MACQUARIE University

Glycolysis, Gluconeogenesis and Glycogen metabolism

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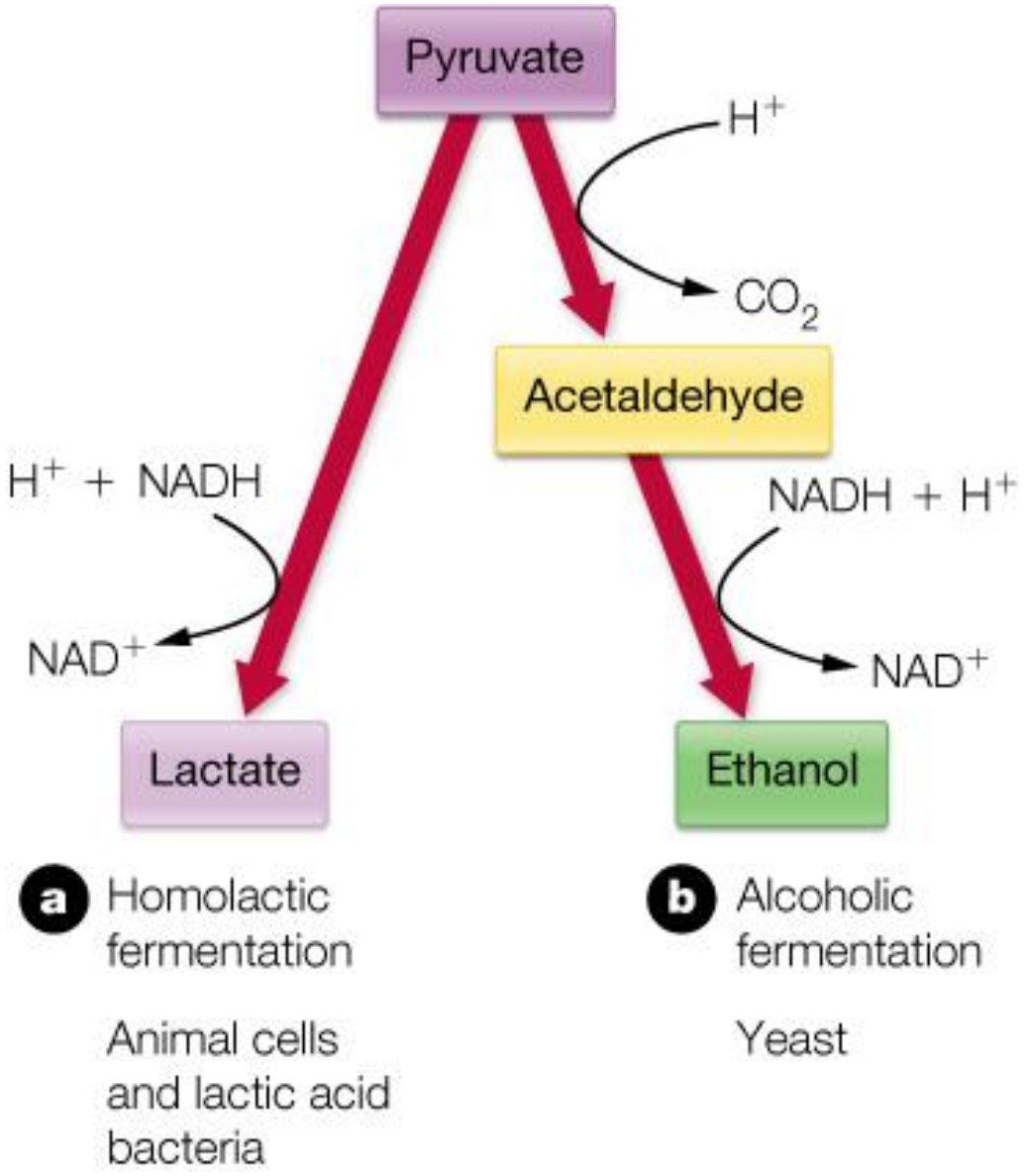
Objectives

- Glycolysis – continued
  - Fermentation: What happens to pyruvate?
  - Regulation of glycolysis
- Gluconeogenesis – how to make glucose (synthesis from pyruvate)
- Other sugars we can use for glycolysis
- Glycogen – the storage form of glucose

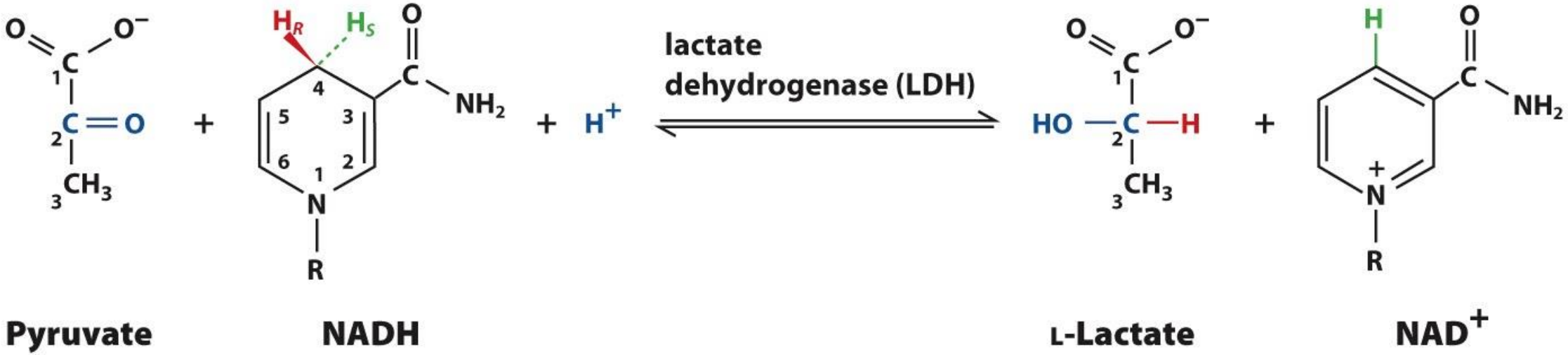
- Textbook Chap. 12

Anaerobic Metabolic Fate of Pyruvate

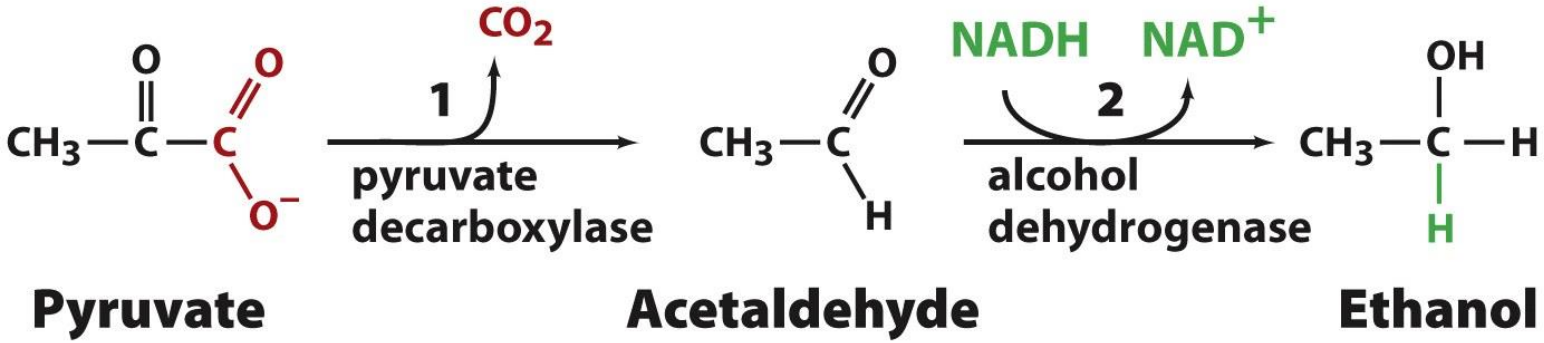
- NADH must be reoxidized to NAD^+ for glycolysis to continue
  - ➢ Homolactic fermentation
  - ➢ Alcoholic fermentation (only yeast)
- Oxidation in mitochondria cannot keep up with the NADH produced by high rates of glycolysis, and anaerobic organisms lack mitochondria



Homolactic Fermentation: lactosis or acidosis: muscle cramps



Reactions of Alcoholic Fermentation



Fermenting yeast cells

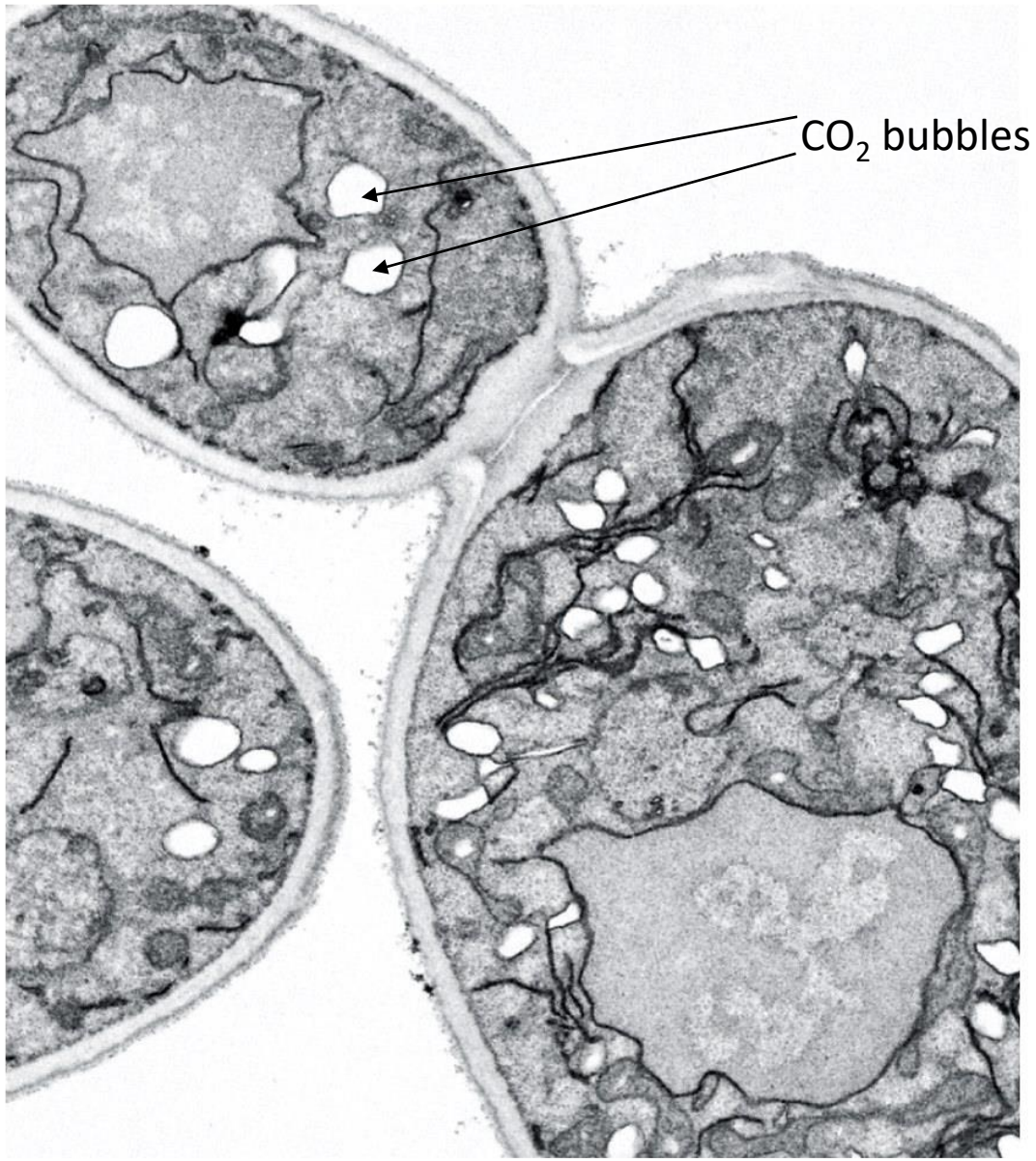


Figure 15-17
Biophoto Associates Photo Researchers

Fermentation: The Anaerobic Fate of Pyruvate - Summary

- **NADH**, a substrate for the GAPDH reaction, must be ***reoxidized for glycolysis to continue.***
- In muscle, **pyruvate** is reduced to **lactate** to regenerate **NAD^+.**
- *Yeast decarboxylates pyruvate to produce CO_2 and ethanol, in a process that requires the cofactor, thiamine pyrophosphate (TPP; Vitamin B1).*

