

1 Catabolism

The catabolism of proteins, fats, and carbohydrates can be divided into 3 stages:

- Stage 1: Oxidation of fatty acids, glucose, and some amino acids yield acetyl-CoA.
- Stage 2: Oxidation of acetyl groups in the citric acid cycle includes 4 steps in which electrons are abstracted.
- Stage 3: Electrons carried by NADH and FADH₂ are funneled into a chain of mitochondrial (in bacteria plasma membrane-bound) electron carriers - the respiratory chain - ultimately reducing O₂ to H₂O. This electron flow drives the production of ATP.

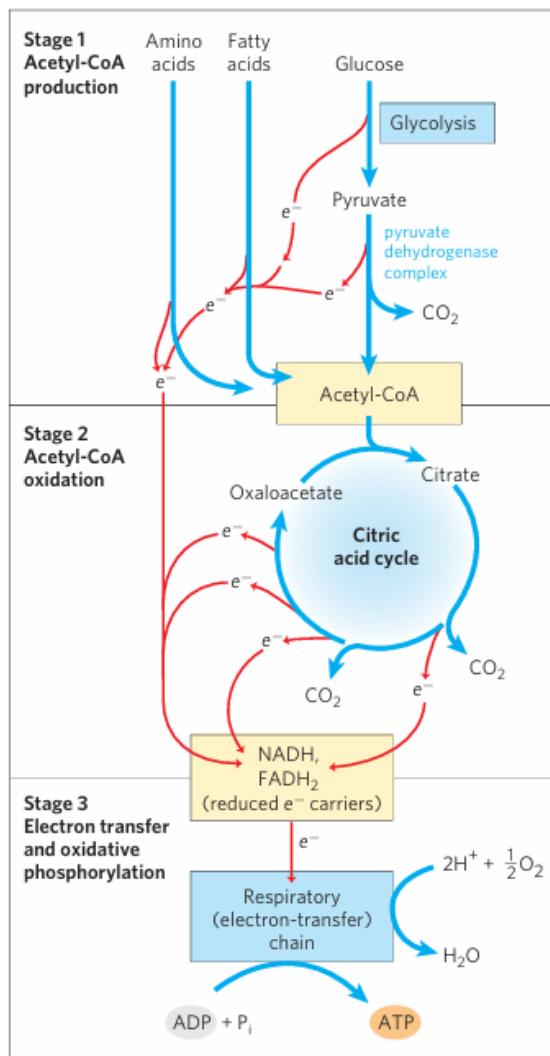


Figure 1: 3 stages of cellular respiration

1.1 Glycolysis

D-Glucose is the major nutrient for a wide range of organisms. It can be stored by cells in the form of polymers and used upon need to generate ATP.

In glycolysis (from the Greek *glycus*, "sugar", and *lysis*, "splitting") a molecule of **glucose** is degraded in a series of enzyme-catalyzed reactions to **two molecules of pyruvate**.

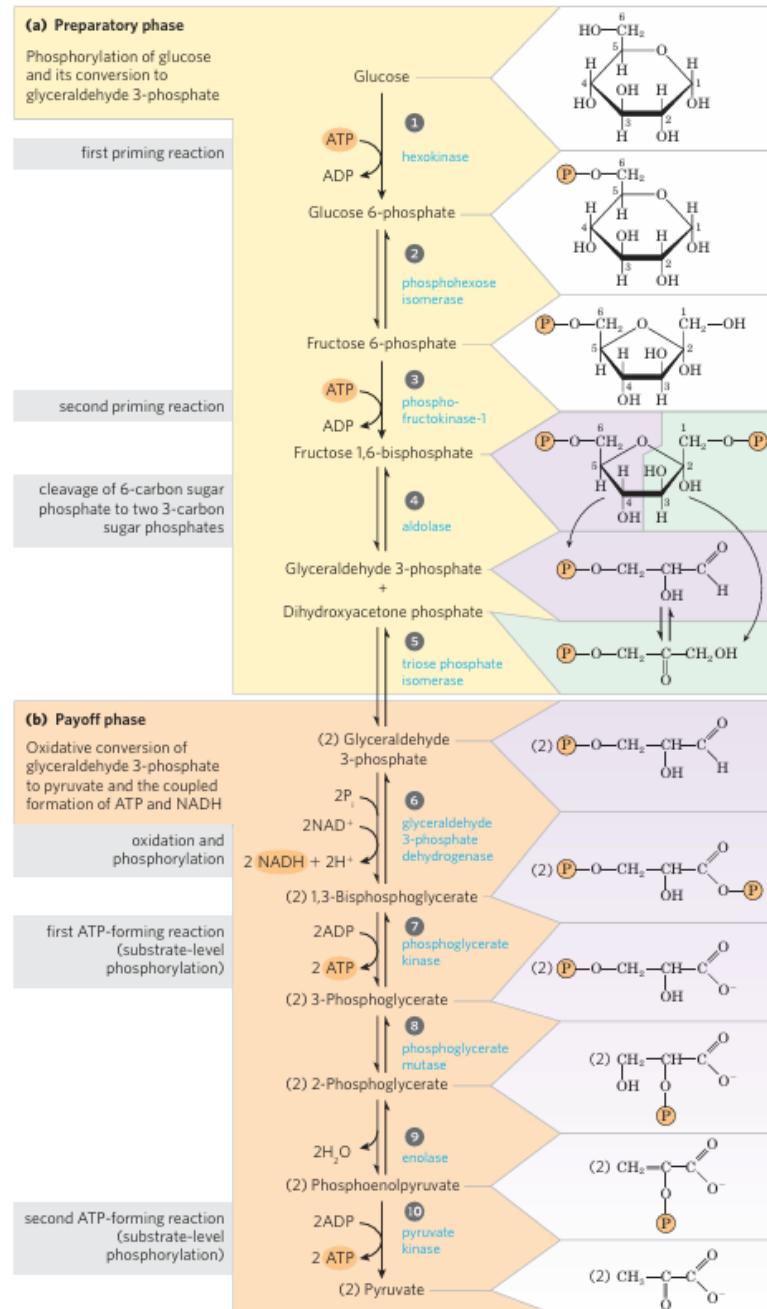


Figure 2: Glycolysis

- $\text{Glucose} + 2\text{ADP} + 2\text{NAD}^+ + 2\text{Pi} \Rightarrow 2 \text{ Pyruvate} + 2\text{ATP} + 2\text{NADH} + 2 \text{ H}^+ + 2 \text{ H}_2\text{O}$

