Welcome to BCII lesson 4

Chimie Biologique II

Biological Chemistry II

BIO-213

Teacher

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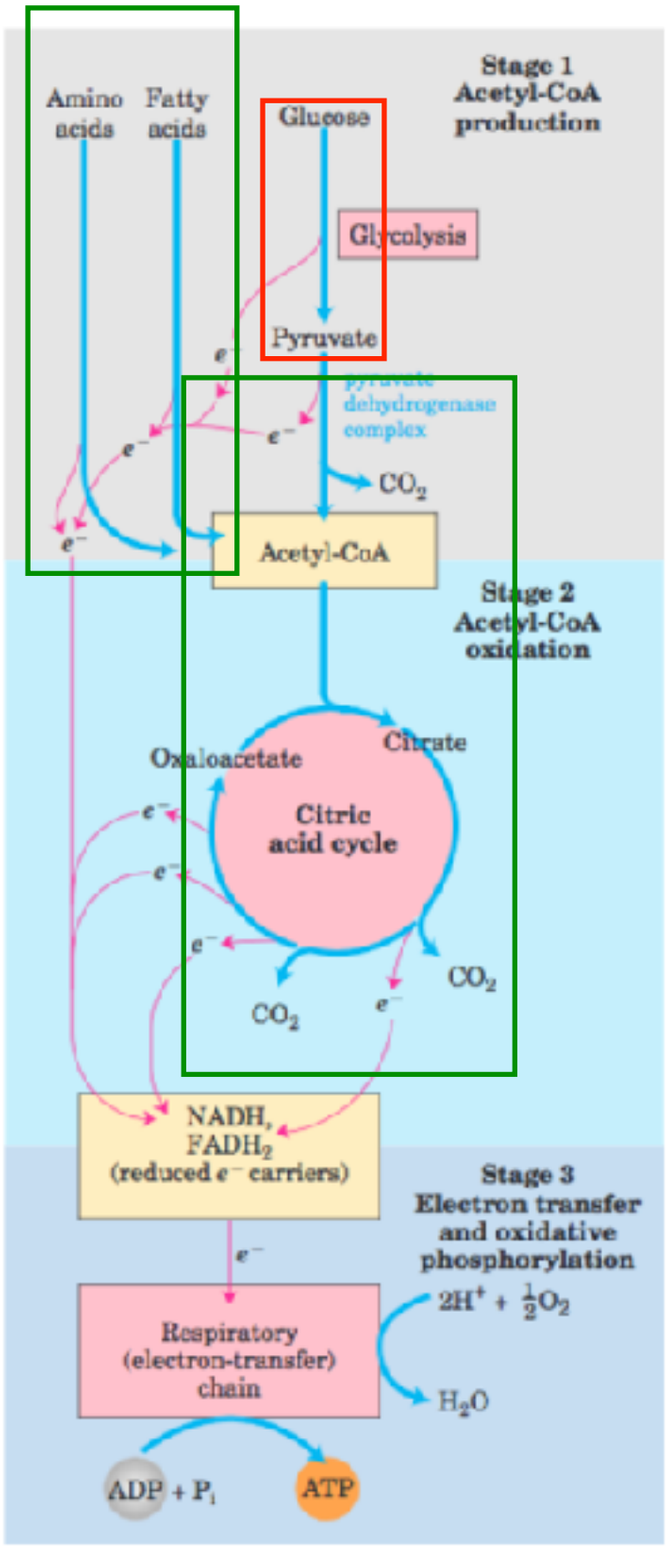
Lecture 4

The TCA Cycle
Fatty acid Oxidation
Amino Acids Oxidation

Catabolic Stages



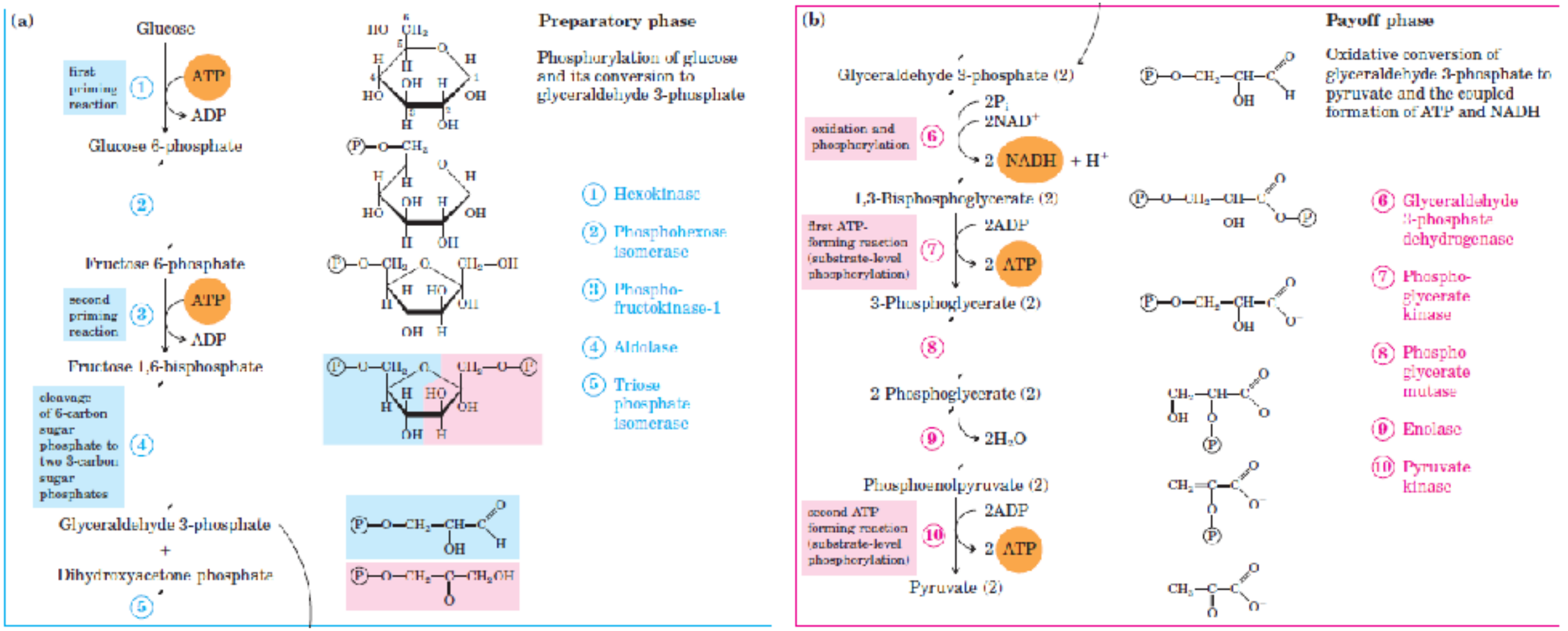
The three stages of the catabolism of proteins fatty acids and carbohydrates.

Stage 1: The oxidation of fatty acids, glucose, and some amino acids yields Acetyl-CoA

Stage 2: The oxidation of the acetyl groups in the citric acid (or Kerbs or tricarboxylic acids or TCA) cycle includes 4 steps where electrons are transferred to electron carriers

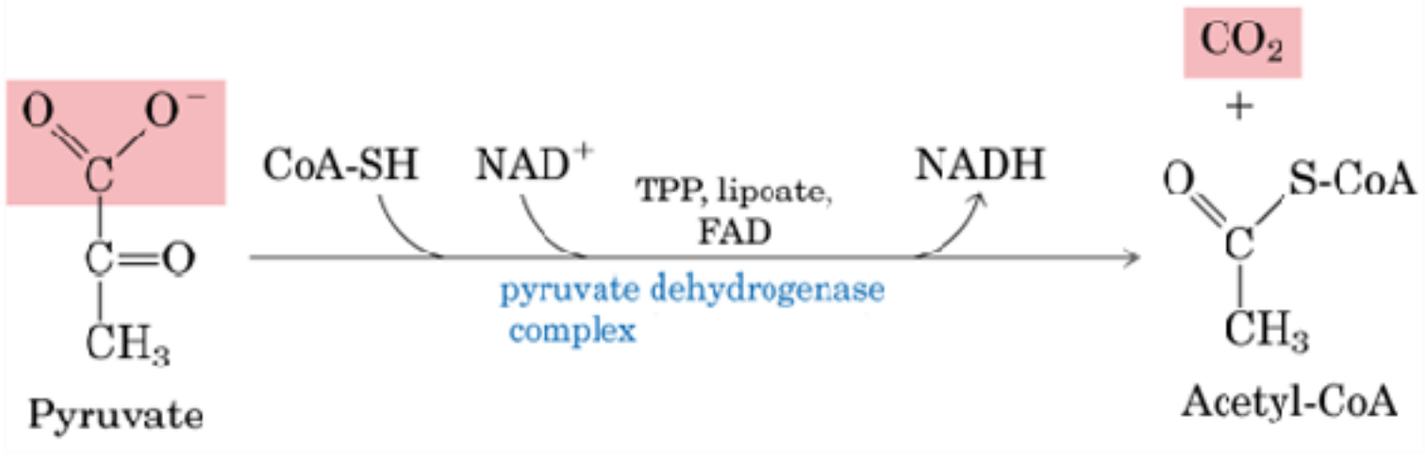
Stage 3: Electrons carried by NADH and $FADH_2$ are funnelled into a chain of mitochondrial (or plasma membrane in bacteria) electron carriers, ultimately reducing O_2 to H_2O. This electron flow produces ATP

From glycolysis to TCA cycle



Glucose + 2ADP + 2NAD⁺ + 2Pi => 2 Pyruvate + 2ATP = 2NADH + 2H⁺ + 2 H_2O

Pyruvate => Acetyl-CoA



Once produced in the cytosol, pyruvate migrates into the mitochondrial matrix through the action of **pyruvate translocases** that mediate the transport of pyruvate across mitochondrial membranes.

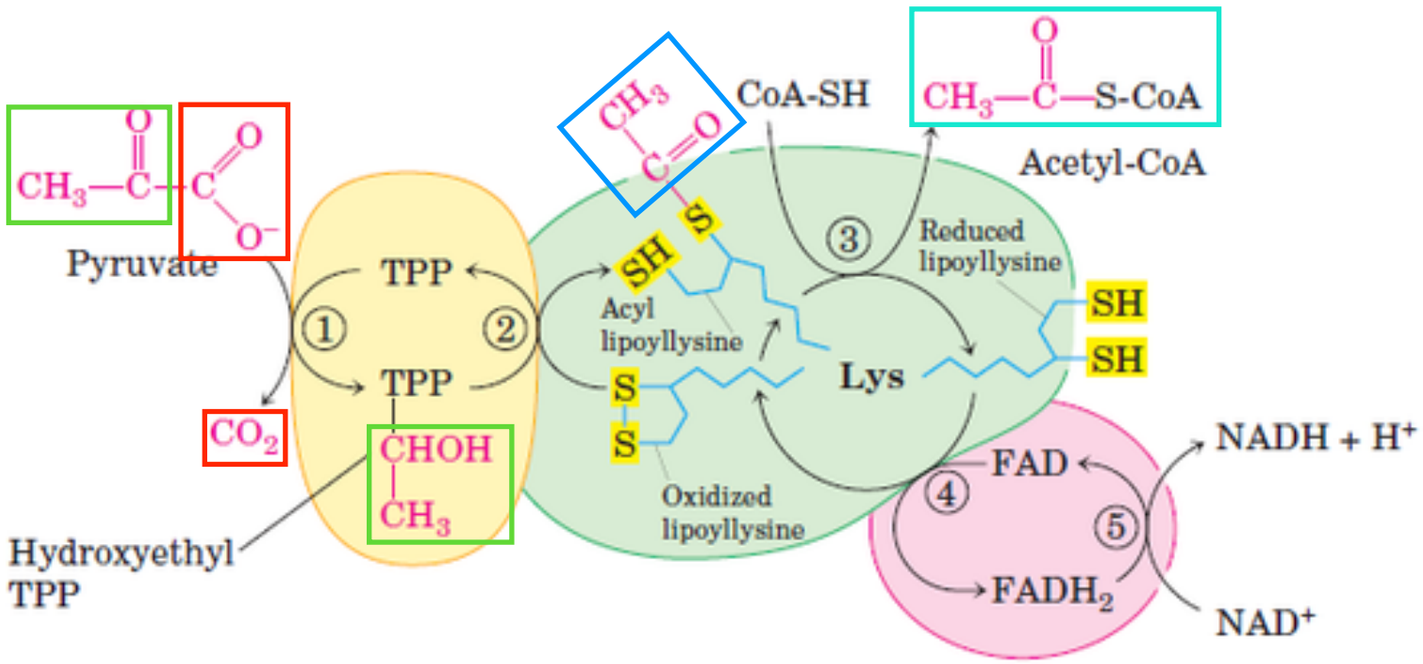
In the mitochondria, pyruvate is decarboxylalated (loses a CO2 molecule) by the action of an enzymatic complex called the **Pyruvate dehydrogenase complex** to produce Acetyl-CoA and an NADH molecule.

The Pyruvate dehydrogenase complex is a large, multi enzymatic complex that consists of **three important enzymes** and uses **five different co-enzymes**.

1. Pyruvate dehydrogenase (E1): Catalyses the redox-decarboxylation reaction.
2. Dihydrolipoyl transacetylase (E2): Catalyses the transfer of the acetyl group.
3. Dihydrolipoyl dehydrogenase (E3): Reforms the oxidised version of lipoamide.

In addition to these enzymes, there are five co-enzymes acting in this process. These are thiamine pyrophosphate (TPP), lipoic acid, FAD, CoA, and NAD+.

Pyruvate => Acetyl-CoA



In the first step, **decarboxylation** takes place (an exergonic reaction), and the C2 atom of what was pyruvate is linked to TPP in the form of a hydroxyethyl derivative (**E1**)

In the second step the hydroxyethyl derivative is **oxidised** to acetate with a concomitant reduction of the S-S bond in lipoic acid bound to a lysine in E2. The acetate here is **esterified** to one of the 2 SH groups of the reduced lipoic acid (**E1**)

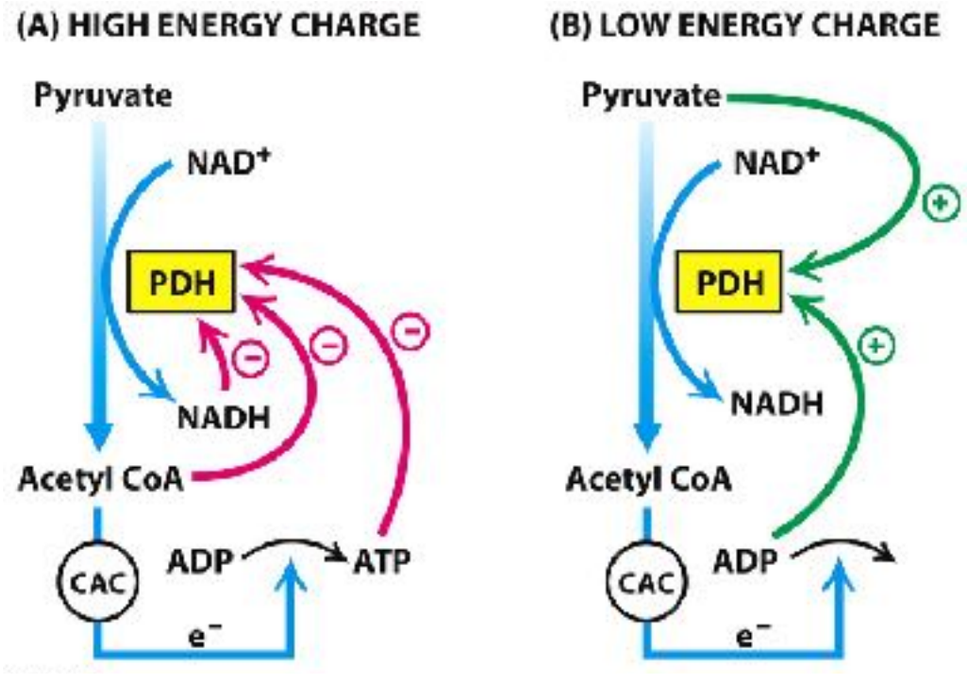
In the third step, the acetate is **trans-esterified** to the SH group of CoA-SH thus generating acetyl-CoA and a fully reduced form of lipoic acid (**E2**)

In the forth step, lipoic acid is **oxidised** to reform thee S-S bond and 2 hydrate groups are transferred to FAD, which is **reduced** to FADH2 (**E3**)

In the fifth step, FADH2 **transfers electrons** to NAD+ to produce NADH and H+ (**E3**)

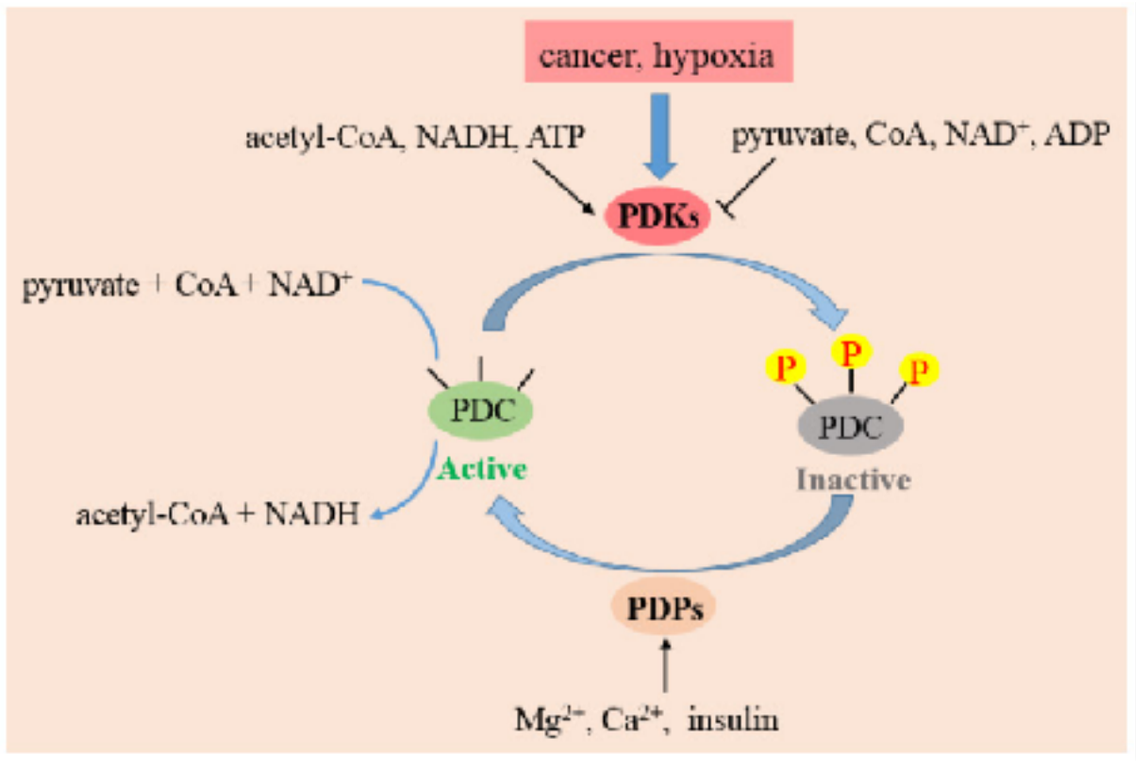
Pyruvate + CoA-SH + NAD+ => Acetyl-CoA + CO2 +NADH + H+

