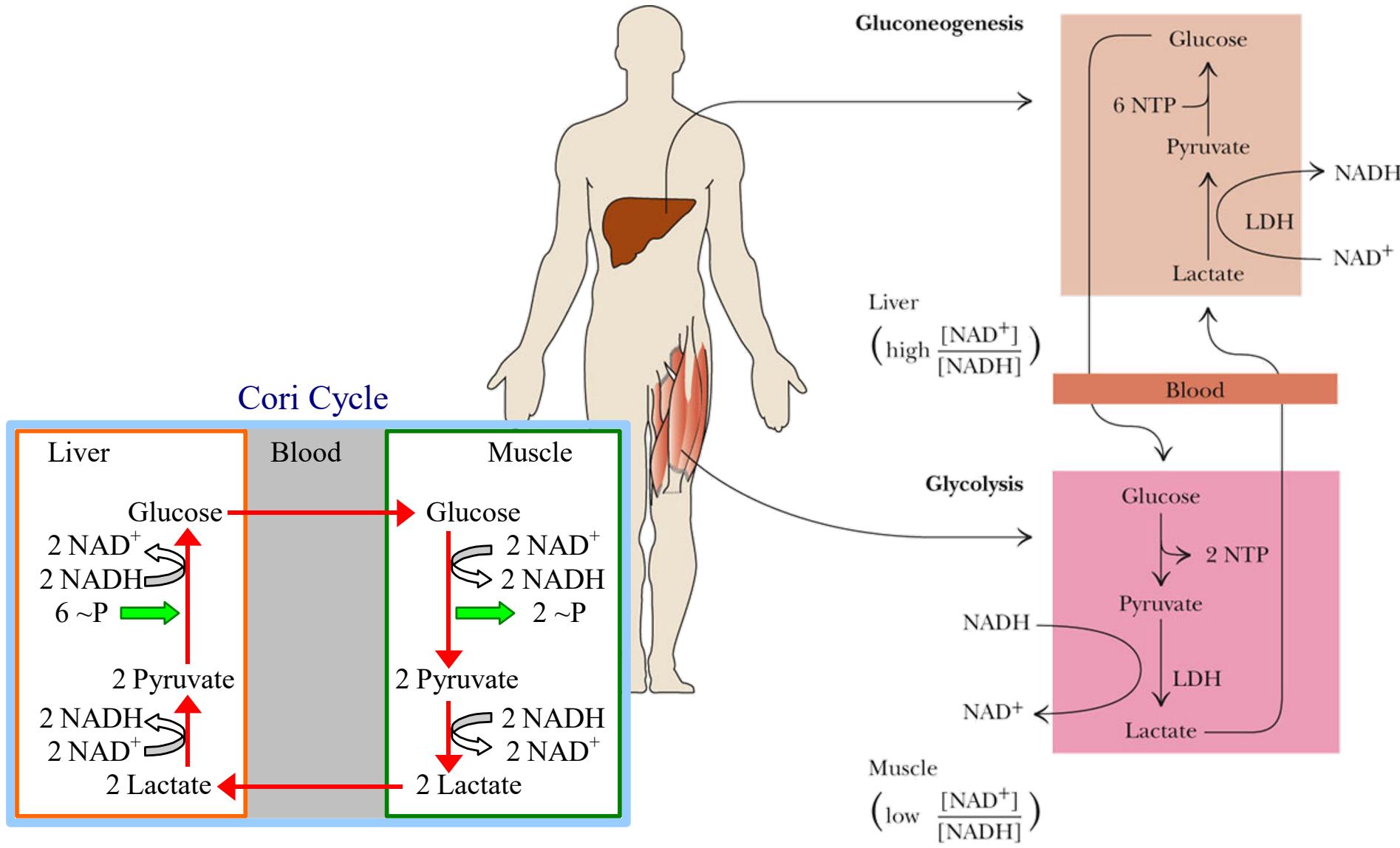
Opposing reactions can be catalyzed by different enzymes and each opposing enzyme or set of enzymes can be independently regulated

Organs Share Carbohydrate Metabolism

The Cori cycle

- Under vigorous exercise, glycolysis in muscle tissue converts glucose to pyruvate; NAD⁺ shortage, NAD⁺ is regenerated by reduction of pyruvate to lactate
- Lactate from muscle is transported to the liver, reoxidized to pyruvate, and converted to glucose
- The liver shares the stress of vigorous exercise