Regulation of Glycolytic Reactions based on Free **Energy Changes**

TABLE 12.1 Summary of Glycolysis

Reaction	Enzyme	ATP yield	$\Delta G^{\circ\prime}$	ΔG
Glucose (G)				
ATP, ATP needed, ADP	Hexokinase (HK)	−1	−18.4	−33.5
Glucose-6-phosphate (G6P)	Glucose-6-phosphate isomerase		+1.7	−2.5
Fructose-6-phosphate (F6P)				
ATP, ATP needed, ADP	Phosphofructokinase (PFK)	−1	−15.9	−22.2
Fructose-1,6-bisphosphate (FBP)				
	Aldolase		+23.9	−1.3
Glyceraldehyde-3-phosphate (GAP) + dihydroxyacetone phosphate (DHAP)				
	Triose phosphate isomerase (TIM)		+7.6	~0

Continue

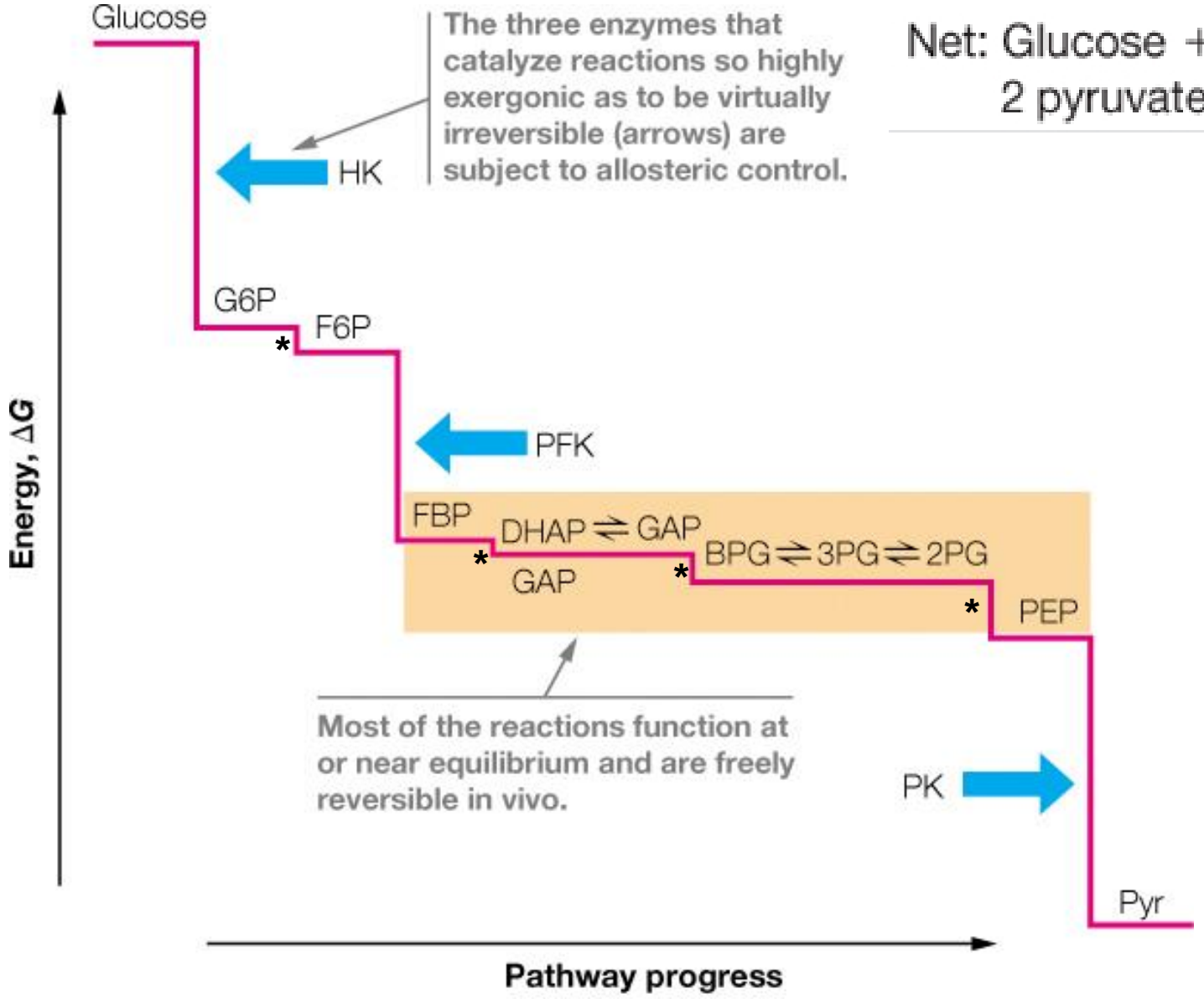
The cell only regulates reactions that have ΔG << 0

TABLE 12.1 Summary of Glycolysis

Reaction	Enzyme	ATP yield	$\Delta G^{\circ\prime}$	ΔG
Two glyceraldehyde-3-phosphate (GAP)				
$NAD^+ + P_i$, $NADH + H^+$	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)		+6.3 (+12.6)	−1.7 (−3.4)
1,3-Bisphosphoglycerate (BPG)				
ADP, ATP	Phosphoglycerate kinase (PGK)	+1(+2)	−17.2 (−34.4)	~0
3-Phosphoglycerate (3PG)				
	Phosphoglycerate mutase		+4.4 (+8.8)	~0
2-Phosphoglycerate (2PG)				
H_2O	Enolase		−3.2 (−6.4)	−3.3 (−6.6)
Phosphoenolpyruvate (PEP)				
ADP, ATP	Pyruvate kinase (PK)	+1(+2)	−29.7 (−59.4)	−16.7 (−33.4)
Pyruvate (Pyr)				
Net: Glucose + 2ADP + 2P_i + 2NAD^+ → 2 pyruvate + 2ATP + 2NADH + 2H^+ + 2H_2O		+2	−79.9	−102.9

Note: $\Delta G^{\circ\prime}$ and ΔG values in kJ/mol. The values in parentheses are based on doubling all the reactions past reaction 5, since the energy generation phase involves 2 three-carbon substrates per glucose molecule. ΔG values are estimated from the approximate intracellular concentrations of glycolytic intermediates in rabbit skeletal muscle.

Regulation of Glycolytic Reactions based on Free Energy Changes



Net: Glucose + 2ADP + 2P_i + 2NAD^+ → 2 pyruvate + 2ATP + 2NADH + 2H^+ + 2H_2O

Steps 2, 4-9 (* in figure) operate close to 0 free energy change in the cell and are therefore self-regulating, equilibrium reactions

Driving force for glycolysis comes from just the 3 big "leaps":

1. Hexokinase (HK);
3. Phosphofructokinase (PFK)

   and

10. Pyruvate kinase (PK)

- Regulating these three enzymes alone will control the flux through the entire pathway.

Daily requirement of glucose

- Human body needs ~160 g/day of glucose
  - 75% (~120 g/day) of this is used by the brain!
  - Body fluids carry 20 g of free glucose
  - **Glycogen** stores 180-200 g of free glucose
  - = about 1 day's requirement + a little more
- So we need to start producing glucose if not provided by food
- Gluco_neo(new)_genesis(generate) gathers pyruvate and lactate and converts them back to glucose
- Another glucose catabolism route is the pentose phosphate pathway (next lecture).

Gluconeogenesis: making **new glucose**

- The liver and kidney can synthesize glucose from ***non-carbohydrate precursors:*** lactate, pyruvate, and amino acids.
- Gluconeogenesis is like glycolysis in reverse, but **three "irreversible" reactions must be reversed**
  - the pyruvate kinase reaction bypassed by the pyruvate carboxylase and phosphoenolpyruvate carboxykinase reactions, and
  - the phosphofructokinase and hexokinase reactions bypassed by phosphatase reactions.
- Glycolysis and gluconeogenesis are reciprocally regulated by allosteric effects, phosphorylation, and changes in enzyme synthesis rates.

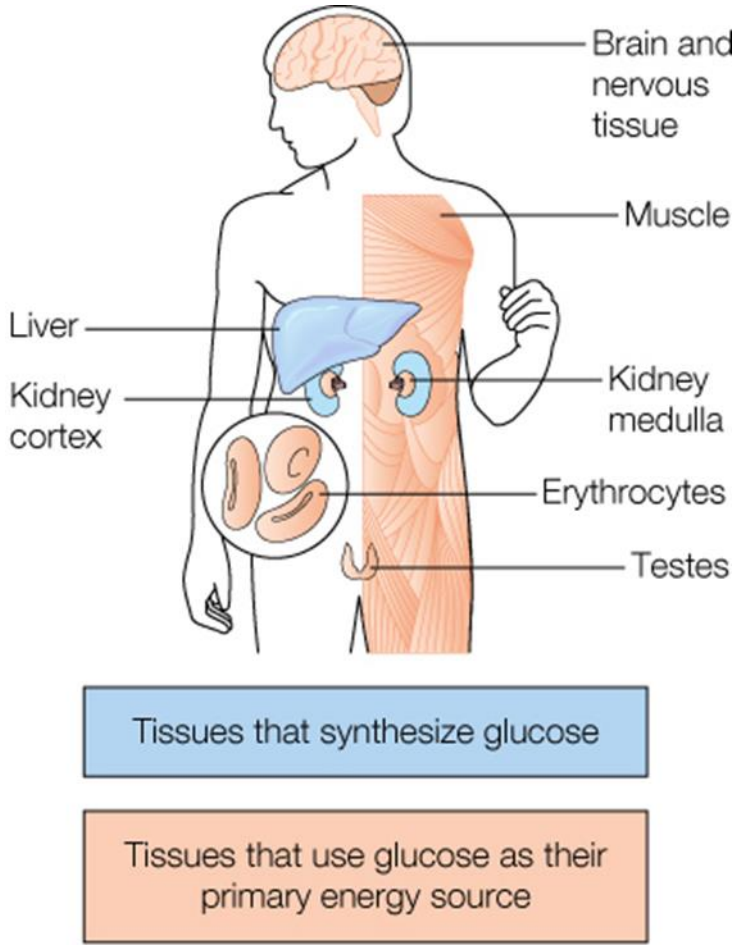


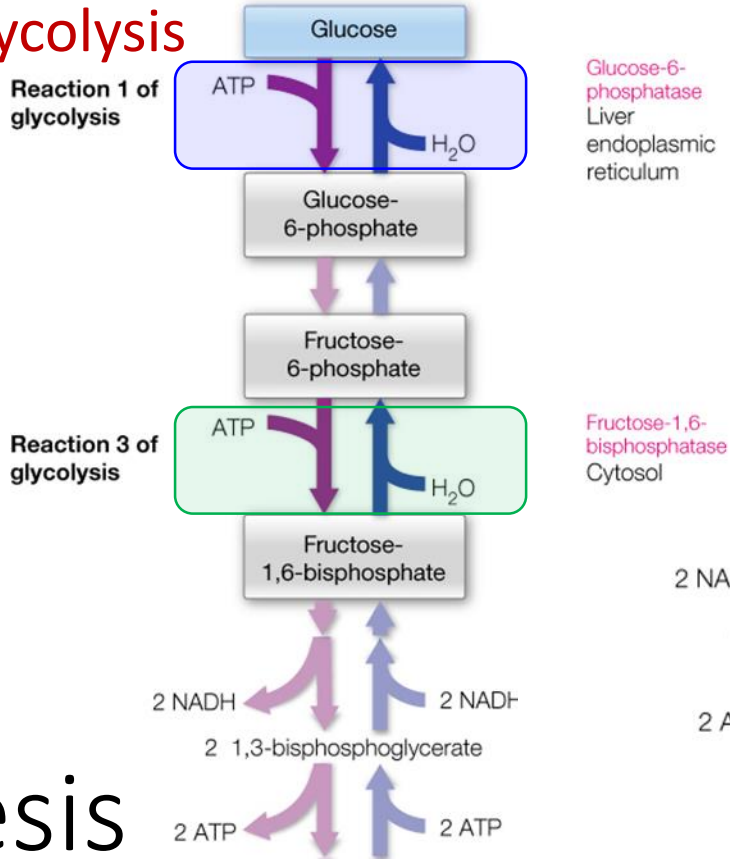
FIGURE 12.9 Synthesis and use of glucose in the human body.

