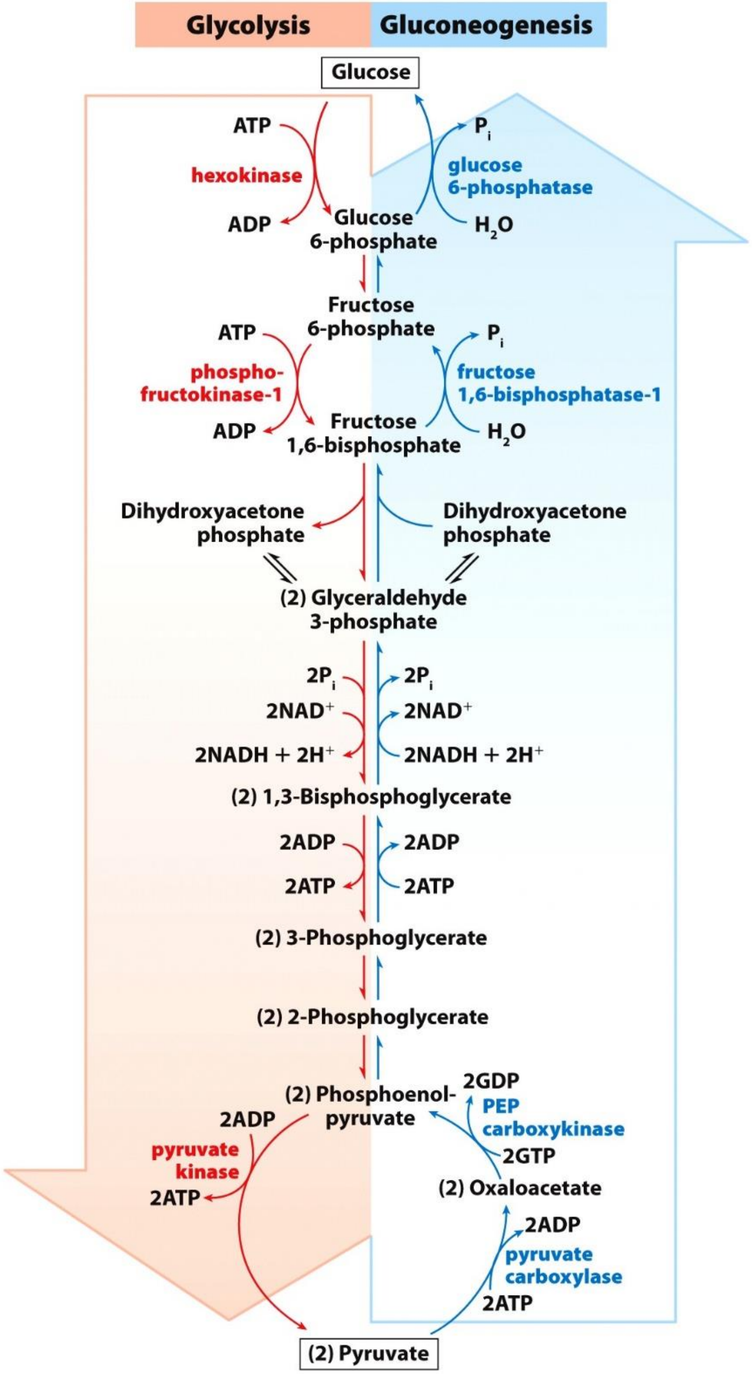
GLYCOLYSIS AND GLUCONEOGENESIS



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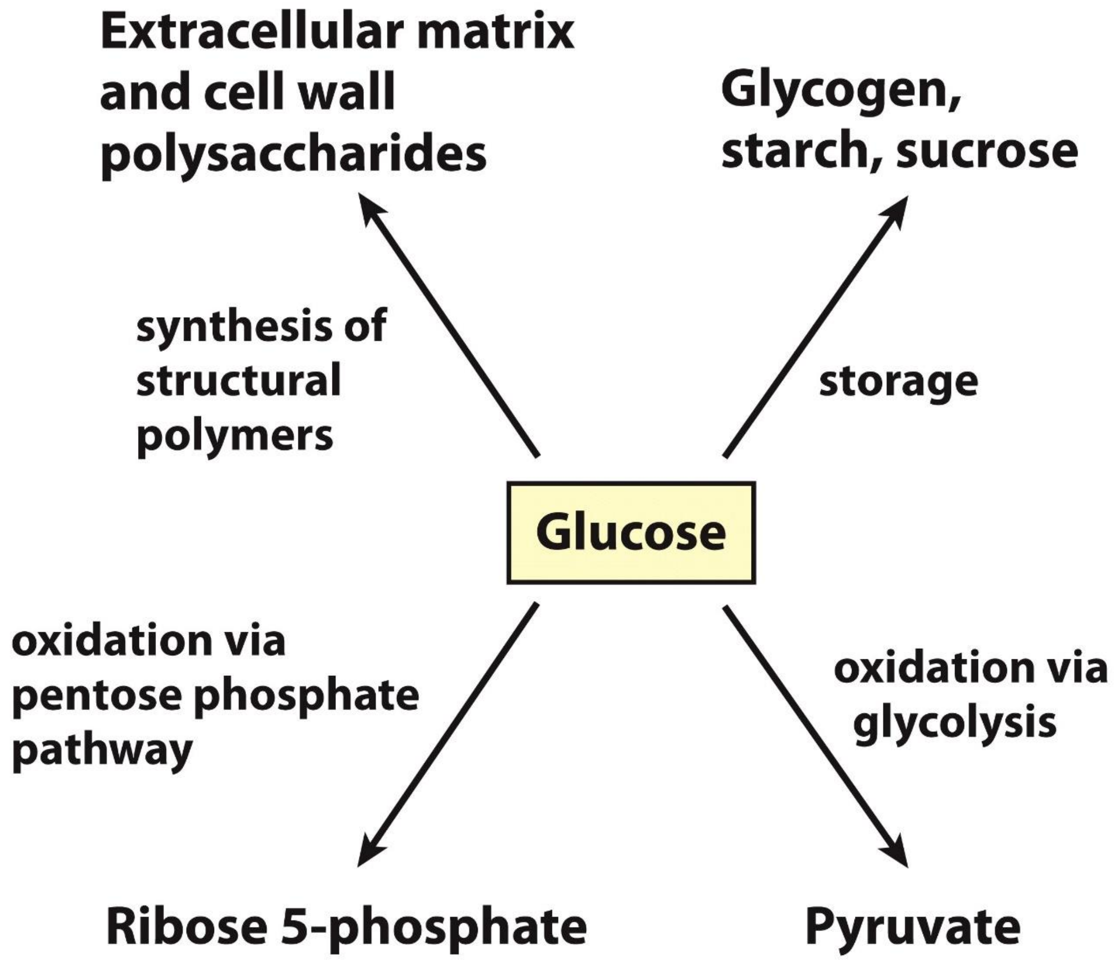
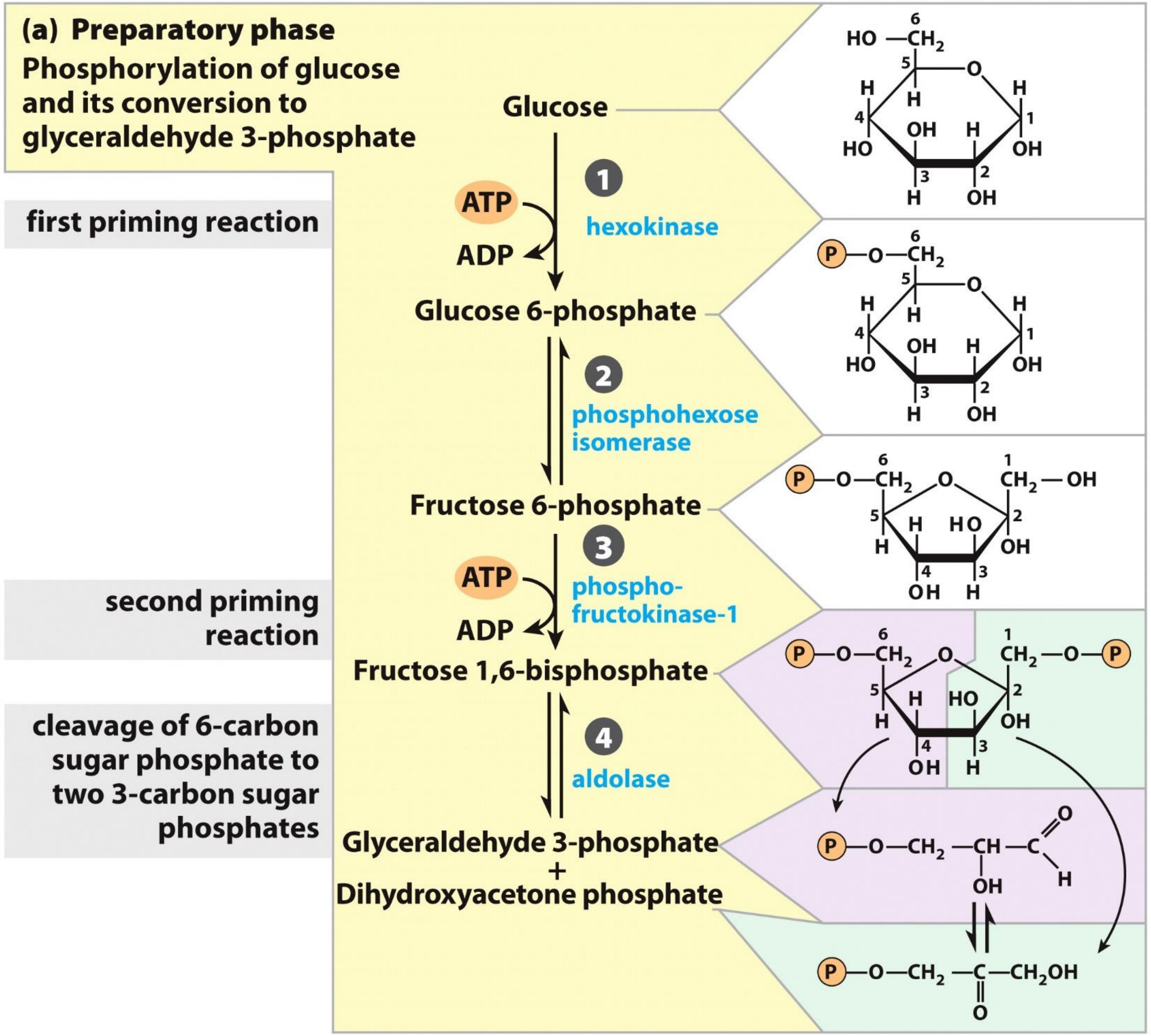
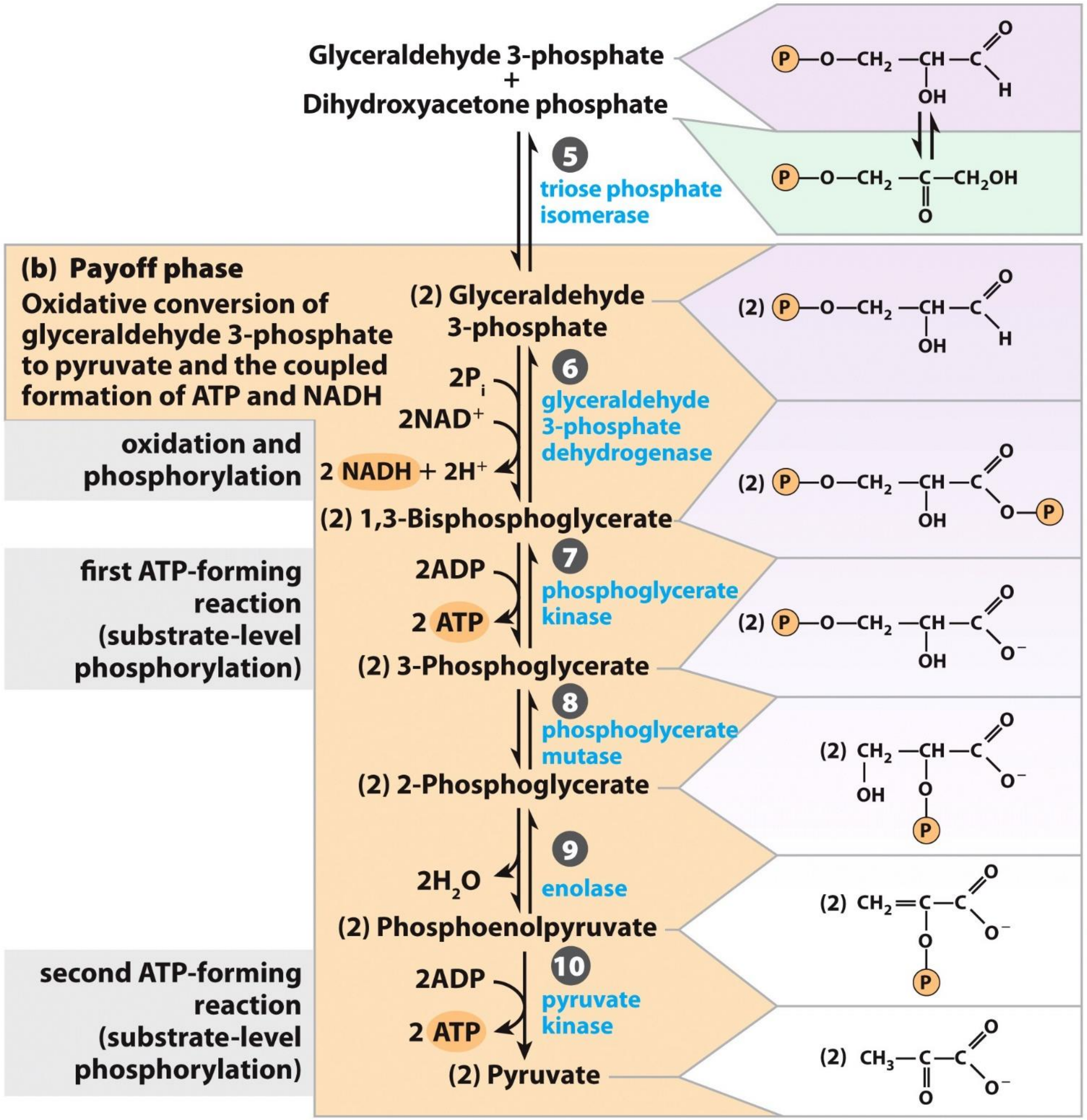
Major pathways of glucose utilization