1.1.0.1 Carbon labeling

Note when labeling GA3P the number do not correspond to the same numbers from the fructose compound. *One always follows the normal rules*

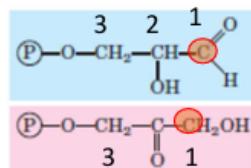
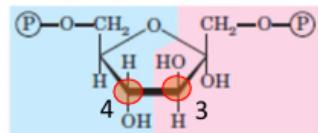


Figure 3: Carbon labeling

Glycolysis can be divided in two stages the preparation phase and the payoff phase.

1.1.1 Stage 1, Preparation Phase

In the preparation phase glucose gets **trapped** inside the cell, "activated", and **broken down** into smaller components.

1.1.1.1 Step1: Phosphorylation of Glucose

D-Glucose moves into the cell with the help of a **membrane transporter**. Once in the cytoplasma it undergoes phosphorylation by **hexokinase** to produce **Glucose 6-phosphate**. This has two consequences:

- **No backsies:** Glucose 6-phosphate is structurally different and thus can not be transported out by the same membrane transporter.
- **More reactive:** The substitution of the hydroxy group with the phosphate group (2 additional charges, etc.) makes the molecule more reactive. But this has to be paid by the **investment** of 1 ATP molecule.

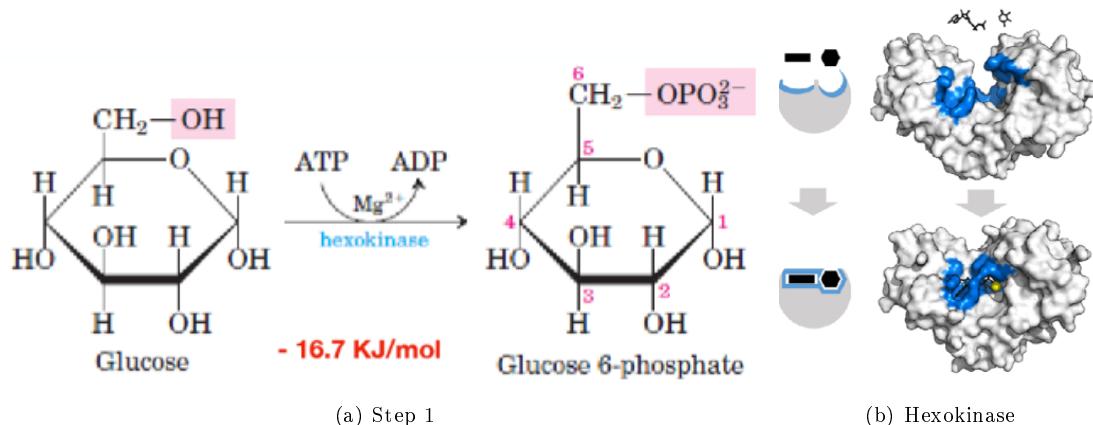


Figure 4: Phosphorylation of Glucose

Remark 1.1 (Hexokinase (HK)). Hexokinase is an enzyme that phosphorylates hexoses (like glucose) using ATP. Like most kinases it requires the presence of the cofactor Mg²⁺ in the active site.

The movement of Glucose into HK active site causes a conformational change whereby two HK lobes rotate by 12 degrees (10 Å) creating an **induced fit**. This makes the **carbon 6 oriented towards ATP** and squeezes out water molecules. (see fig. 4(b))

1.1.1.2 Step2: Isomerization

In the second step, the enzyme **phospho-glucose isomerase** also transforms aldose (glucose) into ketose (fructose). This is done to create more symmetry in preparation for step 3.

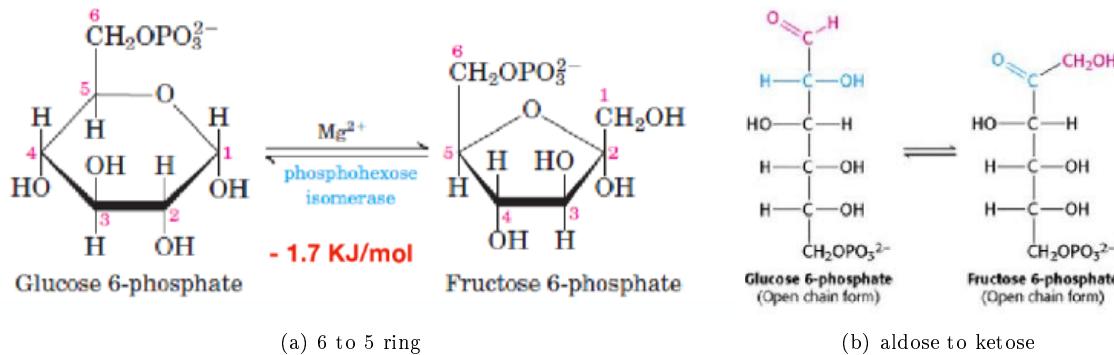


Figure 5: Isomerization

1.1.1.3 Step3: Second phosphorylation

The enzyme **phospho-fructo kinase-1 (PFK-1)** turns Fructose 6-phosphate into Fructose 1,6-biphosphate, completing the symmetry and making the compound even more reactive. This is again paid with the **investment of 1 ATP**. (see fig. 6(a))

Note, that **this step commits the sugar to glycolysis**. This is why **PFK-1 is a highly regulated enzyme** where its activity is modified according to cellular concentration of ATP, ADP, and AMP. (**ATP inhibits - AMP stimulates**).

