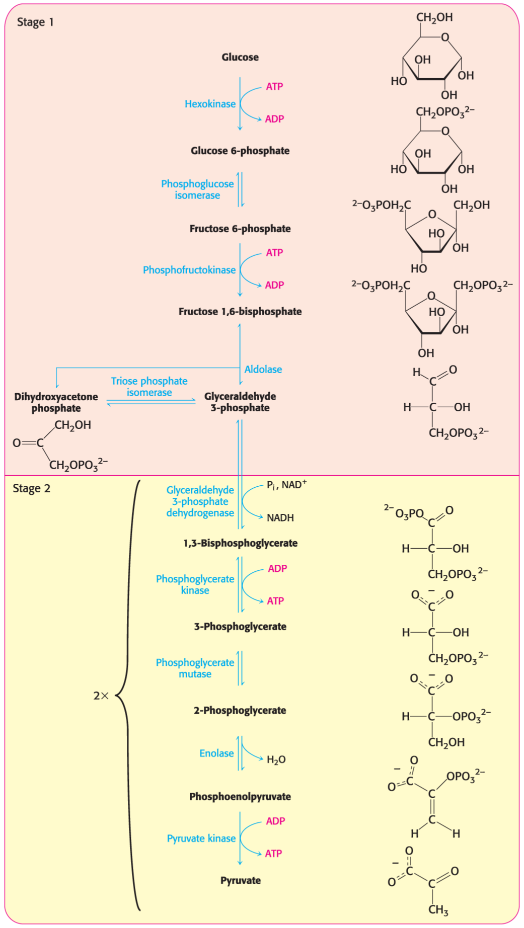
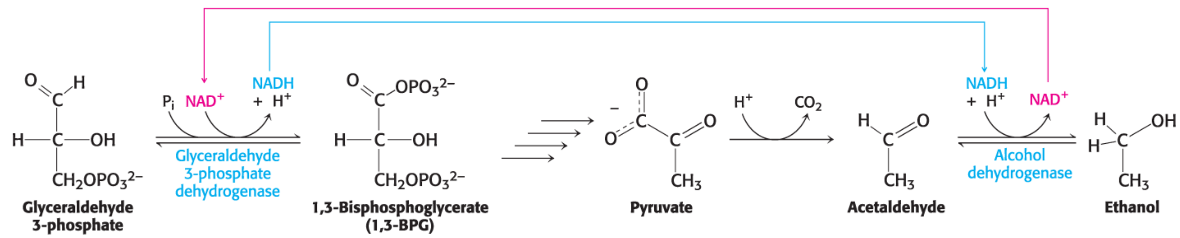
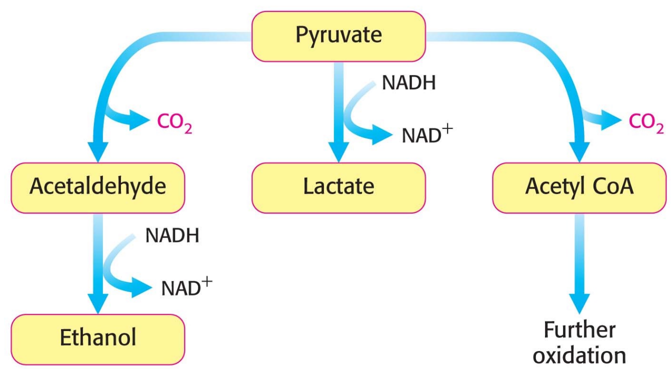
From Last Lecture



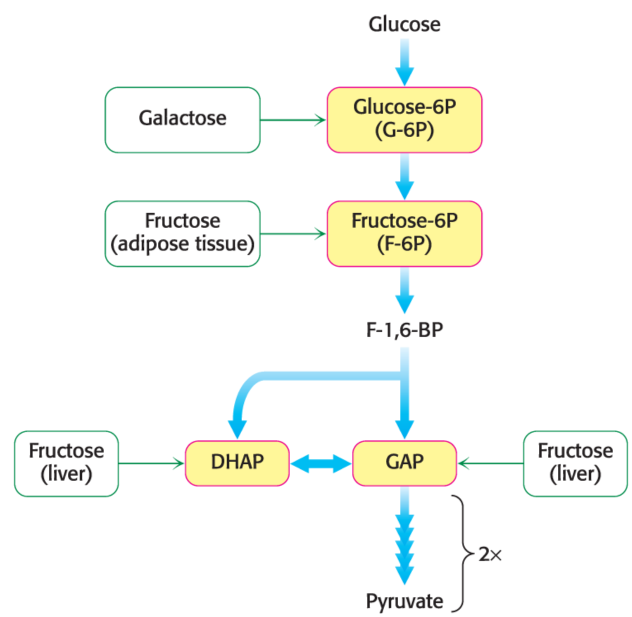


Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company

- Regenerating NAD^+ from pyruvate metabolism keeps glycolysis running by ensuring a steady supply of this essential electron carrier.



- Pyruvate is like a Swiss army knife—it can be converted into many things, depending on what the cell needs to survive or grow.



- Glycolysis is the gateway to understanding how energy flows through a cell
- Phosphorylating glucose adds a molecular 'anchor'—it keeps glucose inside the cell and marks it for metabolism

- Fructose and galactose are like alternate passengers—they take different entry ramps but eventually merge onto the glycolysis highway

In case you did not appreciate Glycolysis yet…

- **Central Role in Metabolism**
  - first step in glucose metabolism, occurring in nearly all living organisms.
  - connects to many other pathways: fermentation, the citric acid cycle, oxidative phosphorylation, and biosynthetic routes.
- **Showcases major biochemical principles**
  - enzyme catalysis
  - energy coupling (e.g., ATP generation and usage)
  - redox reactions (e.g., NAD^+ to NADH)
  - regulation of metabolism (e.g., feedback inhibition)
- **Biomedical Relevance**
  - aberrant glycolysis is involved in diseases like and diabetes.
  - many glycolytic enzymes are drug targets.
- **Evolutionary Perspective**
  - glycolysis is ancient and highly conserved, hinting at early life's metabolic strategies.
  - occurs in the cytoplasm and doesn't require oxygen—useful to discuss anaerobic vs. aerobic life.

Metabolism in Context: Glycolysis Helps Pancreatic Beta Cells Sense Glucose

- Insulin is secreted by β cells of the pancreas in response to high blood levels of glucose. This secretion is stimulated by the metabolism of glucose by the β cells.
- Glucose enters β cells through GLUT2 and is metabolized to pyruvate, and the pyruvate is subsequently oxidized to CO_2 and H_2O.
- The increase in ATP closes a K^+ channel, which alters the charge across the cell membrane. This alteration in turn opens Ca^{2+} channels. The influx of Ca^{2+} ions stimulates the release of insulin.

- Insulin release is controlled by blood glucose levels.
- Glucose metabolism(glycolysis) increases ATP production. Higher ATP closes an ATP-sensitive potassium channel.
- This changes the membrane charge, opening a calcium channel.
- Calcium influx triggers insulin granules to fuse with the membrane.
- Insulin is released into the blood.

