Lecture 3 - Glycolysis and Gluconeogenesis

Chem 454: Regulatory Mechanisms in Biochemistry
University of Wisconsin-Eau Claire

Introduction

Glycolysis converts glucose ($C_6H_{12}O_6$) molecules to two molecules of pyruvic acid ($C_3H_4O_3$).

- Pyruvic acid is more oxidized than glucose
- The energy released from the oxidation is used to create 2 molecules of ATP from 2 ADP and 2 P_i
- This is an **anaerobic** process.
- Under anaerobic conditions the pyruvic acid can be **fermented** to lactic acid or to ethanol plus CO_2.
- Under **aerobic** conditions, glucose is oxidized all the way to $C0_2$ and H_2O.

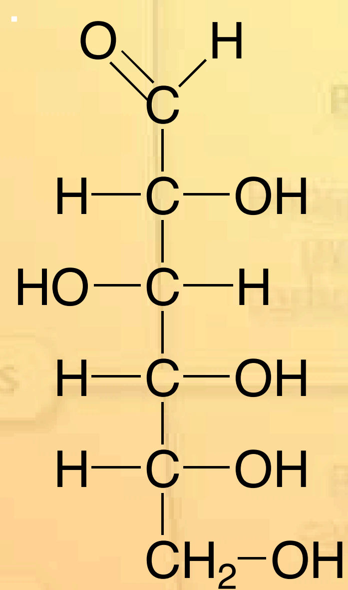
Introduction

Glucose can also be synthesized from molecules such as pyruvic acid or lactic acid.

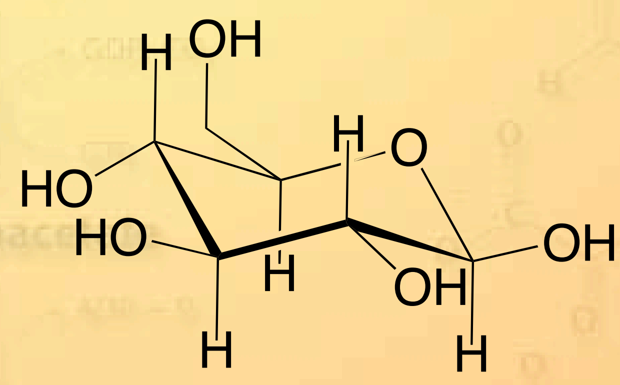
- This process is called **gluconeogenesis.**

Introduction

- Glucose is an important fuel for most organisms.
  - In mammals, glucose is the preferred fuel source for the brain and the only fuel source for red blood cells.
  - Almost all organisms use glucose



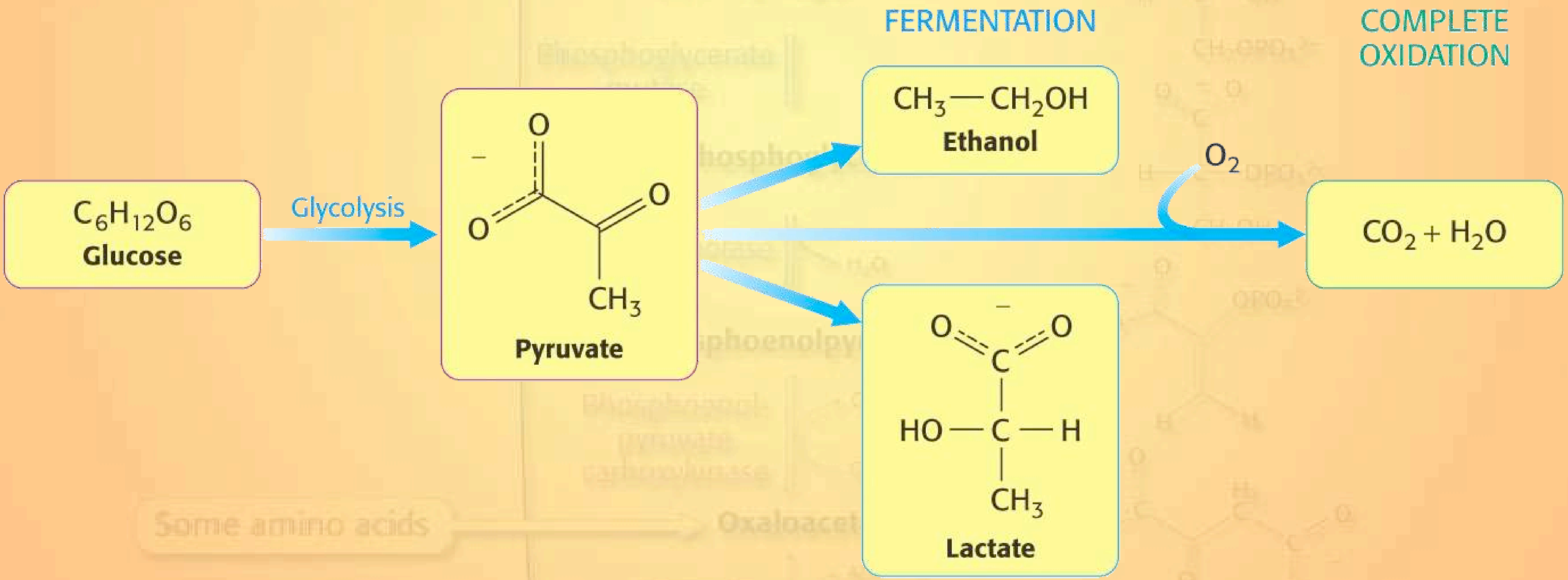
D–Glucose



□–D–Glucose

Introduction

Fermentations provide usable energy in absence of oxygen.



Introduction

Obligate anaerobes

TABLE 16.2 Examples of pathogenic obligate anaerobes

Bacterium	Results of infection
Clostridium tetani	Tetanus (lockjaw)
Clostridium botulinum	Botulism (an especially severe type of food poisoning)
Clostridium perfringens	Gas gangrene (gas is produced as an end point of the fermentation, distorting and destroying the tissue)
Bartonella hensela	Cat scratch fever (flulike symptoms)
Bacteroides fragilis	Abdominal, pelvic, pulmonary, and blood infections

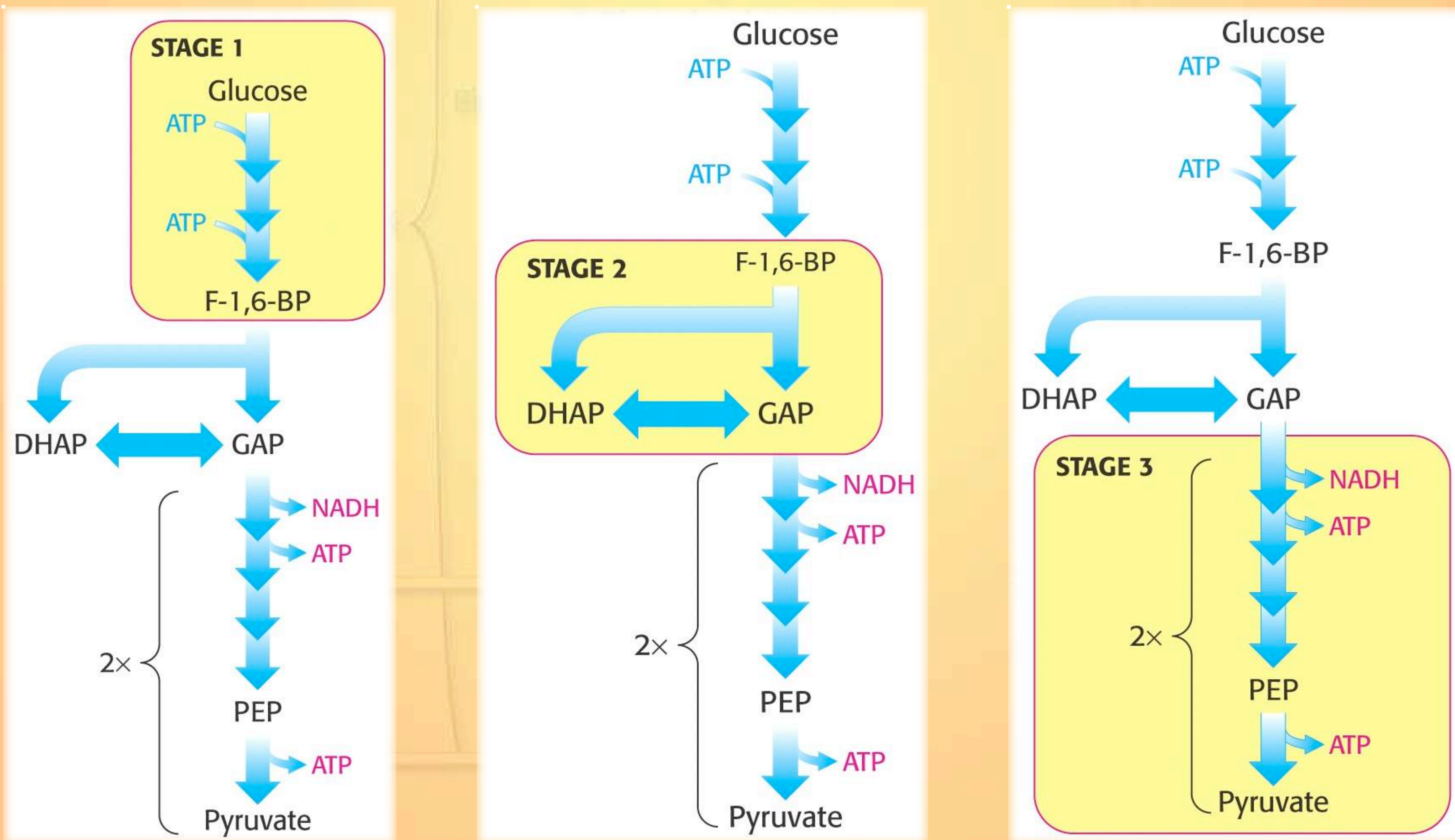
1. Glycolysis is Energy Conversion

Glycolysis is an energy-conversion pathway in many organisms.

- The glycolytic pathway is common to virtually all organisms
- Both eukaryotes and prokaryotes
- In eukaryotes, it occurs in the cytosol

1. Glycolysis is Energy Conversion

The glycolytic pathway is considered in three stages:



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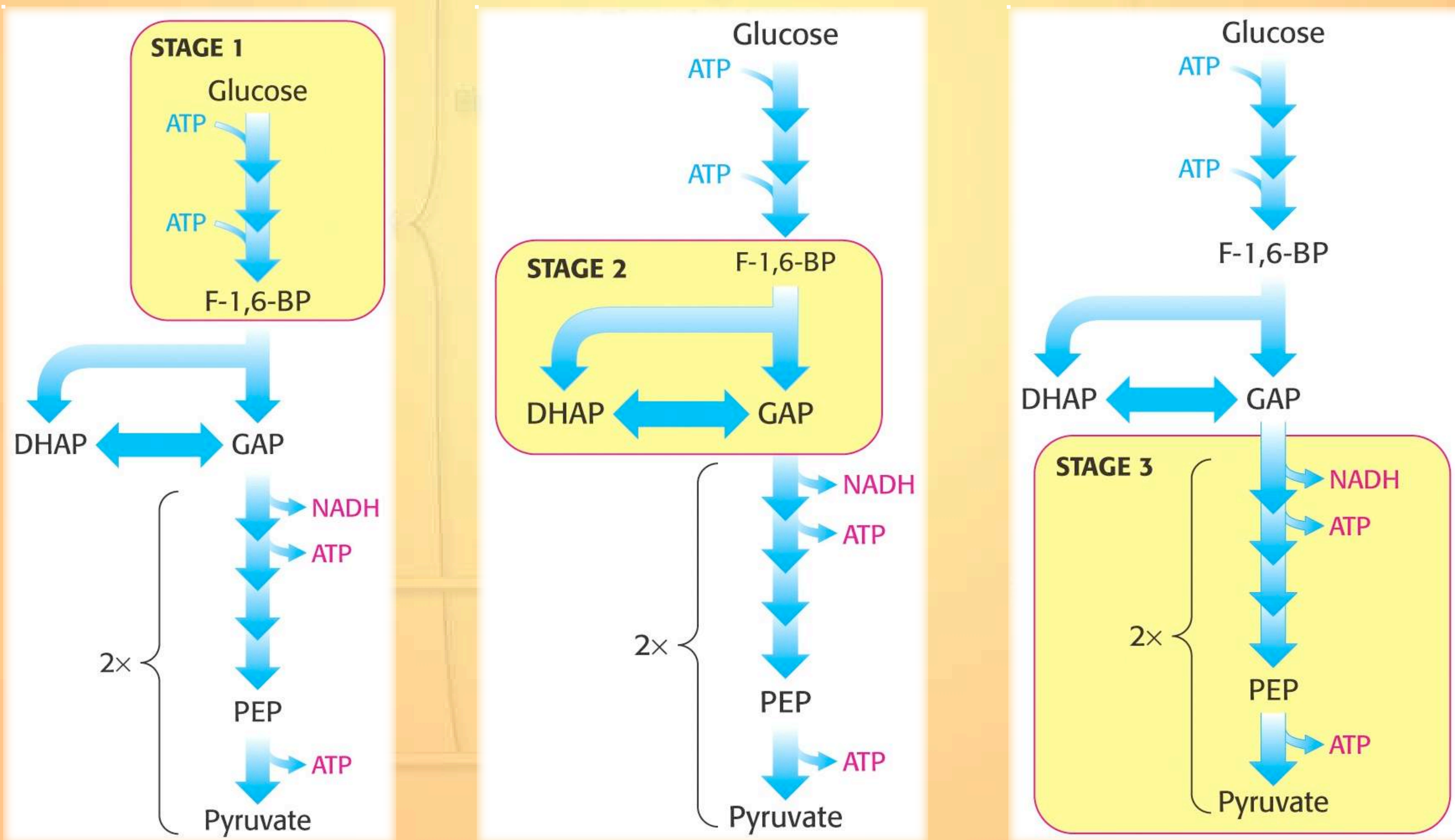

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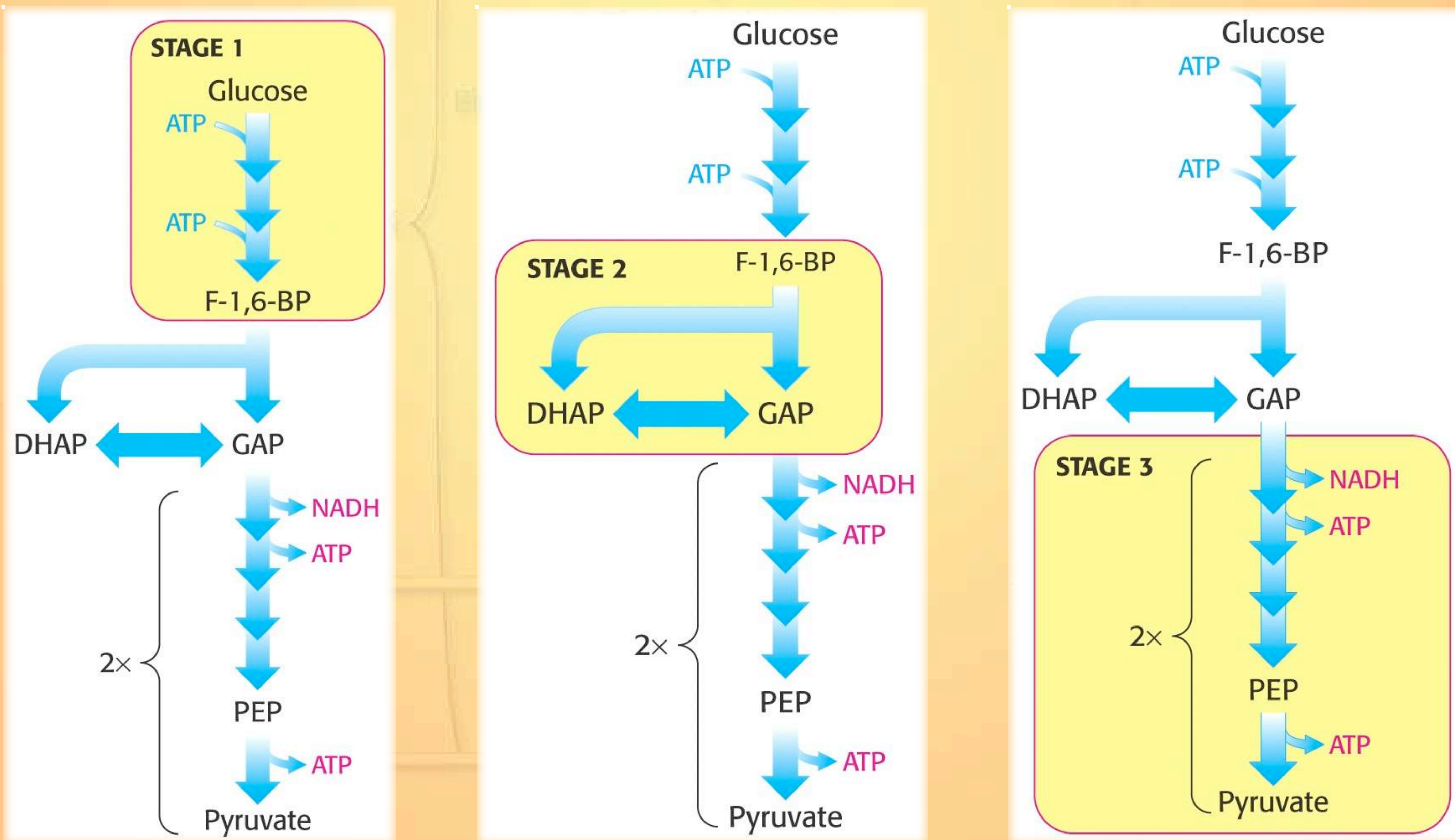
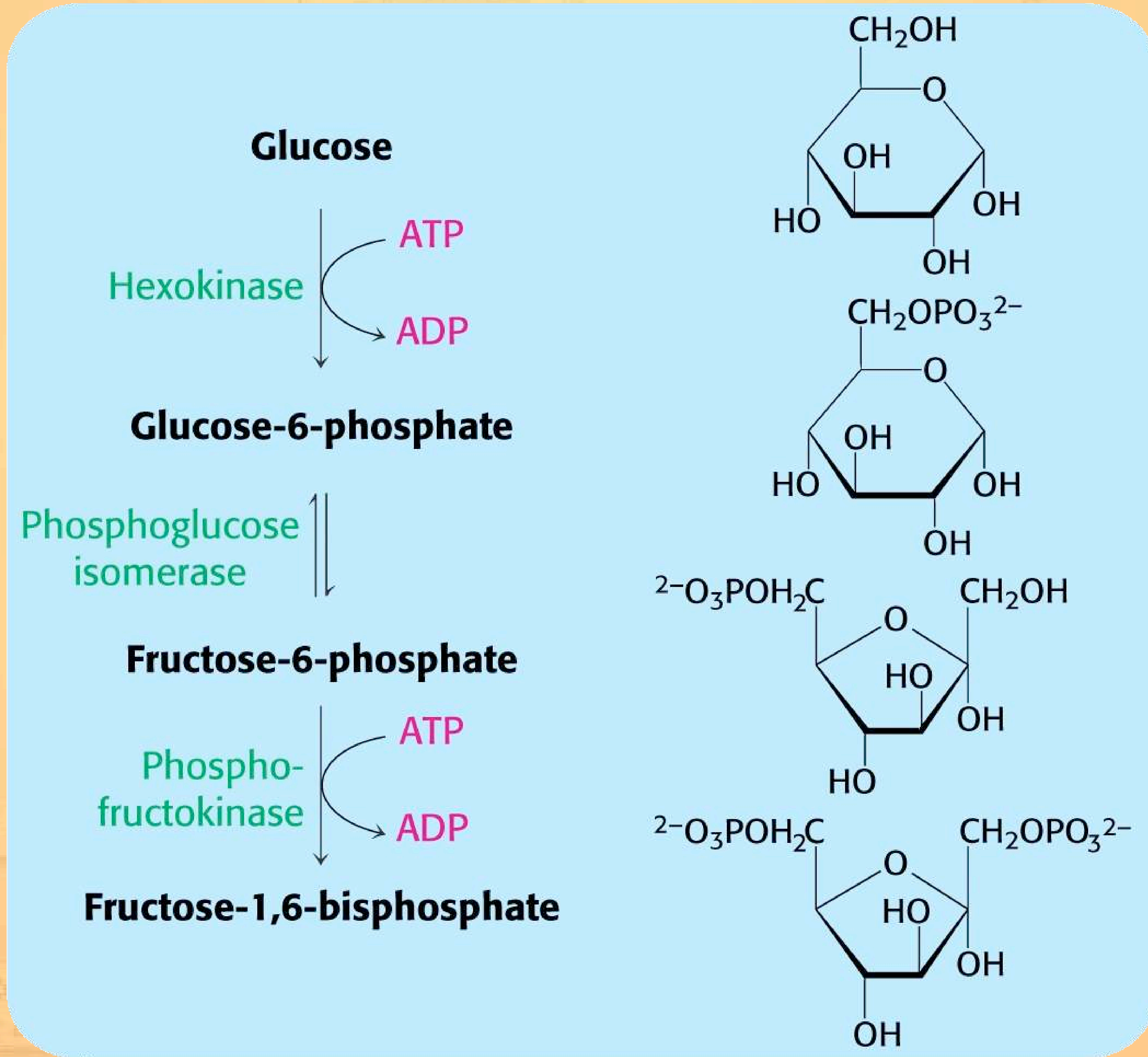