1.1.1.4 Step4: Breakdown of Fructose 1,6-biphosphate

Aldolase catalyses the breakdown of Fructose 1,6-biphosphate into 2 different three-carbon molecules (**GA3P** and **DHAP**). GA3P feeds directly in the glycolytic pathway without any further change while DHAP needs

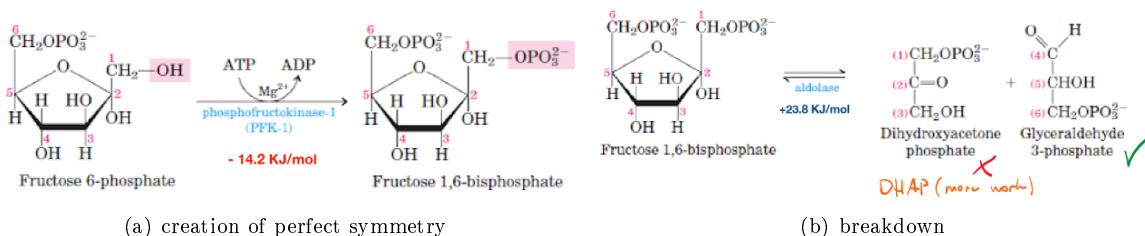


Figure 6: Step3 and Step4

to be first transformed. This is archived by Step5.

1.1.1.5 Step5: Isomerisation of DHAP to GA3P

Triose phosphate isomerase (TPI or TIM) catalyses the rapid and reversible conversion of DAHP to GA3P, ketone to aldehyde. This happens via an intramolecular redox reaction where **an hydrogen is transferred from C1 to C2**.

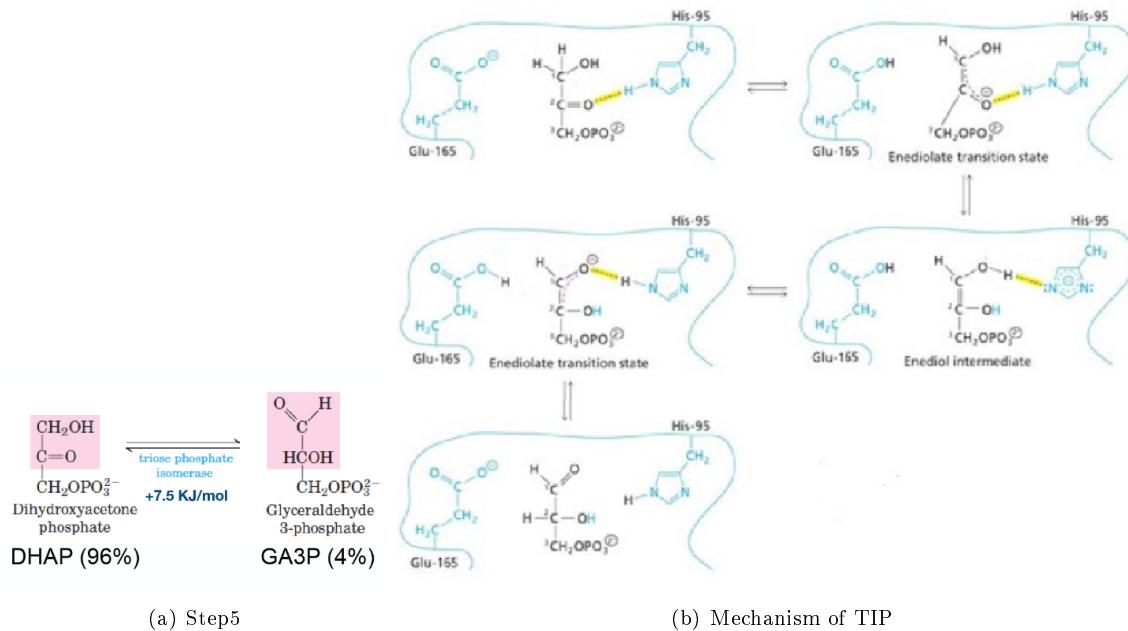


Figure 7: Isomerisation of DHAP to GA3P

Even though TIP increases the rate by 10 billion fold the equilibrium still lies on the unwanted side of DHAP (the **reaction is unfavorable**). But since the reaction is coupled to endergonic reactions (GA3P is always directly used), the **reaction shifts to the side of the product GA3P**.

1.1.2 Stage 2, Payoff Phase

In the payoff phase the components from the stage 1 get **oxidized** in order to produce ATP, NADH, and pyruvate.

1.1.2.1 Step6: Conversion of GA3P to 1,3-BPG

GA3P is converted into 1,3-biphosphoglycerate (1,3-BPG) by the enzyme glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**). Note this reaction produces NADH, which can later be oxidized.