Regulation of Pyruvate Decarboxylation



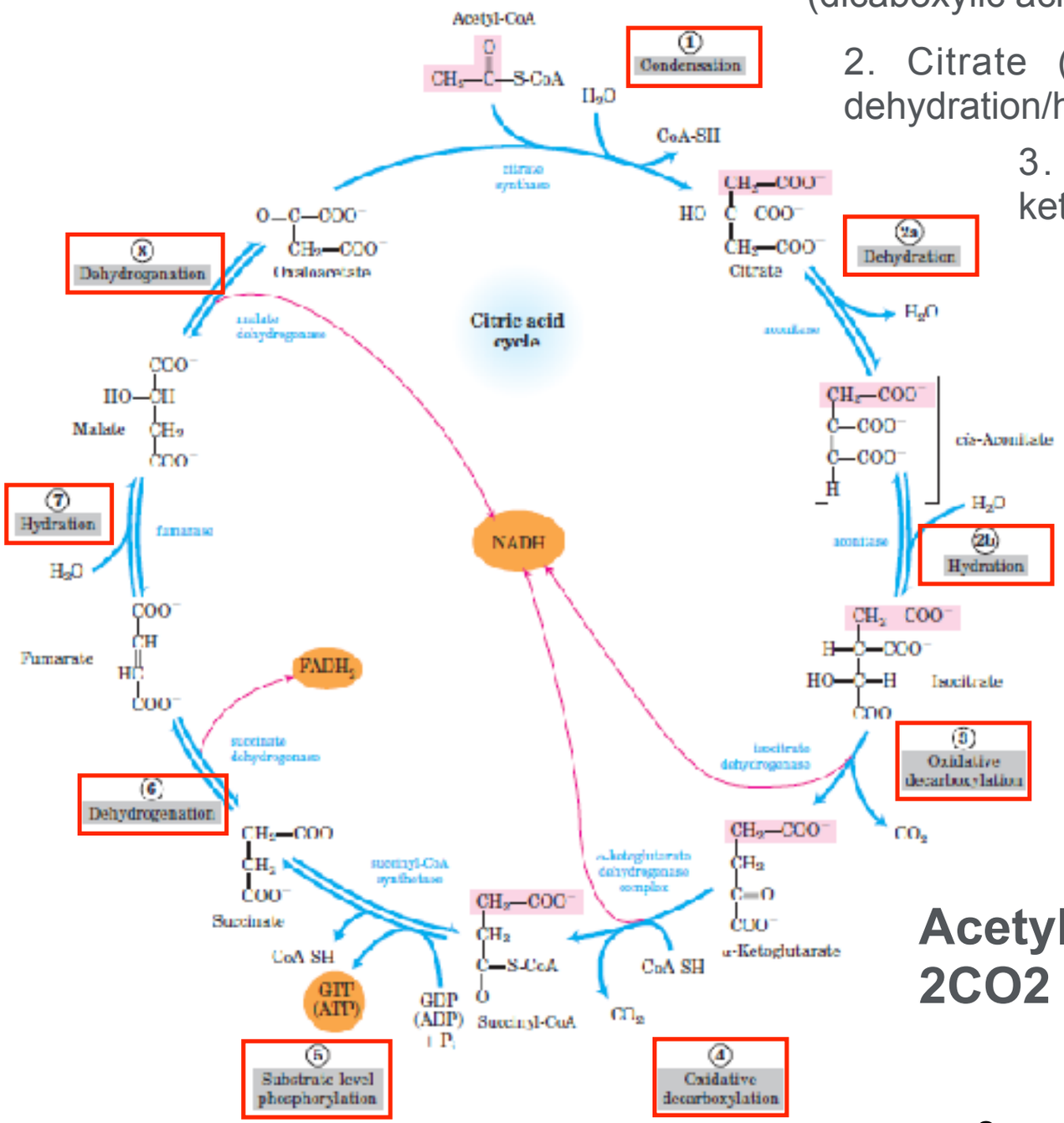
While cytosolic pyruvate can be converted back to glucose, once produced in the mitochondrial matrix Acetyl-COA is **committed** towards either the TCA cycle or lipid synthesis. Thus, the pyruvate dehydrogenase complex (PDC) catalyses a **key and irreversible** step in glucose metabolism. Not surprisingly the PDC is tightly **regulated**. **Acetyl-CoA** and **NADH**, two PDC products, **inhibit** (allosterically) E2 and E3, respectively.

Under resting conditions, the energy charge of the cell is high (high acetyl-CoA, NADH, ATP). These molecules promote the activation of PDC kinases (PDKs) that phosphorylate and inactivate E1. Under exercising conditions the energy charge of the cell is low PDKs are inhibited, also Ca++ influx in the mitochondria is increased, which activates PDC phosphatases (PDPs) that dephosphorylate and activate E1.



The citric acid cycle

The citric acid cycle (also known as the Krebs cycle, tricaboxylic acid cycle or TCA cycle) is the **centre of aerobic respiration**.



1. Acetyl-CoA (2C) is **condensed** with oxaloacetate (4C) (dicaboxylic acid) to form citrate (TCA) (6C).

2. Citrate (6C) is **isomerised** to isocitrate (6C) through dehydration/hydration

3. Isocitrate (6C) is **decarboxylated** to a-ketoglutarate (5C) with production of CO2 and NADH

4. a-ketoglutarate (5C) is **decarboxylated** to Succynil-CoA (4C) with production of CO2 and NADH

5. Succynil-CoA (4C) is **converted** to succinate (4C) with production of GTP and release of CoA-SH

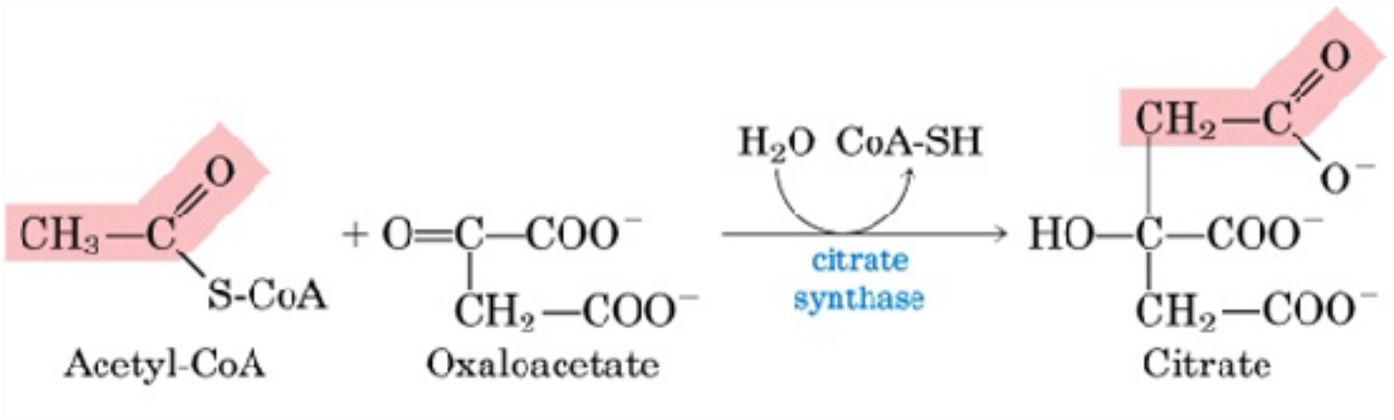
6. Succinate (4C) is **oxidised** to fumarate (4C) with production of FADH2

7. Fumarate (4C) is **hydrated** to form malate (4C)

8. Malate (4C) is **oxidised** to oxaloacetate (4C) with production of NADH

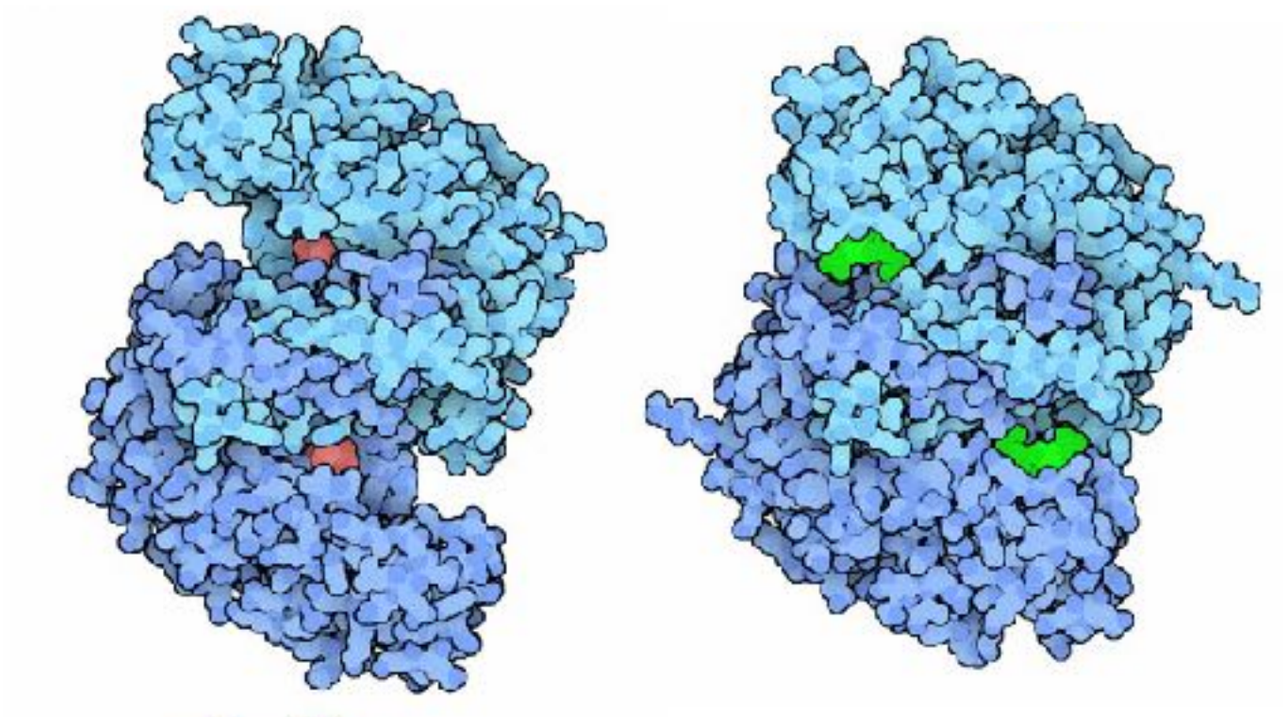
Acetyl-CoA + GDP + Pi + 3NAD+ + FAD => 2CO2 +3NADH + FADH2 + GTP+ CoA-SH

The citric acid cycle- Step1



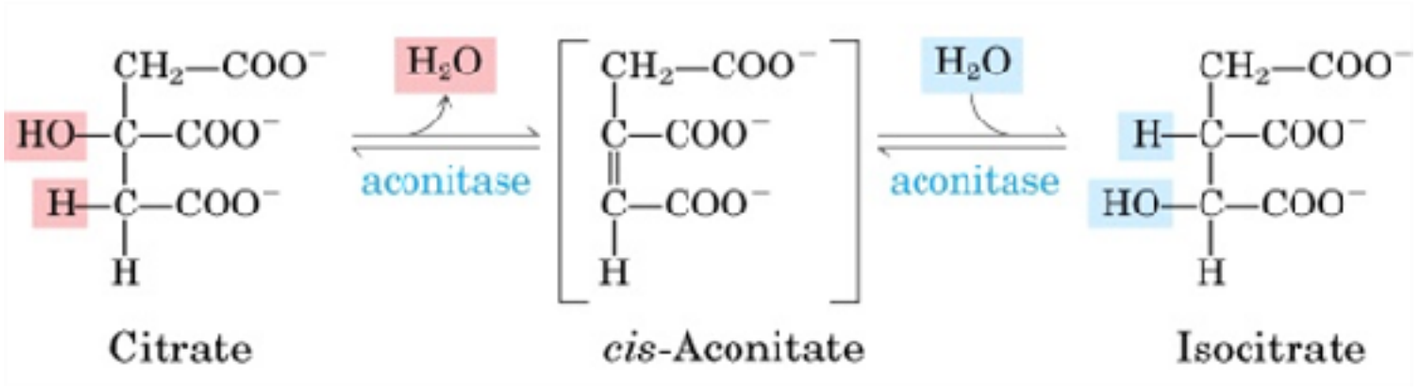
In the first step of the TCA cycle, **citrate synthase** transfers the acetyl group from acetyl-CoA onto oxalacetate to produce citrate

This reaction consists of two phases: **(1)** oxaloacetate is condensed to aceyl-CoA to form citryl-CoA; **(2)** citryl-CoA is hydrolysed to form citrate and CoA-SH. Phase two is highly exergonic and drives he entire reaction.



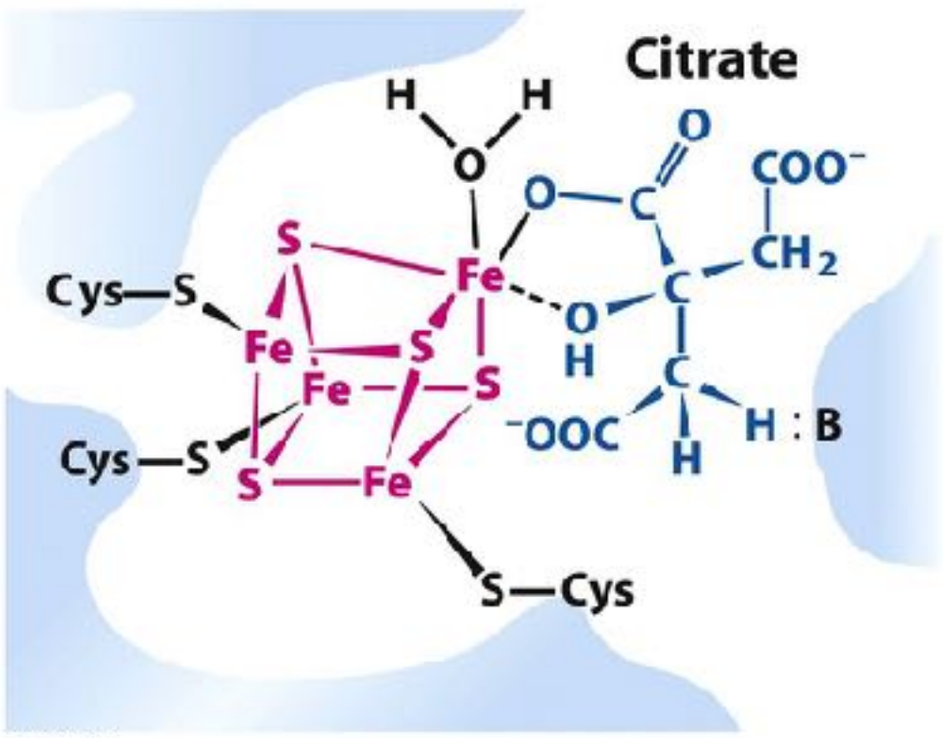
Citrate synthase (a dimer) first binds oxaloacetate into its active site, which causes a conformational change from an ‘open’ to a ‘closed’ conformation. By doing so oxaloacetate binding induces the formation of the acetyl-CoA binding site in citrate synthase and shifts the catalytic residues into their proper positions.

The citric acid cycle- Step 2



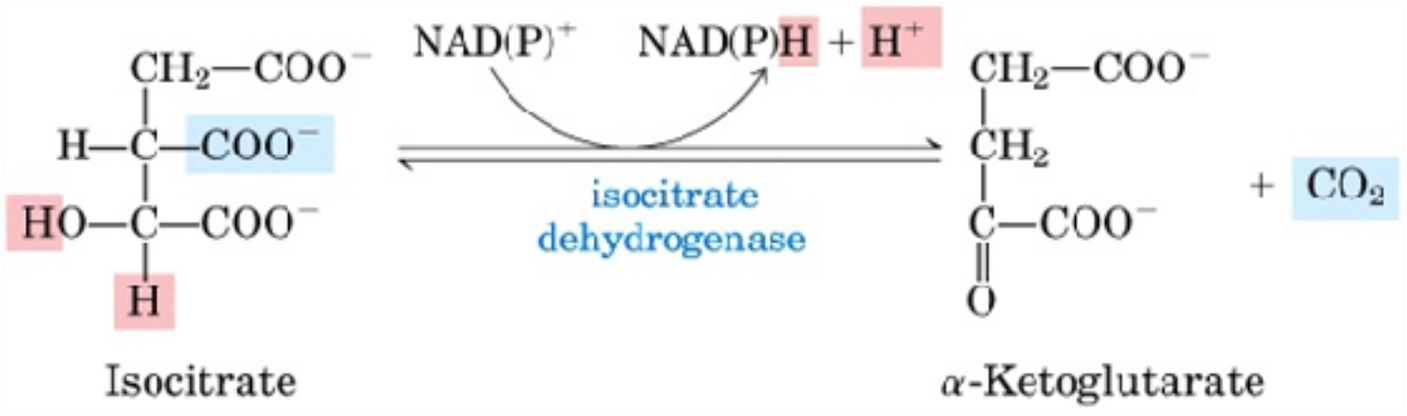
In the second step citrate is converted into isocitrate by the enzyme **aconitase.**

In this reaction an hydroxyl group is moved from the third carbon of citrate to an adjacent carbon via a dehydraion/hydration reaction.