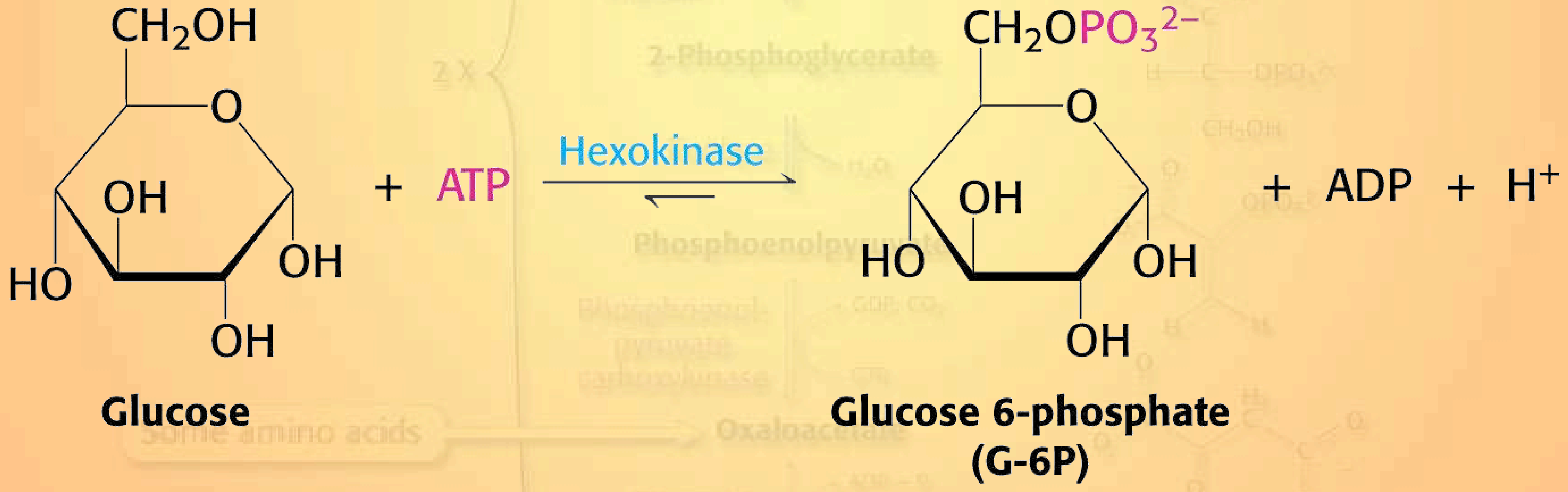
1. Stage 1

Stage 1



1.1. Hexokinase

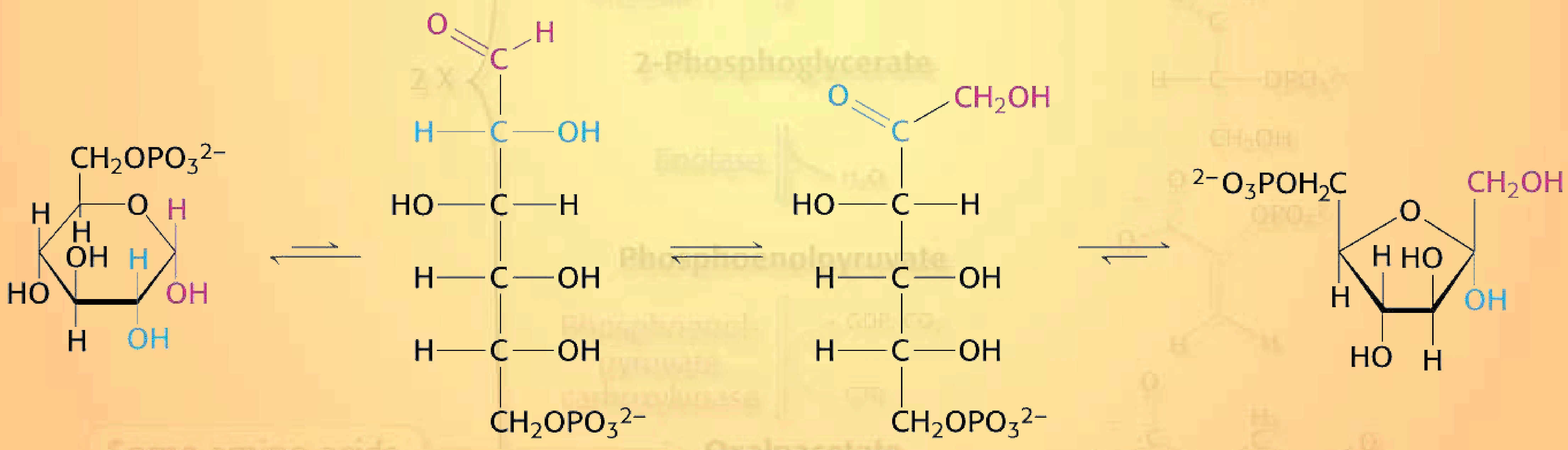
Hexokinase traps glucose in the cell and begins glycolysis.



1.2 Phosphoglucose Isomerase

The formation of fructose 1,6-bisphosphate from glucose 6-phosphate

- Phosphoglucose isomerase

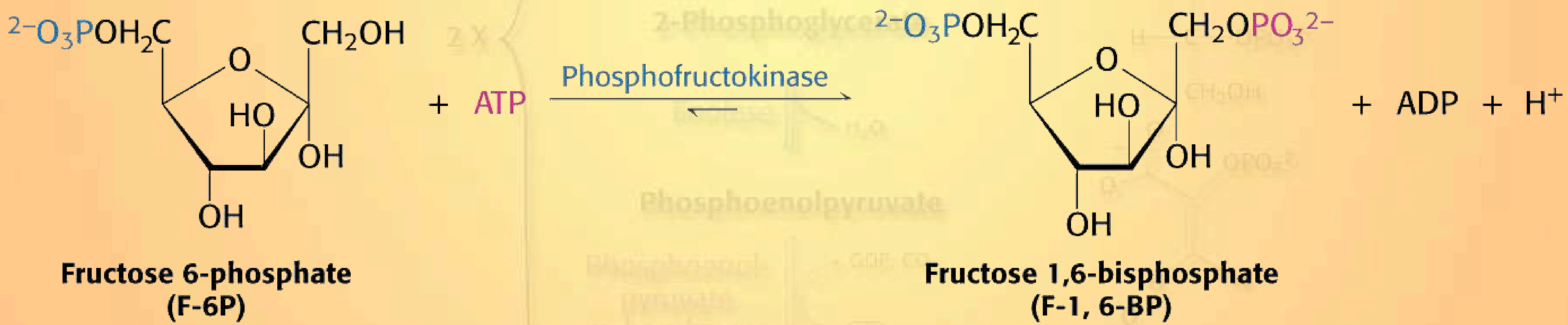


Glucose 6-phosphate (G-6P) | Glucose 6-phosphate (Open chain form) | Fructose 6-phosphate (Open chain form) | Fructose 6-phosphate (F-6P)

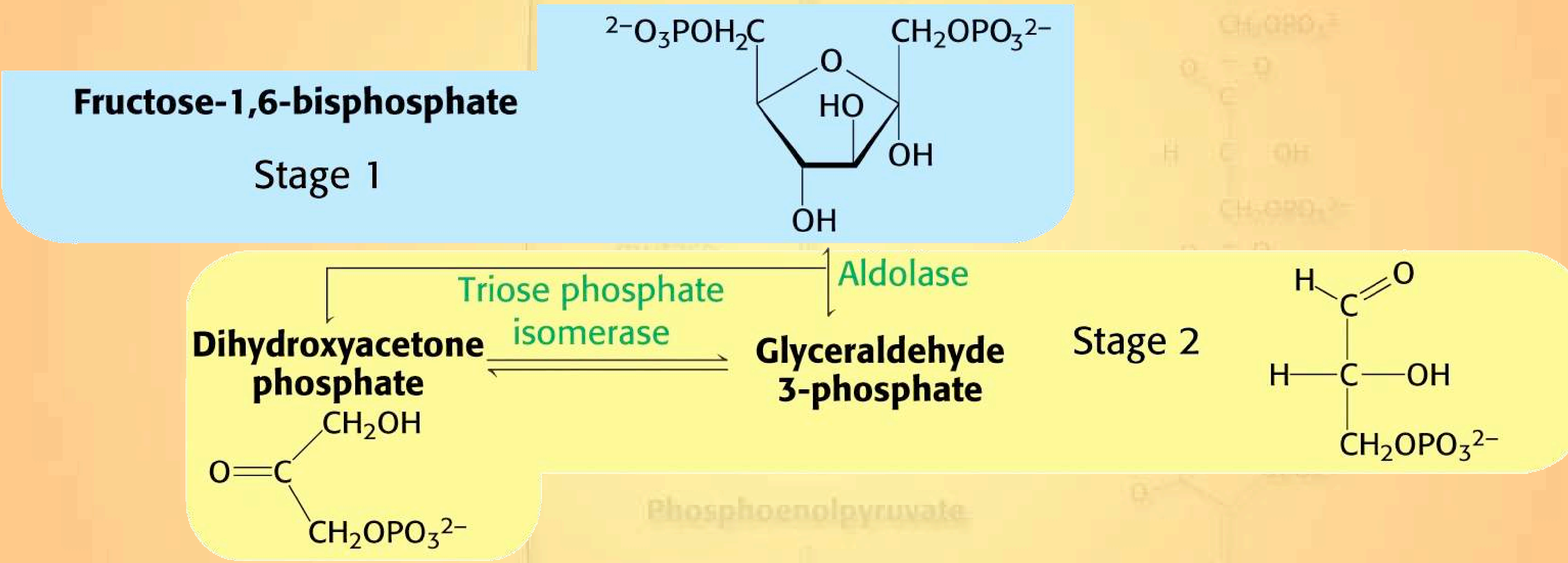
1.2 Phosphofructokinase

The formation of fructose 1,6-bisphosphate from glucose 6-phosphate

- Phosphofructose kinase

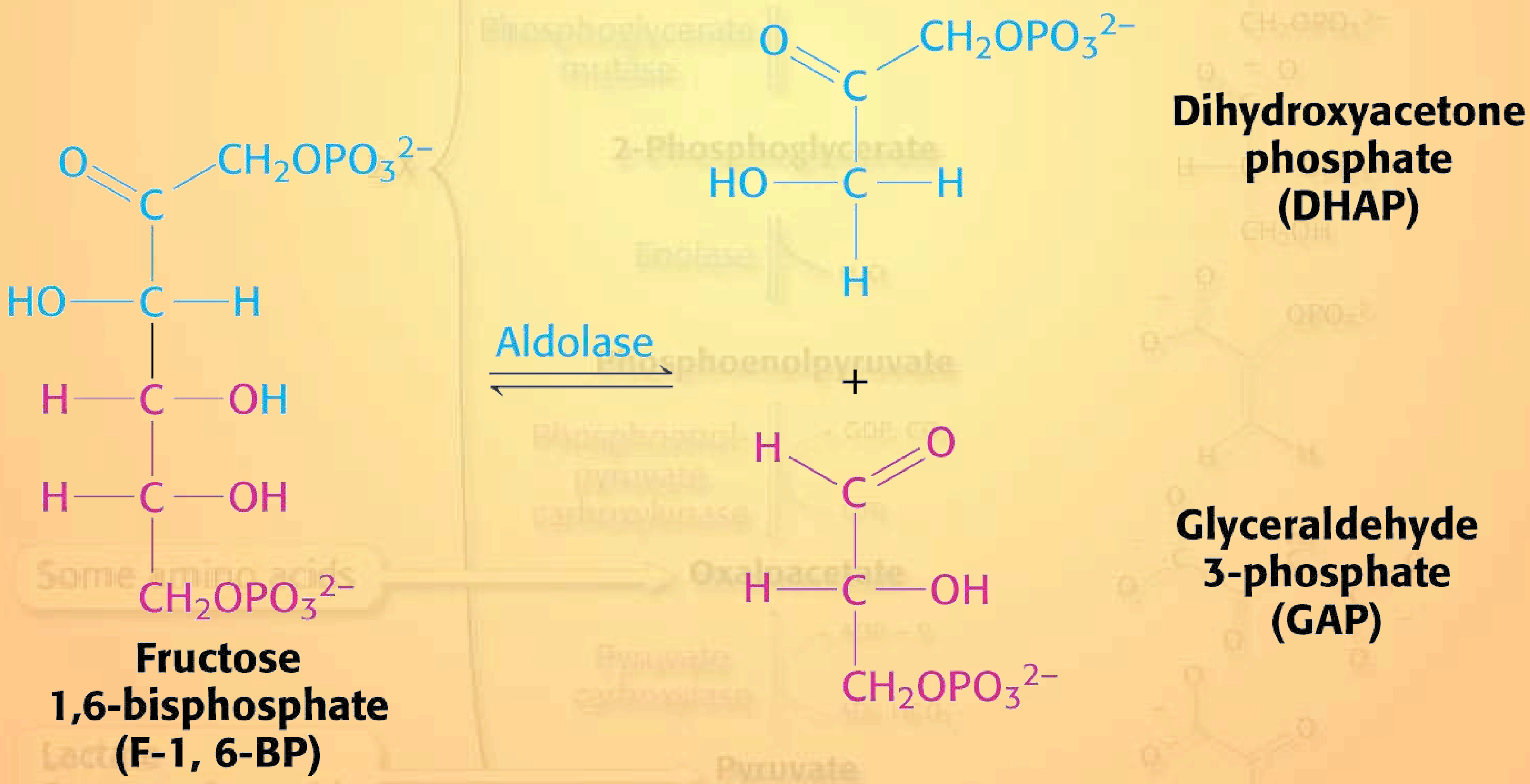


1. Stage 2


Fructose-1,6-bisphosphate
Stage 1
$^{2-}O_3POH_2C$
$CH_2OPO_3^{2-}$
O
HO
OH
OH
Aldolase
Triose phosphate isomerase
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
Stage 2
CH_2OH
O=C
$CH_2OPO_3^{2-}$
H
O
C
H—C—OH
$CH_2OPO_3^{2-}$

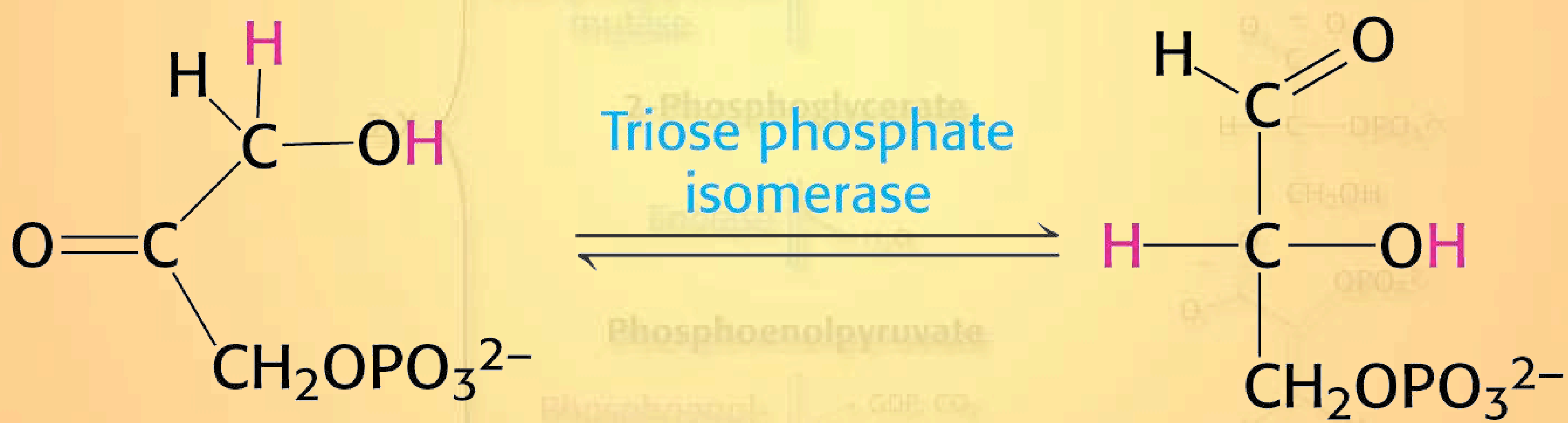
1.3. Aldolase

The six-carbon sugar is cleaved into two three-carbon fragments by aldolase.