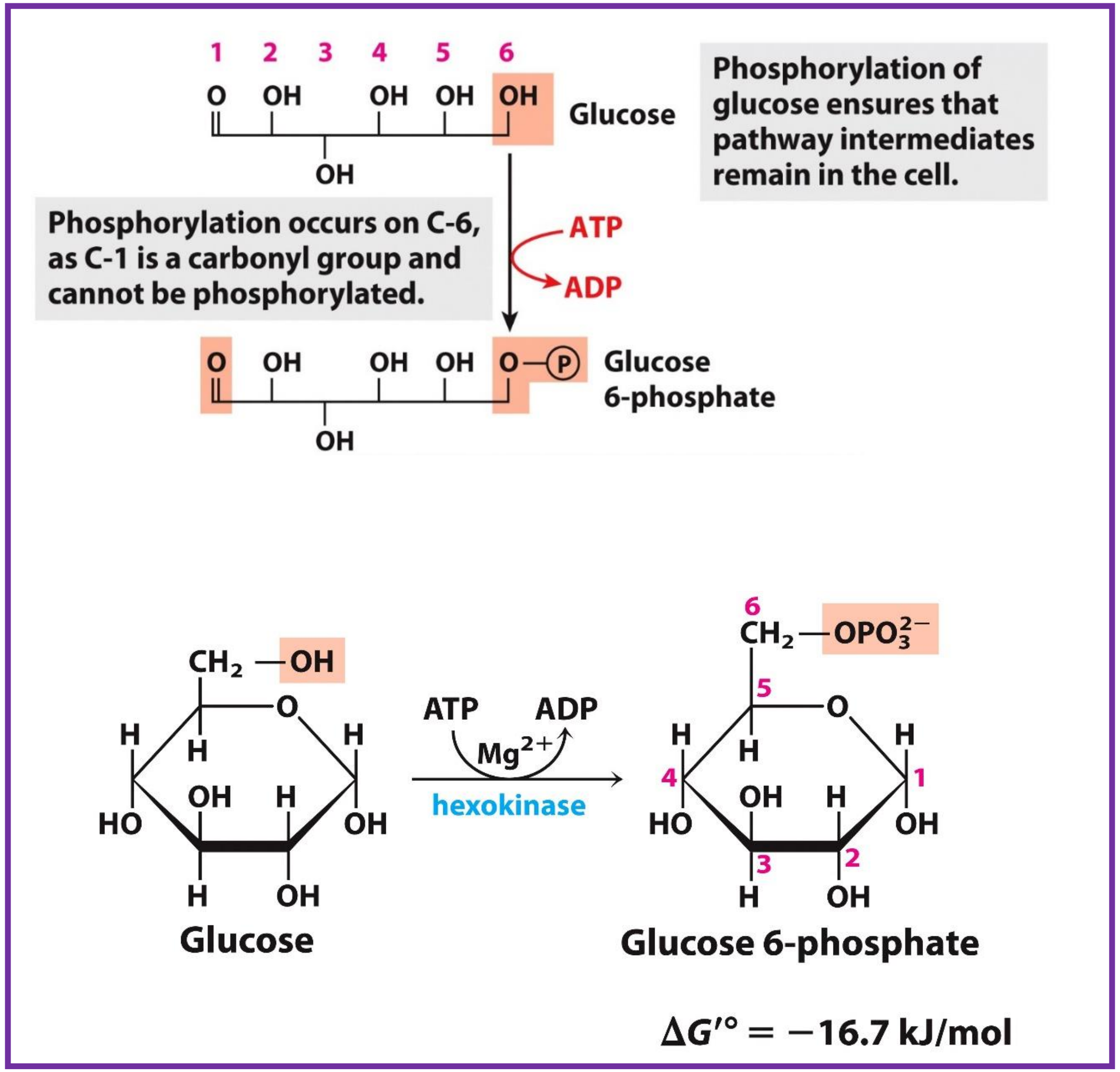
GLYCOLYSIS


(a) Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
first priming reaction
second priming reaction
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
Glucose
ATP
ADP
1
hexokinase
Glucose 6-phosphate
2
phosphohexose isomerase
Fructose 6-phosphate
3
phospho-fructokinase-1
Fructose 1,6-bisphosphate
4
aldolase
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate



Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
triose phosphate isomerase
(b) Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
(2) Glyceraldehyde 3-phosphate
2Pi
2NAD+
2 NADH + 2H+
6
glyceraldehyde 3-phosphate dehydrogenase
oxidation and phosphorylation
(2) 1,3-Bisphosphoglycerate
first ATP-forming reaction (substrate-level phosphorylation)
2ADP
2 ATP
7
phosphoglycerate kinase
(2) 3-Phosphoglycerate
8
phosphoglycerate mutase
(2) 2-Phosphoglycerate
9
enolase
2H2O
(2) Phosphoenolpyruvate
second ATP-forming reaction (substrate-level phosphorylation)
2ADP
2 ATP
10
pyruvate kinase
(2) Pyruvate

①



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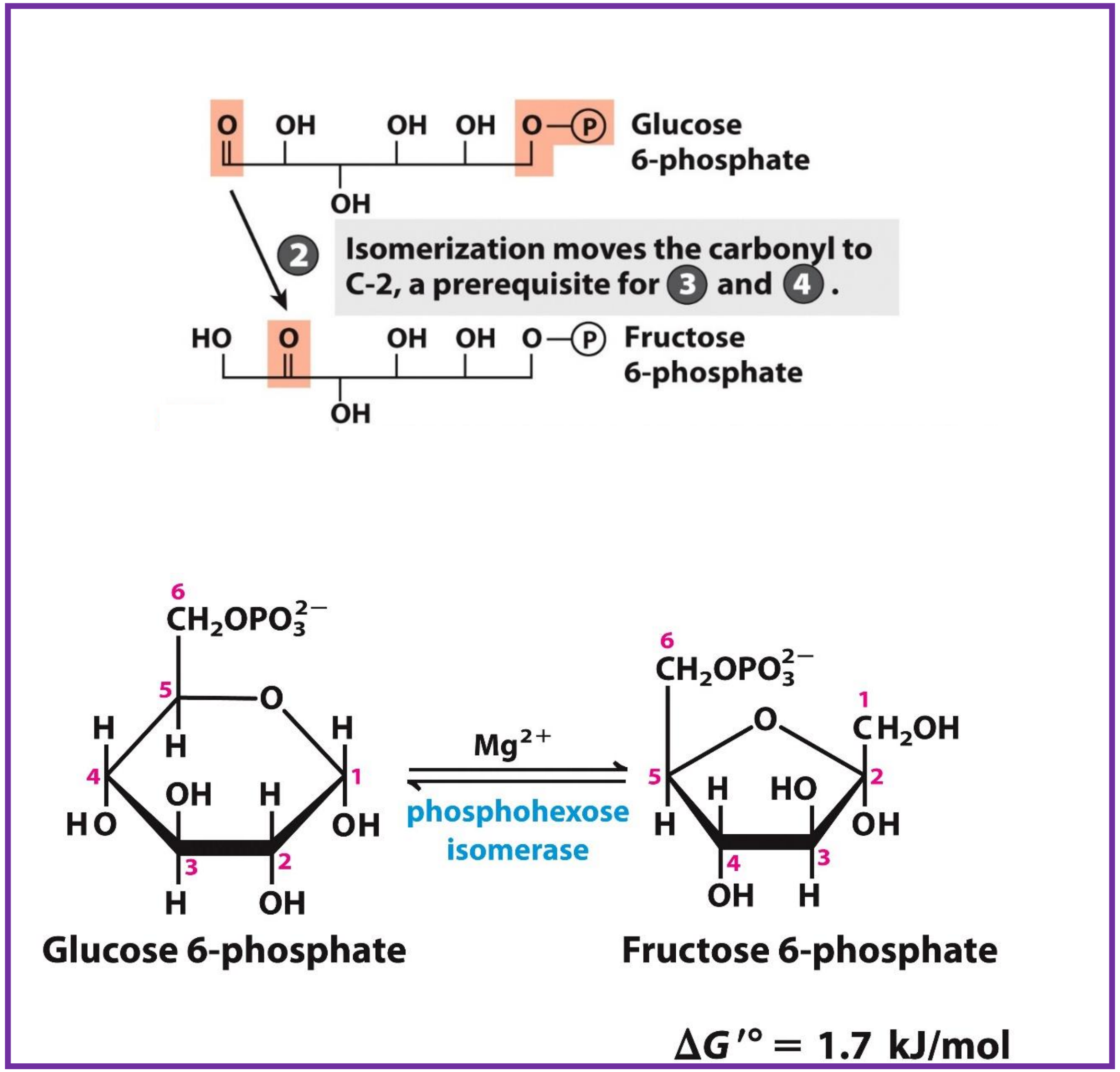
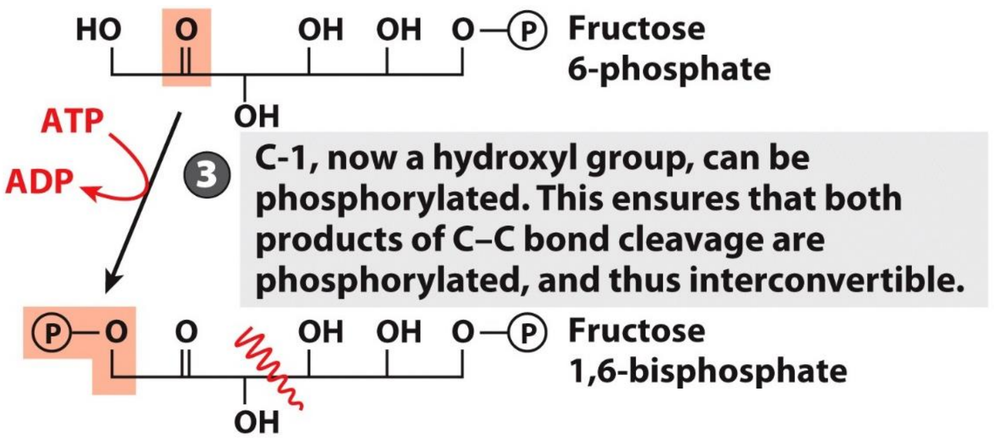
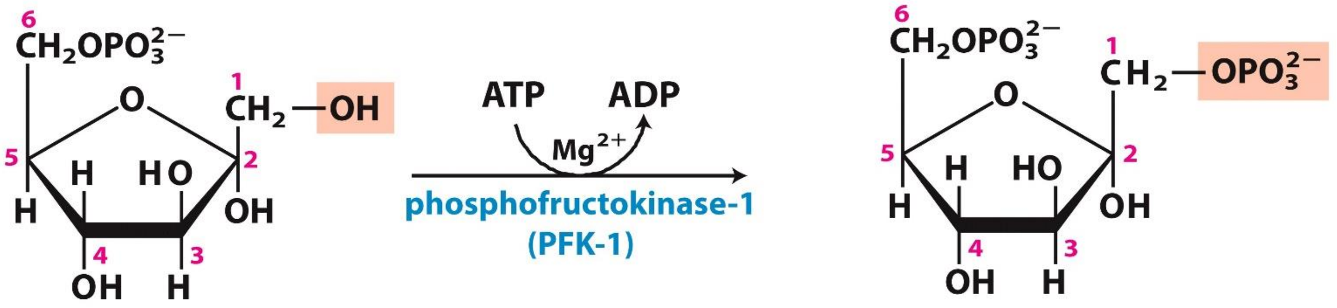


- Enzyme **hexokinase:** 4 isoenzymes (I, II, III i IV) **– in the liver the main isoenzyme is hexokinase IV (glucokinase)**, while other tissues have isoenzymes I, II and III