Glycolysis and Gluconeogenesis

Hydrolytic steps bypass irreversible glycolysis steps

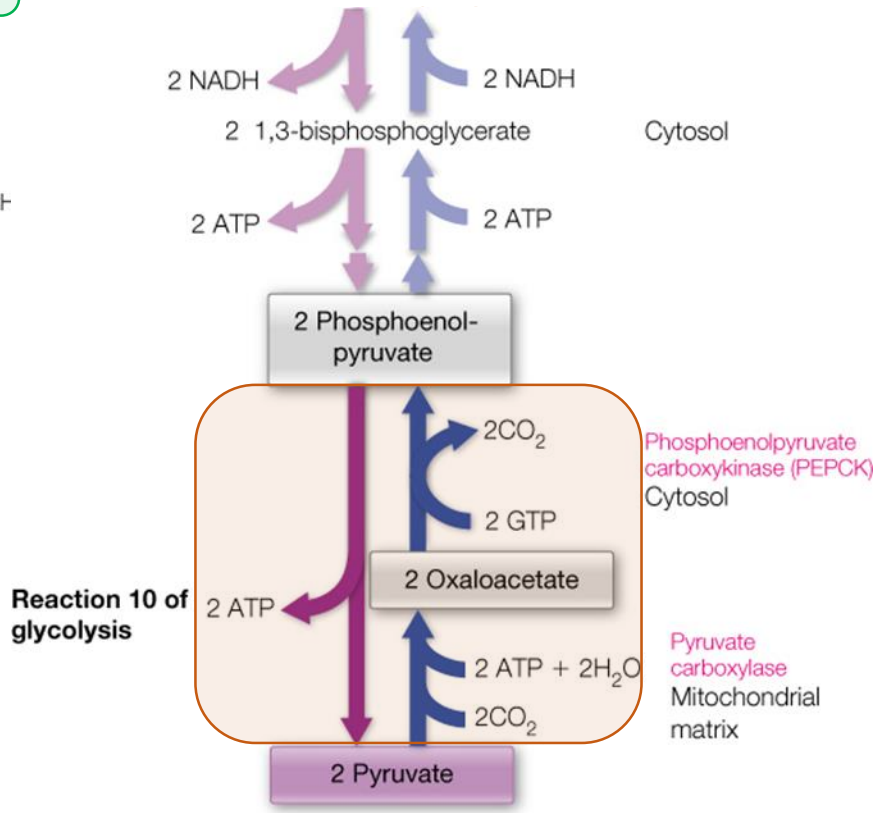
Irreversible Reaction in Glycolysis

- Hexokinase
- Phosphofructokinase
- Pyruvate kinase



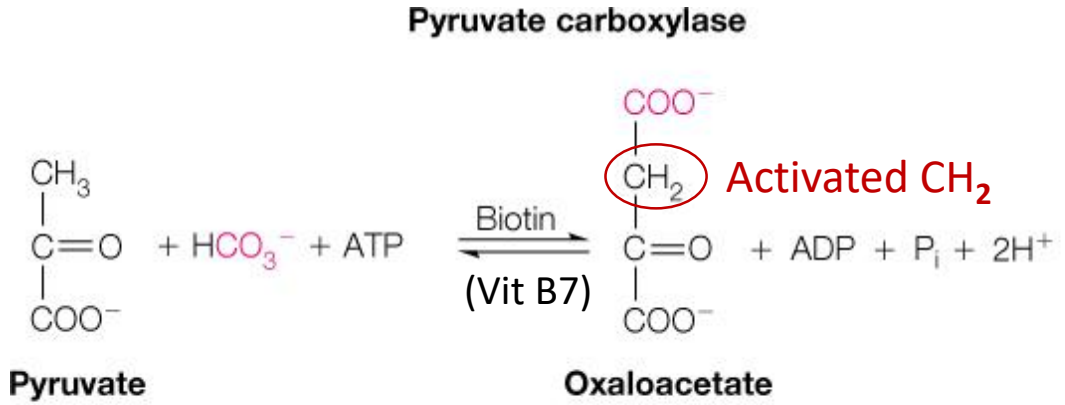
Bypass in Gluconeogenesis

- Glucose-6-phosphatase (only in liver and kidneys)
- Fructose-1,6-bisphosphatase
- Phosphoenol-pyruvate carboxykinase
- Pyruvate carboxylase (mitochondria)

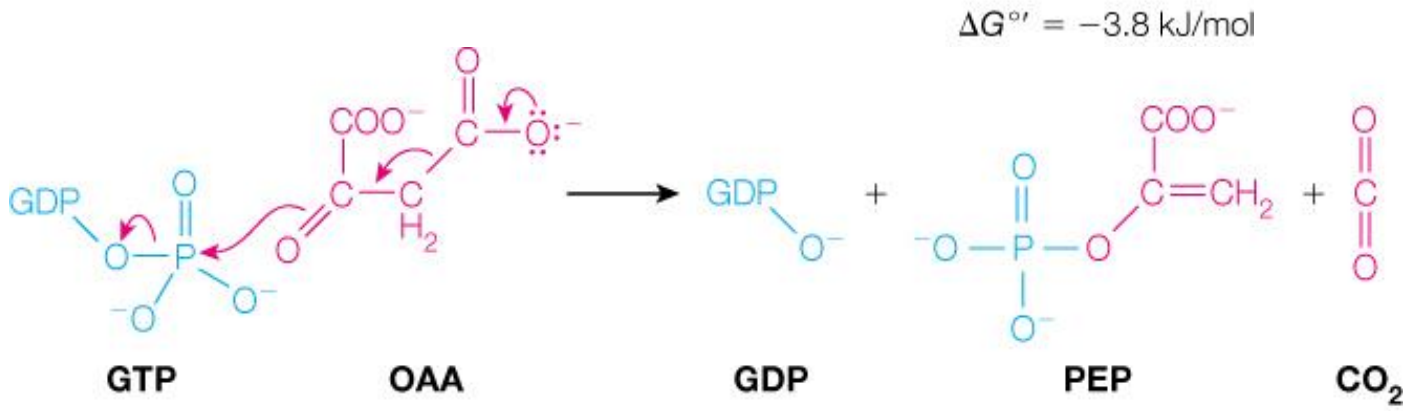


Bypass 1 – Pyruvate Carboxylase and Phosphoenolpyruvate Carboxykinase (PEPCK)

Pyruvate carboxylase



PEPCK



Overall

$$\text{Pyruvate} + \text{ATP} + \text{GTP} \rightarrow \text{phosphoenolpyruvate} + \text{ADP} + \text{GDP} + \text{P}_\text{i} + \text{H}^+ \quad \Delta G^{\circ\prime} = -2.6 \text{ kJ/mol}$$

Compartmentation by placing one critical enzyme in another organelle in the liver (and some in the kidneys)

- Pyruvate carboxylase is in the mitochondrion!
- This pathway shuttles oxaloacetate and reducing equivalents from the mitochondrion to the cytoplasm. Blue spheres indicate inner membrane transporters. MDH = malate dehydrogenase.
- Only pyruvate and malate can cross the mitochondrial membrane – not oxaloacetate!
- Malate cannot be fed back into glycolysis!
- Prevents futile cycling as glycolysis and gluconeogenesis both occur in the cytosol.

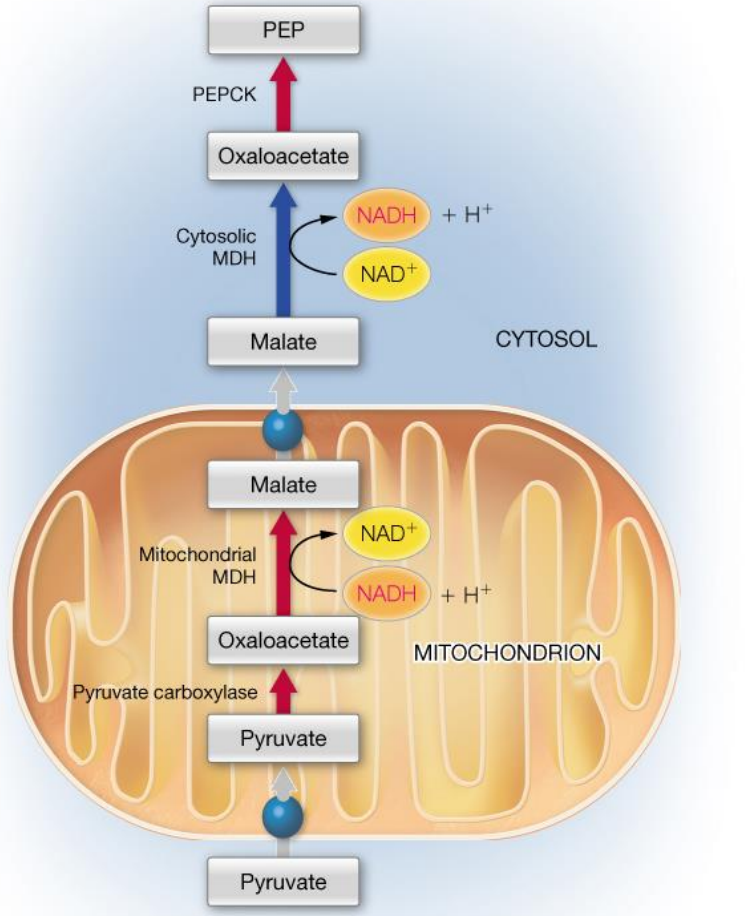


FIGURE 12.11 Compartmentation of bypass 1.