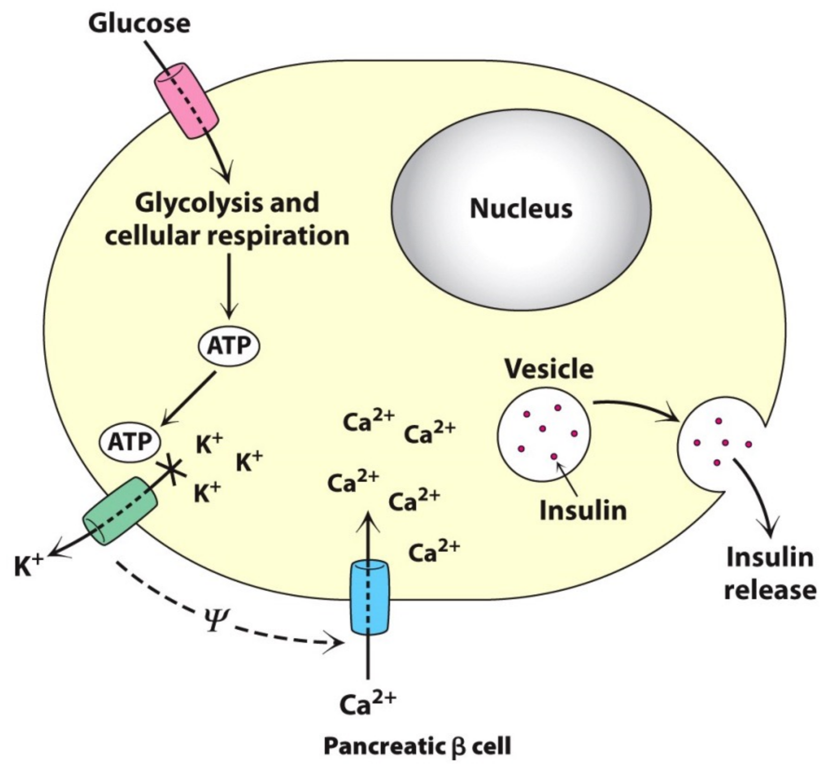


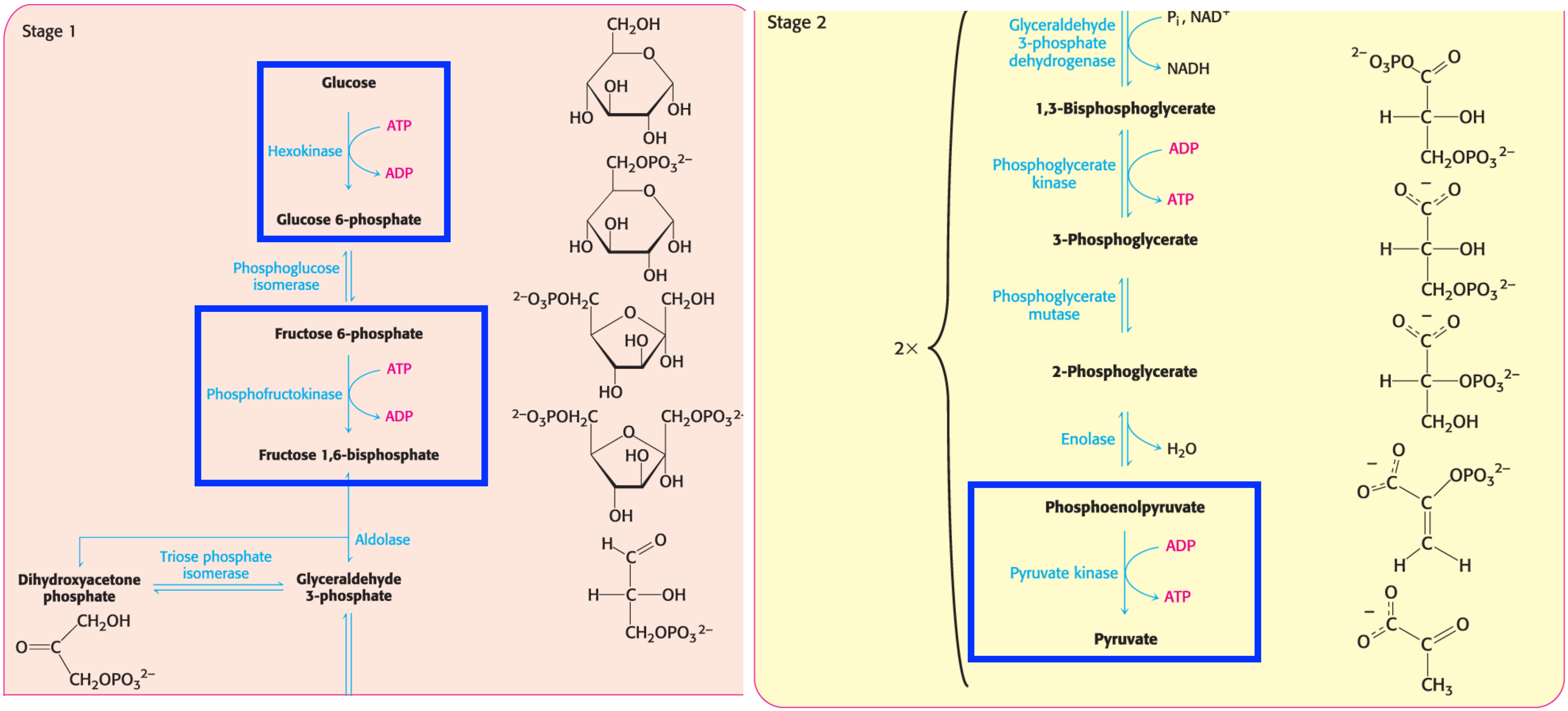
Figure 16.19
Biochemistry: A Short Course, Second Edition

Is pyruvate fermentation to ethanol present in humans?

Why do some people develop insulin resistance?

How is glycolysis regulated?

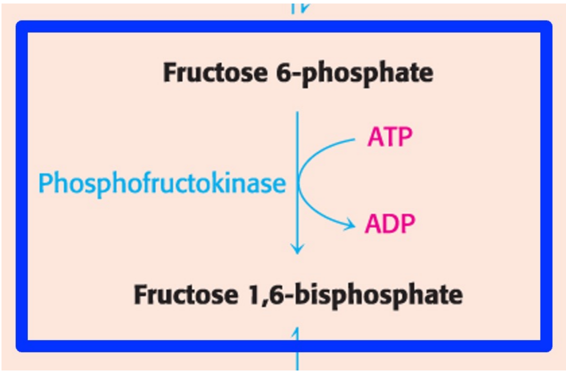
The Glycolytic Pathway is Tightly Controlled



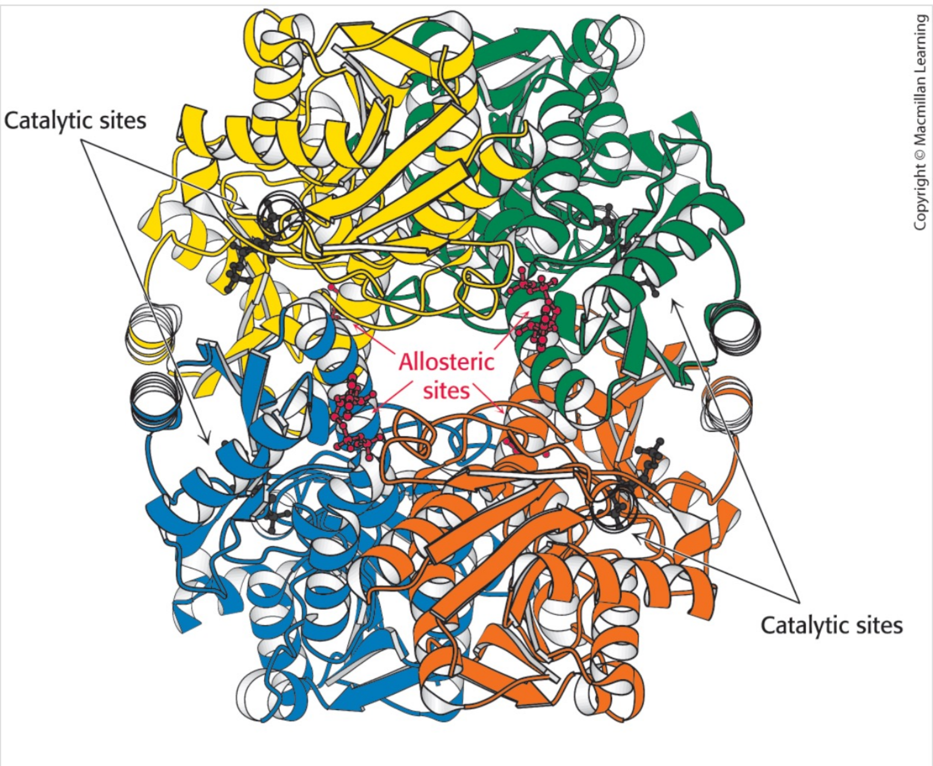
- Irreversible steps in glycolysis are major control points: hexokinase, PFK-1, pyruvate kinase
- These enzymes are regulated in three main ways:
  - binding of allosteric effectors (milliseconds) or covalent modification (seconds)
  - transcriptional control (hours)

- In muscles, signal is AMP/ATP ratio

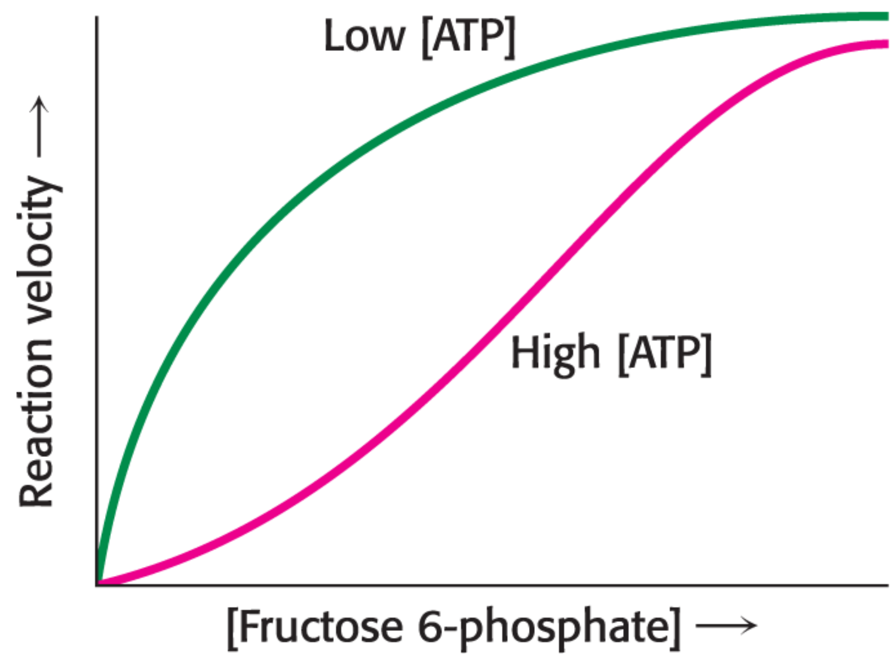
Phosphofructokinase



- The binding of ATP lowers the enzyme's affinity for fructose 6-phosphate.
- AMP reverses the inhibitory action of ATP.
- AMP competes with ATP for the binding site but does not inhibit the enzyme when bound.
- As a result, enzyme activity increases when the ATP/AMP ratio is lowered.



Phosphofructokinase from *E. coli*.



If glycolysis breaks down glucose to make energy, why doesn't the cell just reverse the same steps to make glucose when needed?

Which of the following statements best explains why glycolysis cannot simply run in reverse?

A. The enzymes used in glycolysis are not present in liver cells
B. Glycolysis includes irreversible steps that need alternative pathways
C. Glycolysis requires oxygen, but gluconeogenesis does not
D. Glucose is only made in the mitochondria

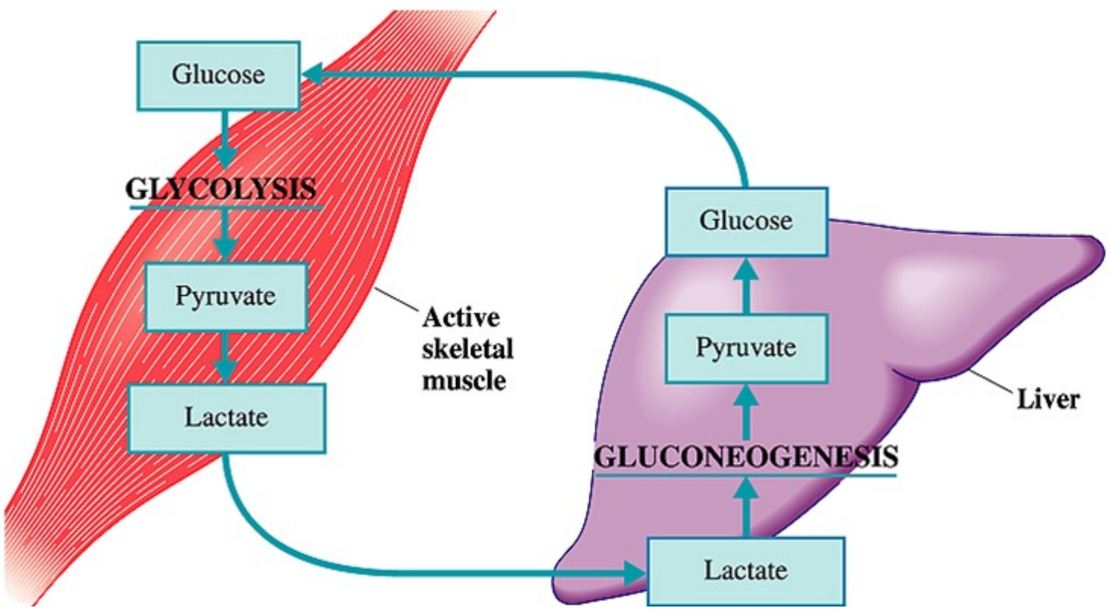
Answer: B

During fasting, the liver performs gluconeogenesis to:
A. Store excess energy
B. Convert glucose into glycogen
C. Maintain blood glucose levels for glucose-dependent tissues
D. Break down fats into glucose