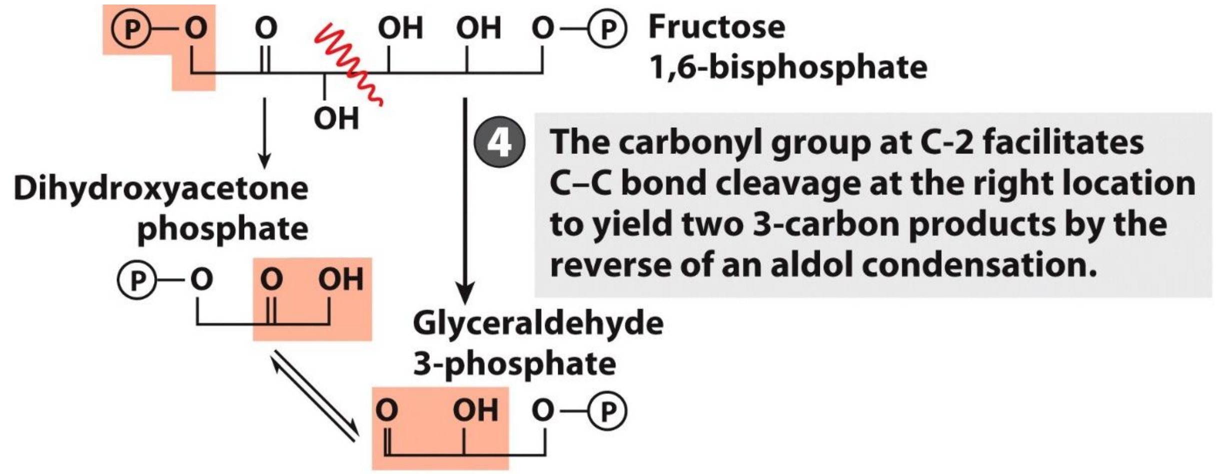
③

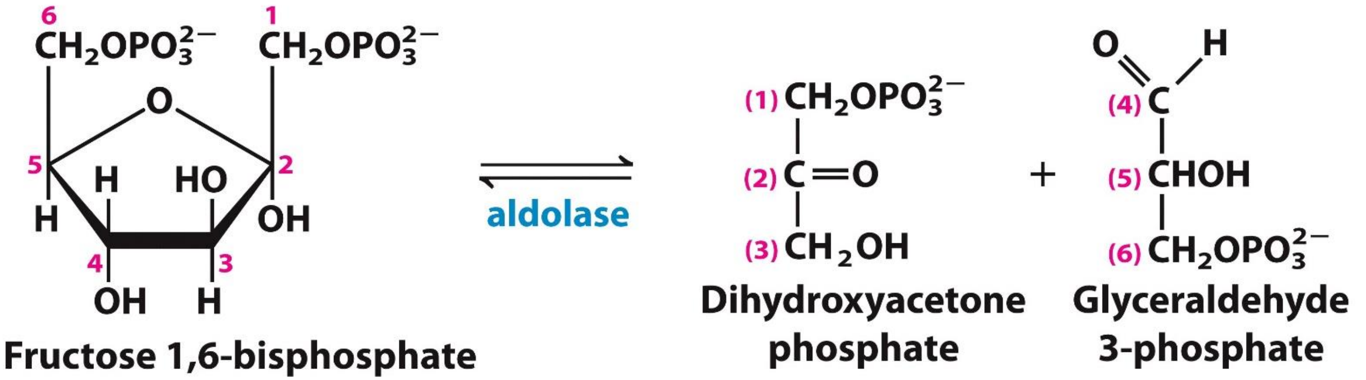




$\Delta G'^{\circ} = -14.2$ kJ/mol

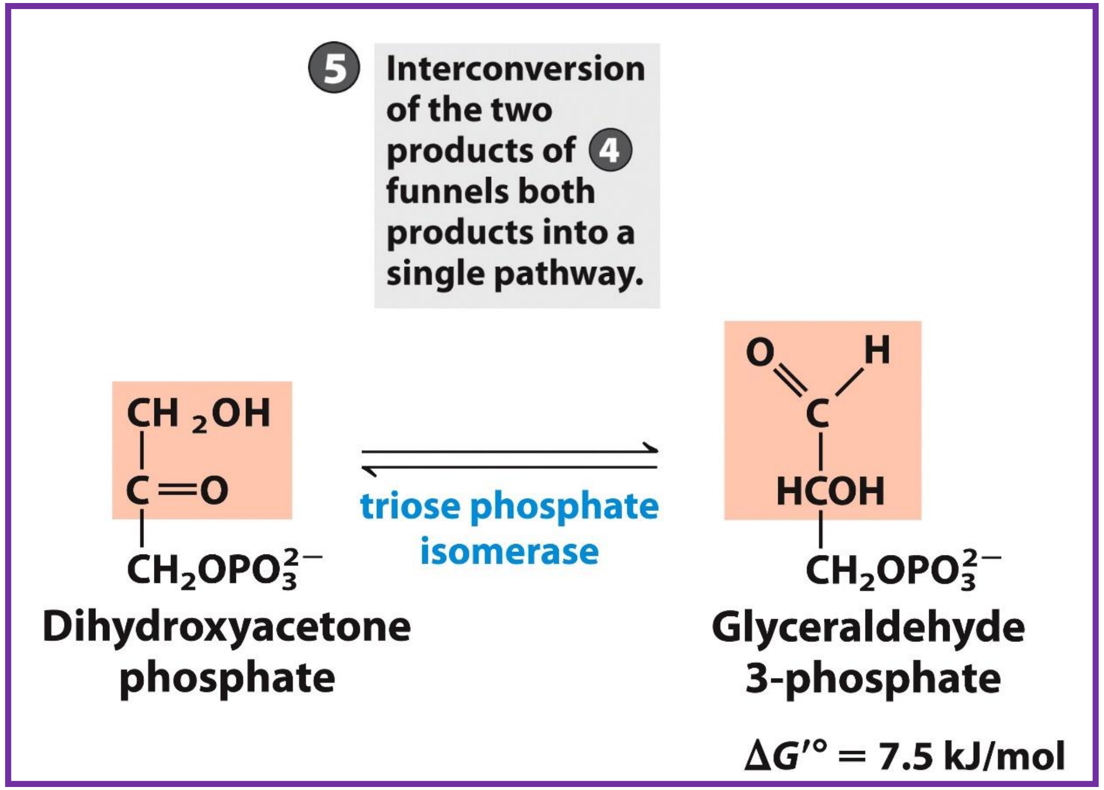
④



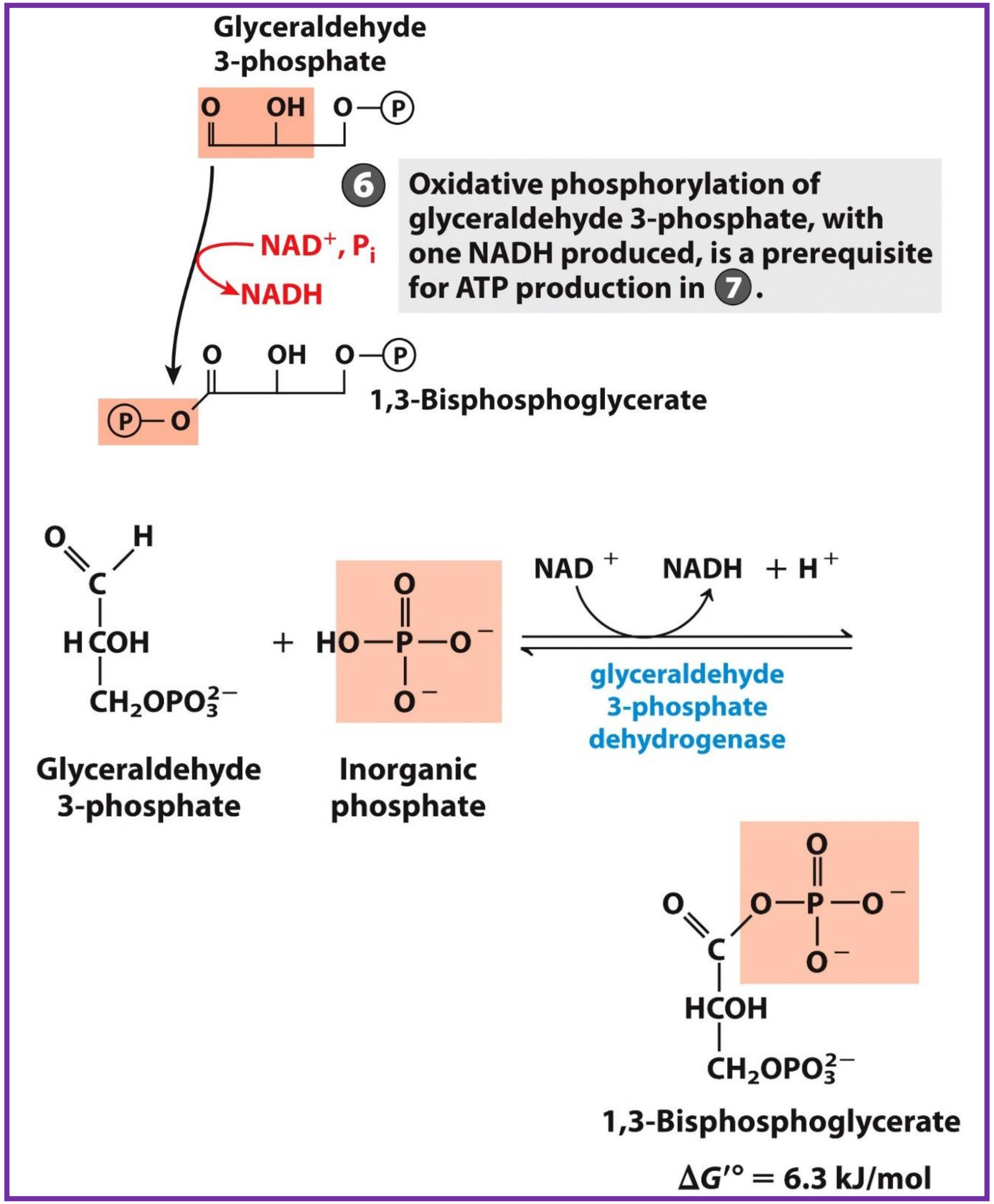


$$\Delta G'^{\circ} = 23.8 \text{ kJ/mol}$$

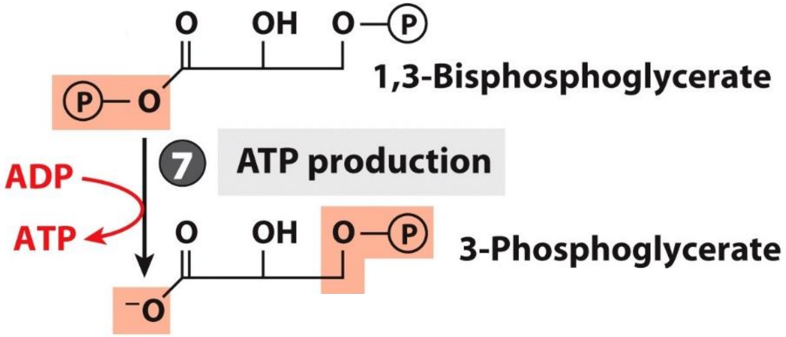
⑤


5 Interconversion of the two products of 4 funnels both products into a single pathway.
CH 2OH
C=O
CH2OPO3 2−
Dihydroxyacetone phosphate
triose phosphate isomerase
O
H
C
HCOH
CH2OPO3 2−
Glyceraldehyde 3-phosphate
ΔG′° = 7.5 kJ/mol