Bypass 2 – Fructose 1,6-bisphosphatase

$$\text{Fructose-1,6-bisphosphate} + H_2O \xrightarrow{Mg^{2+}} \text{fructose-6-phosphate} + Pi$$

$$\Delta G^{\circ\prime} = -16.3 \text{ kJ/mol}$$

Bypass 3 – Glucose 6-phosphatase

$$\text{Glucose-6-phosphate} + H_2O \xrightarrow{Mg^{2+}} \text{glucose} + P_i$$

$$\Delta G^{\circ\prime} = -13.8 \text{ kJ/mol}$$

Energy costs

- Gluconeogenesis:
- 2 pyruvates require 2 ATPs + 2 GTPs (= 4 ATPs) to become OAA
- Reversible step catalysed by phosphoglycerate kinase requires 2 ATPs for 2 3-phospho-glycerate molecules.
- Total 6 ATPs consumed or used up, *compared to 4 ATPs generated in glycolysis*

$$2\ \text{Pyruvate} + 4\text{ATP} + 2\text{GTP} + 2\text{NADH} + 2\text{H}^+ + 4\text{H}_2\text{O} \rightarrow$$

$$\text{glucose} + 4\text{ADP} + 2\text{GDP} + 6\text{P}_\text{i} + 2\text{NAD}^+$$

$$\Delta G^{\circ\prime} = -42.7\ \text{kJ/mol}$$

- Simple Reversal of glycolysis is impossible:

$$2\ \text{Pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O} \rightarrow$$

$$\text{glucose} + 2\text{ADP} + 2\text{P}_\text{i} + 2\text{NAD}^+ \quad \Delta G^{\circ\prime} = +79.9\ \text{kJ/mol}$$

Summary of Gluconeogenetic Reactions

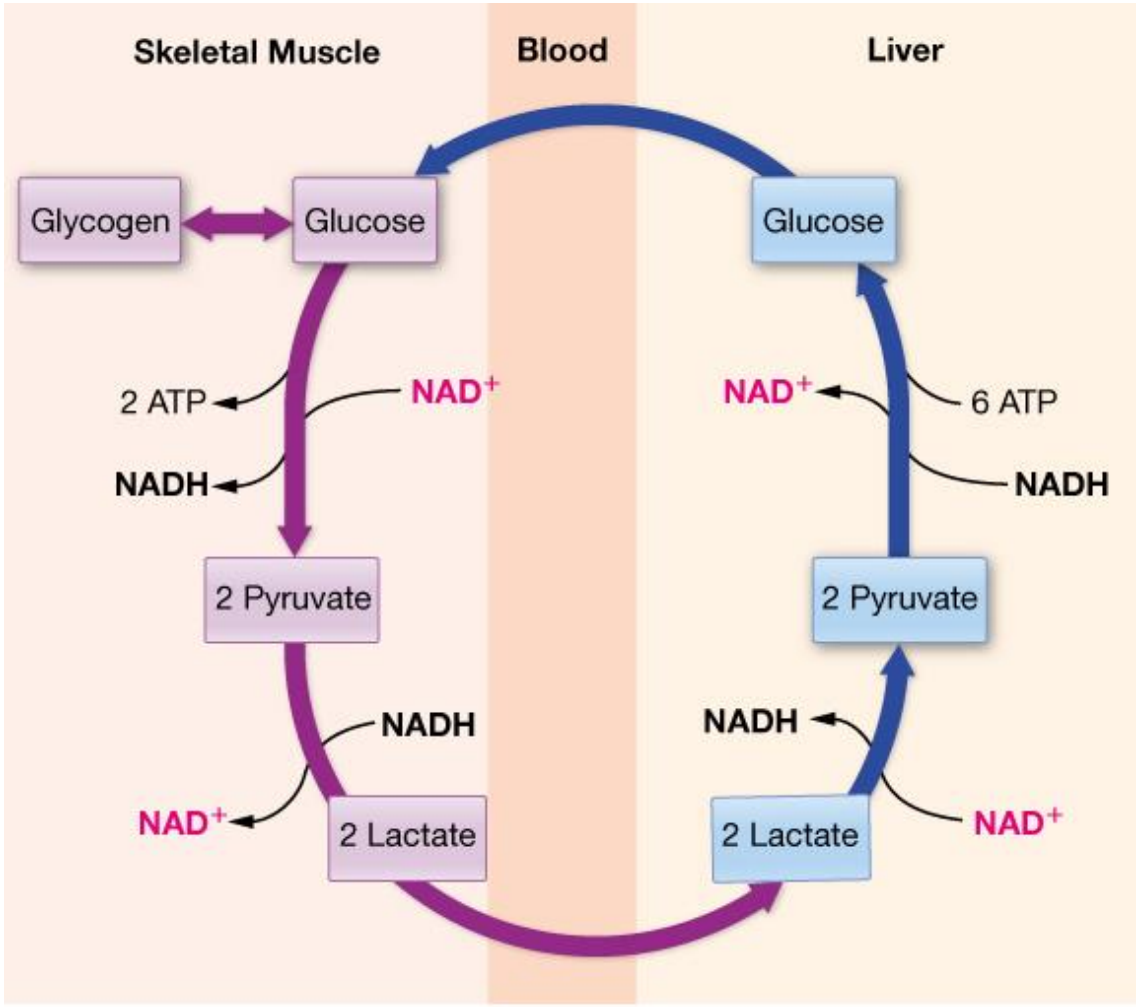
TABLE 12.2 Summary of gluconeogenesis, from pyruvate to glucose

Reaction	$\Delta G^{\circ\prime}$ (kJ/mol)
Pyruvate + HCO_3^- + ATP ⟶ oxaloacetate + ADP + P_i	−3.8 (−7.6)
Oxaloacetate + GTP ⇌ phosphoenolpyruvate + CO_2 + GDP	+1.2 (+2.4)
Phosphoenolpyruvate + H_2O ⇌ 2-phosphoglycerate	+6.4(+12.8)
2-Phosphoglycerate ⇌ 3-phosphoglycerate	−4.4 (−8.8)
3-Phosphoglycerate + ATP ⇌ 1,3-bisphosphoglycerate + ADP	+17.2 (+34.4)
1,3-Bisphosphoglycerate + NADH + H^+ ⇌ glyceraldehyde-3-phosphate + NAD^+ + P_i	−6.3 (−12.6)
Glyceraldehyde-3-phosphate ⇌ dihydroxyacetone phosphate	−7.6
Glyceraldehyde-3-phosphate + dihydroxyacetone phosphate ⇌ fructose-1,6-bisphosphate	−23.9
Fructose-1,6-bisphosphate + H_2O ⇌ fructose-6-phosphate + P_i	−16.3
Fructose-6-phosphate ⇌ glucose-6-phosphate	−1.7
Glucose-6-phosphate + H_2O ⇌ glucose + P_i	−13.8
Net: 2 Pyruvate + 4ATP + 2GTP + 2NADH + $2H^+$ + $4H_2O$ ⟶ glucose + 4ADP + 2GDP + $6P_i$ + $2NAD^+$	−42.7

Note: The reactions in red are those that bypass irreversible glycolytic reactions; the remaining reactions are reversible reactions of glycolysis. The $\Delta G^{\circ\prime}$ values in parentheses are based on doubling the first six reactions because 2 three-carbon precursors are required to make one molecule of glucose. The individual reactions are not necessarily balanced for H^+ and charge.

