Basic concept and design of metabolism

The glycolytic pathway
Oxidative decarboxylation of pyruvate
and of other 2-oxocarboxylic acids

Biochemistry I
Lecture 3

2009 (J.S.)

Living organisms require a **continual input of free energy** for three major purposes:

- the performance of **mechanical work** in cellular movements,
- the **active transport of molecules and ions** across membranes,
- the **synthesis of macromolecules and other biomolecules** from simple precursors.

The free energy used in these processes, which maintain an organism in a state that is far from equilibrium, is **derived from the environment**.

Metabolism **is essentially a series of chemical reactions that provides energy transformations: Energy is being extracted from fuels (nutriments) and used to power biosynthetic processes.**

Catabolism (catabolic reactions) converts chemical energy by decomposing foodstuffs into biologically useful forms.

Anabolism (anabolic reactions) requires energy – useful forms of energy are employed to generate complex structures from simple ones, or energy-rich states from energy-poor ones.

Types of chemical reactions in metabolism

Type of reaction	Description
Oxidation–reduction	Electron transfer
Ligation requiring ATP cleavage	Formation of covalent bonds (i.e., carbon–carbon bonds)
Isomerization	Rearrangement of atoms to form isomers
Group transfer	Transfer of a functional group from one molecule to another
Hydrolytic	Cleavage of bonds by the addition of water
Addition or removal of functional groups	Addition of functional groups to double bonds or their removal to form double bonds

Reactions can occur spontaneously only if they are **exergonic** (if ΔG, the change in free energy, is negative).

The Gibbs free-energy change ΔG

The maximal amount of useful energy that can be gained in the reaction (at constant temperature and pressure).

$$a\,A + b\,B \rightarrow c\,C + d\,D \qquad \Delta G = G_{A+B} - G_{C+D}$$

$$\boxed{\Delta G = \Delta G^{o} + RT\,ln\,\frac{[C]^{c}\,[D]^{d}}{[A]^{a}\,[B]^{b}}}$$

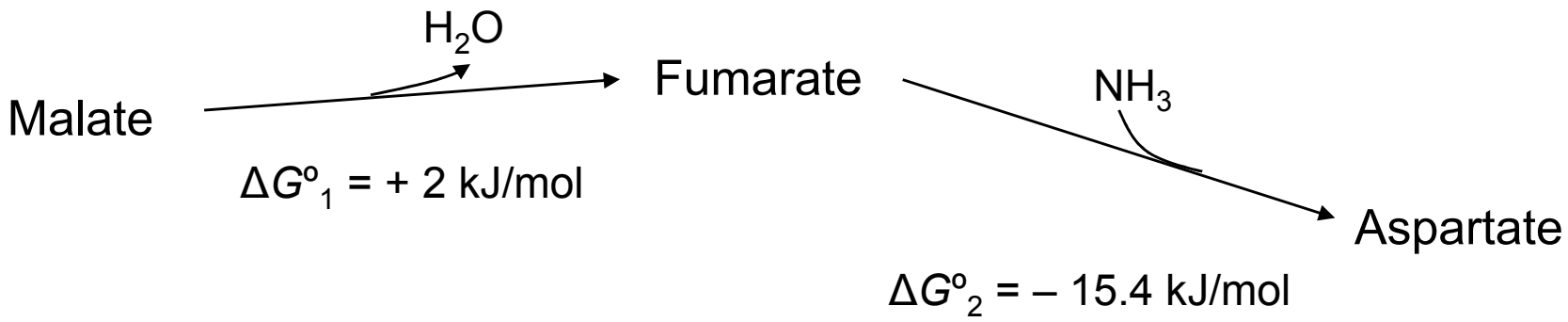
$$\Delta G^{o} = -\,RT\,ln\,K$$

The ΔG of a reaction depends on the **nature** of the reactants (expressed by the ΔG^{o} term) and on their **concentrations** (expressed by the second term).

An endergonic reaction cannot proceed spontaneously, but such a thermodynamically unfavourable reaction can be driven by an exergonic reaction to which it is coupled.

Energetic coupling occurs because the two reactions share **a common reactant or intermediate**.

Example:



The overall net free energy change is negative (ΔG° = – 13.4 kJ/mol), the conversion of malate to aspartate is exergonic.

The reaction which is used to drive endergonic ones is very oft the hydrolysis of ATP.

Example:

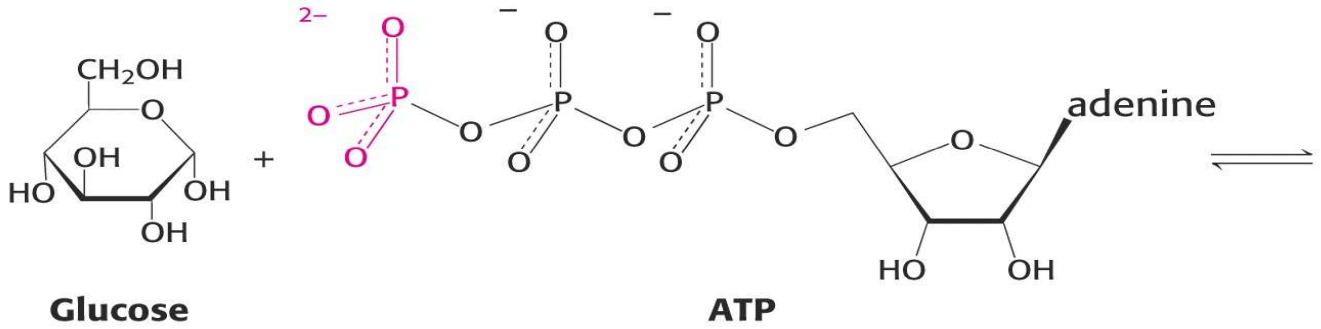
Glucose → Glucose 6-phosphate

ATP → ADP

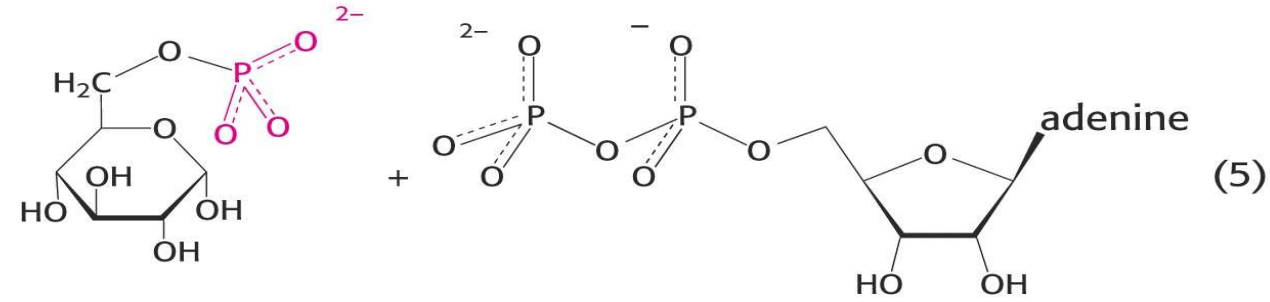
$\Delta G^{o\prime} = +\ 13.8\ \text{kJ mol}^{-1}$

$\Delta G^{o\prime} = -\ 30.5\ \text{kJ mol}^{-1}$

$= -\ 16.7\ \text{kJ mol}^{-1}$



Glucose **ATP**

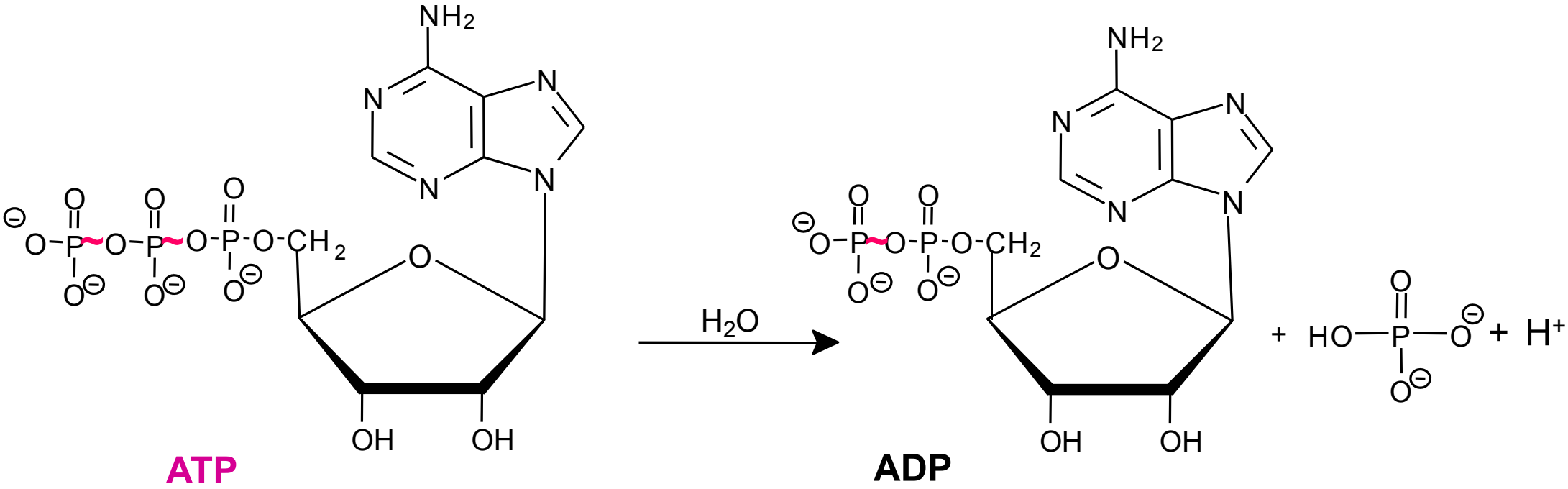


Glucose 6-phosphate (G-6P) **ADP**

(5)

Adenosine triphosphate (ATP)

is a high-energy compound that serves as the "universal currency" of free energy in biological systems. ATP hydrolysis drives metabolism by shifting the equilibrium of coupled reactions.



ATP + H_2O ⟶ ADP + Pi $\quad\quad$ $\Delta G^{\circ\prime}$ (at pH 7) **= – 30,5 kJ mol^{-1}**

The metabolic interplay of living organisms in our biosphere

Living organisms can be divided into two large groups according to the chemical form of carbon they require from the environment.

Autotrophic cells ("self-feeding" cells)

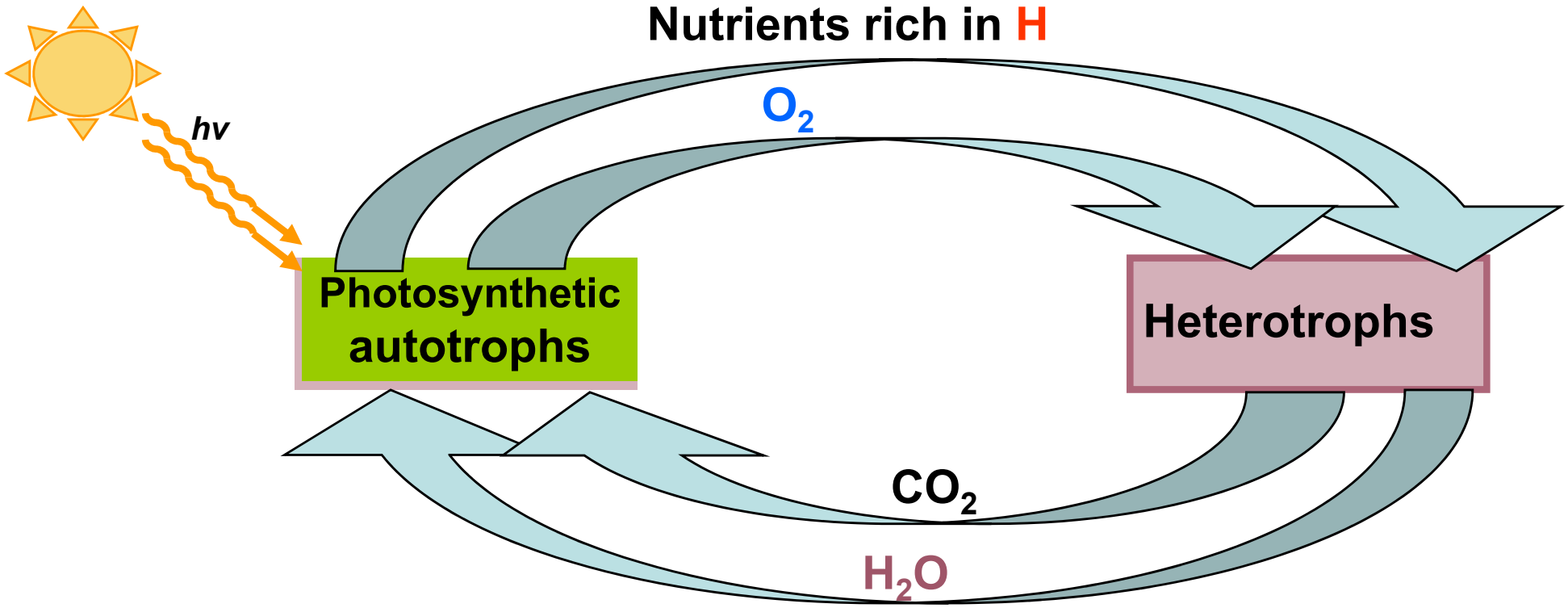
– **green leaf cells of plants** and photosynthetic bacteria – **utilize CO_2** from the atmosphere as the sole source of carbon for construction of all their carbon-containing biomolecules.

They absorb **radiant energy of the sun.** The synthesis of organic compounds is essentially the **reduction (hydrogenation) of CO_2** by means of hydrogen atoms, produced by the photolysis of water (generated dioxygen O_2 is released).

Heterotrophic cells

– cells of higher **animals** and most microorganisms – must obtain carbon in the form of relatively complex **organic molecules** (nutrients such as glucose) formed by other cells. They obtain their **energy from the oxidative (mostly aerobic) degradation of organic nutrients** made by autotrophs and return CO_2 to the atmosphere.

Carbon and oxygen are constantly cycled between the animal and plant worlds, solar energy ultimately providing the driving force for this massive process.



Heterotrophs: O_2 → $2\ O^{2-}$ + $4\ H^+$ = $2\ H_2O$

$4\ e^-$

Reducing equivalents (reducing power)

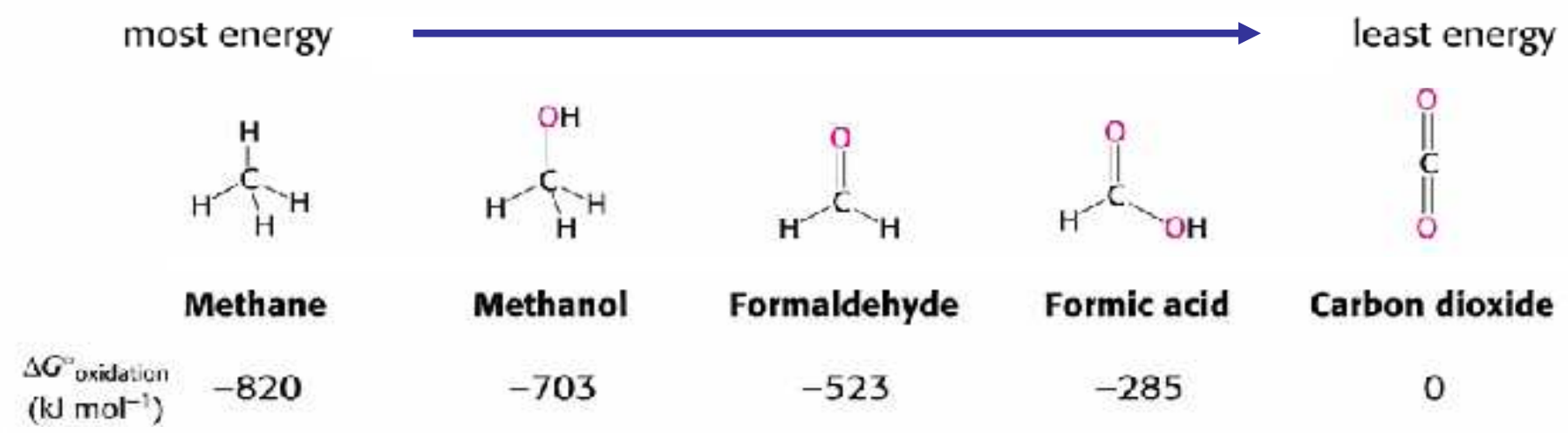
$4\ H$

Nutrients rich in **H** → CO_2

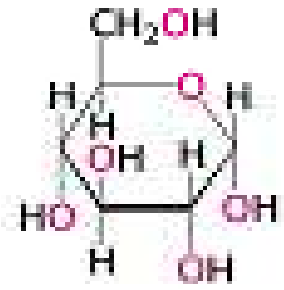
Biological oxidations (dehydrogenations)

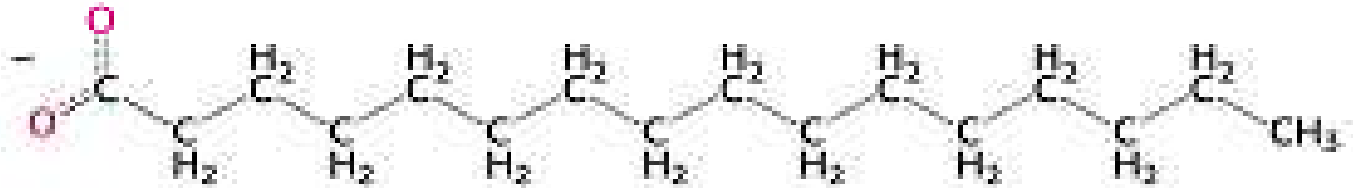
Decarboxylations

Most of the Gibbs´ free energy in the body originates in the exergonic synthesis of water ($2H_2 + O_2 \rightarrow 2H_2O$, 25 °C)**:** $\Delta G° = -474.3$ kJ mol^{-1}

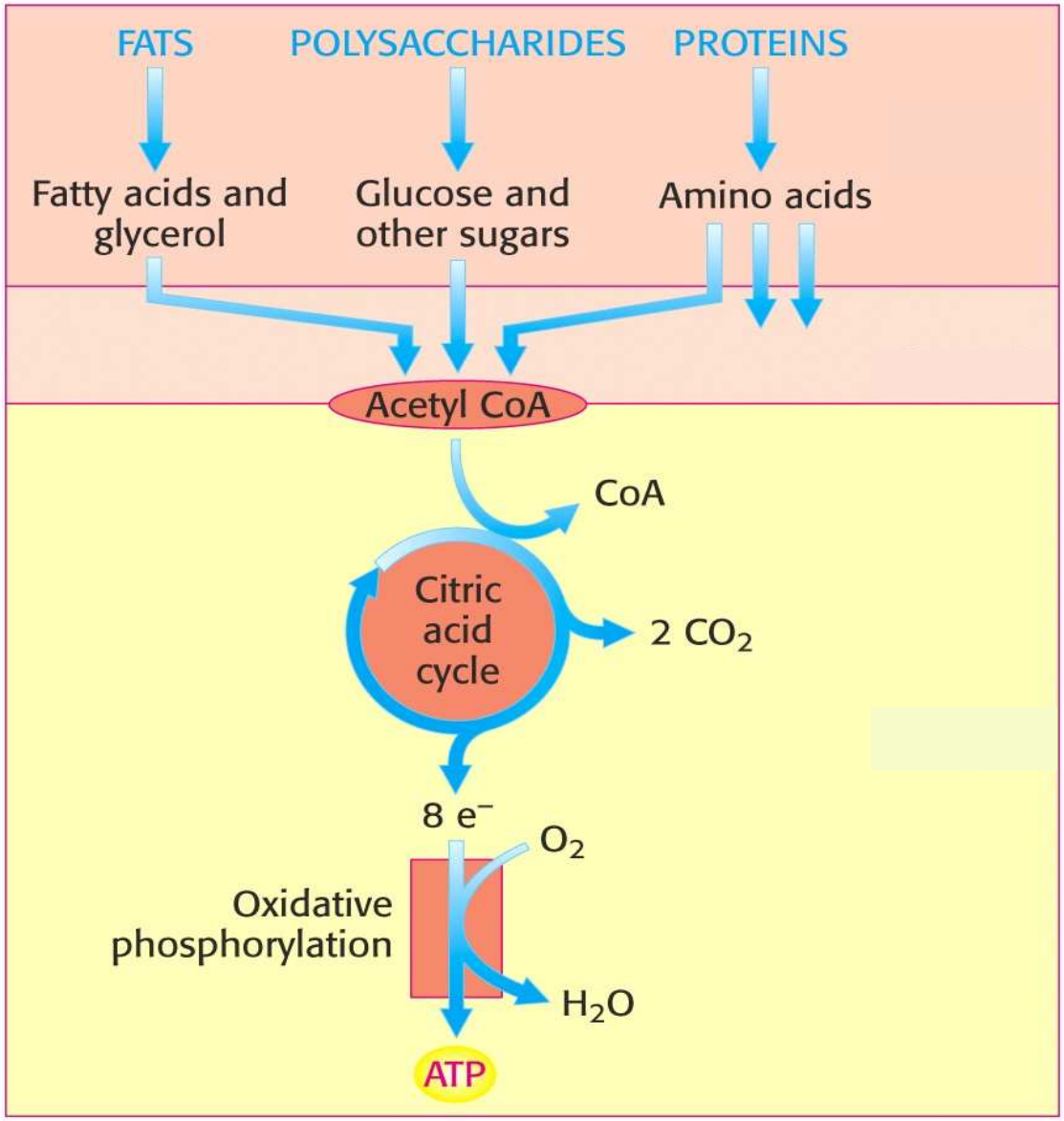


Fatty acids of fats are a more efficient fuel source than saccharides such as glucose because the carbon in fatty acids is **more reduced**