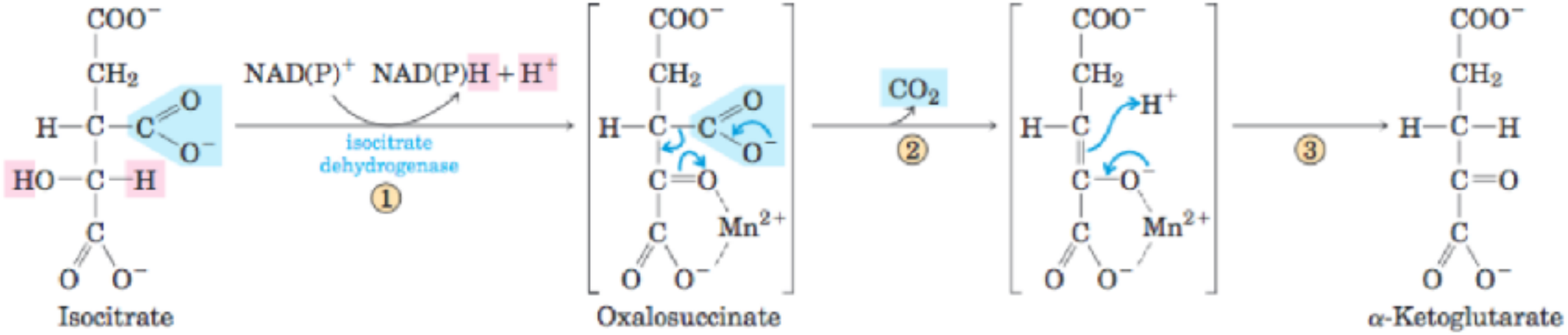


The Aconitase enzyme contains a Iron-Sulfur center (in magenta) that connects to the citrate molecule (in blue). Three Cys residues of the enzyme bind three Fe atoms: the fourth Fe is bound to one of the carboxyl groups of citrate and (non covalently) with a citrate hydroxyl group. The iron-sulfur center acts in both substrate binding and catalysis

The citric acid cycle- Step 3

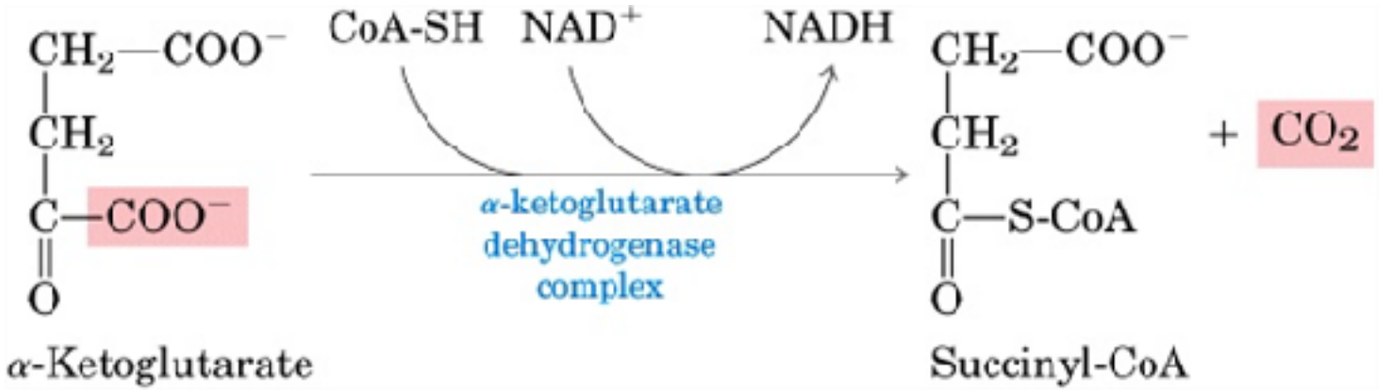


Once isocitrate is formed, it is ready to undergo the first oxidative decarboxylation reaction of the citric acid cycle. This step is catalysed by an enzyme called **isocitrate dehydrogenase** which yields a-ketoglutarate CO2 and NADH



This process is divided in two phases: (1) isocitrate is oxidised to oxalosuccinate and NAD+ is reduced to NADH (2) The highly unstable oxalosuccinate undergoes decarboxylation and a molecule of CO2 is produced.

The citric acid cycle- Step 4



This step represents the second oxidative decarboxylation in the TCA cycled involves the conversion of a-ketoglutarate to succinyl-CoA with concomitant production of CO2 and NADH

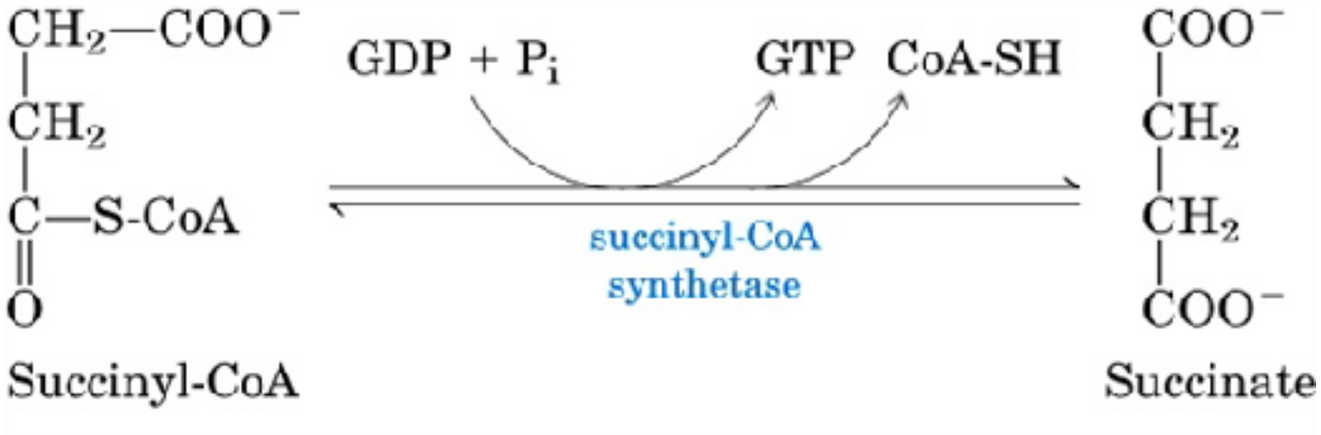
This Step is catalysed by the **a-ketoglutarate dehydrogenase complex**. This is a large enzyme complex that consists of three different subunits each endowed with specific enzymatic activities **[very similar to Pyruvate dehydrogenase complex]**.

E1: a-ketoglutarate dehydrogenase (thiamine pyrophosphate; TPP as a cofactor)

E2: dihydrolipoyl succiniltransferase (lipoic acid as a cofactor)

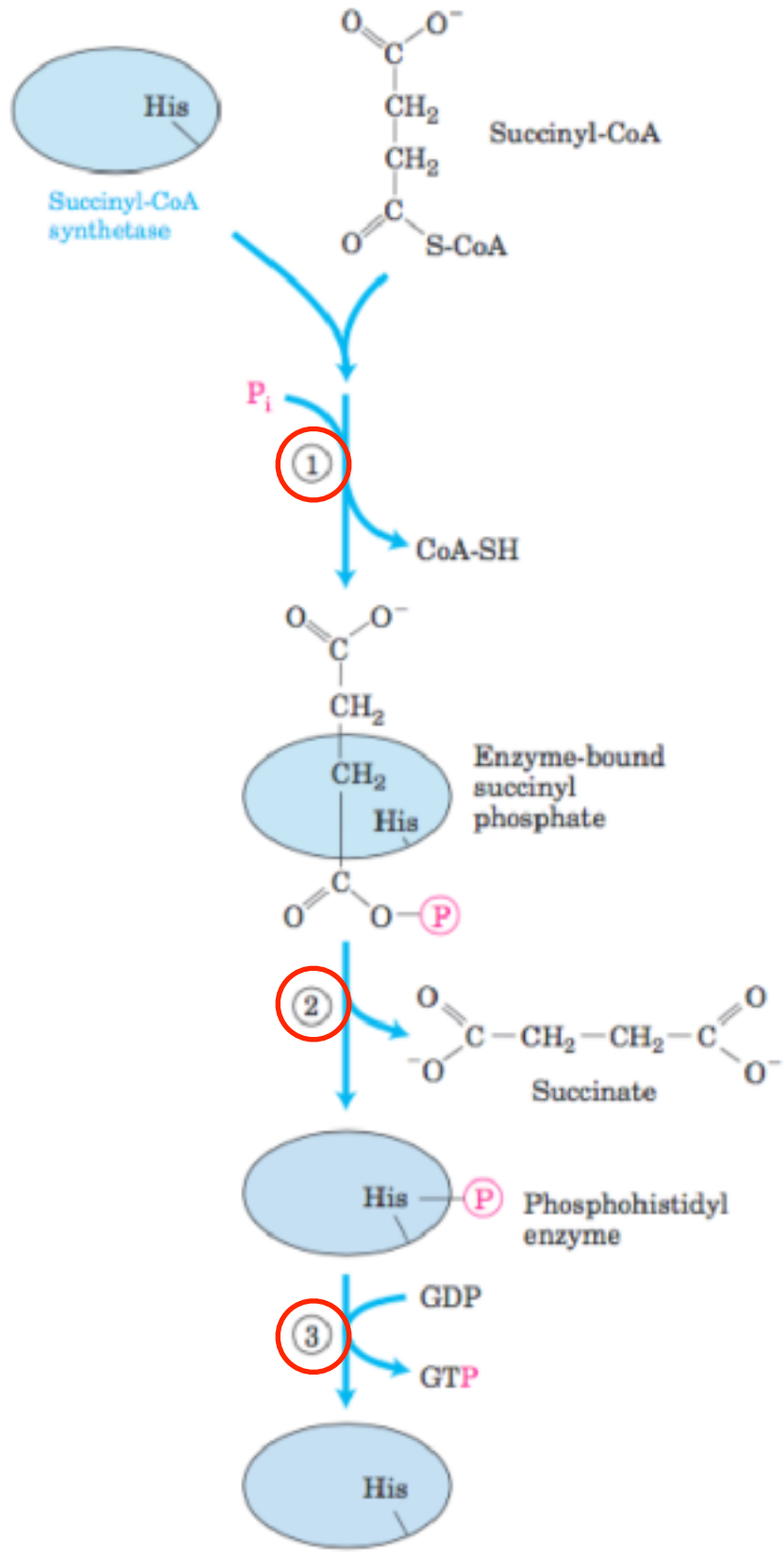
E3: dihydrolipoyl dehydrogenase (FAD as a cofactor)

The citric acid cycle- Step 5

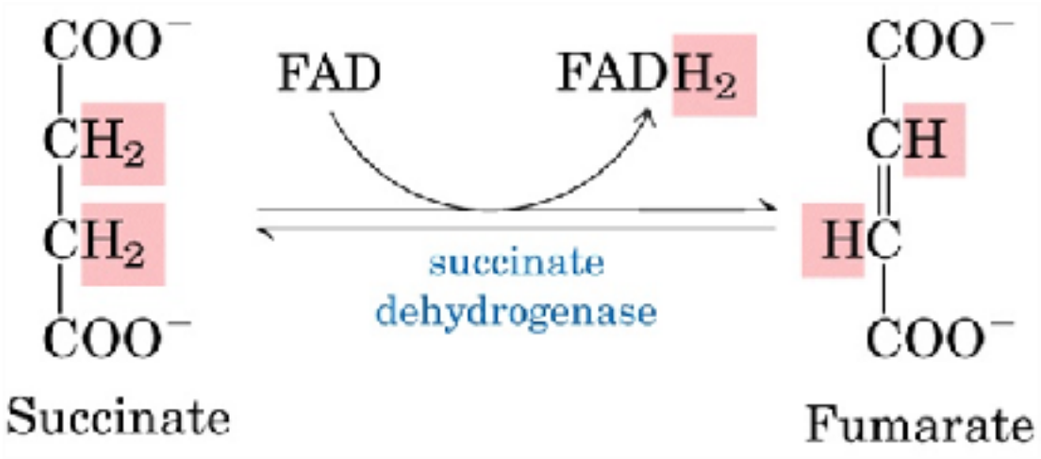


In this step the unstable and high energy thioester bond od succinyl-CoA is cleaved to release the CoA-SH unit. This releases free energy that is used to produce GTP from GDP. This reaction is catalysed by **succinyl CoA synthetase**.

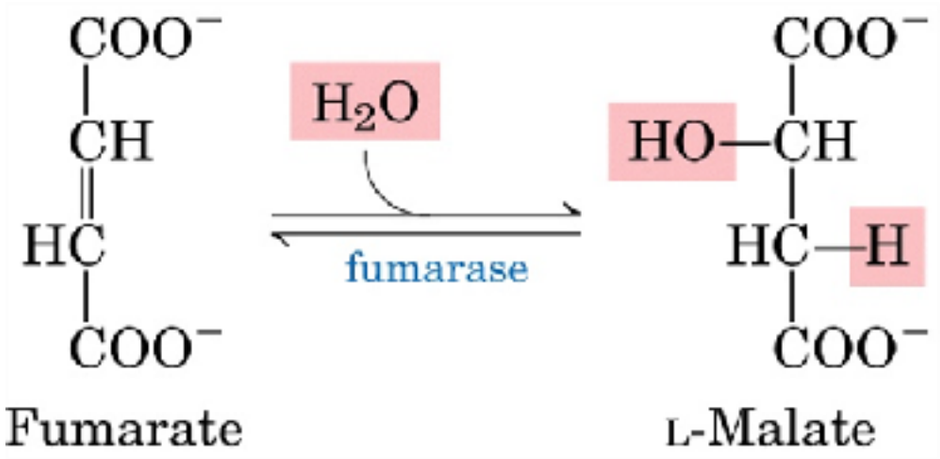
This reaction has 3 phases: (1) a phosphate group substitutes CoA to form succinyl phosphate [a high energy acyl phosphate]; (2) succinyl phosphate donates the phosphate to a His residue in the enzyme [Succinate is formed]; (3) the phosphate group is transferred from the high energy phosphorylated histidine to GDP to form GTP



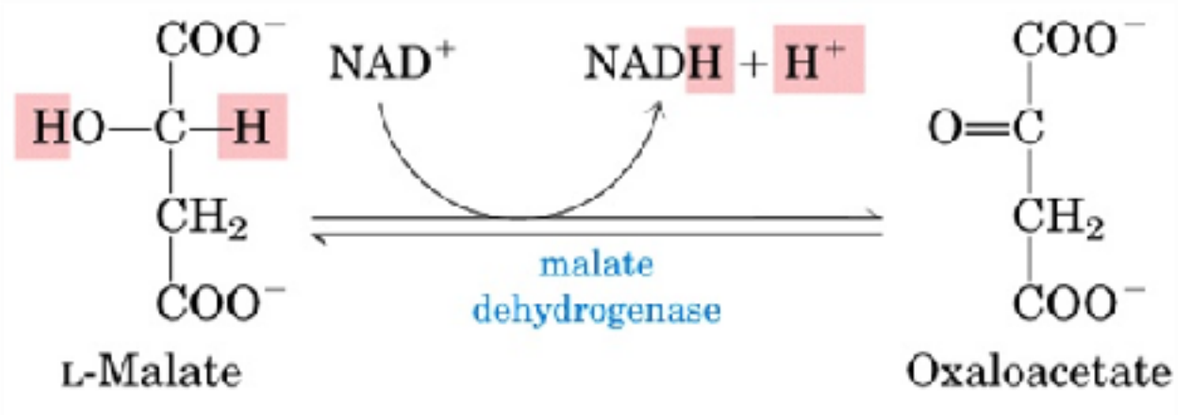
The citric acid cycle- Step 6-8



In step 6, succinate is oxidised to fumarate by the enzyme **succinate dehydrogenase** with the reduction of FAD to FADH2. Succinate dehydrogenase is bound to the inner mitochondrial membrane and its FADH2 passes electron to the electron transport chain.

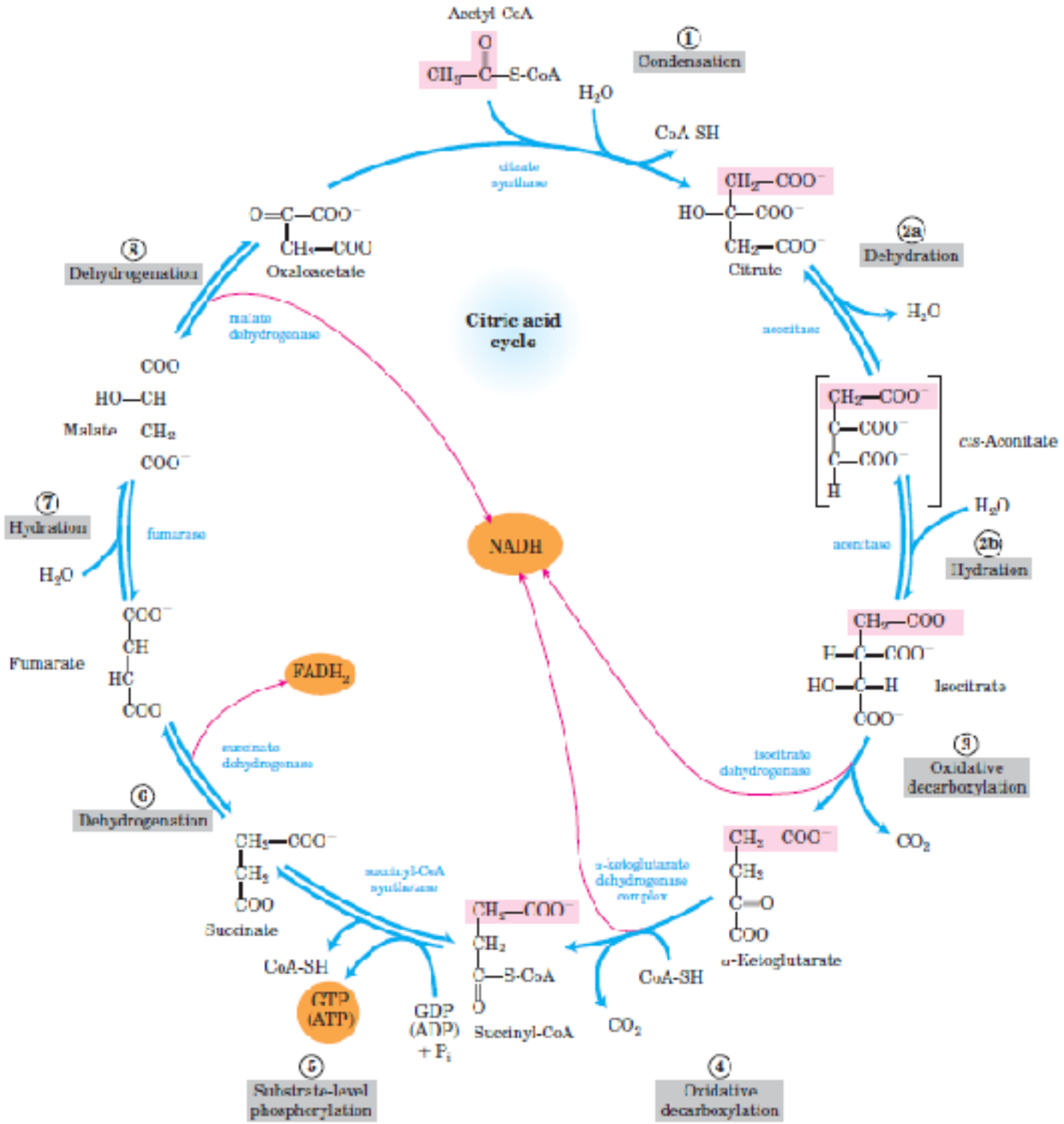


Fumarase catalyses the hydration of fumarate into malate. Note that the water molecule attacks only at a specific site, so only the L-isomer of malate is formed