Cori Cycle

The liver is the most active gluconeogenic organ/tissue

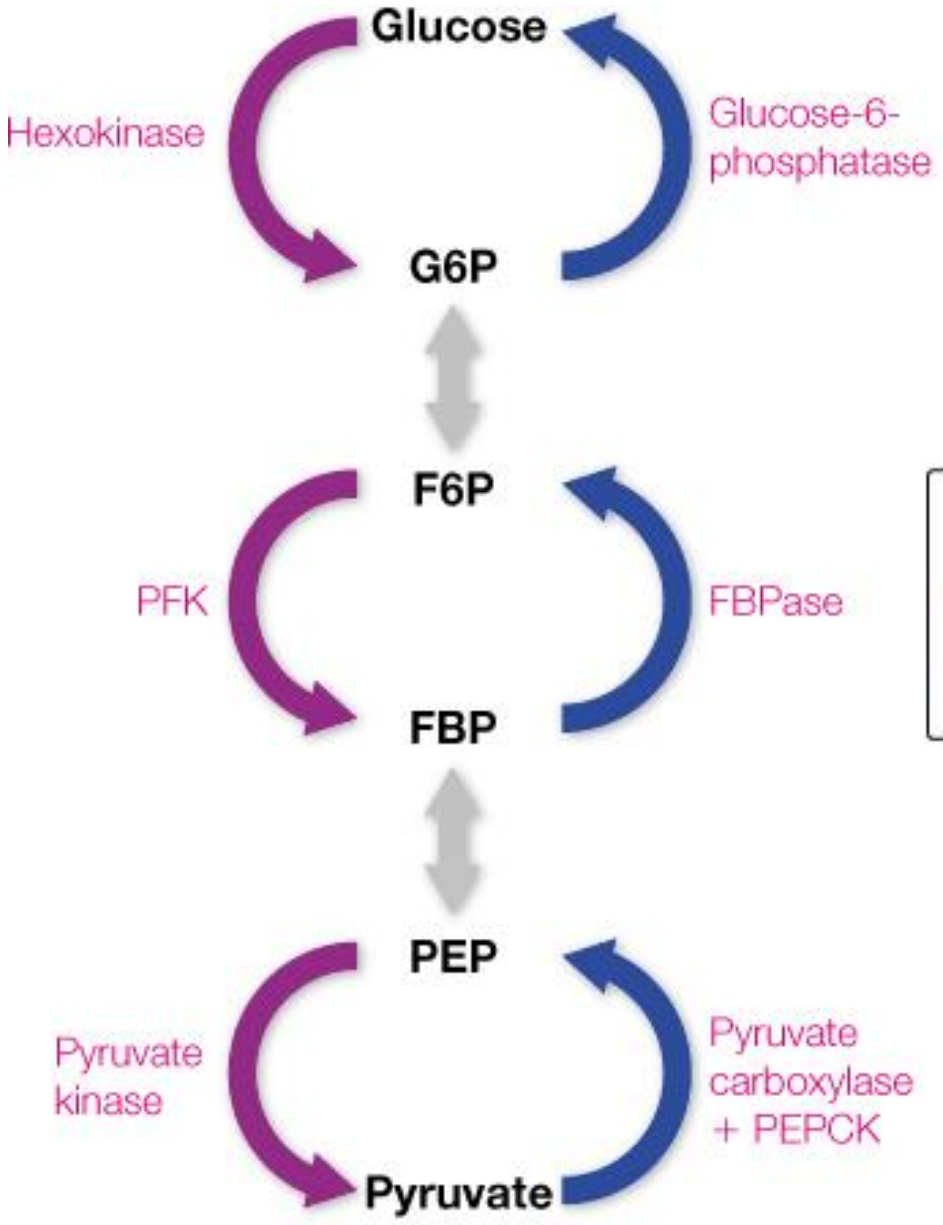


Gluconeogenesis summary

ONLY IN THE LIVER AND KIDNEYS

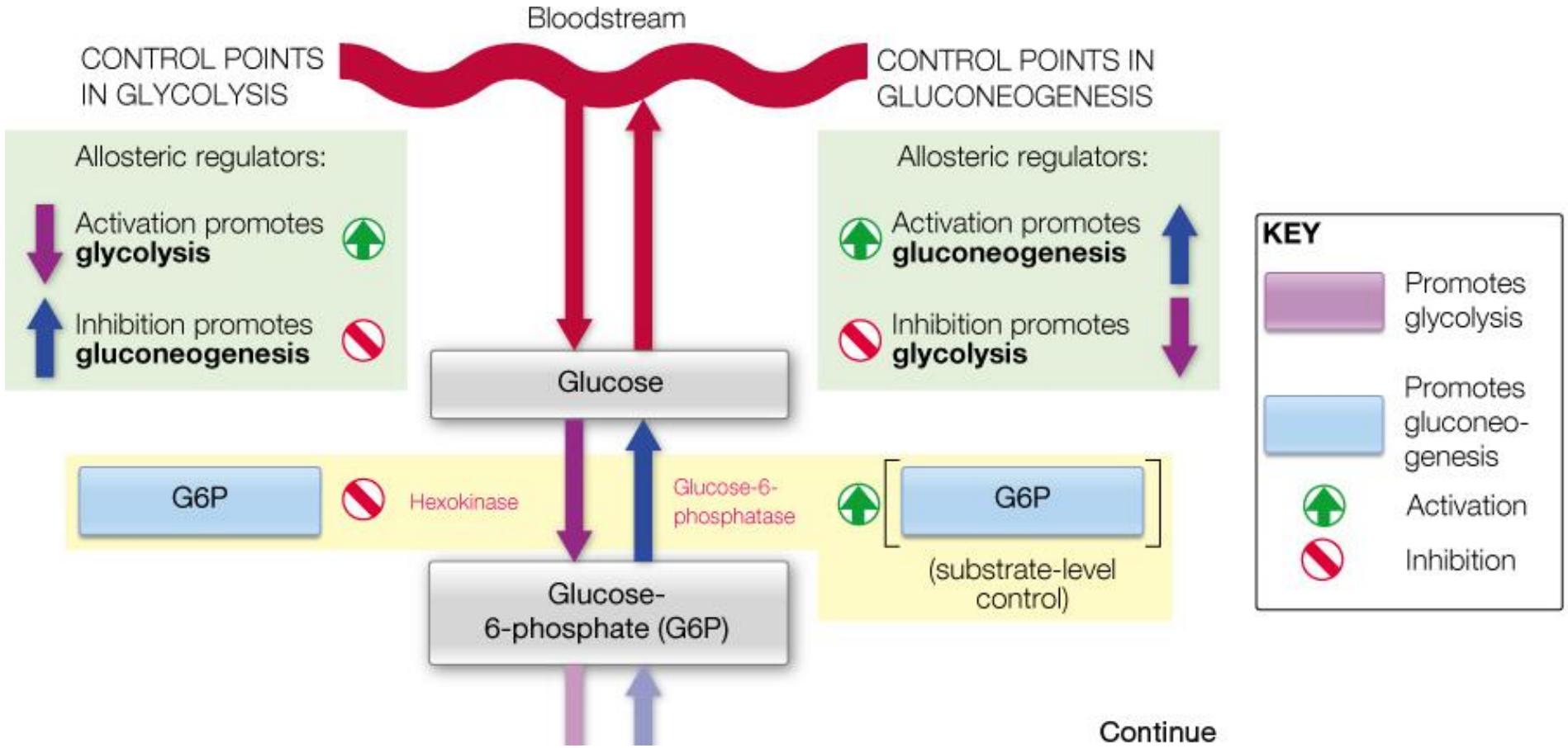
- 3C Pyruvate (or lactate) built up to 6C glucose by enzymes that bypass the three favourable steps of glycolysis:
  1. Pyruvate carboxylase and PEP carboxykinase (PEPCK) bypass pyruvate kinase (mitochondria),
  2. fructose-1,6-bisphosphatase (FBPase) bypasses phosphofructokinase, and
  3. glucose-6-phosphatase (in the liver and kidneys) bypasses hexokinase.
- Gluconeogenesis is regulated by changes in enzyme synthesis and by allosteric effectors.

Coordinated regulation of Glucose Breakdown and Synthesis



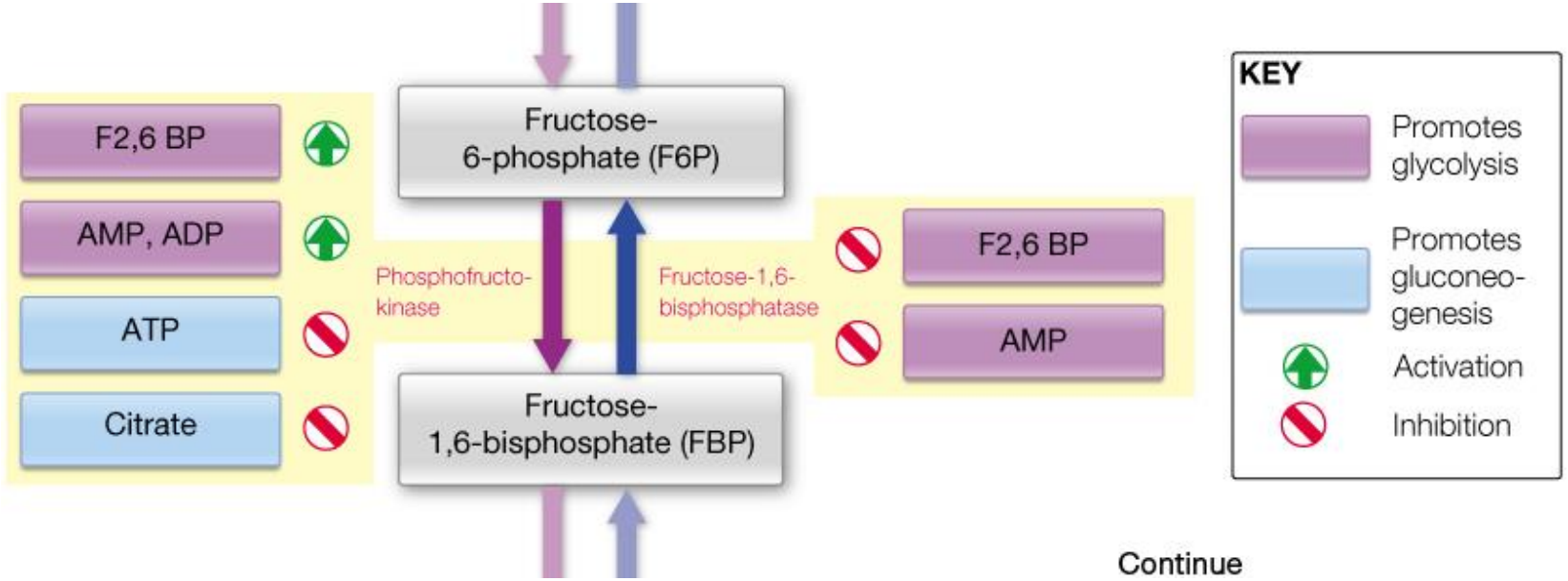
- Glycolysis and gluconeogenesis must be controlled reciprocally to prevent futile cycles
- Regulation must also take into account the need to maintain pools of intermediates for other biosynthetic purposes

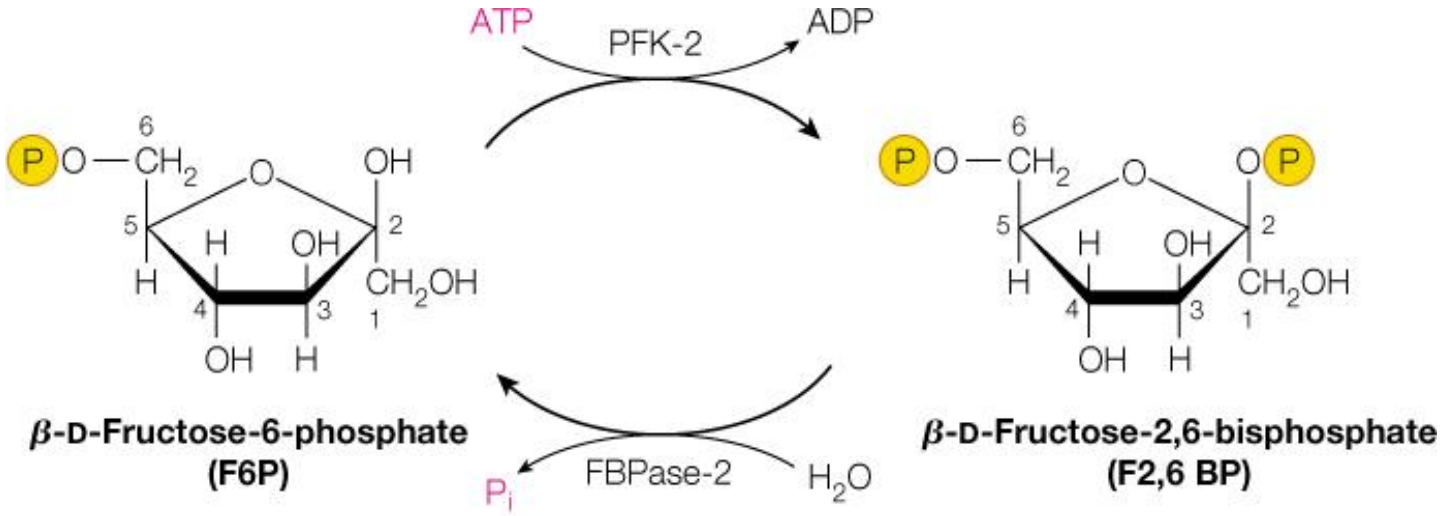
Control Point 1: Allosteric regulation by Glucose-6-phosphate



Control Point 2

- Energy charge: ATP inhibits glycolysis; AMP/ADP activate glycolysis; AMP inhibits gluconeogenesis.
- Citrate inhibits glycolysis

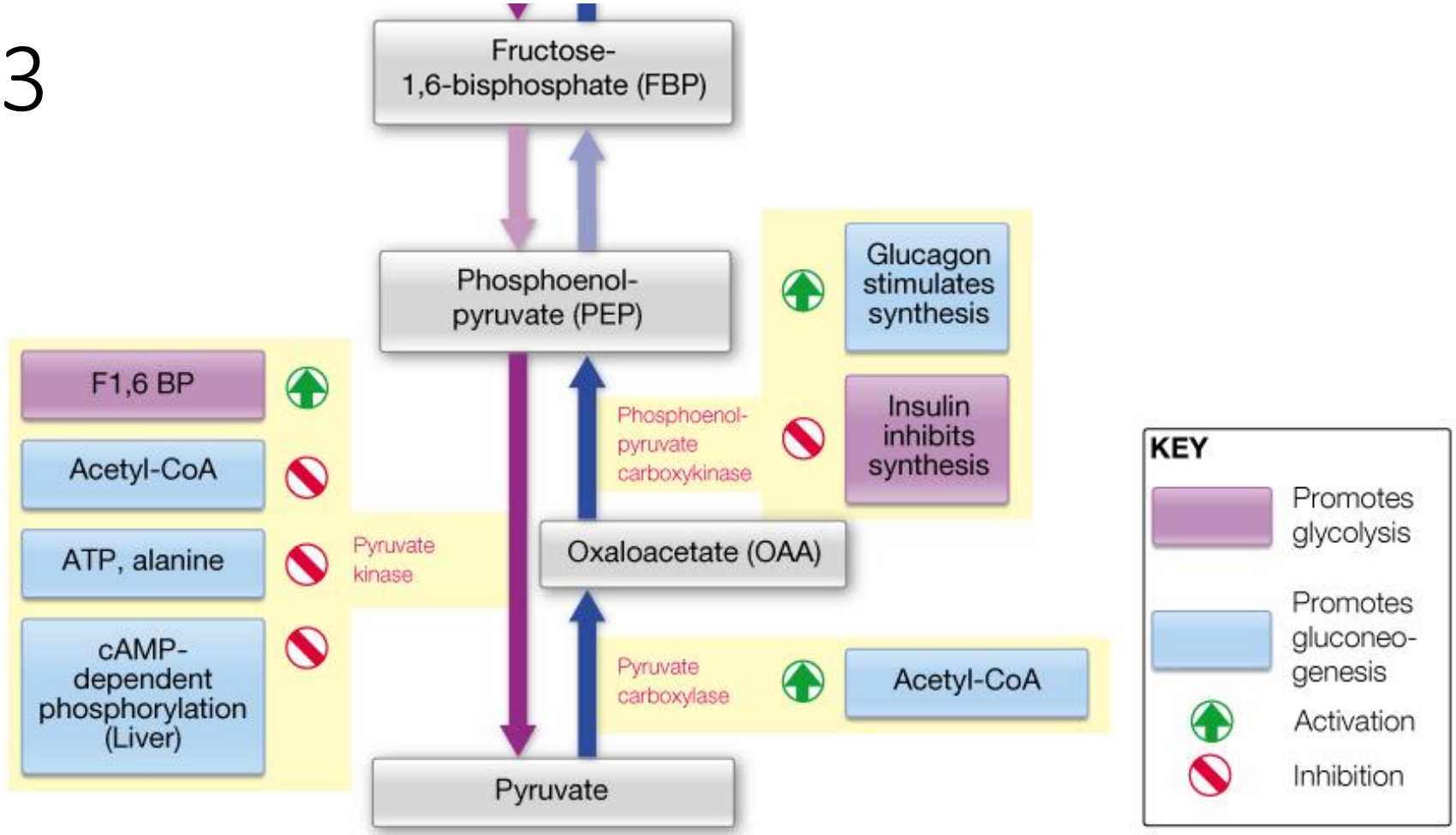




- F2,6BP activates glycolysis and inhibits gluconeogenesis. F2,6BP is synthesized by the bifunctional PFK-2/FBPase-2, which is distinct from the enzymes found in glycolysis and gluconeogenesis

Control Point 3

- ATP inhibits glycolysis
- F1,6BP activates glycolysis
- Acetyl-CoA (made from pyruvate) inhibits glycolysis and activates gluconeogenesis
- Alanine (from aa breakdown) makes pyruvate; inhibits glycolysis
- Hormones: glucagon activates and insulin inhibits gluconeogenesis.