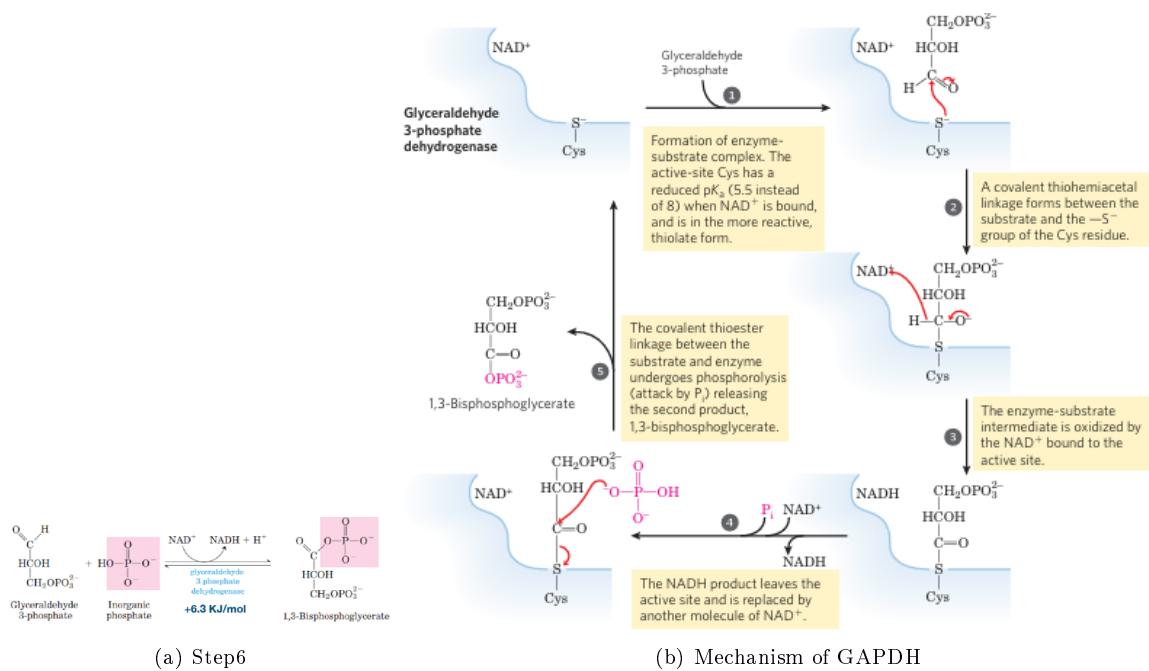


Figure 8: Conversion of GA3P to 1,3-BPG

1.1.2.2 Step7: Phosphotransfer from 1,3-BPG to ADP

Step7 is the **break-even point**. 1, 3-BPG is used as a phosphate doner to ADP. This reaction is catalyzed by **glycerophosphate kinase** and produces 3-Phosphoglycerate and ATP. (see fig. 9(a))

1.1.2.3 Step8: Conversion to 2-Phosphoglycerate

Phosphoglycate mutase catalyses the transfer of the phosphate group from C3 of 3-phosphoglycerate to C2 to form 2-phosphoglycerate.

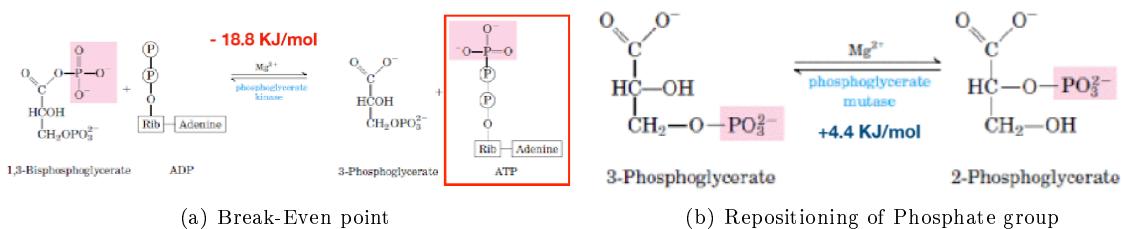


Figure 9: Step7 and Step8

1.1.2.4 Step9: Conversion to Phosphoenolpyruvate (PEP)

Enolase converts 2-phosphoglycerate into phosphoenolpyruvate (PEP). This **dehydration reaction increases the phosphoryltransfer potential** of the molecule.

1.1.2.5 Step10: Conversion to Pyruvate

The phosphoryltransfer potential of **PEP** is exploited to create ATP and pyruvate. The enzyme **pyruvate kinase** catalyses the phosphoric transfer. At this point we have gained a **total of 2 ATP and 2 NADH**.

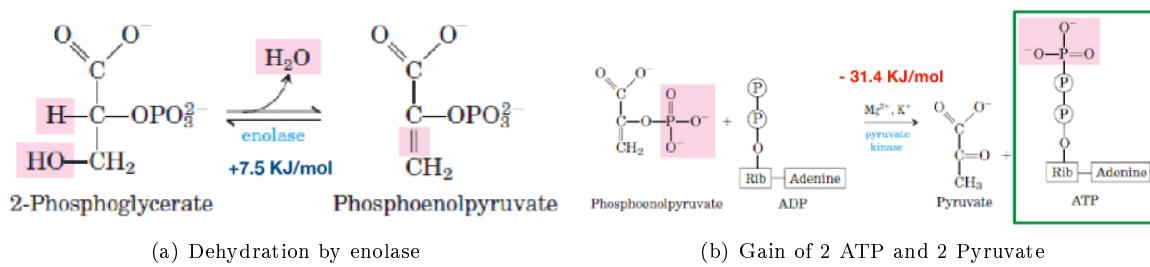


Figure 10: Step 9 and Step10

1.1.3 The fates of Pyruvate

Pyruvate is a three-carbon molecule that is the end product of glycolysis.

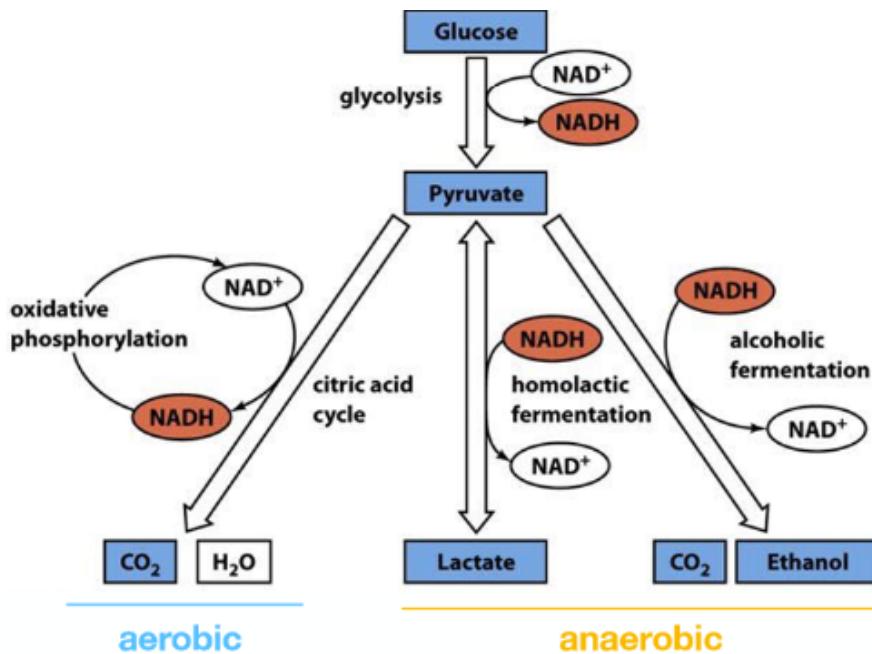


Figure 11: The fates of Pyruvate

Definition 1.2 (Facultative Anaerobic Organism). A Facultative Anaerobic Organism is able to produce ATP by aerobic respiration if oxygen is present, but is also capable of switching to fermentation if oxygen is absent. For example *E.coli* or some muscle cells (temporarily in humans).