Answer: C

Gluconeogenesis – The Basics

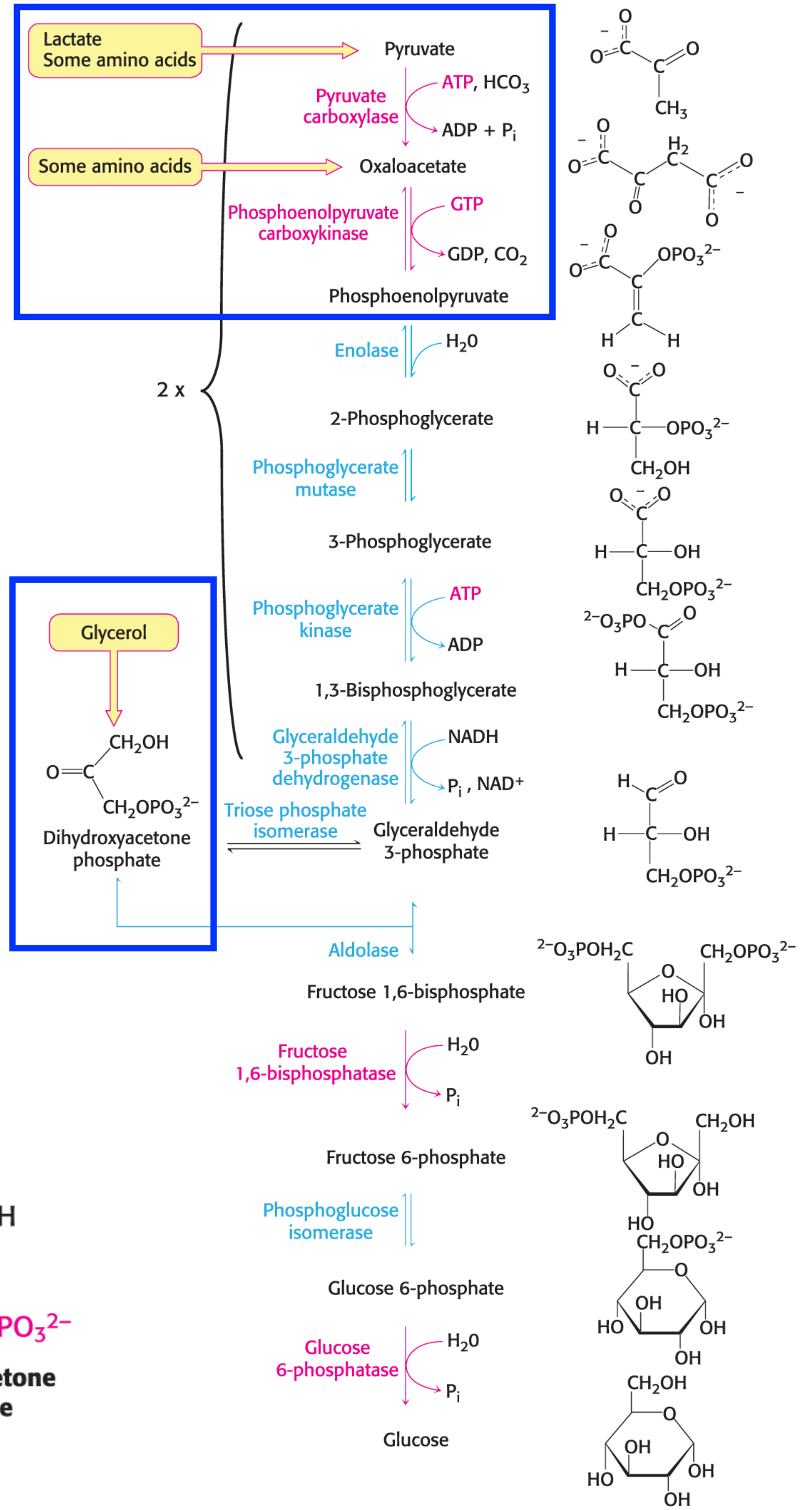
- Gluconeogenesis synthesizes glucose from noncarbohydrate precursors–an essential “recycling” process.
- Vital during fasting to supply glucose to the brain and red blood cells.
- Not simply the reverse of glycolysis
    - Irreversible glycolysis steps are bypassed with alternative exergonic reactions.
- Glycolysis and gluconeogenesis are reciprocally regulated to prevent energy-wasting futile cycles.

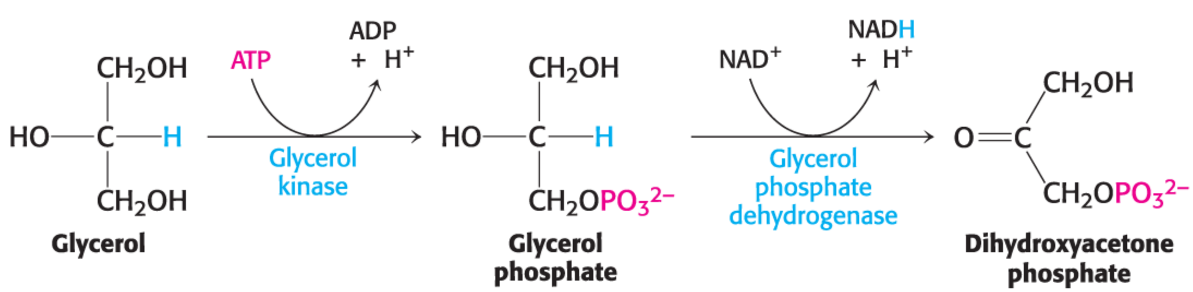


- Also recycles lactate produced by active tissues.
- Primary site: liver; minor sites: kidney and other tissues.
- Not active in: brain, skeletal muscle, or heart–these rely on blood glucose.

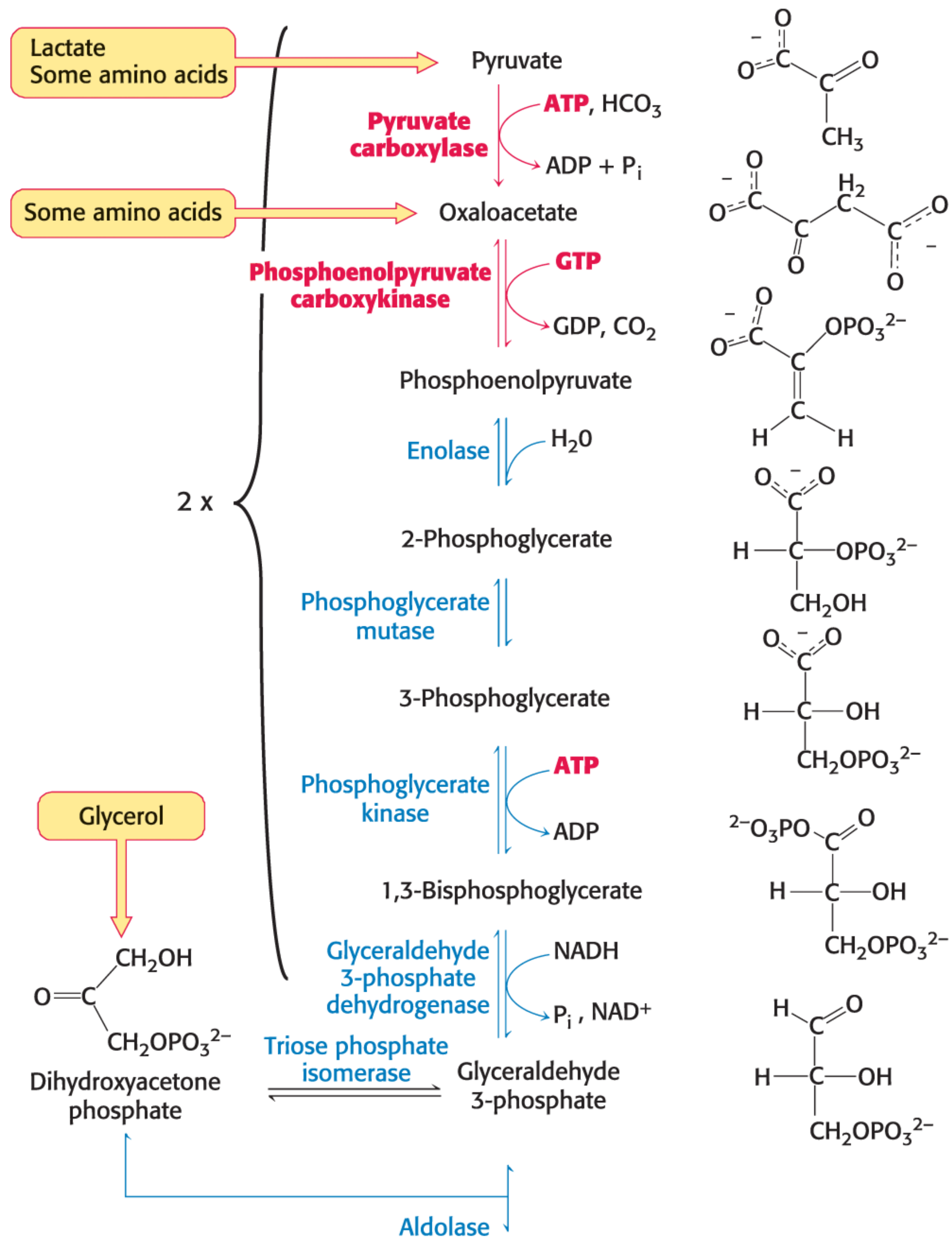
The Gluconeogenic Pathway

- Gluconeogenesis synthesizes glucose from noncarbohydrate precursors.