Glucose


Fatty acid

Stages in the extraction of energy from foodstuffs

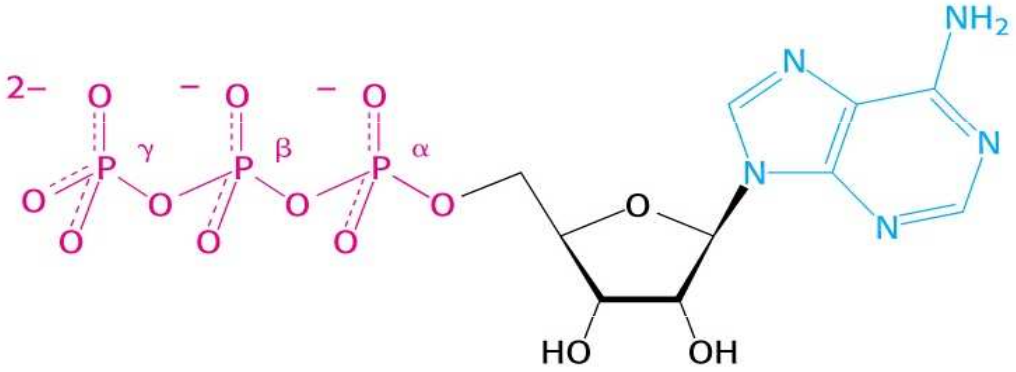


The first stage of catabolism
Large molecules in food are broken down into smaller units

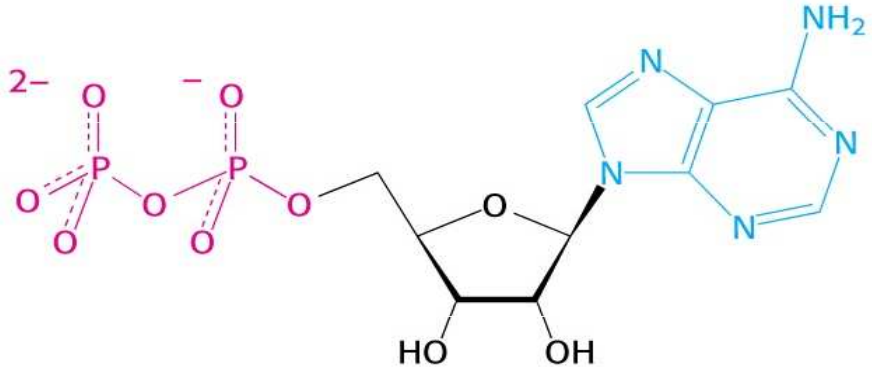
Stage II
Degradation to a few amphibolic intermediates

Stage III
The final common pathways – most of the ATP is produced from the complete oxidation of the acetyl unit of acetyl CoA

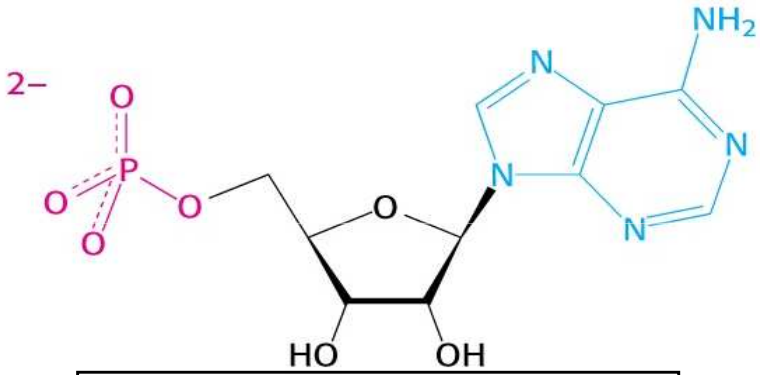
High-energy compounds



Adenosine triphosphate (ATP)



Adenosine diphosphate (ADP)



Adenosine monophosphate (AMP)

is <u>not</u> a high-energy compound
(no anhydride bond)

GTP, CTP, UTP, TTP are quite analogous to ATP. as well as GDP, CDP, UDP, TDP are analogous to ADP.

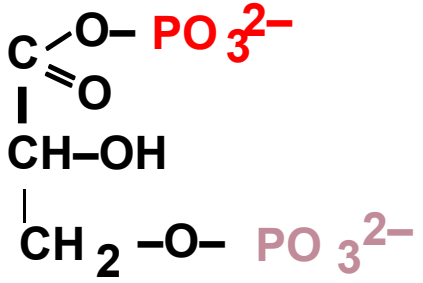
Different types of high-energy compounds

Anhydrides

di– and triphosphates **ATP, ADP, UTP, CTP** etc.

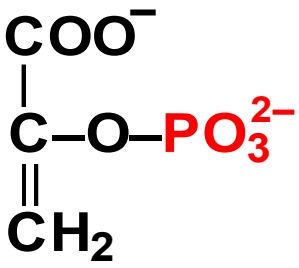
phosphosulfate phosphoadenosyl-phosphosulfate (PAPS)

acylphosphates **1,3-bisphosphoglycerate**



Ester

phosphoenolpyruvate

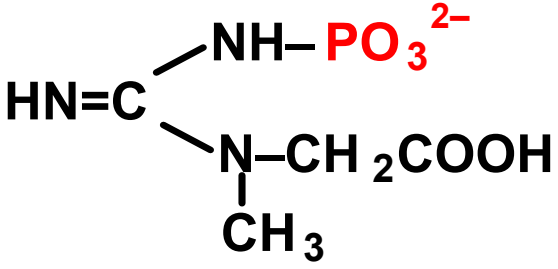


Thioesters

acyl coenzymes A

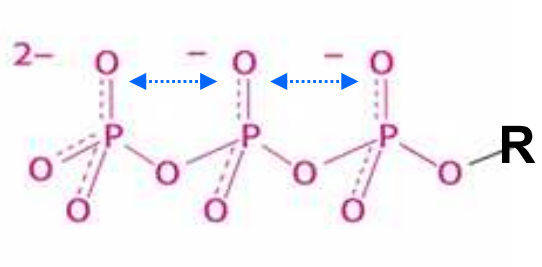
Amides

phosphocreatine

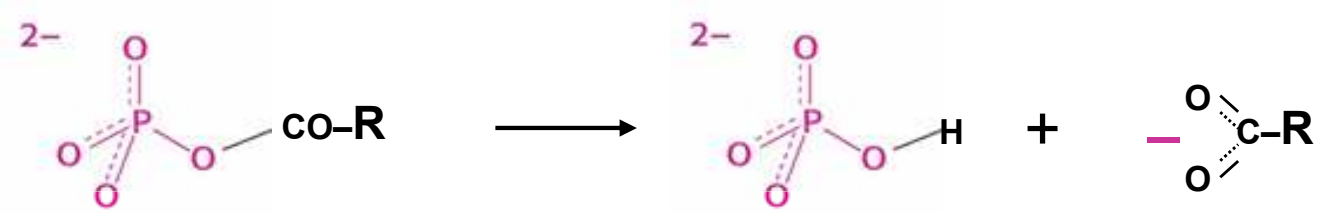


Factors contributing to the large change in ΔG^{o} of hydrolysis:

1 Electrostatic repulsion of negatively charged groups



2 Products of hydrolysis are more stable than the reactant because of greater resonance possibilities



3 and the groups in the products are more prone to isomerization or they exhibit more states of ionization

Phosphoenolpyruvate^{-} ⟶ Hydrogenphosphate^{2-} + pyruvate^{-}

More negative el. charges and
tautomerization enolpyruvate to the ketoform

Synthesis of ATP by phosphorylation of ADP in the cell

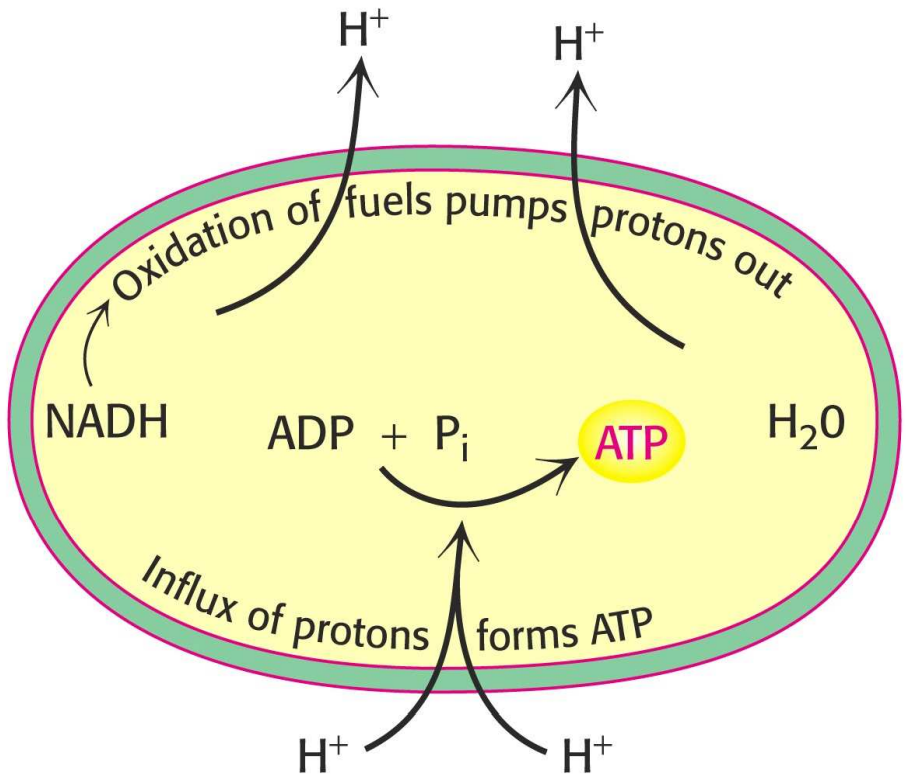
1 Oxidative phosphorylation in mitochondria

accounts for more than 90 % of ATP generated in animals.

The synthesis of ATP from ADP and Pi is driven by the **electrochemical potential of proton gradient** across the inner mitochondrial membrane.

This gradient is generated by the **terminal respiratory chain**, in which **hydrogen atoms**, as NADH + H+ and FADH2 produced by the oxidation of carbon fuels, **are oxidized to water**.

The oxidation of hydrogen by O_2 is coupled to ATP synthesis.



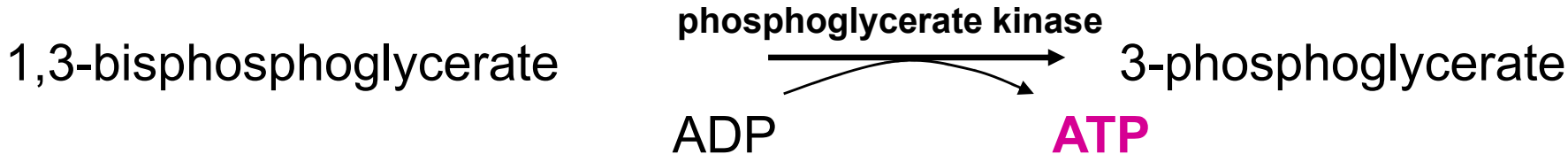
2 Phosphorylations of ADP on the substrate level

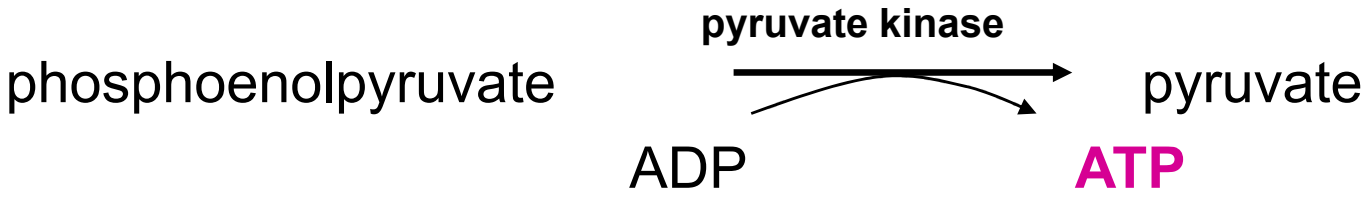
are provided by few reactions, in which a nucleoside triphosphate is synthesized by utilization of the free energy of hydrolysis of a soluble energy-rich compound.

- Energy released by certain **carbon oxidations** can be converted into high phosphoryl-transfer potential and so the favourable oxidation is coupled with the unfavourable synthesis (phosphorylation) of ATP.
- The high phosphoryl-transfer potential of phosphoenolpyruvate arises primarily from the large driving force of the **subsequent enol-ketone conversion**. Dehydration of 2-phosphoglycerate "traps" the molecule of the product in its unstable enol form.

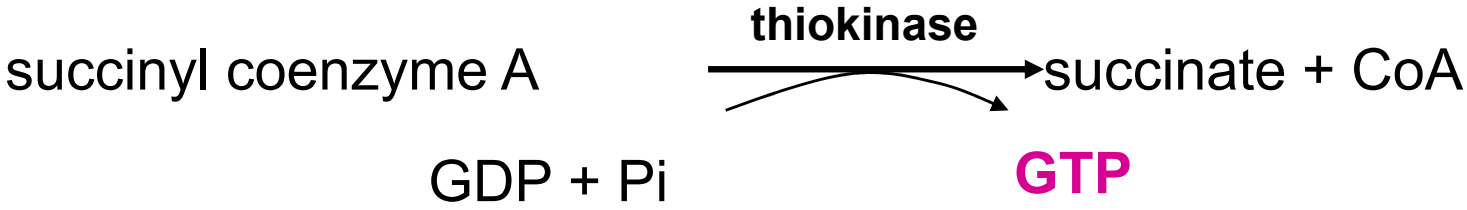
Examples of substrate-level phosphorylations

In glycolysis

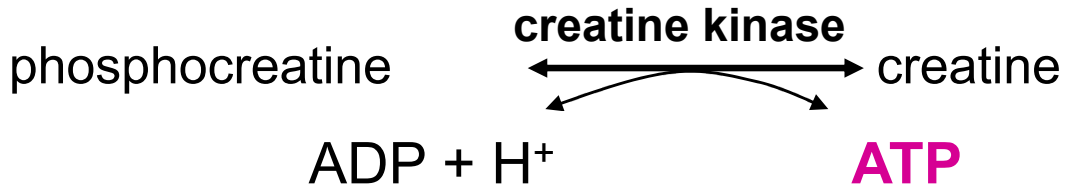




In the citrate cycle



In skeletal muscle phosphocreatine serves as a reservoir of high-potential phosphoryl groups that can be readily transferred to ATP:



Control of metabolism

Metabolism is regulated by controlling

- **catalytic activity of enzymes**

  allosteric and cooperative effects, reversible covalent modification, substrate concentration

- **the amount of enzymes**

  synthesis of adaptable enzymes

- **the accessibility of substrates**

  compartmentalization segregates biosynthetic and degradative pathways, the flux of substrates depends on controlled transfer from one compartment of a cell to another

- **the energy status of the cell**

  of which the energy charge or the phosphorylation potential are used as indexes

- **communication between cells**

  hormones, neurotransmitters, and other extracellular molecular signals often regulate the reversible modification of key enzymes

$$\textbf{Energy charge} = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

can have a value ranging from 0 (all AMP) to 1 (all ATP).

Catabolic (ATP-generating) pathways are inhibited by an energy charge, whereas anabolic (ATP-utilizing) pathways are stimulated by a high-energy charge.

The energy charge of most cells ranges **from 0.80 to 0.95**.

$$\textbf{Phosphorylation potential} = \frac{[ATP]}{[ADP] \times [P_i]}$$

is an alternative index of the energy status of a cell.

In contrast with the energy charge, it depends on the concentration of P_i and is directly related to the free energy storage available from ATP.

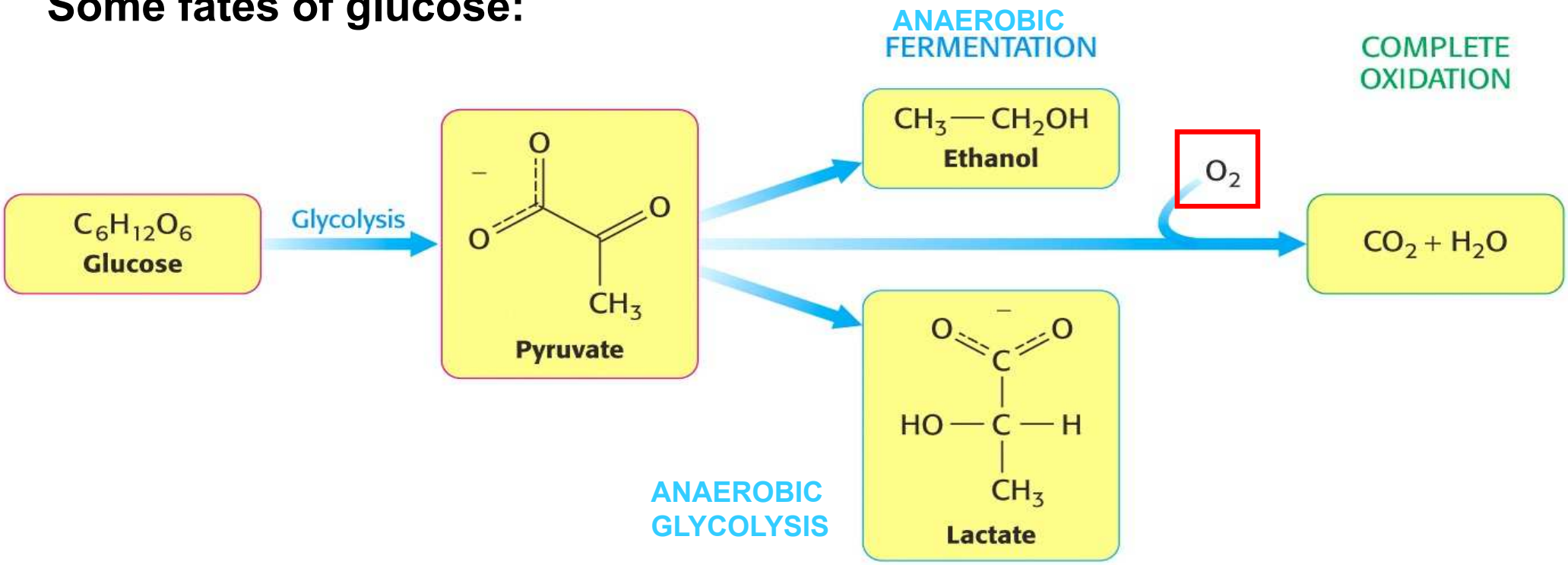
The glycolytic pathway

Glucose is an important and common nutrient for most organisms.

In mammals

glucose is the only fuel that the brain uses under non-starvation conditions and the only fuel that red blood cells can use at all.

Some fates of glucose:



Glucose transporters

mediate the thermodynamically downhill movement of glucose across the plasma membranes of animal cells.

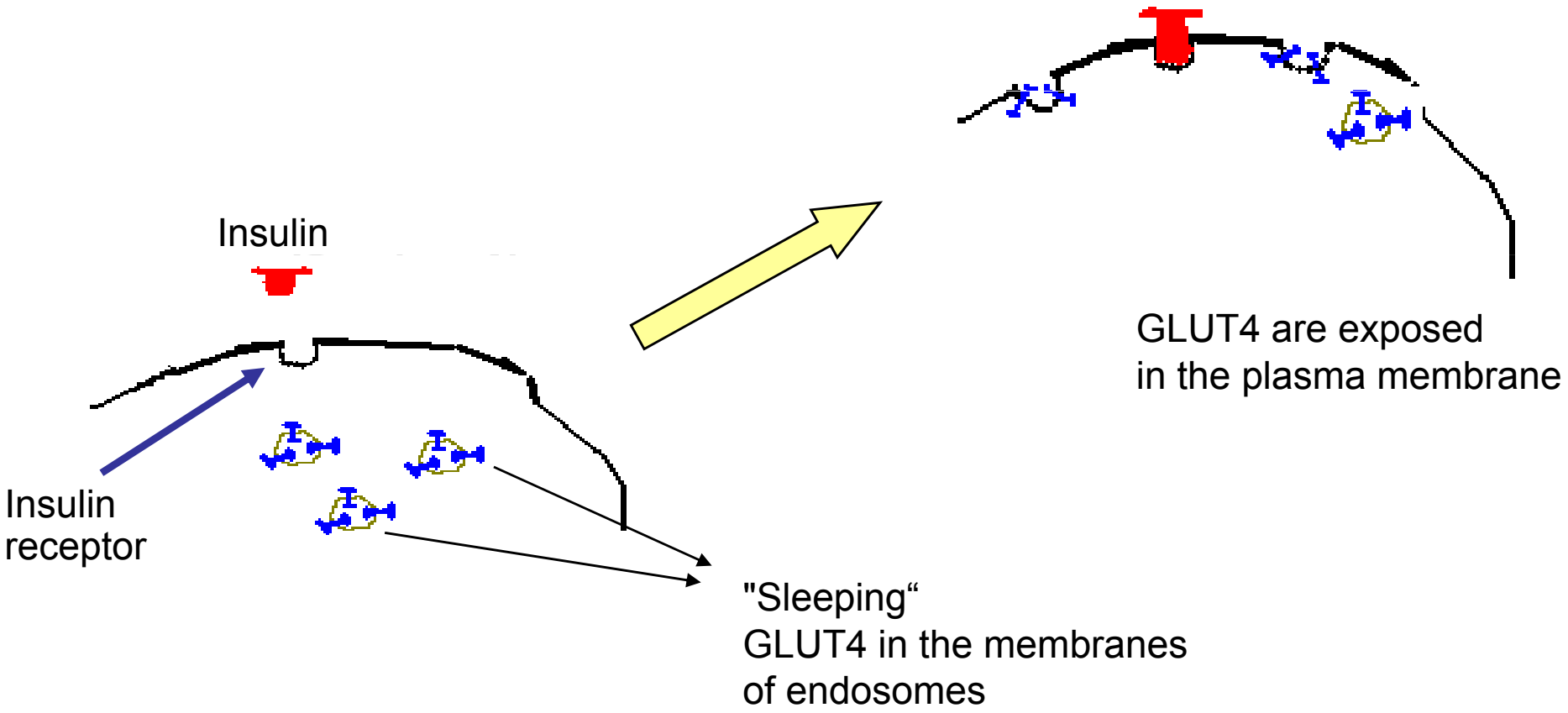
The members of the family of transporters have distinctive roles.