Remark 1.3 (Soy Sauce). Soy sauce is produced by fermenting a salted mixture of soy beans. Soybeans contain starch which will be broken down to glucose and then degraded via glycolysis to pyruvate. And the ferment in the absence of oxygen. However if oxygen were present pyruvate would be oxidized to acetyl-CoA entering the citric acid cycle. But some acetyl-CoA would get hydrolyzed to acetic acid (vinegar) which would result in a undesired strong vinegar taste.

1.1.3.1 Ethanol Fermentation

Yeast and several bacteria utilise ethanol (alcoholic) fermentation to regenerate NAD^+ and to transform pyruvate into ethanol and carbon dioxide.

In a first step **pyruvate decarboxylase** catalyses a decarboxylation reaction. The enzymes needs the **coenzyme TPP**, a vitamin B1 derivative, and cofactor Mg²⁺

- Note, that the **C3 & C4 carbons of glucose will be cut away** in form of CO₂.

In the second step **alcohol dehydrogenase** will regenerate NAD⁺ in reducing acetaldehyde to ethanol. Note alcohol dehydrogenase contains a **zinc ion** in the active site to help polarize the carbonyl double bond that promotes hydride transfer from NADH.

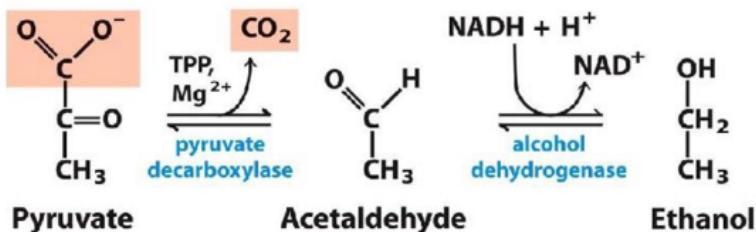


Figure 12: Ethanol Fermentation

- Glucose + 2ADP + 2Pi => 2 Ethanol + **2ATP** + 2 CO₂ + 2 H₂O

1.1.3.2 Lactic Fermentation

Many prokaryotic and eukaryotic organisms can use lactic fermentation. Like ethanol fermentation it is necessary to regenerate NAD⁺. Lactic fermentation is catalysed by **lactate dehydrogenase (LDH)**.

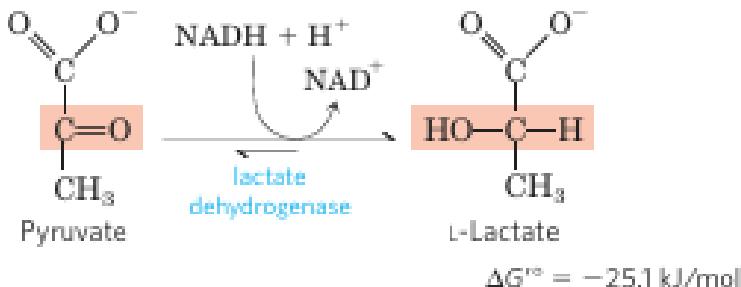


Figure 13: Lactic Fermentation

Remark 1.4 (Cancer, PET scan). Cancer cells often rely on aerobic glycolysis, known as the **Warburg effect**, where they preferentially use glycolysis followed by lactic acid fermentation, even in the presence of oxygen. This allows them to rapidly generate ATP and biosynthetic precursors for growth. Positron Emission Tomography (PET scans) exploit this metabolic shift by using **fluorodeoxyglucose (FDG)**, a radiolabeled glucose analog. Since cancer cells have a higher glucose uptake due to increased glycolysis, they accumulate FDG, which emits positrons detectable by **PET imaging**.

1.2 TCA cycle

In aerobic organisms, glucose and other sugars, fatty acids, and most amino acids are ultimately oxidized to CO₂ and H₂O via the citric cycle and the respiratory chain.

Remark 1.5 (TCA cycle). The TCA cycle (TCA = tricarboxylic acid) is also called **Krebs cycle** or **citric acid cycle**

Before entering the citric acid cycle, the carbon skeleton of sugars and fatty acids are degraded to the acetyl group of acetyl-CoA

1.2.0.1 Pyruvate \Rightarrow Acetyl-CoA

Remark 1.6 (pyruvate translocase). 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.

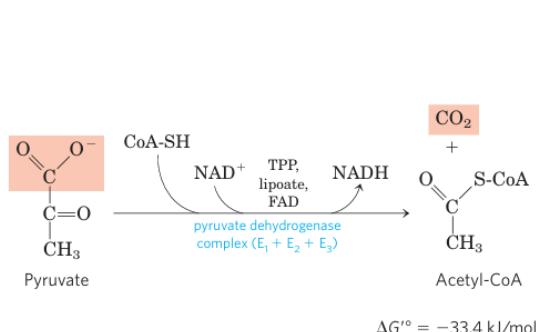
In the mitochondria pyruvate is converted to acetyl-CoA in order to enter the TCA cycle. This is done by the **pyruvate dehydrogenase complex** which catalyses an **oxidative decarboxylation**, an **irreversible** oxidation process in which the carbonyl group is removed from pyruvate as a molecule of CO₂.

The combined dehydrogenation and decarboxylation of pyruvate requires the sequential action of 3 different enzymes:

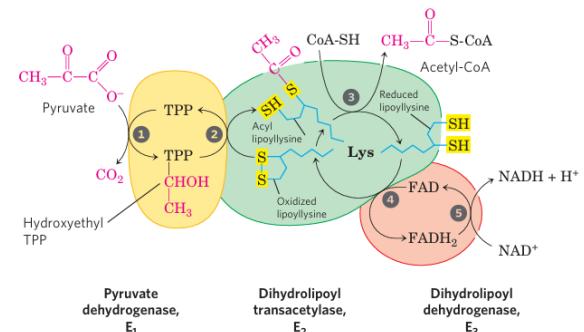
- E1 - Pyruvate dehydrogenase: Catalyses the redox-decarboxylation reaction.
- E2 - Dihydrolipoyl transacetylase: Catalyses the transfer of the acetyl group.
- E3 - Dihydrolipoyl dehydrogenase: Reforms the oxidised version of lipoamide.