1.4. Triose Phosphate Isomerase

Triose phosphate isomerase salvages a three-carbon fragments

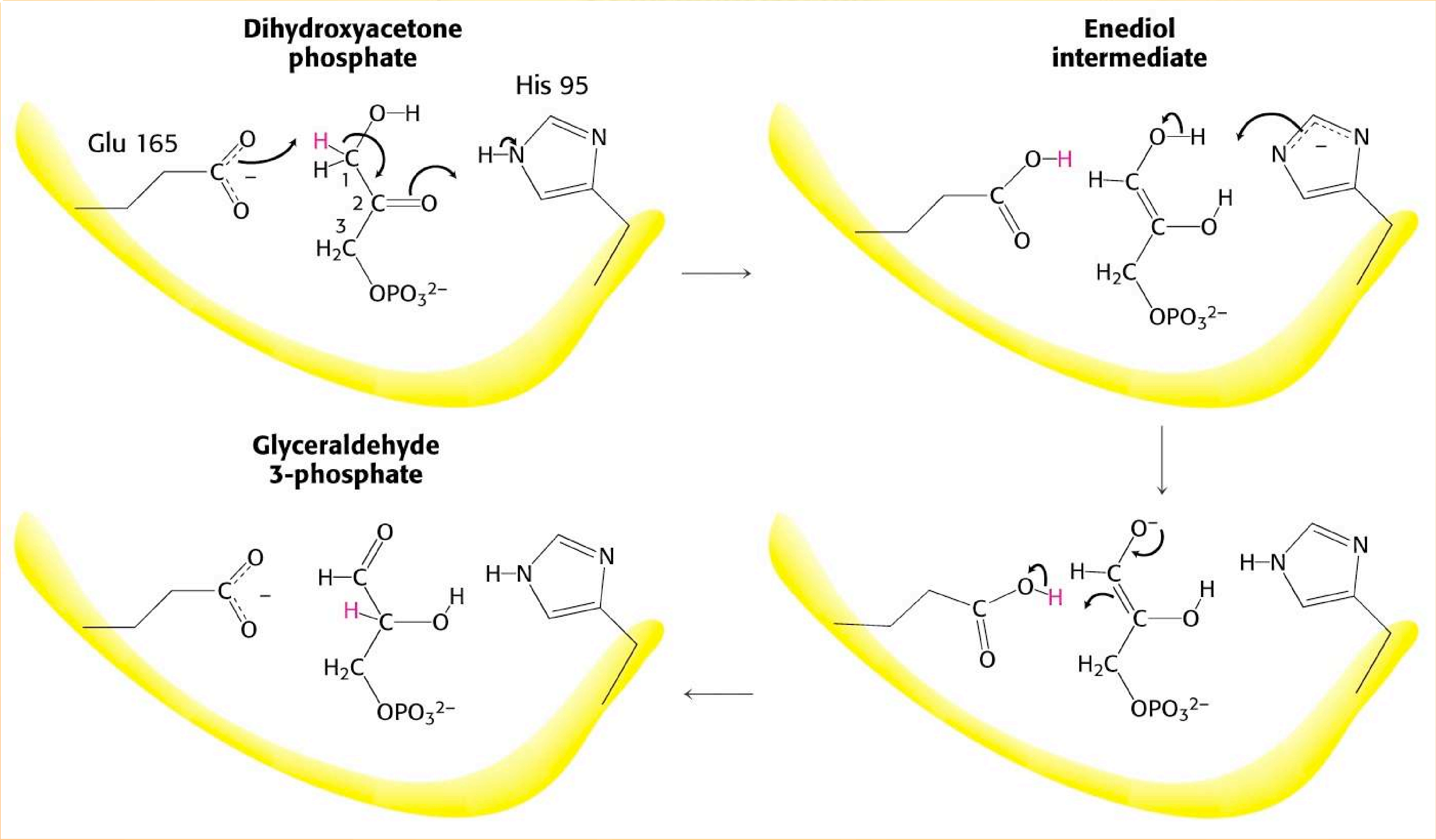


Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

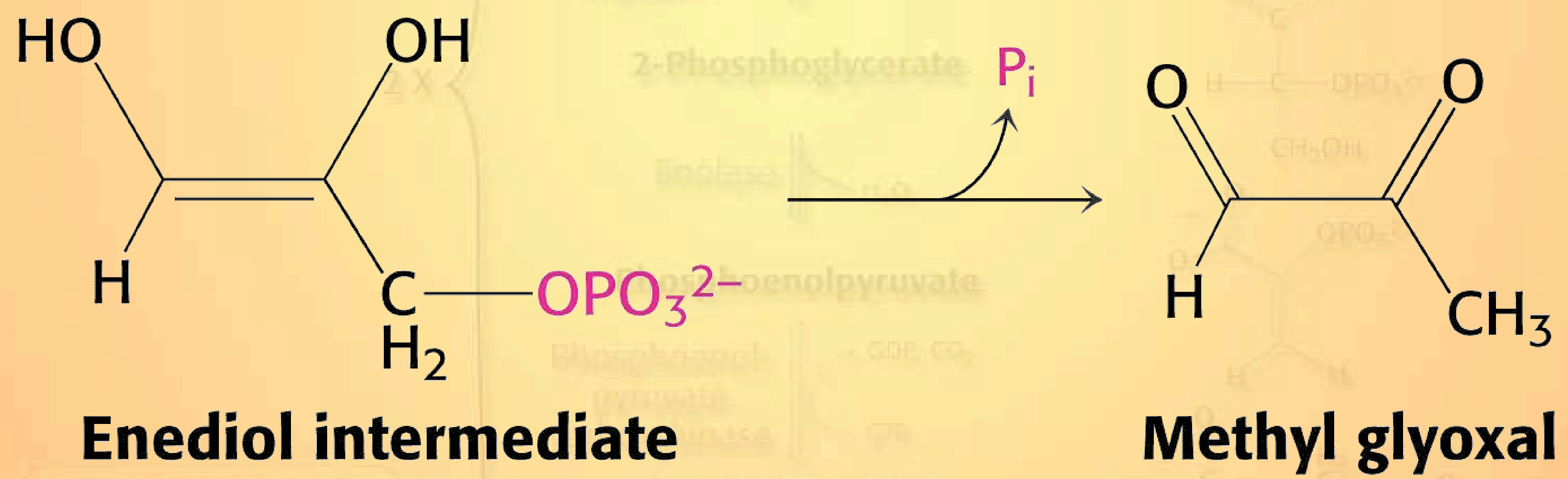
1.4. Triose Phosphate Isomerase

Triose phosphate isomerase salvages a three-carbon fragments

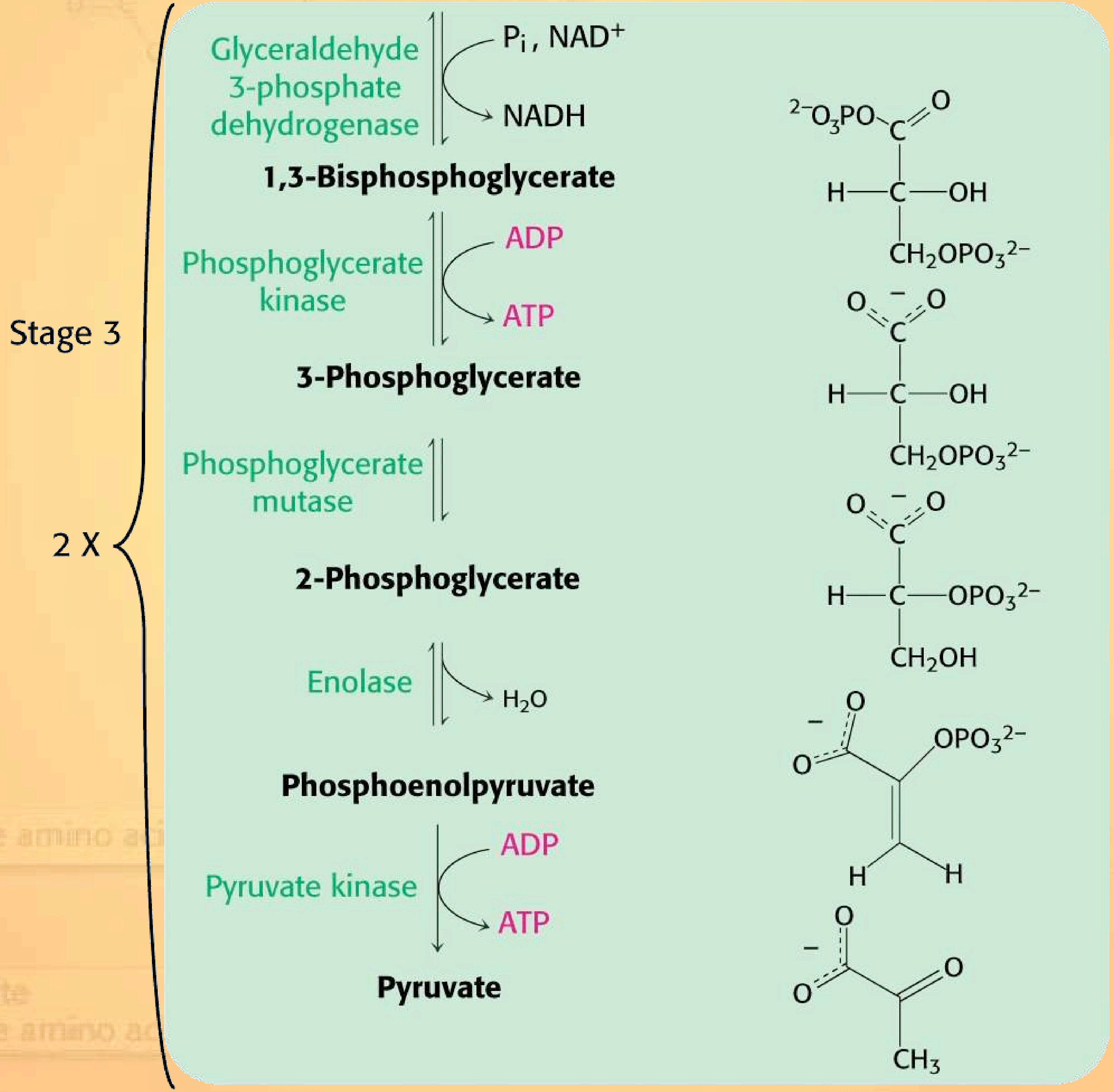


1.4. Triose Phosphate Isomerase

Triose phosphate isomerase is an example of a kinetically perfect enzyme.



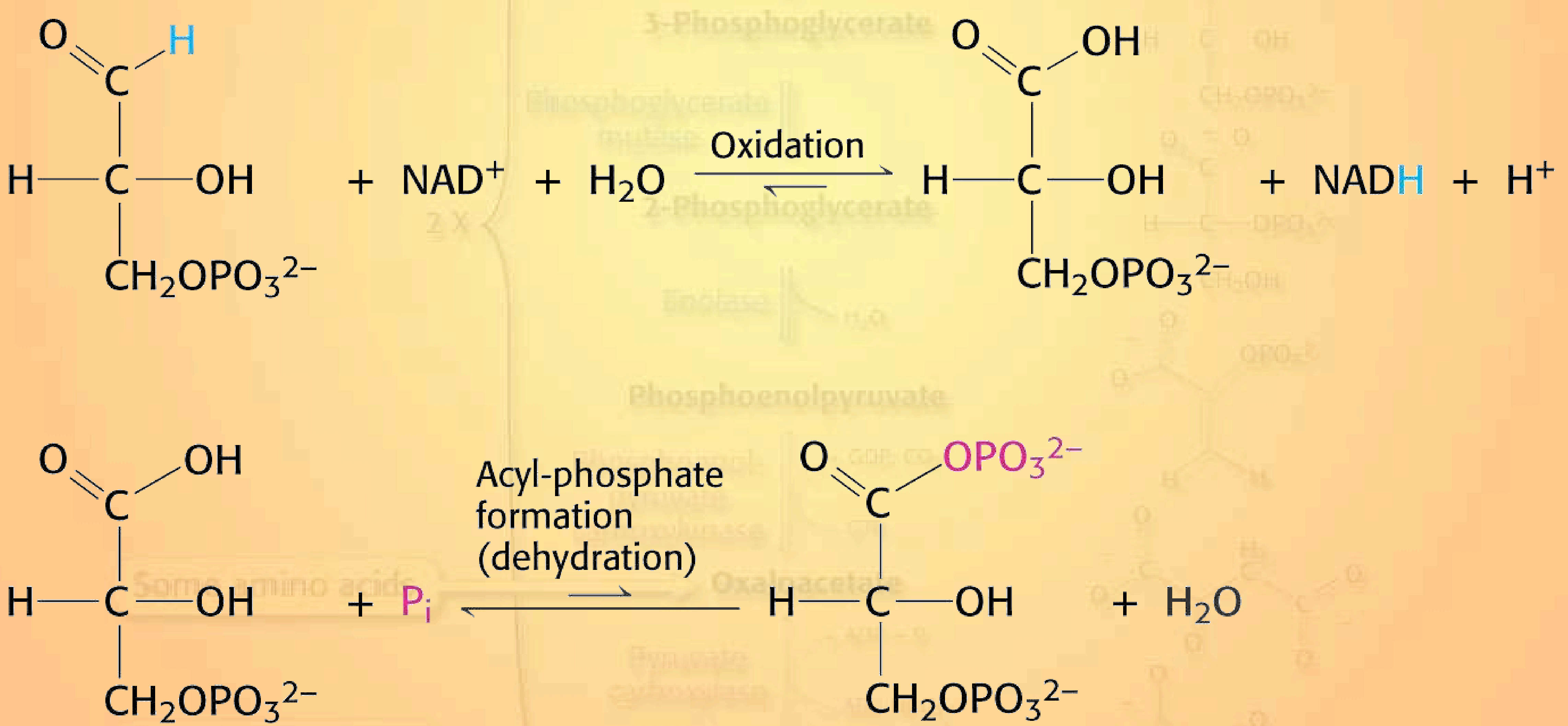
1. Stage 3



Stage 3
2 X
Glyceraldehyde 3-phosphate dehydrogenase
Pi, NAD+
NADH
1,3-Bisphosphoglycerate
Phosphoglycerate kinase
ADP
ATP
3-Phosphoglycerate
Phosphoglycerate mutase
2-Phosphoglycerate
Enolase
H2O
Phosphoenolpyruvate
Pyruvate kinase
ADP
ATP
Pyruvate

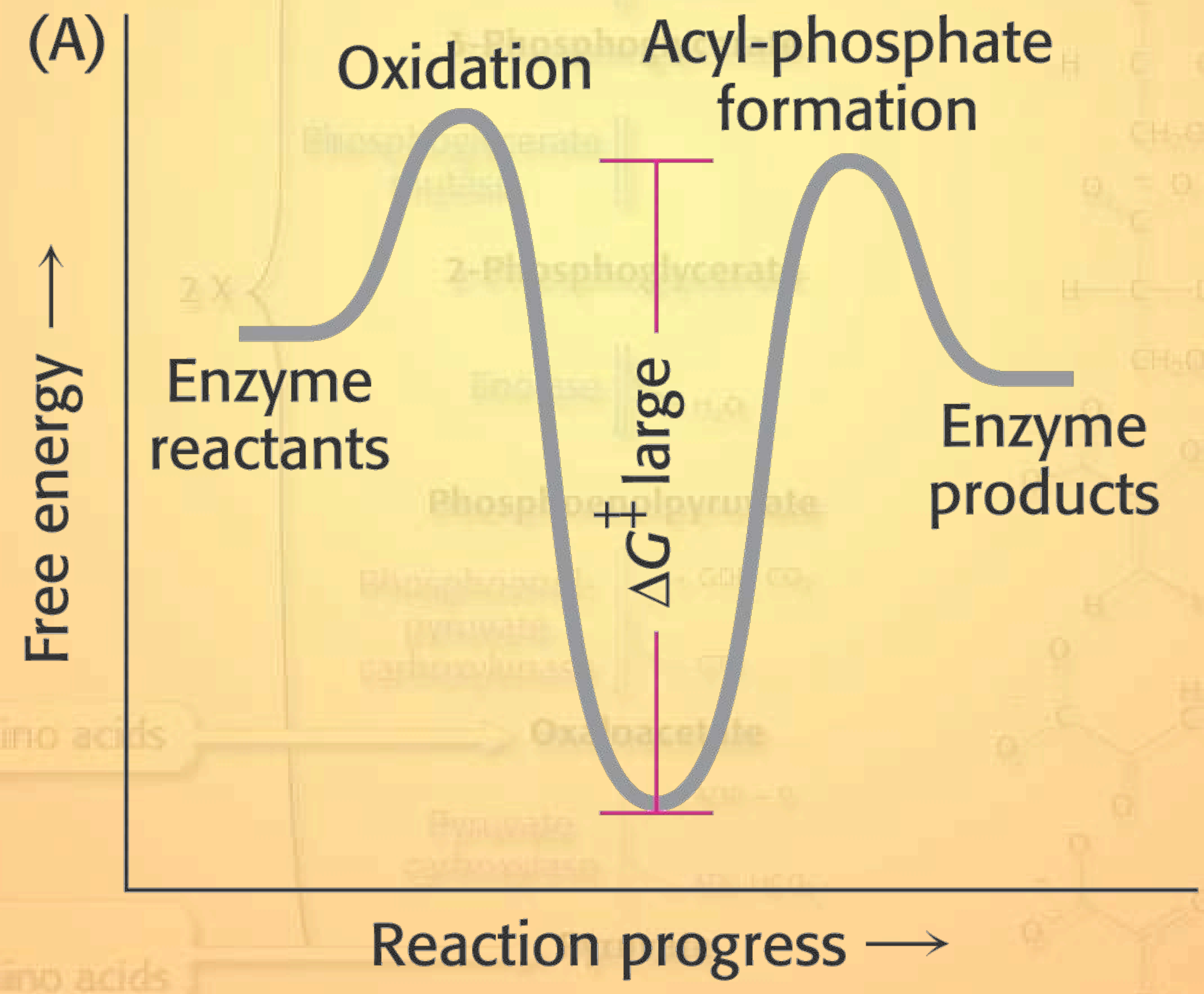
1.5. Glyceraldehyde 3-Phosphate Dehydrogenase

Energy transformation: Phosphorylation is coupled to the oxidation of glyceraldehyde 3-phosphate.

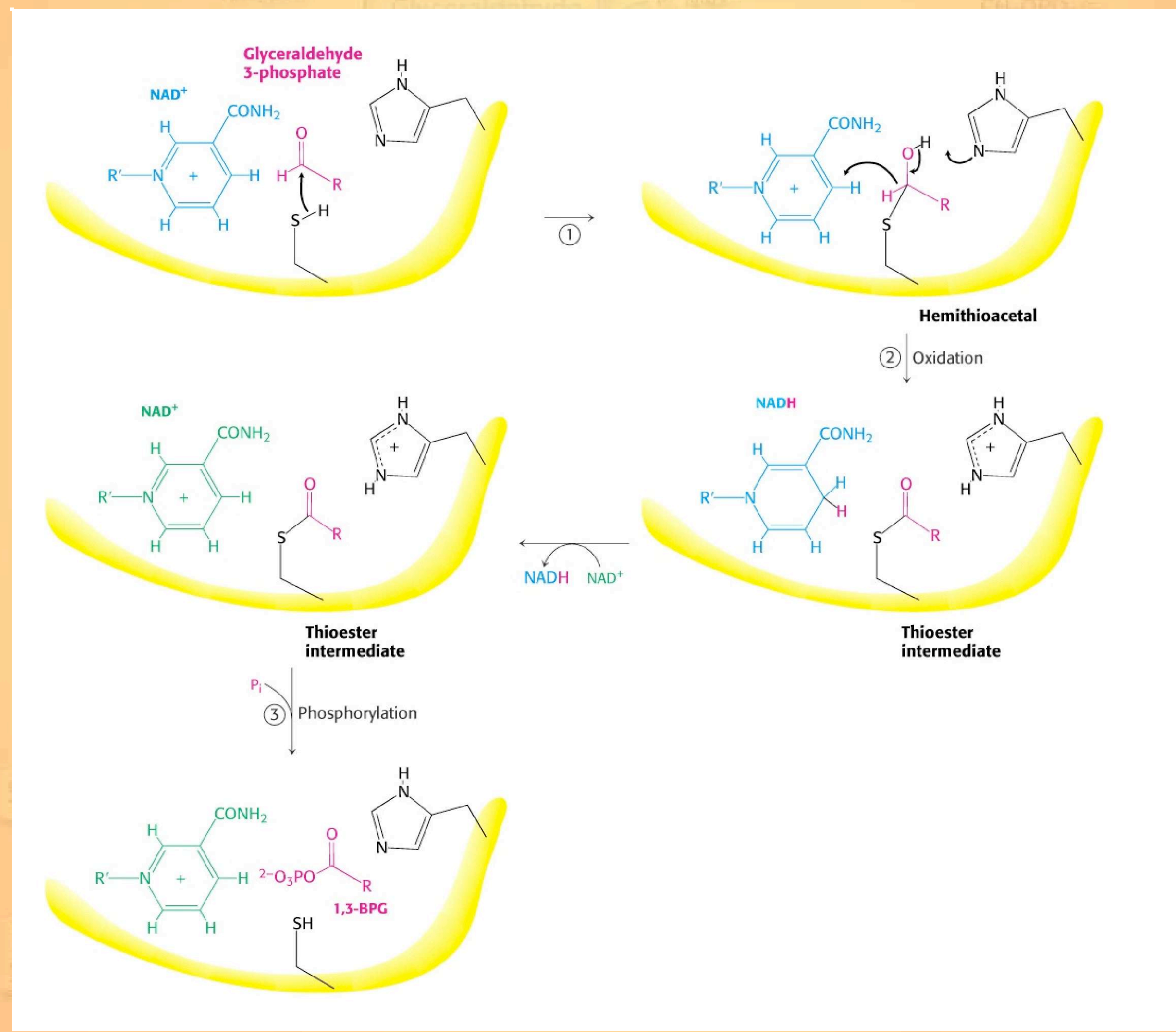


1.5. Glyceraldehyde 3-Phosphate Dehydrogenase

Energy transformation: Phosphorylation is coupled to the oxidation of glyceraldehyde 3-phosphate.

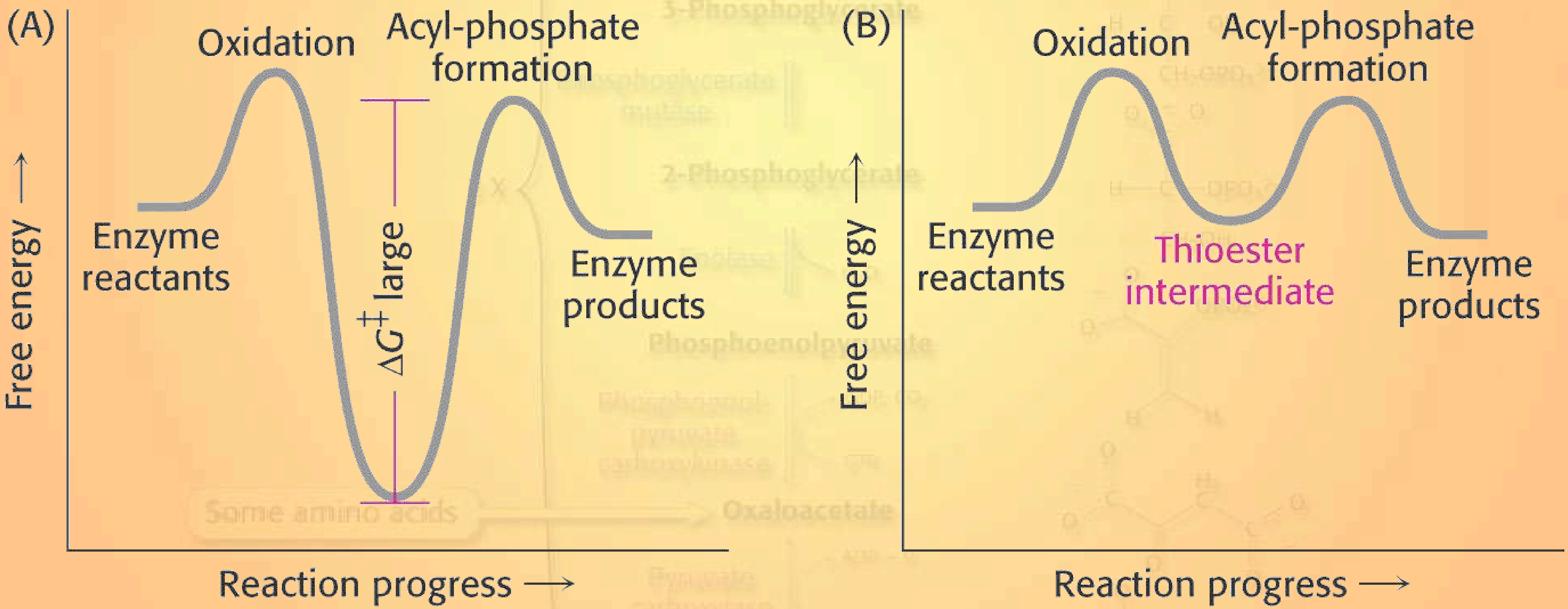


1.5. Glyceraldehyde 3-Phosphate Dehydrogenase



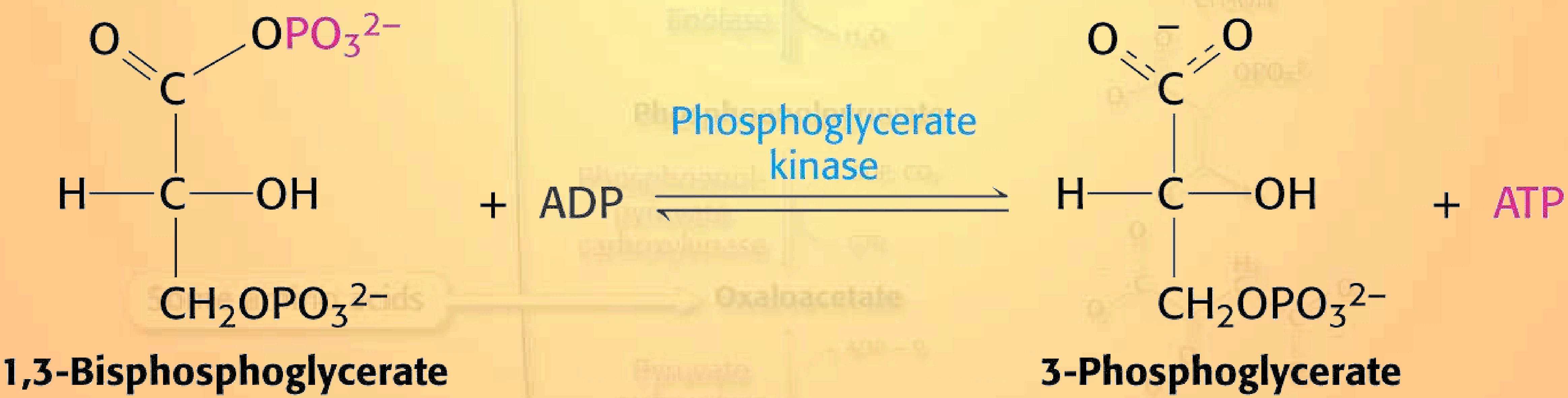
1.5. Glyceraldehyde 3-Phosphate Dehydrogenase

The enzyme-bound thioester intermediate reduces the activation energy for the second reaction:



1.6. Phosphoglycerate Kinase

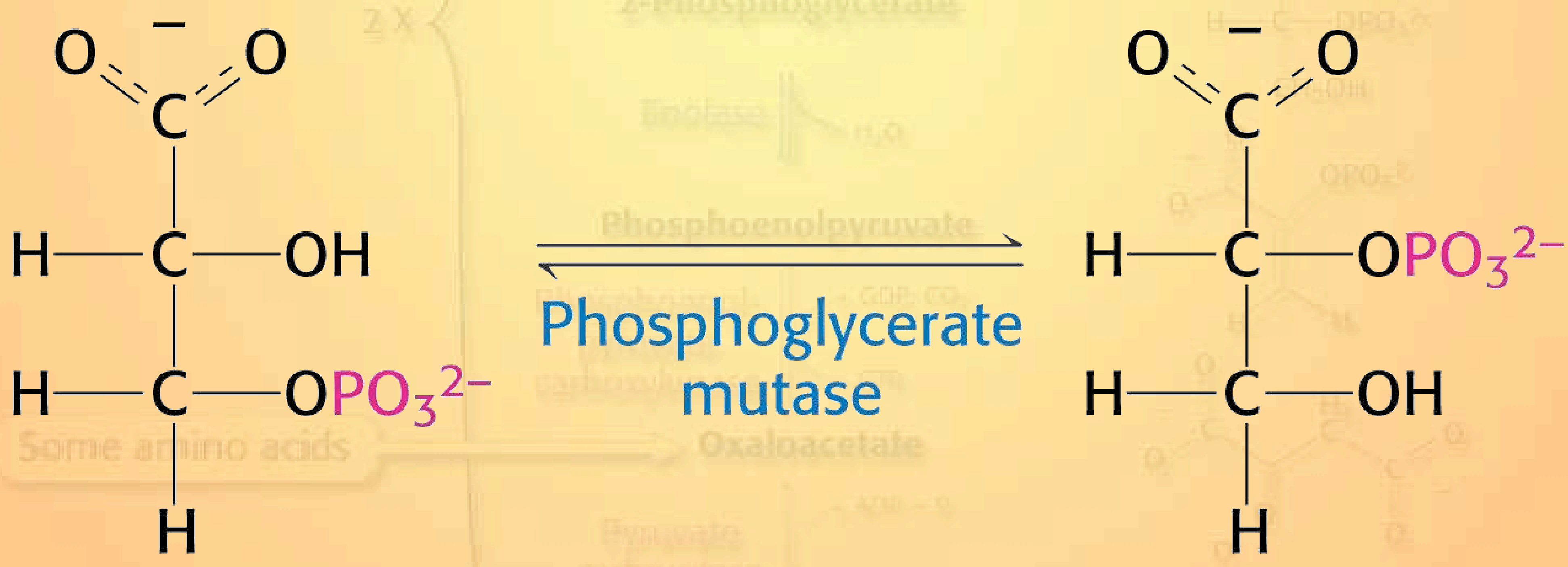
The acyl phosphate in 1,3-bisphosphoglycerate has a high enough phosphoryl transfer potential to phosphorylate ADP to produce ATP:



1.7. Phosphoglycerate Mutase

The next two reactions convert the remaining phosphate ester into a phosphate having a high phosphoryl transfer potential

- The first is an isomerization reaction

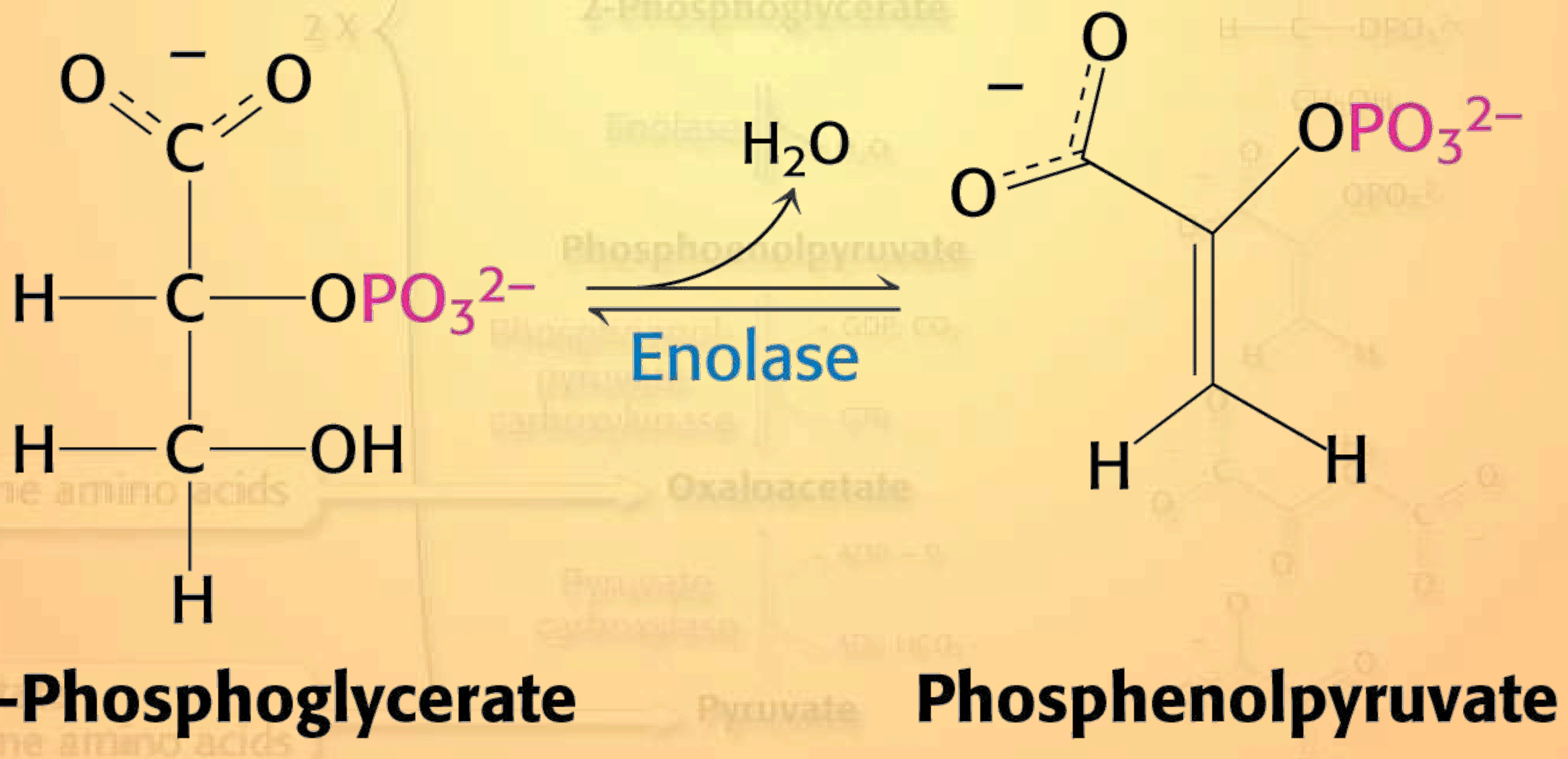


3-Phosphoglycerate **2-Phosphoglycerate**

1.7. Enolase

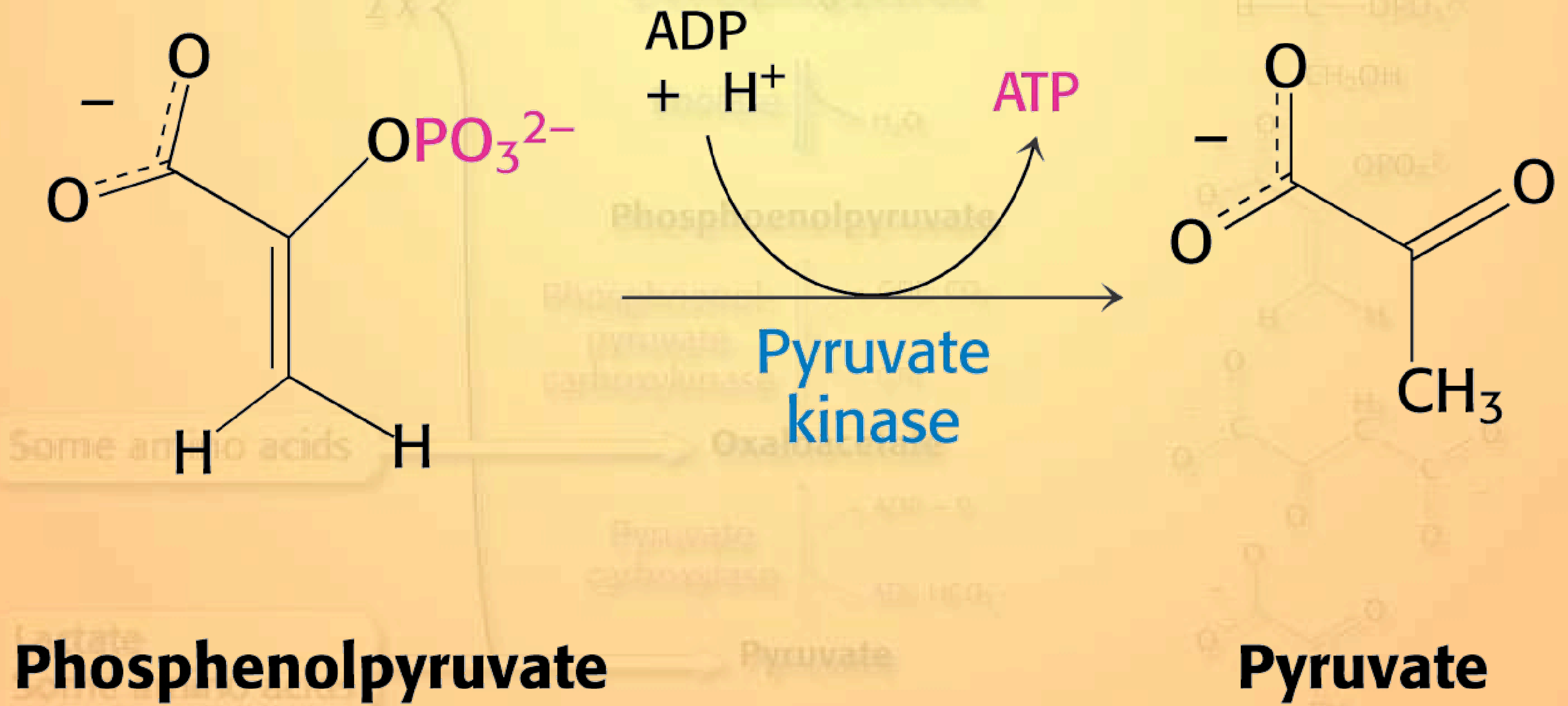
The next two reactions convert the remaining phosphate ester into a phosphate having a high phosphoryl transfer potential

- The second is a dehydration (lyase) reaction



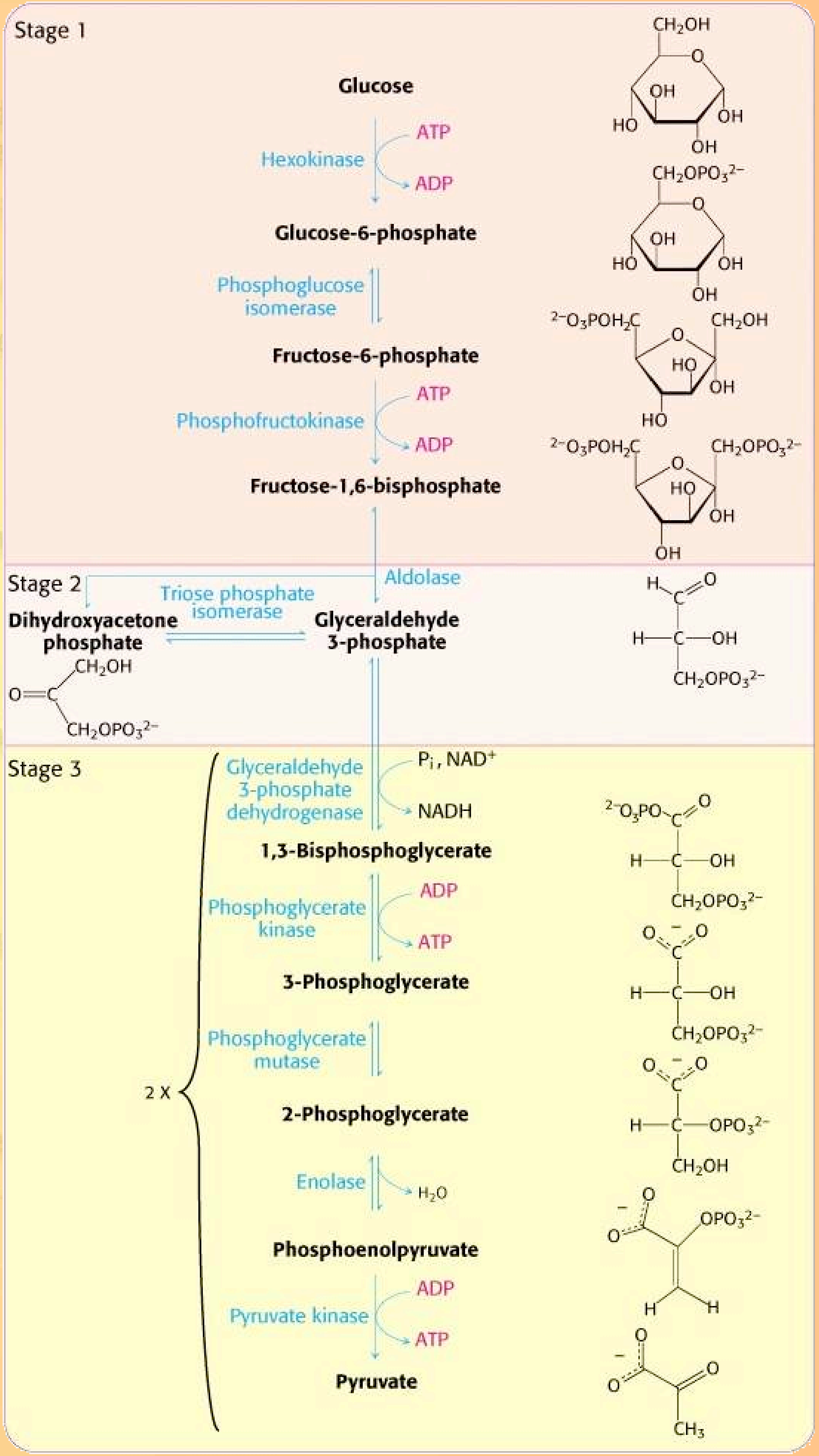
1.7. Pyruvate Kinase

The final reaction in the glycolytic pathway transfers the phosphate from phosphoenolpyruvate to ADP to produce ATP:



1.8 The Net Reaction

$$\text{Glucose} + 2\ NAD^{+} + 2\ ADP + 2\ P_i \longrightarrow \text{Pyruvate} + 2\ NADH + 2\ H^{+} + 2\ ATP$$



1.8. The Net Reaction

Summary of the reactions in the glycolytic pathway:

TABLE 16.3 Reactions of glycolysis

Step	Reaction	Enzyme	Reaction type	$\Delta G^{\circ\prime}$ in kcal mol^{-1} (kJ mol^{-1})	ΔG in kcal mol^{-1} (kJ mol^{-1})
1	Glucose + ATP $\longrightarrow$ glucose 6-phosphate + ADP + H^+	Hexokinase	Phosphoryl transfer	−4.0 (−16.7)	−8.0 (−33.5)
2	Glucose 6-phosphate $\rightleftharpoons$ fructose 6-phosphate	Phosphoglucose isomerase	Isomerization	+0.4 (+1.7)	−0.6 (−2.5)
3	Fructose 6-phosphate + ATP $\longrightarrow$ fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	Phosphoryl transfer	−3.4 (−14.2)	−5.3 (−22.2)
4	Fructose 1,6-bisphosphate $\rightleftharpoons$ dihydroxyacetonephosphate + glyceraldehyde 3-phosphate	Aldolase	Aldol cleavage	+5.7 (+23.8)	−0.3 (−1.3)
5	Dihydroxyacetone phosphate $\rightleftharpoons$ glyceraldehyde 3-phosphate	Triose phosphate isomerase	Isomerization	+1.8 (+7.5)	+0.6 (+2.5)
6	Glyceraldehyde 3-phosphate + P_i + NAD^+ $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	Phosphorylation coupled to oxidation	+1.5 (+6.3)	+0.6 (+2.5)
7	1,3-Bisphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	Phosphoryl transfer	−4.5 (−18.8)	+0.3 (+1.3)
8	3-Phosphoglycerate $\rightleftharpoons$ 2-phosphoglycerate	Phosphoglycerate mutase	Phosphoryl shift	+1.1 (+4.6)	+0.2 (+0.8)
9	2-Phosphoglycerate $\rightleftharpoons$ phosphoenolpyruvate + H_2O	Enolase	Dehydration	+0.4 (+1.7)	−0.8 (−3.3)
10	Phosphoenolpyruvate + ADP + H^+ $\longrightarrow$ pyruvate + ATP	Pyruvate kinase	Phosphoryl transfer	−7.5 (−31.4)	−4.0 (−16.7)

Note: ΔG, the actual free-energy change, has been calculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiologic conditions. Glycolysis can proceed only if the ΔG values of all reactions are negative. The small positive ΔG values of three of the above reactions indicate that the concentrations of metabolites in vivo in cells undergoing glycolysis are not precisely known.

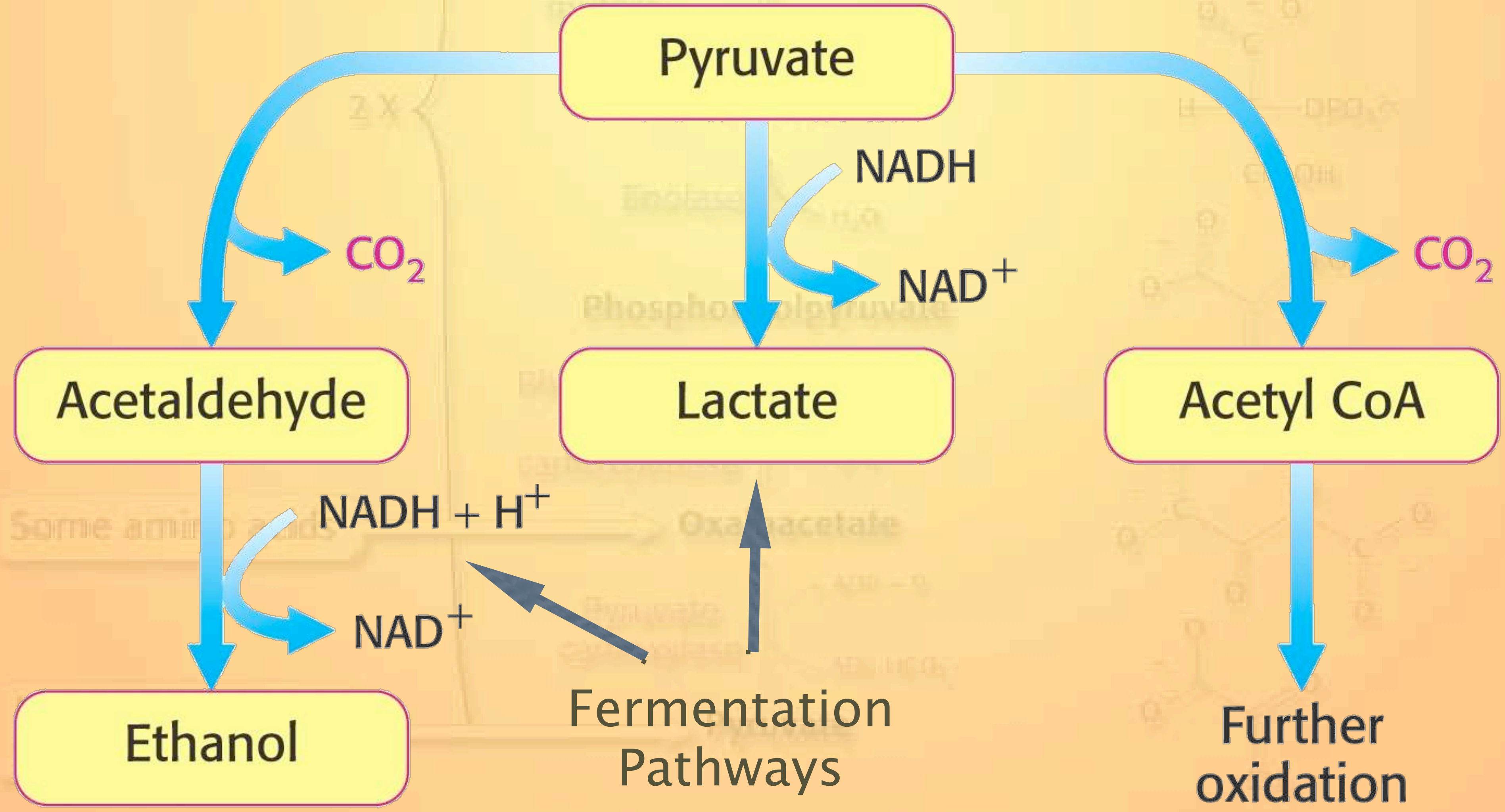
1.9.Maintaining Redox Balance

There is a problem:

- There are only catalytic quantities of NAD^+ in the cell.
- In order to continue to use glycolysis to generate ATP, there needs to be some means of reoxidizing the NADH + H^+ that is produced in glycolysis

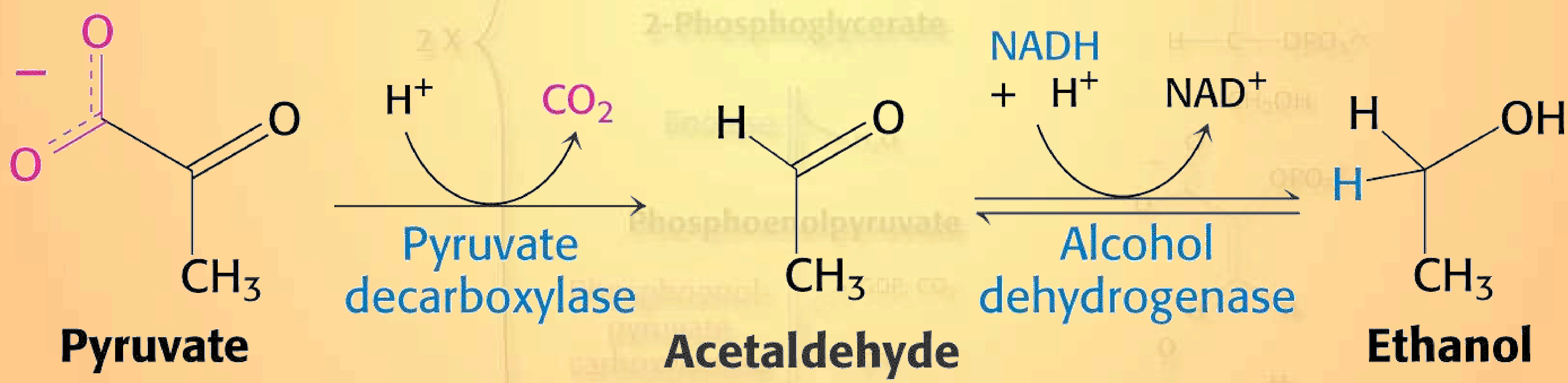
1.9.Maintaining Redox Balance

The solution to this problem lies in what happens to the pyruvate that is produced in glycolysis:



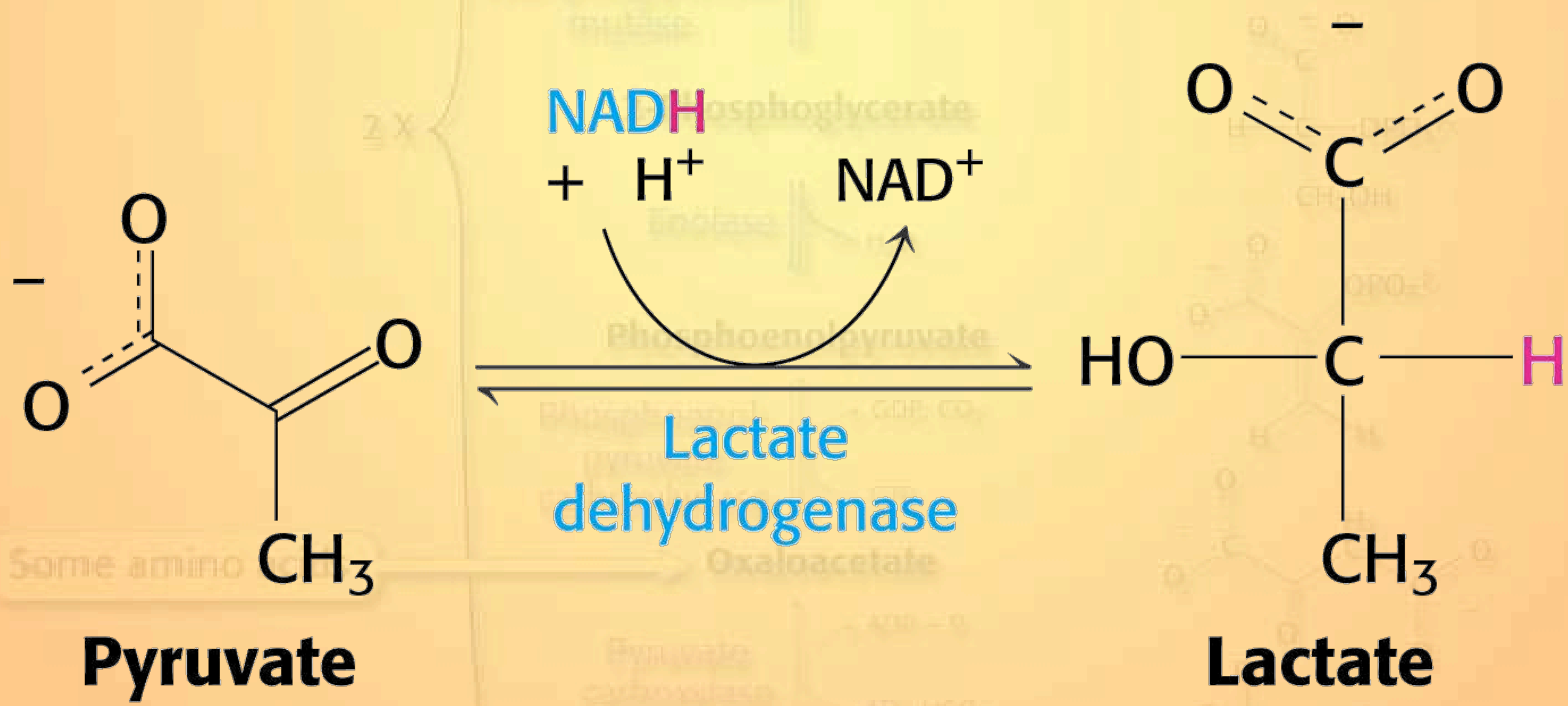
1.9.Maintaining Redox Balance

Ethanol fermentation is use by yeast and produces ethanol and CO_2.