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

Glucose transporter GLUT4

transports glucose into muscle and fat cells. The presence of **insulin**, which signals the fed state leads to a **rapid increase in the number of GLUT4** transporters in the plasma membrane. Hence, insulin promotes the uptake of glucose by muscle and adipose tissue.



The glycolytic pathway

(also known as the Embden-Meyerhof pathway)

The conversion of glucose into two molecules of **pyruvate** is anaerobic with the concomitant net production of two molecules of ATP.

Under **anaerobic** conditions, pyruvate can be processed to **lactate**.

Under **aerobic** conditions, pyruvate can be decarboxylated to **acetyl CoA** and completely oxidized to **CO_2**, generating much more ATP.

Glycolysis is common to all types of cells.

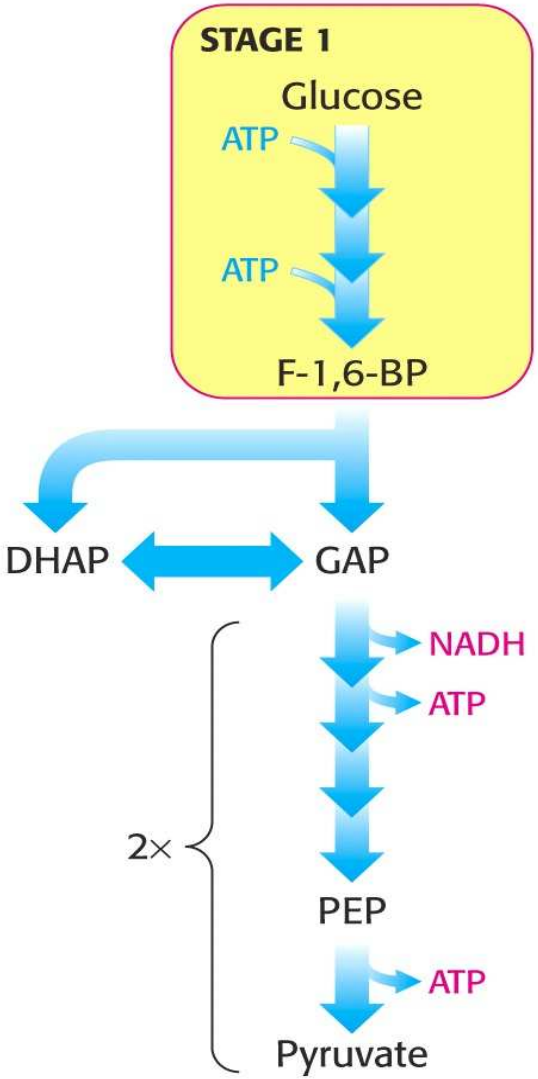
In eukaryotic cells, glycolysis takes place **in the cytosol**.

Reactions of glycolysis are catalyzed by enzymes.

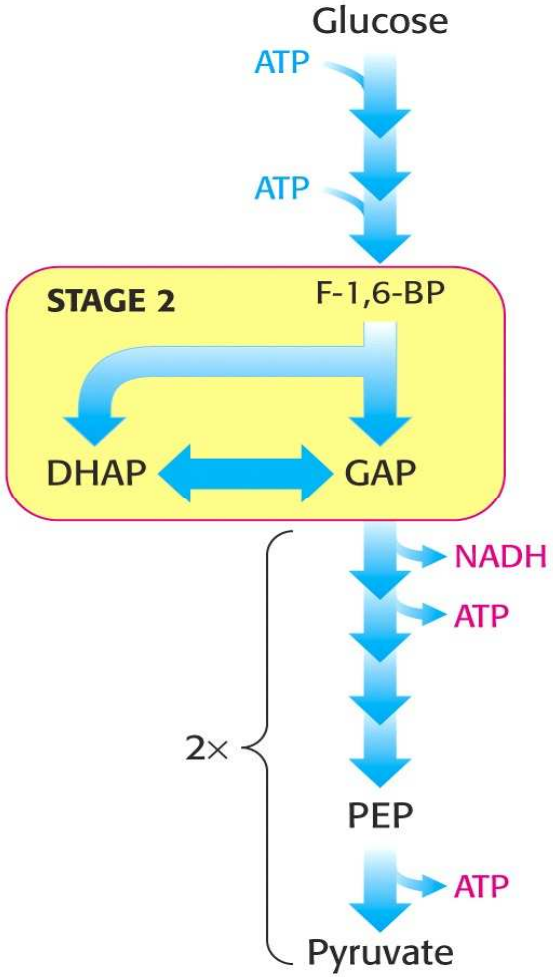
Three of them are irreversible. (In gluconeogenesis, pyruvate is converted to glucose: those three reactions differ and are catalyzed by different enzymes.)

Fructose and galactose also enter into glycolysis.

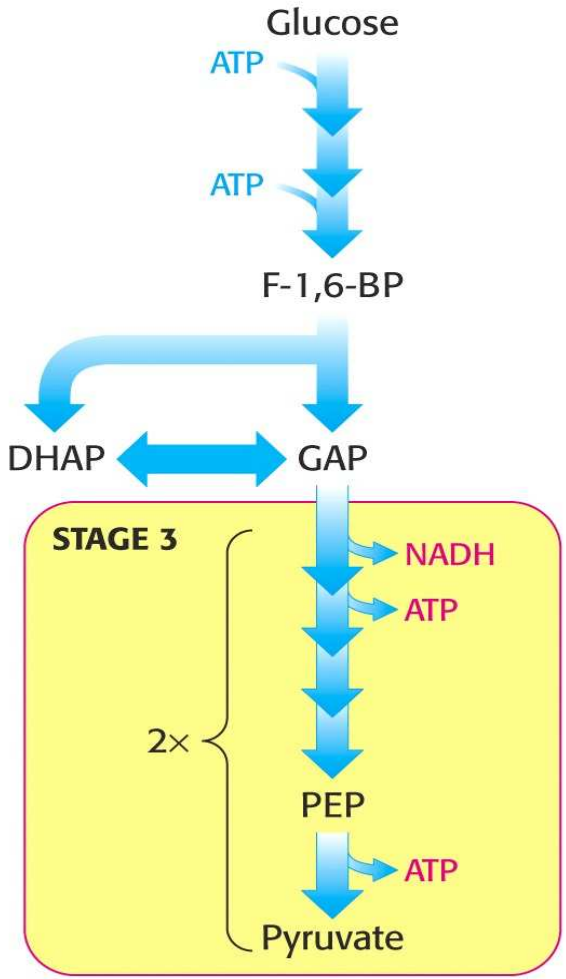
The glycolysis can be thought of as comprising three stages:



Trapping the glucose in the cell and destabilization by phosphorylation.

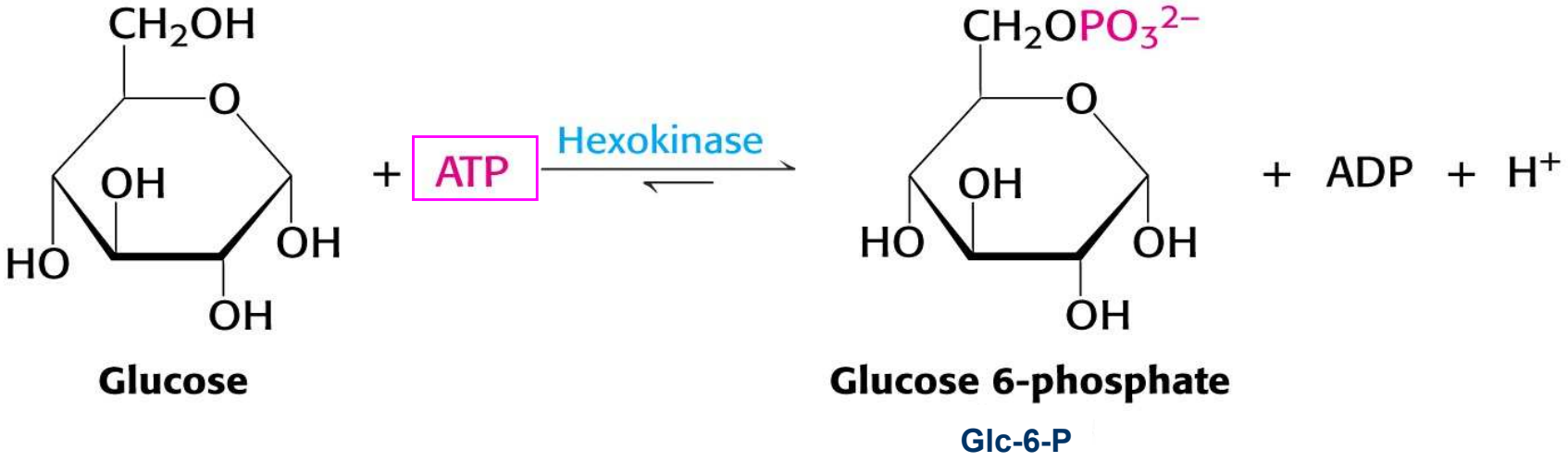


Cleavage into two three-carbon units.



Oxidative stage in which new molecules of ATP are formed by substrate-level phosphorylation of ADP.

The phosphorylation of glucose by ATP:



Glucose **Glucose 6-phosphate**

Glc-6-P

Hexokinase reaction **traps glucose in the cell**, Glc-6-P cannot diffuse through the membrane, because of its negative charges.

Conversion of Glc-6-P to glucose catalysed by glucose 6-phosphatase takes place only in the liver (and to a lesser extent in the kidney).

The addition of the phosphoryl group begins to **destabilize glucose**,
thus **facilitating its further metabolism:**

- through further reactions of glycolysis,

but also through reactions starting

- synthesis of glycogen (glycogenesis)
- the pentose phosphate pathway (supplying NADPH),
- synthesis of other saccharides (e.g. mannose, galactose, amino sugars, glucuronic acid).

The phosphorylation of glucose in the cytosol **accelerates the entry of glucose into the cell**.

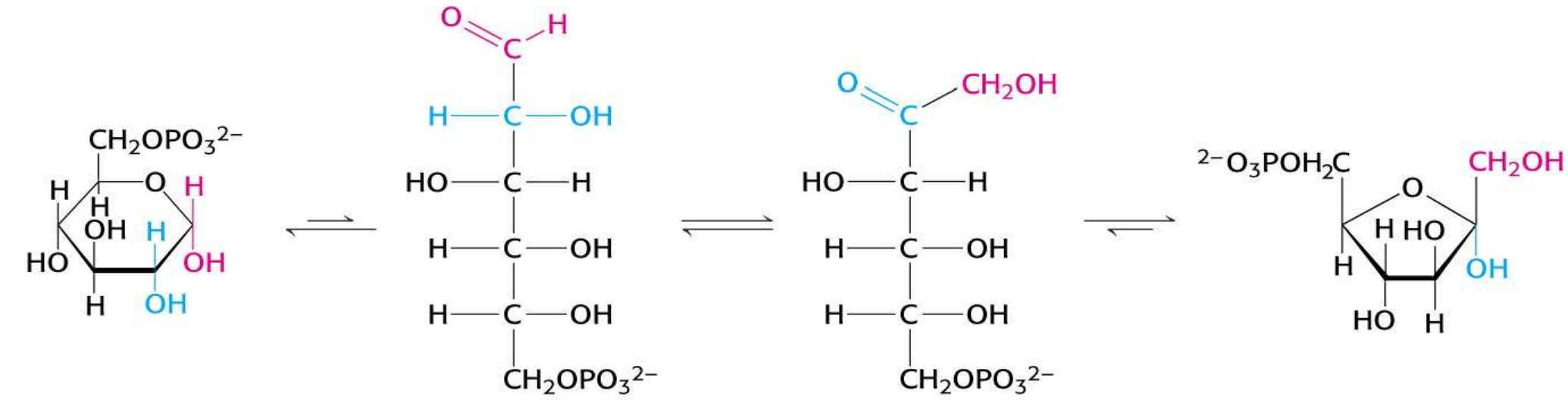
On the contrary to other tissues, **the liver cells** (and the pancreatic β-cells) comprise a specialized isoenzyme of hexokinase called **glucokinase**. The enzyme is very efficient, but its affinity for glucose is low (value of Michaelis constant is high, K_m = 10 mmol/l). It means that the uptake of glucose by the liver cells (as well as β-cells of pancreatic islets secreting insulin) shall predominate, if there is a steep rise in blood glucose. The role of glucokinase is to provide glucose for the synthesis of glycogen and for the formation of fatty acids. Glucose will not be wasted in other tissues when it is abundant.

Hexokinases present in the **other tissues** are inhibited by glucose 6-phosphate, the reaction product. High concentration of this molecule signal that the cell no longer requires glucose for energy, for storage in the form of glycogen, or as a source of biosynthetic precursors, and the glucose will be left in the blood.
High affinities of hexokinases for glucose (Michaelis constant $K_m \leq 0{,}1$ mmol/l) will ensure the constant and preferential flow of glucose into the extrahepatic tissues, if the blood glucose level is low..

Glucokinase	Hexokinases
In the liver, specific for glucose	In extrahepatic tissues, broad specifity for hexoses
Not inhibited by Glc-6-P	Inhibited by Glc-6-P
Low affinity for glucose	High affinity for glucose
Inducible (in the liver) by insulin	Not inducible by insulin

The isomerization of Glc-6-P to fructose 6-phosphate

catalysed by phosphoglucose isomerase:

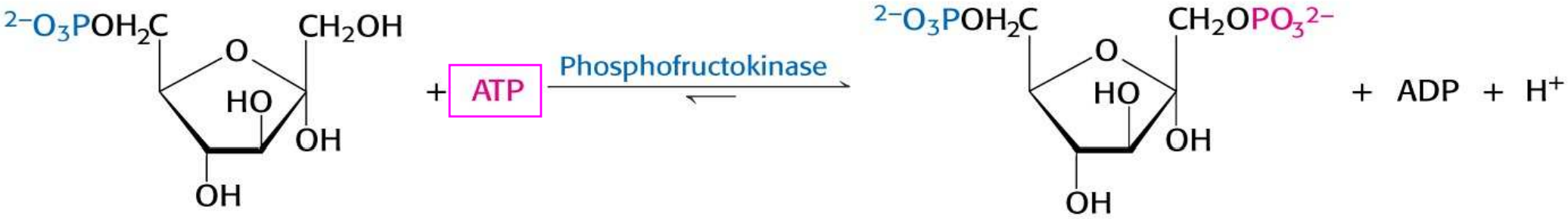


The second phosphorylation catalysed by phosphofructokinase

is the **rate-limiting step** and a **major control point of glycolysis** :

Common features of the rate-limiting step of a metabolic pathway:

- The molar activity (turnover number, k_{cat}) of the particular enzyme is smaller than those of other enzymes taking part in the metabolic pathway.
- The reaction rate does not usually depend on substrate concentration [S] because it reaches the maximal value V_{max}.
- The reaction is practically irreversible. The process can be reversed only by the catalytic action of a separate enzyme.



Fructose 6-phosphate
(Fru-6-P)

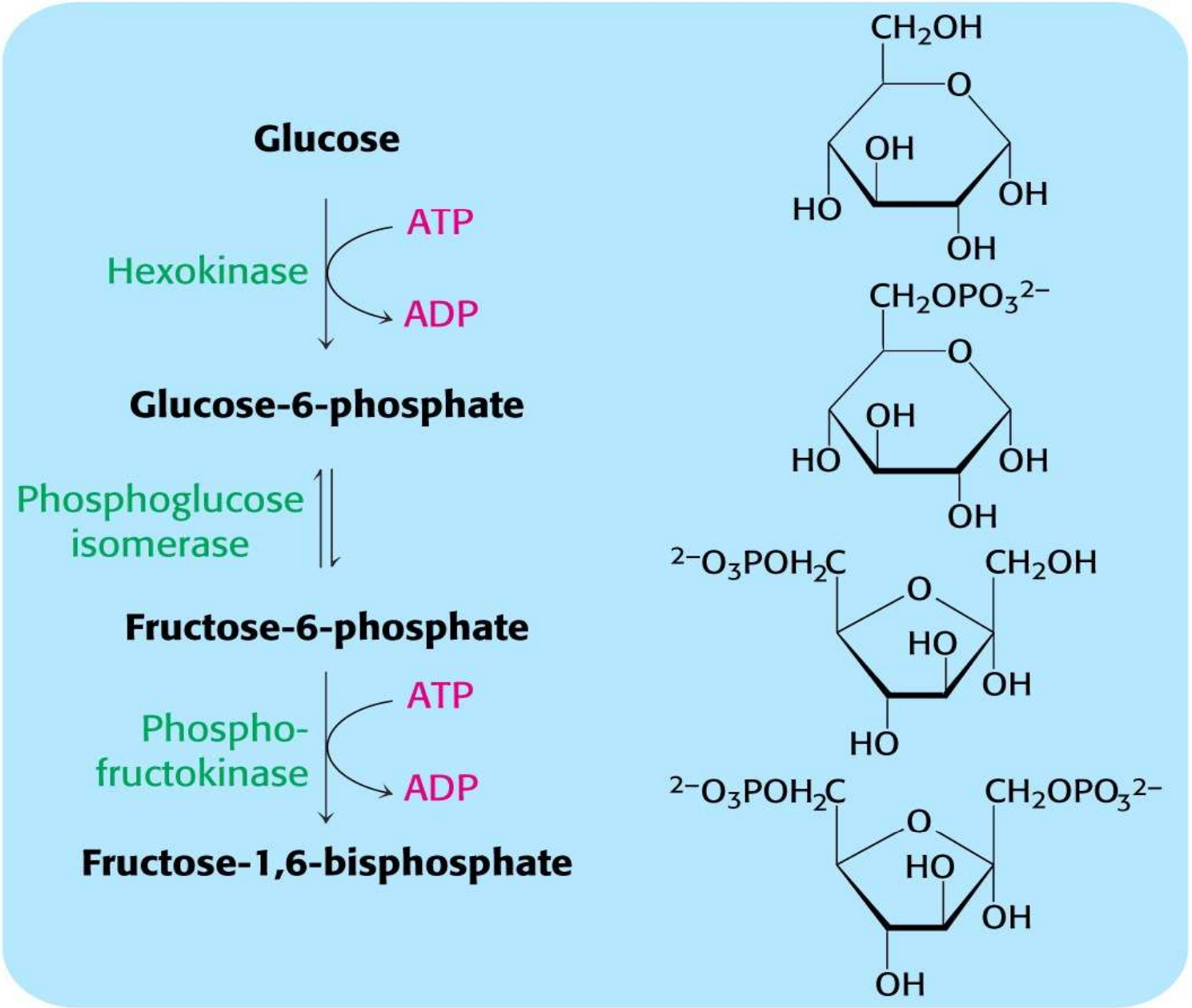
Fructose 1,6-bisphosphate
(Fru-1,6-P_2)

Allosteric control of phosphofructokinase:

- allosteric **inhibition** by ATP and citrate,
- allosteric **activation** by AMP, ADP, and in the liver by fructose **2**,6-bisphosphate

Stage 1

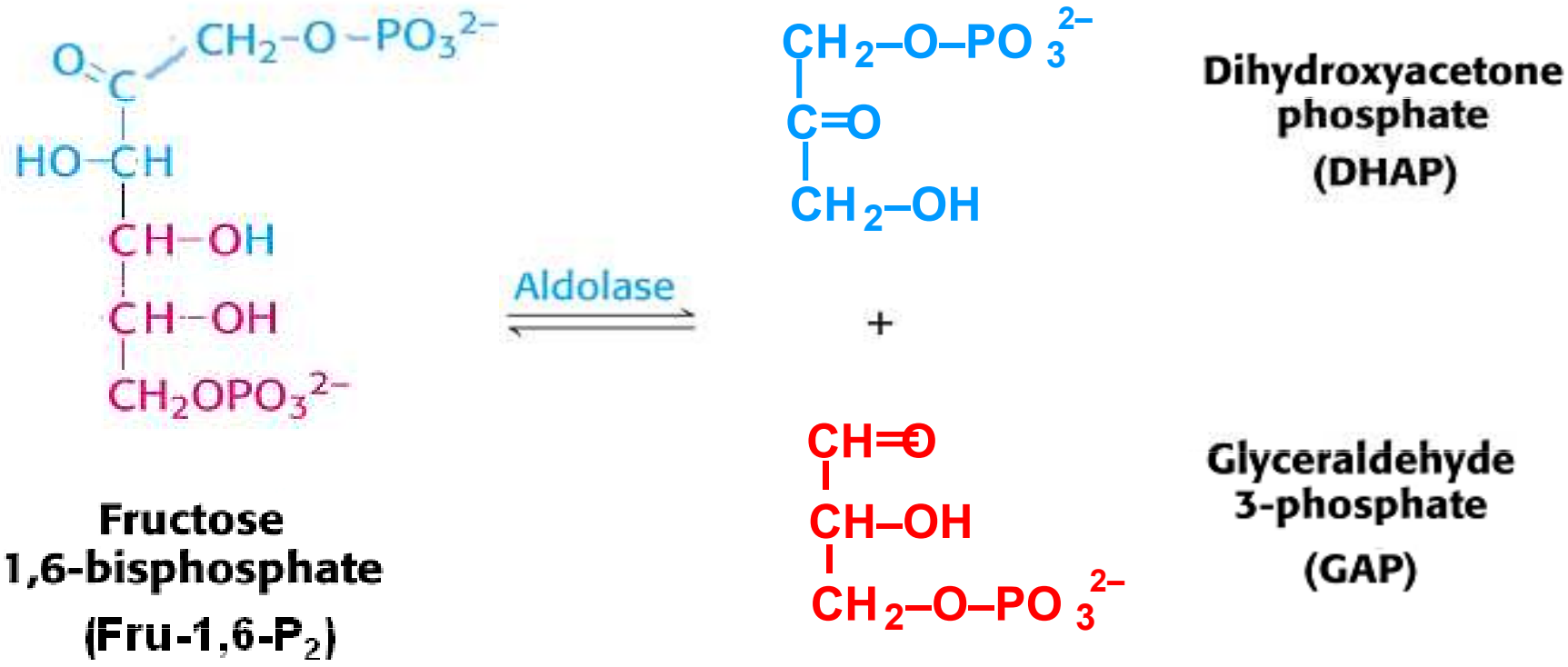
- summary



Stage 2

The splitting of fructose 1,6-bisphosphate into two triose phosphates

catalysed by aldolase:

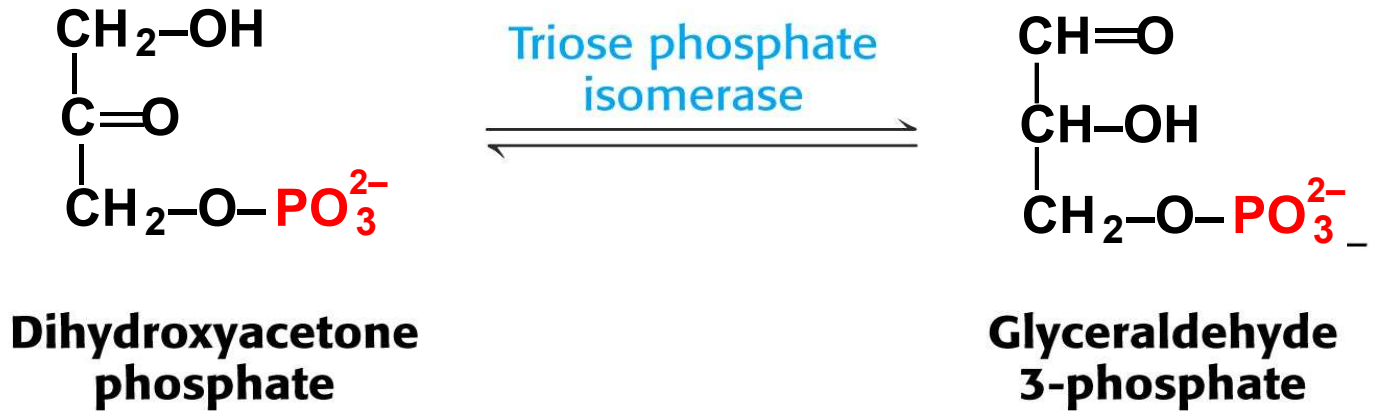


Fructose 1,6-bisphosphate (Fru-1,6-P_2)

Dihydroxyacetone phosphate (DHAP)

Glyceraldehyde 3-phosphate (GAP)

In the following stage 3, only glyceraldehyde 3-phosphate is oxidized. Dihydroxyacetone phosphate does not accumulate because it is continuously converted to glyceraldehyde phosphate by triose phosphate isomerase:



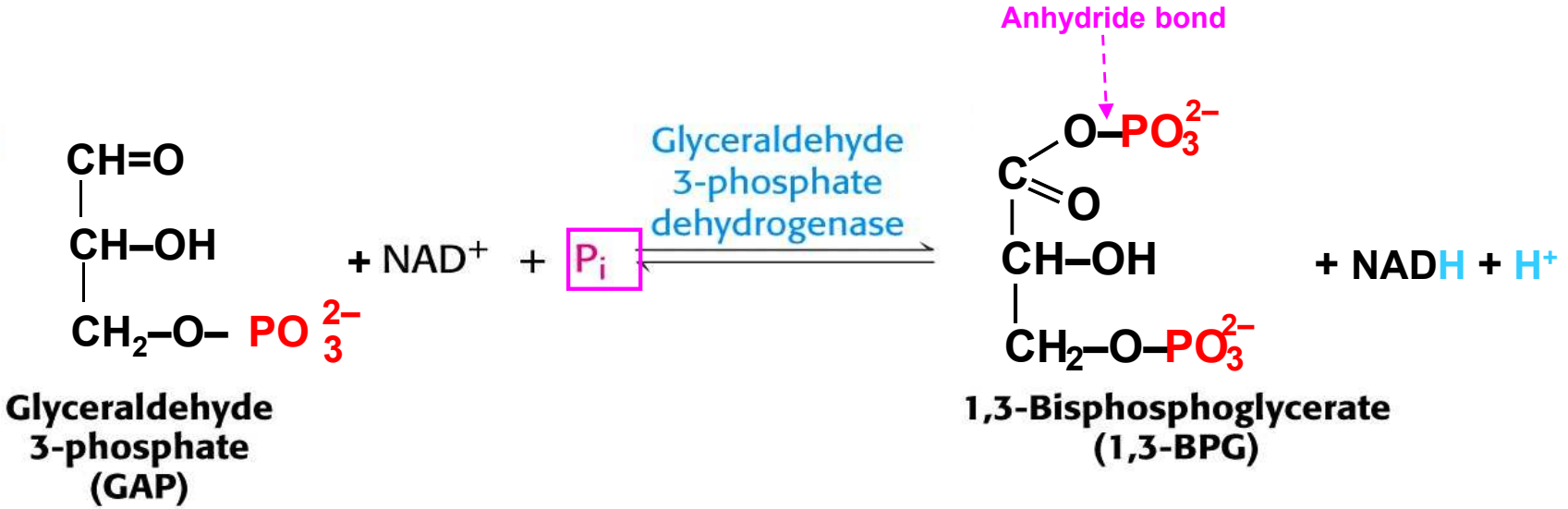
Stage 2 – summary

Fructose 1,6-bisphosphate ⇌ 2 molecules of **glyceraldehyde 3-phosphate**

Stage 3

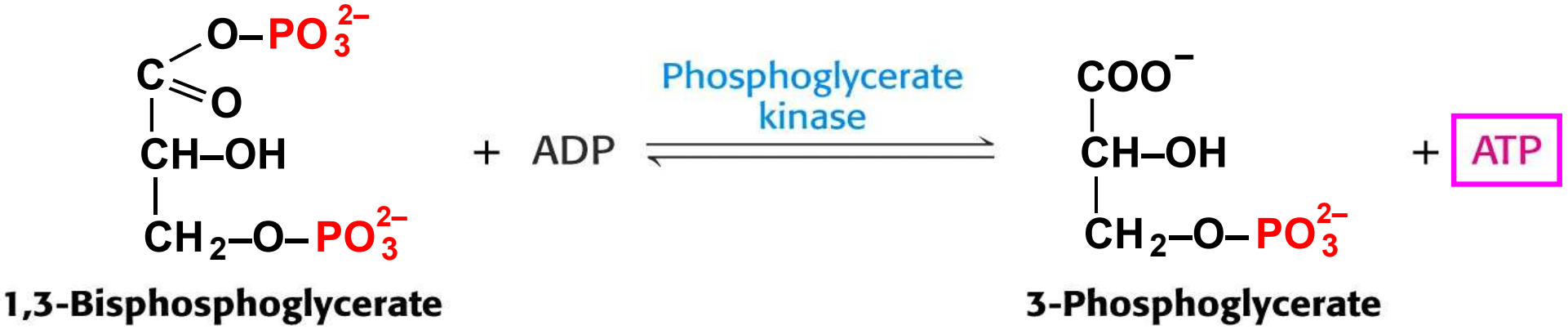
Oxidative stage – new molecules of ATP are formed by substrate-level phosphorylation of ADP

Oxidation of GAP by NAD⁺ to 1,3-bisphosphoglycerate:



The reaction is the only oxidative step in the glycolytic pathway, it produces NADH and is highly exergonic. The product 1,3-BPG is a **high-energy intermediate** (a mixed anhydride of 3-phosphoglycerate and phosphate).
This reaction is coupled energetically with the following step in which the large negative free energy of hydrolysis of 1,3-BPG is utilized in an endergonic phosphorylation of ADP to ATP.

In the reaction catalysed by phosphoglycerate kinase the energy-rich anhydride **1,3-bisphosphoglycerate is hydrolysed**, and at the same time the energy-rich **ATP is formed** by the phosphorylation of ADP:

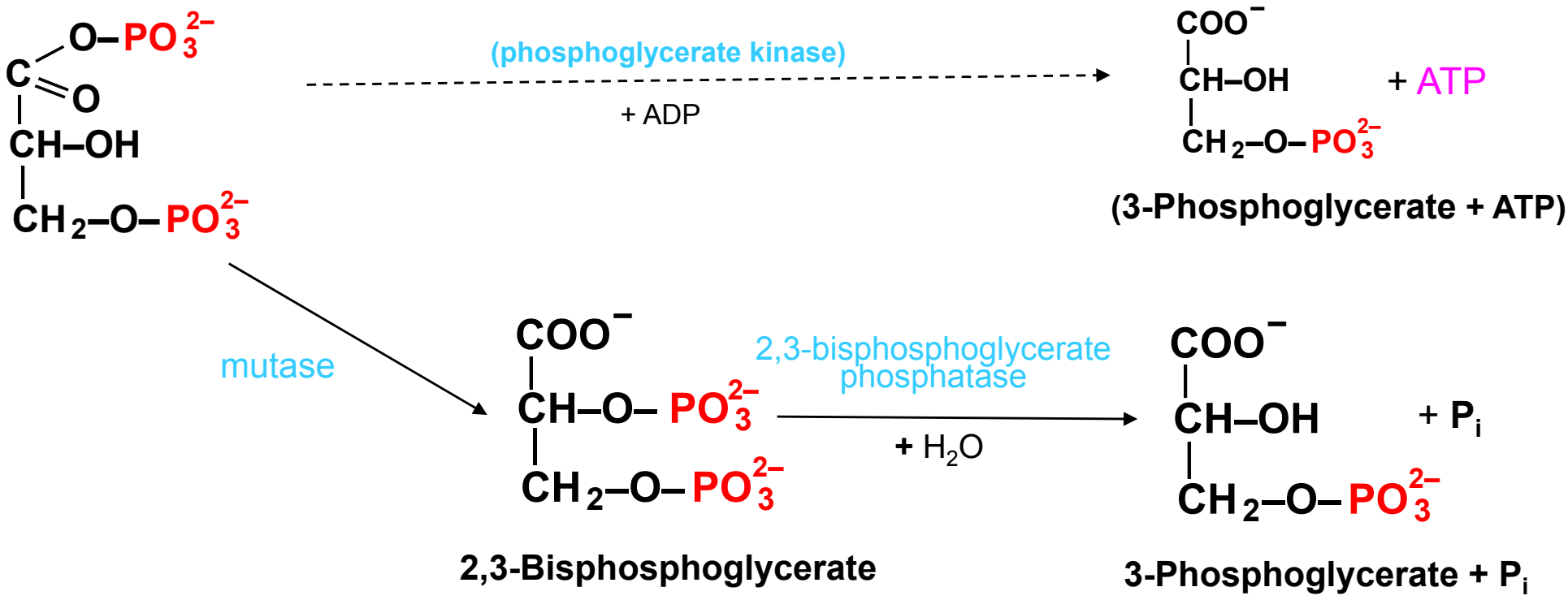


1,3-Bisphosphoglycerate **3-Phosphoglycerate**

The oxidation of GAP to 1,3-BPG thus drives the synthesis of ATP from ADP.
This is an example of **substrate-level phosphorylation** of ADP.

In **red blood cells** (the demand of ATP is lower when compared to other cells) the reaction can be passed by without the gain of ATP:

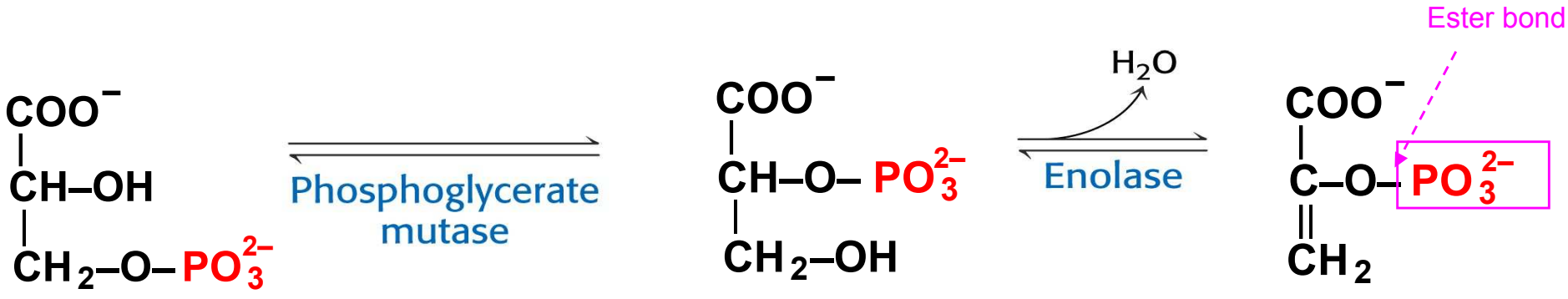
The by-pass of phosphoglycerate kinase reaction **in red blood cells:**



2,3-Bisphosphoglycerate is an important effector of oxygen binding by haemoglobin.

Formation of phosphoenolpyruvate

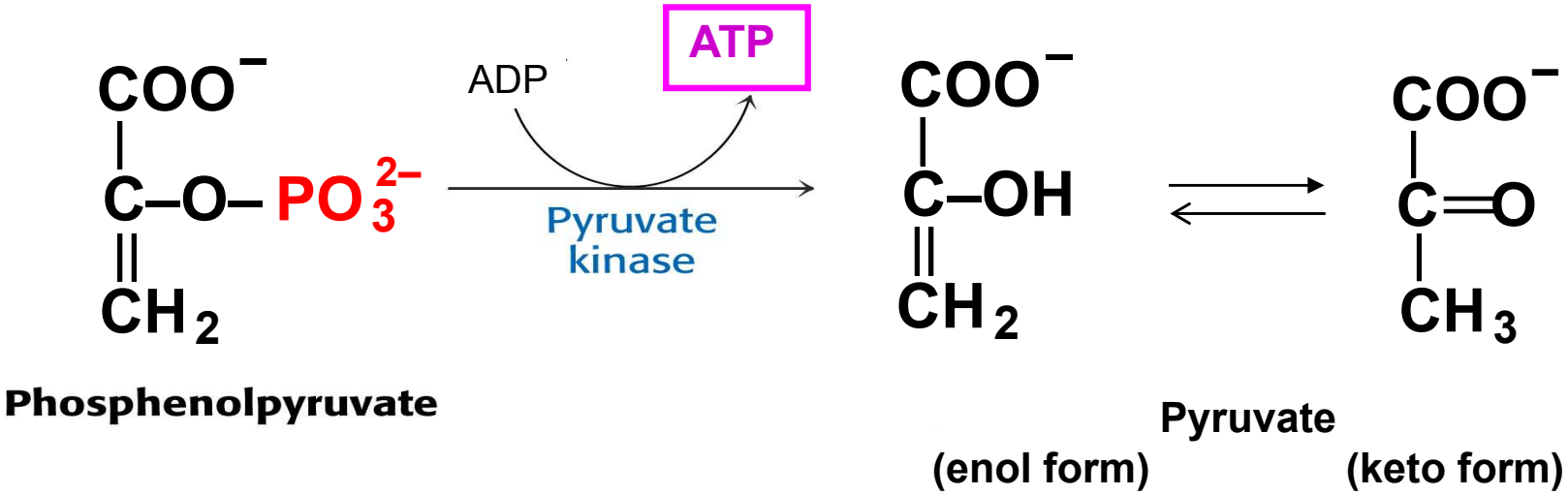
is catalysed by phosphoglycerate mutase and by enolase:



3-Phosphoglycerate **2-Phosphoglycerate** **Phosphoenolpyruvate (PEP)**

Both reactions are readily reversible.
The product phosphoenolpyruvate is a **high-energy intermediate** (an ester of the enol form of pyruvate and phosphate).

In the reaction catalysed by pyruvate kinase the energy-rich ester **phosphoenolpyruvate is hydrolysed**, and at the same time the energy-rich **ATP is formed** by the phosphorylation of ADP:

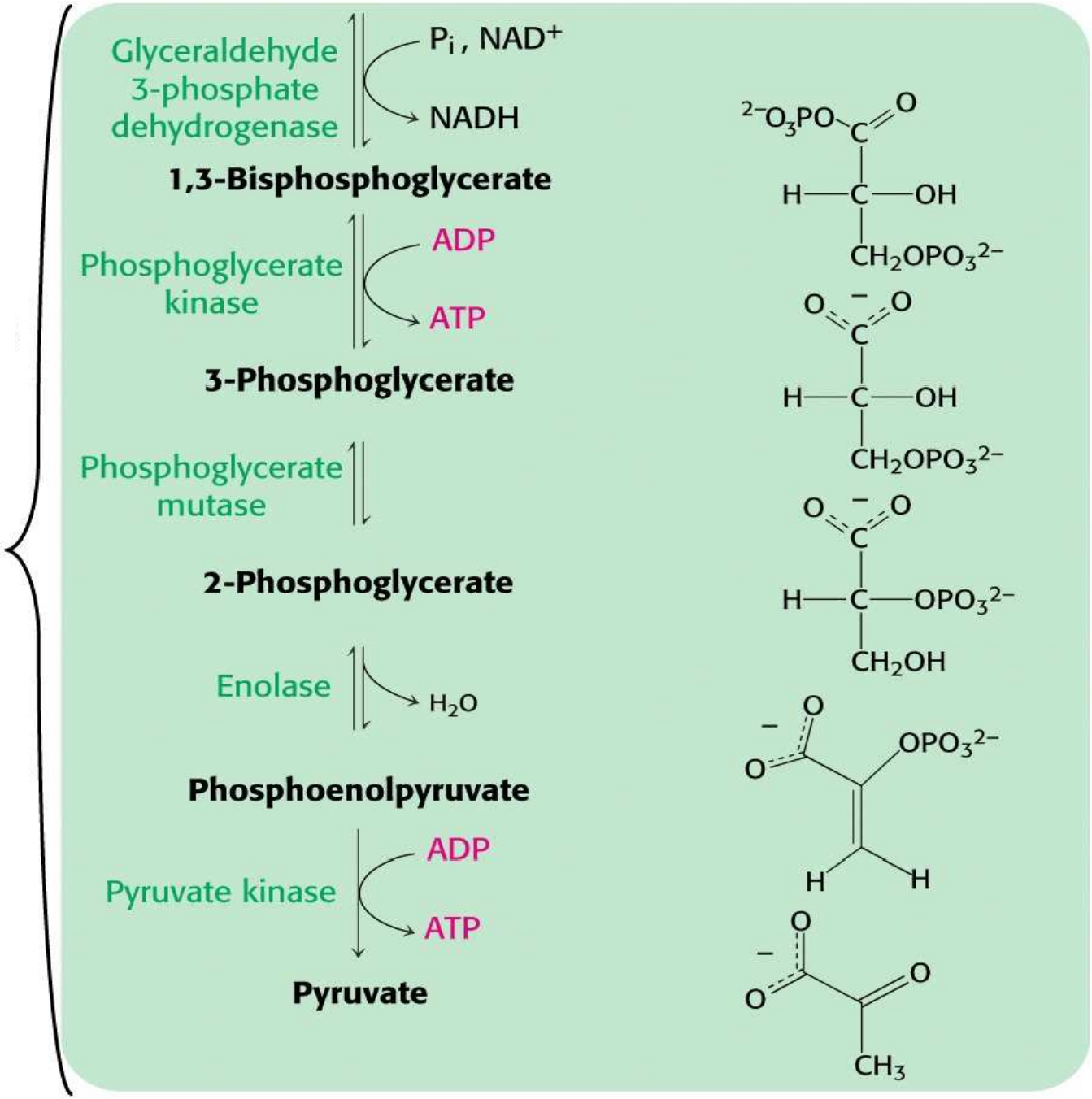


This reaction (essentially irreversible) is a **substrate-level phosphorylation**, the second one of the 3rd stage of glycolysis.
The synthesis of ATP from ADP is driven by the dehydration of 2-phosphoglycerate to phosphoenolpyruvate (PEP) in the previous reaction.

Pyruvate kinase reaction is the **3rd control point** of the glycolytic pathway. Pyruvate kinase is
- allosterically activated by fructose-1,6-bisphosphate (the product of an earlier step),
- and in liver cells inhibited by hormone glucagon through phosphorylation.