The Cori Cycle

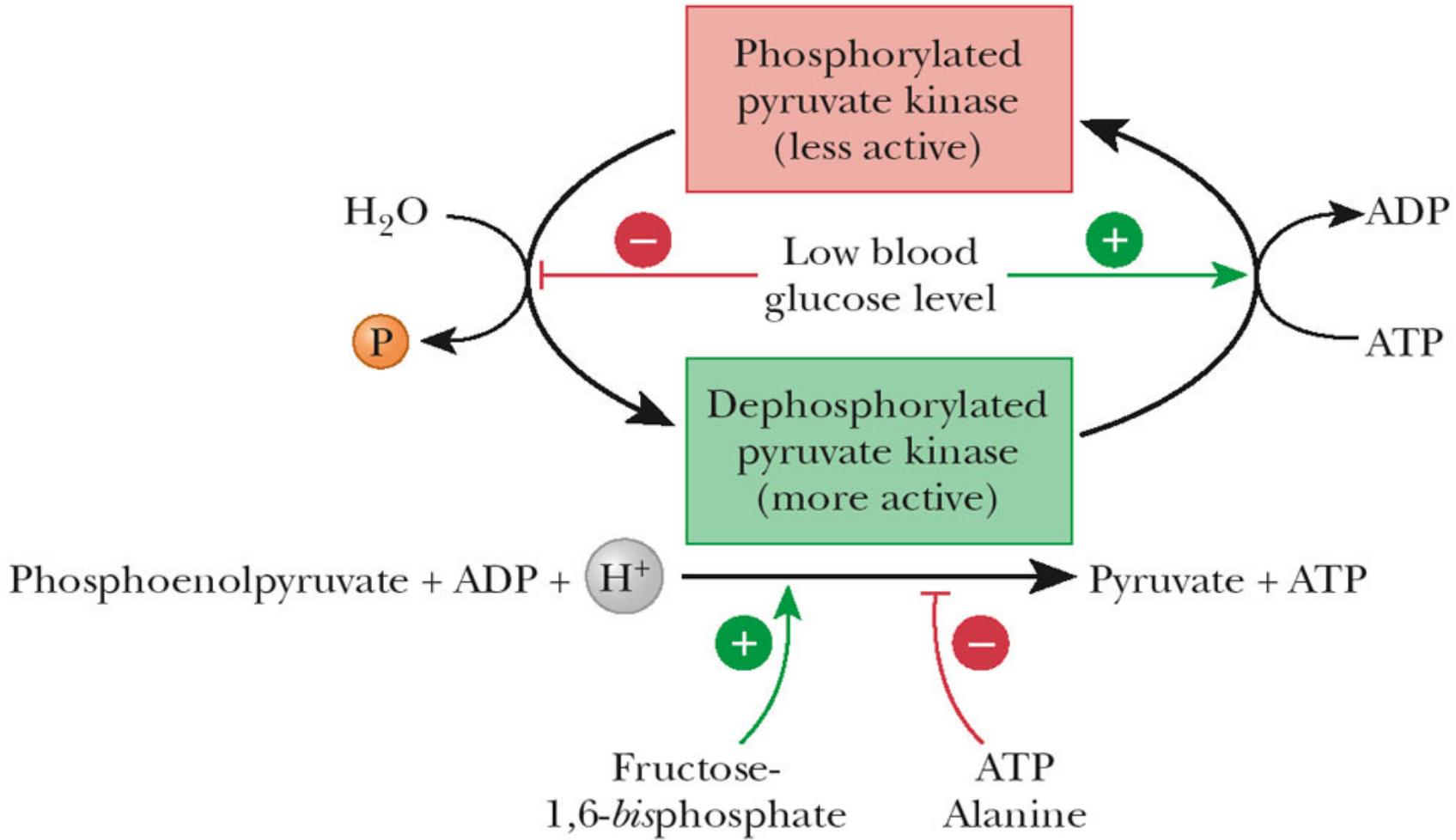


Control Points in Carbohydrate Metabolism

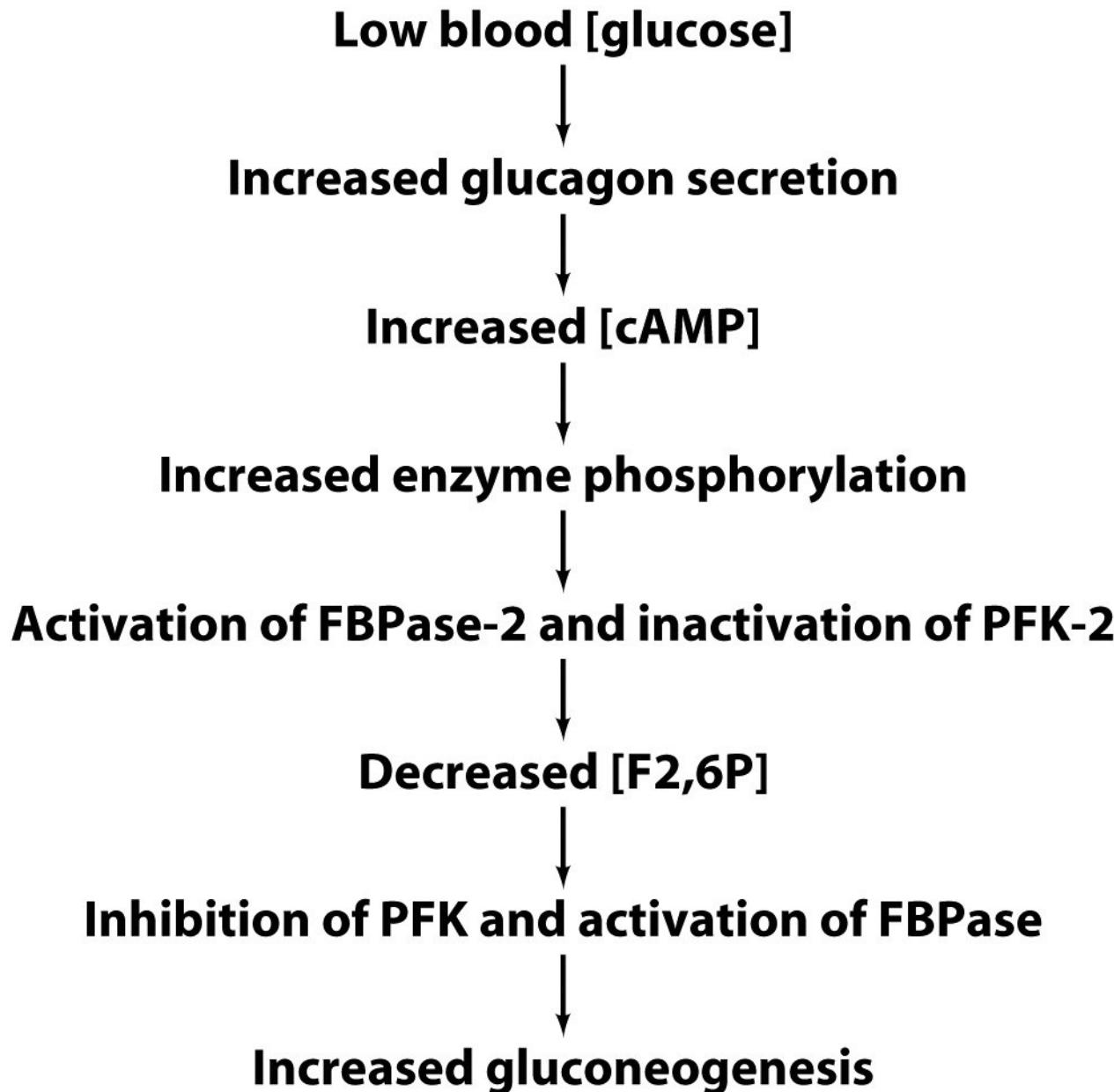
- First and last steps in glycolysis are major control points in glucose metabolism
- Hexokinase
 - Inhibited by high levels of glucose 6-phosphate
 - When glycolysis is inhibited through phosphofructokinase, glucose 6-phosphate builds up, shutting down hexokinase
- Glucokinase in liver is activated by high glucose in liver
 - Glucose ---- G6P
 - This will activate glucogenesis
 - (glycogen formation not needed for energy release)

Control Points in Carbohydrate Metabolism

- First and last steps in glycolysis are major control points in glucose metabolism
- Pyruvate kinase (PK) is an allosteric enzyme
 - Inhibited by ATP and alanine (a.a. version of pyruvate)
 - Activated by fructose-1,6-bisphosphate
- Pyruvate kinase have 3 different subunits
 - Native PK is a tetramer
 - Liver isoenzymes are subject to covalent modification
 - phosphorolyation make PK less active



- Control of liver pyruvate kinase by phosphorylation:
- When blood glucose is low, phosphorylation of pyruvate kinase is favored.
- The phosphorylated form is less active, thereby slowing glycolysis and allowing pyruvate to produce glucose by gluconeogenesis