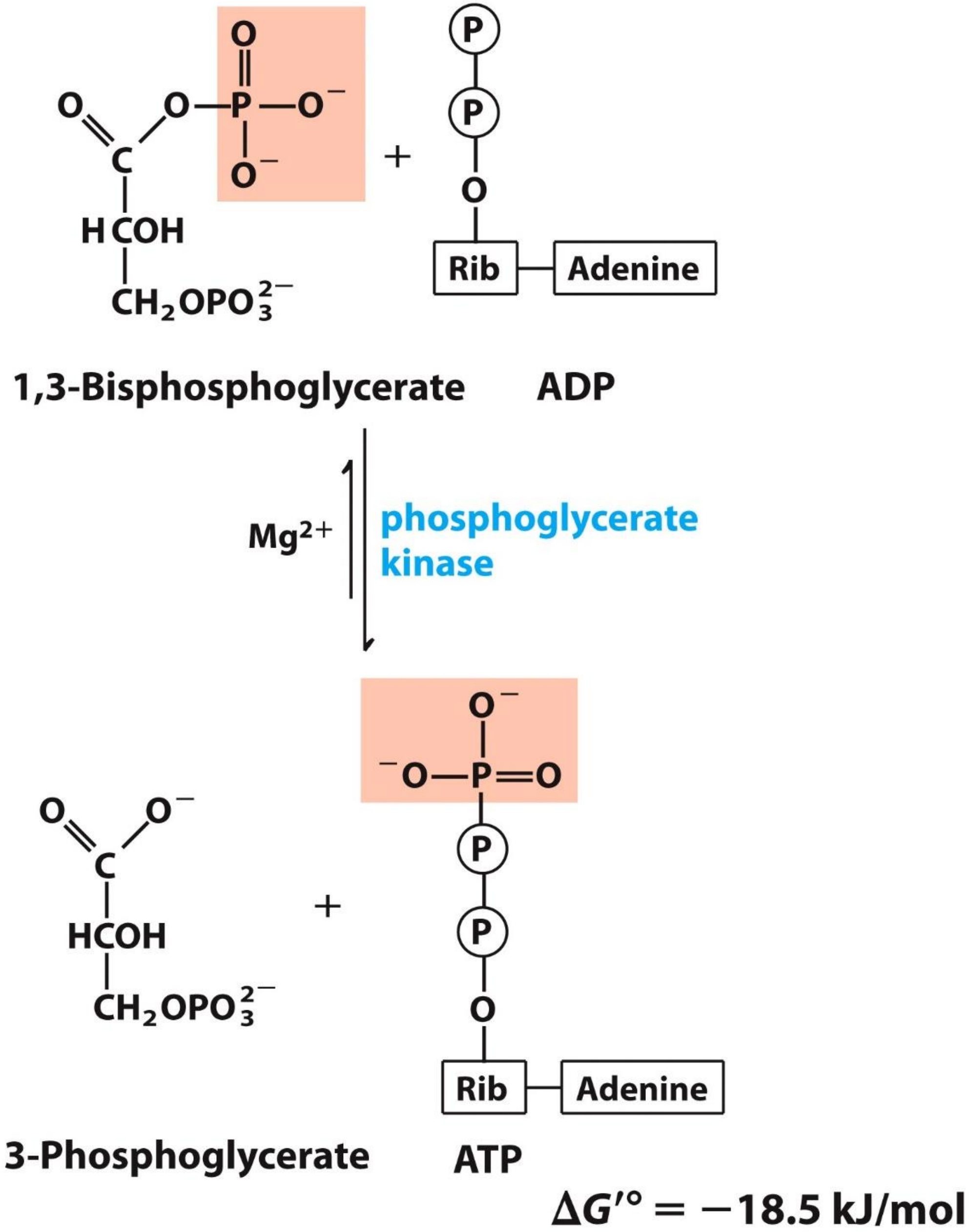
⑥


Glyceraldehyde 3-phosphate
O OH O—P
6 Oxidative phosphorylation of glyceraldehyde 3-phosphate, with one NADH produced, is a prerequisite for ATP production in 7.
NAD+, Pi
NADH
P—O
O OH O—P
1,3-Bisphosphoglycerate
O
H
C
HCOH
CH2OPO3 2−
+
HO—P—O−
O
O−
Glyceraldehyde 3-phosphate
Inorganic phosphate
NAD +
NADH + H +
glyceraldehyde 3-phosphate dehydrogenase
O
C
O—P—O−
O
O−
HCOH
CH2OPO3 2−
1,3-Bisphosphoglycerate
ΔG′° = 6.3 kJ/mol

⑦

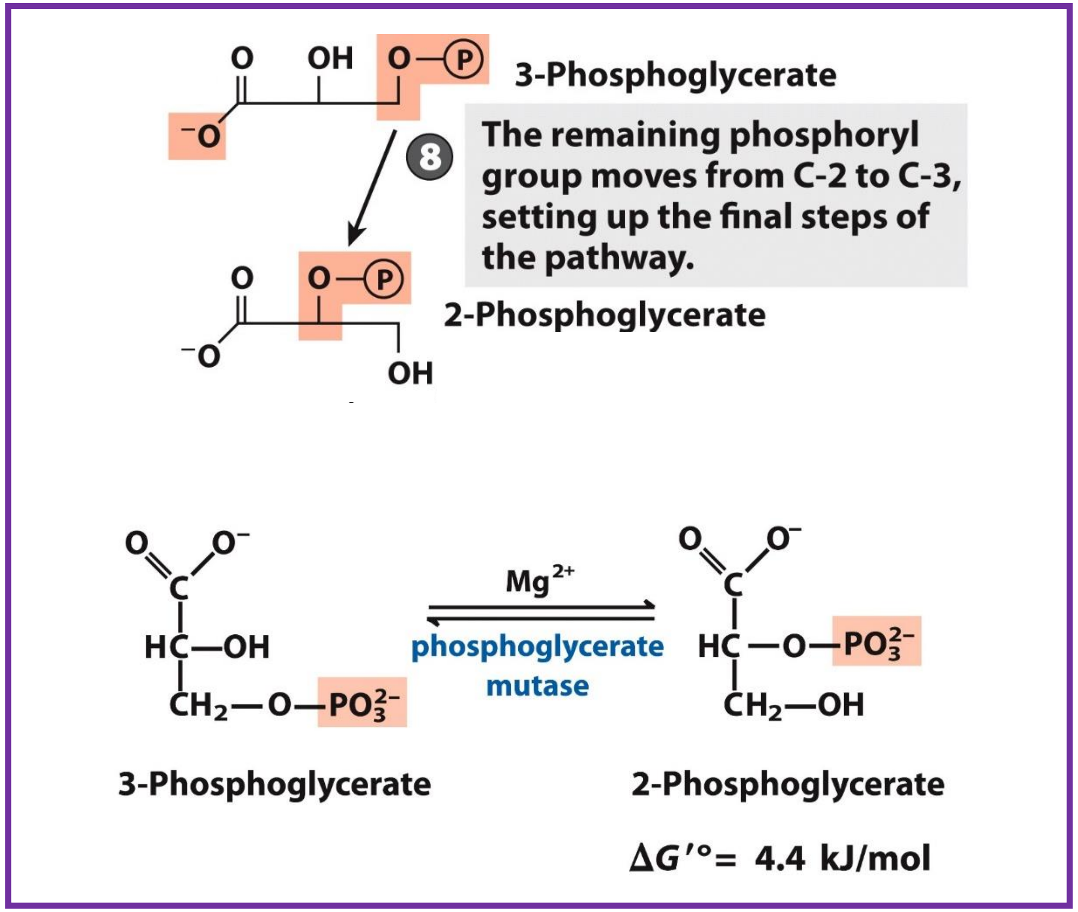


O
OH
O
P
P
O
1,3-Bisphosphoglycerate
ADP
ATP
7
ATP production
O
OH
O
P
⁻O
3-Phosphoglycerate

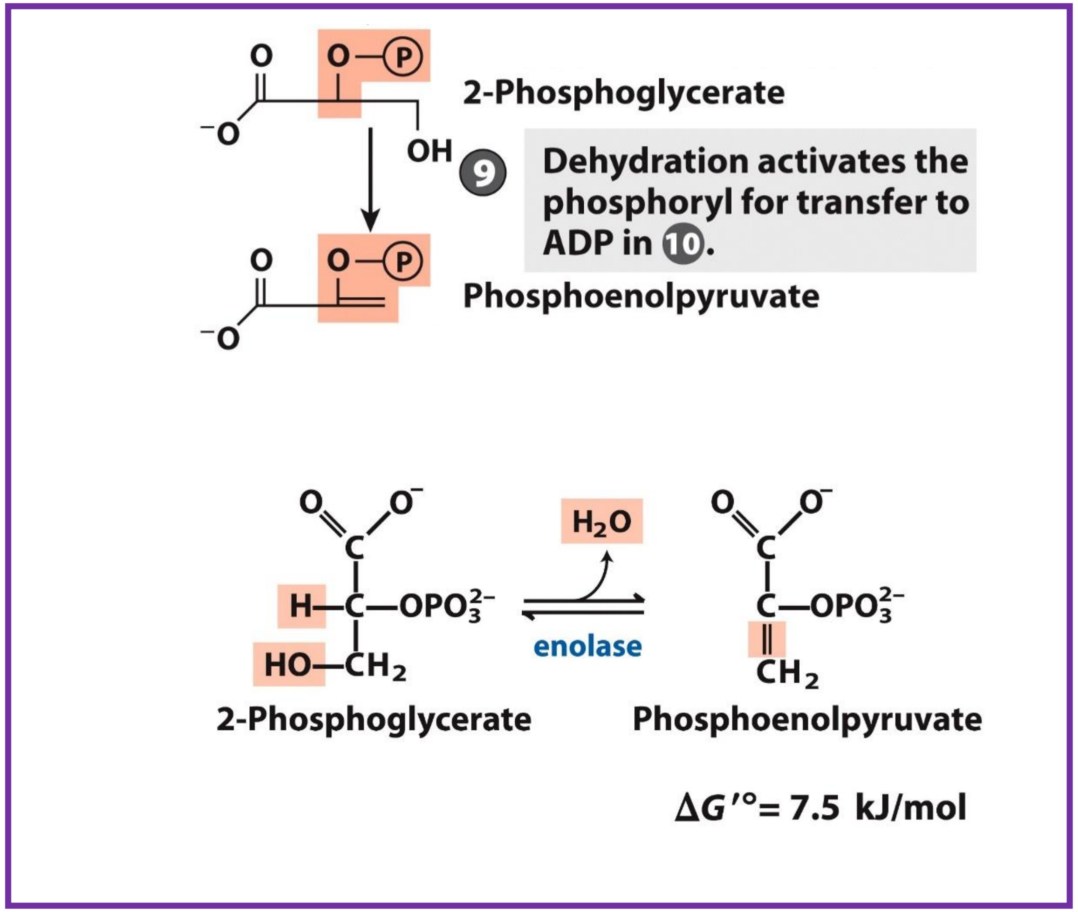


1,3-Bisphosphoglycerate
ADP
Rib
Adenine
Mg2+
phosphoglycerate kinase
3-Phosphoglycerate
ATP
ΔG′° = −18.5 kJ/mol

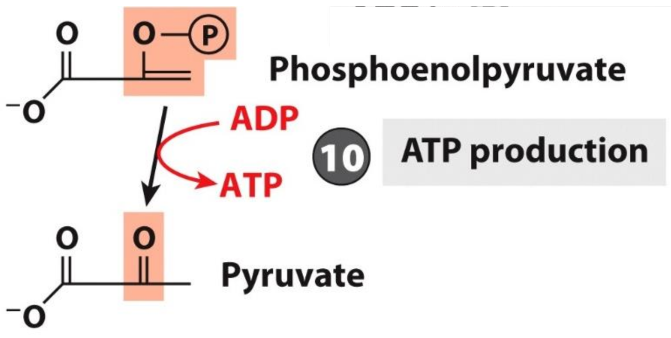
⑧


3-Phosphoglycerate
The remaining phosphoryl group moves from C-2 to C-3, setting up the final steps of the pathway.
2-Phosphoglycerate
Mg^{2+}
phosphoglycerate mutase
3-Phosphoglycerate
2-Phosphoglycerate
$\Delta G'^{\circ} = 4.4$ kJ/mol

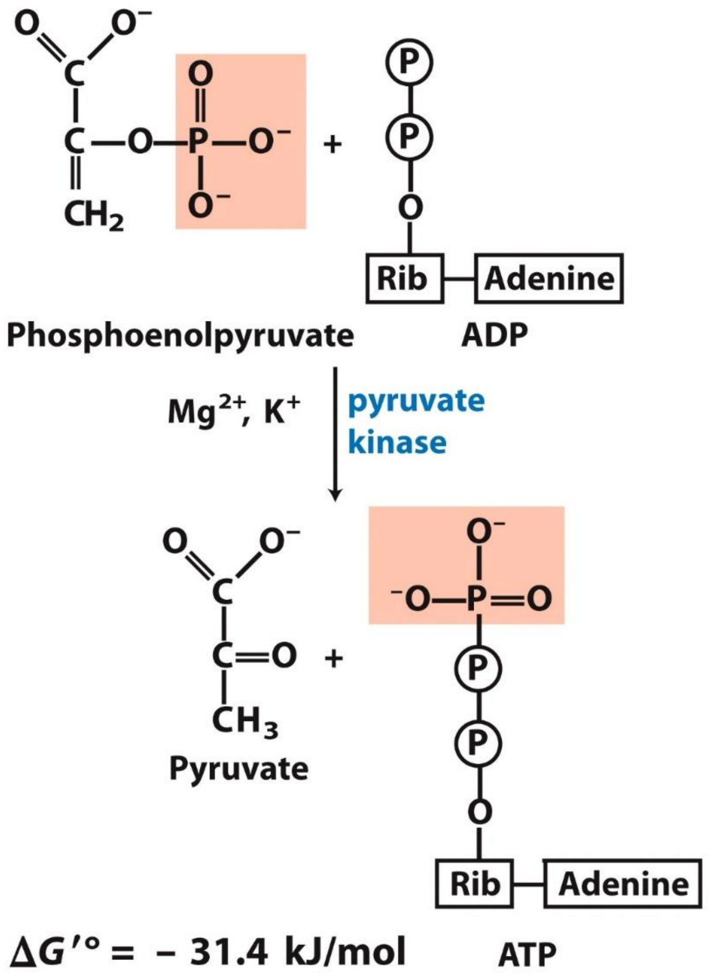
⑨


2-Phosphoglycerate
Dehydration activates the phosphoryl for transfer to ADP in 10.
Phosphoenolpyruvate
H_2O
enolase
2-Phosphoglycerate
Phosphoenolpyruvate
$\Delta G'^{\circ} = 7.5$ kJ/mol