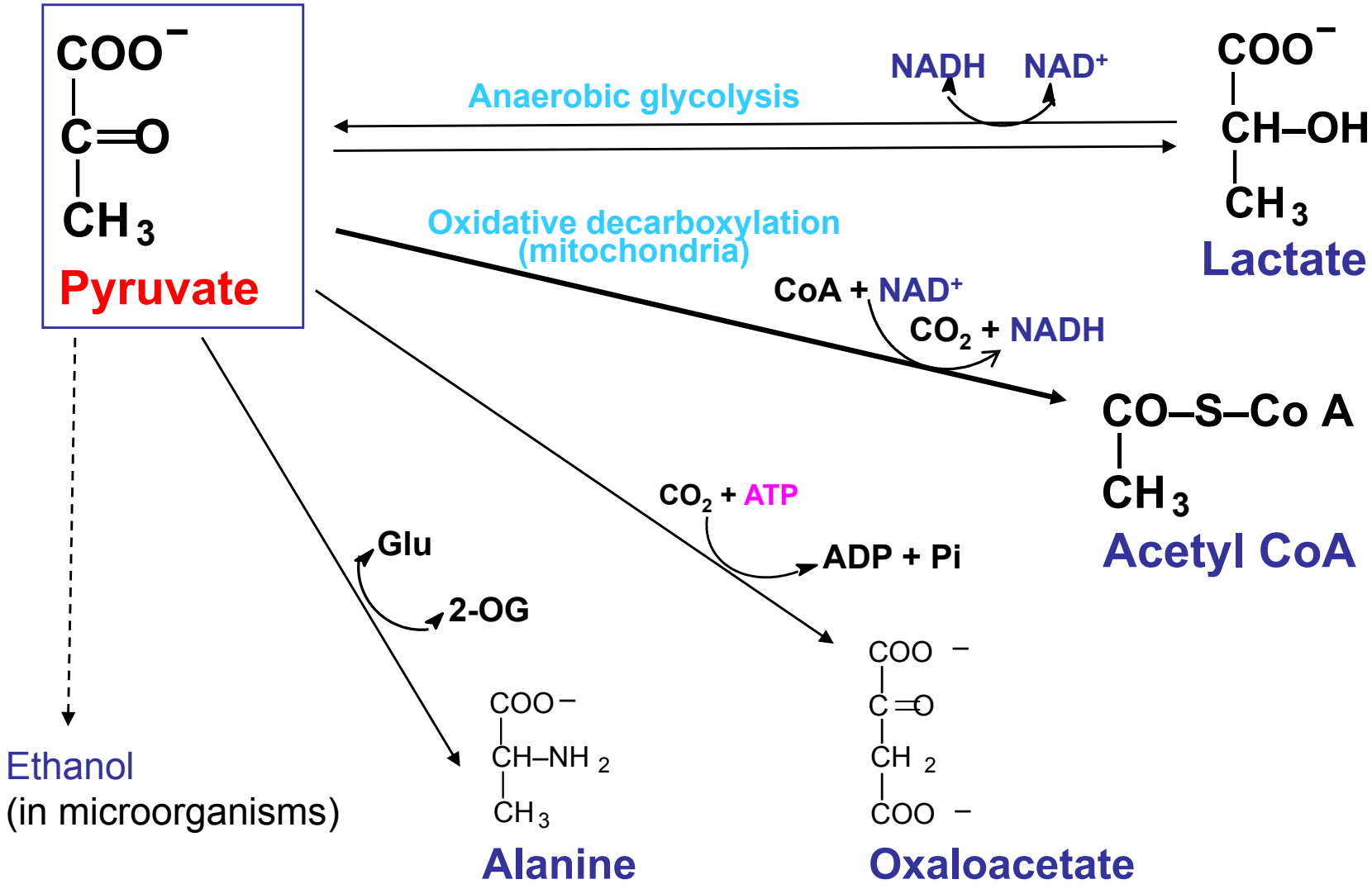
Stage 3
- summary

2 ×



The diverse fates of pyruvate

Pyruvate is a pivotal intermediate in saccharide metabolism

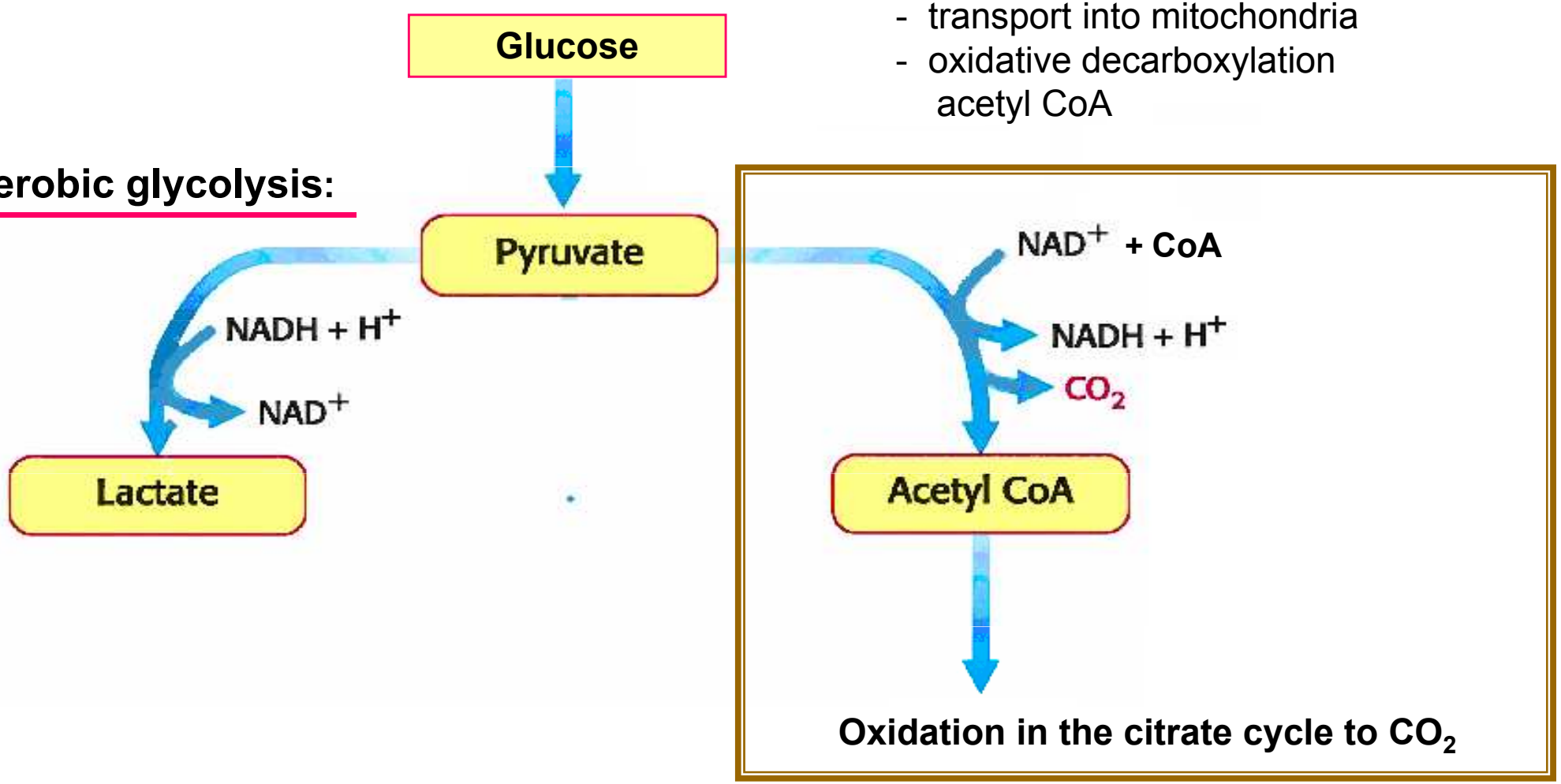


Pyruvate catabolism

in animals is an **aerobic pathway** located **in the mitochondrial matrix**.

The initial steps are
- transport into mitochondria
- oxidative decarboxylation
  acetyl CoA

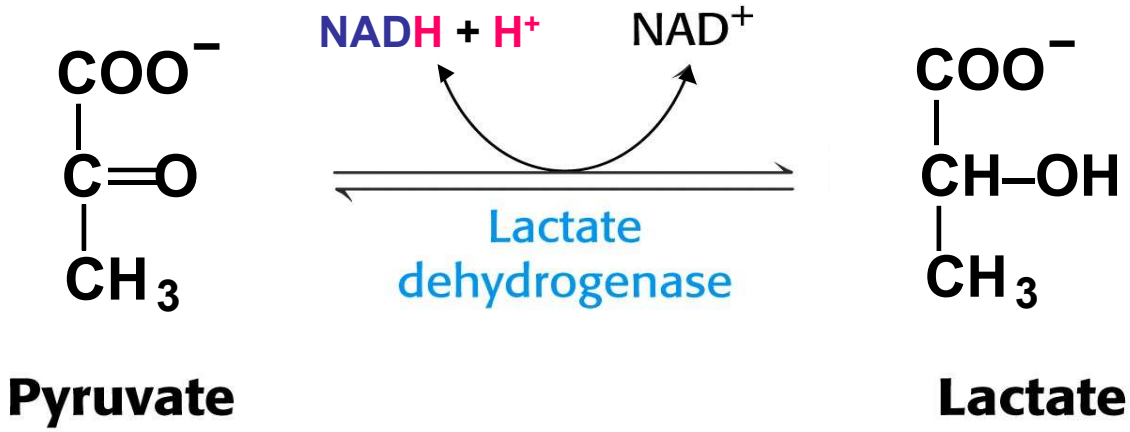
Anaerobic glycolysis:



or **conversion to fatty acids or cholesterol**

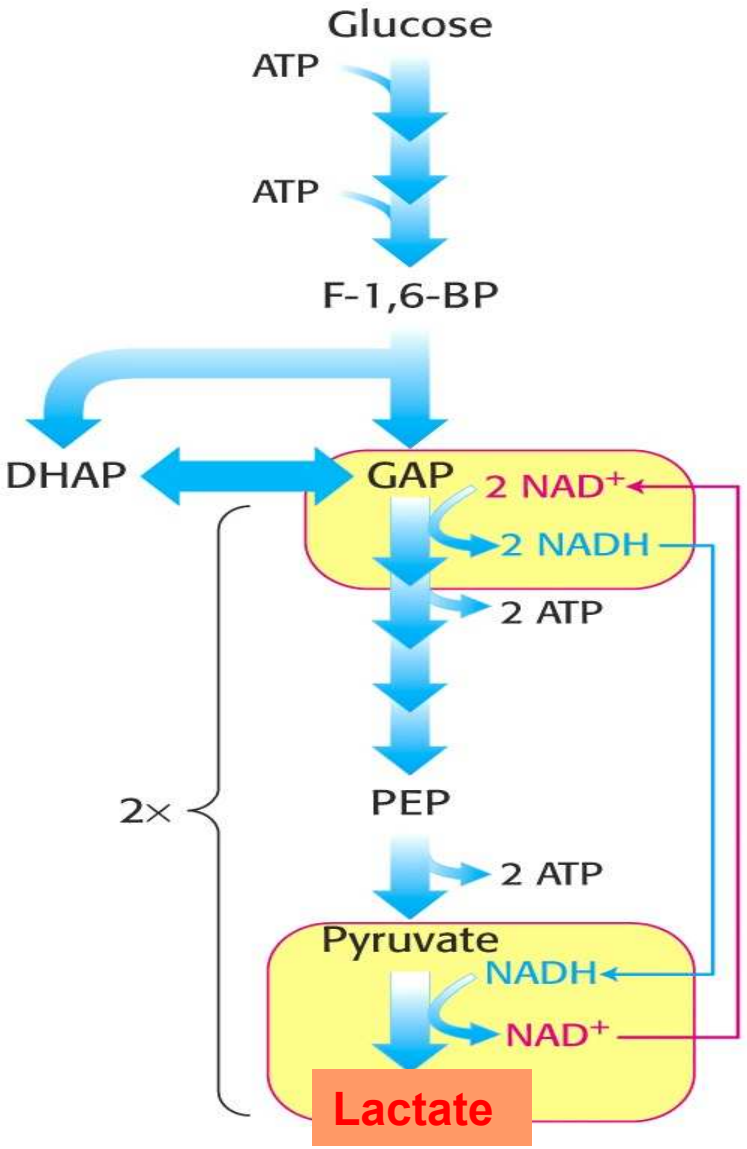
Anaerobic glycolysis

When the oxidative decarboxylation of pyruvate is stopped under anaerobic conditions, **pyruvate is reduced to lactate**. The reaction is catalysed by lactate dehydrogenase, and it is readily reversible.:



The purpose of this final reduction is **to regenerate NAD^+** consumed in dehydrogenation of 3-phosphoglyceraldehyde to 1,3-bisphosphoglycerate. At insufficient concentration of NAD^+, molecules of glucose cannot enter the glycolytic pathway.

Reoxidation of NADH in anaerobic glycolysis:



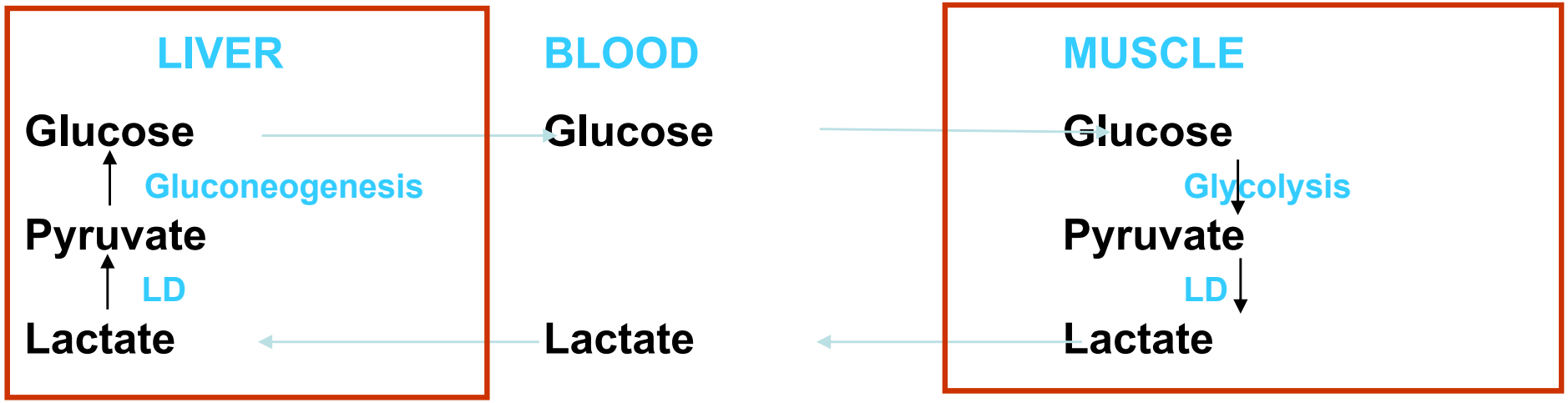
In fact, the anaerobic glycolysis produces **lactic acid** (lactate anion as well as H^+).
The intense lactate production may be a cause of its accumulation associated with a decrease in pH that could stop the glycolytic pathway.

The total lactate formation in man (70 kg) ≈ 1.3 mol / d

Of this, 25 % comes from erythrocytes,
25 % from skin,
about 14 % each from muscle,
brain and
renal medulla,
8 % from intestinal mucosa.

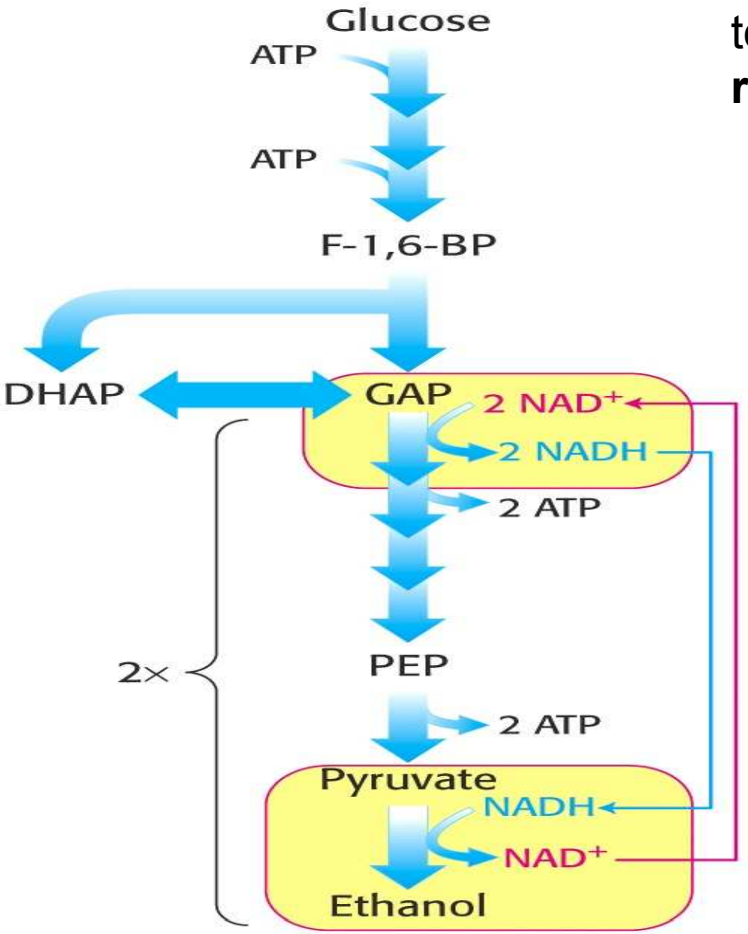
The lactate concentration in blood is normally around **1 mmol / l**; it can rise to about 30 mmol / l during vigorous exercise, but quickly falls when exercise ceases.

The reconversion of lactate (gluconeogenesis) in the liver - the Cori cycle

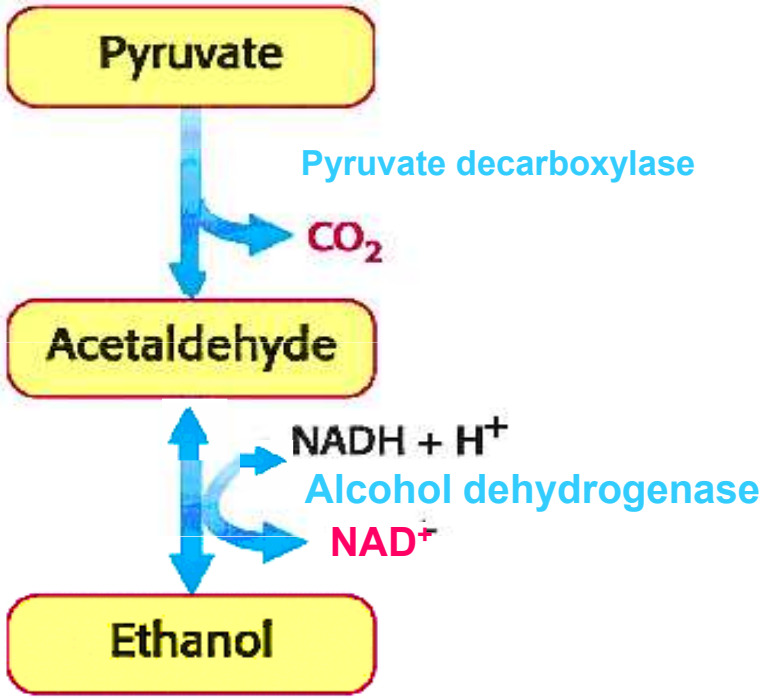


Alcoholic fermentation of glucose

in yeasts (obligatory anaerobic organisms) also produces pyruvate. The difference between anaerobic glycolysis and alcoholic fermentation is in the process of **reoxidation of NADH**:



Pyruvate is a subject of simple decarboxylation to acetaldehyde, NADH is reoxidized through **reduction of acetaldehyde to ethanol.**



Energetic yield of glycolysis and aerobic breakdown of glucose

GLYCOLYSIS

Stage 1: two molecules ATP are consumed
Stage 3: four molecules ATP are formed by substrate-level phosphorylations

Net yield: **2 molecules ATP / 1 molecule glucose** (i.e. 2 pyruvates)

AEROBIC BREAKDOWN of glucose to CO_2

Glycolysis:	(by substrate-level phosphorylations)	2 molecules ATP
	and 2 molecules NADH *) ⇒	6 molecules ATP
The possible loss due to redox shuttle transport		– 2 molecules ATP

Oxidative decarboxylation of two pyruvates:

	2 molecules NADH ⇒	6 molecules ATP

Decomposition of 2 acetyl CoA in the citrate cycle:

⇒	the overall yield	24 molecules ATP

Net yield: **36 – 38 molecules ATP / 1 molecule glucose**

*) Supposing that reoxidation of NADH will give 3 ATP and $FADH_2$ 2 ATP (in spite of the lower values are referred to in recent literature).

The control of glycolysis

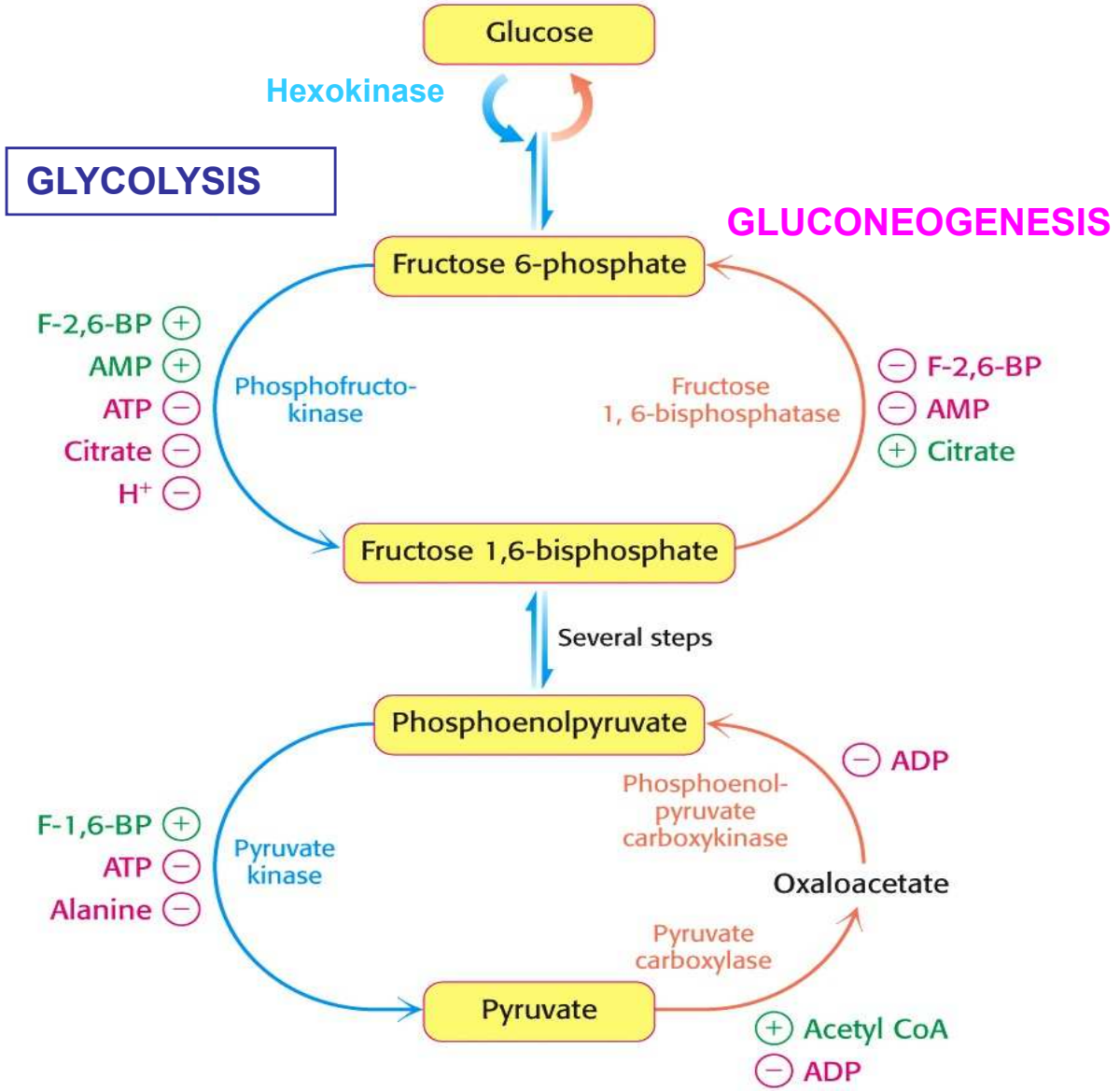
Three control points

are the three irreversible reactions of glycolysis catalysed by

1 hexokinase,

2 phosphofructokinase 1,

3 pyruvate kinase.