Moreover 5 different co-enzymes are acting across the 5 different steps (see fig. 14(b)):

- Step 1: Pyruvate reacts with the coenzyme **glstpp** bound to E1, and undergoes decarboxylation to the hydroxyethyl derivative.
- Step 2: E1 also carries out step 2, transferring 2 electrons and then the acetyl group (oxidized form of hydrocyenthanyl group) from TPP to the oxidized and then reduced form of the coenzyme **lipoyllysine** of E2. This reduces the disulfid bond of lipoyllysine and binds the acetyl group covalently as a thioester. *Note that lipoyllysine has two thiol groups that can undergo reversible oxidation to a disulfid bond, similar to that between two Cys residues in a protein. Therefore it can serve as both electron carrier and as an acyl carrier.*
- Step 3: The acetate is **trans-estrified** to the SH group of **CoA-SH**.
- Step 4: Iionic acid is oxidised to reform the S-S bond and 2 hydride groups are transferred to **FAD**, the coenzyme bound to E3. FAD is reduced to FADH₂.
- Step 5: FAD is regenerated, transferring electrons to the coenzyme **NAD⁺** to produce NADH and H⁺.



(a) Overall reaction



(b) PDH complex

Figure 14: Pyruvate + CoA-SH + NAD⁺ \Rightarrow Acetyl-CoA + CO₂ + NADH + H⁺

Remark 1.7 (pyruvate dehydrogenase complex (PDH complex or PDC)). PDH complex is a classic, much-studied example of a multi-enzyme complex in which a series of chemical intermediates remain bound to the enzyme molecules as a substrate is transformed into the final product. It has **3 important enzymes** and uses **5 different co-enzymes**, four derived from vitamins, participate in the reaction mechanism.

Moreover, the **PDH complex is the prototype** for two other important exzmae compexes: α -ketogutarate dehydrogenase, of the TCA cylce, and α -keto acid dehydrogenase, of the oxidative pathway of several amino acids.

Note that the number of copies of each enzyme varies and therefore also the size of the complex.

While cytosolic pyruvate can be converted back to glucose, onece produced in the mitochondrial matrix Acetyl-CoA is **committed** towards the TCA cycle or lipid synthesis. Therefore PDC is catalysis a **key and irreversible step** in the glucose metabolism. Thus **PDC is tightly regulated**.

- Acetyl-CoA and NADH, two 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 activity of **PDC kinases (PDKs)** that phosphorylate and inactivate E1.

Under exercising conditions the energy charge of the cell is low and PDKs are inhibited, also **Ca⁺⁺ influx** in the mitochondria is increased, which activates **PDC phosphatases (PDPs)** that dephosphorylate and activate E1.

- E1 is inhibited by PDKs under resting conditions and activated by PDPs under exercising conditions.

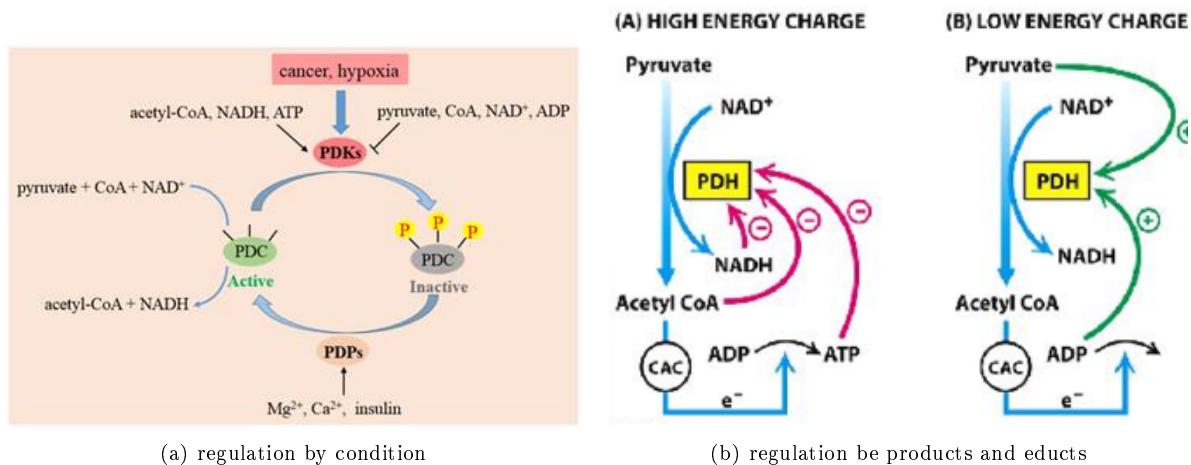


Figure 15: Regulation of PDC/PDH

1.2.1 TCA cylce steps

The TCA cylce is a game of decarboxylation, generating energy by reduction of electron carriers. The formula:

- $\text{Acteyl-CoA} + \text{GDP} + \text{Pi} + 3 \text{ NAD}^+ + \text{FAD} \Rightarrow 2 \text{ CO}_2 + 3 \text{ NADH} + \text{FADH}_2 + \text{GTP} + \text{CoA-SH}$

In the bigger picture, this is were the CO₂ is produced that we breve out.