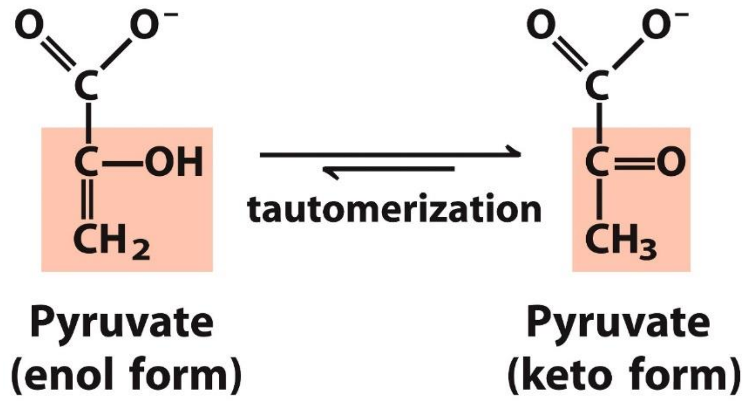
⑩



Phosphoenolpyruvate
ADP
ATP
10
ATP production
Pyruvate



Phosphoenolpyruvate
ADP
Rib
Adenine
Mg²⁺, K⁺
pyruvate kinase
Pyruvate
ATP
ΔG′° = – 31.4 kJ/mol

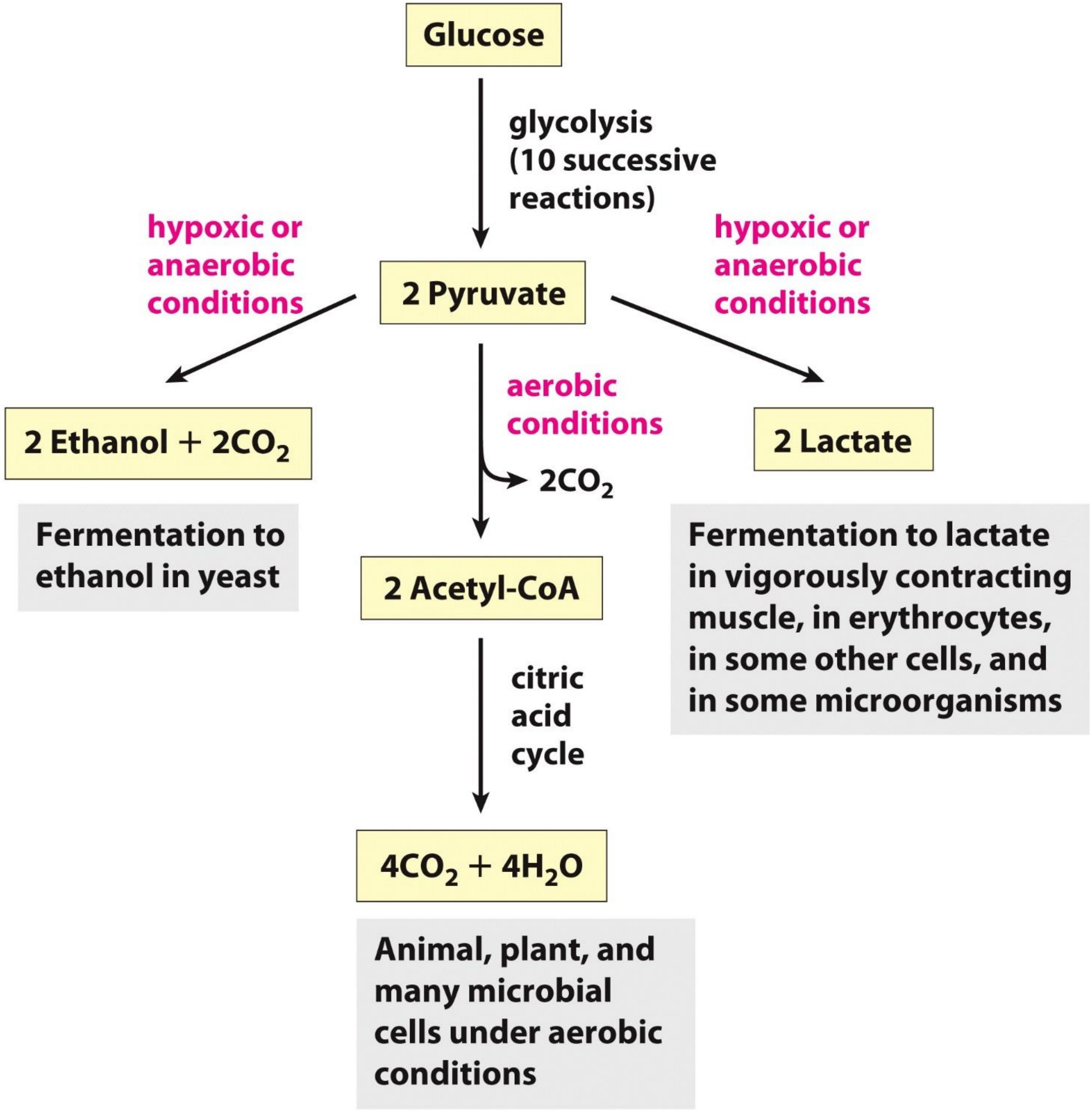


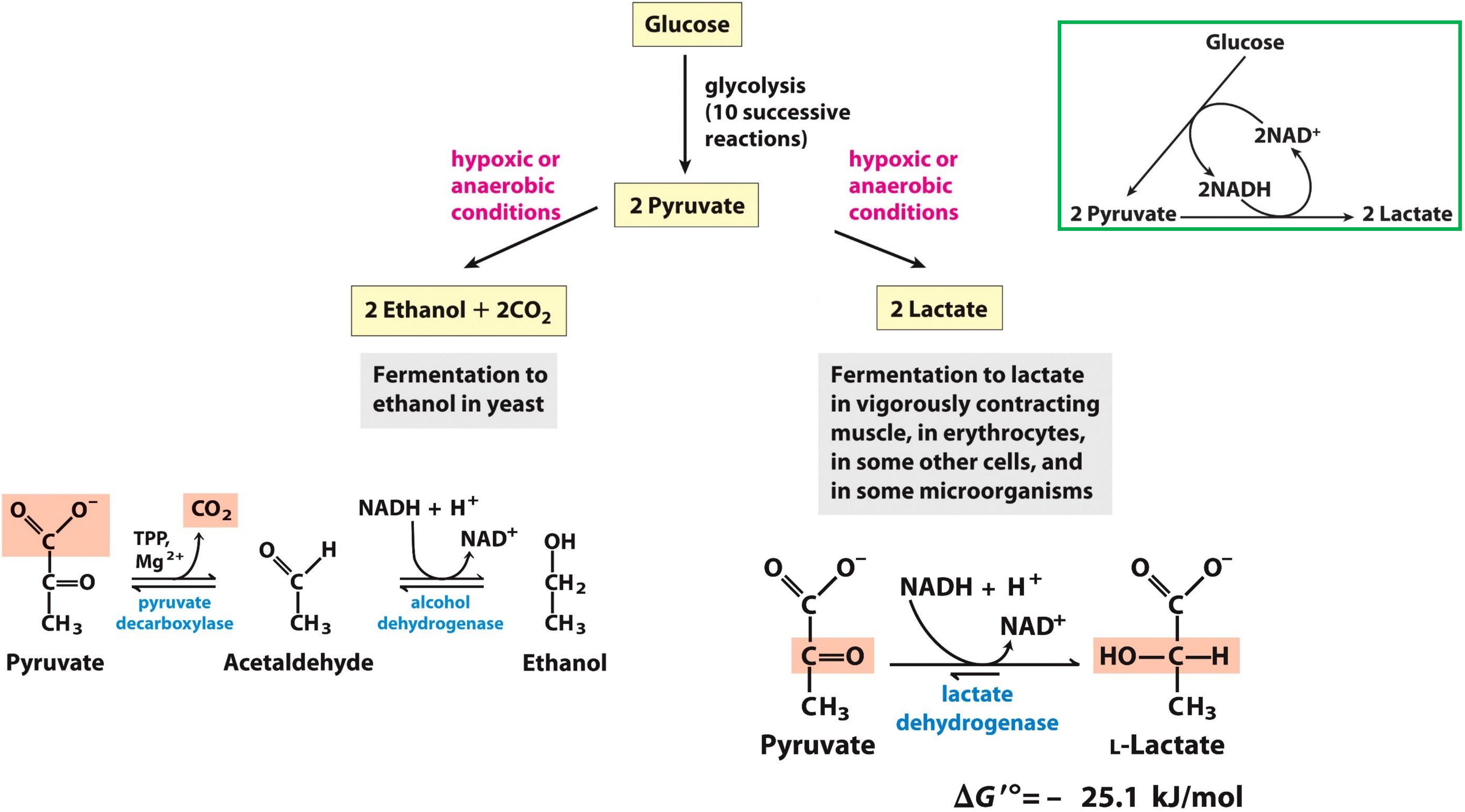
tautomerization
Pyruvate (enol form)
Pyruvate (keto form)

Overall equation for glycolysis:

$$\text{glucose} + 2NAD^+ + 2ADP + 2P_i$$

$$\rightarrow 2\ \text{pyruvate} + 2NADH + 2\ H^+ + 2\ ATP + 2H_2O$$


Glucose
glycolysis (10 successive reactions)
hypoxic or anaerobic conditions
2 Pyruvate
hypoxic or anaerobic conditions
aerobic conditions
2 Ethanol + $2CO_2$
$2CO_2$
2 Lactate
Fermentation to ethanol in yeast
2 Acetyl-CoA
Fermentation to lactate in vigorously contracting muscle, in erythrocytes, in some other cells, and in some microorganisms
citric acid cycle
$4CO_2 + 4H_2O$
Animal, plant, and many microbial cells under aerobic conditions



Glucose
glycolysis (10 successive reactions)
hypoxic or anaerobic conditions
2 Pyruvate
hypoxic or anaerobic conditions
2 Ethanol + 2CO2
2 Lactate
Fermentation to ethanol in yeast
Fermentation to lactate in vigorously contracting muscle, in erythrocytes, in some other cells, and in some microorganisms
Glucose
2NAD+
2NADH
2 Pyruvate
2 Lactate
CO2
TPP, Mg2+
pyruvate decarboxylase
NADH + H+
NAD+
alcohol dehydrogenase
Pyruvate
Acetaldehyde
Ethanol
NADH + H+
NAD+
lactate dehydrogenase
Pyruvate
L-Lactate
ΔG'° = – 25.1 kJ/mol

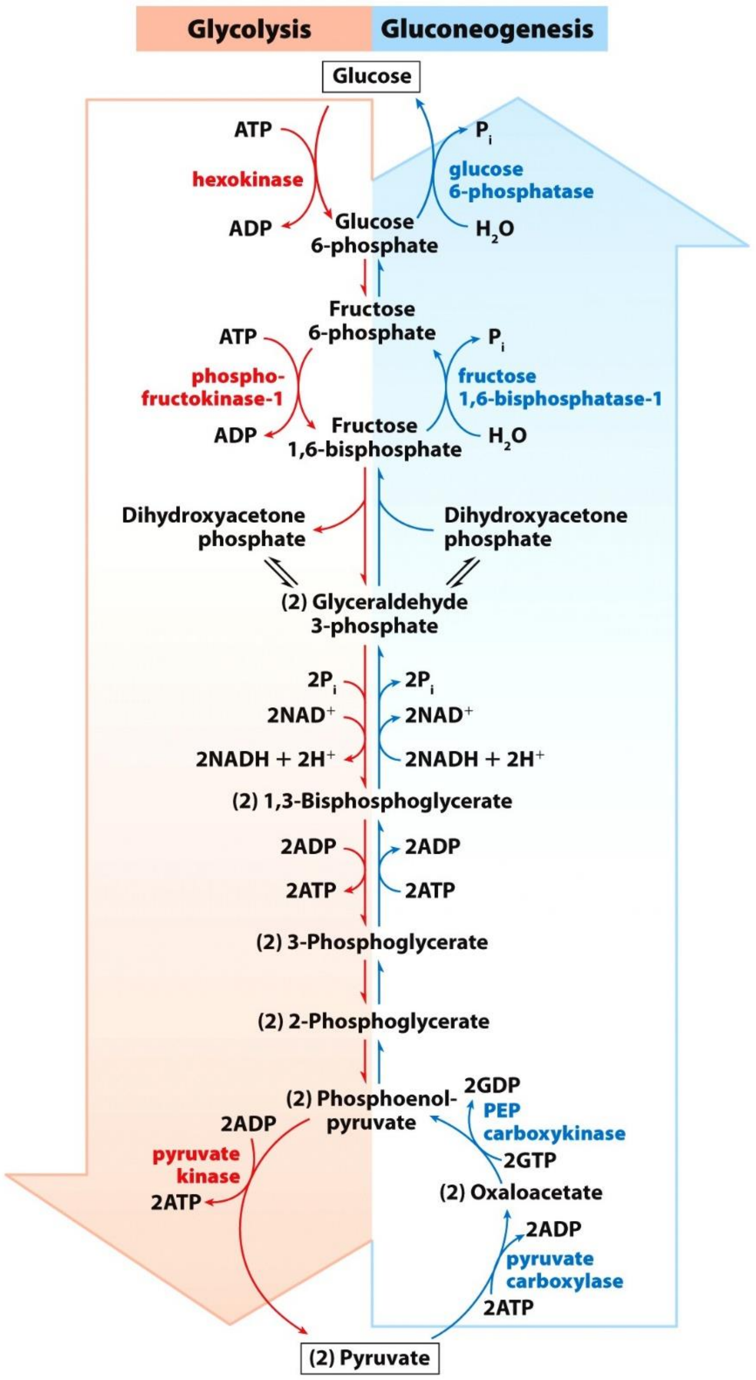
GLUCONEOGENESIS

- glucose synthesis from noncarbohydrate precursors: **pyruvate, lactate, glucogenic amino acids, glycerol**