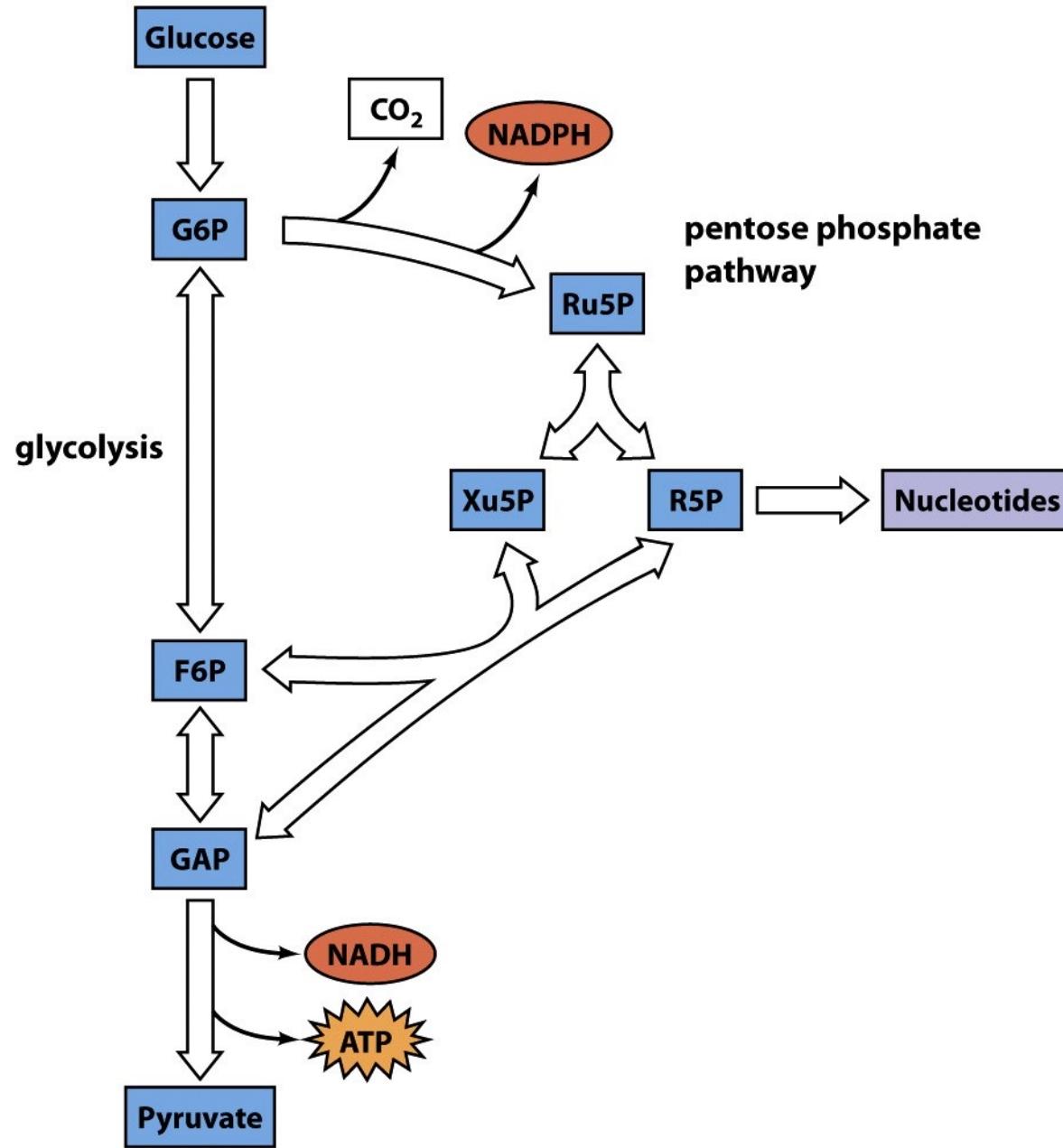


Pentose Phosphate Pathway

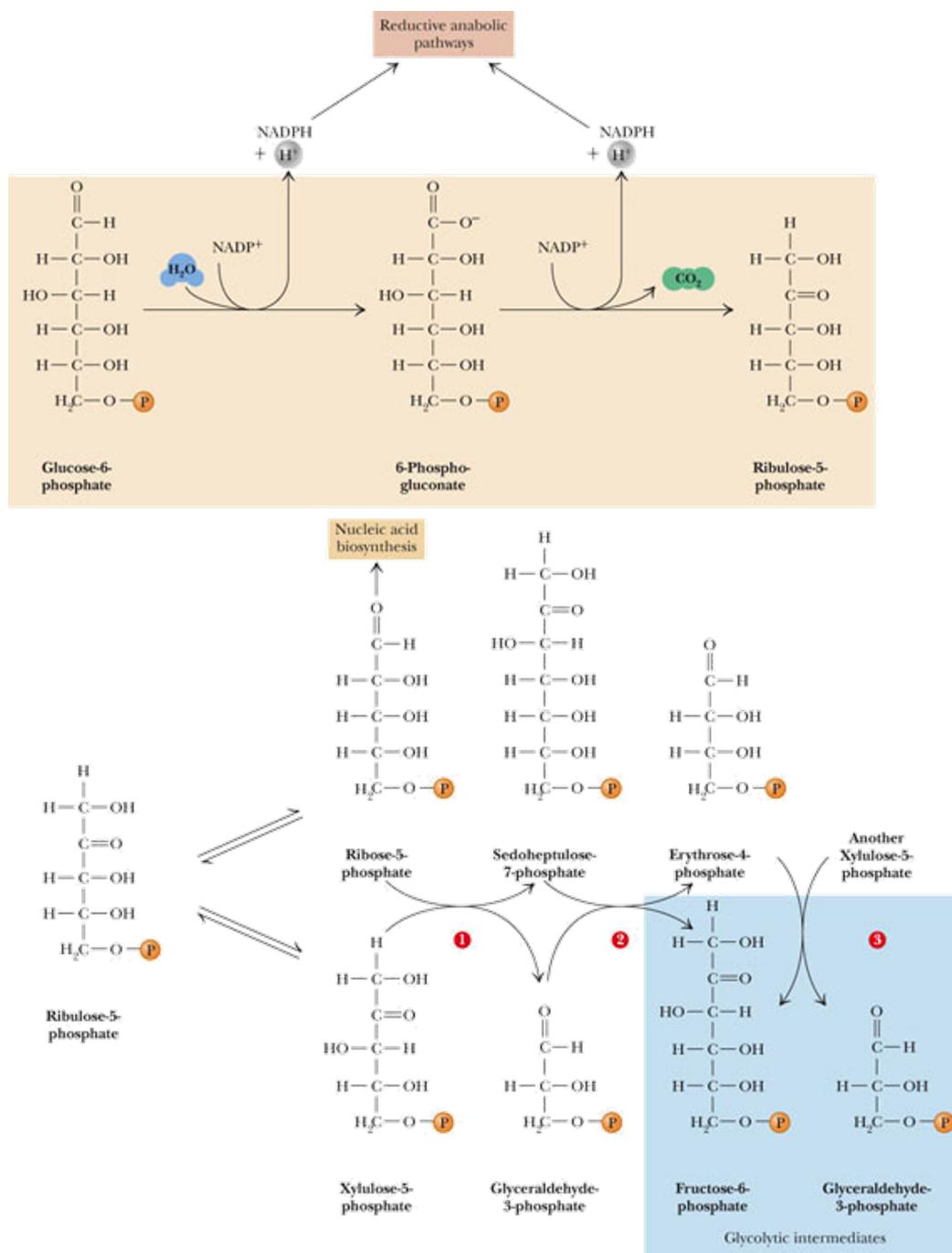
- Consist of two irreversible oxidative reactions and a series of reversible sugar phosphate inter-conversion
- CO_2 is released +2 NADPH
- Other names:
 - **Phosphogluconate Pathway**
 - **Hexose Monophosphate Shunt**
-

The Pentose Phosphate Pathway

- The Pentose Phosphate Pathway (PPP) is an alternative to glycolysis and differs in several ways
 - In glycolysis, ATP production is important, in PPP, is not
 - As the name implies, five carbon sugars, including ribose, are produced from glucose
 - Oxidizing agent is NADP^+ ; it is reduced to NADPH, which is a reducing agent in biosyntheses
 - Begins with two oxidation steps (NADP^+) to give NADPH and five carbon sugars
 - Following this, a series of non-oxidative reshuffling of the carbon skeleton during which three-, four-, five-, six-, and seven-carbon monosaccharide phosphates are produced