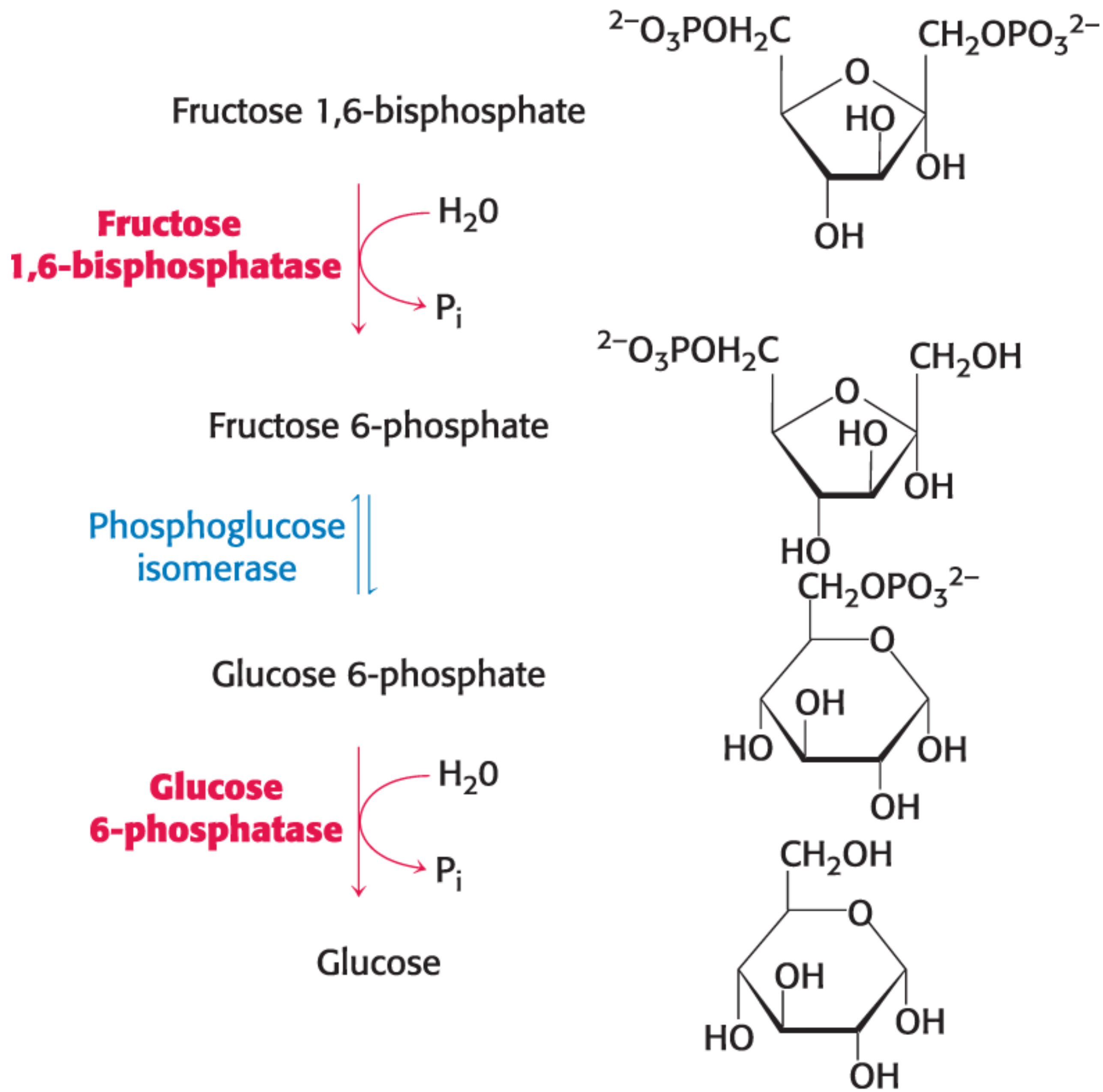




The Gluconeogenic Pathway



The Gluconeogenic Pathway



Gluconeogenesis is NOT the reversal of Glycolysis

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{glucose 6-phosphate} + \text{ADP}$$

$$\Delta G = -33 \text{ kJ mol}^{-1}\left(-8.0 \text{ kcal mol}^{-1}\right)$$

$$\text{Fructose 6-phosphate} + \text{ATP} \xrightarrow{\text{Phosphofructokinase}} \text{fructose 1,6-bisphosphate} + \text{ADP}$$

$$\Delta G = -22 \text{ kJ mol}^{-1}\left(-5.3 \text{ kcal mol}^{-1}\right)$$

$$\text{Phosphoenolpyruvate} + \text{ADP} \xrightarrow{\text{Pyruvate kinase}} \text{pyruvate} + \text{ATP}$$

$$\Delta G = -17 \text{ kJ mol}^{-1}\left(-4.0 \text{ kcal mol}^{-1}\right)$$

Three irreversible steps in glycolysis that must be bypassed in gluconeogenesis.

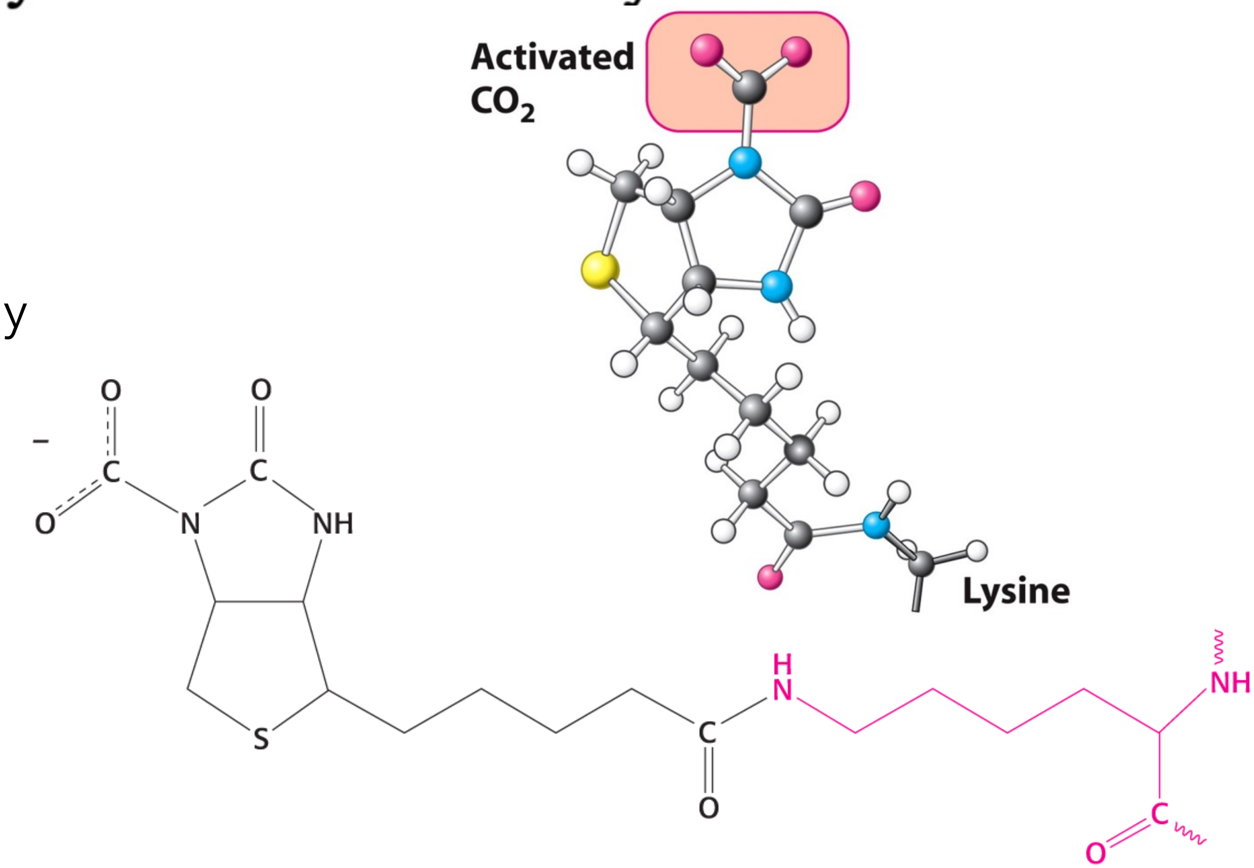
First Step in Gluconeogenesis

$$HCO_3^- + ATP \rightleftharpoons HOCO_2\text{-}PO_3^{2-} + ADP$$

$$\text{Biotin-enzyme} + HOCO_2\text{-}PO_3^{2-} \rightarrow CO_2\text{-biotin-enzyme} + P_i$$

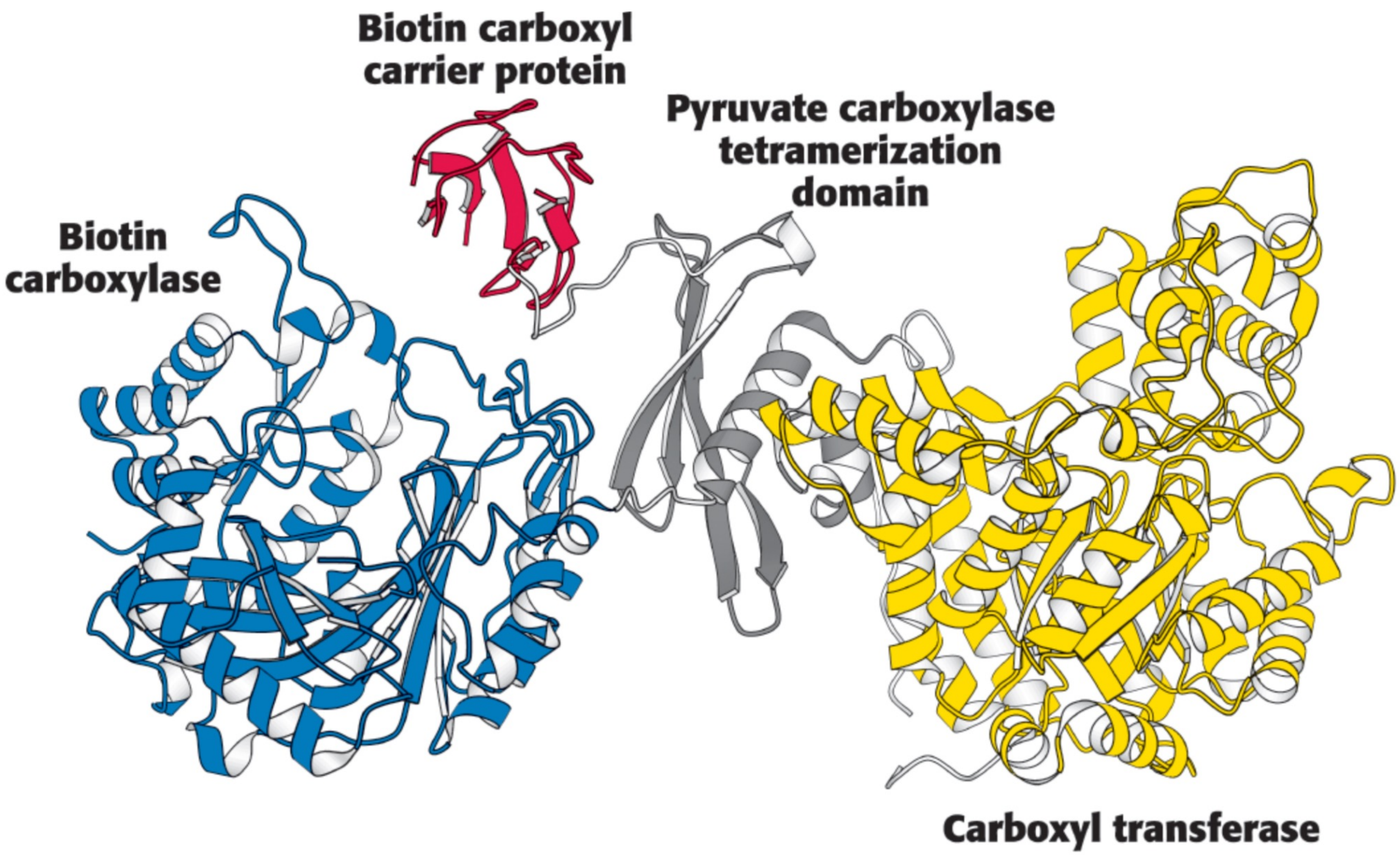
$$CO_2\text{-biotin-enzyme} + \text{pyruvate} \rightleftharpoons \text{biotin-enzyme} + \text{oxaloacetate}$$

- in mitochondria, catalyzed by pyruvate carboxylase (PC), which…
- requires biotin (vitamin B7) as a prosthetic group.
- Biotin carries activated CO_2 in carboxylation reactions.
- Note that acetyl-CoA must be bound to the enzyme for biotin carboxylation.