- takes place mainly in the liver (also the renal cortex and intestinal cells)

- glucose is major or sole fuel source for human brain, erythrocytes, testes, renal medulla (brain needs approx. 120 g of glucose daily)

- gluconeogenesis and glycolysis are not identical pathways running in opposite directions; 7 of 10 glycolytic reactions are reversible and occur in gluconeogenesis; **3 irreversible glycolytic reactions** are **bypassed in gluconeogenesis** by a separate set of enzymes



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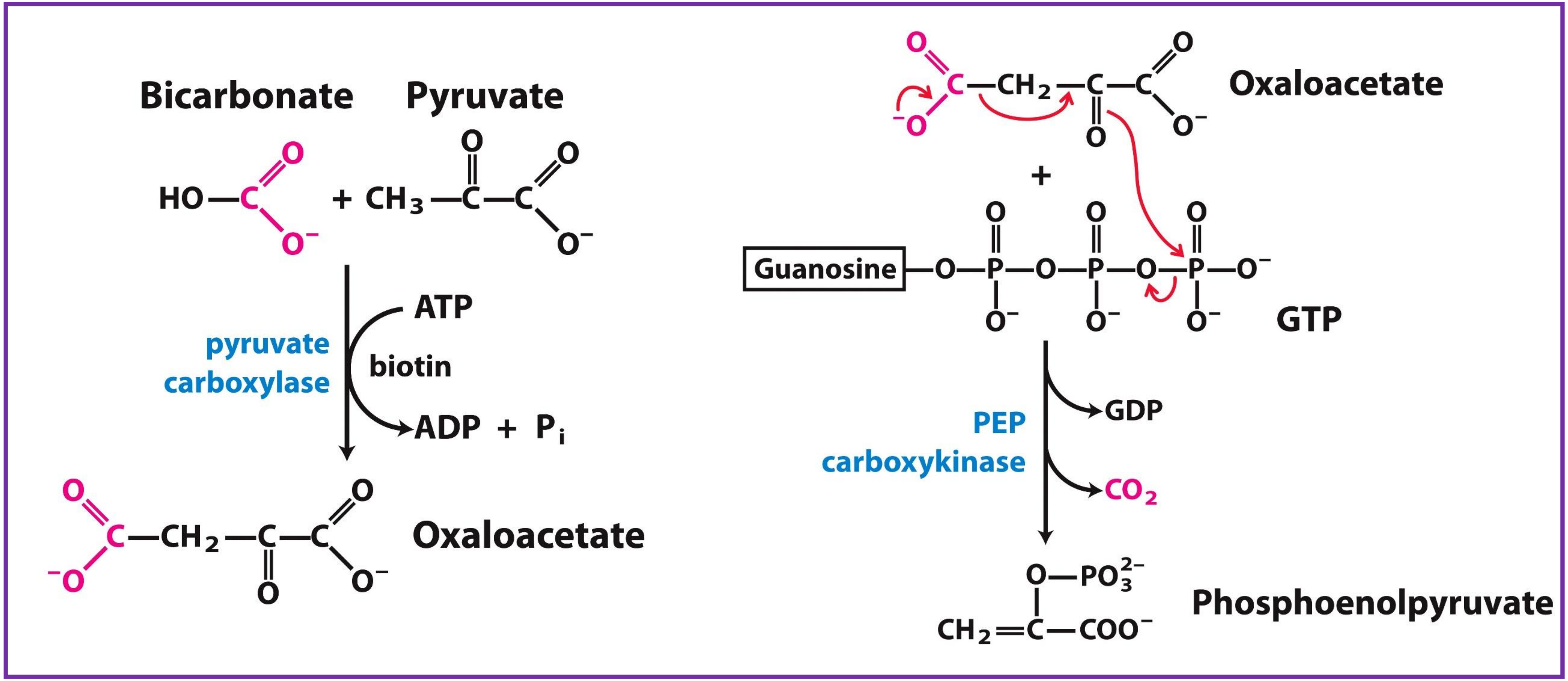


Carboxylation-decarboxylation sequence „activates" pyruvate and facilitates formation of phosphoenolpyruvate.
(Similar reaction sequence of carboxylation-decarboxylation is used for activation of acetyl-CoA for fatty acid biosynthesis.)

Pyruvate carboxylase requires coenzyme **biotin** (acts as a carrier of activated HCO_3^-). The same reaction is used to replenish intermediates of citric acid cycle (anaplerotic reactions)!

②

Fructose 1,6-bisphosphate + H_2O $\xrightarrow{\text{Fructose 1,6-bisphosphatase}}$ fructose 6-phosphate + P_i

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

③

Glucose 6-phosphate + H_2O $\xrightarrow{\text{glucose 6-phosphatase}}$ glucose + P_i

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

*Glucose 6-phosphatase is not expressed in muscle and brain tissue!

Overall equation for gluconeogenesis:

$$2\ \text{pyruvate} + 4\ ATP + 2\ GTP + 2\ NADH + 6\ H_2O \rightarrow$$

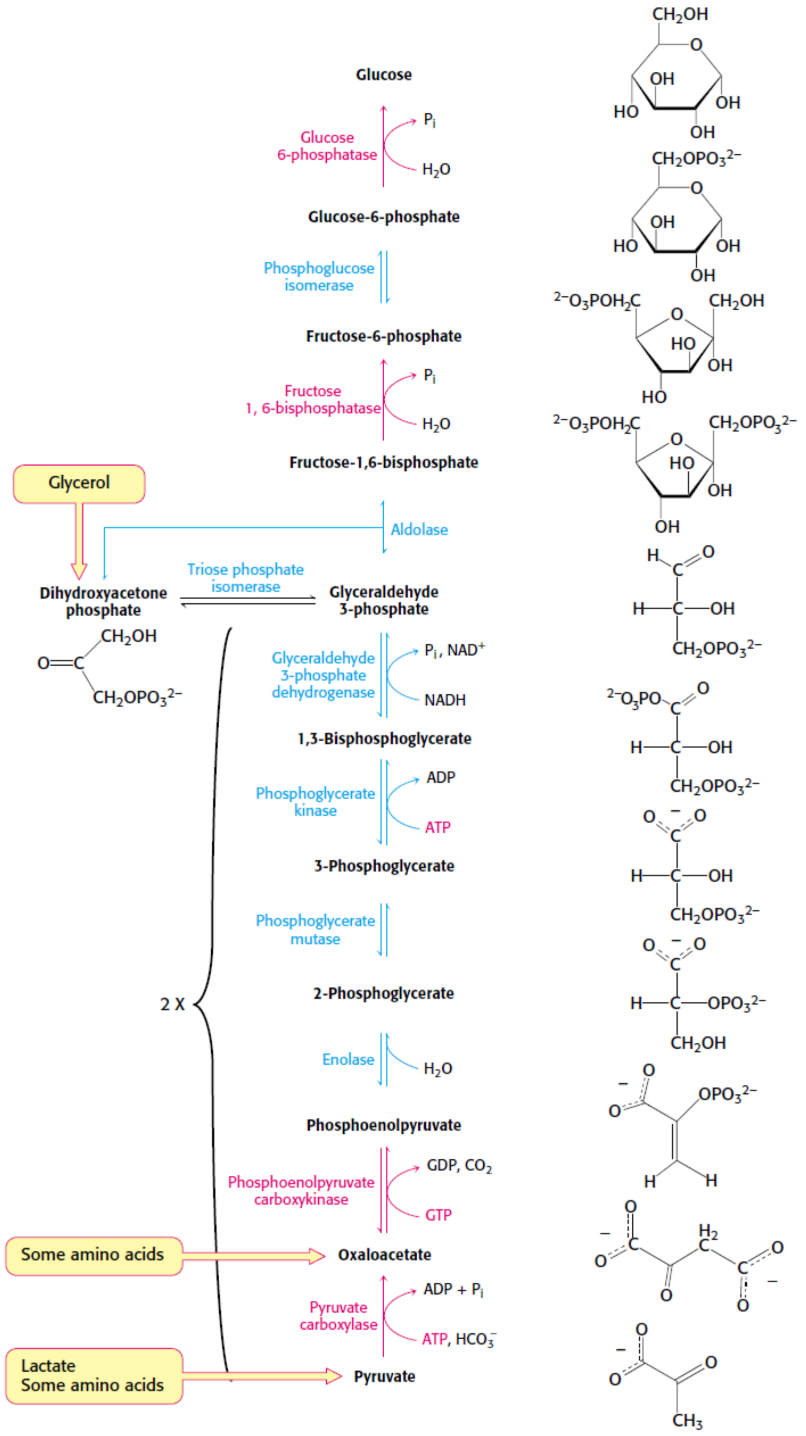
$$\text{glucose} + 4\ ADP + 2\ GDP + 6\ P_i\ +$$

$$2\ NAD^+ + 2\ H^+$$

CORI CYCLE;
GLUCOSE-ALANINE CYCLE;
REGULATION OF GLYCOLYSIS AND GLUCONEOGENESIS

Noncarbohydrate precursors for gluconeogenesis

- **Pyruvate** – entry point for **lactate** and **alanine**
- **Oxaloacetate** – entry point for **some aminoacids**
- **Dihydroxyacetone phosphate** – entry point for **glycerol**



PYRUVATE AND ALANINE AS GLUCONEOGENESIS SUBSTRATES – GLUCOSE-ALANINE CYCLE

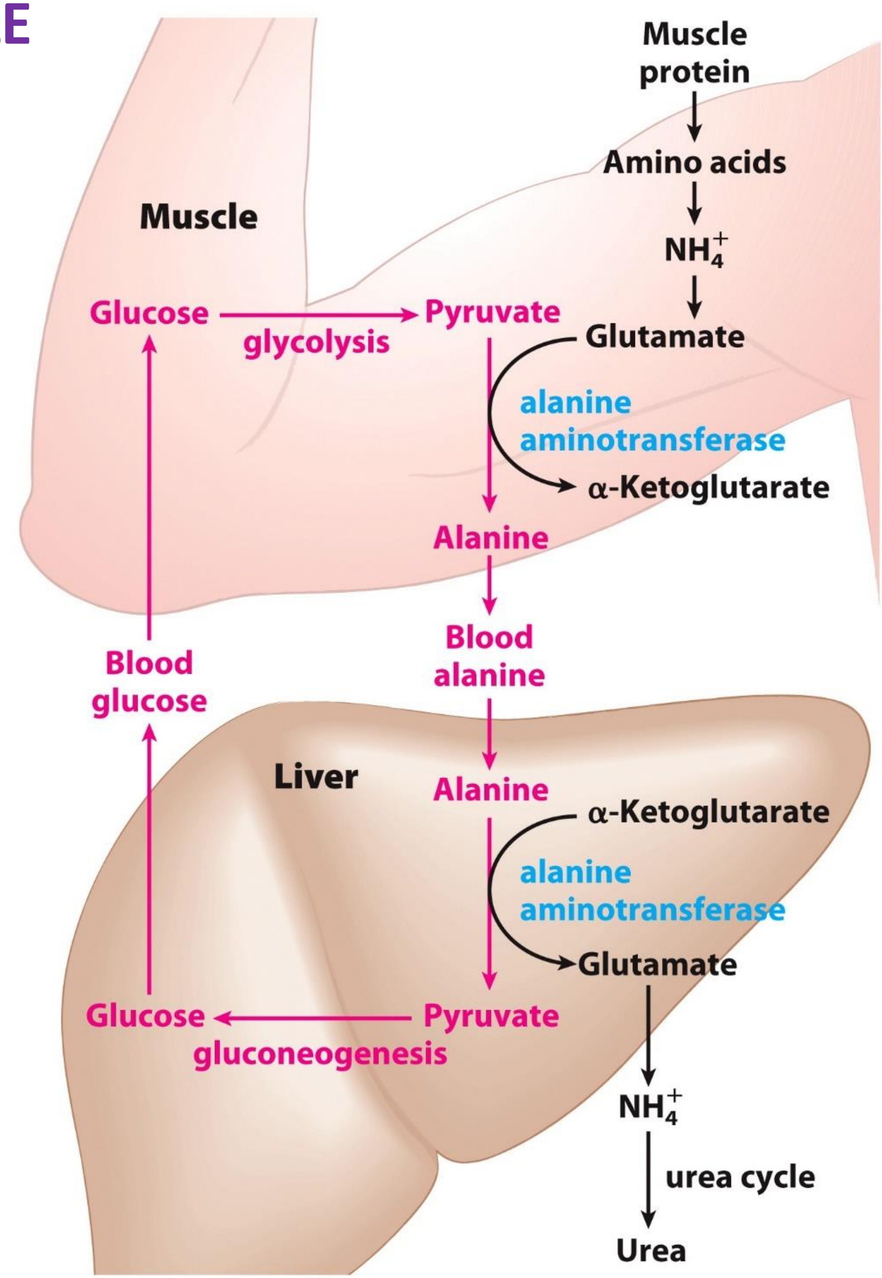
- Between meals, or during fasting, or during muscle exercise, muscle proteins are degraded to amino acids which are converted to glutamate by transamination reaction.

- Pyruvate produced in glycolysis is converted to alanine by a transamination reaction involving glutamate.

$$\text{alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}$$

- Glucose-alanine cycle ensures availability of gluconeogenesis precursors.