Towards exam prep

- Compare and contrast glycolysis and gluconeogenesis
  - Hormonal control of glycolysis and gluconeogenesis
  - Also glycogen metabolism
- Which pathway to choose, when there is
  - low blood glucose level
  - high blood glucose level

Utilization of Sugars Other Than Glucose

- Glycolysis can use other commonly available hexoses:
  - ✓ Fructose (fruit sugar),
  - ✓ Galactose (milk sugar), and
  - ✓ Mannose (from liver cells)
- Glycerol from fat metabolism can also be used by glycolysis
- Complex carbohydrates (starch from plants and glycogen stored by animals) are also hydrolysed and enter glycolysis as G1P.

❖ **3 ATPs** generated as 1st investment step bypassed

- ➢ Glycogen and Galactose as G1P
- ➢ also mannose and fructose that enter the pathway with 1 phosphate group

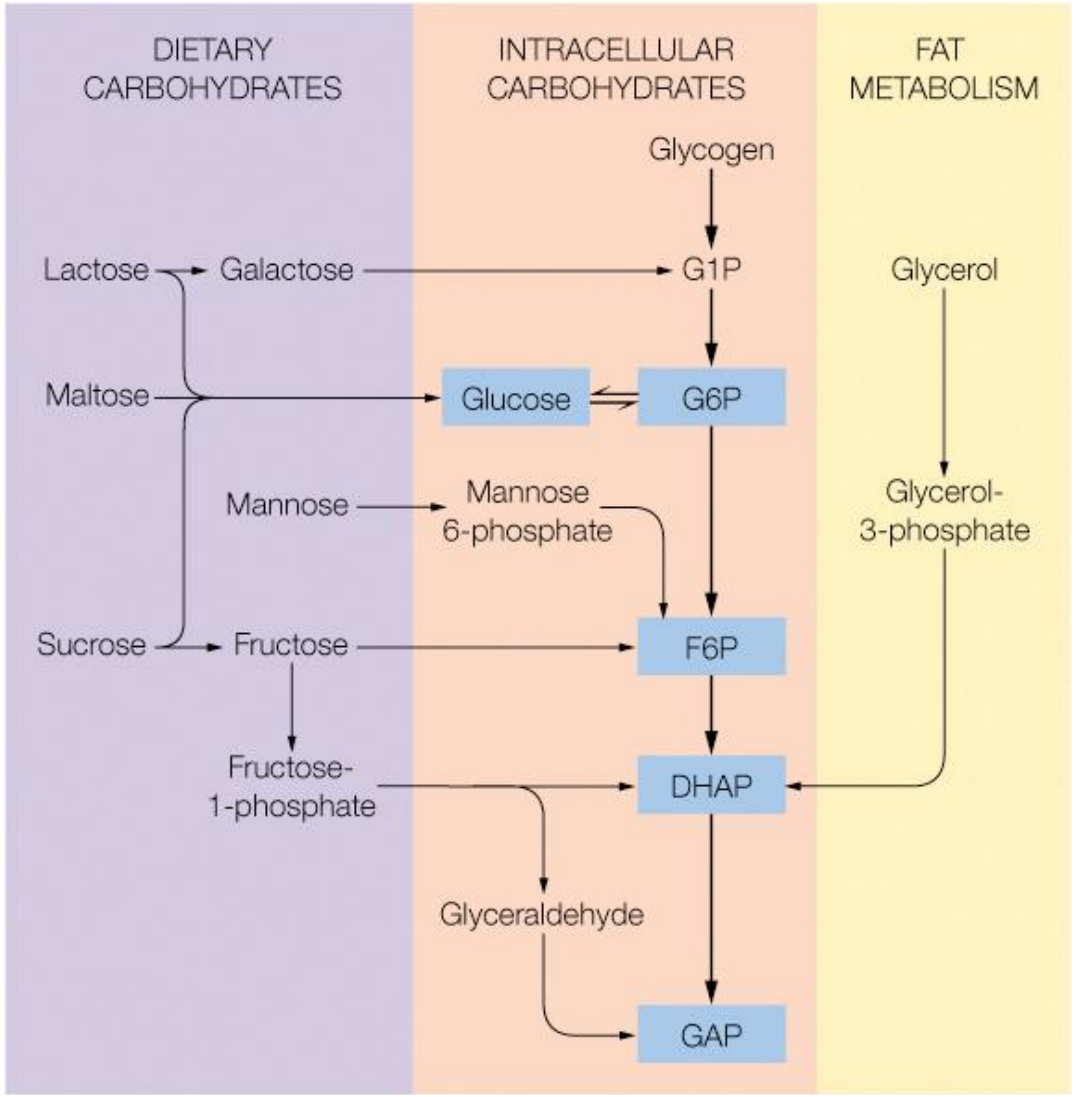


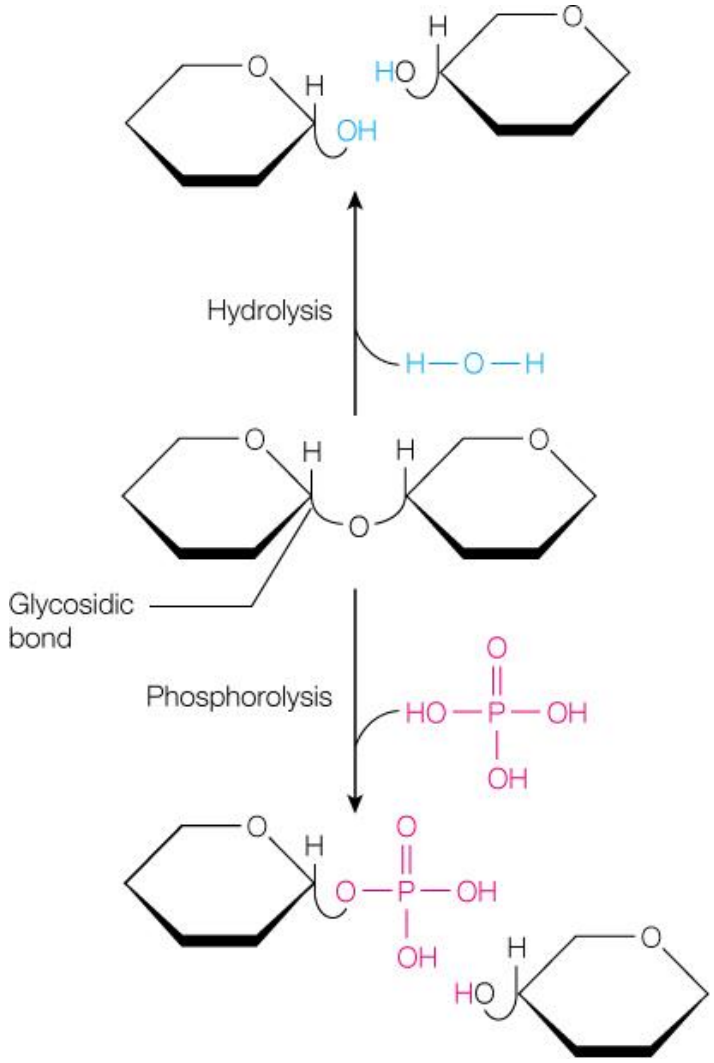
FIGURE 12.19 Routes for utilizing substrates other than glucose in glycolysis.

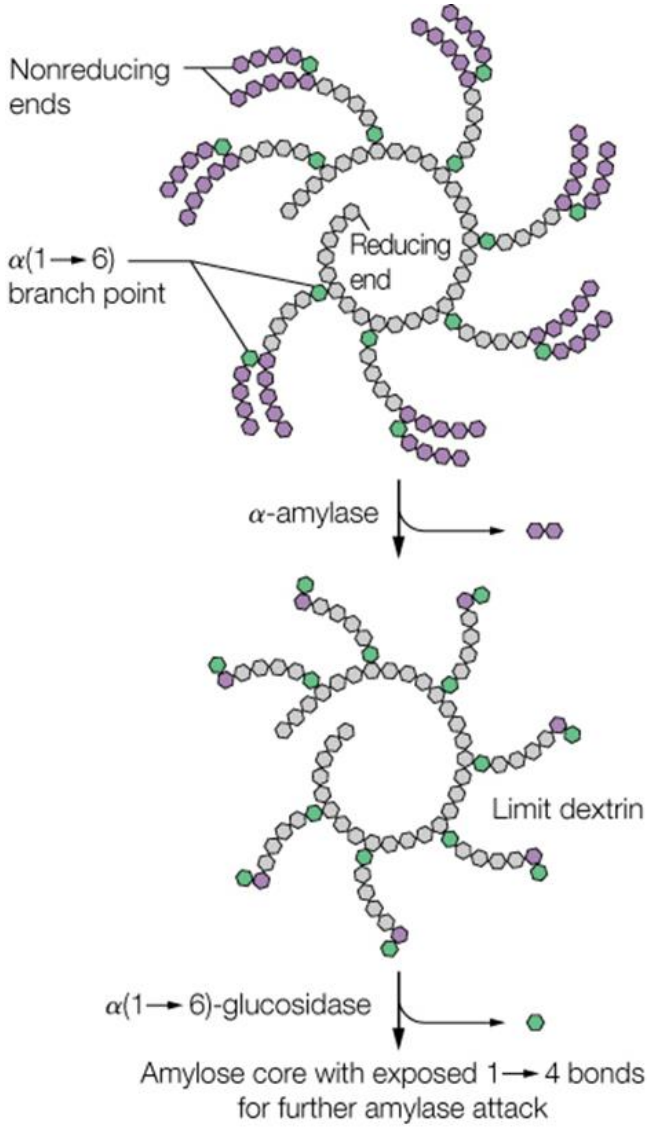
Substrates for gluconeogenesis

- Metabolites need to be converted to the 4C oxaloacetate to enter gluconeogenesis pathway
- The noncarbohydrate precursors to gluconeogenesis include:
  1. Glycolysis products: pyruvate, lactate
  2. Citric acid cycle intermediates
  3. Amino acids except leucine and lysine (these form acetyl-CoA) – these are called glucogenic amino acids