The Pentose Phosphate Pathway



Group Transfer Reactions

Table 18.2

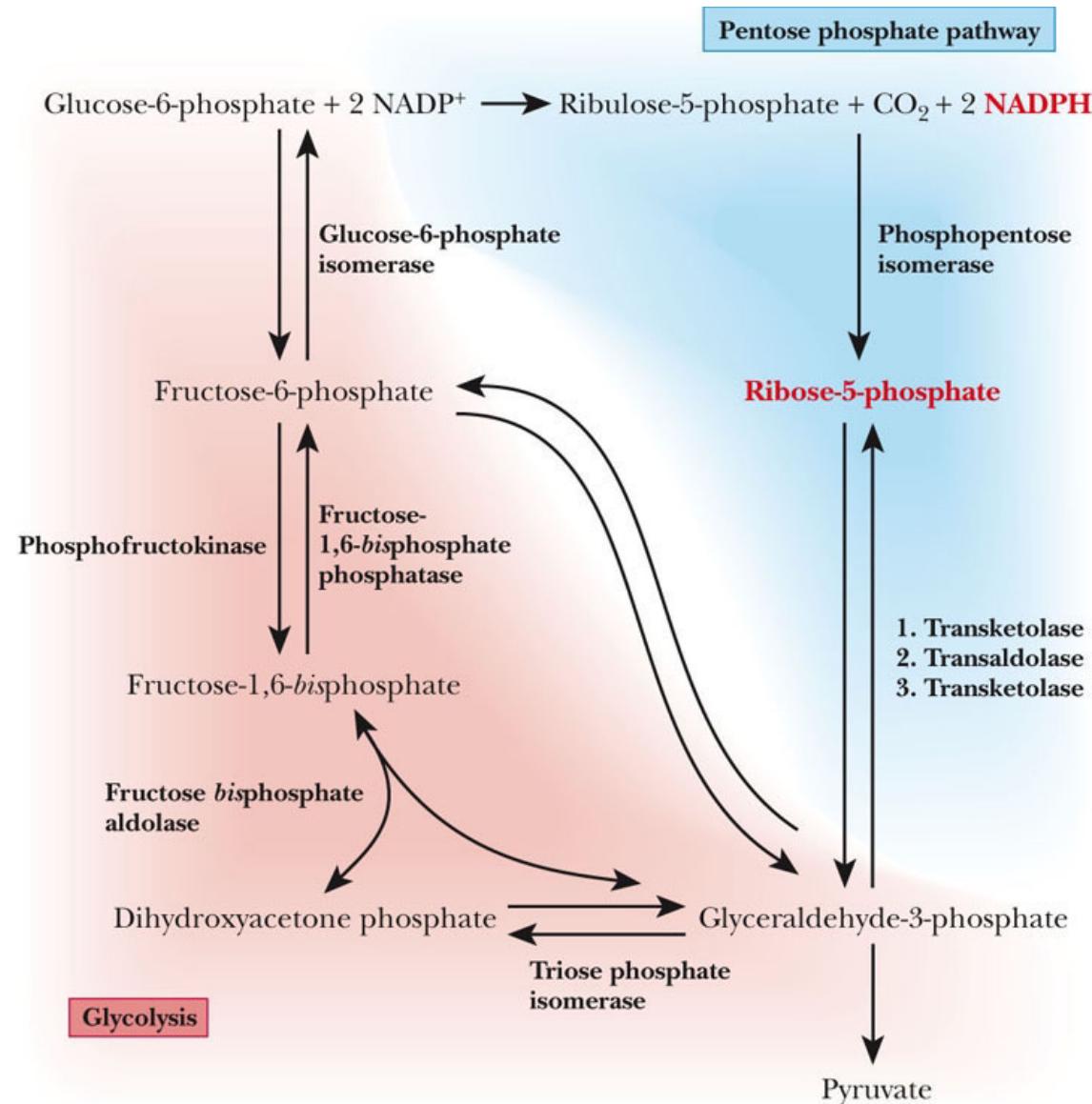
Group-Transfer Reactions in the Pentose Phosphate Pathway

	Reactant	Enzyme	Products
Two-carbon shift	$C_5 + C_5$	Transketolase ↔	$C_7 + C_3$
Three-carbon shift	$C_7 + C_3$	Transaldolase ↔	$C_6 + C_4$
Two-carbon shift	$C_5 + C_4$	Transketolase ↔	$C_6 + C_3$
Net reaction	$3C_5$	↔	$2C_6 + C_3$