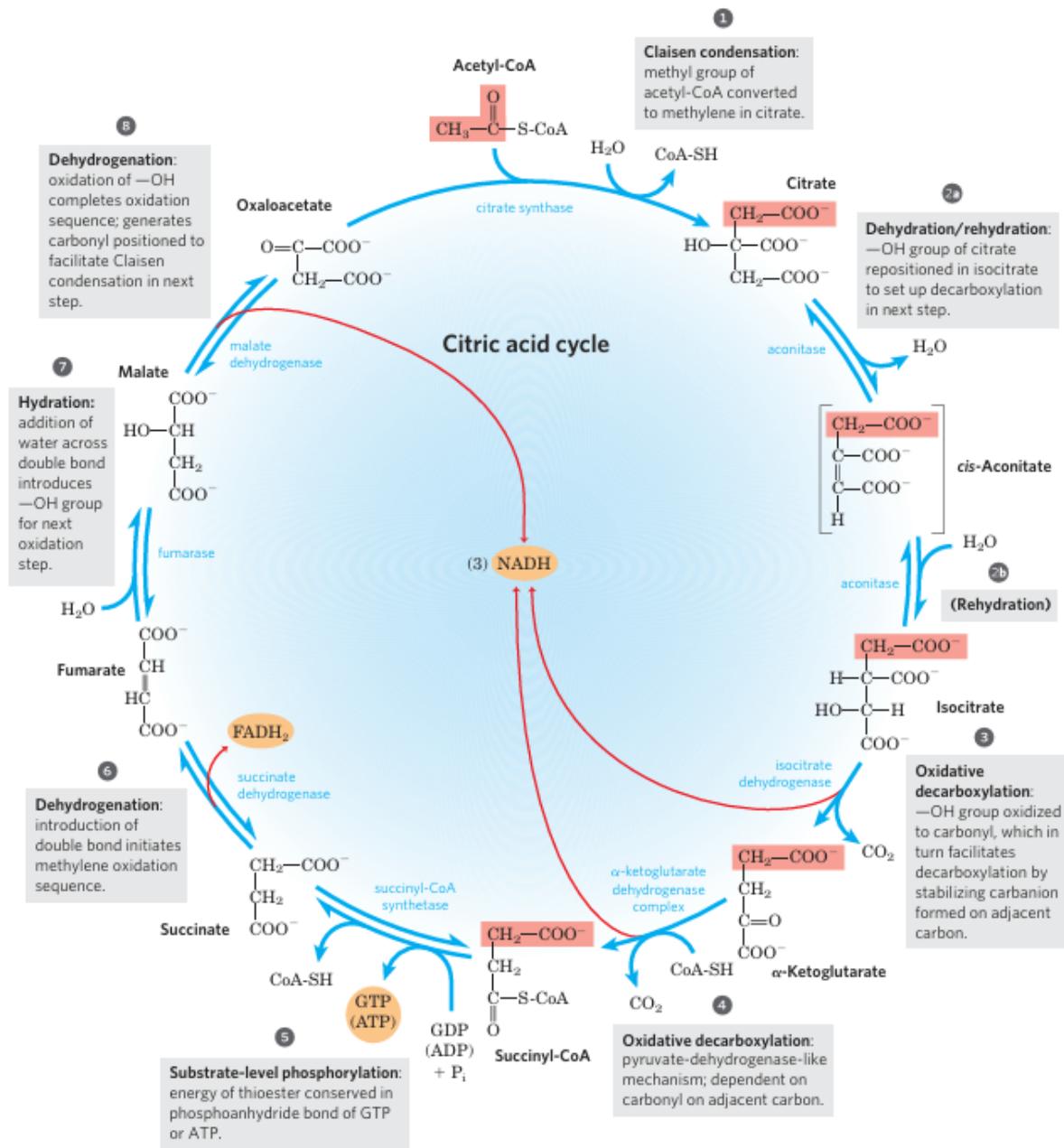


Figure 16: Overview of the TCA cycle: The carbons shaded in red are those derived from the acetate of acetyl-CoA in the first turn.

Remark 1.8 (What happens to 3-14C-pyruvate). The Resulting Acetyl-CoA will be labeled at C2 (methyl group). Therfore, at the end of the first cycle: Oxaloacetate will be labelled in C2 or C3 (because of symmetry in succinate). After two more cycles, half of the label will be released. (the label of C2 ends up at the second cycle at C2 or C3, while the lable on C3 ends up at C4, which is released in the following clycle) But il will take an infinite number of cycles to release all the label.

1.2.1.1 Step1: Formation of Citrate

In the first step the **acetyl group** is transferred to **oxalacetate** to produce citrate. The reaction consists of two phases: (1) oxalacetate is condensed to acetyl-CoA to form citryl-CoA; (2) citryl-CoA is hydrolyzed to form citrate and CoA-SH. Whereby **phase 2 is highly exergonic** and therefore drives the entire reaction.

Remark 1.9 (Citrate synthase). Step 1 is carried out by citrate synthase. Citrate synthase is a **dimer**. It first binds oxalacetate into its active site, which causes a conformational change from open to closed conformation. By doing so oxalacetate binding induces the formation of the acetyl-CoA binding site and shifts the catalytic residue into proper position.

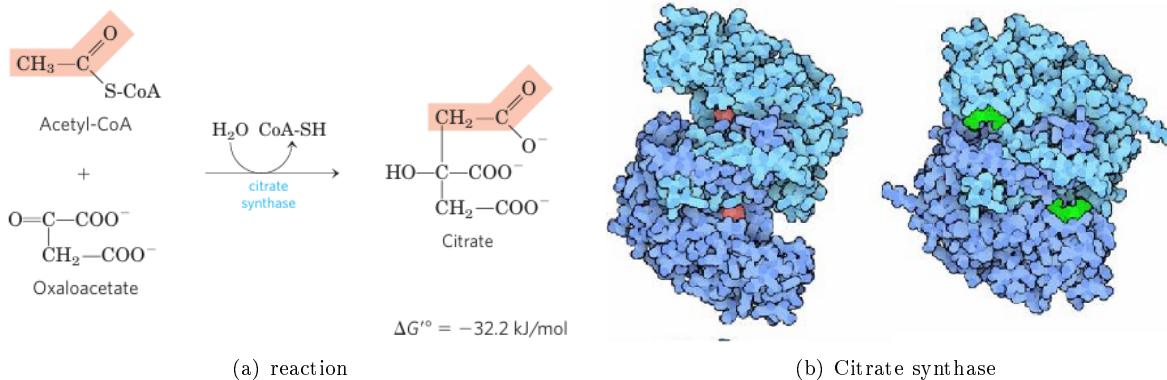


Figure 17: Formation of Citrate

1.2.1.2 Step2: Formation of Isocitrate

Citrate is converted into isocitrate. In this reaction an hydroxyl group is moved from the third carbon of citrate to an adjacent carbon via a dehydration/hydration reaction.