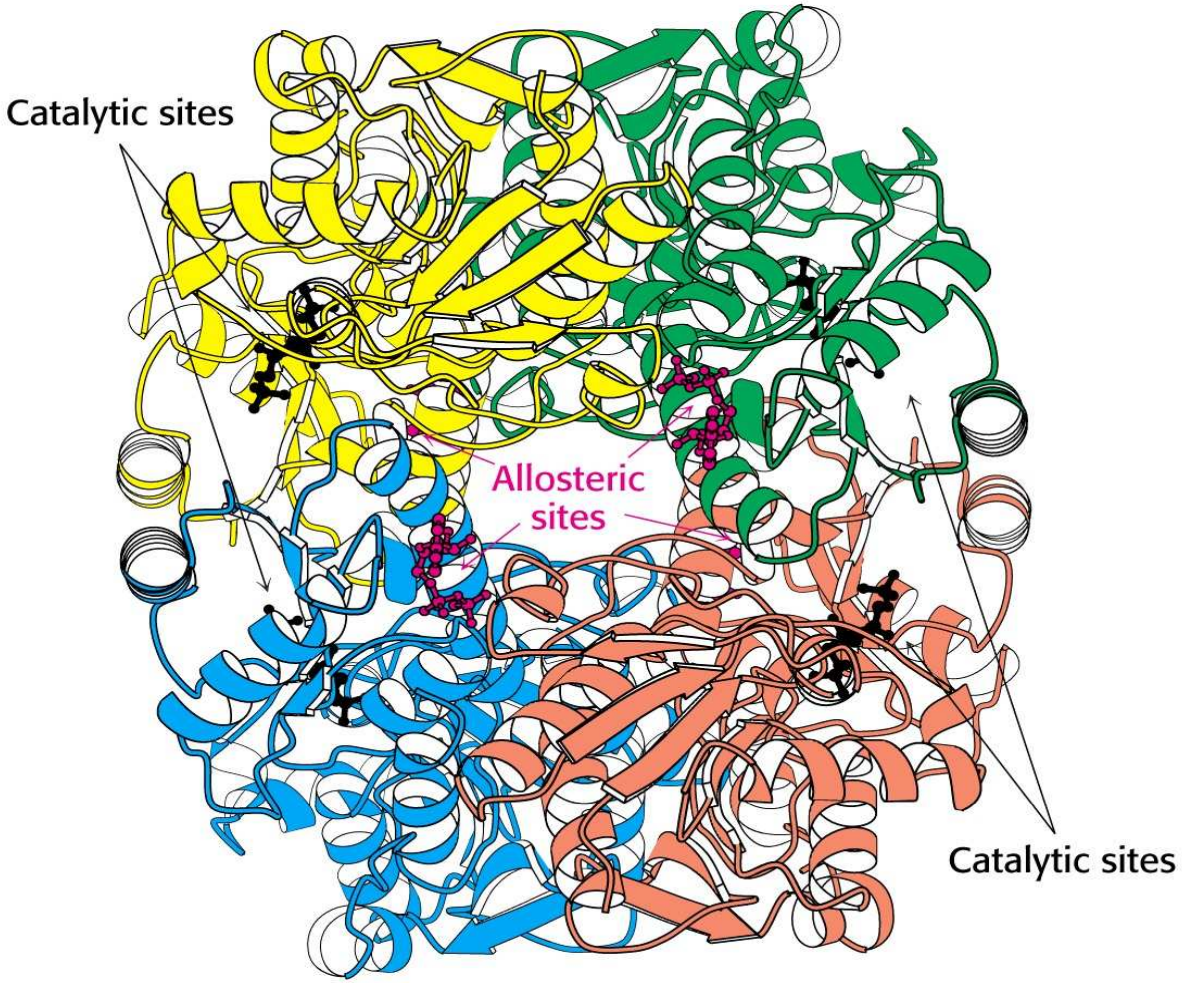


1 Hexokinase(s)

present **in the extrahepatic tissues** are **inhibited by glucose 6-phosphate, the reaction product.**

High concentration of this molecule signal that the cell no longer requires glucose for energy, for storage in the form of glycogen, or as a source of biosynthetic precursors, and the glucose will be left in the blood.

2 Phosphofructokinase is the key enzyme in the control of glycolysis



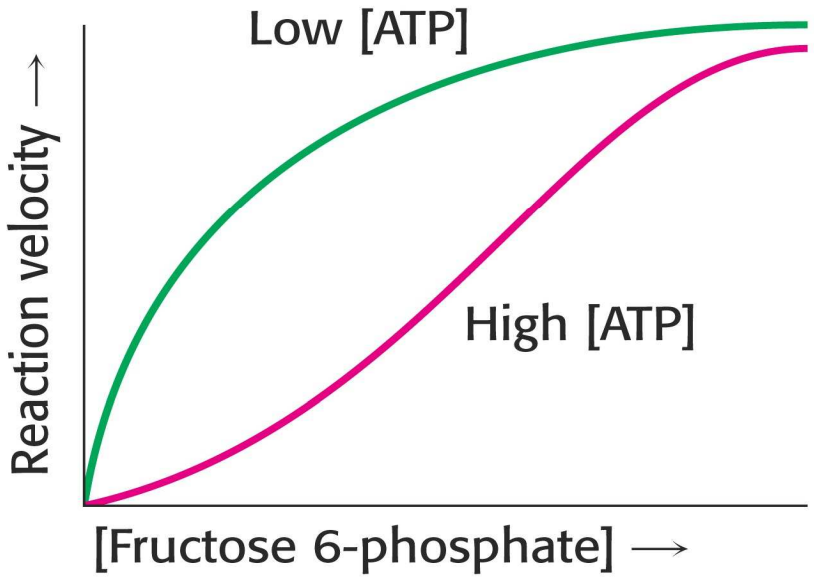
Phosphofructokinase (PFK) in the liver is a tetramer of four identical subunits.

The positions of **catalytic** and **allosteric sites** are indicated.

Allosteric inhibition of PFK by ATP

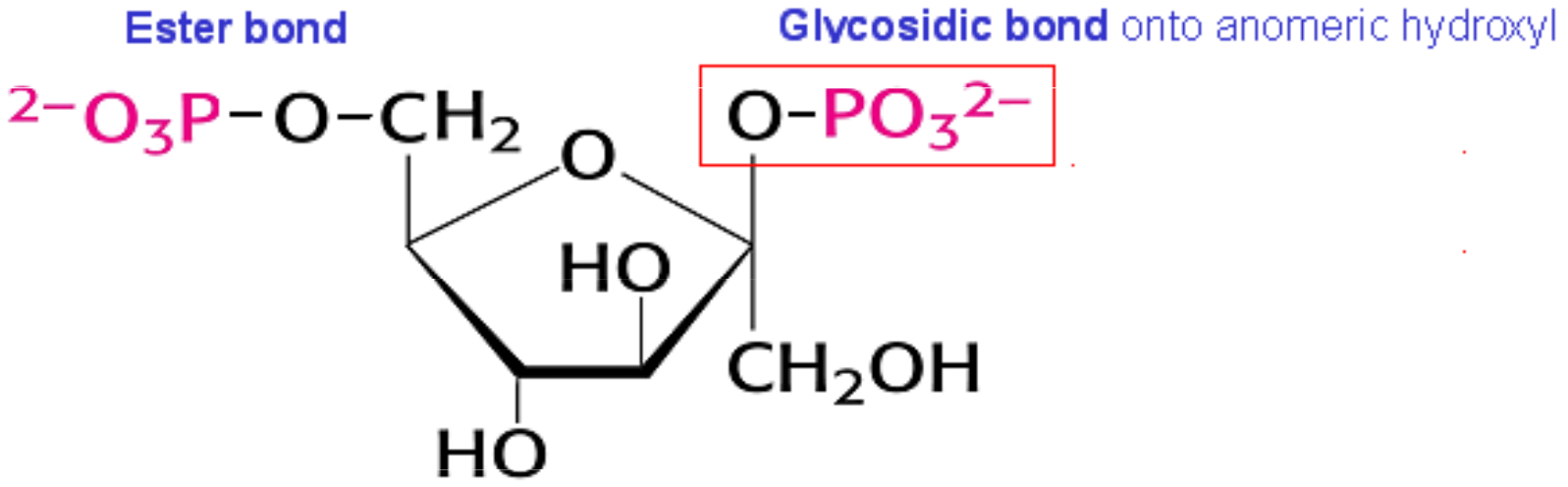
ATP as a substrate of the PFK catalyzed reaction binds to the catalytic site.
At high concentration of ATP it also binds to a specific regulatory site that is distinct from the catalytic site and allosterically inhibits the PFK activity.

AMP reverses the inhibitory action of ATP –
glycolysis is stimulated as the energy charge falls.

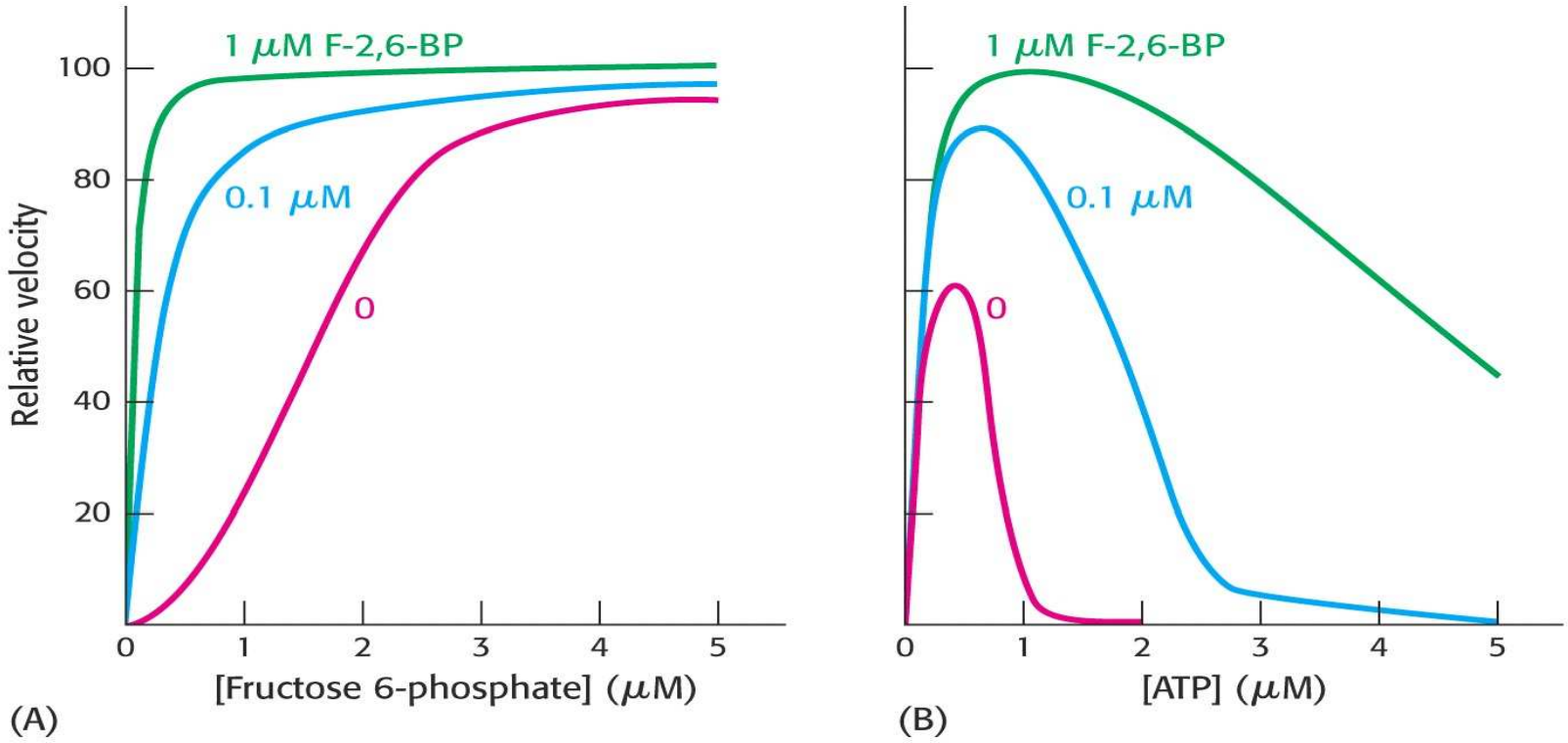


A fall in pH value also **inhibits PFK** activity – inhibition by H^+ prevents excessive formation of lactic acid and a drop in blood pH.

Allosteric activation of phosphofructokinase by fructose 2,6-bisphosphate



Fructose 2,6-bisphosphate (Fru-2,6-P_2)



(A) Allosteric activation of PFK by Fru-2,6-P_2

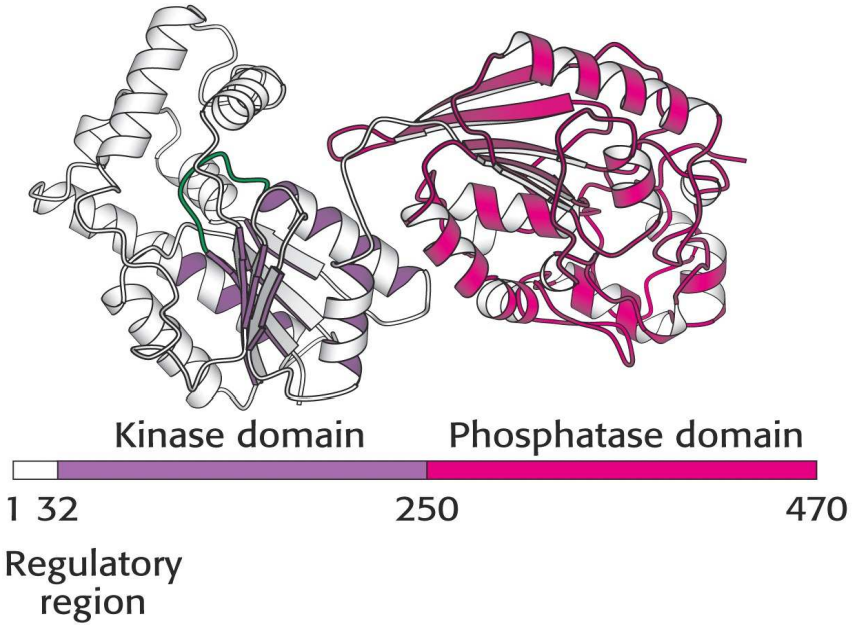
(B) The inhibitory effect of ATP is reversed by Fru-2,6-P_2

The concentration of Fru-2,6-P_2 is controlled by a **regulated bifunctional enzyme.**

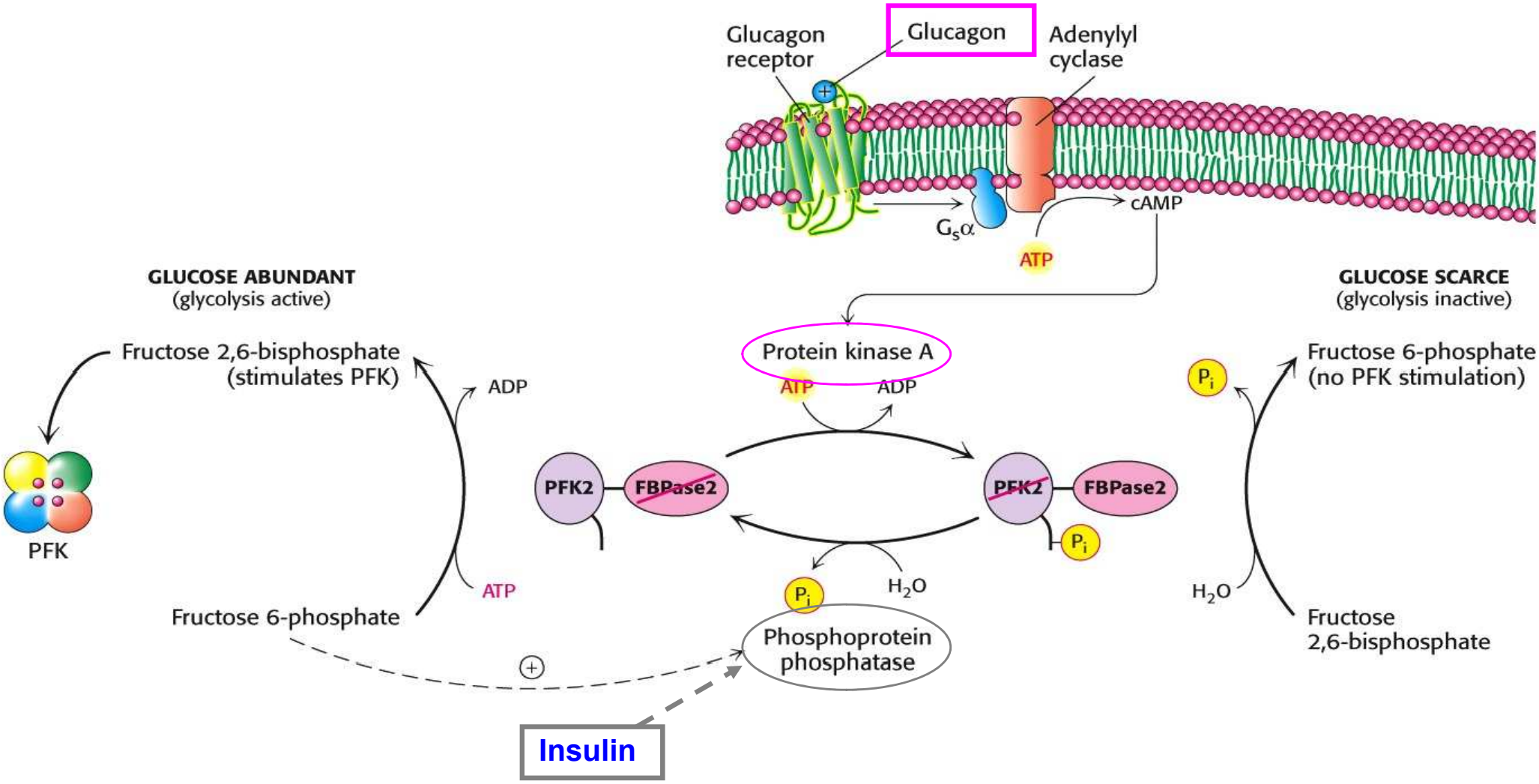
Fru-2,6-P_2 is formed in a reaction catalyzed by **phosphofructokinase 2**,

and hydrolyzed to Fru-6-P by a specific phosphatase **fructose bisphosphatase 2**.