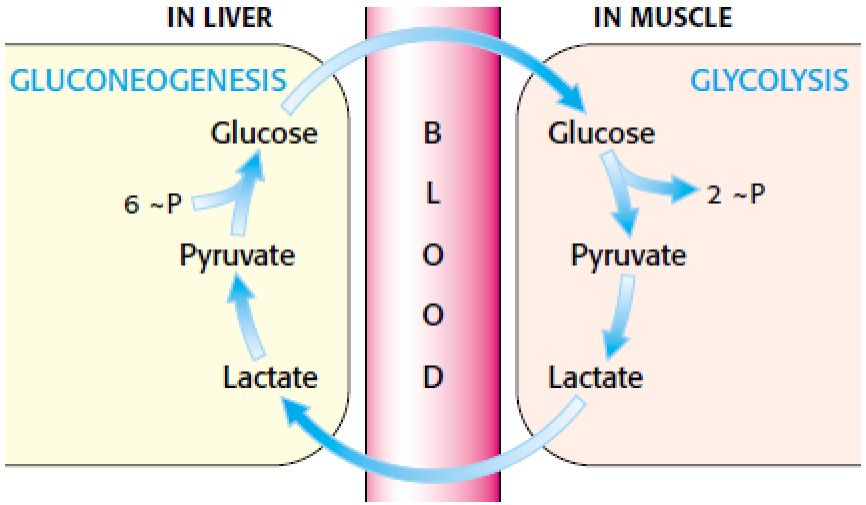
- Also, it serves as a mechanism for transporting amino acid nitrogen to the liver (link to urea cycle).

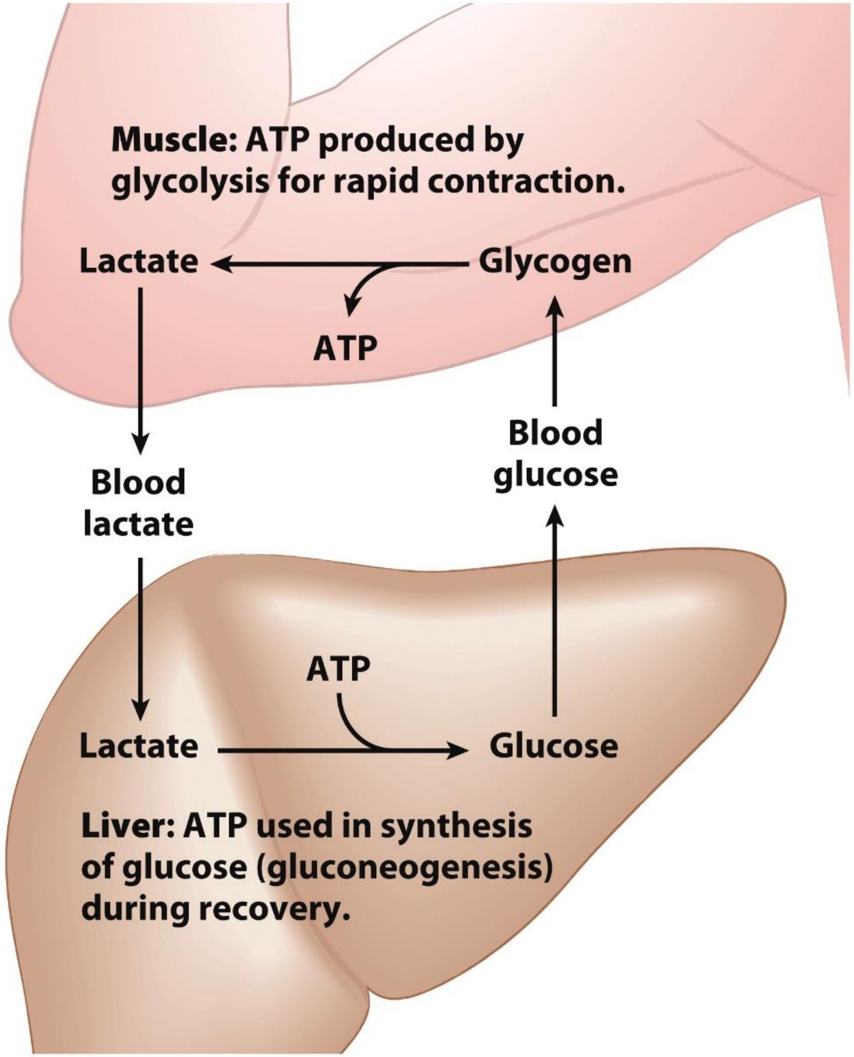


CORI CYCLE –
UTILISATION OF LACTATE AS GLUCONEOGENESIS SUBSTRATE

- During strenuous exercise, lactate is produced anaerobically in muscle cells.

- After passing through blood to the liver, lactate is converted to glucose by gluconeogenesis.

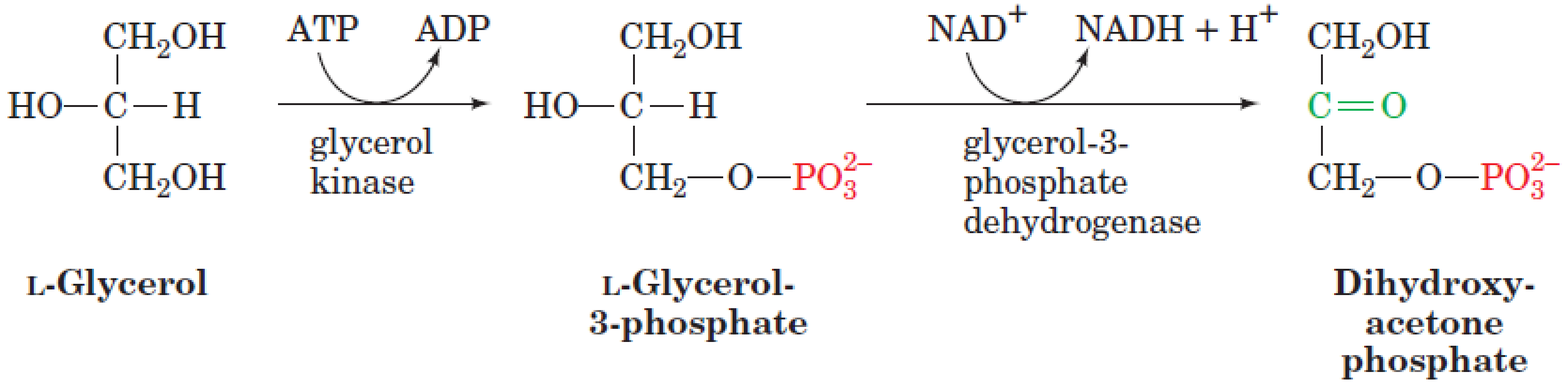




Glycerol as a substrate for gluconeogenesis

- Glycerol is a product of fat metabolism in adipose tissue.

- In the liver, glycerol is used for gluconeogenesis due to catalytic activity of two enzymes: **glycerol kinase** and **glycerol-3-phosphate-dehydrogenase**



Glucogenic amino acids are converted either to pyruvate or citric acid cycle intermediates and serve as gluconeogenesis precursors

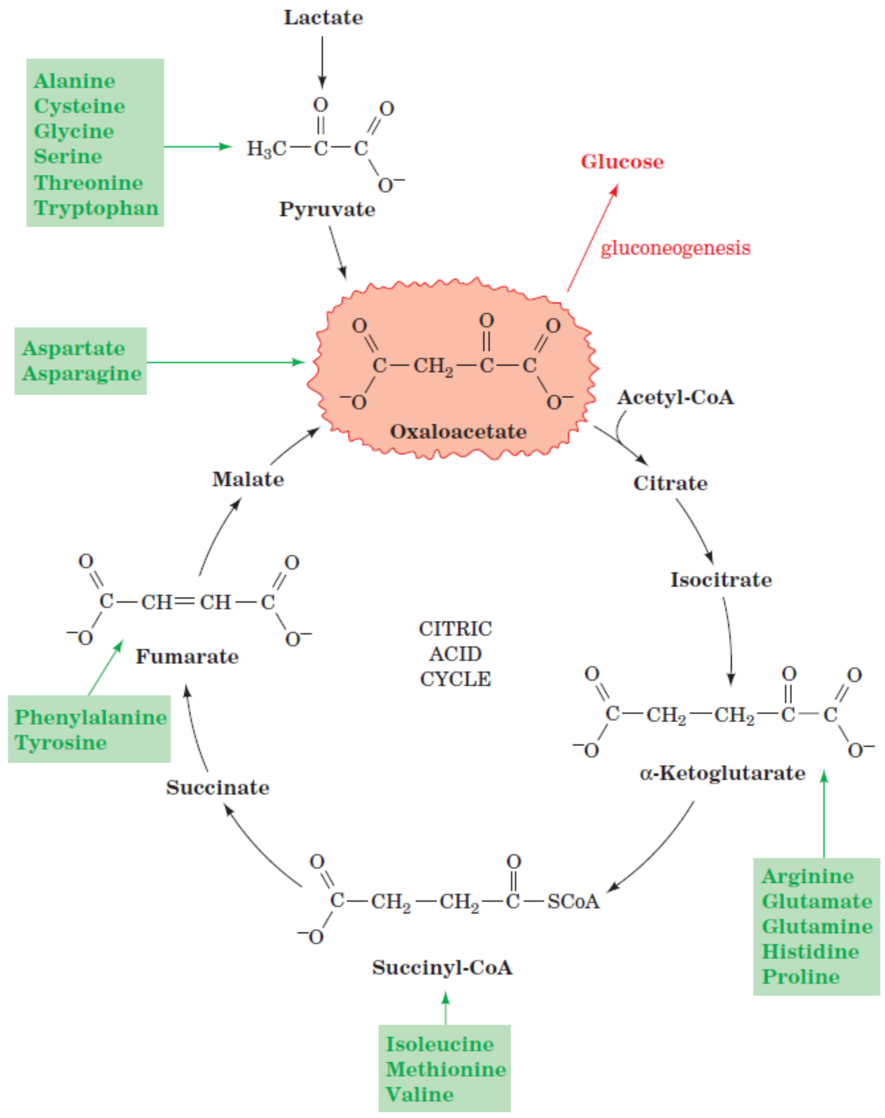
Pyruvate – alanine, cysteine, glycine, serine, triptophane

Succinyl-CoA – isoleucine, methionine, threonine, valine

α-ketoglutarate – arginine, glutamate, glutamine, histidine, prolin

Fumarate – phenylalanine, tyrosine

Oxaloacetate – asparagine, aspartate

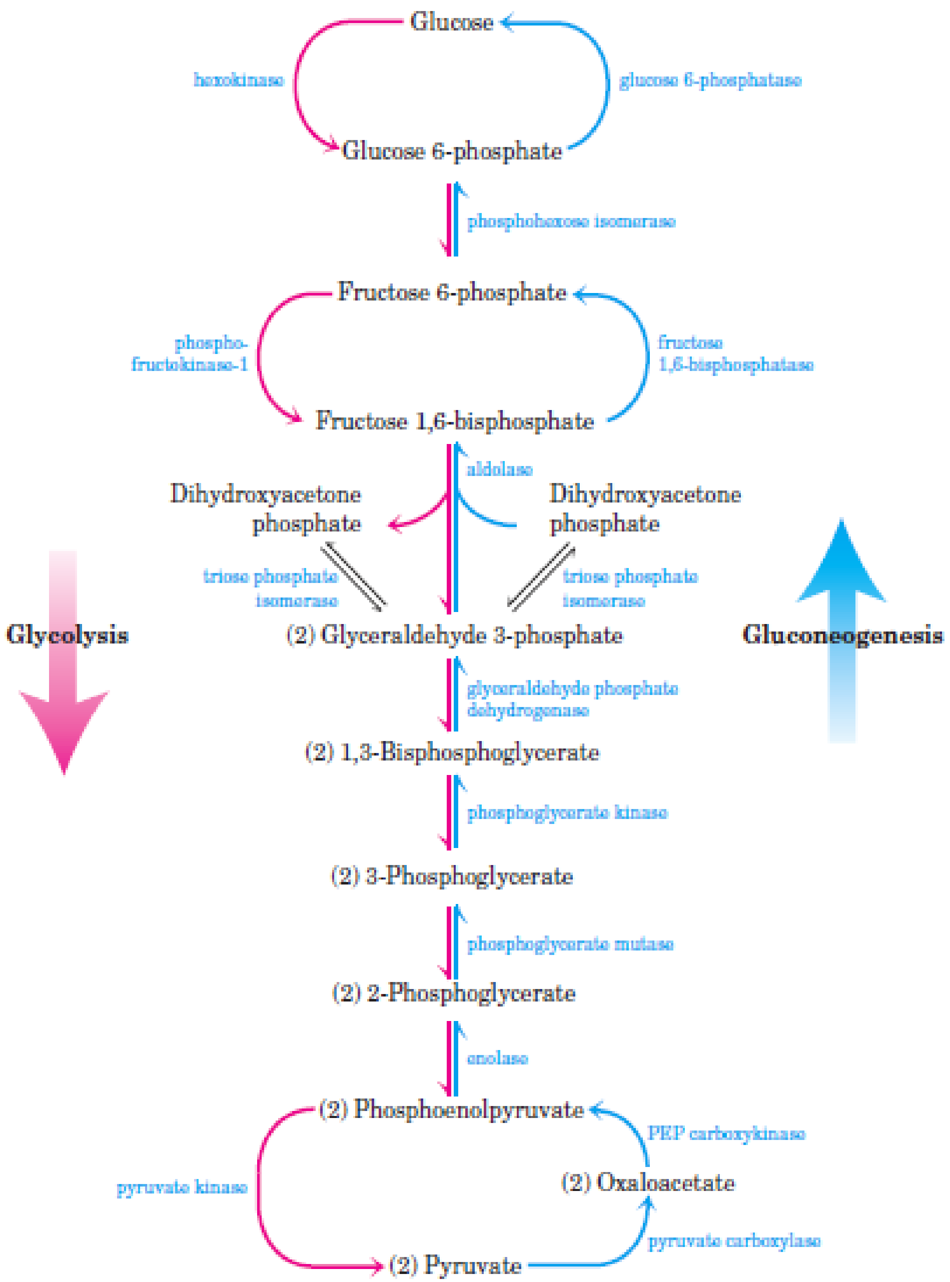


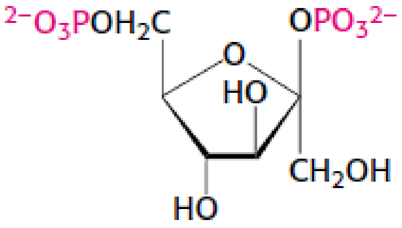
COORDINATED REGULATION OF GLYCOLYSIS AND GLUCONEOGENESIS

- Glycolysis and gluconeogenesis cannot proceed simultaneously, otherwise the energy would be dissipated (**futile cycle**); **they are reciprocally regulated.**

- Glycolysis and gluconeogenesis proceed as substrate cycles, sharing 7 (reversible) of 10 reactions, and are precisely and coordinately regulated.