Carboxybiotin covalently bound to ε-amino group of a lysine

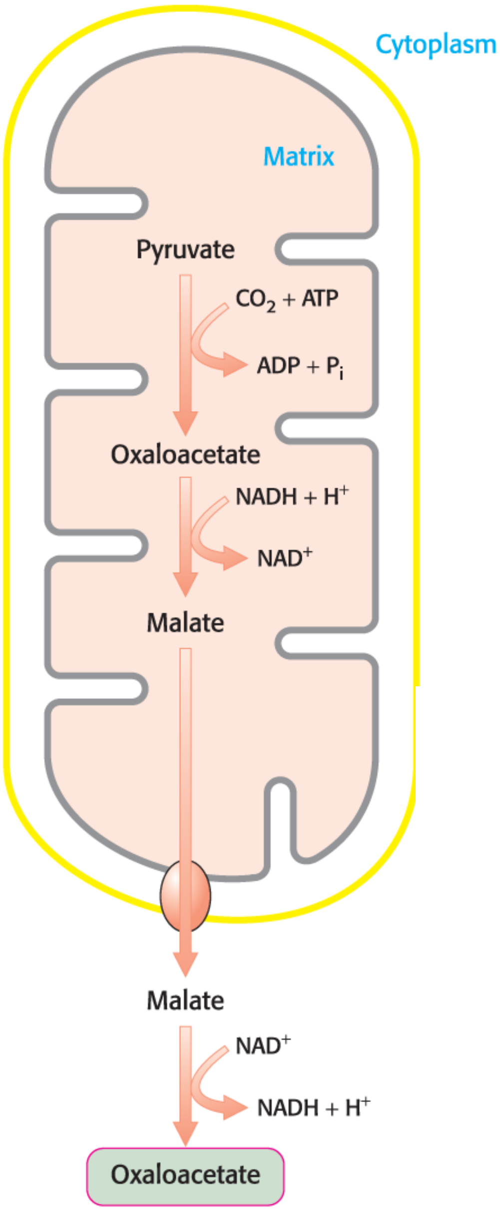
First Step in Gluconeogenesis



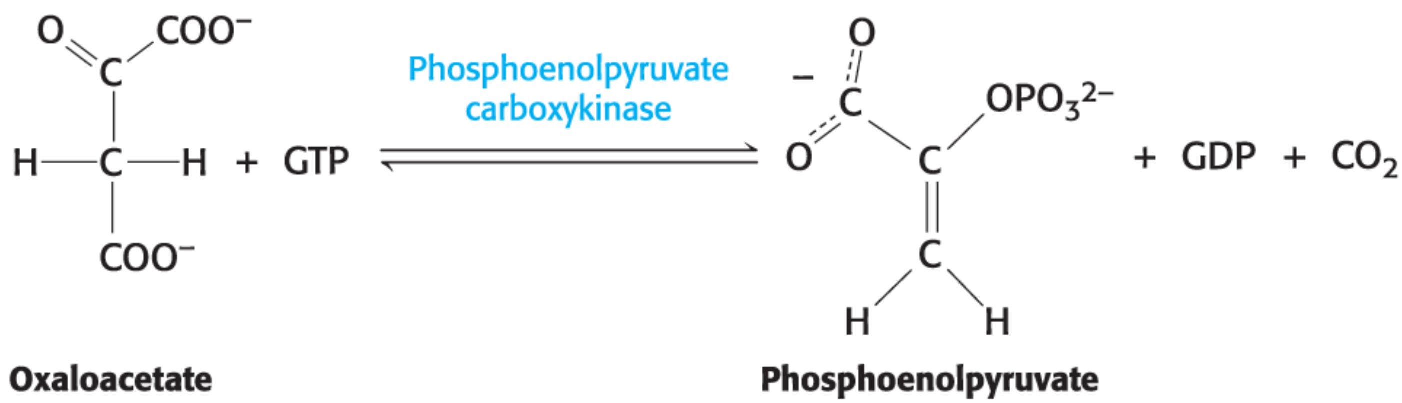
Charles Bruglag/FeaturePics.

- Biotin carboxylase forms carboxyphosphate and attaches CO_2 to biotin.
- Biotin carboxyl carrier protein carries covalently bound biotin.
  - Swings ~75 Å to reach the pyruvate carboxylase domain.
- Pyruvate carboxylase domain transfers CO_2 to pyruvate → oxaloacetate.
- Tetramerization domain helps subunits assemble.

Oxaloacetate → Phosphoenolpyruvate



- Oxaloacetate must move to the cytoplasm
  - Is reduced to malate by mitochondrial malate dehydrogenase
- Malate crosses the mitochondrial membrane, then is reoxidized to back to oxaloacetate in the cytoplasm (by cytoplasmic NAD^+-linked malate dehydrogenase).

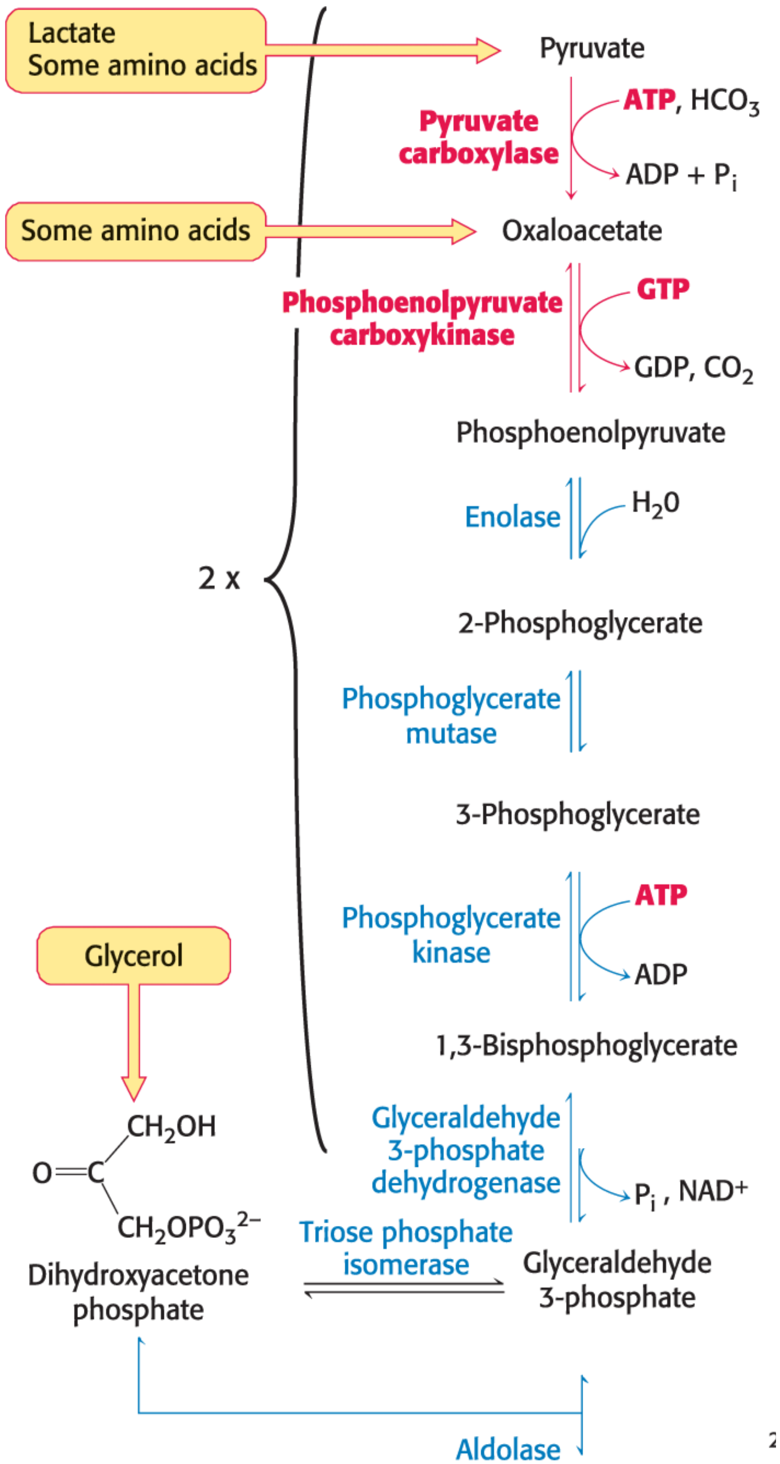


Note: This is both a decarboxylation and a phosphorylation reaction catalyzed by PEPCK.