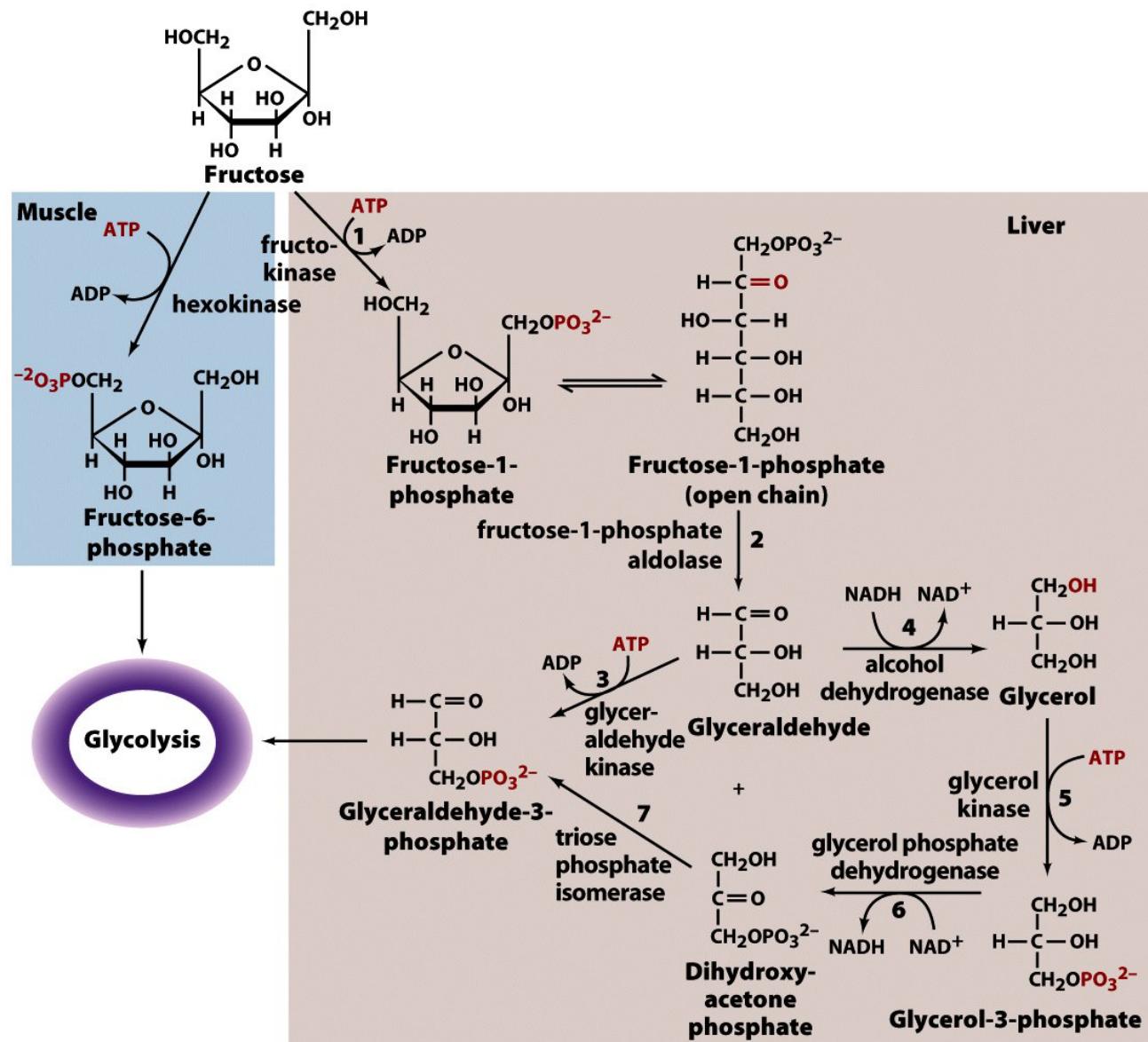
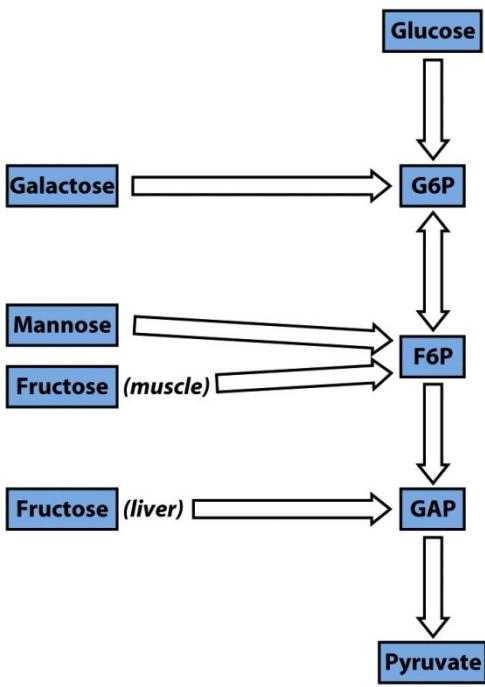
Control of the Pentose Phosphate Pathway