Both activities are present in a single polypeptide chain:

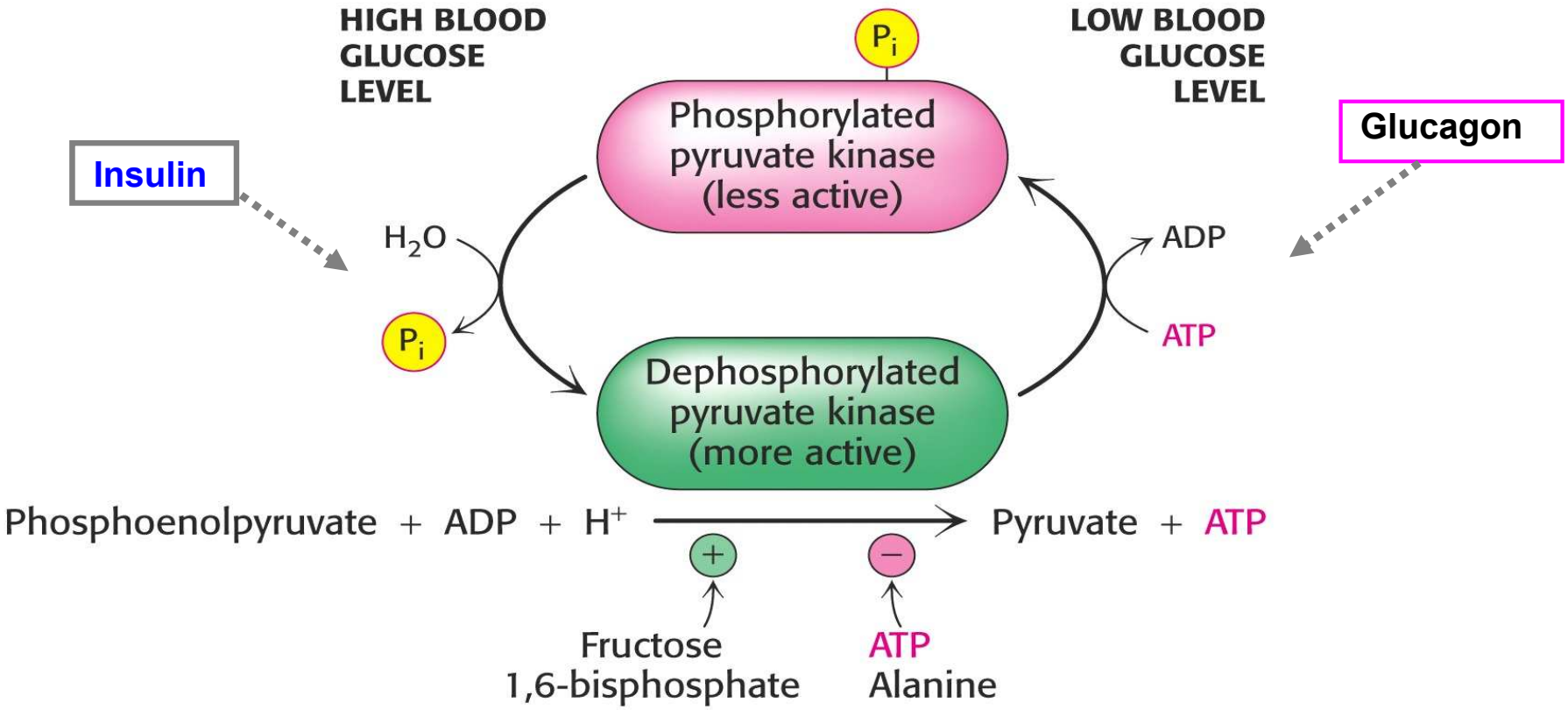


Control of the bifunctional enzyme by phosphorylation and dephosphorylation



3 Control of pyruvate kinase activity

- by phosphorylation and dephosphorylation
- by allosteric effectors



Oxidative decarboxylation of pyruvate

and of other 2-oxocarboxylic acids

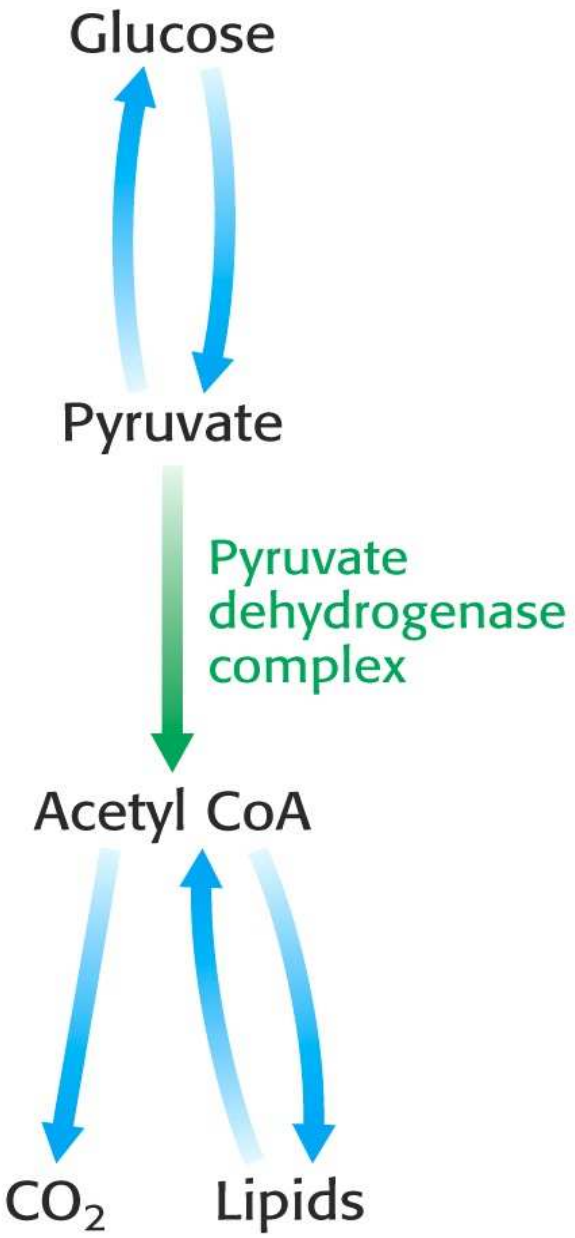
The synthesis of acetyl-CoA by the pyruvate dehydrogenase complex Is a key irreversible step in the metabolism of glucose.

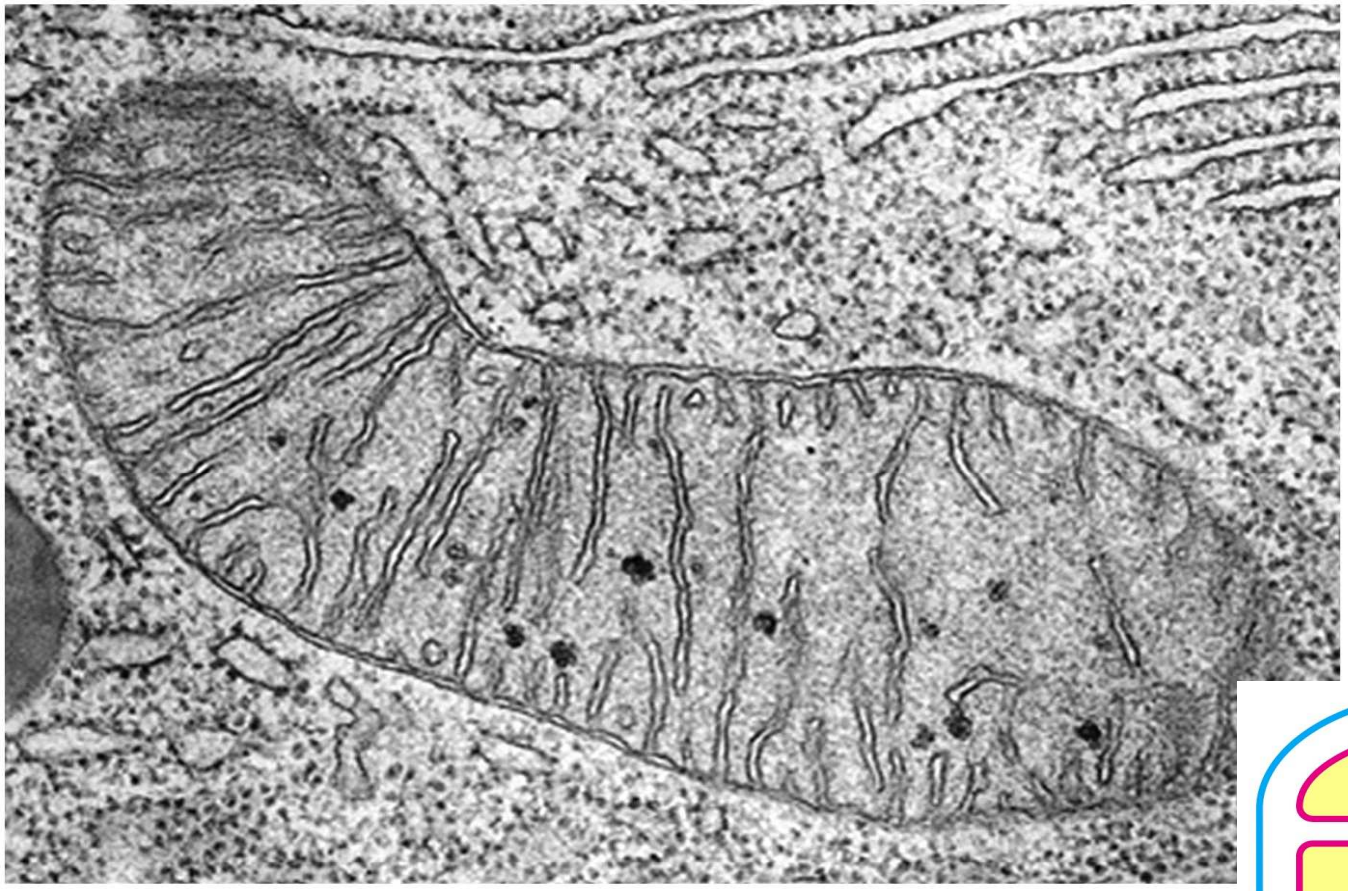
The oxidative decarboxylation of pyruvate takes place within the matrix of mitochondrion.

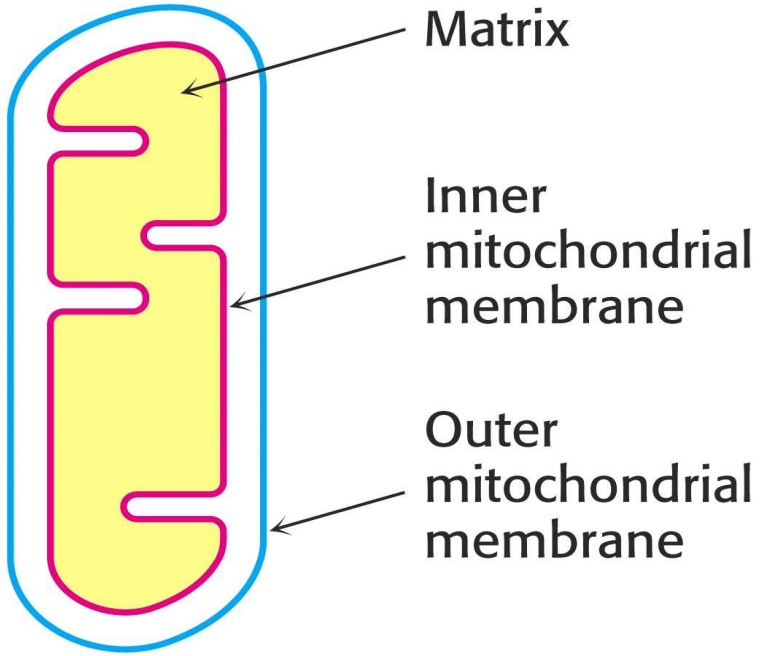
Under aerobic conditions, the pyruvate is **transported into mitochondria** in exchange for OH^- by the pyruvate carrier, an antiporter.

Pyruvate + CoA + NAD^+ $\rightarrow$

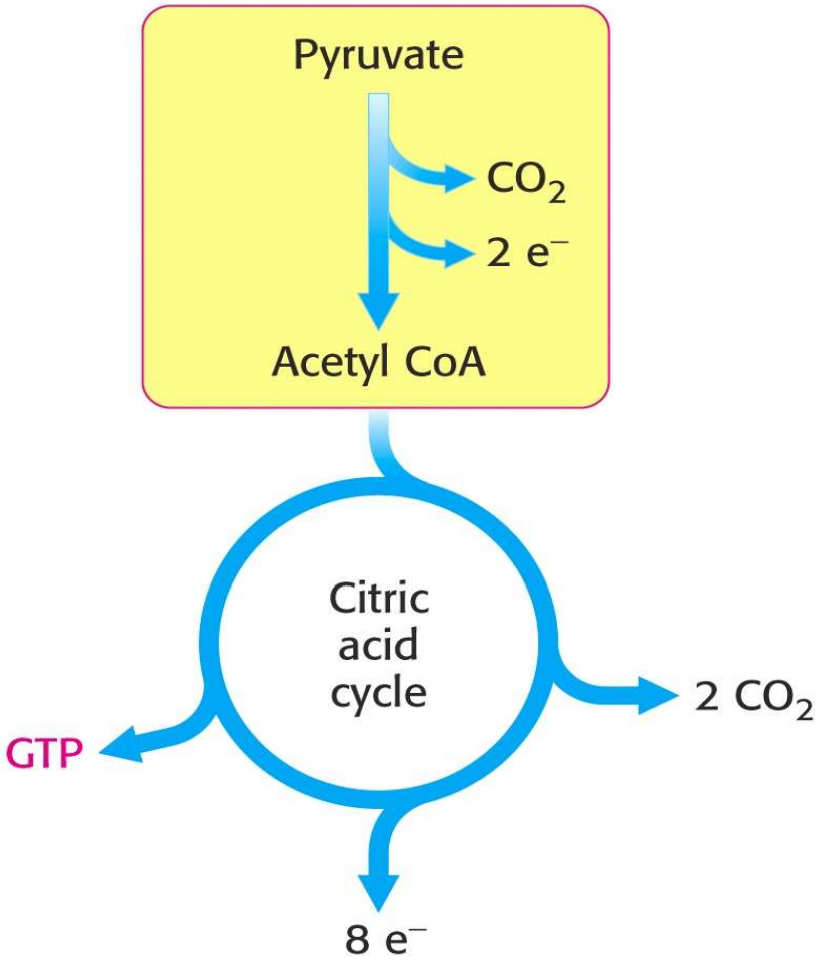
$\rightarrow$ acetyl CoA + CO_2 + NADH



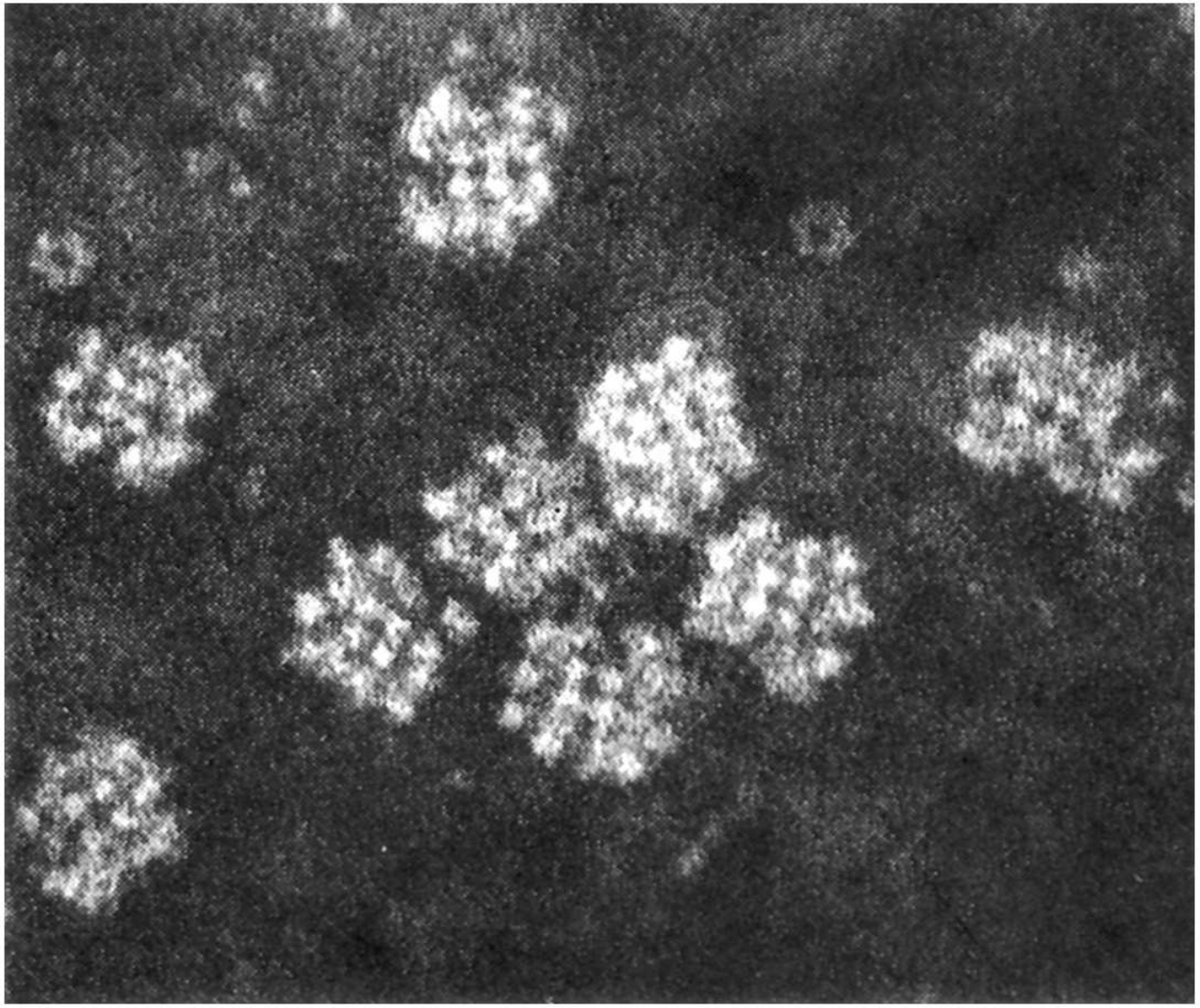



Matrix
Inner mitochondrial membrane
Outer mitochondrial membrane

Oxidative decarboxylation of pyruvate represents the link between glycolysis and the citric acid cycle.

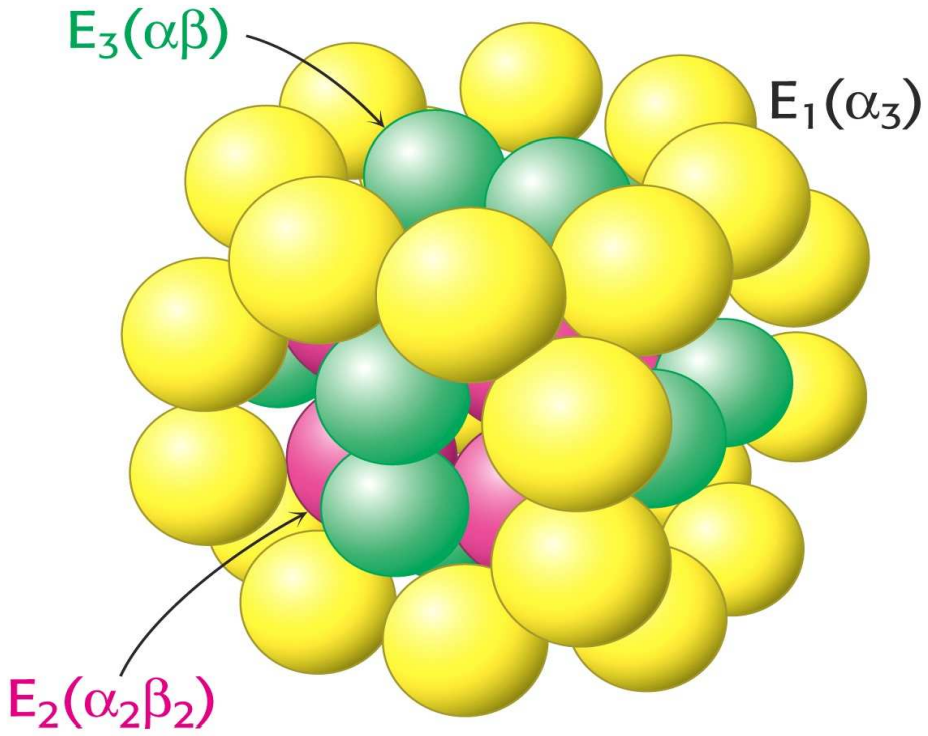


Pyruvate produced by glycolysis is converted into acetyl CoA, the substrate (fuel) for the citric acid cycle.



Electron micrograph of the **pyruvate dehydrogenase complex** from *E. coli*

Pyruvate dehydrogenase complex – schematic representation



The three enzymes of the complex:

E_1 – the **decarboxylating component** of the dehydrogenase
E_2 – the **transacetylase** core
E_3 – **dihydrolipoyl dehydrogenase**

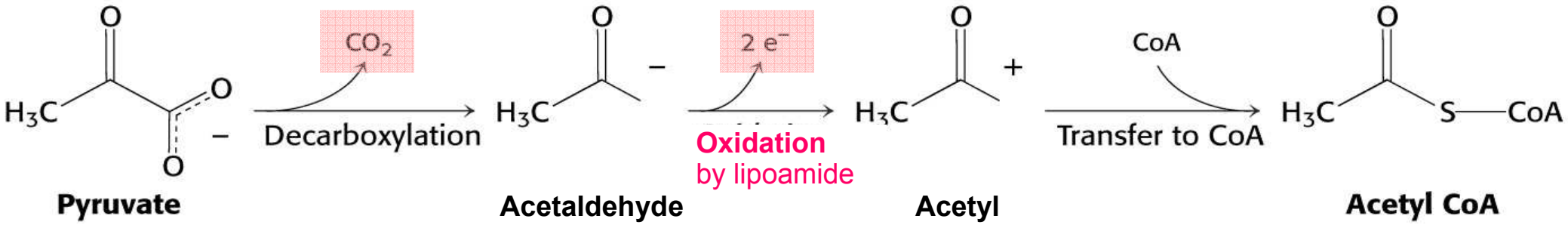
Pyruvate dehydrogenase complex of *E. coli*

Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	24	Lipoamide	Transfer of the acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	12	FAD	Regeneration of the oxidized form of lipoamide

The enzyme complex requires the participation of **five coenzymes**:

Thiamine diphosphate
Lipoamide (lipoate attached to the E_2 by an amide linkage to lysyl)
Coenzyme A
FAD (flavin adenine dinucleotide)
NAD^+

Steps in the oxidative decarboxylation of pyruvate



Decarboxylating component **Transacetylase**

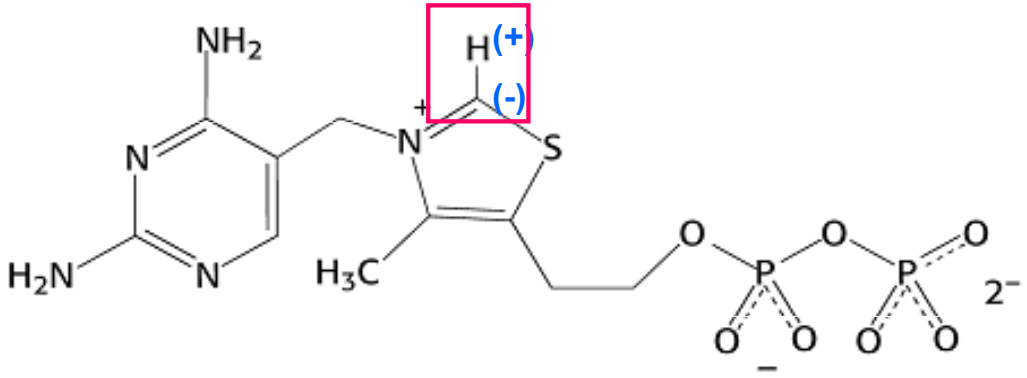
E_1 **E_2**

Reoxidation of dihydrolipoamide to lipoamide
(2 hydrogen atoms accepted by FAD and then
by NAD^+ resulting in NAD**H** + **H^+**)