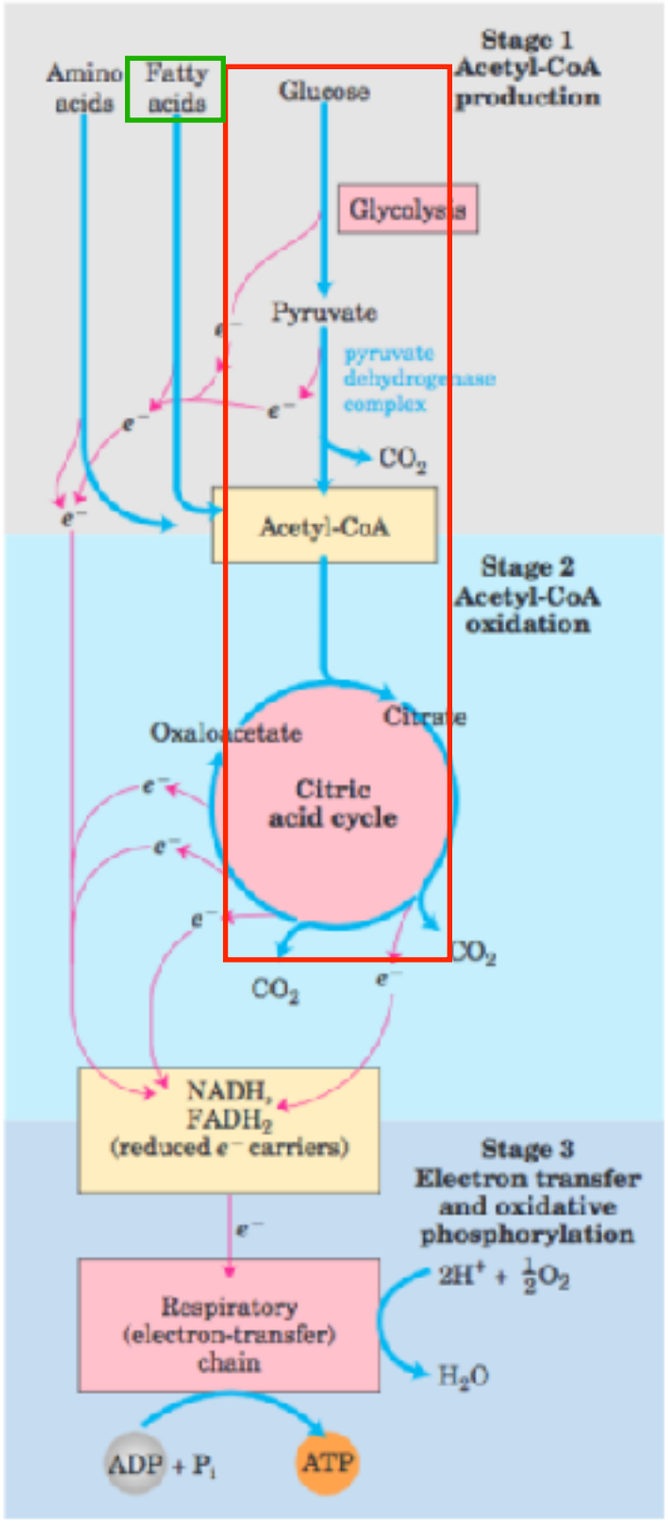
Malate dehydrogenase then oxidises malate into oxaloacetate and reduces NAD+ to NADH. This process is highly endoergonoic and needs to be coupled with other exergonic steps

The citric acid cycle



To summarise, our cells uptake glucose (and other nutrients) from the extracellular environment and decompose it in the cytosol through glycolysis. The yield of glycolysis is pyruvate, which, in the presence of oxygen, is transported to the mitochondrial matrix thanks to pyruvate translocase. In the mitochondrial matrix, pyruvate is transformed into acetyl-CoA by the highly regulated enzyme PDC. Acetyl-CoA then enters the TCA cycle by being complexed to the oxaloacetate to form citrate. Then, through a series of 7 reactions citrate is converted back to oxaloacetate ready to be complexed to a new acetyl-CoA molecule. In the process 3 NADH, 1 FADH2 and 1 GTP molecule are produces along with 2 CO2 molecules.

Fatty acid oxidation



Adipose tissue cells (adipocytes) are specialised cells that store energy in the form of triglycerides.

Upon demand triglycerides are hydrolysed to glycerol and fatty acids that are transported to target tissues.

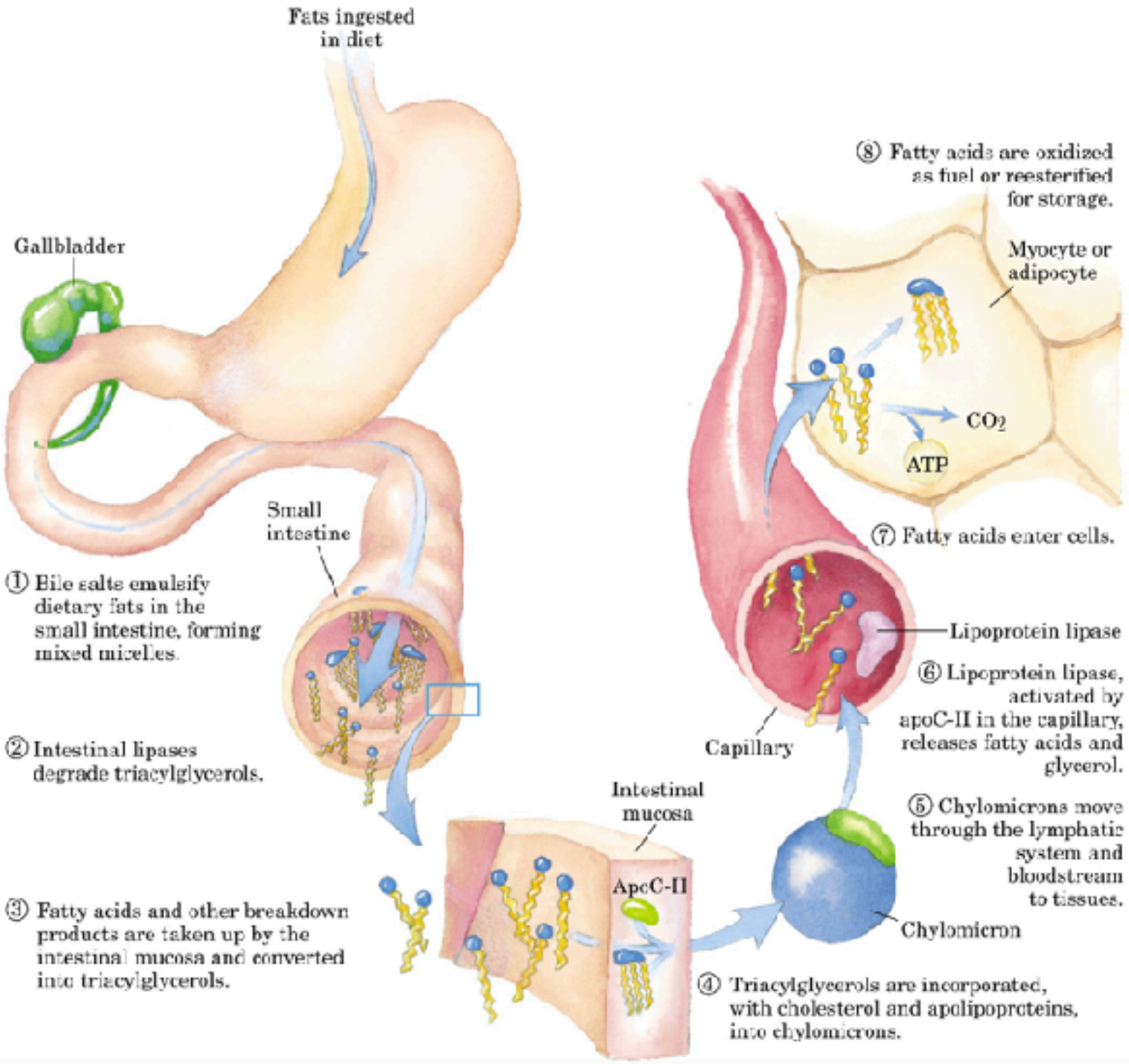
Following this step fatty acids can be activated and transported to the mitochondrial matrix of our cells.

Once activated, fatty acids (bound to CoA) make it to the mitochondrial matrix where they iteratively undergo a series of **4 reactions** that shorten the acyl chain by 2 carbon atoms and release acetyl-CoA (that can feed in the TCA cycle).

This process recurs until the fatty acid is completely degraded.

This entire process is referred to as **fatty acid oxidation**

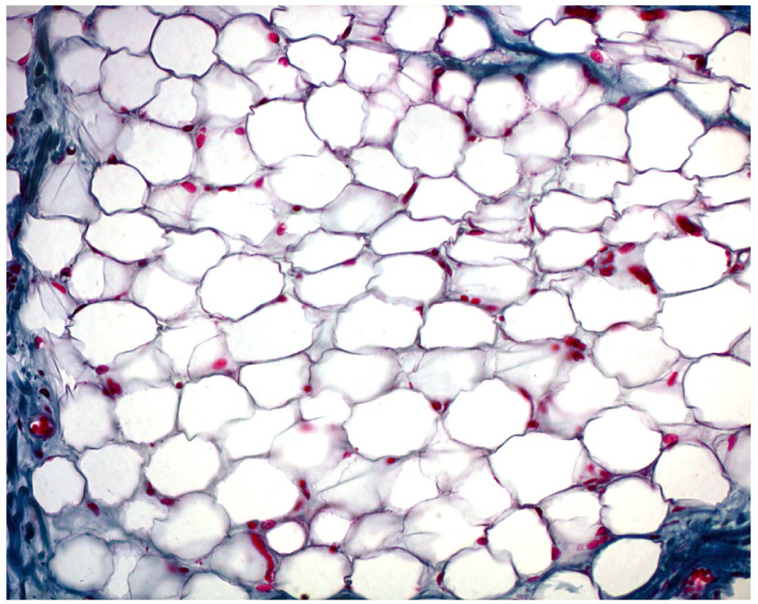
Fatty acid oxidation



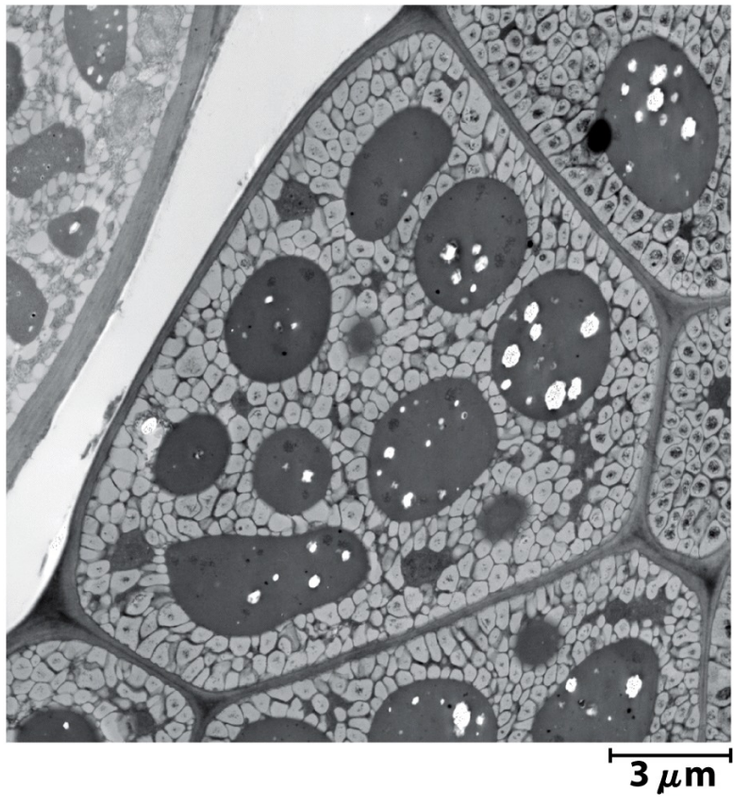
Dietary fats are absorbed in the small intestine. Bile acids, synthesized from cholesterol in the liver and stored in the gallbladder, are released in the small intestine following a fat-rich meal. They act as biological detergents, resuspending triglycerides into fine micelles. In this form, triglycerides are accessible to water-soluble lipases in the intestinal lumen. The products of lipases are absorbed by the intestinal mucosa and converted back to triglycerides. These are then mobilized through the bloodstream and stored in dedicated adipose cells (adipocytes) in specialized organelles named lipid droplets.

Fatty acid oxidation

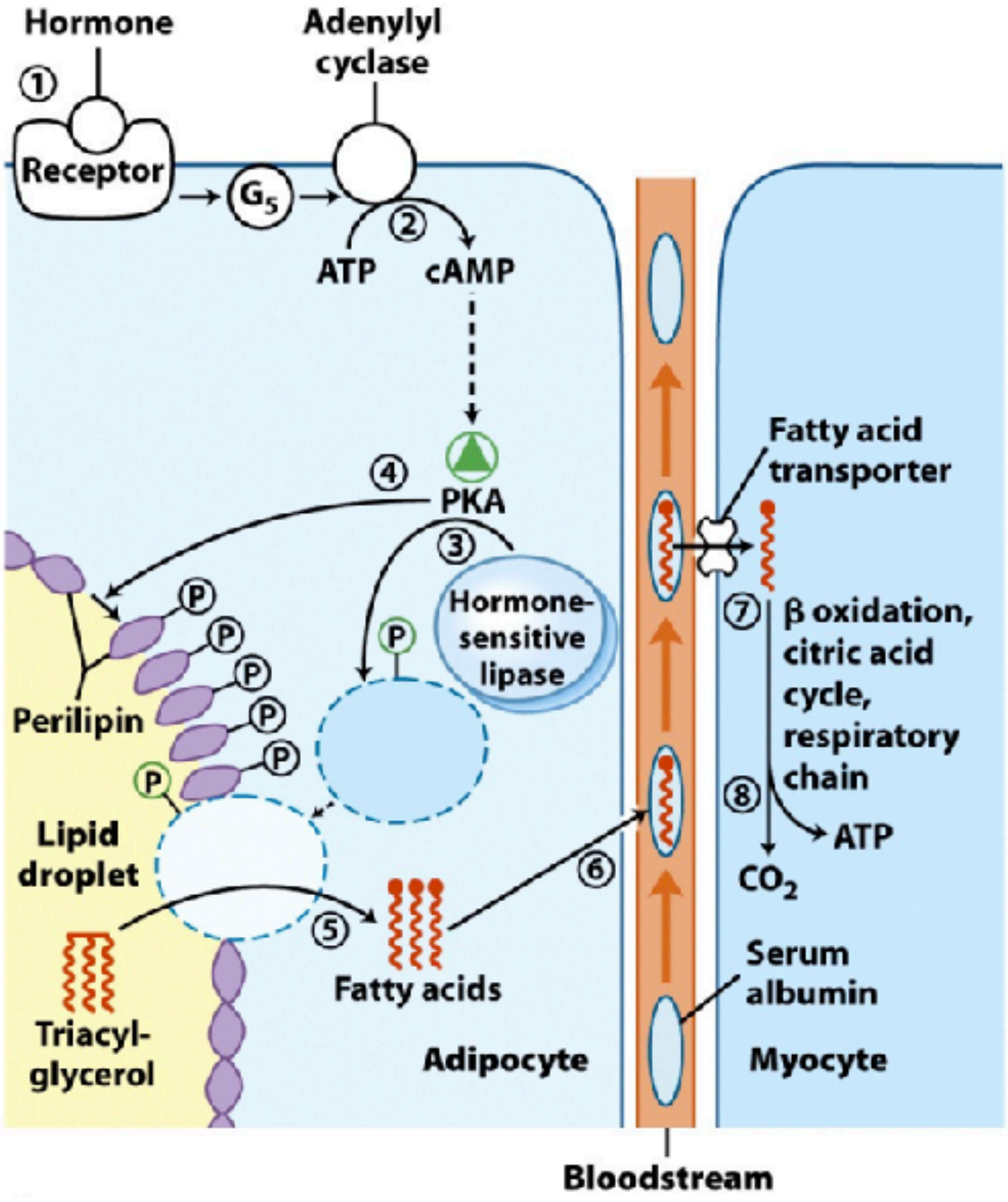
In most eukaryotic cells, triacylglycerols form microscopic, oily droplets in the aqueous cytosol, serving as **metabolic fuel**. In vertebrates, specialized cells called adipocytes or fat cells store large amounts of triacylglycerols as fat droplets that nearly fill the cell.



Triacylglycerols contain **more energy per gram than polysaccharides**. Additionally, they are unhydrated, and the organism does not have to carry extra weight in the form of water as with stored polysaccharides. In some animals, such as seals, penguins, and bears, fat stores under the skin also serve as insulation against cold temperatures

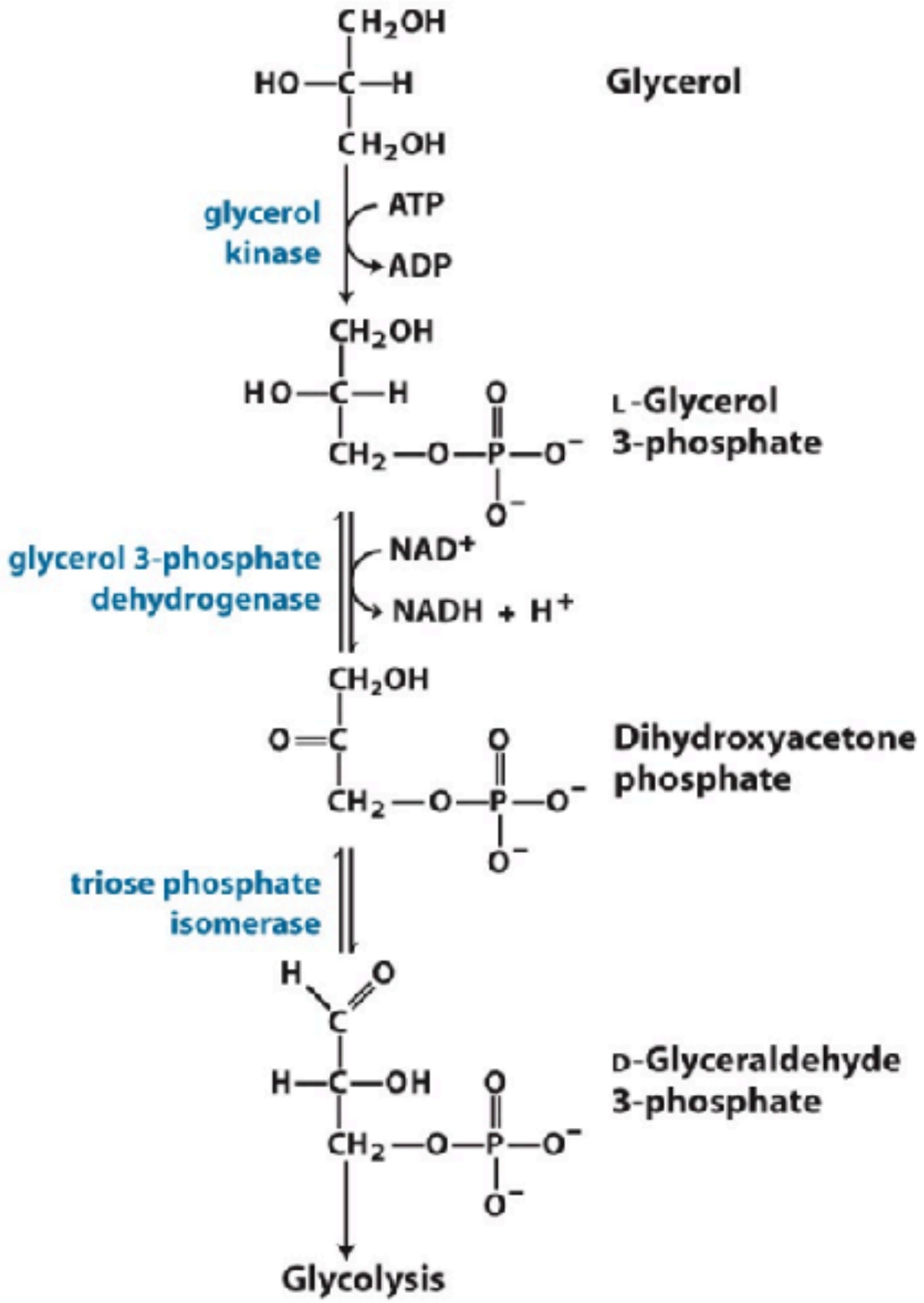


Fatty acid oxidation



When blood glucose levels are low, some hormones (glucagon/adrenaline) are released into the circulation, activating adenylyl cyclase on the surface of adipocytes. Adenylyl cyclase leads to the production of cAMP and the activation of protein kinase A (PKA), which triggers intracellular triglyceride lipases to produce fatty acids and glycerol. The produced fatty acids are then released into the bloodstream and complexed with the serum protein Albumin. In this form, fatty acids reach muscle cells (myocytes) where they are oxidized for the production of ATP.