- **Allosteric effectors and hormones** (insulin, glucagon, cortisol, adrenaline) are involved in regulation of glycolysis and gluconeogenesis.





Fructose 2,6-bisphosphate
(F-2,6-BP)

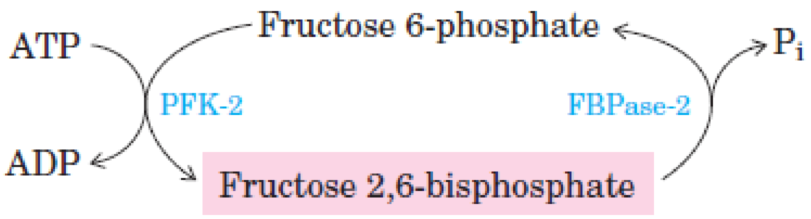
FRUCTOSE 2,6-BISPHOSPHATE: A REGULATORY MOLECULE

- **fructose 2,6-bisphosphate (F-2,6**-BP) has an important role in signalling blood glucose concentration

- it is synthesized in a reaction catalyzed by **phosphofructokinase 2 (PFK-2)** which is a bifunctional enzyme

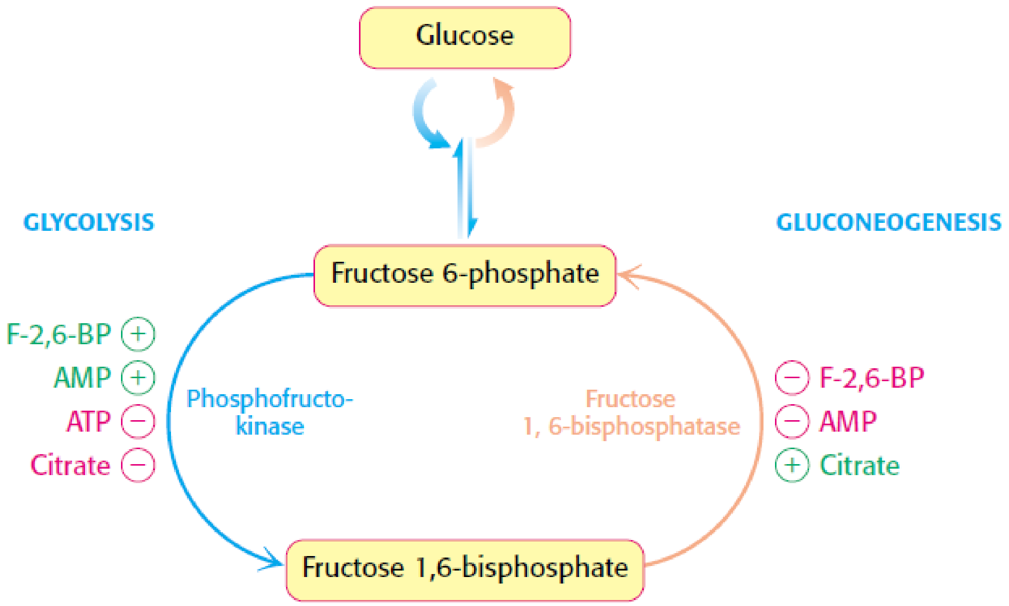
- **bifunctional enzyme: kinase and phosphatase domain**
    - **phosphofructokinase-2 (PFK-2)** – catalyzes the phosphorylation of F-6-P to F-2,6-BP
    - **fructose 2,6-bisphosphatase (FBPase-2)** – catalyzes the dephosphorylation of F-2,6-BP to F-6-P

- cellular concentration of **fructose 2,6-bisphosphate (F-2,6**-BP) depends on action of hormones insulin and glucagon



PHOSPHOFRUCTOKINASE 1 AND FRUCTOSE 1,6-BIPHOSPHATASE REGULATION

- these two enzymes are main regulatory points for glycolysis and gluconeogenesis



Glycolysis and gluconeogenesis regulation		
Enzyme	**Activator**	**Inhibitor**
PFK-1	AMP, F-2,6-BP	ATP and citrate
F-1,6-BISPHOSPHATASE	citrate	AMP, F-2,6-BP

How does F-2,6-BP concentration change depending on glucose blood concentration?

LOW GLUCOSE CONCENTRATION
IN BLOOD
(overnight or in starvation)

↓

GLUCAGON concentration in blood rises

↓

signalling cascade triggered (through second messenger cAMP) which leads to **PHOSPHORYLATION** of the bifunctional enzyme (PFK/FBPase-2)

↓

phosphorylation activates FBPase-2, and inhibits PFK-2

↓

concentration of F-2,6-BP drops

↓

GLUCONEOGENESIS

HIGH GLUCOSE CONCENTRATION
IN BLOOD
(after a meal)

↓

INSULIN concentration in blood rises

↓

signalling cascade triggered which leads to **DEPHOSPHORYLATION** of the bifunctional enzyme (PFK/FBPase-2)

↓

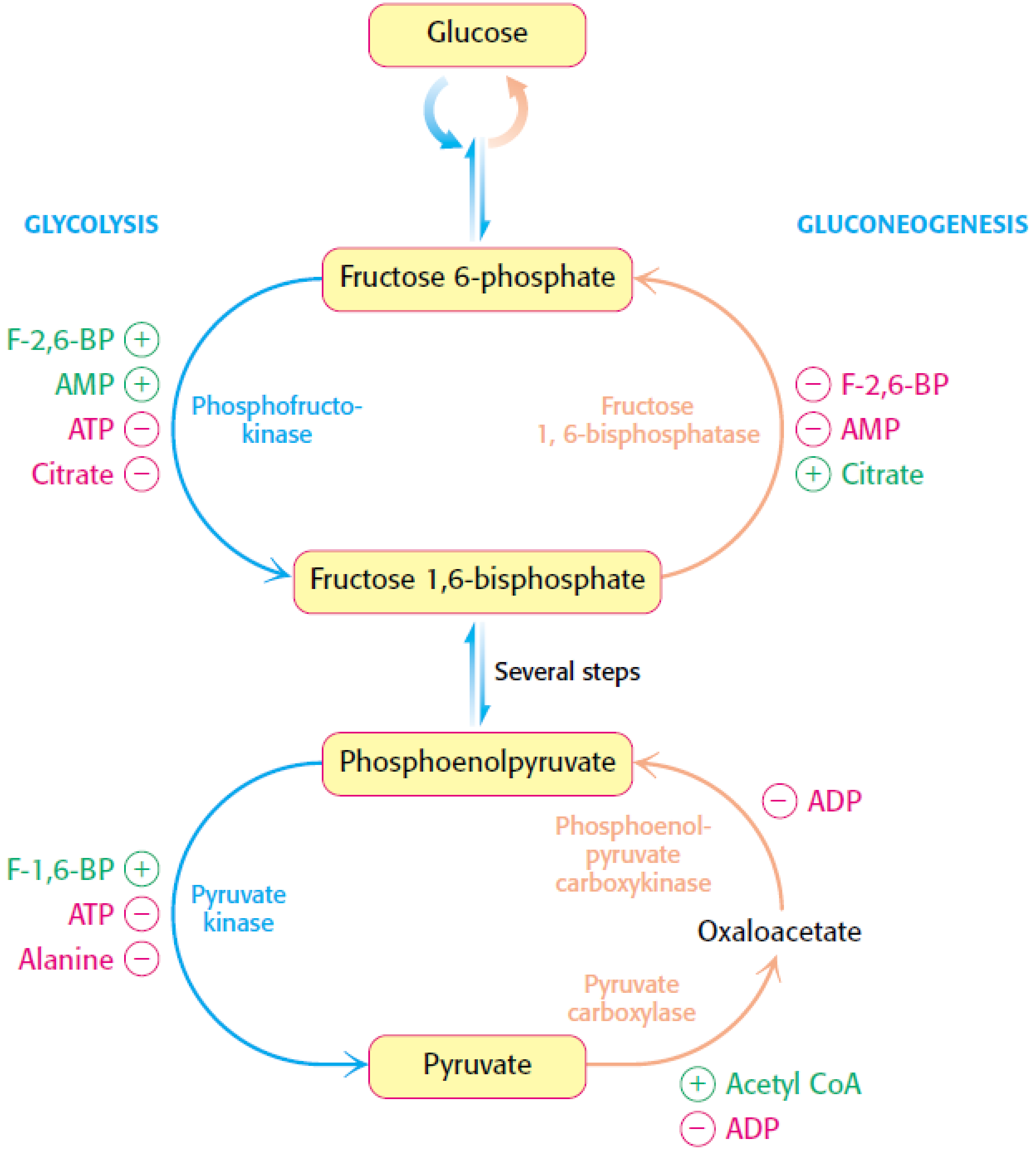
dephosphorylation activates PFK-2, and inhibits FBPase-2

↓

concentration of F-2,6-BP rises

↓

GLYCOLYSIS


Glucose
GLYCOLYSIS
GLUCONEOGENESIS
Fructose 6-phosphate
F-2,6-BP +
AMP +
ATP −
Citrate −
Phosphofructo-kinase
Fructose 1, 6-bisphosphatase
− F-2,6-BP
− AMP
+ Citrate
Fructose 1,6-bisphosphate
Several steps
Phosphoenolpyruvate
− ADP
Phosphoenol-pyruvate carboxykinase
F-1,6-BP +
ATP −
Alanine −
Pyruvate kinase
Oxaloacetate
Pyruvate carboxylase
Pyruvate
+ Acetyl CoA
− ADP

In short

Enzyme	Allosteric activators	Allosteric inhibitors	Phosphorylation effect
PFK-1	AMP, F-2,6-BP	ATP, citrate	
F-1,6-BISPHOSPHATASE	Citrate	AMP, F-2,6-BP	
PYRUVATE KINASE	F-1,6-BP	ATP, alanine	Inhibiting
PYRUVATE CARBOXYLASE	Acetyl-CoA	ADP	
PEPCK		ADP	
PFK-2	AMP, F-6-P, P_i	Citrate	Inhibiting
F-2,6-BISPHOSPHATASE	Glycerol-3-P	F-6-P	Activating

Literature used to prepare the presentation

1. J.M. Berg, J.L. Tymoczko and L. Stryer: **Biochemistry**, 7 th edition, W.H. Freeman and Company, USA, 2010.

2. D.L. Nelson and M.M. Cox: **Lehninger Principles of Biochemistry**, 6 th edition, W.H. Freeman and Company, USA, 2013.

3. R.A. Harvey and D.R. Ferrier: **Lippincott's Illustrated Reviews: Biochemistry**, 5th edition, Wolters Kluwer – Lippincott Williams & Wilkins, USA, 2011.

4. D. Voet i J.G. Voet: **Biochemistry**, 4th edition, John Wiley & Sons Inc., USA, 2010.

ANIMATION:
http://www.wiley.com/college/fob/anim/

Review questions/questions you should know the answers to:

1. What are regulatory reactions for glycolysis? Represent the substrates and products of those reactions by structural formulas and name the enzymes that catalyze them.

2. What reactions are different between glycolysis and gluconeogenesis?

3. What reaction is a key (major) regulatory reaction for glycolysis and gluconeogenesis?

4. What noncarbohydrate precursors can glucose be synthesized from *via* gluconeogenesis?

5. Write the net equations for glycolysis and gluconeogenesis.

6. Shortly describe the role of fructose 2,6-bisphosphate in glucose and gluconeogenesis.

7. What is the effect of glucagon on glycolysis and gluconeogenesis (what pathway does glucagon inhibit and what pathway does it stimulate)?

8. What is the effect of insulin on glycolysis and gluconeogenesis (what pathway does insulin inhibit and what pathway does it stimulate)?