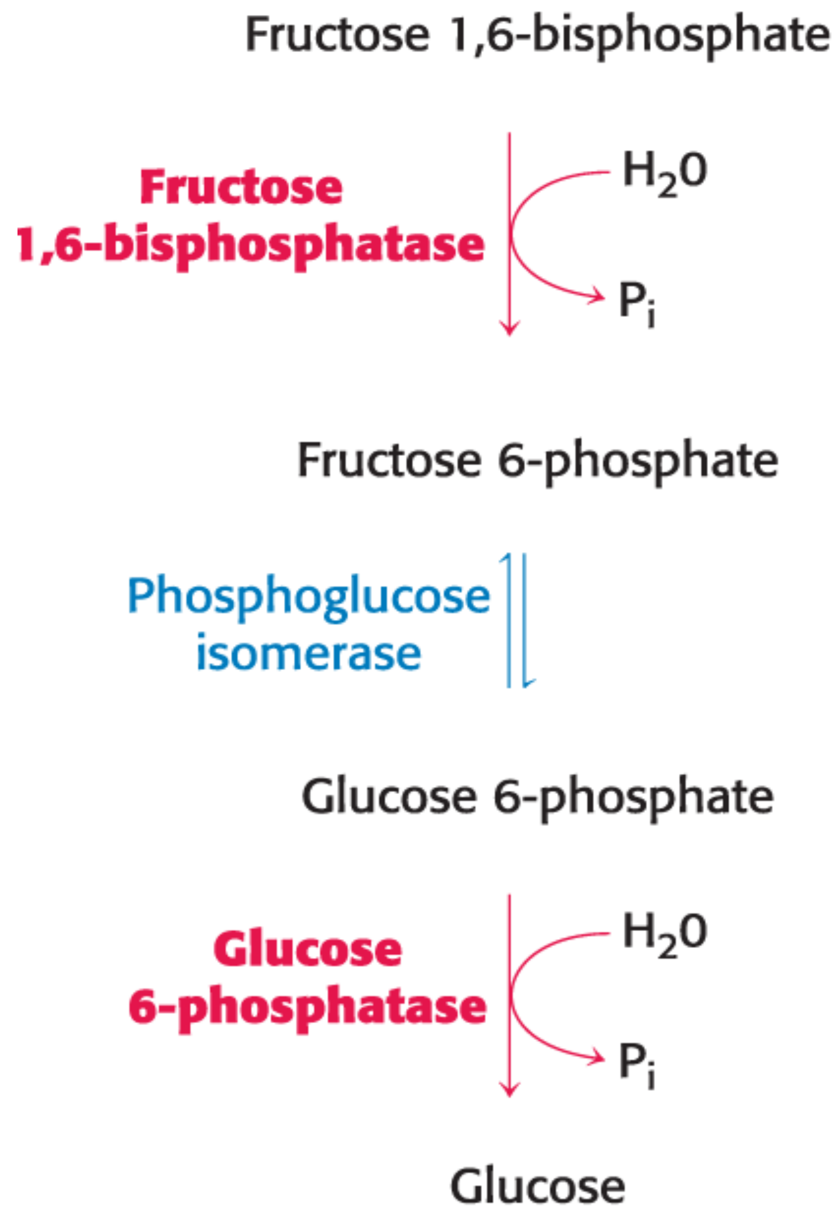
Quick Quiz 1

Glucose is an essential fuel for the brain. In times of starvation, glucose is generated from non-diet precursors. As skeletal muscle breaks down, _________ is/are generated and enter the gluconeogenic pathway to generate fuel for the brain.

A. nitrogen
B. lactate
C. urea
D. amino acids
E. glycerol



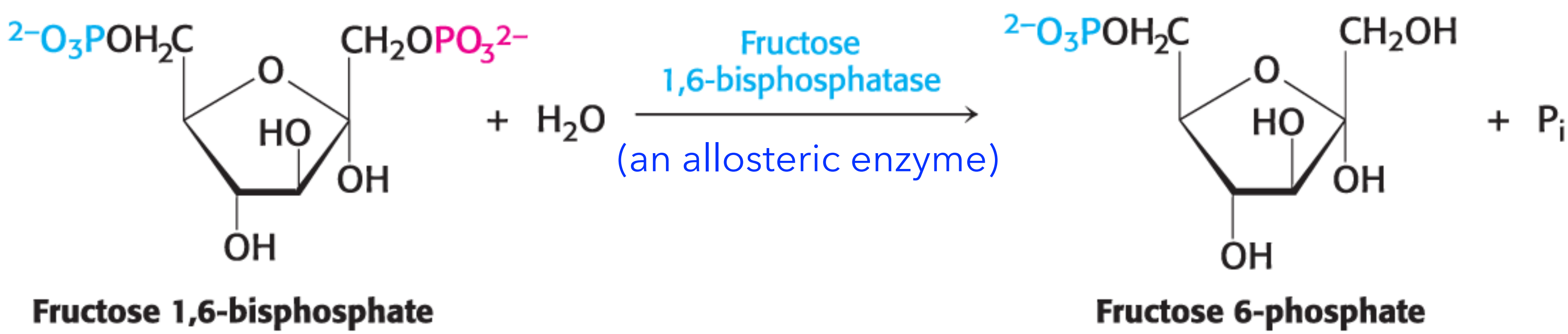
Lactate
Some amino acids
Pyruvate
Pyruvate carboxylase
ATP, HCO3
ADP + Pi
Some amino acids
Oxaloacetate
Phosphoenolpyruvate carboxykinase
GTP
GDP, CO2
Phosphoenolpyruvate
Enolase
H2O
2 x
2-Phosphoglycerate
Phosphoglycerate mutase
3-Phosphoglycerate
Phosphoglycerate kinase
ATP
ADP
1,3-Bisphosphoglycerate
Glycerol
Glyceraldehyde 3-phosphate dehydrogenase
Pi , NAD+
CH2OH
O=C
CH2OPO3 2−
Dihydroxyacetone phosphate
Triose phosphate isomerase
Glyceraldehyde 3-phosphate
Aldolase



Fructose 1,6-bisphosphate
Fructose 1,6-bisphosphatase
H2O
Pi
Fructose 6-phosphate
Phosphoglucose isomerase
Glucose 6-phosphate
Glucose 6-phosphatase
H2O
Pi
Glucose

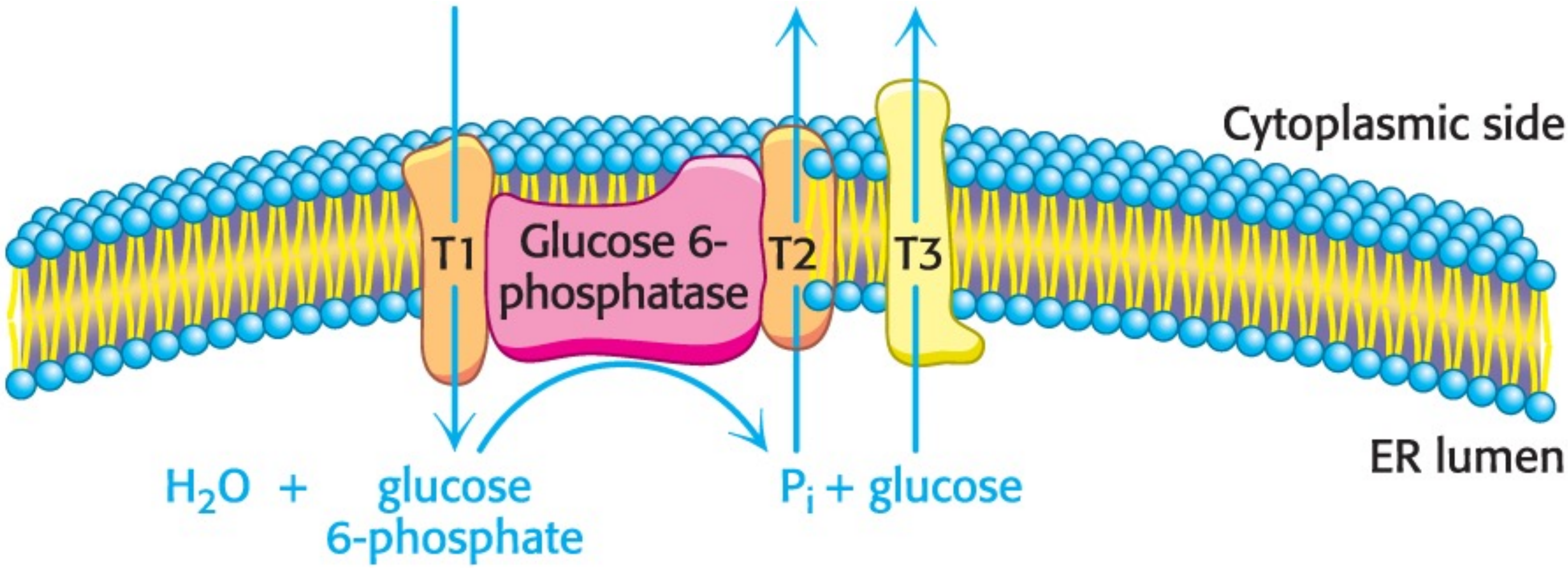
Fructose 1,6-bisphosphate ➜ Fructose 6-phosphate

- Phosphoenolpyruvate is metabolized by the enzymes of glycolysis in the reverse direction until the next irreversible step, the hydrolysis of fructose 1,6-bisphosphate.



- FBPase is an allosteric enzyme ➜ primary regulation point of gluconeogenesis.
- Fructose 6-phosphate produced ➜ Glucose 6-phosphate

Generation of free glucose is an important control point



- The generation of free glucose, which occurs essentially only in the liver, is the final step in gluconeogenesis.
- Glucose 6-phosphate is transported into the lumen of the endoplasmic reticulum.
- Glucose 6-phosphatase, an integral membrane on the inner surface of the endoplasmic reticulum, catalyzes the formation of glucose from glucose 6-phosphate.
- Transporters transport Pi (T2) and glucose (T3) back to the cytoplasm.

Let's do some accounting....