Stored Sugars: Starch and Glycogen Digestion

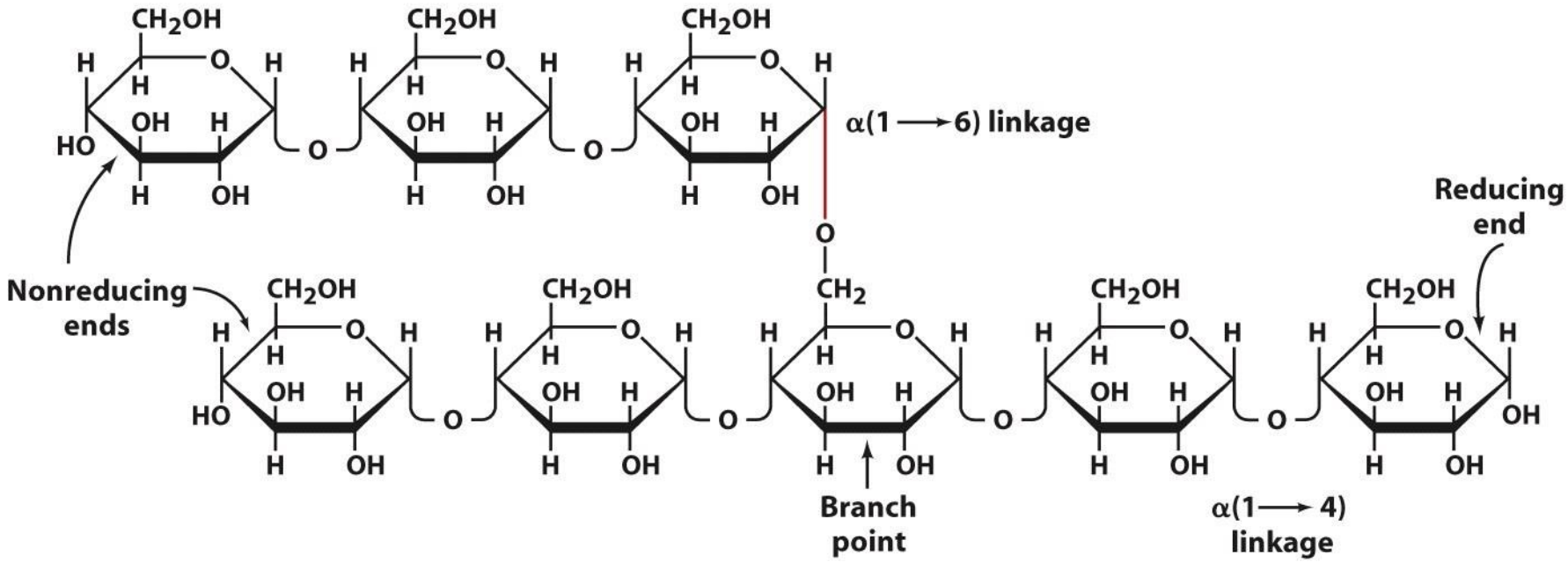
- Dietary polysaccharides are metabolized by hydrolysis to monosaccharides
- Starch forms maltose (glucose dimer), which is then broken up onto glucose
- Glycogen is mobilized as phosphorylated G1P by phosphorylation





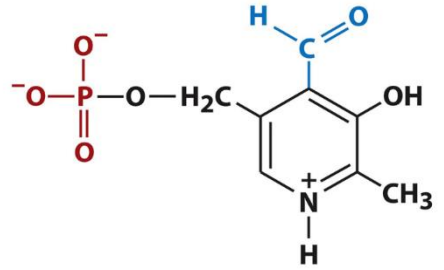
Structure of Glycogen (recap)

- **Glycogen** (*like starch*) is a ***polymer of glucose*** residues linked by **α linkages**

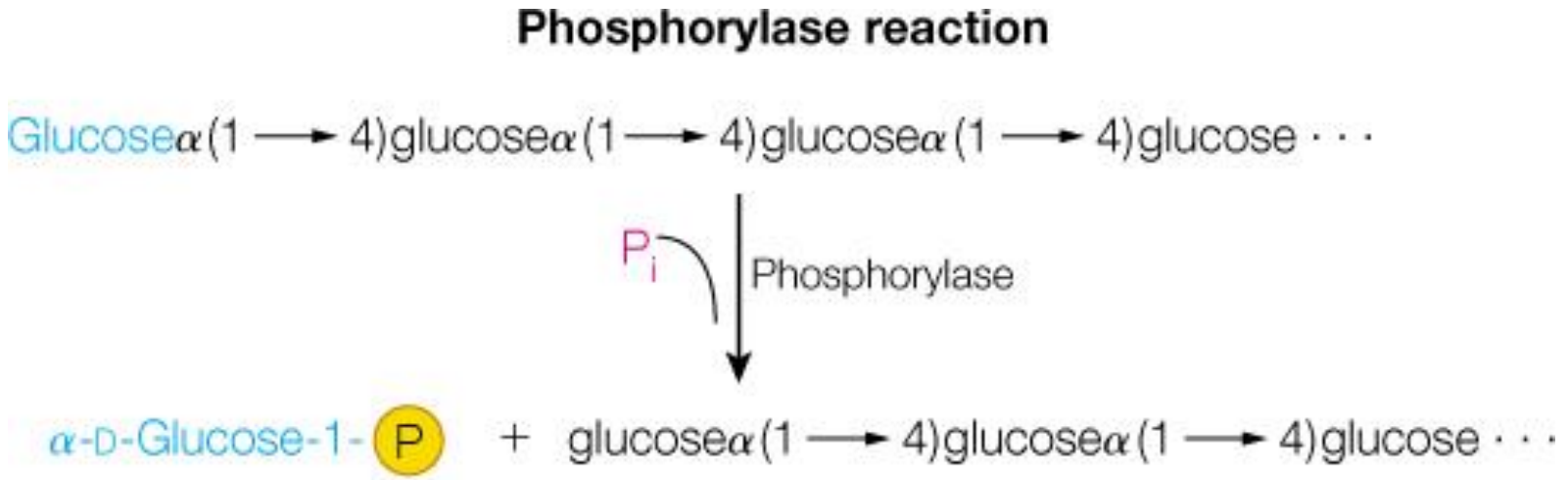


Glycogenolysis: 3 enzymatic steps of glycogen breakdown

1. **Glycogen phosphorylase** cleaves α(1→4) bonds via phosphorolysis, yielding **α-D-glucose-1-phosphate (G1P)**.
   - This enzyme requires PLP (Vitamin B6) as cofactor
   - Also can only remove glucose to within 4 units from a branch point!

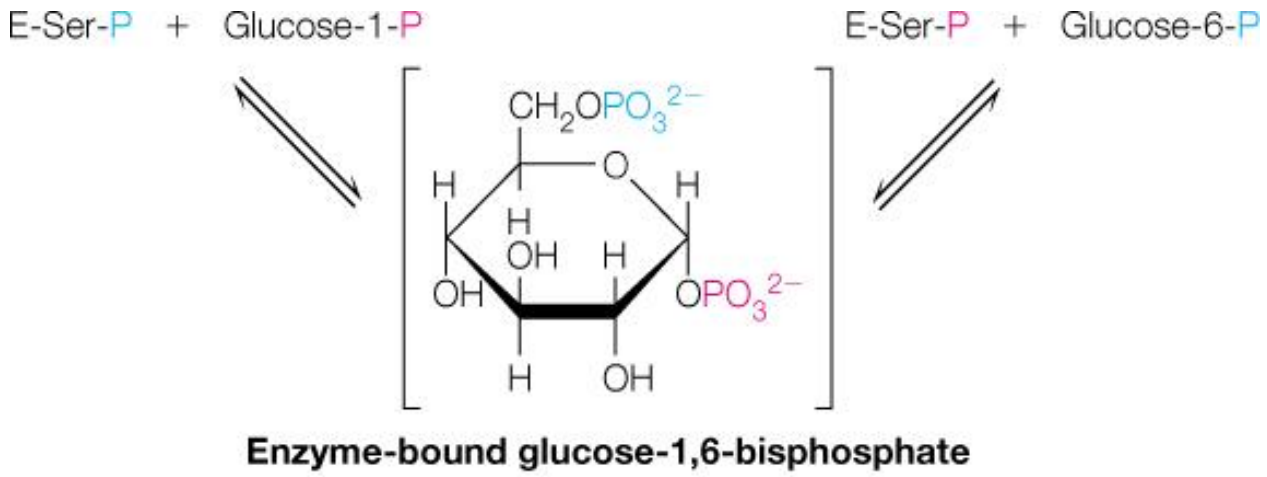


Pyridoxal-5′-phosphate (PLP)



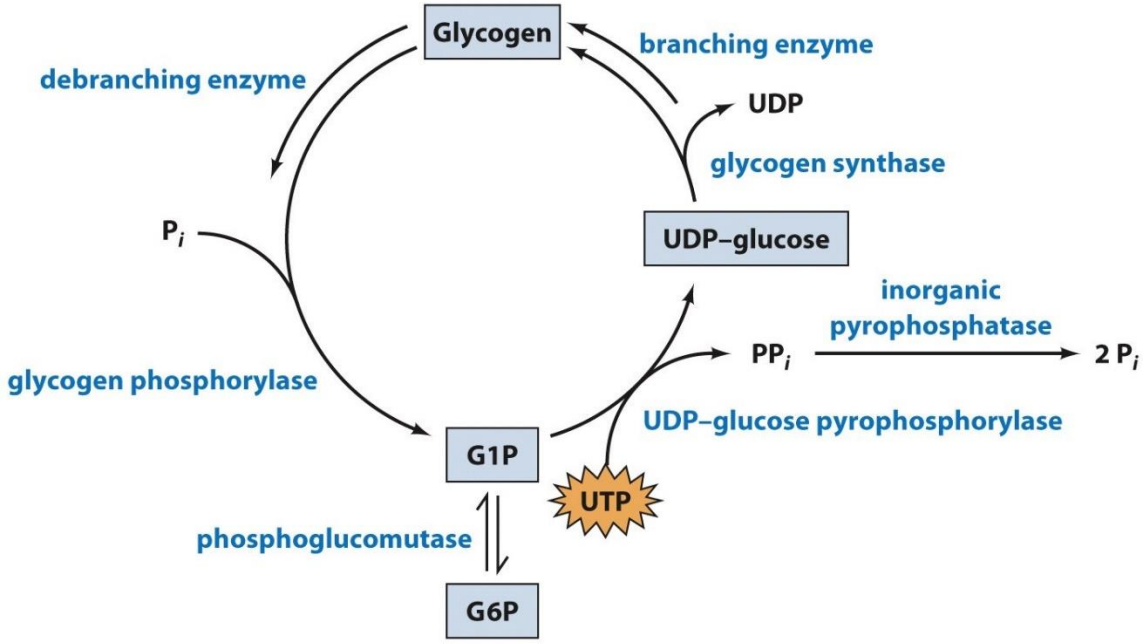
3 enzymatic steps of glycogen breakdown

2. **Glycogen debranching enzyme:** removes glycogen's branches, making **additional glucose residues available** for breakdown by glycogen phosphorylase (step 1).
3. **Phosphoglucomutase** converts G1P to G6P, which can be used in metabolic pathways:
   - **Glycolysis** or **glucose-6-phosphatase** generates free glucose in the liver
   - **Pentose phosphate pathway** or **Back to glycogen**!