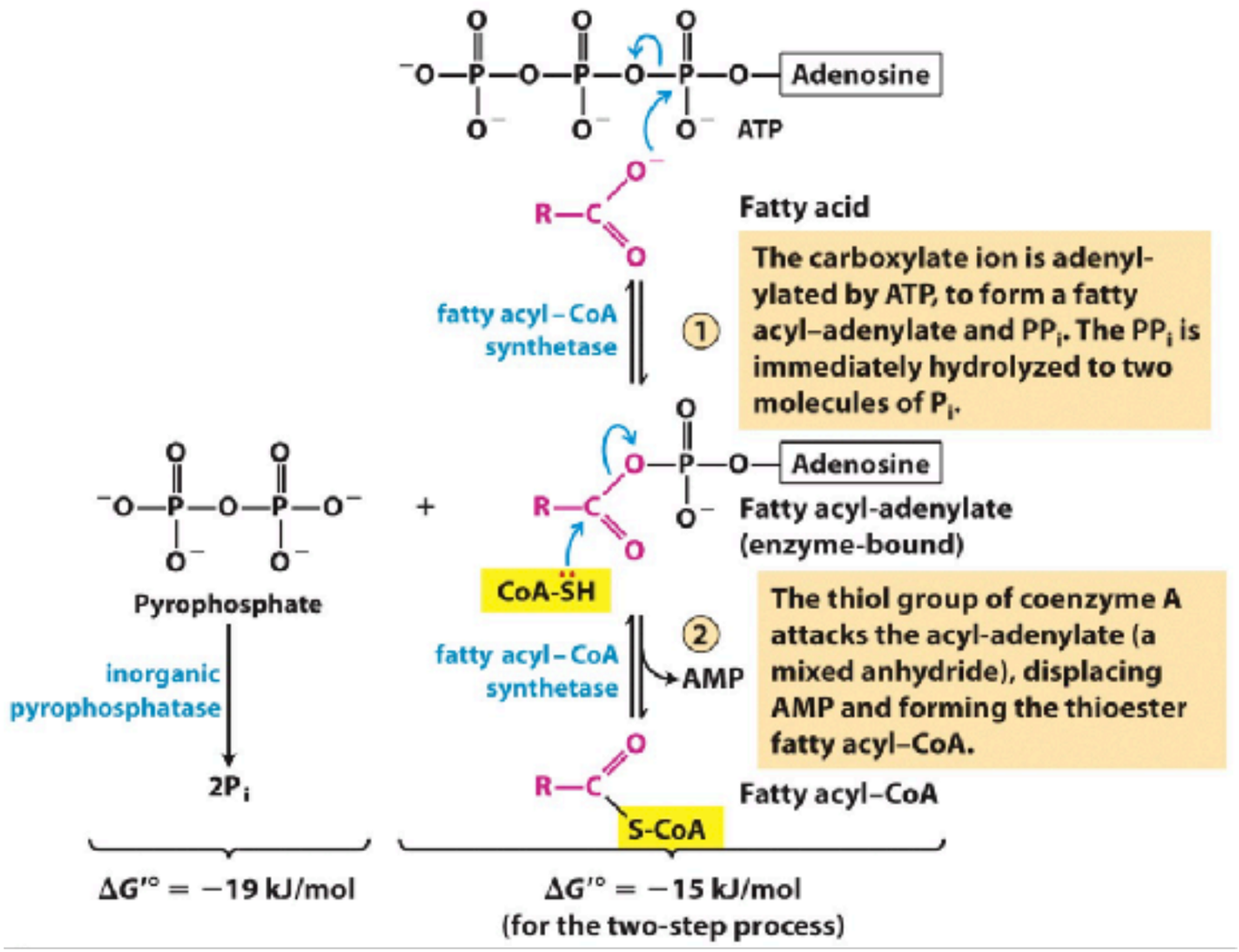
what about glycerol?



About 95% of the energy derived from triglycerides comes from the oxidation of their fatty acid chains, while 5% comes from glycerol. Glycerol, released by triglyceride lipases, is phosphorylated to glycerol 3-phosphate by the enzyme **glycerol kinase** (with the consumption of an ATP molecule). Glycerol 3-phosphate is then oxidized by the enzyme glycerol 3-**phosphate dehydrogenase** to dihydroxyacetone phosphate (DHAP), thus entering the glycolytic pathway.

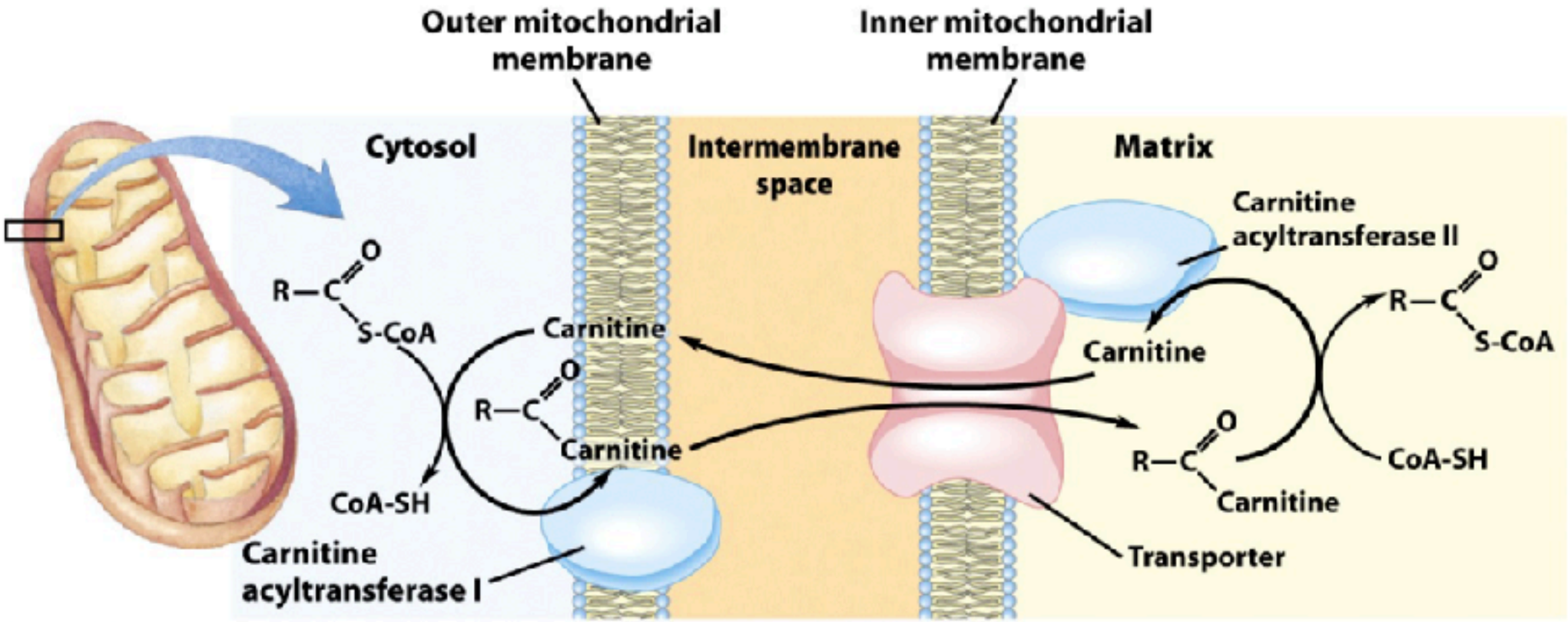
How much ATP do we get from glycerol in the glycolytic pathway?

Fatty acids are transported within mitochondria



Fatty acid oxidation takes place in the mitochondrial matrix; thus, fatty acids need to be transported within mitochondria to be oxidized. For this purpose, they are 'activated' by being linked to CoA by a family of enzymes named **fatty acyl-CoA synthetases**. These enzymes have a two-phase mechanism: (1) the fatty acid is complexed with an AMP from ATP, producing PPi; (2) CoA-SH attacks the fatty acyl adenylate to form AMP and fatty acyl-CoA. The PPi produced in this reaction is promptly dissociated into phosphate molecules by the **inorganic pyrophosphatase**.

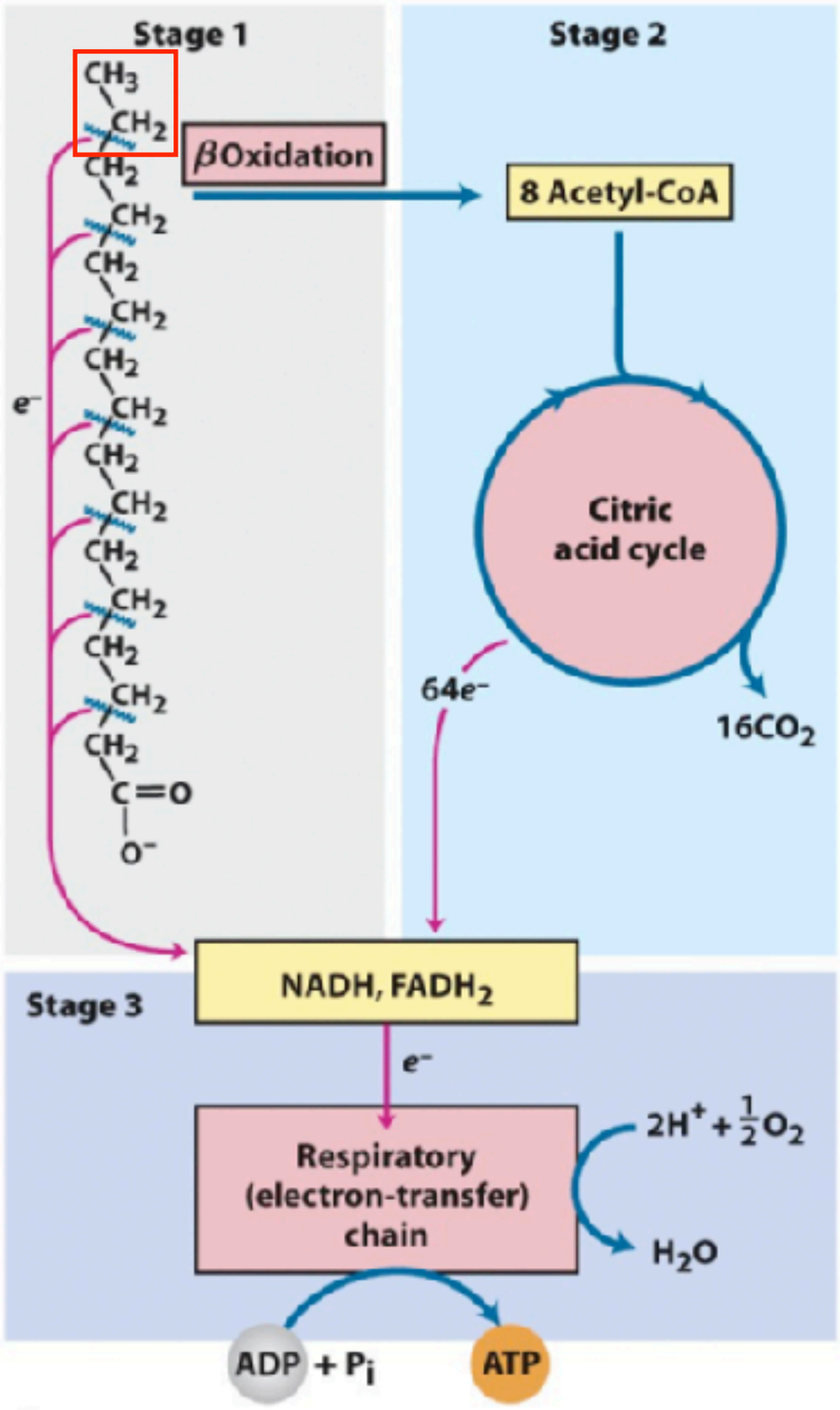
Fatty acids are transported within mitochondria



Once formed in the cytosolic environment, fatty acyl-CoA crosses the mitochondrial membrane by transiently exchanging its CoA with carnitine and exploiting the carnitine/acyl-carnitine transporter. This process requires the action of two enzymes, **carnitine acyltransferase I** and **carnitine acyltransferase II**, which are associated with the outer and inner mitochondrial membranes, respectively. The transport of fatty acids into the mitochondria is the rate-limiting step in β-oxidation

10’ Break

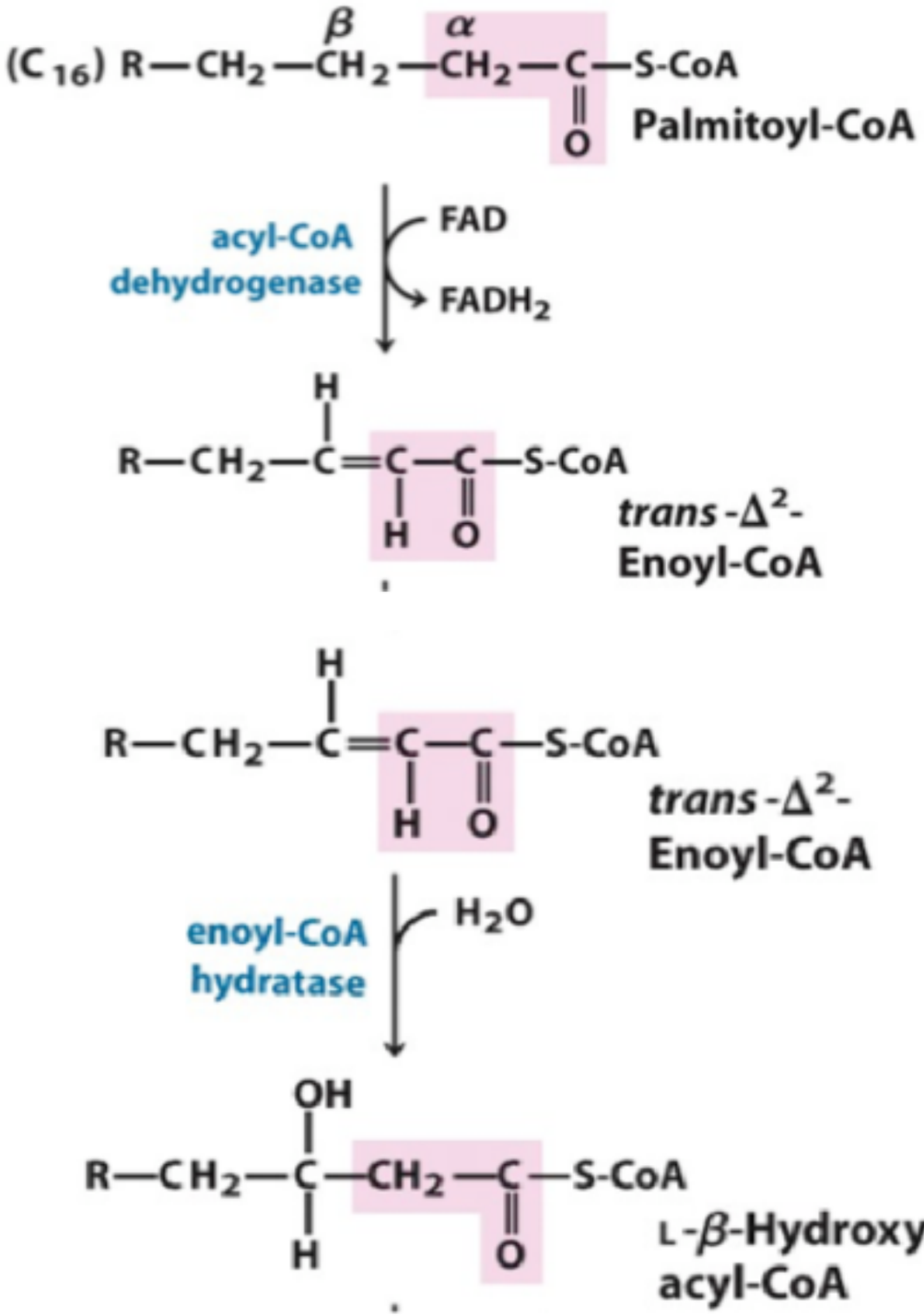
Fatty acid oxidation



Once in the mitochondrial matrix, fatty acyl-CoAs are progressively oxidised by an iterative sequence of four reactions that produce acetic units in the form of acetyl-CoA (Stage1). The newly formed acetyl-CoA can thus feed in the TCA cycle (Stage 2) and then the electrons subtracted in these oxidative reactions are used to produce ATP in the oxidative phosphorylation chain (Stage 3).

Let us have a quick look at the 4 reactions involved in B-oxidation!