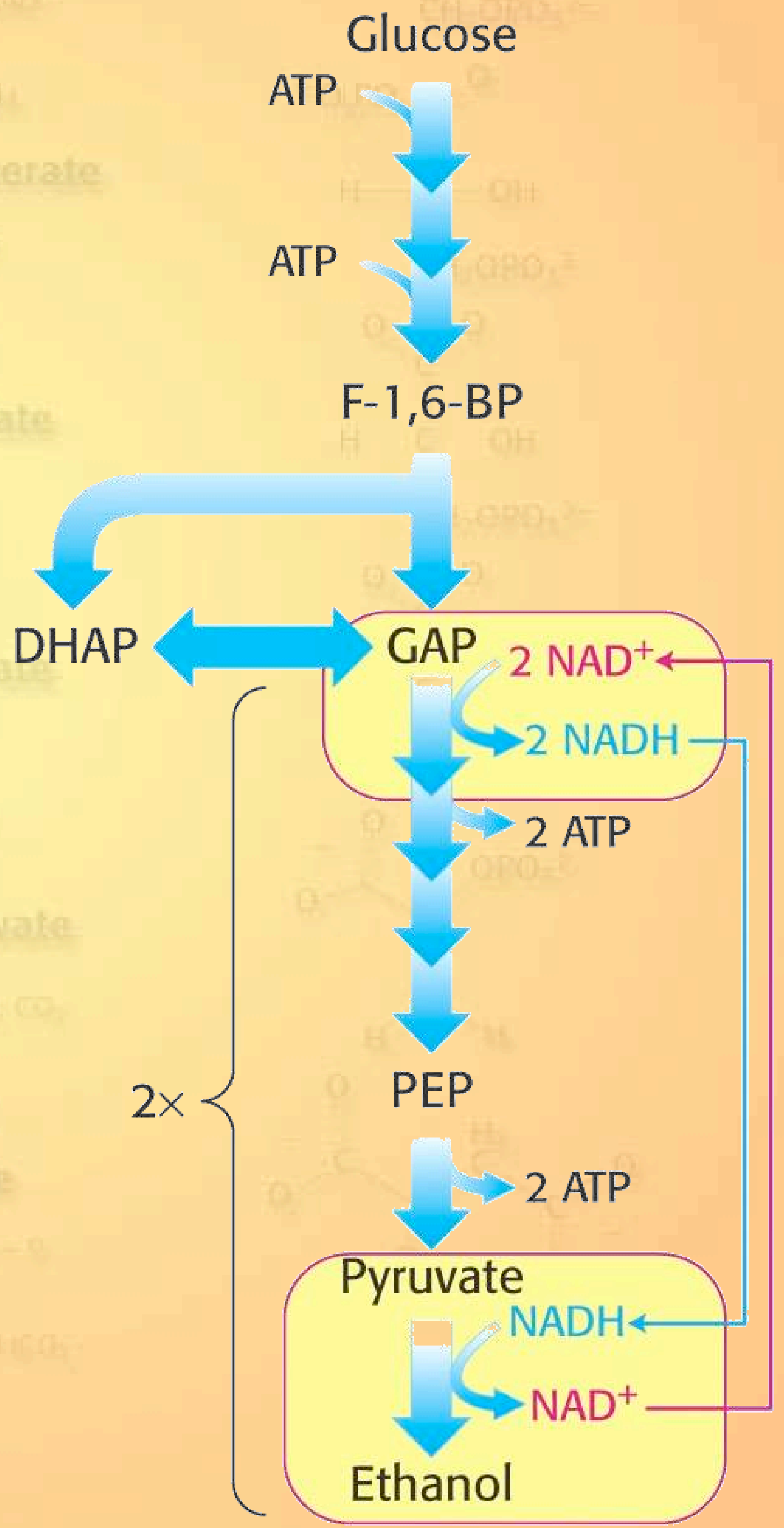
1.9.Maintaining Redox Balance

Lactic acid fermentation is use by bacteria and human muscles and produces lactate.



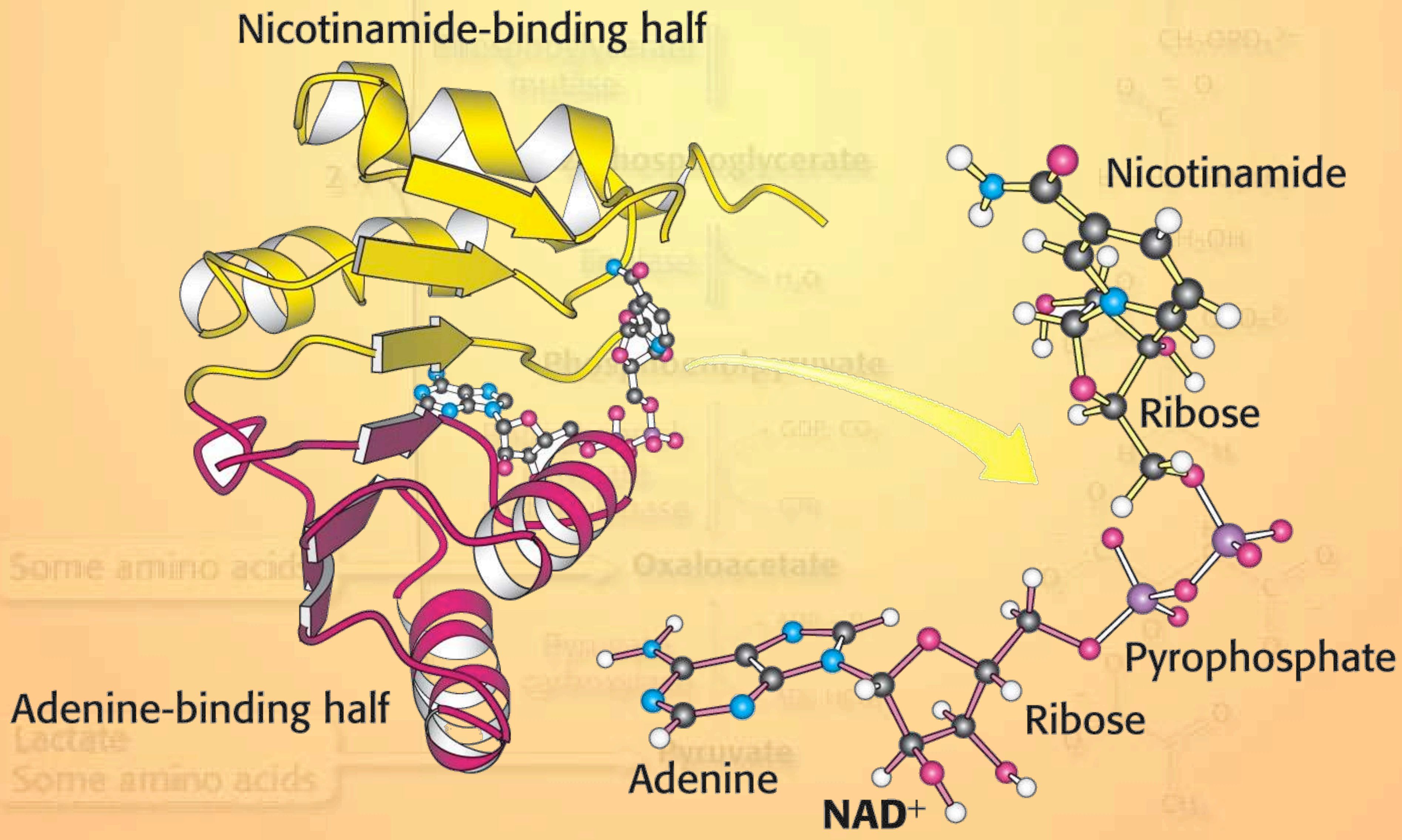
1.9.Maintaining Redox Balance

The fermentation pathways restore the redox balance:



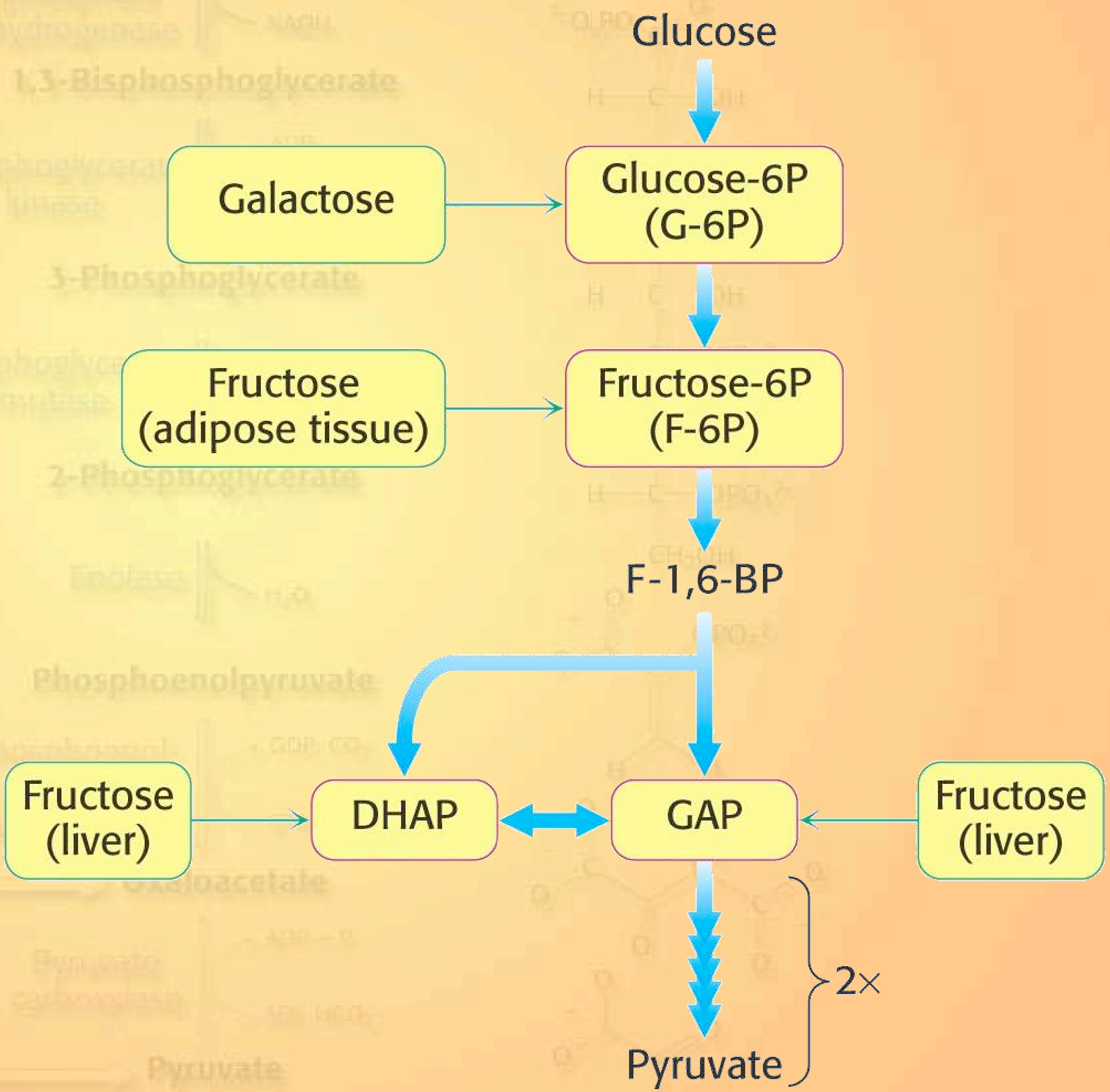
1.10 NAD^+ Binding

All three of the dehydrogenase in glycolysis and the fermentation pathways share a common domain for binding NAD^+.



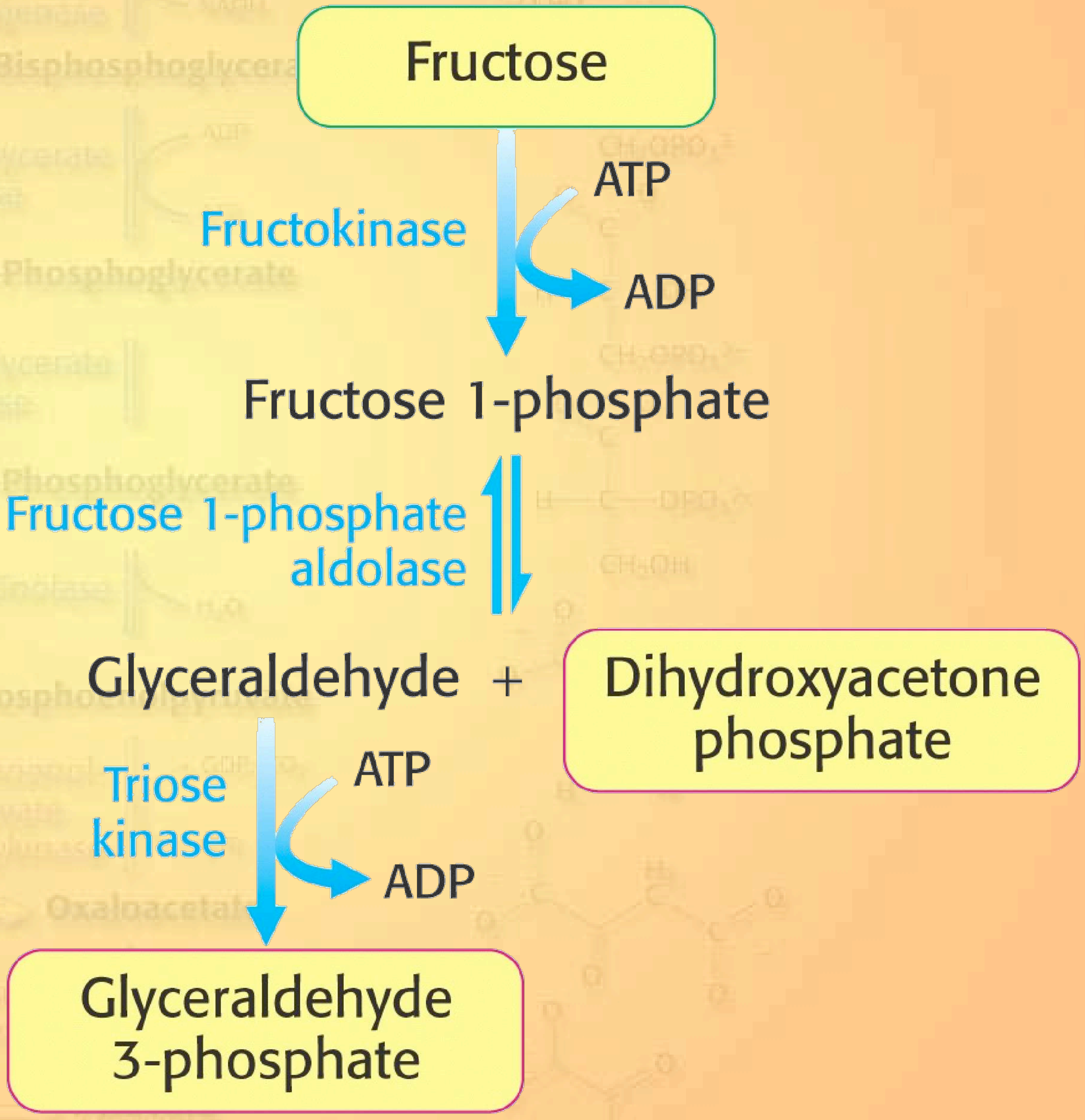
1.11 Other Points of Entry

The entry of fructose and galactose into glycolysis.



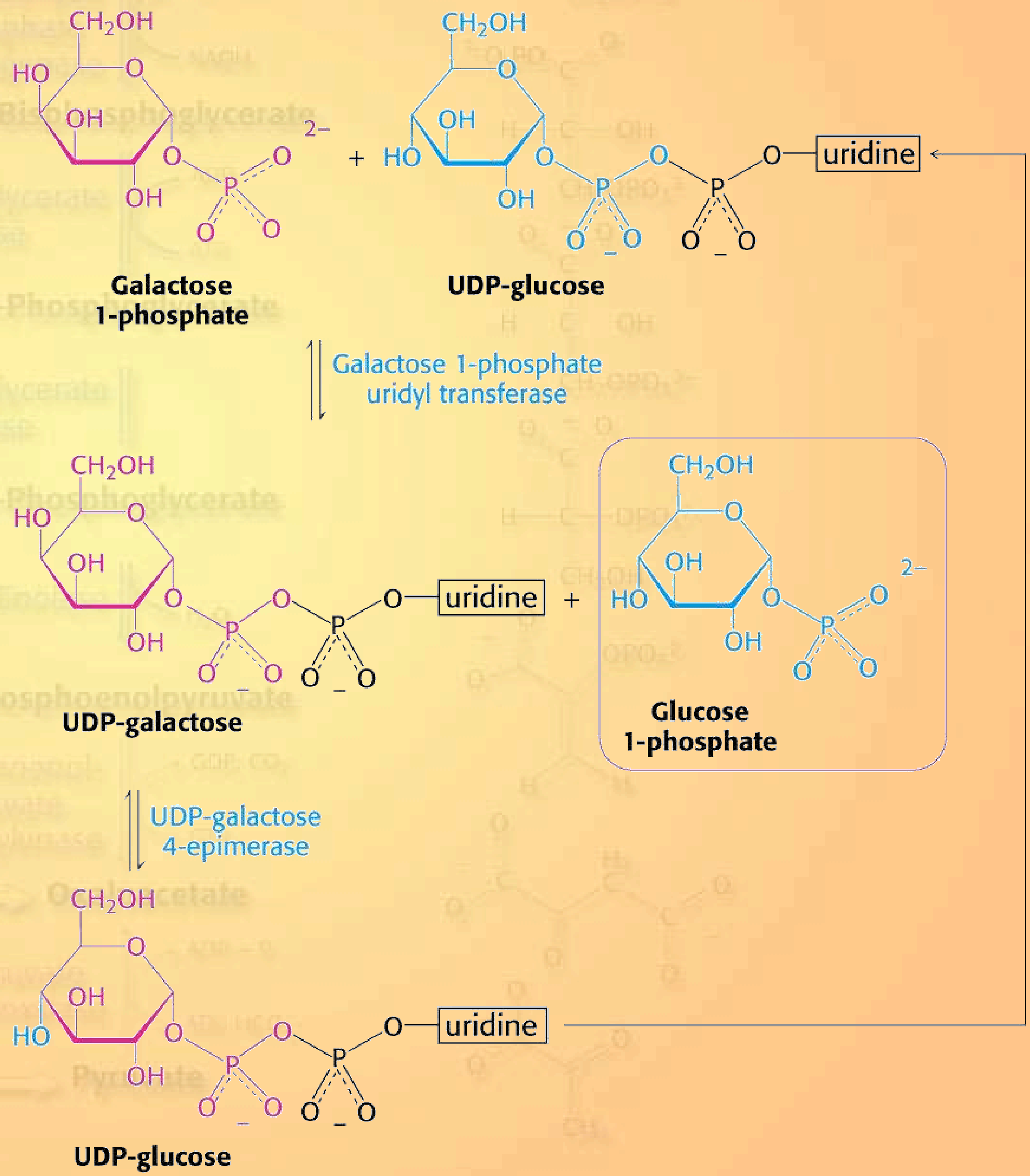
1.11 Other Points of Entry

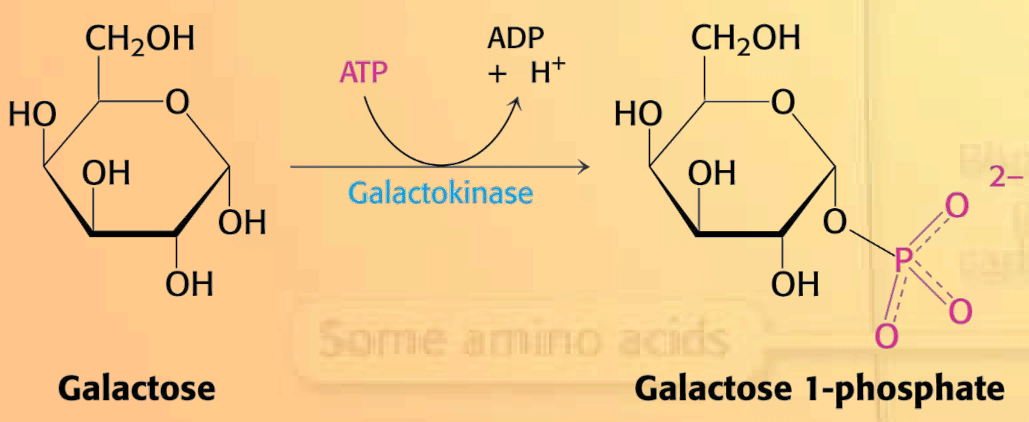
Fructose is phosphorylated to fructose 1-phosphate in the liver.