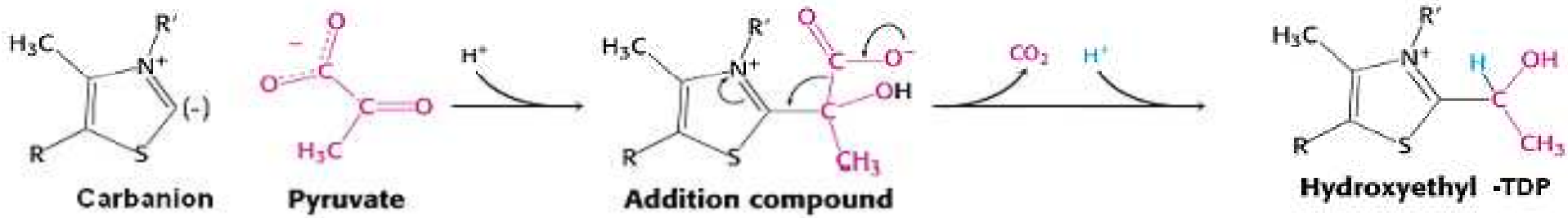
Dihydrolipoyl dehydrogenase

E_3

Decarboxylating component of pyruvate dehydrogenase E_1 contains bound **thiamine diphosphate (TDP):**

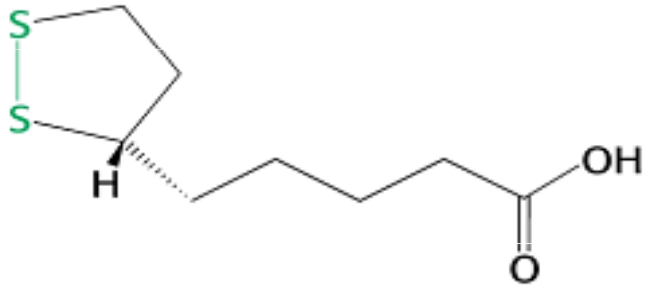


The thiazole ring of the coenzyme TDP binds pyruvate. The product of decarboxylation is **acetaldehyde** bound onto TDP in the form of **α-hydroxyethyl:**

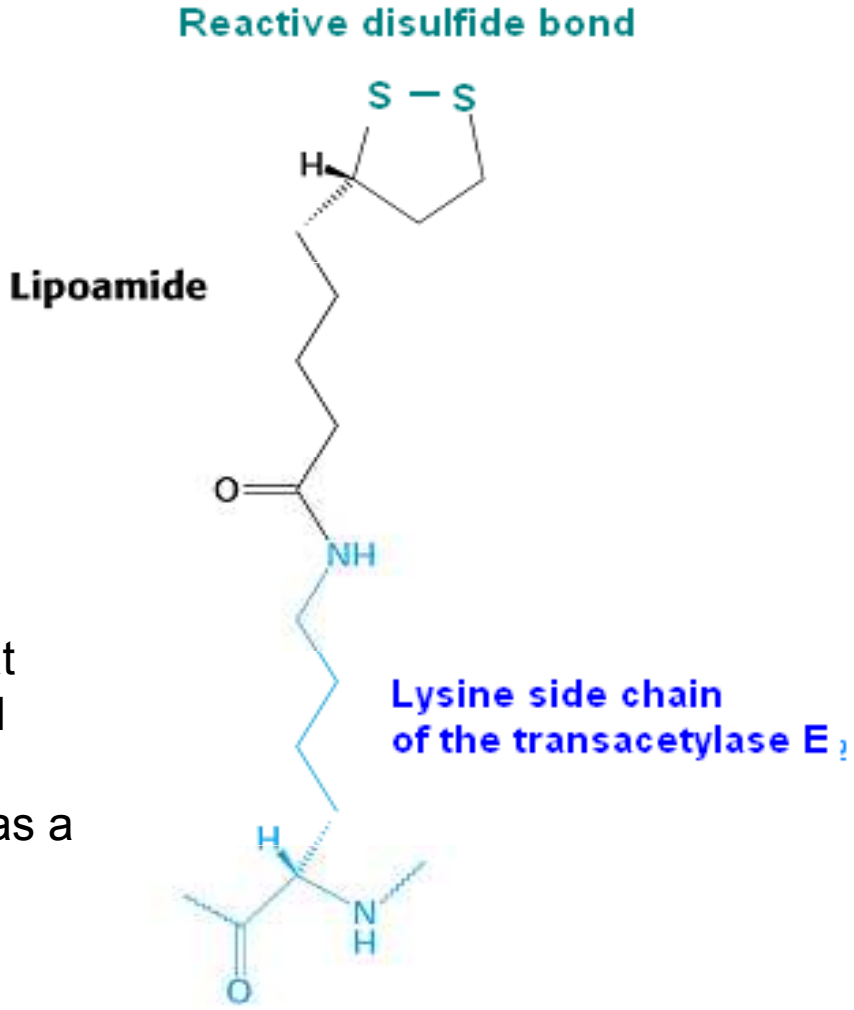


E_1 catalyses the **transfer of α-hydroxyethyl to the lipoyl** arm of transacetylase E_2.

Transacetylase E_2 contains bound lipoic acid that is attached to the amino group of the side chain of certain lysyl residue. That is why it is named **lipoamide**.



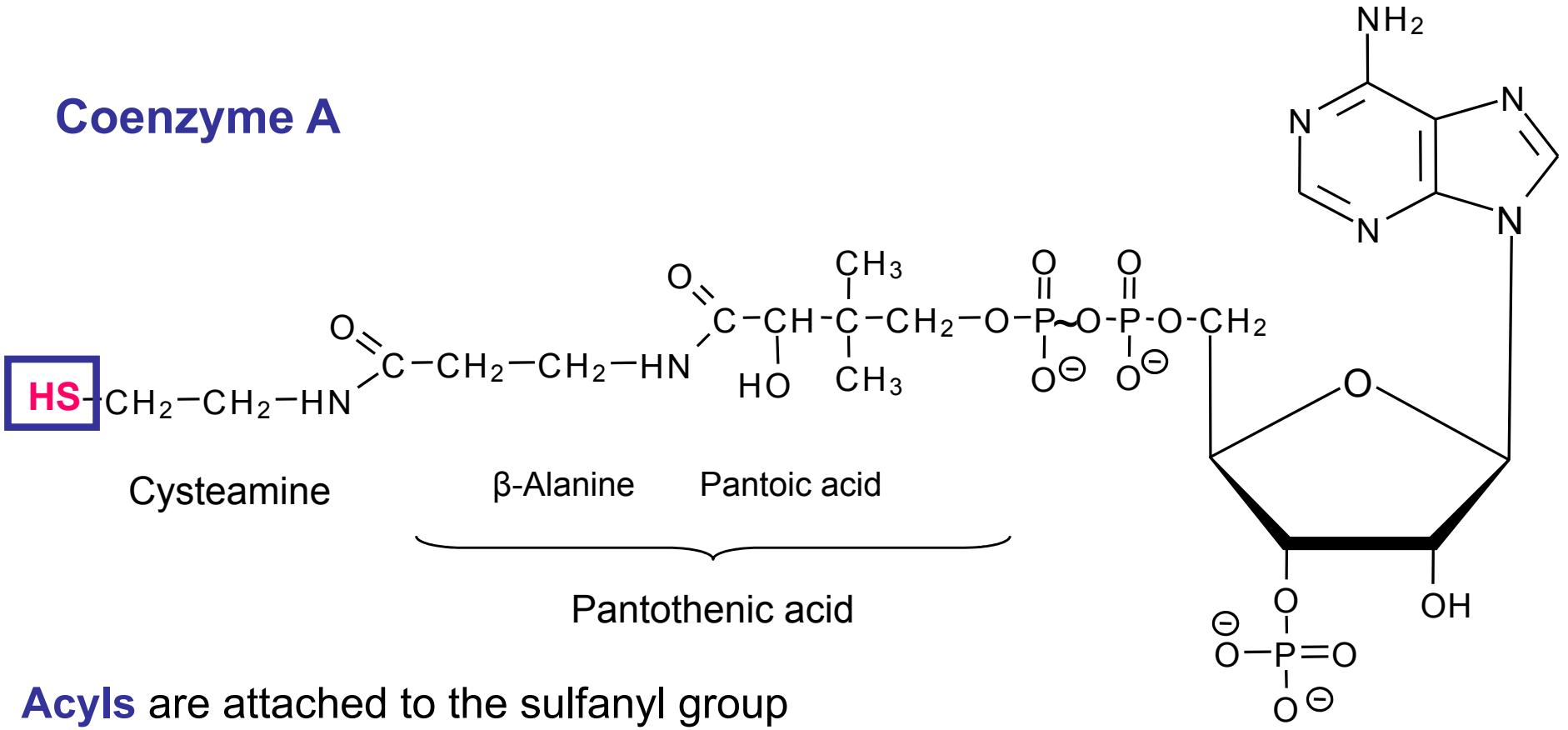
Lipoic acid



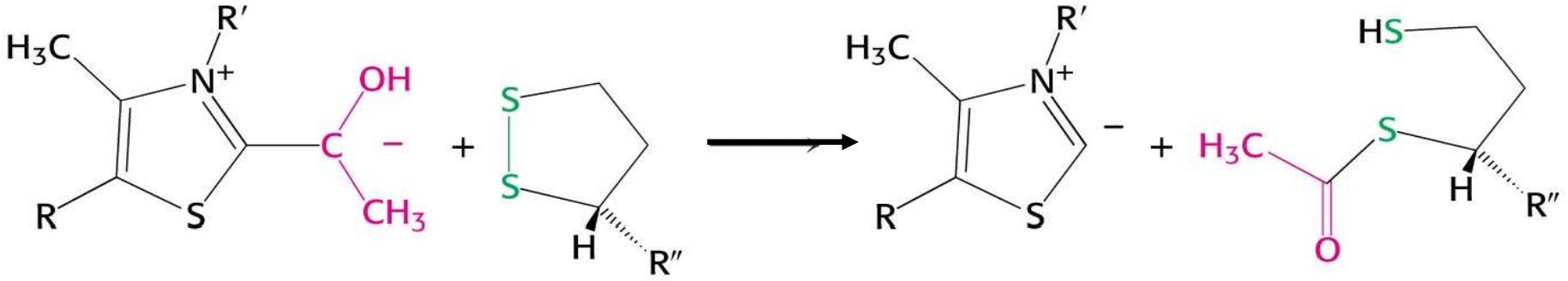
Lipoamide (oxidized form, a disulfide) acts as an arm that accepts the hydroxyethyl group from TDP. **Hydroxyethyl group** ("activated acetaldehyde") reduces lipoamide to dihydrolipoamide and thus **is oxidized to acetyl** bound as a thioester - 6-acetyllipoamide.

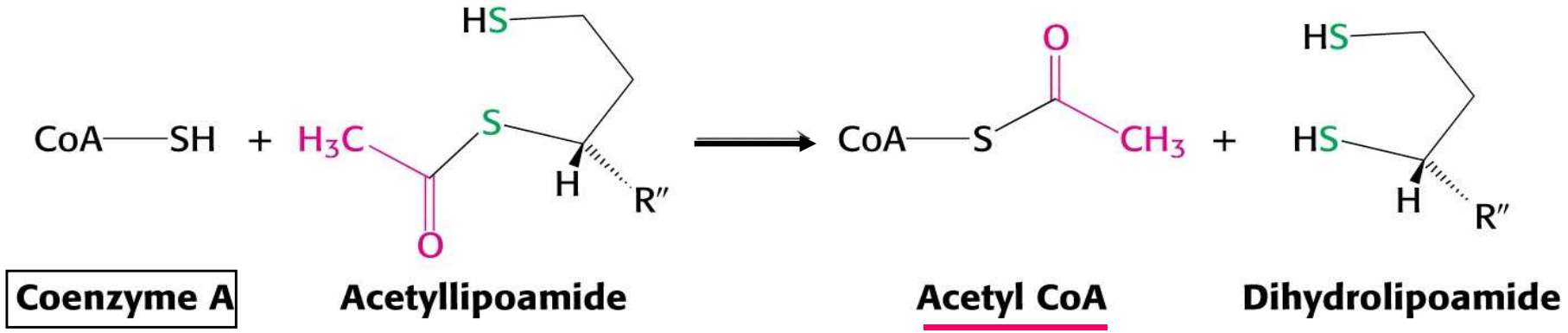
The acetyl is then transferred to coenzyme A :

Coenzyme A



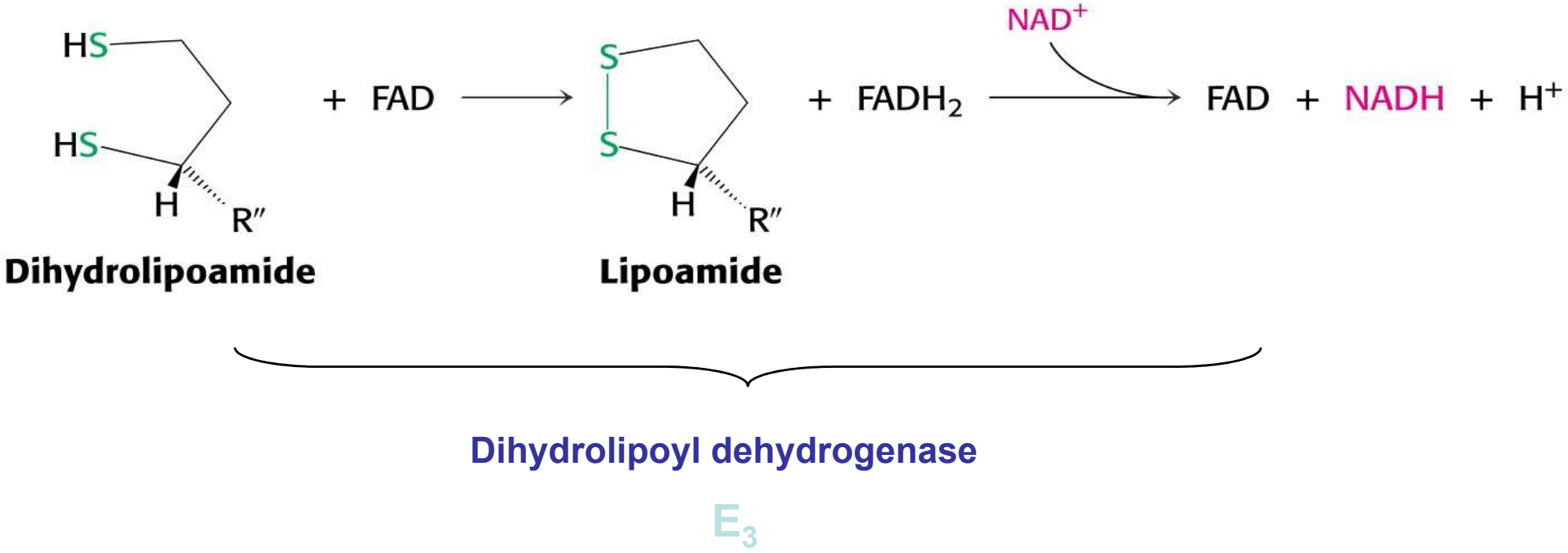
Acyls are attached to the sulfanyl group by means of a **thioester bond**.


Hydroxyethyl· TDP
(ionized form)
Lipoamide
Carbanion of TDP
Acetyllipoamide

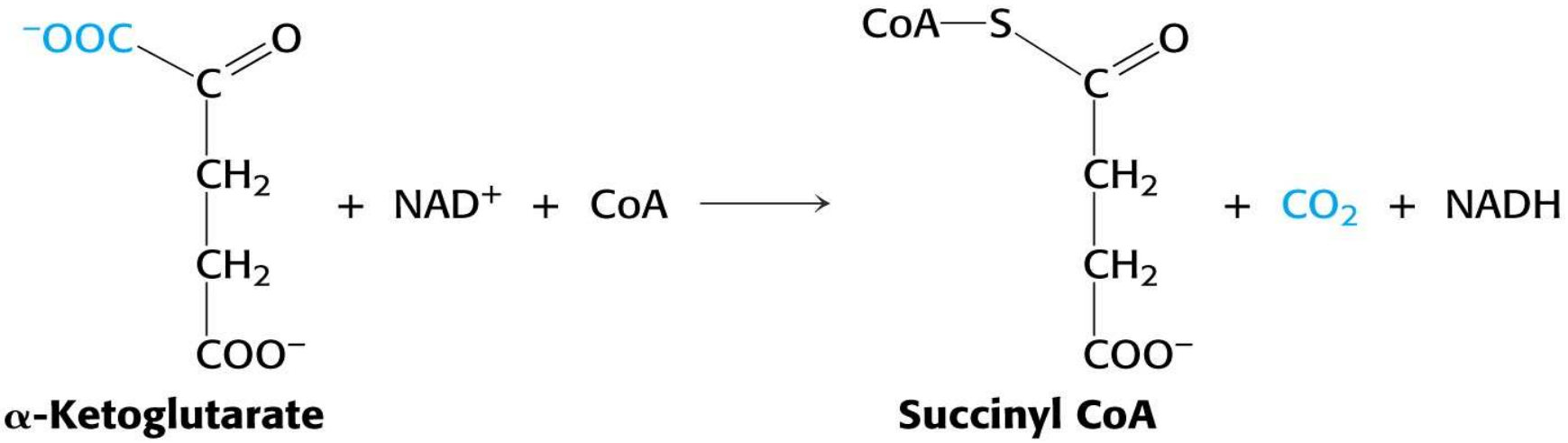

Coenzyme A
Acetyllipoamide
Acetyl CoA
Dihydrolipoamide

The dihydrolipoyl arm then swings to E3, where it is reoxidized.

Dihydrolipoyl dehydrogenase E_3 contains bound **coenzyme FAD** that accepts two hydrogen atoms which are passed on to **NAD^+**..

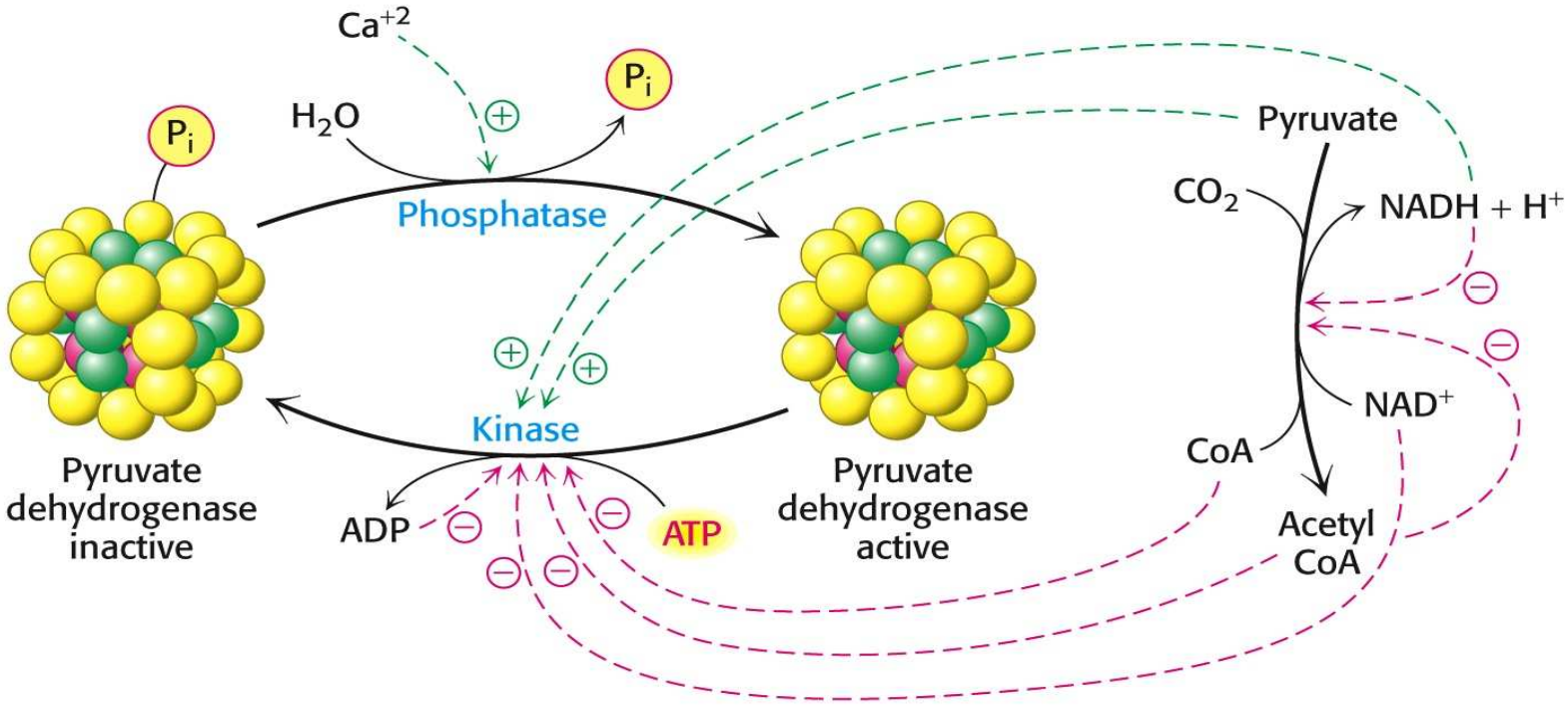


In the citrate cycle, the **oxidative decarboxylation of 2-oxoglutarate** (to succinyl CoA) closely resembles that of pyruvate:



The **2-oxoglutarate dehydrogenase complex** consists of $\mathbf{E_1}$ (decarboxylating 2-oxoglutarate) and $\mathbf{E_2}$ (**transsuccinylase**) components different from but homologous to the corresponding enzymes in the pyruvate dehydrogenase complex, whereas $\mathbf{E_3}$ (**dihydrolipoyl dehydrogenase**) components of the two complexes are identical.

Regulation of the pyruvate dehydrogenation complex



Inhibition - by the immediate products **NADH** and **acetyl-CoA**,
- by **ATP**, and
- by **phosphorylation** (depending e.g. on glucagon)

Activation by **dephosphorylation** (depending on insulin)