Gluconeogenesis:

$$2\ \text{Pyruvate} + 4\ \text{ATP} + 2\ \text{GTP} + 2\ \text{NADH} + 2\ \text{H}^+ + 6\ \text{H}_2\text{O} \rightarrow$$

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

$$\Delta G^{\circ\prime} = -48\ \text{kJ mol}^{-1}(-11\ \text{kcal mol}^{-1})$$

Reversal of Glycolysis:

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

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

$$\Delta G^{\circ\prime} = +90\ \text{kJ mol}^{-1}(+22\ \text{kcal mol}^{-1})$$

Quick Quiz 2

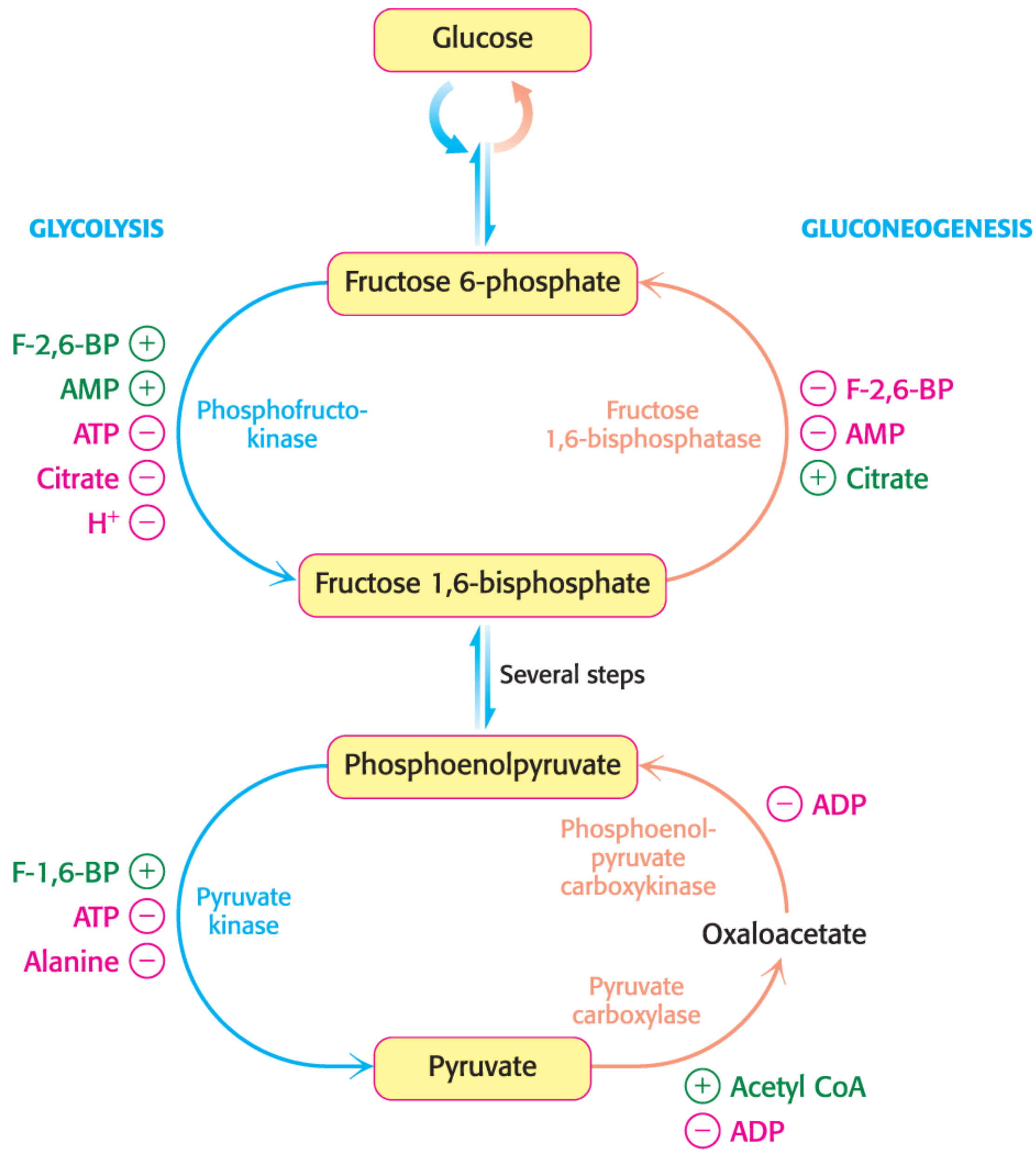
Which of the following is true for both glycolysis and gluconeogenesis?

A. Some steps in the pathway consume ATP.
B. Some steps in the pathway produce ATP.
C. Glucose-6-phosphate is an intermediate in the pathway.
D. All of the above.
E. A & C

Gluconeogenesis and glycolysis in the liver are under reciprocal regulation

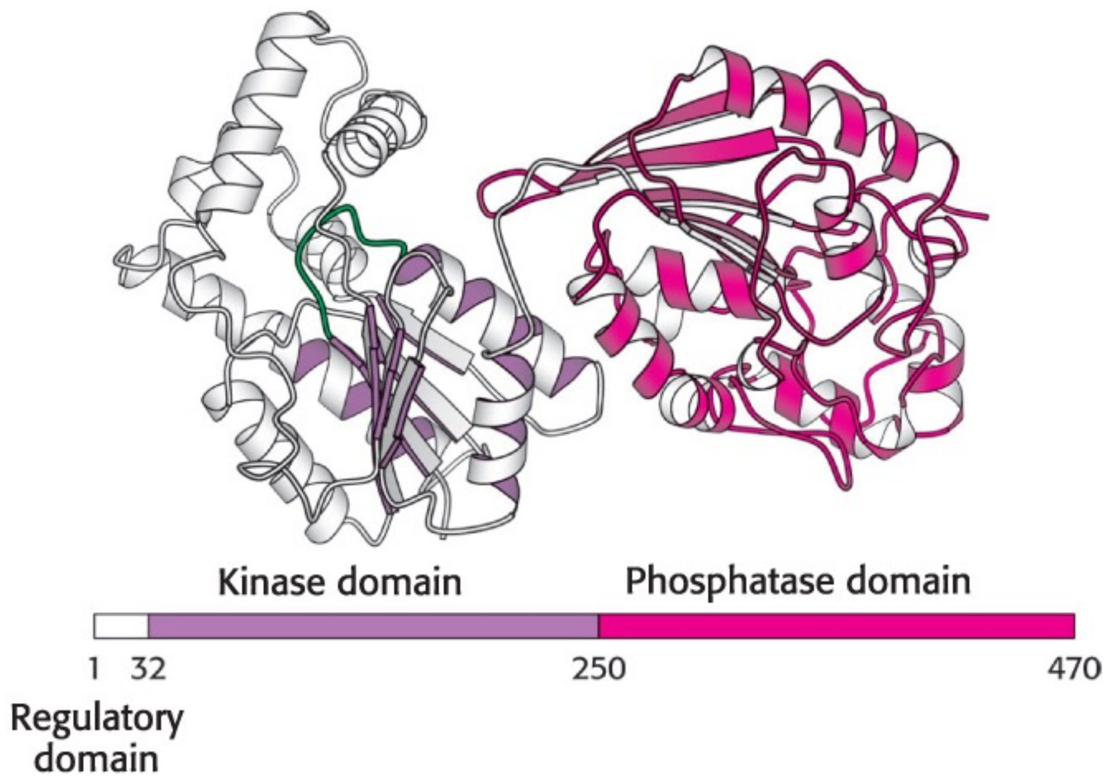
Reciprocal regulation → glycolysis will predominate when glucose is abundant, and gluconeogenesis takes over when glucose is scarce.

Activities of distinctive enzymes are controlled (both pathways are not concurrently active) and rate is regulated by glucose (glycolysis) and lactate/other precursors (gluconeogenesis).



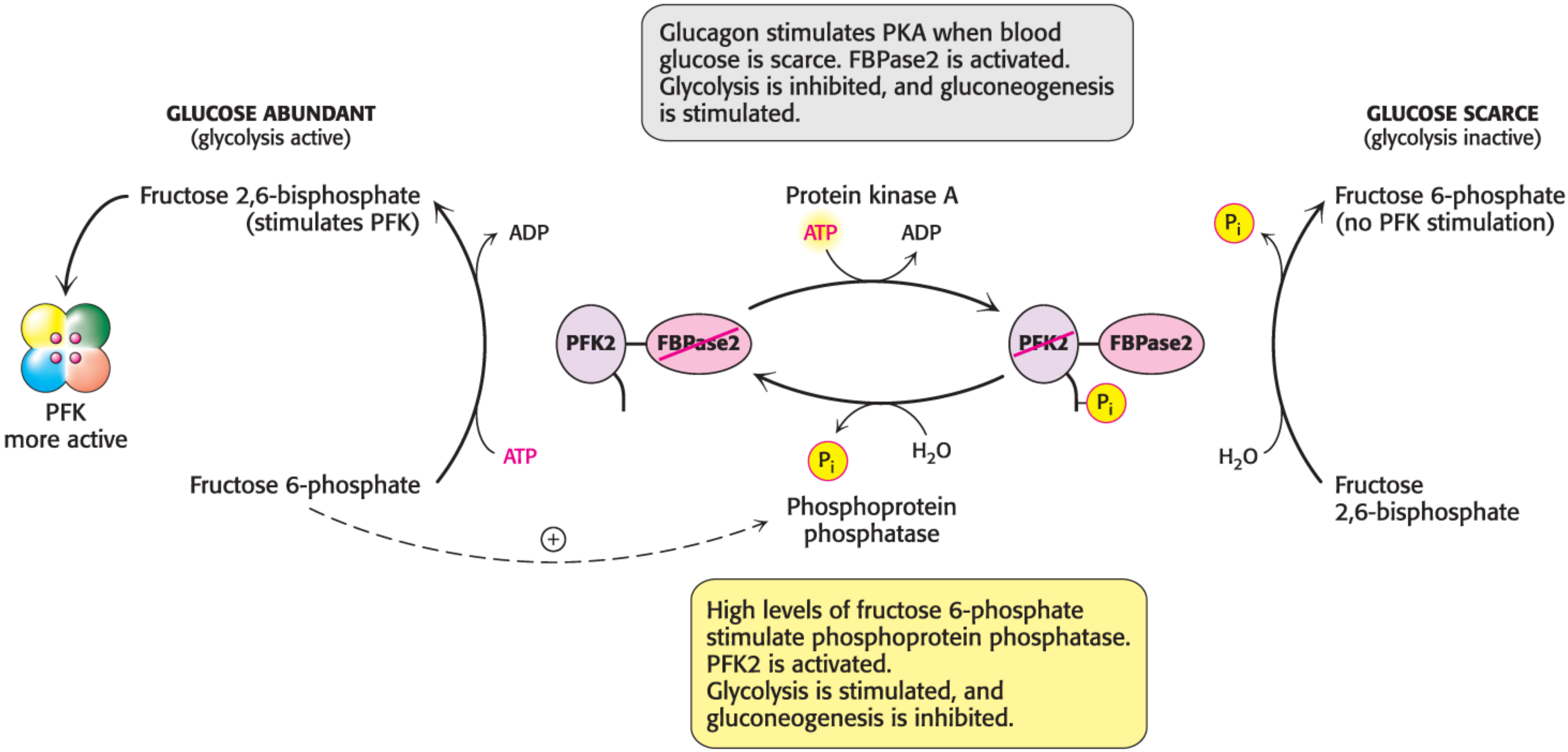
Gluconeogenesis and glycolysis in the liver are under reciprocal regulation

- The balance between glycolysis and glycogenesis in the liver is sensitive to blood-glucose concentration.
- In liver, the rates of glycolysis and gluconeogenesis are adjusted to maintain blood glucose levels.
- The key regulator of glucose metabolism in liver is fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate stimulates phosphofructokinase and inhibits fructose 1,6-bisphosphatase.