1.11 Other Points of Entry

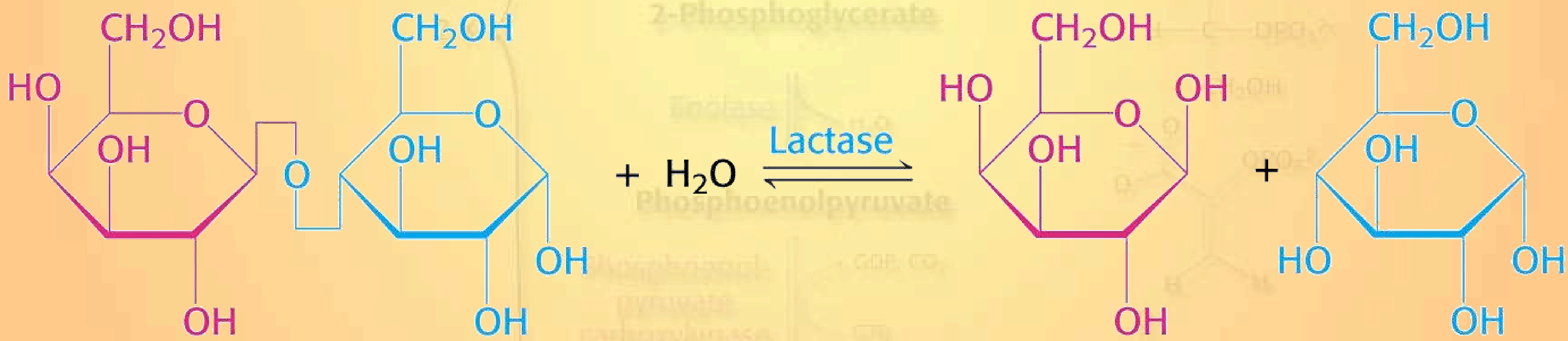
Galactose is urdidylated before epimerization to UDP-glucose:





1.12 Lactose Intolerance

Many adults are intolerant of milk because they are deficient in lactase



Lactose Galactose Glucose

1.13 Galactose is Highly Toxic

- Disruption of galactose metabolism is called *galactosemia*.
  - Usually due to loss of uridyl transferase activity
- Symptoms include
  - Failure to thrive infants
  - Enlarged liver and jaundice, sometimes cirrhosis
  - Cataracts
  - Mental retardation

2. Control of Glycolysis

Two major needs of the the cell influence the flow of material from glucose to pyruvate:

- The need for ATP (energy)
- The need for building blocks for biosynthesis

2. Control of Glycolysis

In metabolic pathways, control is focused on those steps in the pathway that are irreversible.

TABLE 16.3 Reactions of glycolysis

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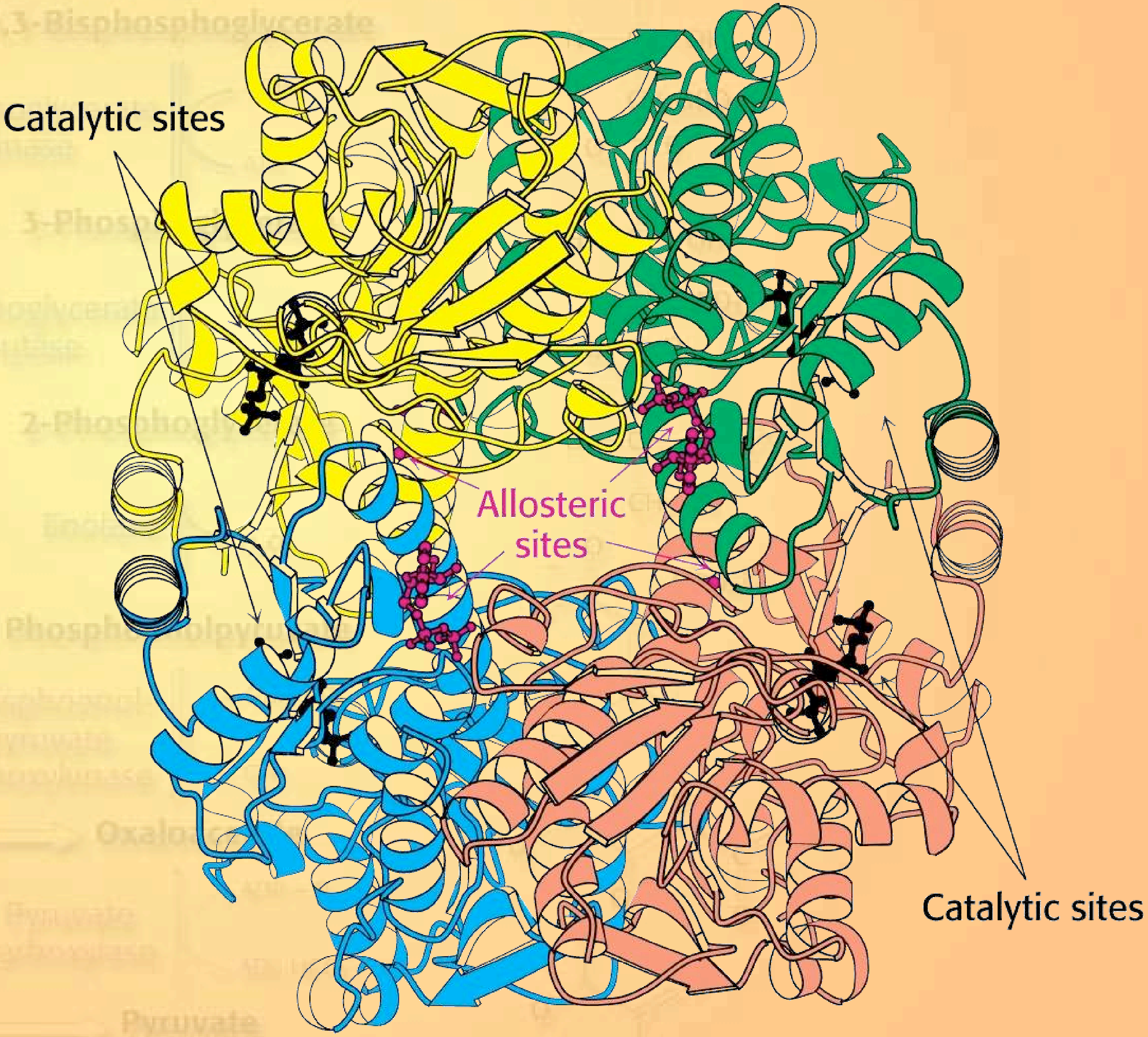
2. Control of Glycolysis

The different levels of control have different response times:

Level of Control	Response Time
Allosteric	milleseconds
Phosphorylation	seconds
Transcriptional	hours

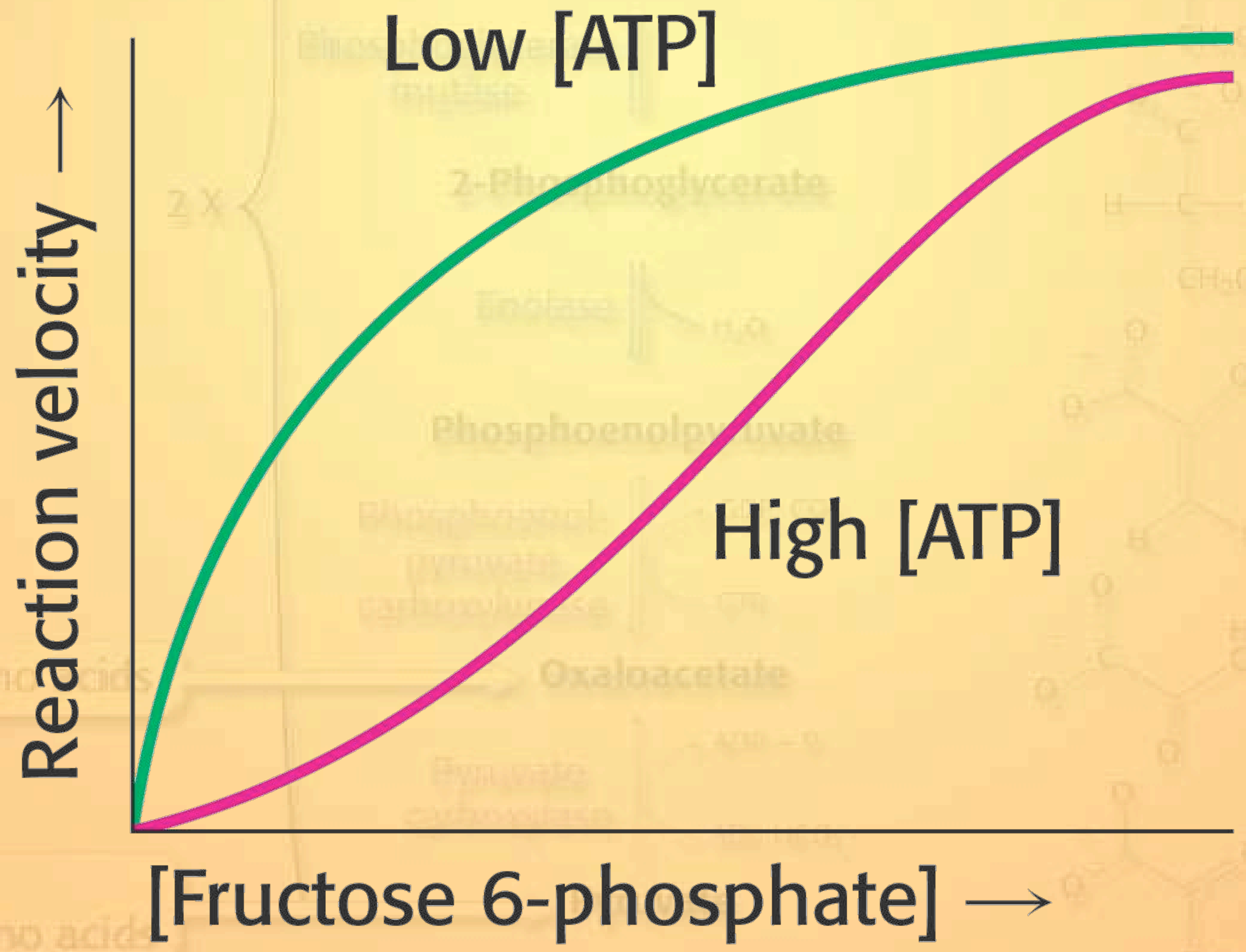
2.1 Phosphofructokinase

Phosphofructokinase is the key enzyme in the control of glycolysis.



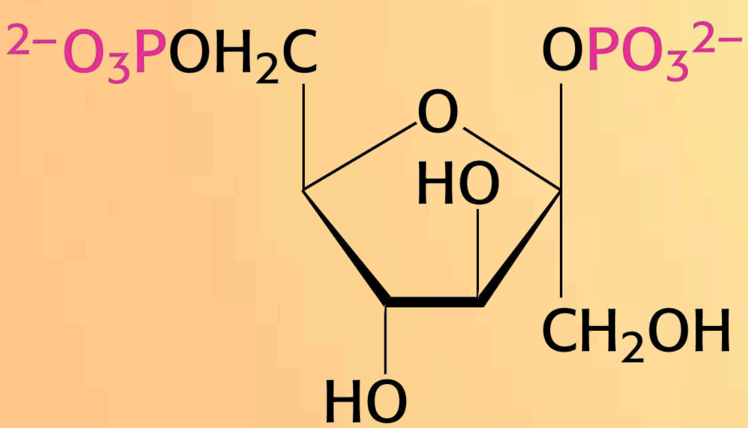
2.1 Phosphofructokinase

Allosteric regulation of phosphofructo-kinase by ATP

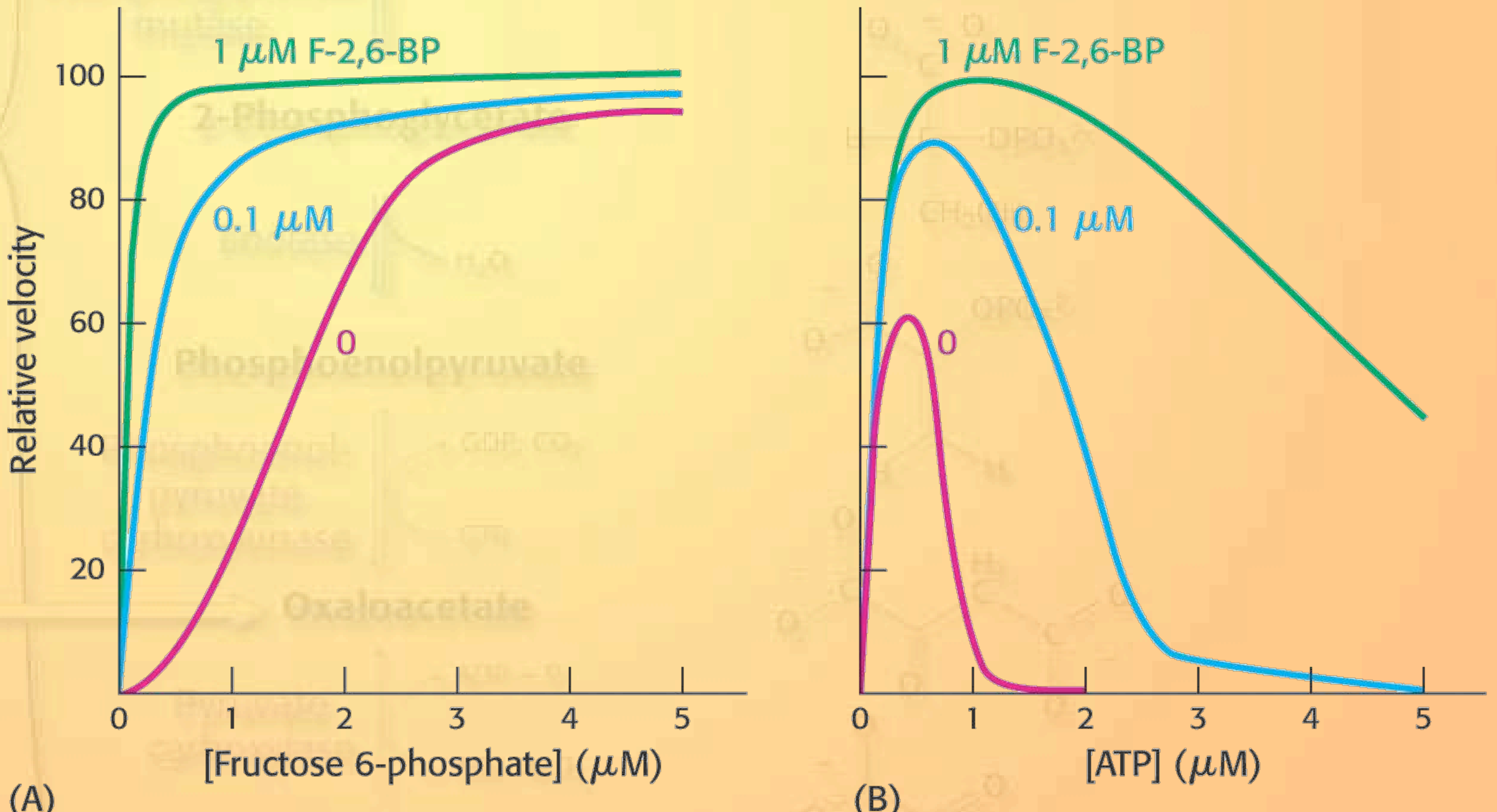


2.1 Phosphofructokinase

Phosphofructokinase is also regulated by fructose 2,6-bisphosphate:



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

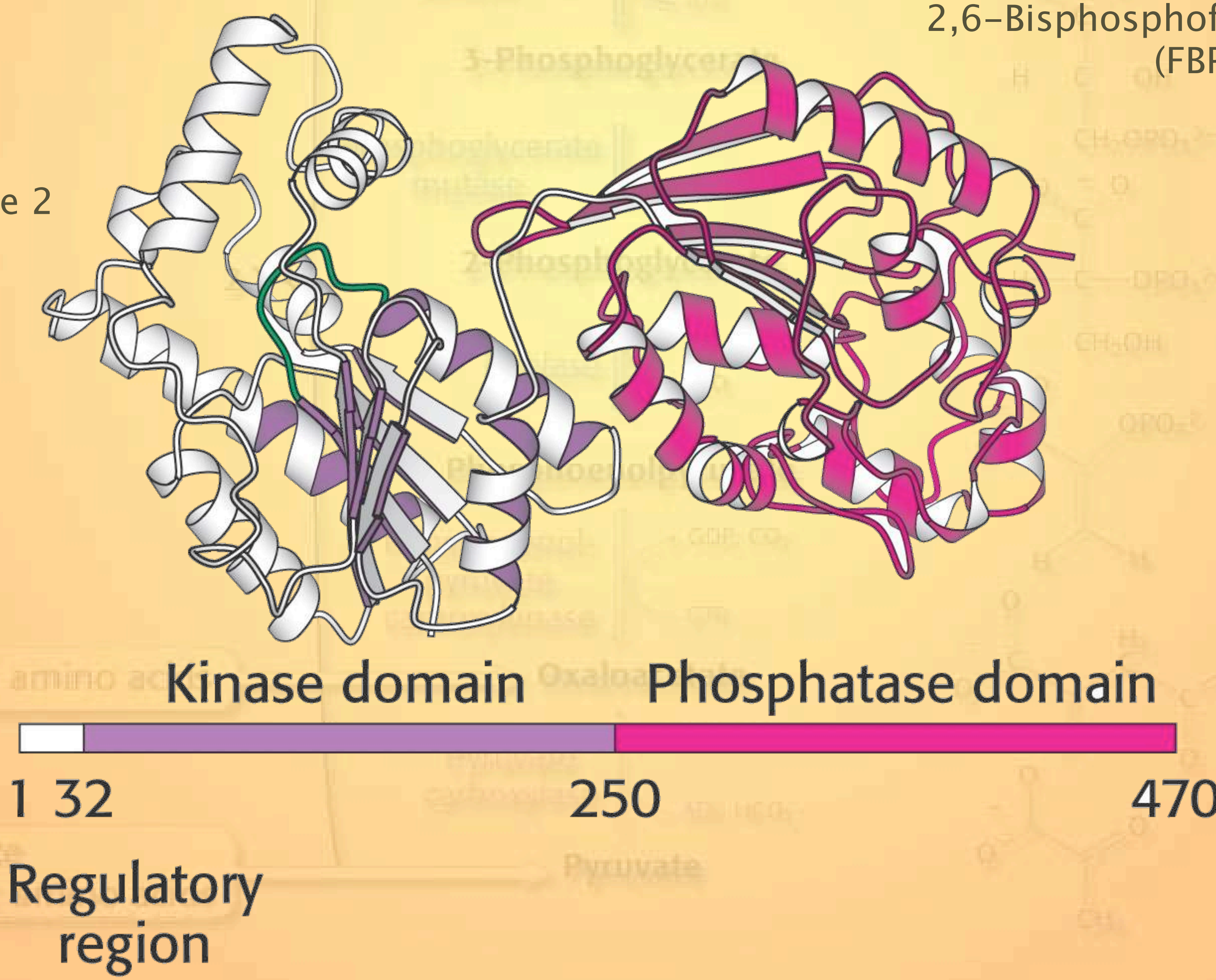


2.2. Fructose 2,6-bisphosphate

A regulated bifunctional enzyme synthesizes and degrades fructose 2,6-bisphosphate:

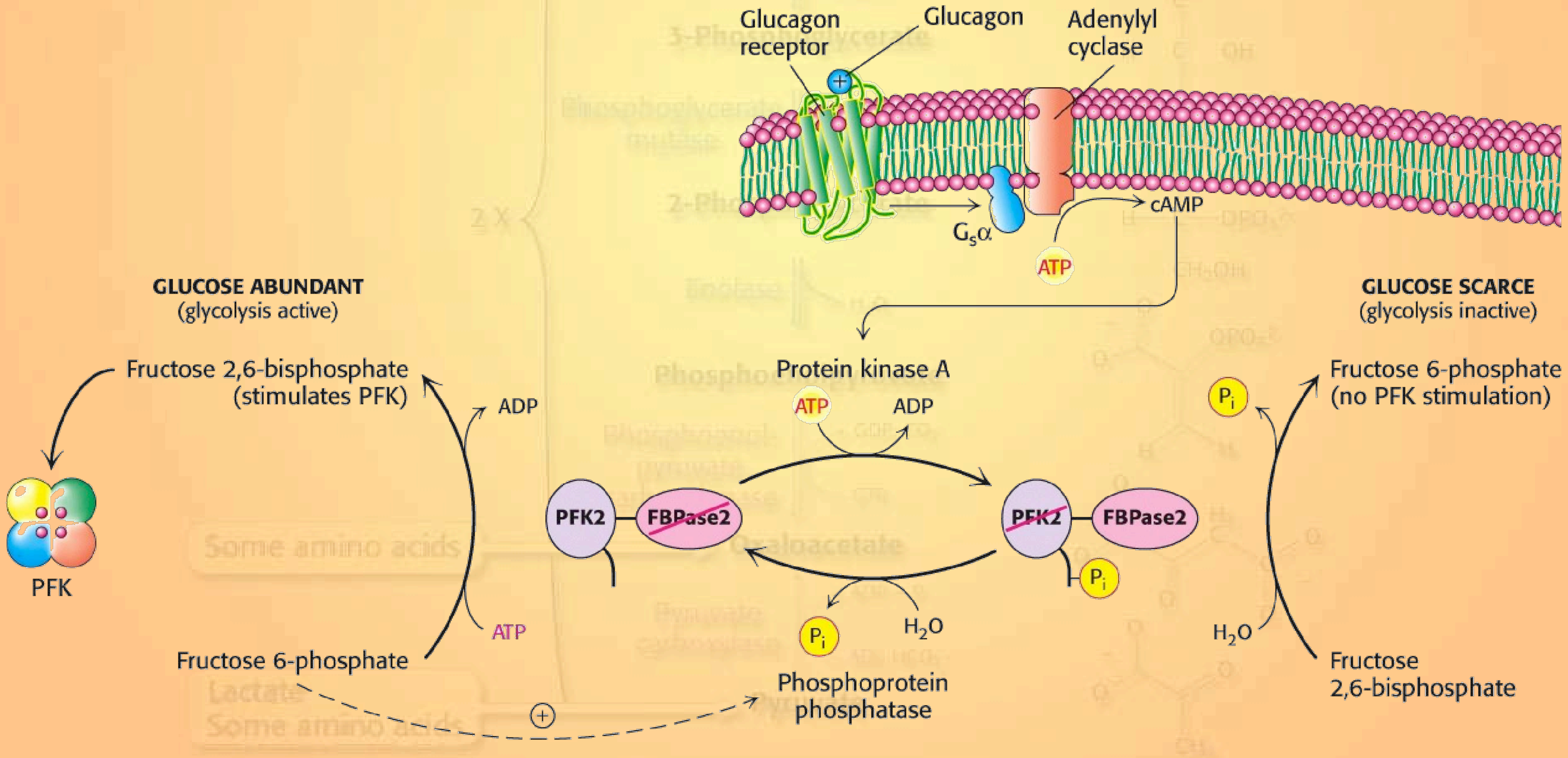
Phosphofructokinase 2 (PFK2)

2,6–Bisphosphofructophosphatase (FBPase2



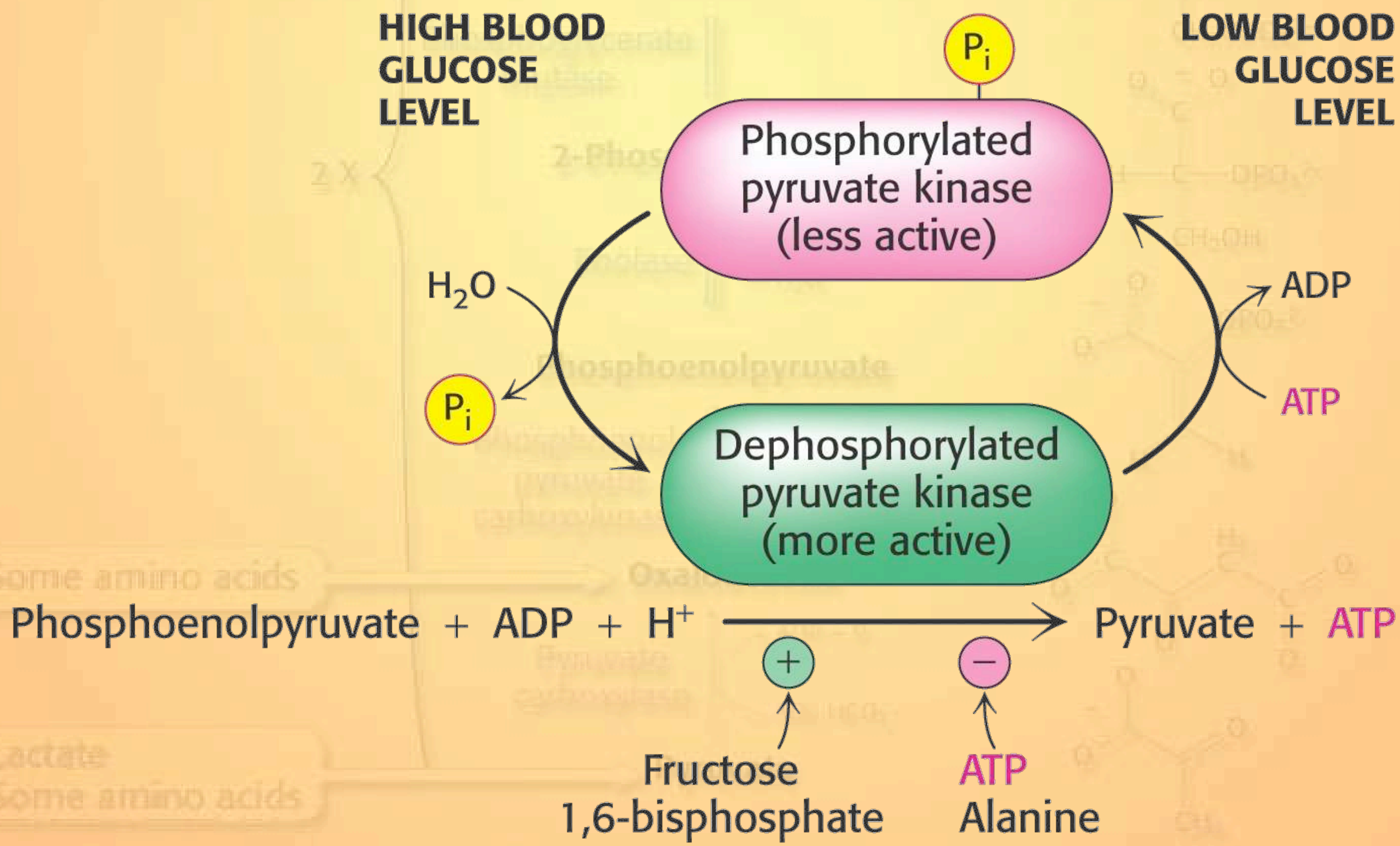
2.2. Fructose 2,6-bisphosphate

A regulated bifunctional enzyme synthesizes and degrades fructose 2,6-bisphosphate:



2.3 Hexokinase and Pyruvate Kinase

Hexokinase and pyruvate kinase also set the pace of glycolysis.



2.4. Glucose Transporters

A family of transporters enables glucose to enter and leave animal cells.

TABLE 16.4 Family of glucose transporters

Name	Tissue location	K_m	Comments
GLUT1	All mammalian tissues	1 mM	Basal glucose uptake
GLUT2	Liver and pancreatic β cells	15–20 mM	In the pancreas, plays a role in regulation of insulin In the liver, removes excess glucose from the blood
GLUT3	All mammalian tissues	1 mM	Basal glucose uptake
GLUT4	Muscle and fat cells	5 mM	Amount in muscle plasma membrane increases with endurance training
GLUT5	Small intestine	—	Primarily a fructose transporter

3. Gluconeogenesis

Glucose can be synthesized from noncarbohydrate precursors.

- The brain has a strong preference for glucose, while the red blood cells have and absolute requirement for glucose.
- The brain needs 120 g of glucose/day
- The liver has about a 190 g store of glucose as glycogen. (About a 1 day’s supply)
- Glucose can be synthesized in the liver from pyruvate, glycerol and amino acids.

3.1. Gluconeogenesis

Gluconeogenesis is not the reverse of glycolysis.

$$2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ H^{+} + 2\ H2O \rightarrow \text{Glucose} + 2\ \text{ADP} + 2\ \text{Pi} + 2\ NAD^{+}$$

- The ΔG°' for this reaction is +20 kcal/mol

3.1. Gluconeogenesis

The three kinase reactions are the ones with the greatest positive free energies in the reverse directions

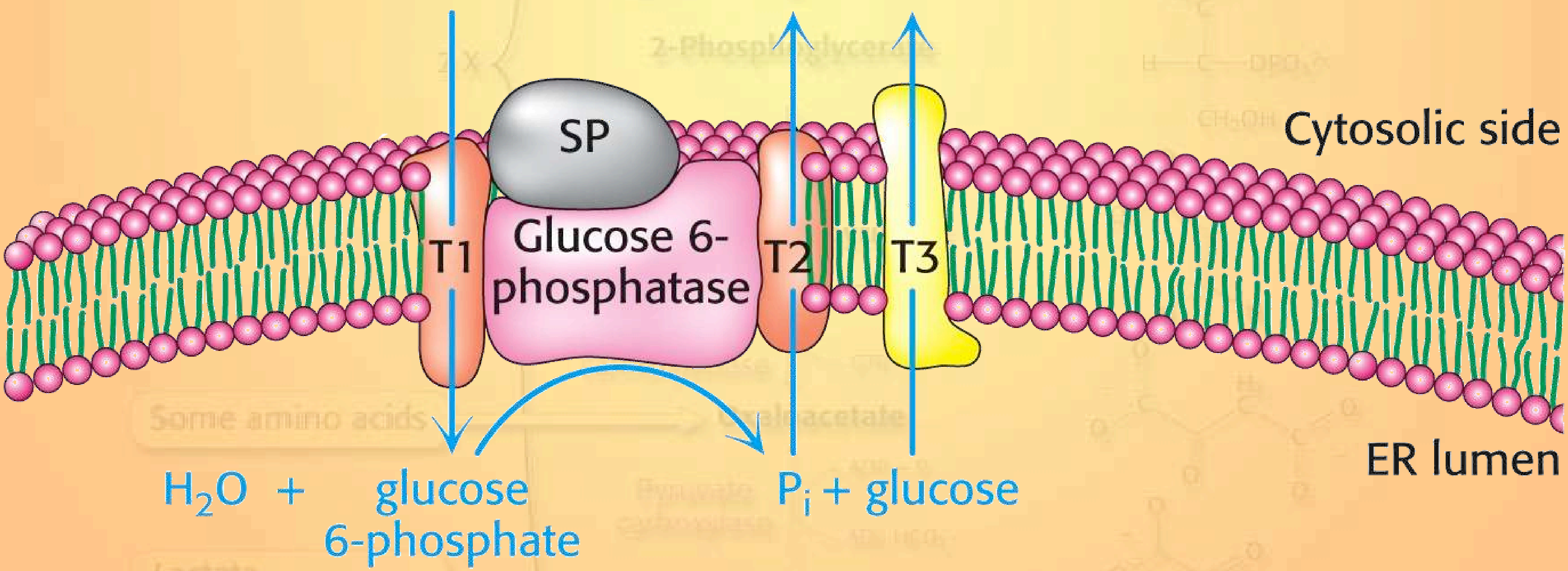
TABLE 16.3 Reactions of glycolysis

Step	Reaction	Enzyme	Reaction type	$\Delta G^{\circ\prime}$ in kcal mol^{-1} (kJ mol^{-1})	ΔG in kcal mol^{-1} (kJ mol^{-1})
1	Glucose + ATP $\longrightarrow$ glucose 6-phosphate + ADP + H^+	Hexokinase	Phosphoryl transfer	−4.0 (−16.7)	−8.0 (−33.5)
2	Glucose 6-phosphate $\rightleftharpoons$ fructose 6-phosphate	Phosphoglucose isomerase	Isomerization	+0.4 (+1.7)	−0.6 (−2.5)
3	Fructose 6-phosphate + ATP $\longrightarrow$ fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	Phosphoryl transfer	−3.4 (−14.2)	−5.3 (−22.2)
4	Fructose 1,6-bisphosphate $\rightleftharpoons$ dihydroxyacetonephosphate + glyceraldehyde 3-phosphate	Aldolase	Aldol cleavage	+5.7 (+23.8)	−0.3 (−1.3)
5	Dihydroxyacetone phosphate $\rightleftharpoons$ glyceraldehyde 3-phosphate	Triose phosphate isomerase	Isomerization	+1.8 (+7.5)	+0.6 (+2.5)
6	Glyceraldehyde 3-phosphate + P_i + NAD^+ $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	Phosphorylation coupled to oxidation	+1.5 (+6.3)	+0.6 (+2.5)
7	1,3-Bisphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	Phosphoryl transfer	−4.5 (−18.8)	+0.3 (+1.3)
8	3-Phosphoglycerate $\rightleftharpoons$ 2-phosphoglycerate	Phosphoglycerate mutase	Phosphoryl shift	+1.1 (+4.6)	+0.2 (+0.8)
9	2-Phosphoglycerate $\rightleftharpoons$ phosphoenolpyruvate + H_2O	Enolase	Dehydration	+0.4 (+1.7)	−0.8 (−3.3)
10	Phosphoenolpyruvate + ADP + H^+ $\longrightarrow$ pyruvate + ATP	Pyruvate kinase	Phosphoryl transfer	−7.5 (−31.4)	−4.0 (−16.7)

Note: ΔG, the actual free-energy change, has been calculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiologic conditions. Glycolysis can proceed only if the ΔG values of all reactions are negative. The small positive ΔG values of three of the above reactions indicate that the concentrations of metabolites in vivo in cells undergoing glycolysis are not precisely known.

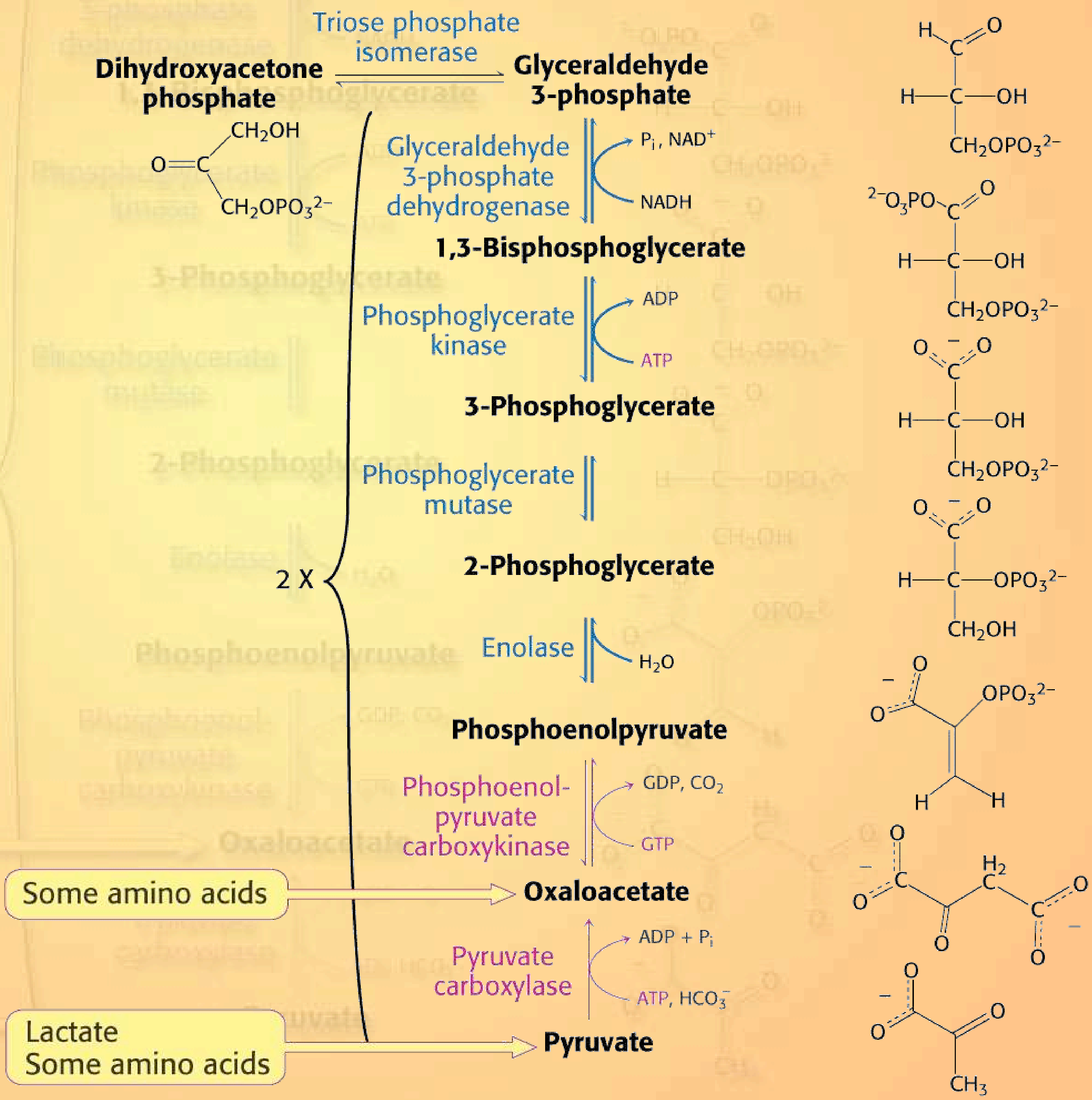
3.1. Gluconeogenesis

The hexokinase and phosphofructokinase reactions can be reversed simply with a phosphatase



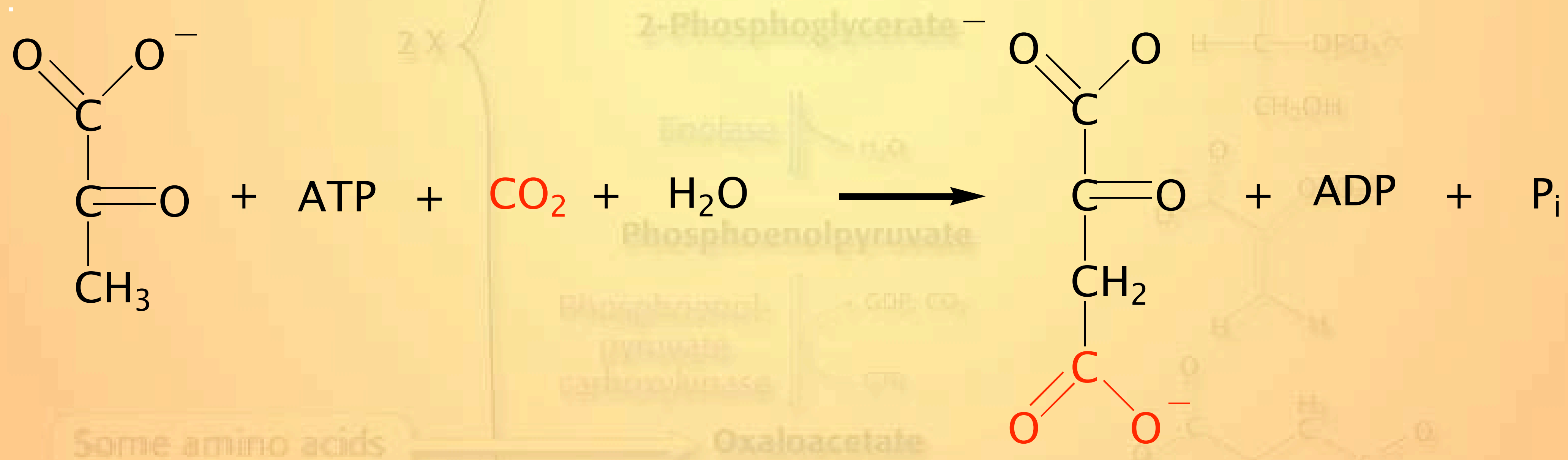
3.2. Formation of Phosphoenopyruvate

Reversing the pyruvate kinase reaction is not as easily done



3.2. Formation of Phosphoenopyruvate

The conversion of pyruvate into phosphoenolpyruvate begins with the formation of oxaloacetate.