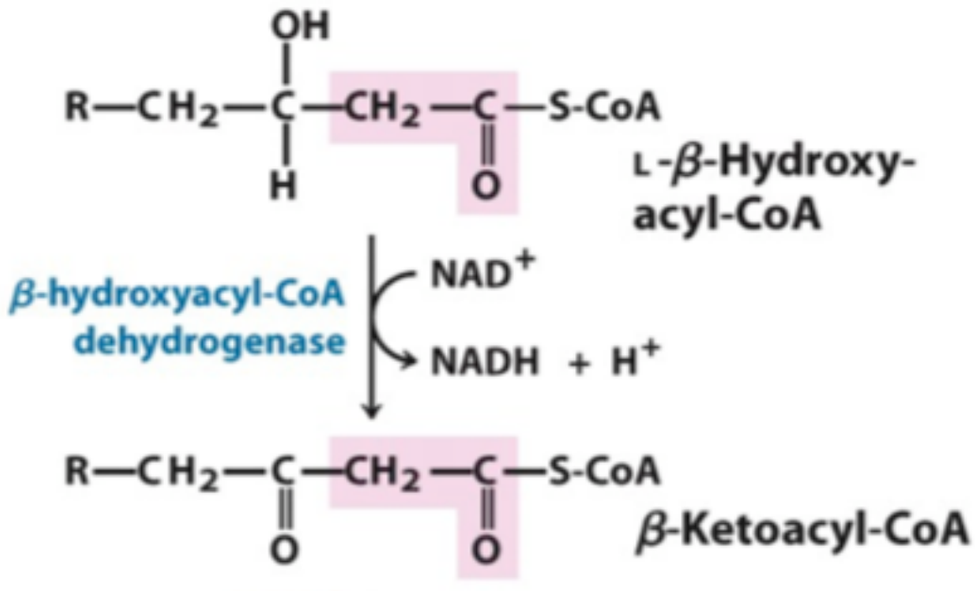
Fatty acid oxidation- Steps 1-2



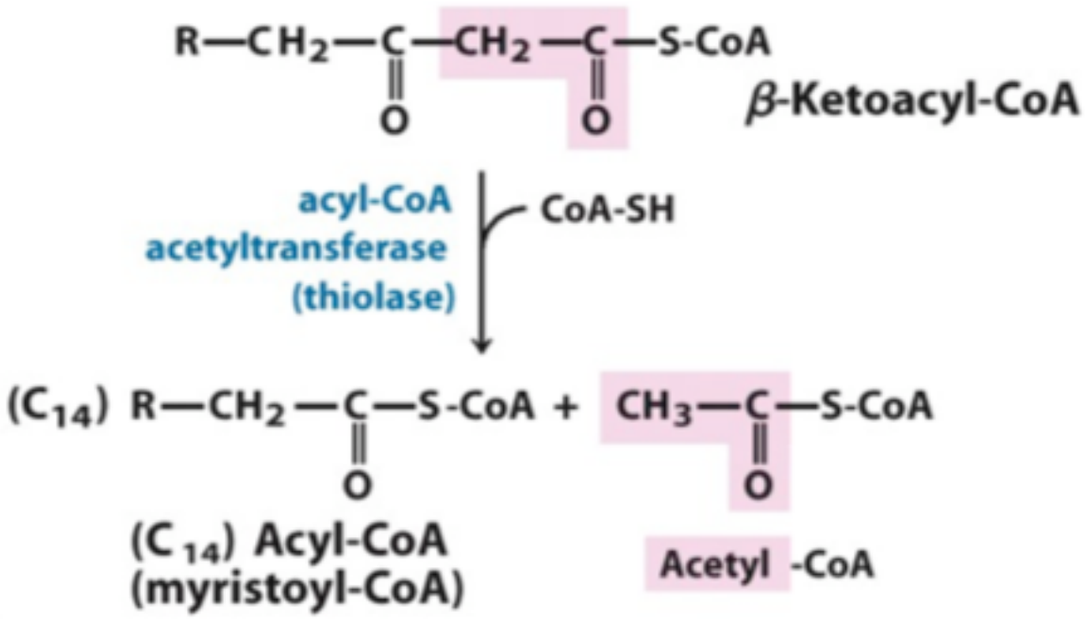
Step 1: The first step of fatty acid oxidation is a dehydrogenation that produces a double bond between the carbons alpha and beta (C2 and C3). This reaction is catalyzed by the enzyme **acyl-CoA dehydrogenase** (very similar to the succinate dehydrogenase from the TCA cycle). The electrons subtracted from the fatty acid are loaded into an FAD molecule to produce FADH2.

Step 2: The second step of fatty acid oxidation is hydration (very similar to that catalyzed by fumarase in the TCA cycle), whereby a water molecule is added to the alpha-beta double bond. This reaction is catalyzed by the enzyme **enoyl-CoA hydratase** and produces a β-hydroxyacyl-CoA.

Fatty acid oxidation- Steps 3-4

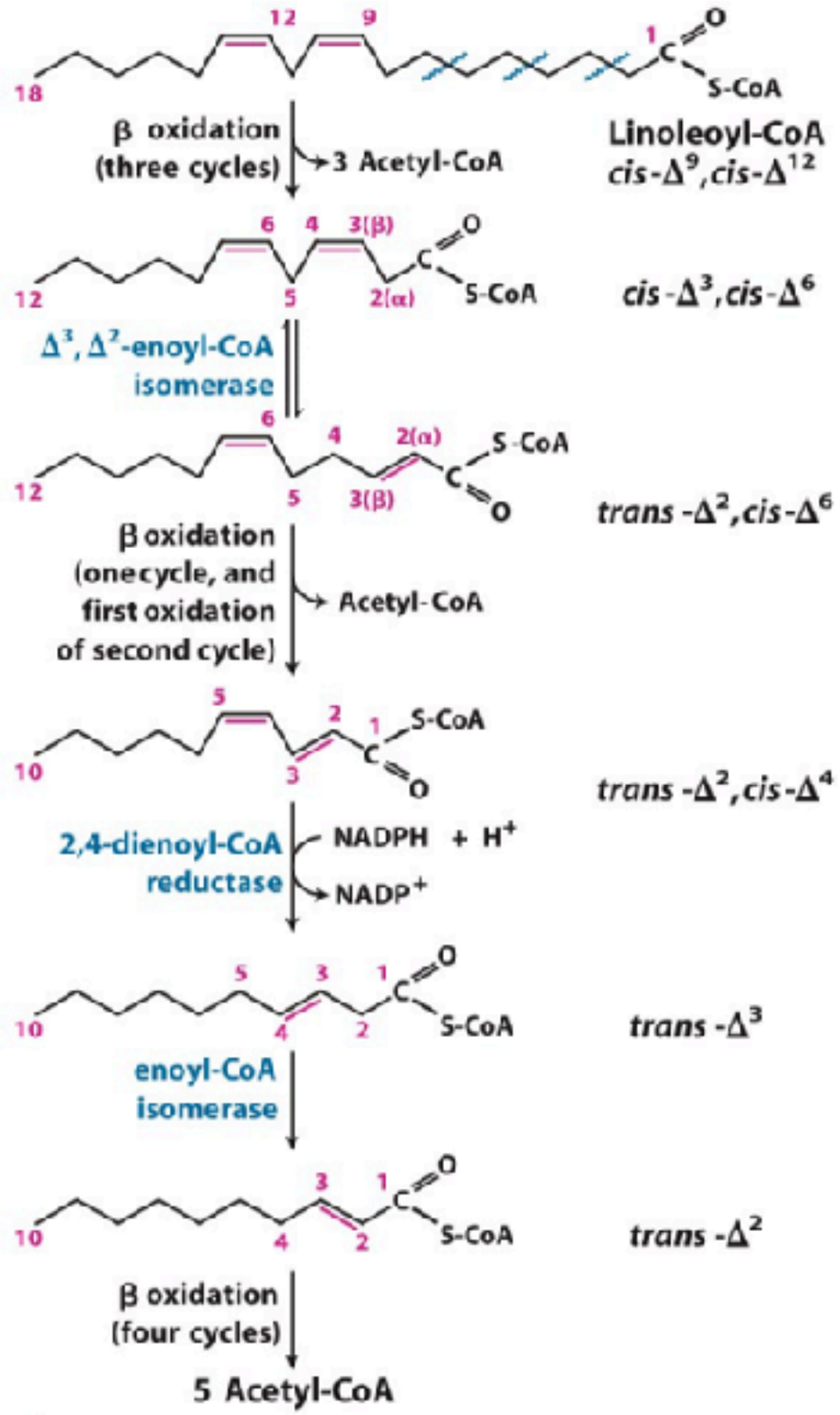


Step 3: The third step of fatty acid oxidation is again a dehydrogenation whereby the β-hydroxyacyl-CoA is oxidized to produce a β-ketoacyl-CoA with the concomitant production of an NADH molecule. This reaction is catalyzed by **β-hydroxyacyl-CoA dehydrogenase**, whose action is homologous to that of malate dehydrogenase in the TCA cycle.



Step 4: The last step of fatty acid oxidation is catalyzed by the enzyme **acyl-CoA acetyltransferase** (aka thiolase), whereby a free CoA-SH molecule reacts with the β-ketoacyl-CoA to form an acetyl-CoA and an acyl-CoA, where the acyl chain is 2C shorter than that of the original fatty acid. This new acyl-CoA is ready to undergo a new cycle of β-oxidation.

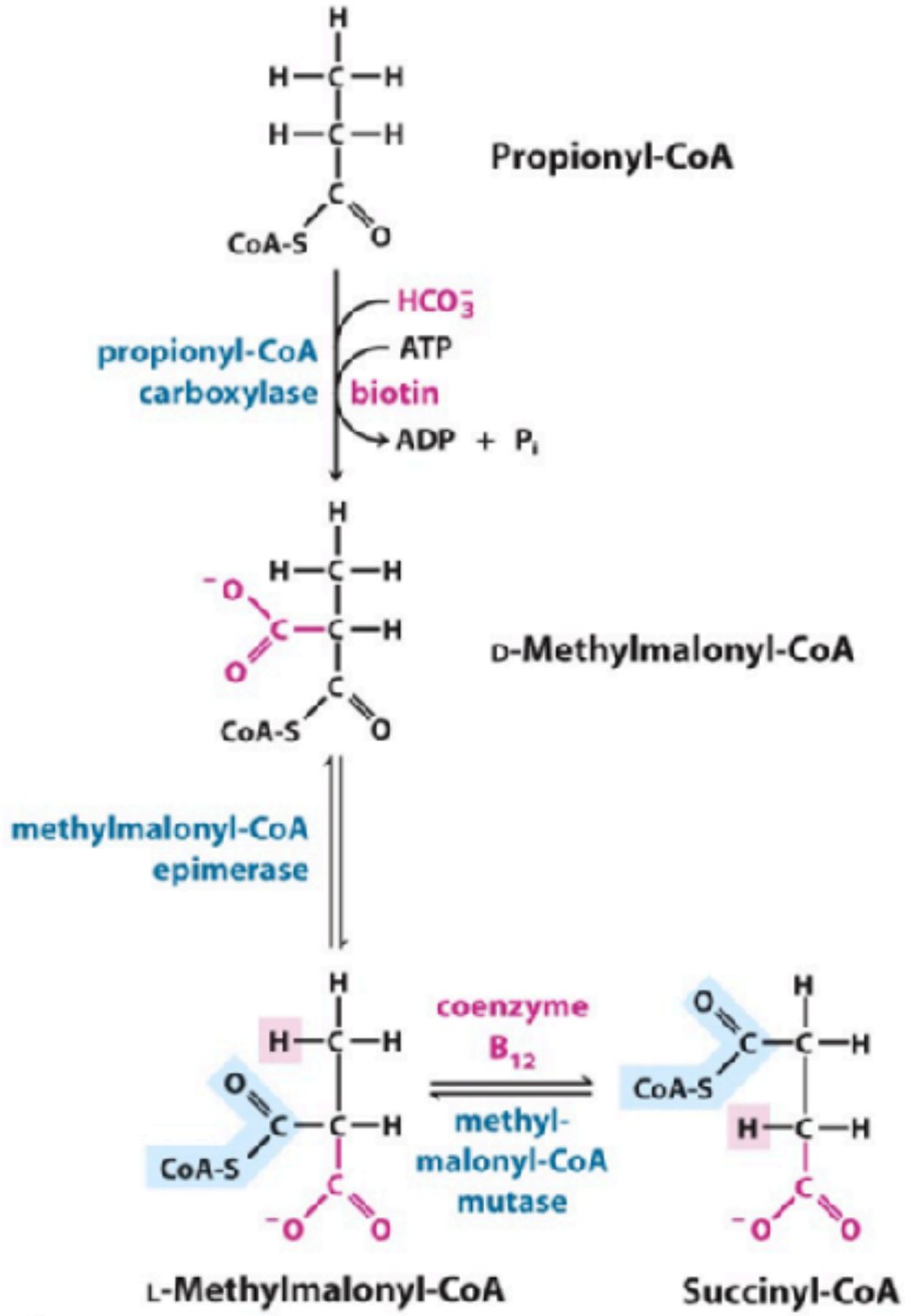
Fatty acid oxidation- unsaturated FAs



The The oxidative reaction we have described here works on saturated fatty acids with an even number of carbon atoms. But how do our cells deal with unsaturated and odd-numbered fatty acids?

In the case of unsaturated fatty acids, two more enzymes are required to make them degradable by β-oxidation. One is **enoyl-CoA isomerase**, which converts the cis-isomer into a trans-isomer (that can be acted upon by the **enoyl hydratase**). The second is **2,4-dienoyl-CoA reductase**, which reduces the unsaturated fatty acid, consuming an NADPH molecule. Following this step, the **enoyl-CoA isomerase** can act on the substrate and make it degradable by β-oxidation.

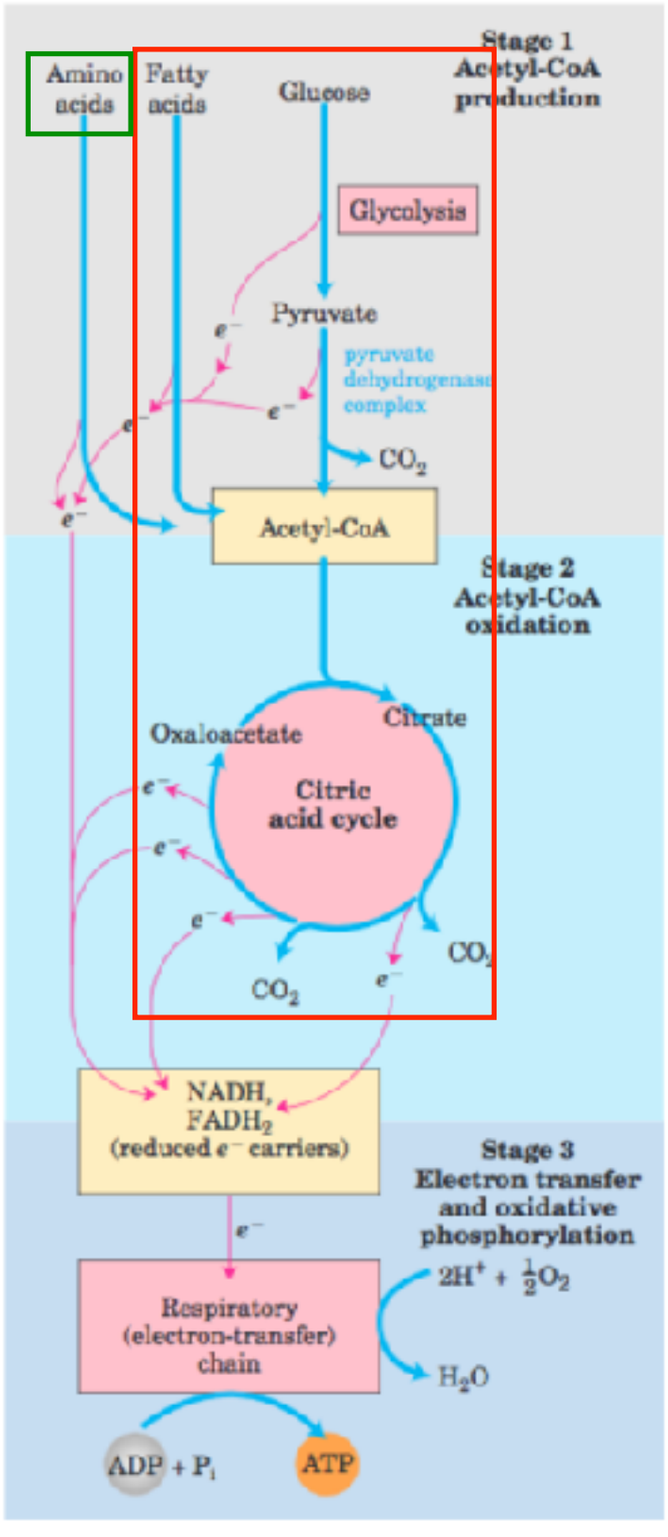
Fatty acid oxidation- Odd numbered FAs



The vast majority of fatty acids are constituted by an even number of carbon atoms; however, odd-numbered fatty acids exist and need to be degraded. Odd-numbered fatty acids are degraded in the same way as even-numbered fatty acids, but in the last iteration of β-oxidation, they yield propionyl-CoA instead of acetyl-CoA.

The propionyl-CoA is carboxylated by the enzyme p**ropionyl-CoA carboxylase** to produce D-methylmalonyl-CoA. Through two further reactions, D-methylmalonyl-CoA is then converted into succinyl-CoA, which enters the TCA cycle.

Amino acid oxidation and Urea production



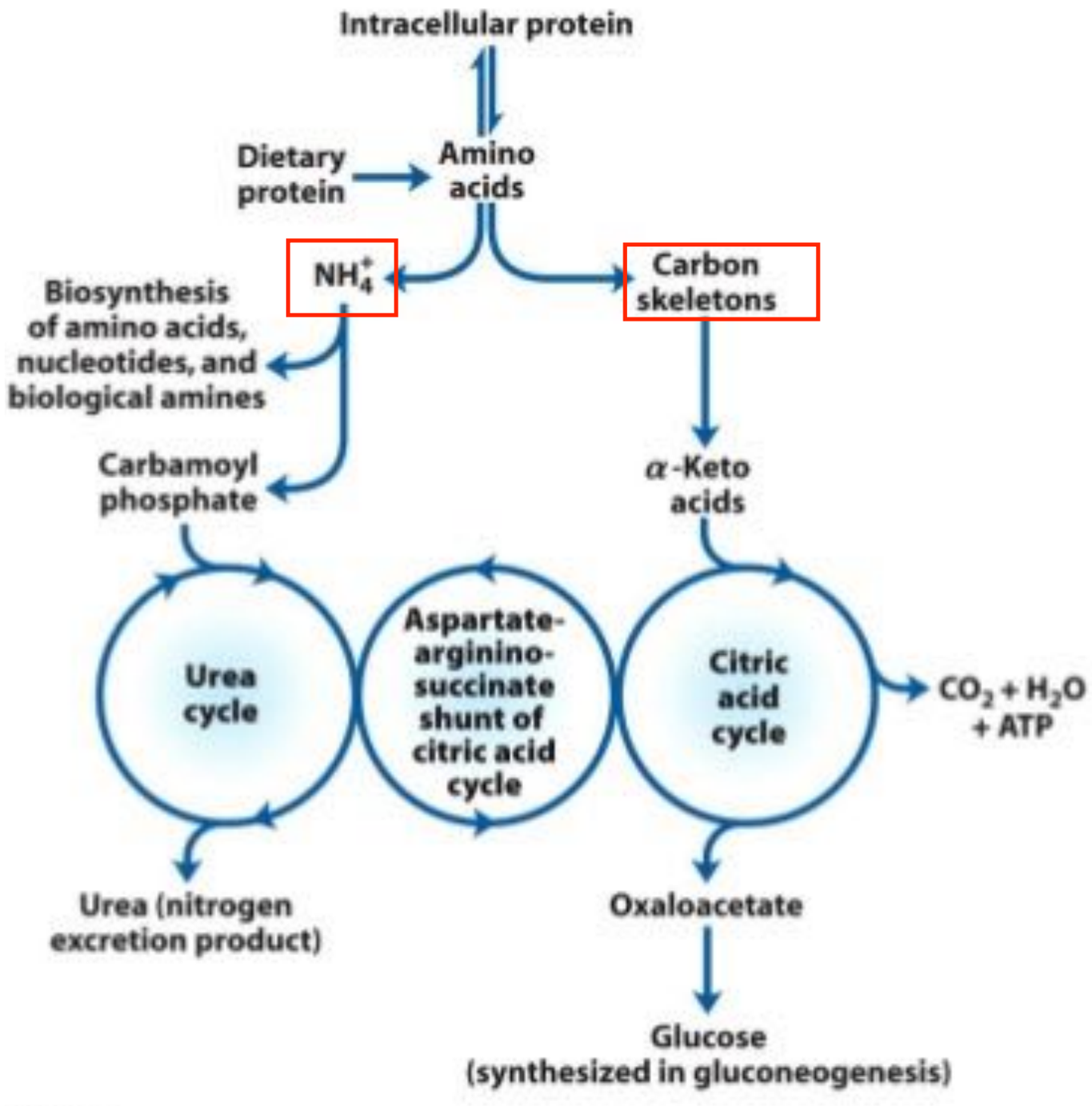
Amino acids that are derived from the degradation of proteins are the third class of biomolecules (after carbohydrates and fatty acids) that significantly contribute cellular energy metabolism. The relative contribution of amino acids to the energy needs varies among organisms and physiological conditions. In a meat-eating animal after a meal, amino acids can satisfy up to 90% of the energy needs. In animals amino acids are oxidised in three different metabolic conditions.