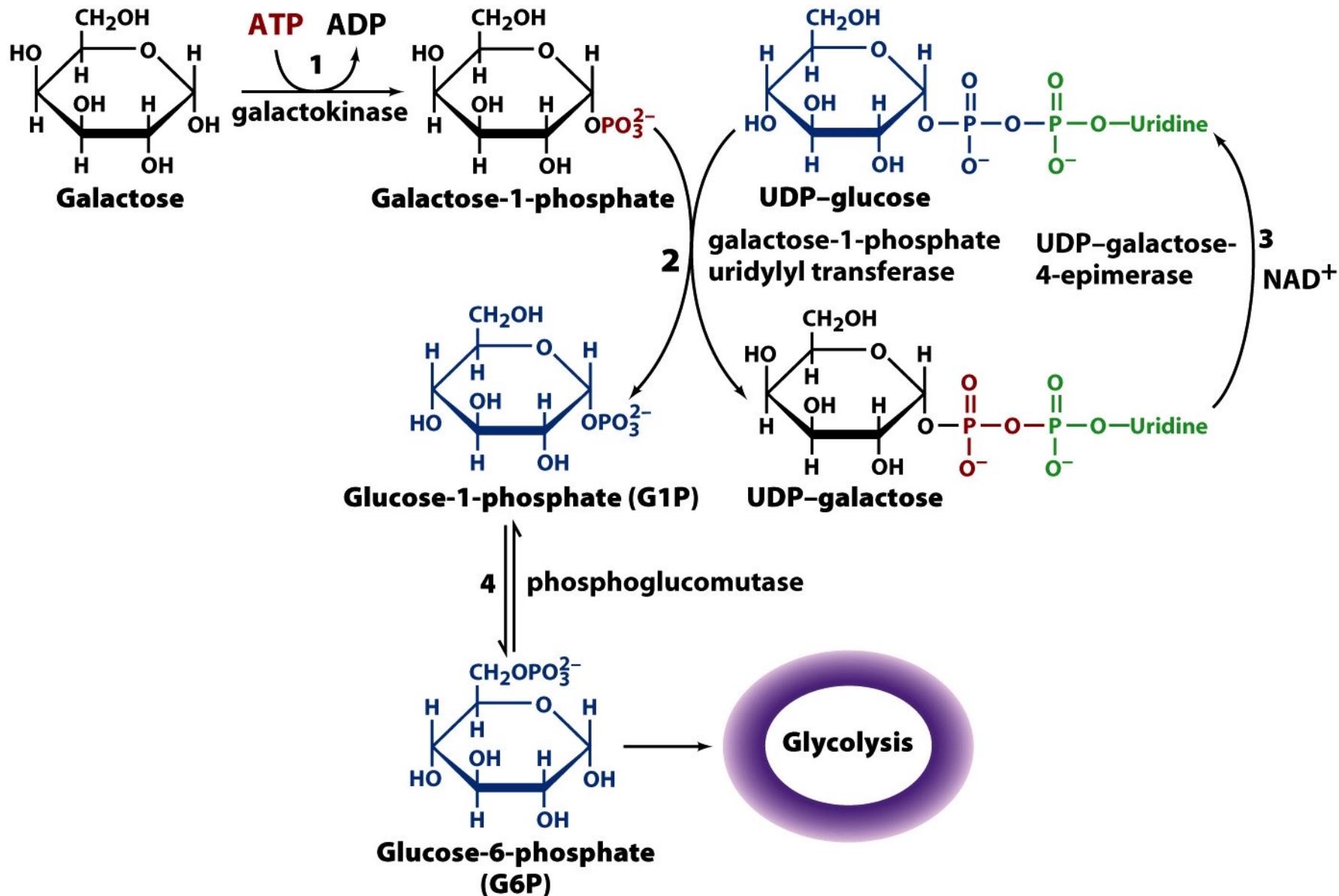
- The carbon-shuffling reaction are catalyzed by:
 - **Transketolase** for the transfer of two-carbon units
 - **Transaldolase** for the transfer of three-carbon units
- **Control of the PPP is maintained by:**
 - Glucose-6-phosphate (G6P) can be channeled into either glycolysis or the pentose phosphate pathway
 - G6P channeling into glycolysis, if ATP is needed
 - G6P channeling into the pentose phosphate pathway, if NADPH or Ribose 5 phosphate are needed
 - Ribose 5 phosphate can be obtained from other sources (away from oxidation occur in PPP)
 - Glycolytic intermediates

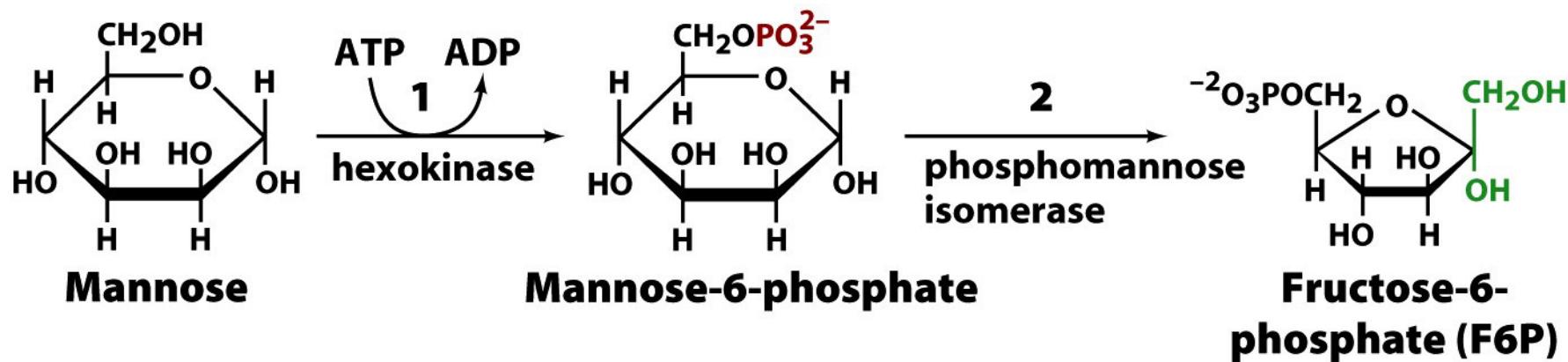
Relationship between PPP and Glycolysis



Other Hexoses







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