- The kinase that synthesizes fructose 2,6-bisphosphate and the phosphatase that hydrolyzes this molecule are located on the same polypeptide chain. Such an arrangement is called a bifunctional enzyme.

Gluconeogenesis and glycolysis in the liver are under reciprocal regulation



- Rates of glycolysis and gluconeogenesis in the liver are sensitive to blood-glucose concentration and are adjusted to maintain blood glucose levels.

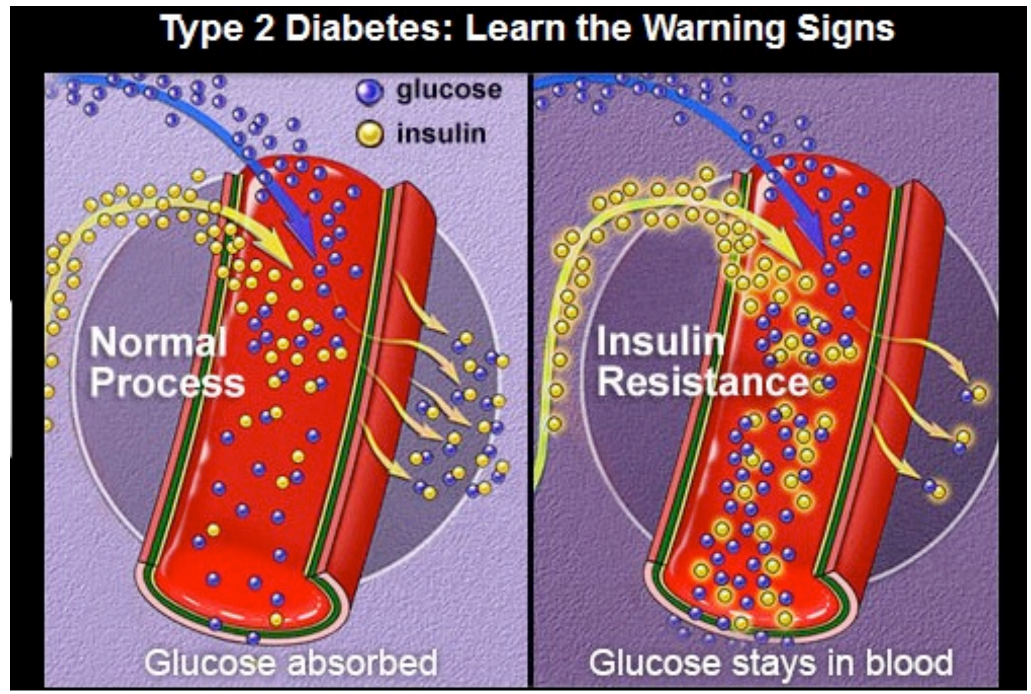
- The key regulator of glucose metabolism in liver is fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate stimulates phosphofructokinase and inhibits fructose 1,6-bisphosphatase.

Gluconeogenesis and glycolysis are reciprocally regulated

- Insulin normally inhibits gluconeogenesis. In type 2 diabetes, insulin fails to act, a condition called insulin resistance.
- The enzymes of gluconeogenesis, especially PEPCK (phosphoenolpuruvatecarboxy-kinase), are active leading to abnormally high levels of blood glucose.
- Exercise and diet can enhance insulin sensitivity

Clinical Insight

Insulin Fails to Inhibit Gluconeogenesis in Type 2 Diabetes



http://www.medicinenet.com/type_2_diabetes_pictures_slideshow/article.htm

Why do some people develop insulin resistance?

- Insulin resistance ➜ cells no longer respond well to insulin, so glucose uptake is impaired, even though insulin is present.

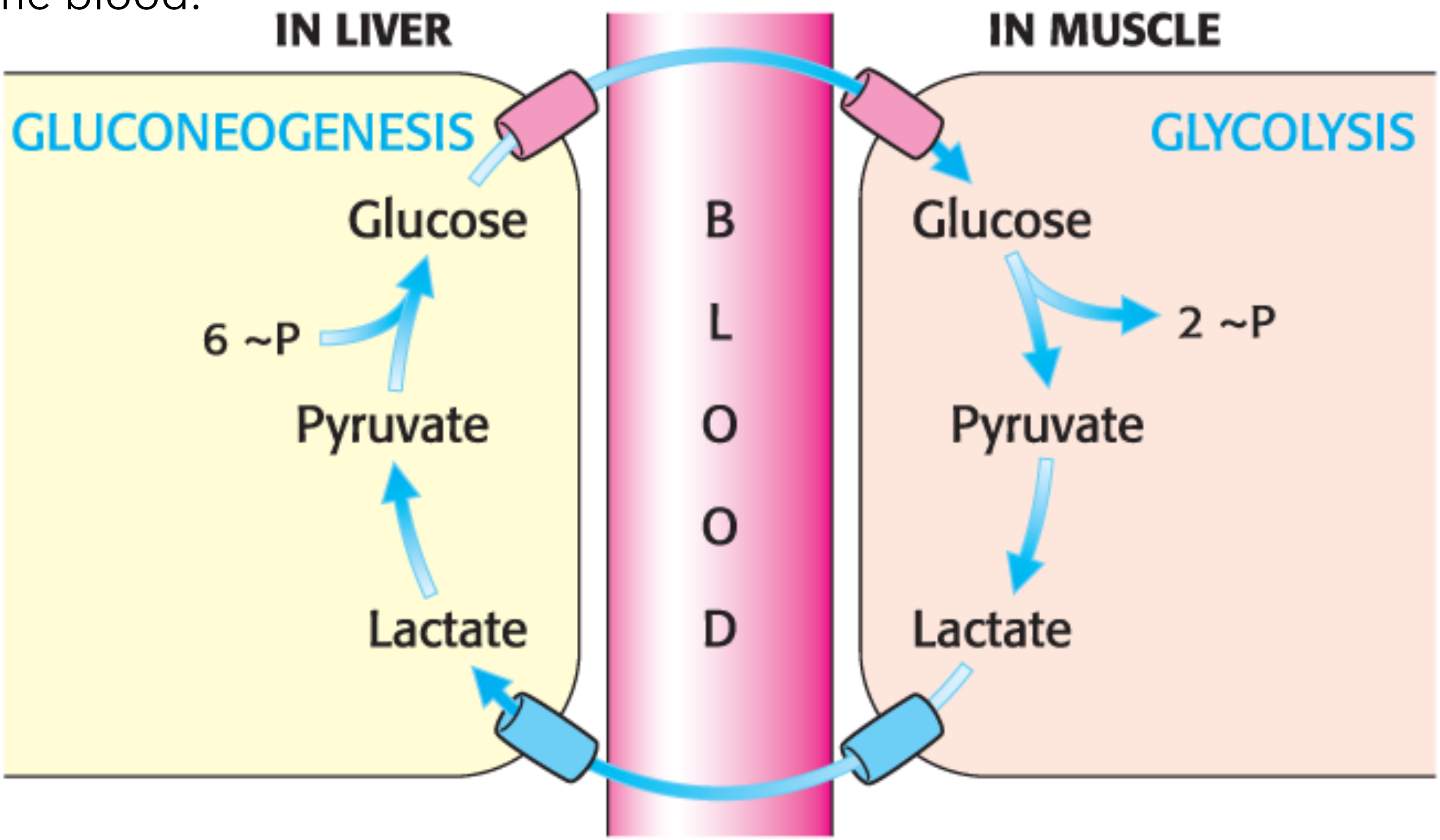
- Insulin binds to its receptor
  - normally activates a signaling cascade to insert GLUT4 transporters on the membrane.
- In insulin resistance
  - the signaling pathway is disrupted.
  - GLUT4 transporters don’t reach the surface, so glucose can't enter the cell efficiently.

- Causes: fat buildup in liver and muscle cells, genetics, chronic high insulin levels, inflammation and oxidative stress

- As a result, blood glucose stays high, pancreas works harder ➜ insulin increases; over time ➜ Beta-cell fatigue ➜ Type 2 Diabetes

Metabolism in context: Precursors Formed by Muscle Are Used by Other Organs

- Muscle and liver display inter-organ cooperation in a series of reactions called the ***Cori cycle***.
- Lactate produced by muscle during contraction is released into the blood.
- Liver removes the lactate and converts it into glucose, which can be released into the blood.



Quick Quiz 3

What is the name of the enzyme that catalyzes the conversion of pyruvate to lactate in muscle cells?

A. pyruvate decarboxylase
B. lactate decarboxylase
C. pyruvate dehydrogenase
D. lactate dehydrogenase
E. None of the above.

Quick Quiz 4

Which of the following occurs during glycolysis?

A. A six-carbon molecule is split into two different three-carbon molecules.
B. A three-carbon molecule combines with another three-carbon molecule to form a six-carbon molecule.
C. NAD^+ participates in an oxidation reaction.
D. A & C
E. B & C

From Glycolysis to Gluconeogenesis

Glycolysis – Energy Generation

→ breakdown of glucose to pyruvate
→ occurs in the cytoplasm
→ produces ATP and NADH
→ active during fed state
→ Key enzymes:

- Hexokinase
- Phosphofructokinase-1 (PFK-1)
- Pyruvate kinase

While glycolysis breaks down glucose to meet immediate energy demands, gluconeogenesis allows the body to maintain glucose levels when dietary intake is low

Gluconeogenesis – Glucose Synthesis

→ synthesis of glucose from non-carbohydrate precursors
→ occurs mainly in liver (and kidney)
→ consumes ATP and NADH
→ active during fasting or prolonged exercise
→ bypasses glycolysis's irreversible steps using:

- Pyruvate carboxylase
- PEP carboxykinase
- Fructose 1,6-bisphosphatase
- Glucose 6-phosphatase

Assigned Problems

Chapter	Tymochko, Berg, Stryer, Biochemistry, 2^{nd} Edition,	**Chapter**	Tymochko, Berg, Stryer, Biochemistry, 2^{nd} Edition,
16	1, 3, 9, 10, 15, 17, 19, 22, 24, 25, 29, 31.	17	2, 4, 5, 8, 10, 11, 17, 20, 21, 22.
Chapter	Tymochko, Berg, Stryer, Biochemistry, 3^{rd} Edition, 4^{th} Edition (bottom line)	**Chapter**	Tymochko, Berg, Stryer, Biochemistry, 3^{rd} Edition, 4^{th} Edition (bottom line)
16	2, 4, 10, 11, 15, 17, 19, 22, 24, 26, 31, 33.	17	2, 4, 5, 8, 10, 11, 17, 22, 23, 24.