(1) during protein turnover some amino acids can be oxidised if not required for the synthesis of other proteins.

(2) in a protein rich diet, food-derived amino acids can exceed the needs of protein biosynthesis. As, differently than for carbohydrates (glycogen) and fat acids (triglycerides), animals do not have ways to store amino acids they are degraded.

(3) In starvation when carbohydrates are not available (or in diabetes) amino acids from endogenous proteins are used as an energy source

Amino acid oxidation and Urea production



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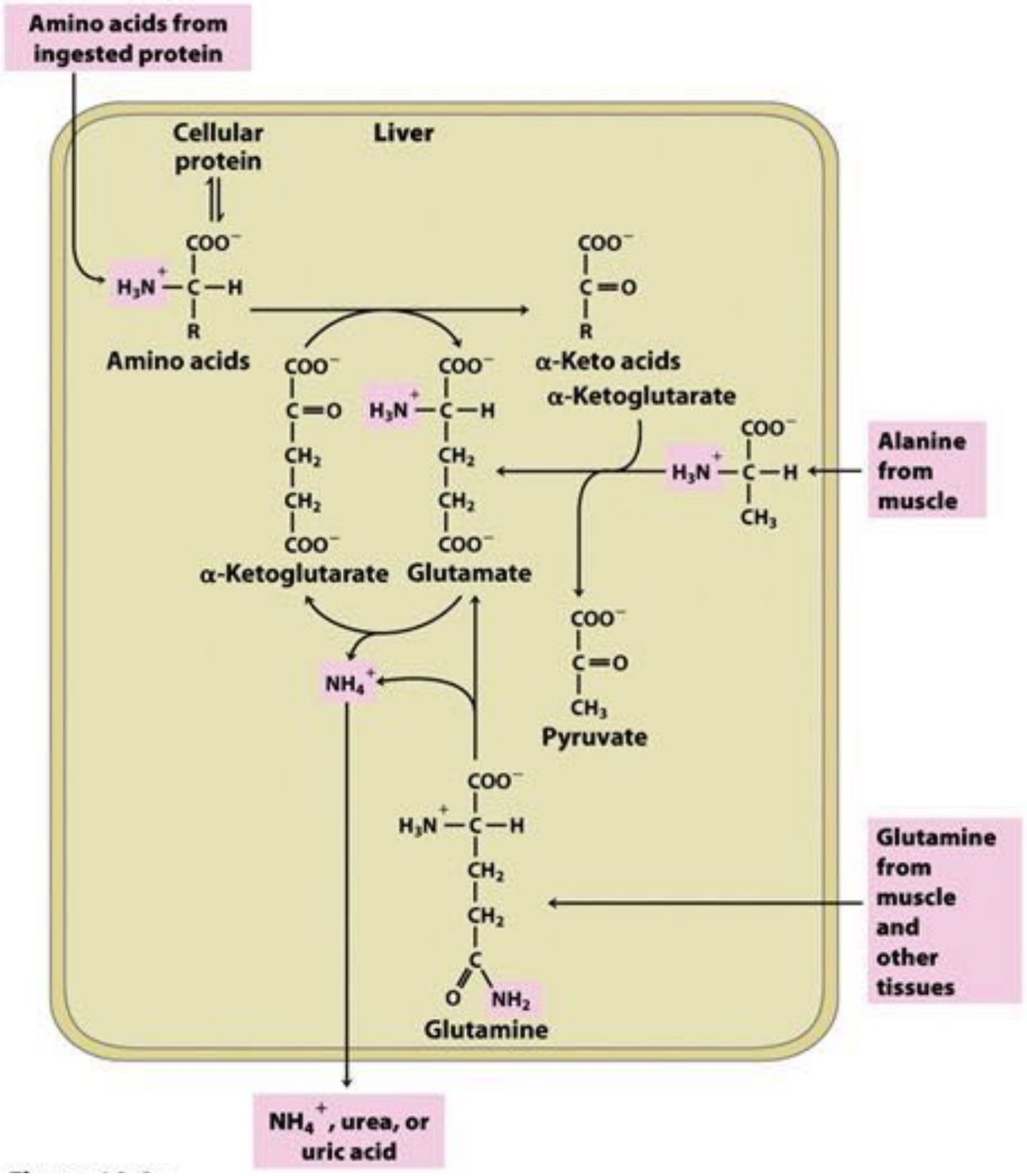
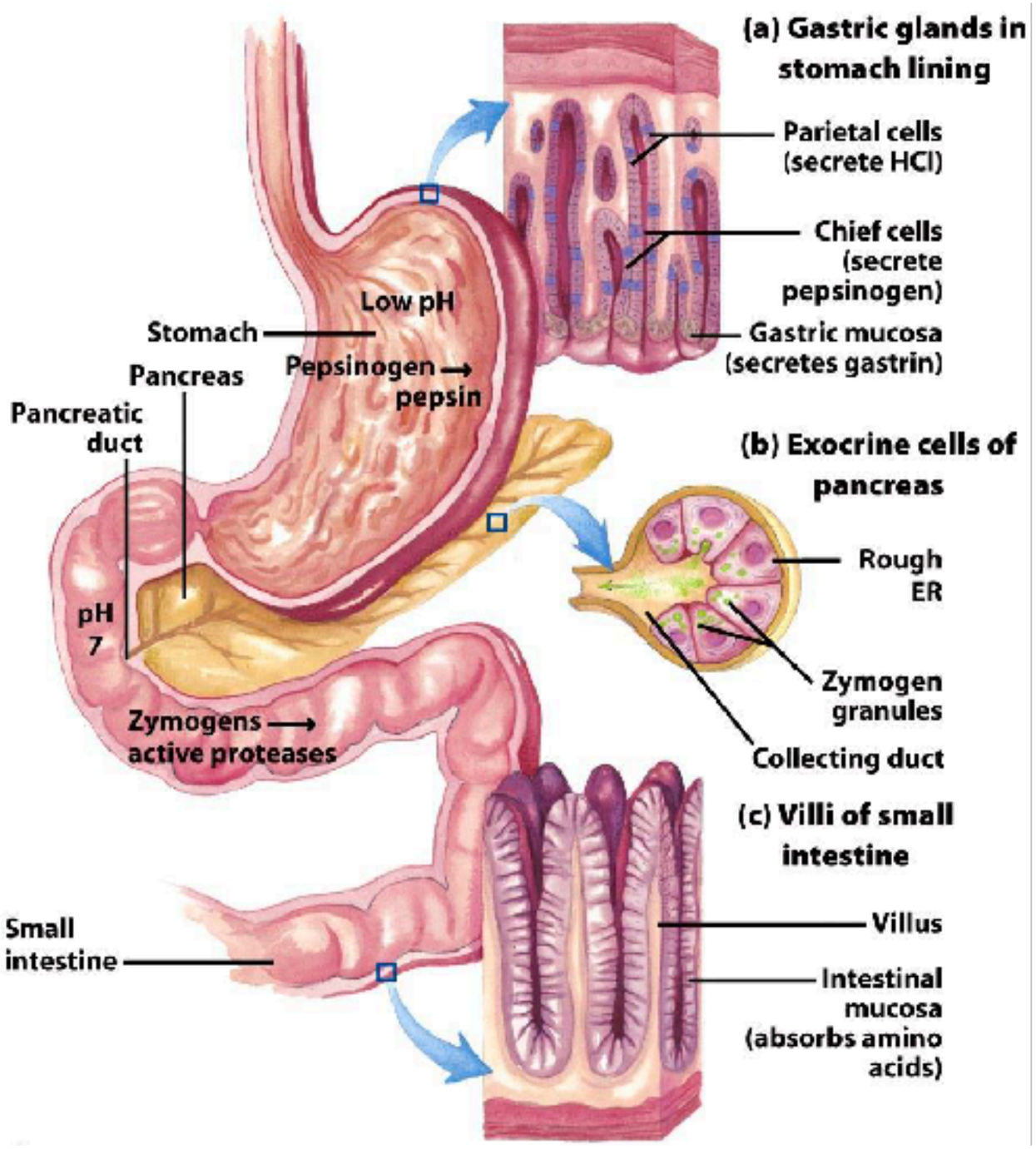


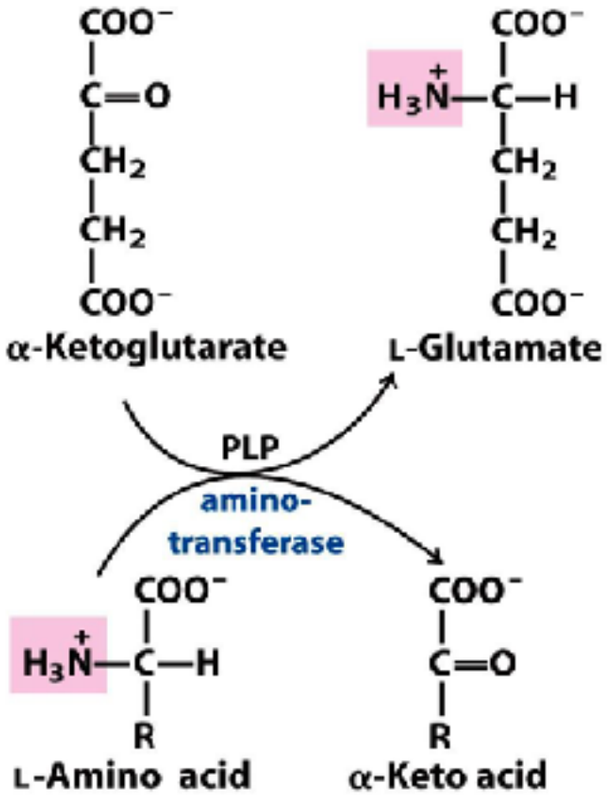
Amino acids derived from food proteins are the major source of amino groups in animals. Part of these amino groups are recycled in biosynthetic processes, but the excess needs to be expelled either in the form ammonia or as uric acid or urea (depending on the organism). the amino acids glutamate and glutamine have special role in the ammonia cycle. Amino groups from amino acids are first transferred to a-ketoglutarate to form glutamate. Glutamate is transferred in the mitochondria where the amine group is displaced to glutamine that is transported to liver mitochondria. In muscle cell excess amino groups are transferred to pyruvate to form alanine

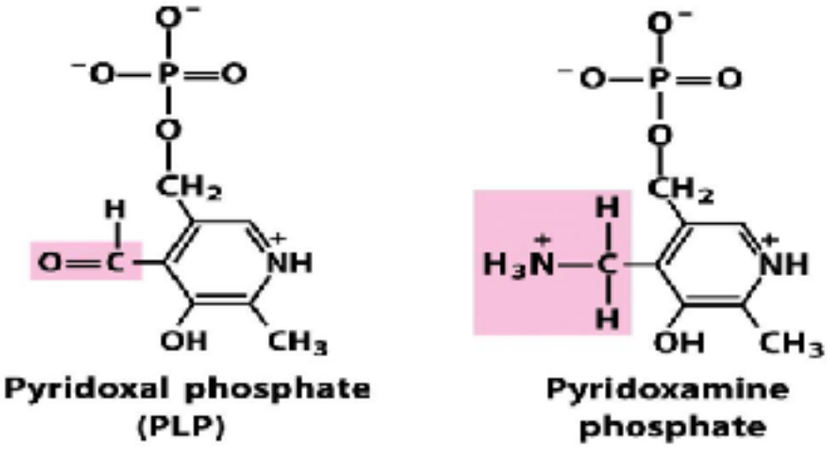
Amino acid oxidation and Urea production



In our stomachs gastric glands secrete pepsin and HCl in response to the hormone gastrin. Pancreatic cells also secrete zymogens (trypsinogen and chymotryspinogen) in the small intestine that are converted to active proteolytic enzymes (trypsin/chymotrypsin). The combined action of pepsin in the stomach and of trypsin/chymotrypsin in the small intestine digest food-derived into amino acids that are absorbed along the lower intestinal tract by epithelial cells of the intestinal mucosa.

Amino acid oxidation and Urea production



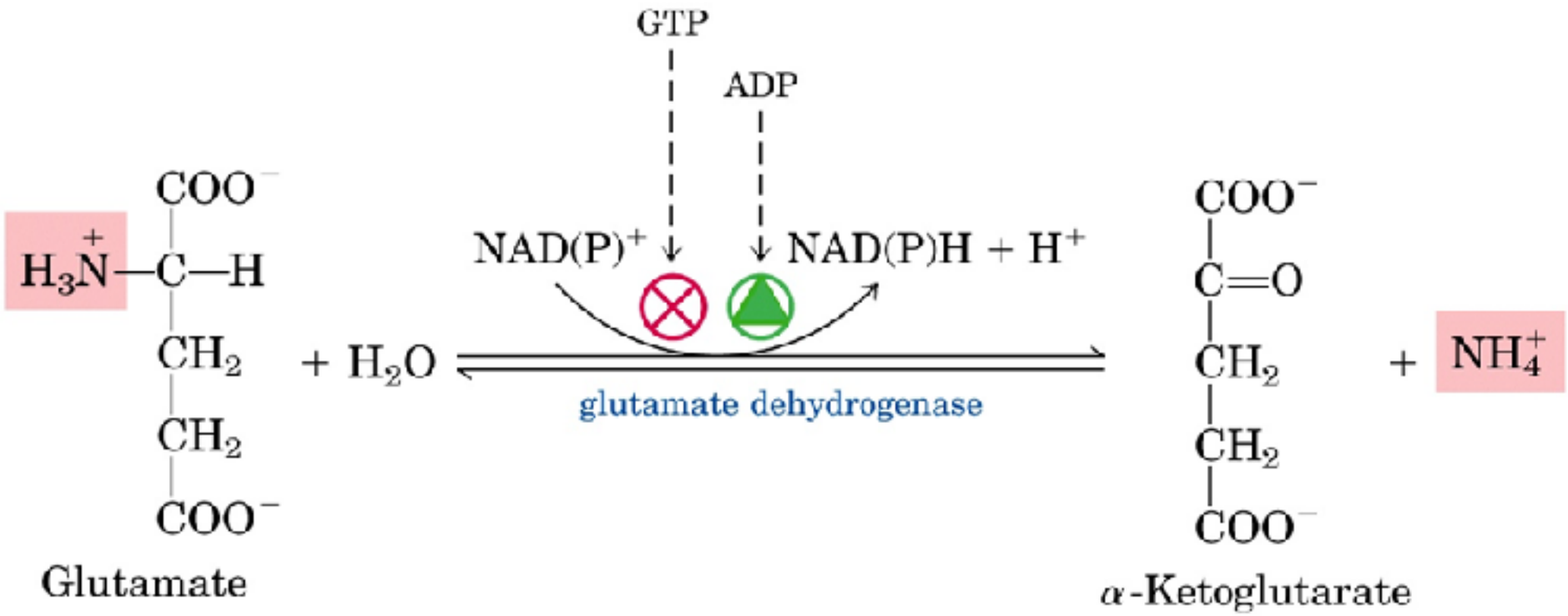


The alpha amino group of the 20 amino acids are removed in the process of oxidative degradation and conveyed in a process that produces a single excretion product. Most vertebrates excrete ammonia, terrestrial vertebrates mostly excrete urea, birds and reptiles excrete uric acid.

The detachment of the alpha amino group is the first step in the catabolism of most amino acids and it is catalysed by **amino transferases** that transfer the amino group to the a-ketoglutarate to form the a-keto acid corresponding to the given amino acid and glutamate.

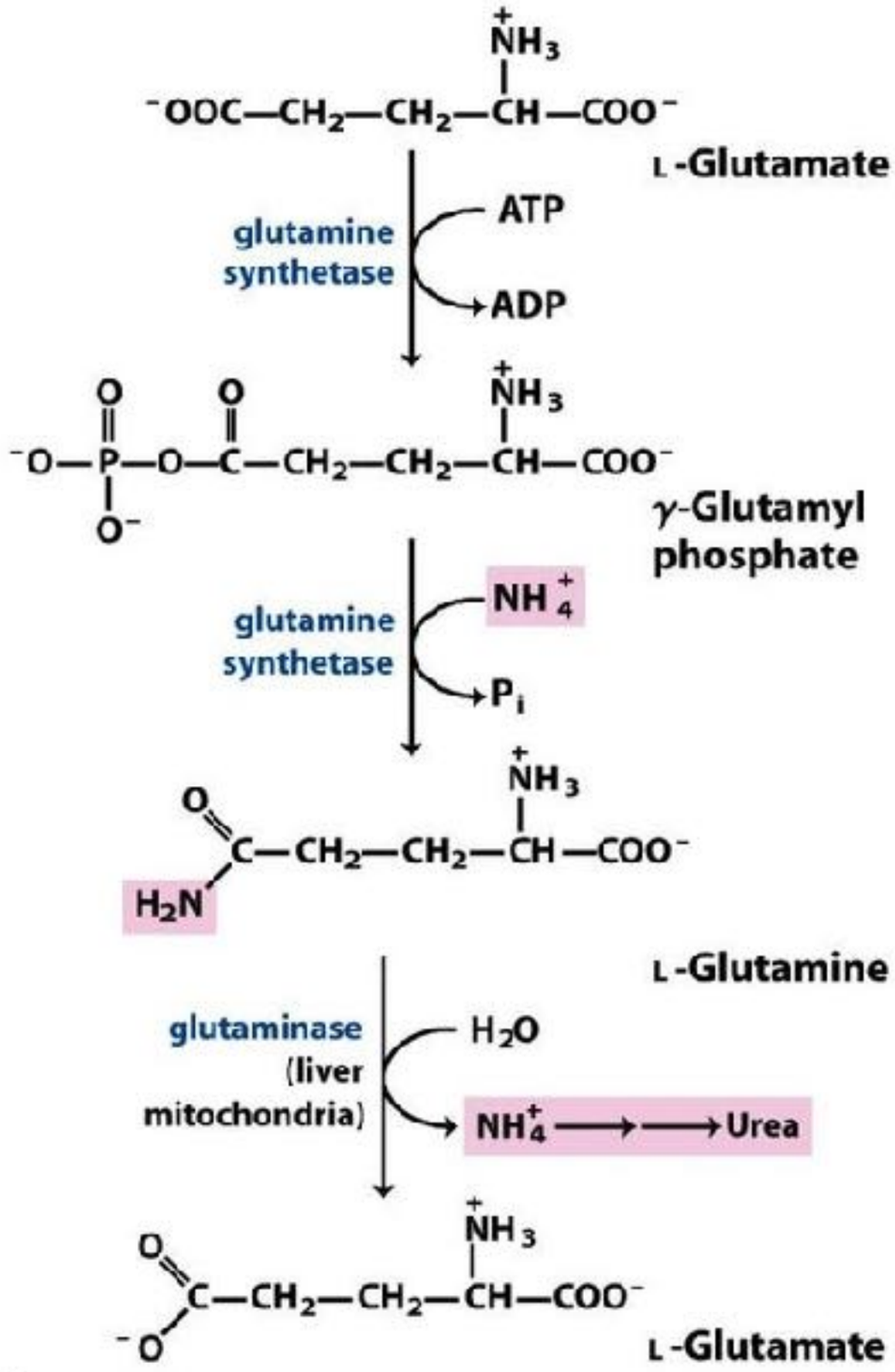
amino transferases have as a cofactor the pyridoxal phosphate (PLP), a derivative of Vitamine B6, that acts in theses reactions as a transitory transporter of amino groups

Amino acid oxidation and Urea production



Once formed in the cytoplasm, glutamate is transported in the mitochondria where it undergoes **oxidative deamination** catalysed by the enzyme **glutamate dehydrogenase** that oxidises glutamate to a-ketoglutarate and NH4+ with the concomitant reduction of NAD(P)H from NAD(P)+. This reaction is tightly controlled with GTP [indicating a high energy charge] acting as an inhibitor and ADP [indicating low energy charge] as a stimulator.