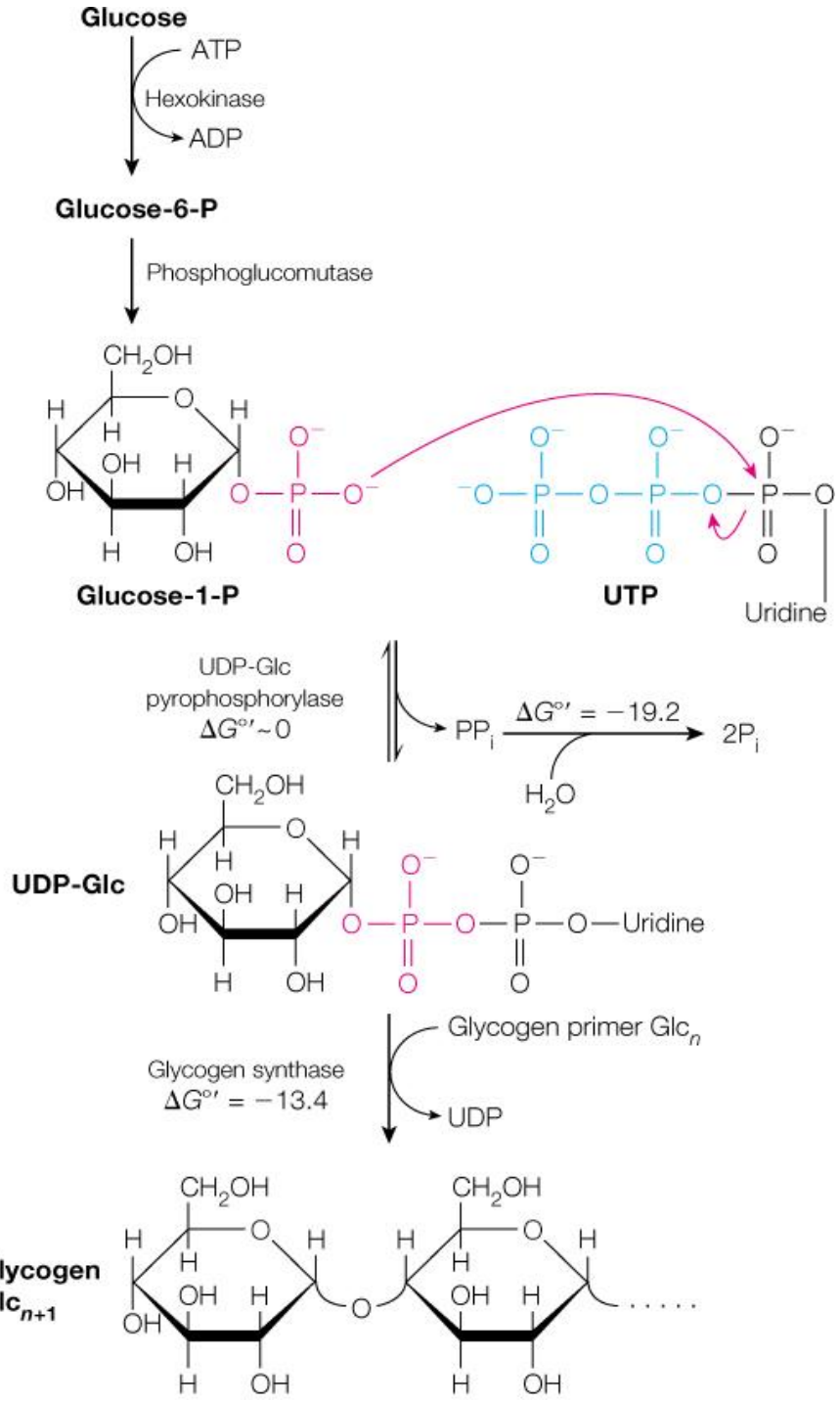


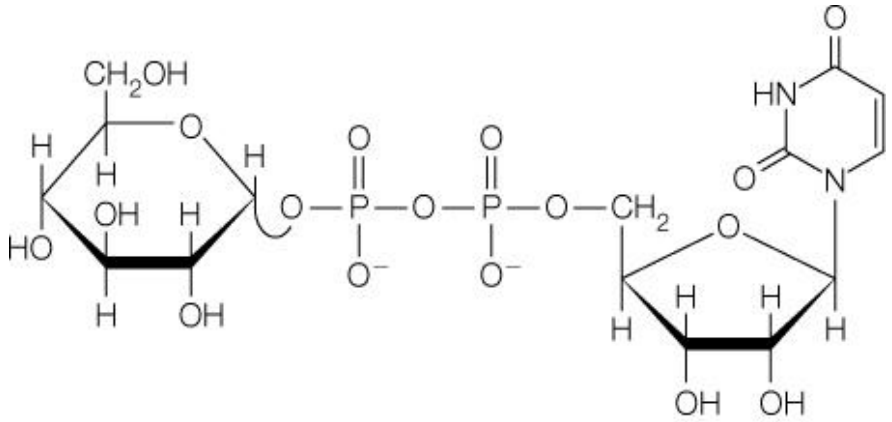
Glycogen Synthesis

- Glycogen synthesis involves a series of conversions from glucose to glucose-6-phosphate, to glucose-1-phosphate, to UDP–glucose, and finally to glycogen.
- **UTP** provides energy for this process.
- **UDP–glucose** is an activated form of the glucose molecule.
- Glycogen is extended from a primer built on and by the protein glycogenin.



1. Synthesis of UDP-Glucose



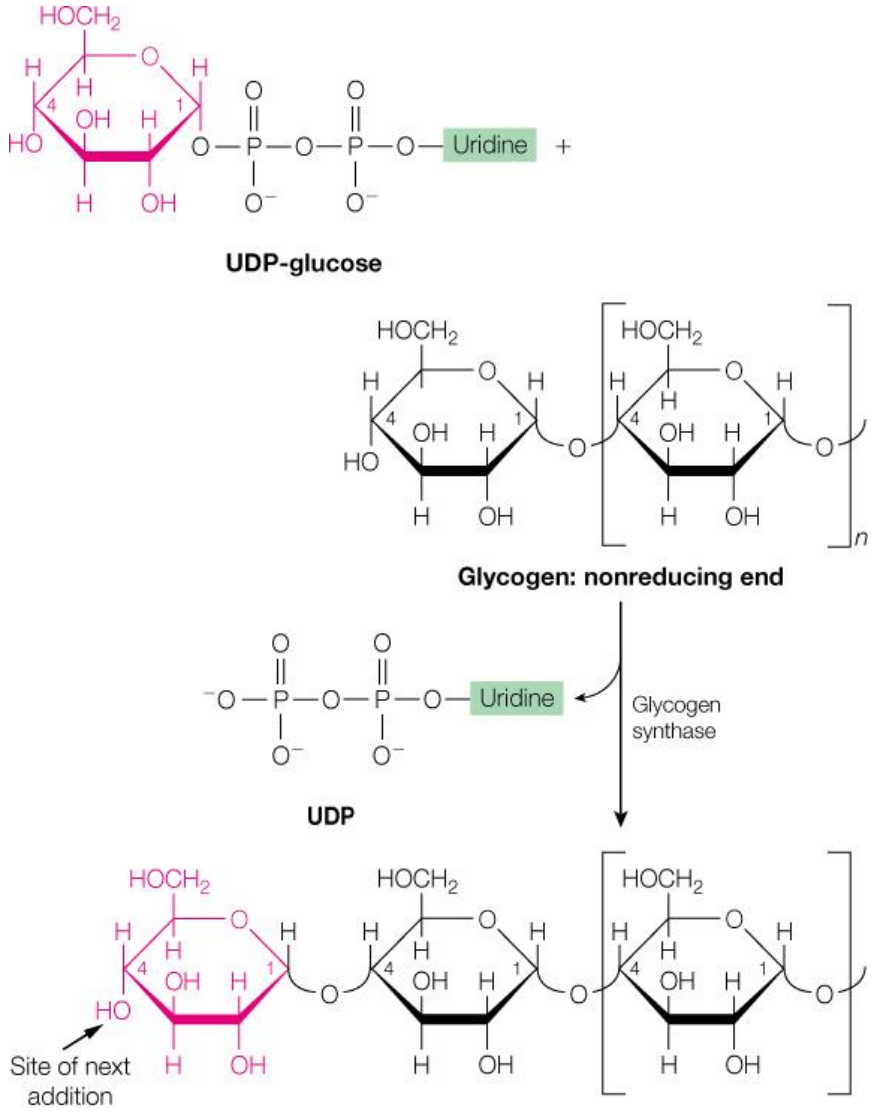


Uridine diphosphate glucose (UDP-Glc)

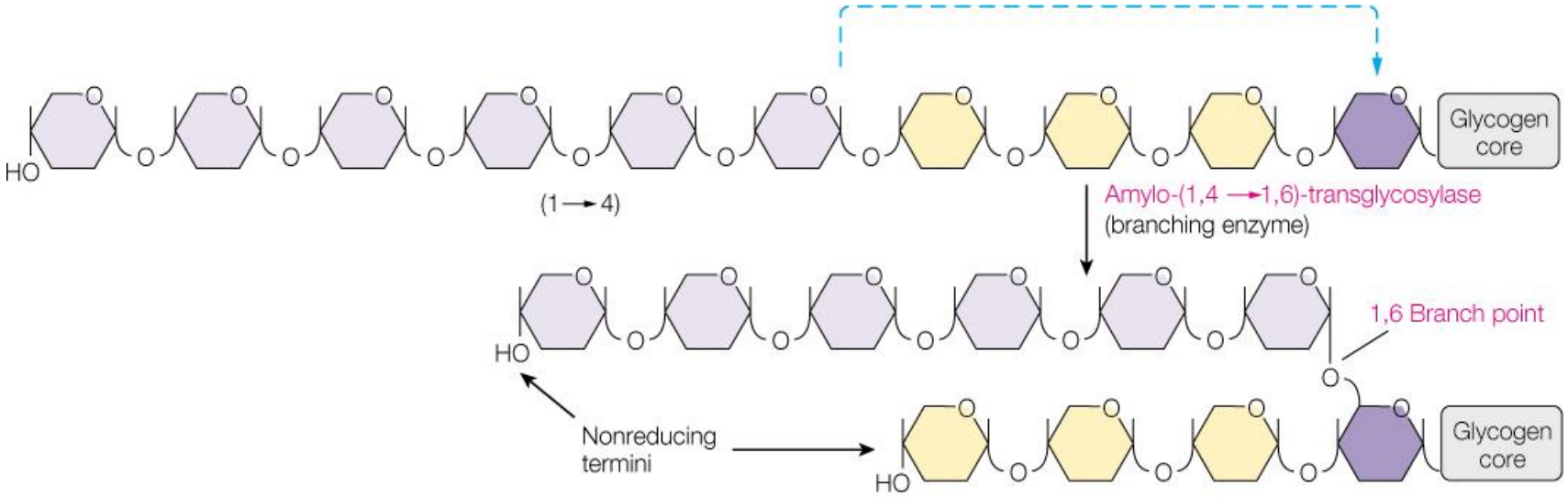
UDP-glucose is an activated form of glucose for glycogen synthesis

2. Synthesis of Glycogen from UDP-Glucose

Using UDP-glucose, α(1⟶4)-linked glycogen is synthesized by **glycogen synthase**



The Glycogen Branching Process



Glycogen branches are added by an amylo-(1,4→1,6) transglycosylase, which transfers 6 to 7 residues from a branch terminus at least 11 residues long

Glycogen synthesis summary

- Glycogen synthesis requires a different pathway in which G1P is activated by UTP to form UDP-glucose.
- Glycogen synthase adds glucosyl monomer units to the end of a growing glycogen molecule.
- Mostly in the liver but also in muscle cells, when there is excess glucose.
- Branching increases the density of glucose monomers in glycogen.

Glycogen Metabolism and Human Disease

In the liver: mainly hepatomegaly (enlarged liver) and hypoglycemia (low blood sugar) – others below cause muscle cramps and weakness:

TABLE 12.3 Human congenital defects of glycogen metabolism

Type	Common Name	Enzyme Deficiency	Glycogen Structure	Organ Affected
Ia	von Gierke disease	Glucose-6-phosphatase (ER)	Normal	Liver, kidney, intestine
Ib		Glucose-6-phosphate transporter (ER)	Normal	Liver
III	Cori or Forbes disease	Debranching enzyme	Short outer chains	Liver, heart, muscle
IV	Andersen disease	Branching enzyme	Abnormally long unbranched chains	Liver and other organs
V	McArdle disease	Muscle glycogen phosphorylase	Normal	Skeletal muscle
VI	Hers disease	Liver glycogen phosphorylase	Normal	Liver, leukocytes
VII	Tarui disease	Muscle phosphofructokinase	Normal	Muscle
IX		Liver phosphorylase kinase	Normal	Liver
—		Glycogen synthase	Normal	Liver