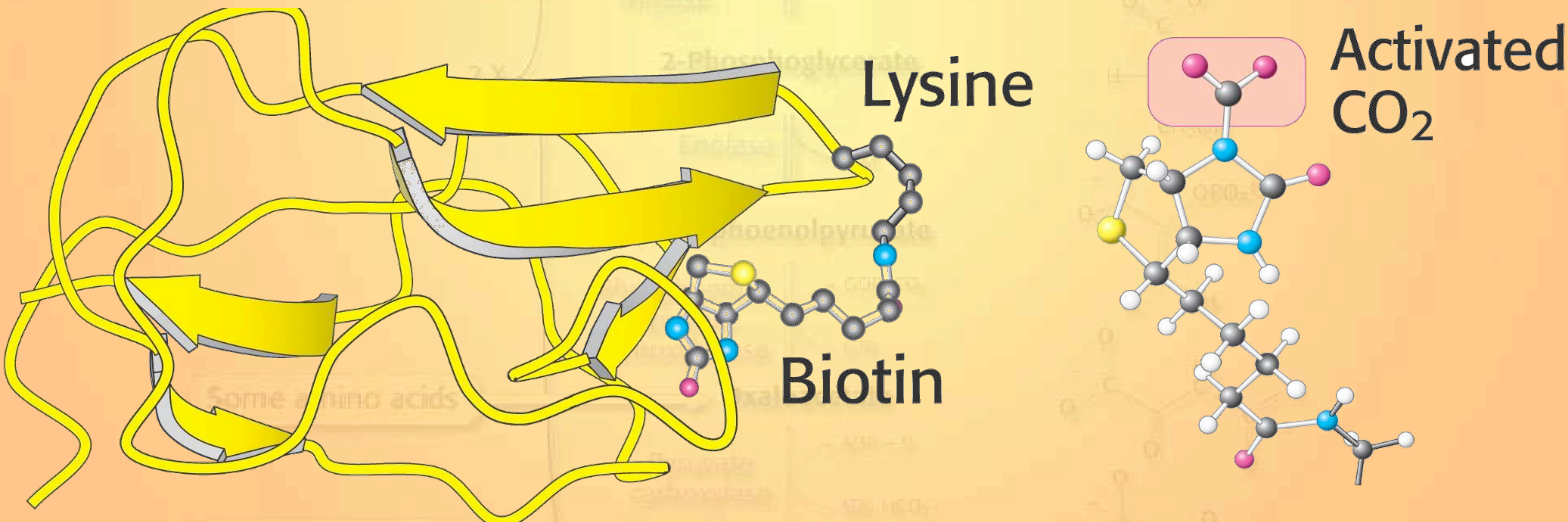


Pyruvate

Oxaloacetate

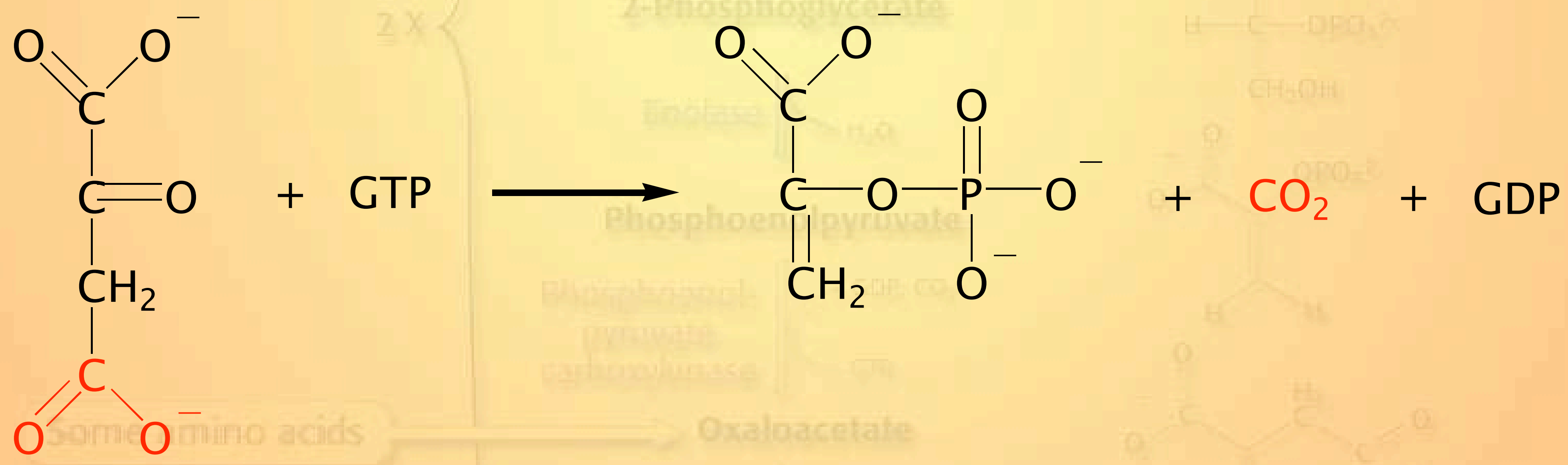
3.2. Formation of Phosphoenolpyruvate

Pyruvate kinase uses the biotin cofactor to activate the CO_2



3.2. Formation of Phosphoenolpyruvate

The formation of phosphoenolpyruvate from oxaloacetate is driven both by the hydrolysis of GTP and a decarboxylation

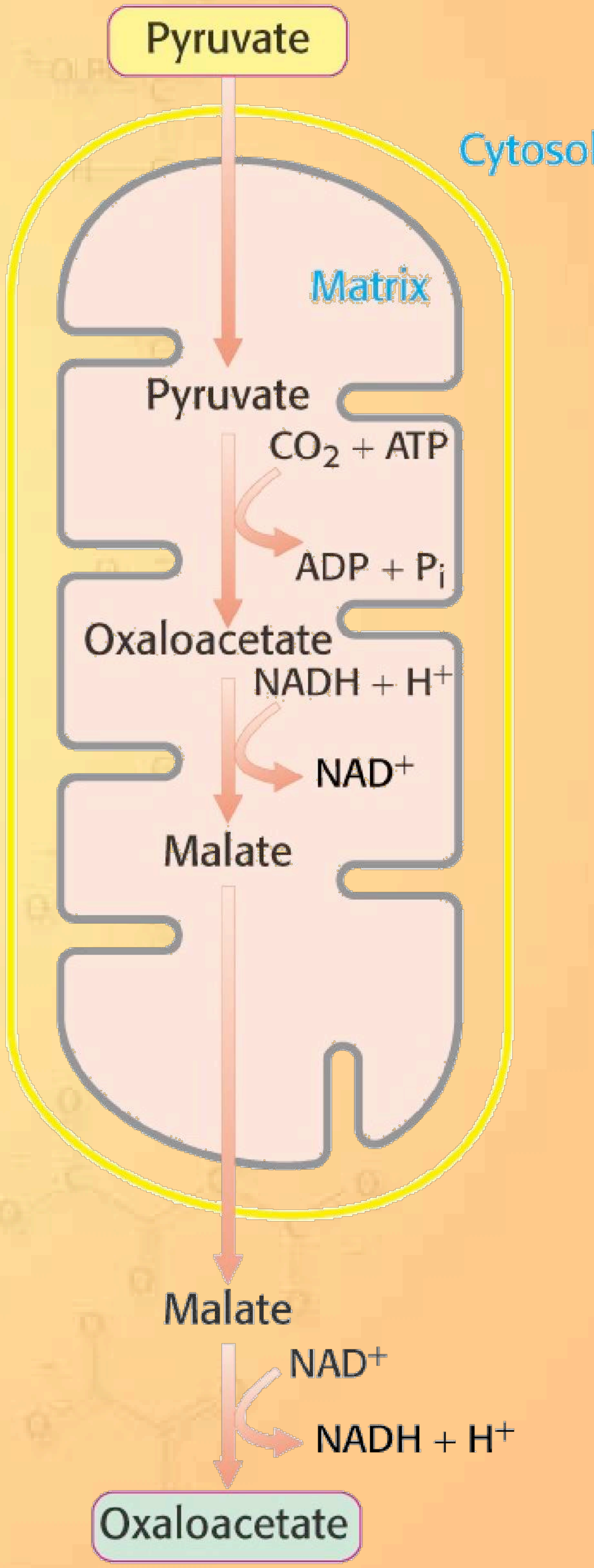


Oxaloacetate

Phosphoenolpyruvate

3.3. Oxaloacetate Shuttle

Oxaloacetate is synthesized in the mitochondria and is shuttled into the cytosol where it is converted into phosphoenolpyruvate



3.6. "High-Energy" Phosphate Bonds

Six high-energy phosphate bonds are spent in synthesizing glucose from pyruvate.

Gluconeogenesis:

2 Pyruvate + 4 ATP + 2GTP + 2 NADH + 2 H^+ + 6 H2O →
Glucose + 4 ADP + 2 GDP + 6 Pi + 2 NAD^+

ΔG°' = −9 kcal/mol

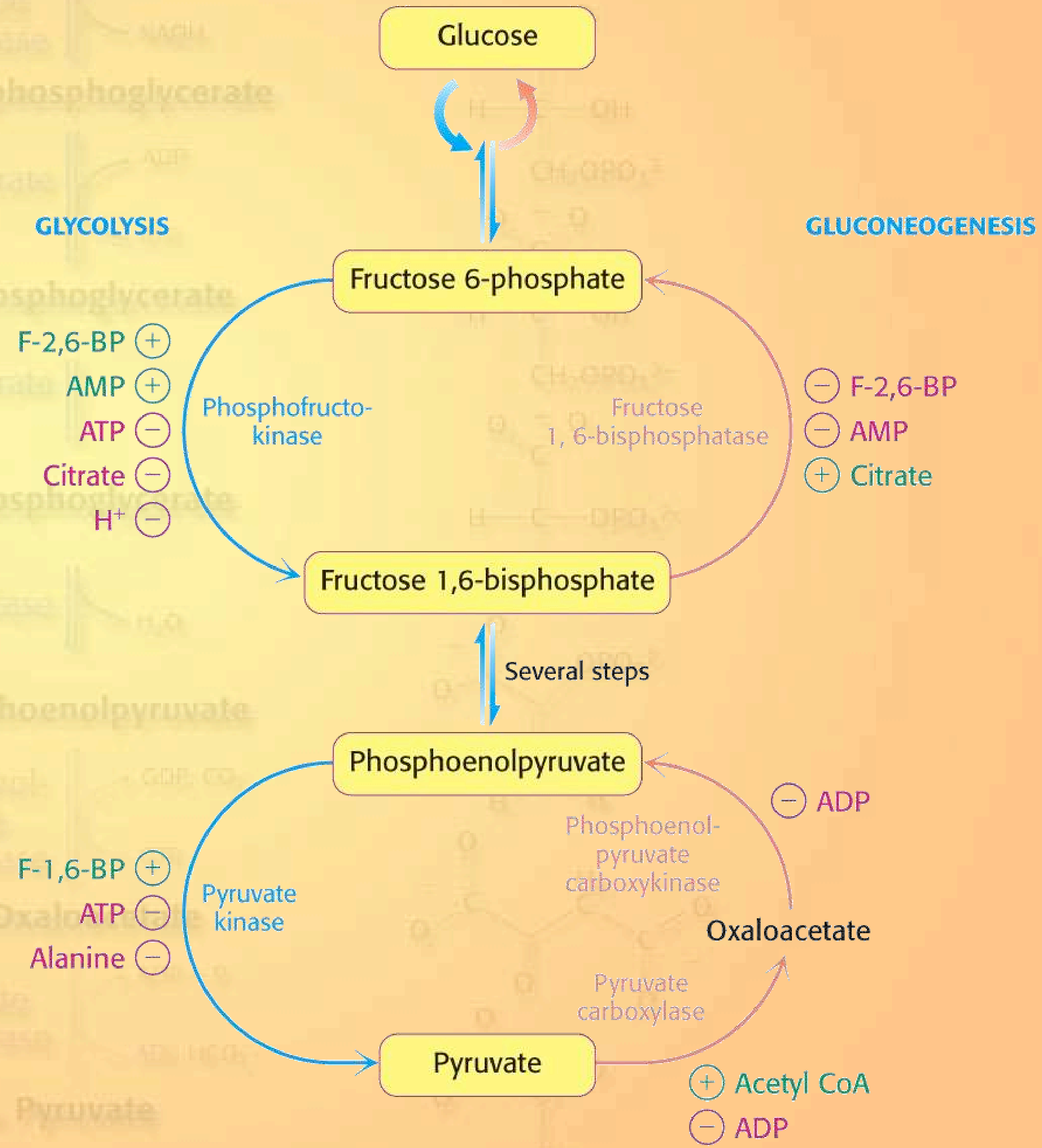
Reverse of Glycolysis:

2 Pyruvate + 2 ATP + 2 NADH + 2 H^+ + 2 H2O →
Glucose + 2 ADP + 2 Pi + 2 NAD^+

ΔG°' = +20 kcal/mol

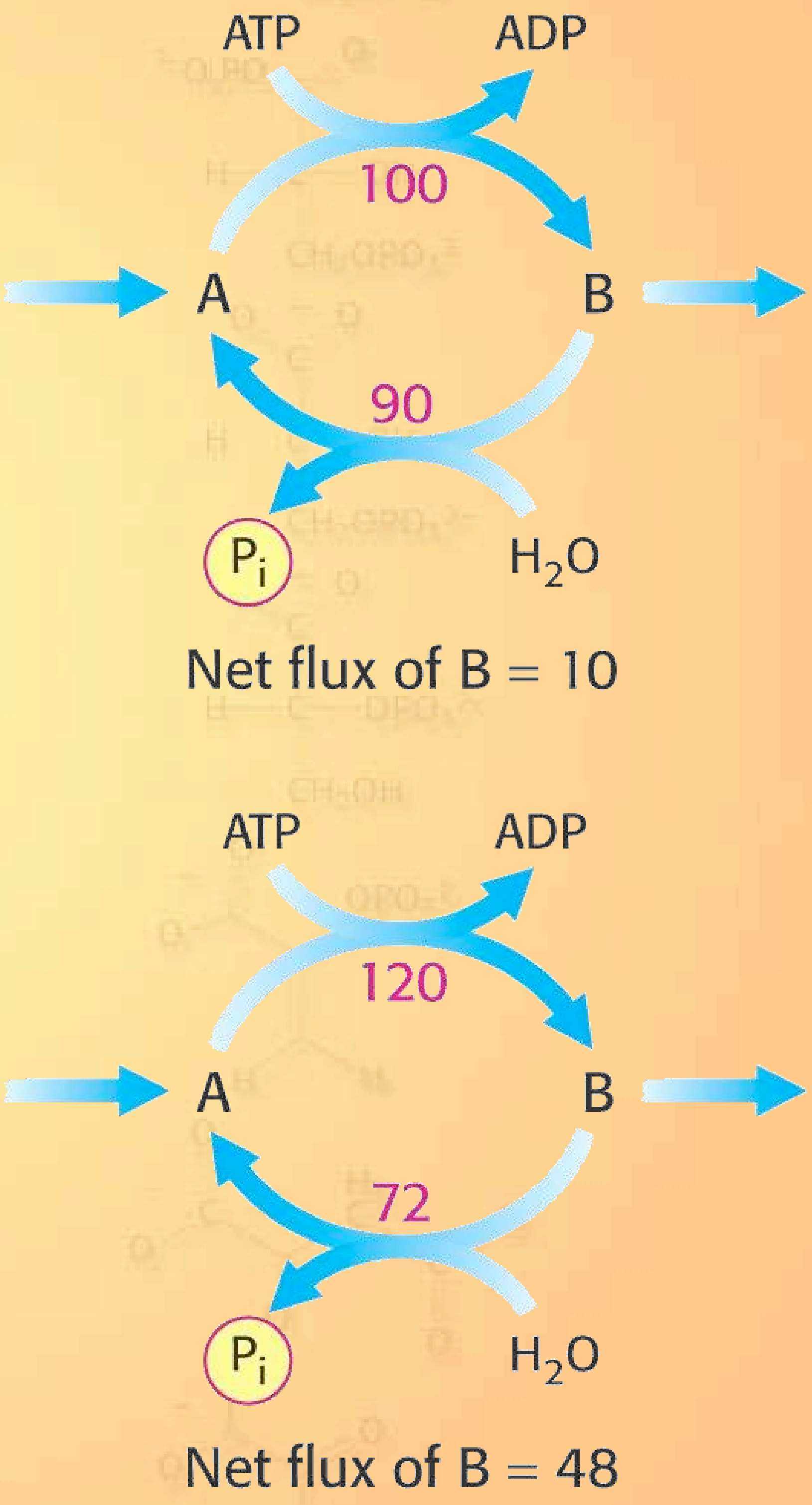
4. Regulation of Glycolysis and Gluconeogenesis

Reciprocal regulation of glycolysis and gluconeogenesis in the liver



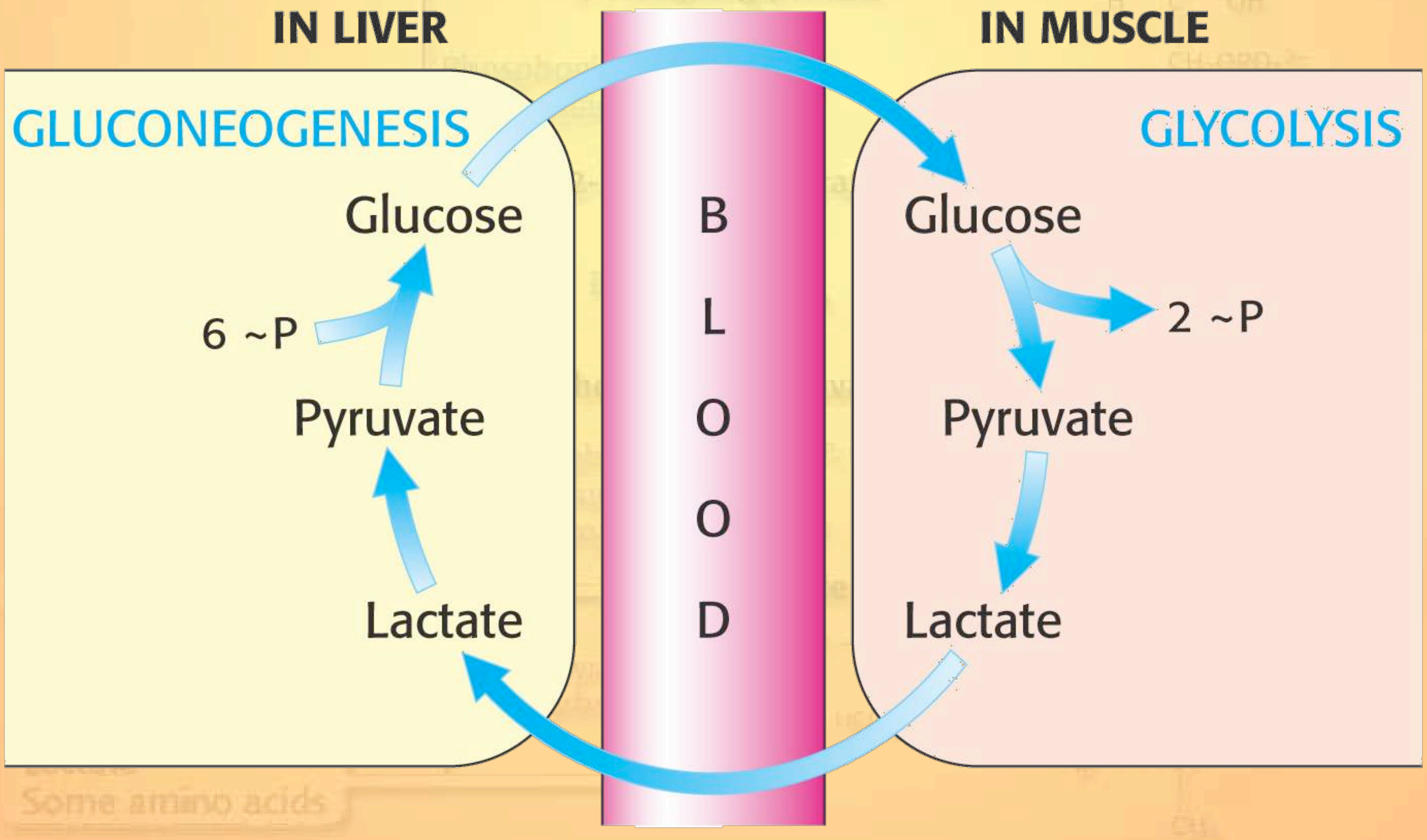
4.1. Substrate Cycles

Substrate cycles amplify metabolic signals and produce heat.



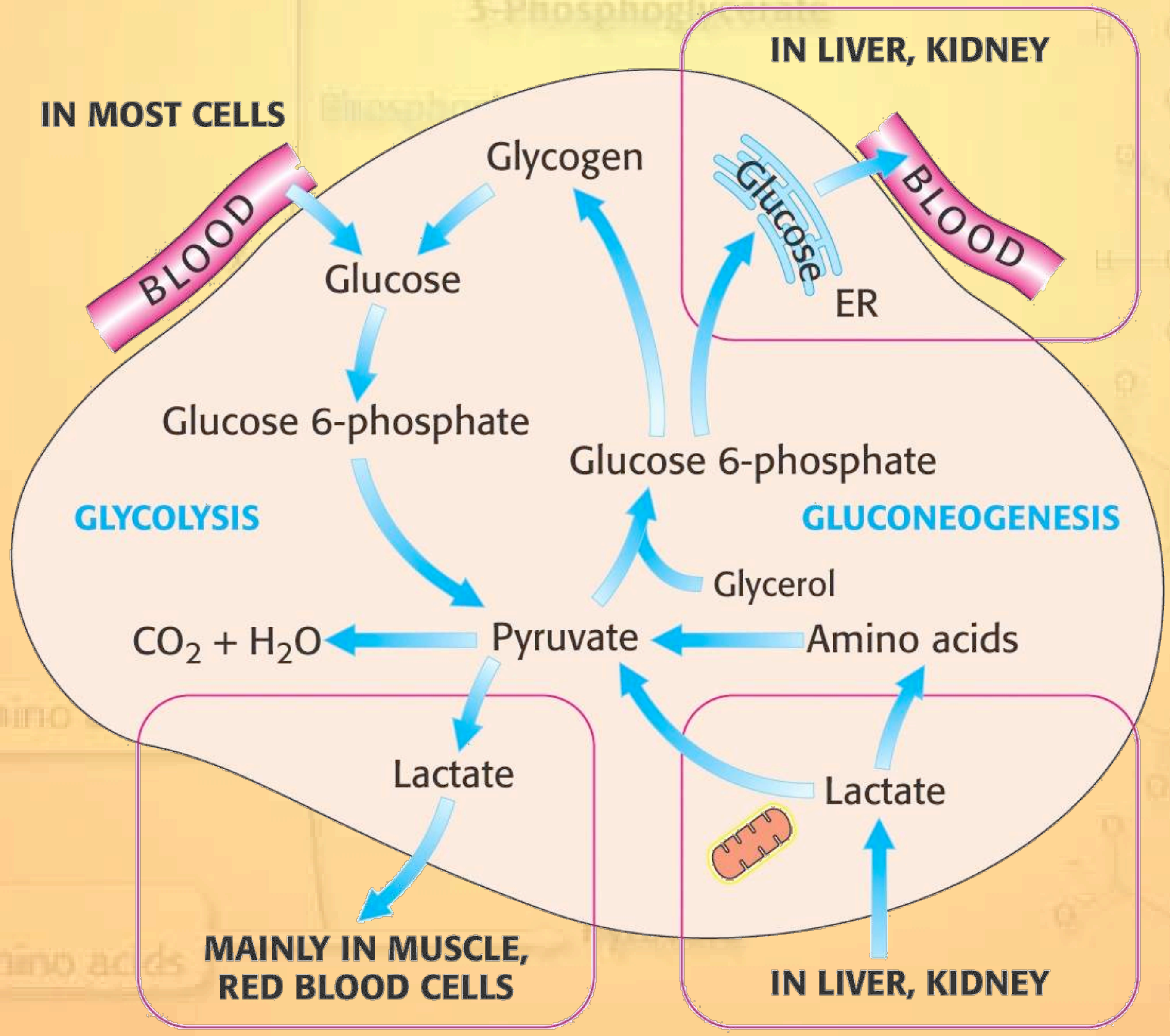
4.2. Lactate and Alanine

Lactate and alanine formed by contracting muscle are used by other organs



4.2. Lactate and Alanine

Lactate and alanine formed by contracting muscle are used by other organs



4.3. Evolution of Glycolysis and Gluconeogenesis

- Glycolysis and Gluconeogenesis are evolutionarily intertwined.