Amino acid oxidation and Urea production

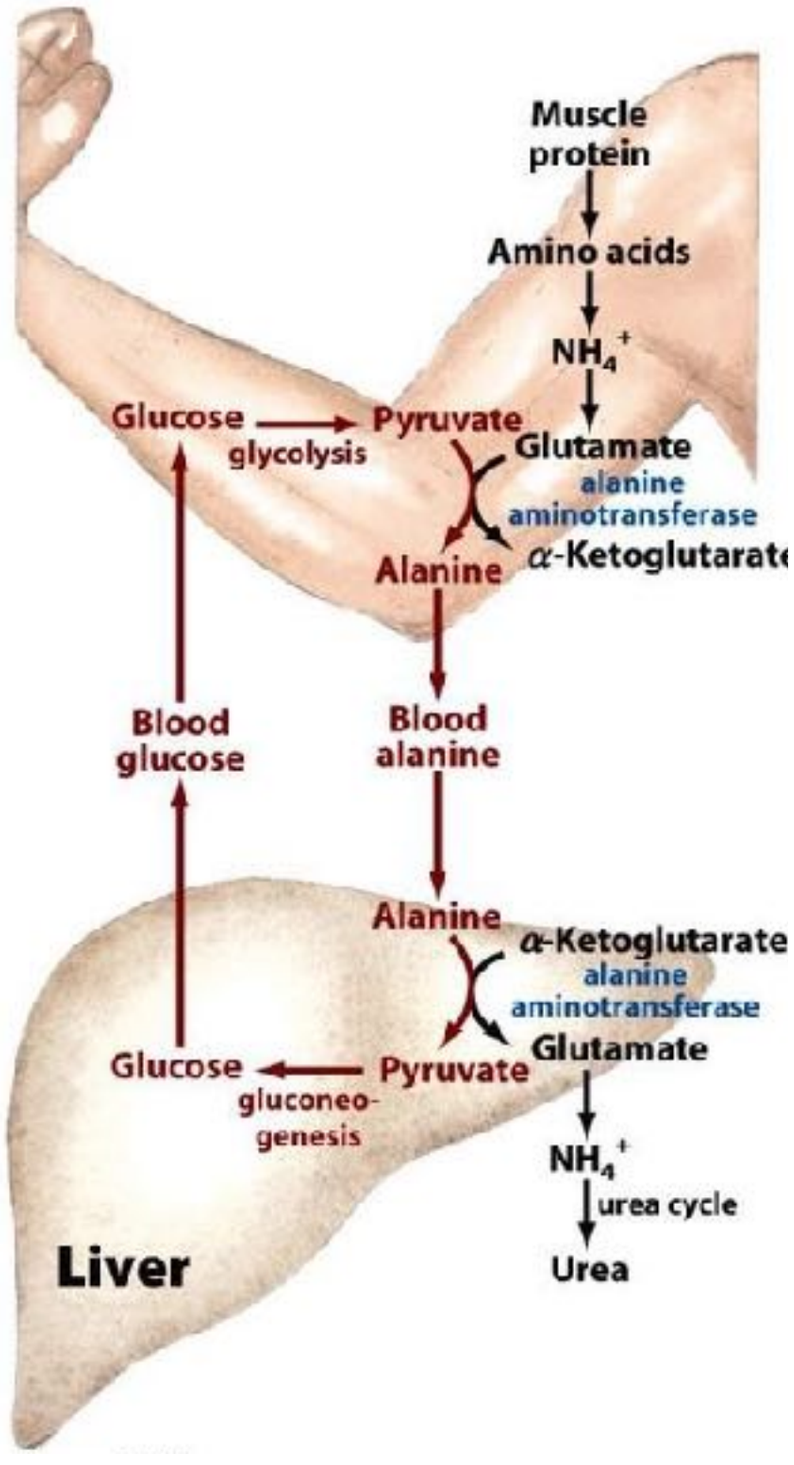


Ammonia is toxic for most tissues, thus, in order to be transported to the liver where it is converted into urea needs two be incorporated into a non-toxic compound. In most cases this non-toxic compound is glutamine.

In most tissues ammonia is complexed to glutamate to produce glutamine by the enzyme **glutamine synthetase** in a reaction that requires ATP.

We actually encountered this reaction in our first lesson to explain how ATP hydrolysis fosters otherwise unfavourite reactions. Here ATP dependent phosphorylation of glutamate renders glutamate highly reactive and able to substitute phosphate with an amino group.

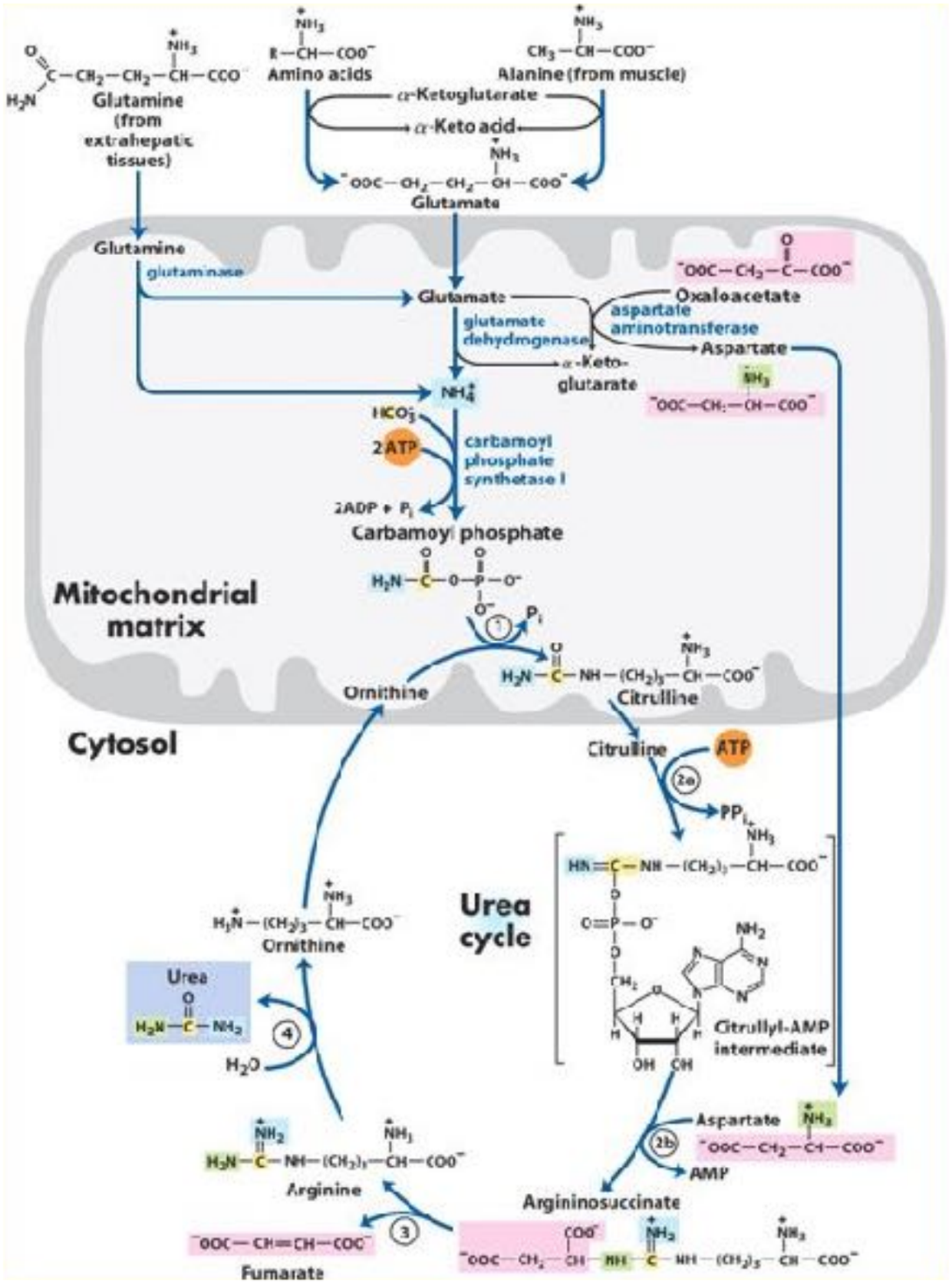
Amino acid oxidation and Urea production



In the muscles and in other tissues where amino acids are intensively used for the production of energy the amino group from glutamate can be transferred to pyruvate to form alanine. Alanine is non-toxic and is then transported to the liver to discharge NH4+ for its excretion through the urea cycle.

In the glucose-alanine cycle alanine serves as a pyruvate and ammonia transporter to the liver where pyruvate can be used in the gluconeogenesis to produce glucose that can be used in the muscle through glycolysis to produce back pyruvate and ATP

Amino acid oxidation and Urea production



The ammonia groups are then excreted as urea that is produced by hepatocytes (liver cells) in a series of 4 reactions one of which happens in the mitochondrial matrix while the remaining three happen in the cytosol.

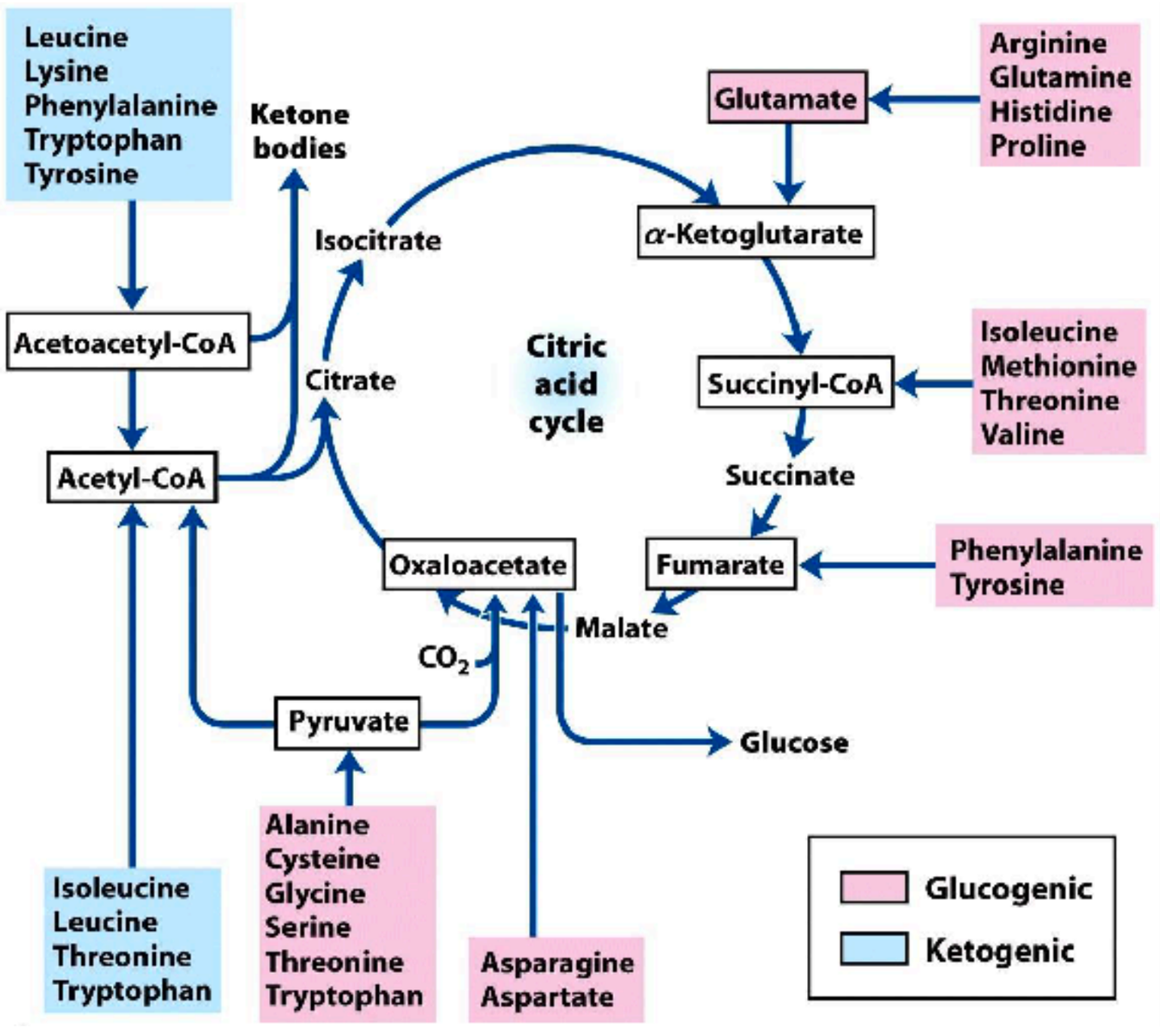
(1) Citrulline is formed from Ornithine by the addition of a carbamoyl group from glutamate derived carbamoyl-phosphate.

(2) Argininosuccinate is formed by the condensation of citrulline and aspartate

(3) Argininosuccinate is decomposed in fumarate [that feeds in the TCA cycle] and arginine

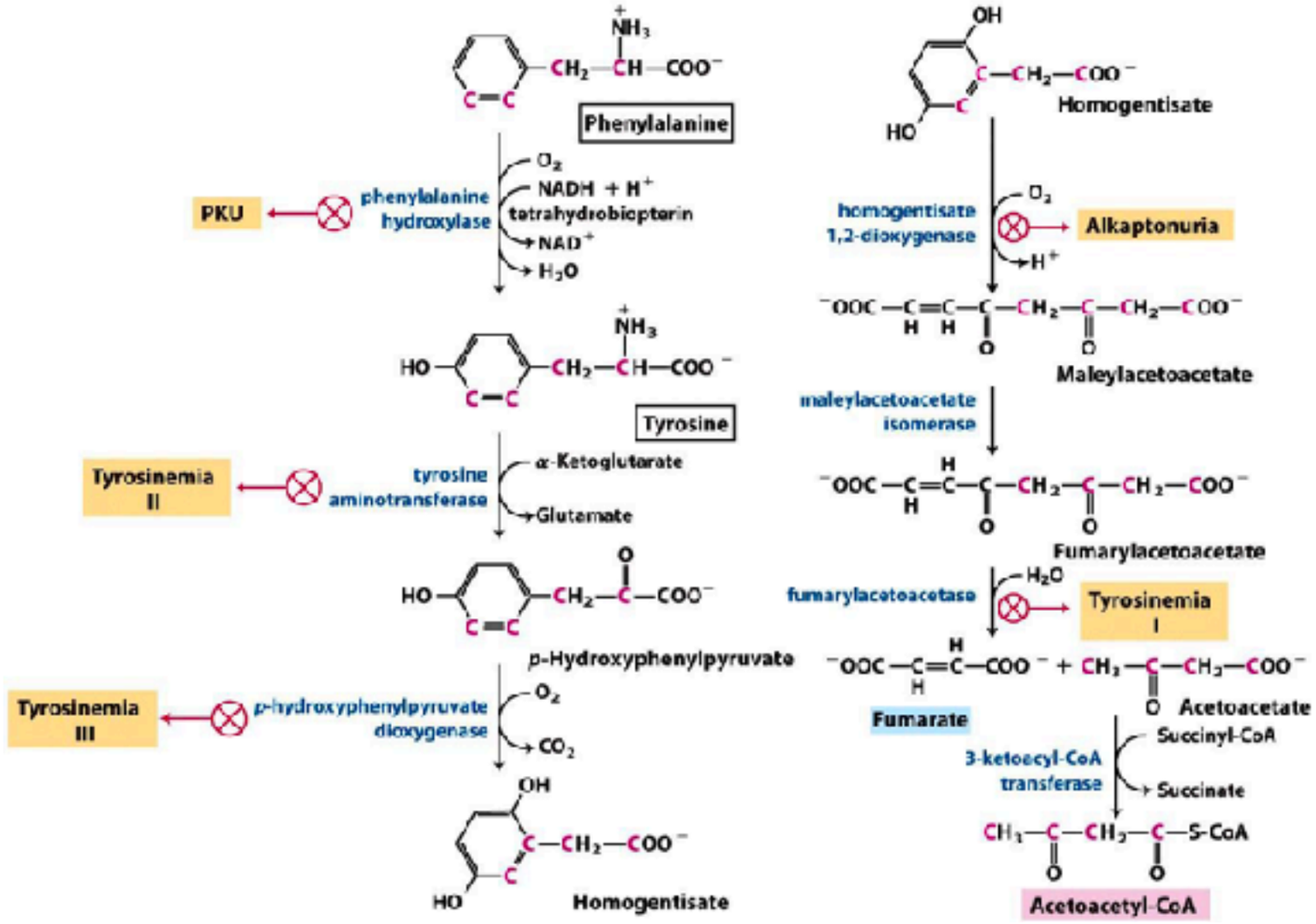
(4) Arginine is decomposed into urea and ornithine by the enzyme **arginase**

Amino acid oxidation and Urea production



As there are 20 amino acids there are 20 catabolic ways for these amino acids. of the 20 amino acids 10 feed in the TCA cycle through acetyl-CoA, 5 through a-ketoglutarate, 4 through succinyl-CoA, 2 through fumarate and 2 through oxaloacetate..

Phenylalanine catabolism



Several genetic defects have been identified in humans that impact the amino acid metabolism. these enzymatic defects lead two the accumulation of neurotoxic intermediates leading to intellectual disability. The first enzyme in the catabolism of phenylalanine (i.e., **phenylalanine hydroxylase**) mediates the conversion of phenylalanine to tyrosine. a genetic defect of this enzymes leads to a condition known as phenylketonuria (PKU). In PKU high levels of phenylalnine are found in the blood stream. When phenylalnine accumulates into tissues it can be converted to phenylpyruvate, phenylacetate and phenyllactate.

Take Home Messages

- The glycolysis product pyruvate feeds in the TCA cycle
- The TCA cycle yields a number of reduced electron carriers that will feed in the oxidative phosphorylation chain fo ATP production
- Fatty acids are oxidatively decarboxylated in the beta-oxidation reactions
- Amino acids are also oxidised and release amino groups that are extorted as urea in vertebrates through the urea cycle.
- The carbon backbones of amino acids feed in the TCA cycle using 5 different entry points