Remark 1.10 (Aconitase enzyme). Aconitase enzyme catalyzed this dehydration/hydration reaction. It contains a **Iron-Sulfur center**. 3 Cys residues of the enzyme bind 3 Fe atoms, the 4th 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.**

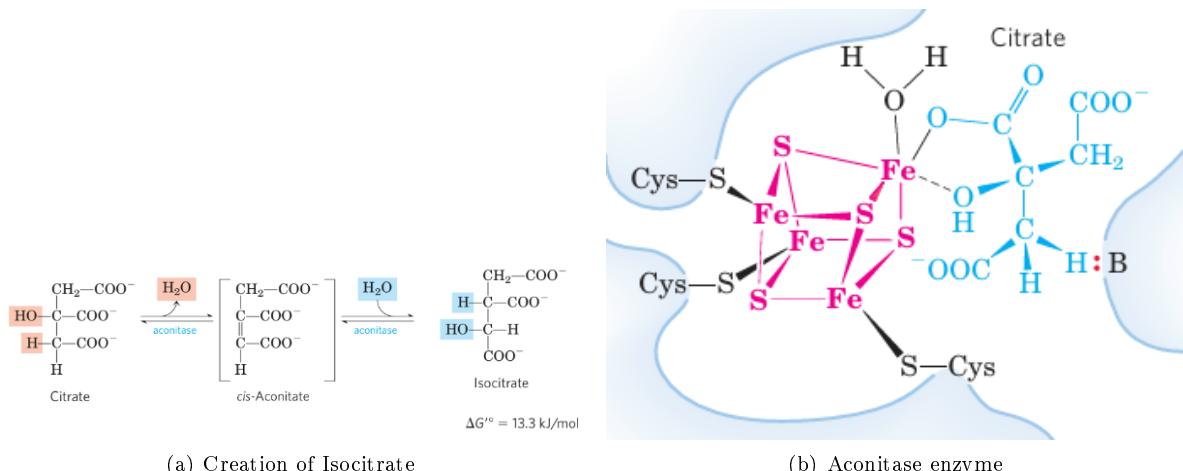


Figure 18: Formation of Isocitrate via cis-Aconitate

1.2.1.3 Step3: Decarboxylation of Isocitrate

Once isocitrate is formed, it is ready to undergo the **first oxidative decarboxylation reaction** of the citric acid cycle. This step is catalyzed by **isocitrate dehydrogenase** which yields α -ketoglutarate, CO_2 and NADH

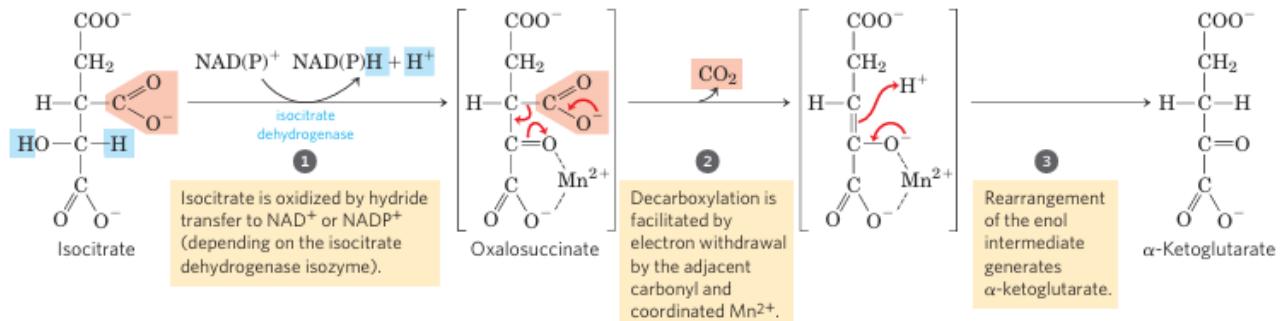


Figure 19: formation of α -ketoglutarate by isocitrate dehydrogenase

1.2.1.4 Step4 Decarboxylation of α -ketoglutarate

This is the **second oxidative decarboxylation reaction** in the TCA cycle. α -ketoglutarate is converted to succinyl-CoA with concomitant production of CO_2 and NADH. Note that the energy of the oxydation is conserved in the thioester bond.

Remark 1.11 (α -ketoglutarate dehydrogenase complex). α -ketoglutarate dehydrogenase complex is build very similar to the pyruvate dehydrogenase complex. Like PDC it has 3 has 3 important enzymes:

- E1: α -ketoglutarate dehydrogenase with TPP as a cofactor
- E2: dihydrolipyl succinyltransferase with lipoic acid as a cofactor
- E3: dihydrolipyl dehydrogenase with FAD as cofactor.

This is a clear case of **divergent evolution**.

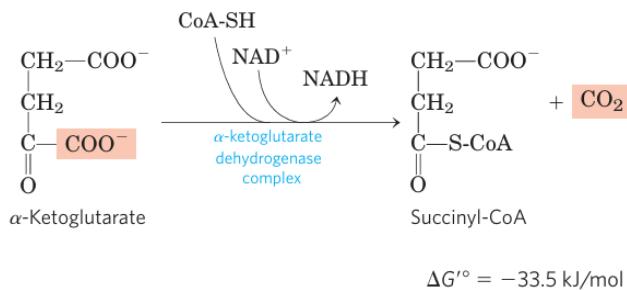


Figure 20: oxidation of α -Ketoglutarate to Succinyl-CoA and CO_2

1.2.1.5 Step5: Conversion of Succinyl-CoA to Succinate

The unstable and high energy thioester bond of 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 catalyzed by **succinyl CoA synthetases**.

The reaction has 3 phases as illustrated by fig. 21(b):

- i) A phosphate group substitutes CoA to form succinyl phosphate (a high energy acyl phosphate).
- ii) Succinyl phosphate donates the phosphate to a His residue in the enzyme (Succinat is formed).
- iii) The phosphate group is transferred from the high energy phosphorylated histidine to GDP to form GTP.

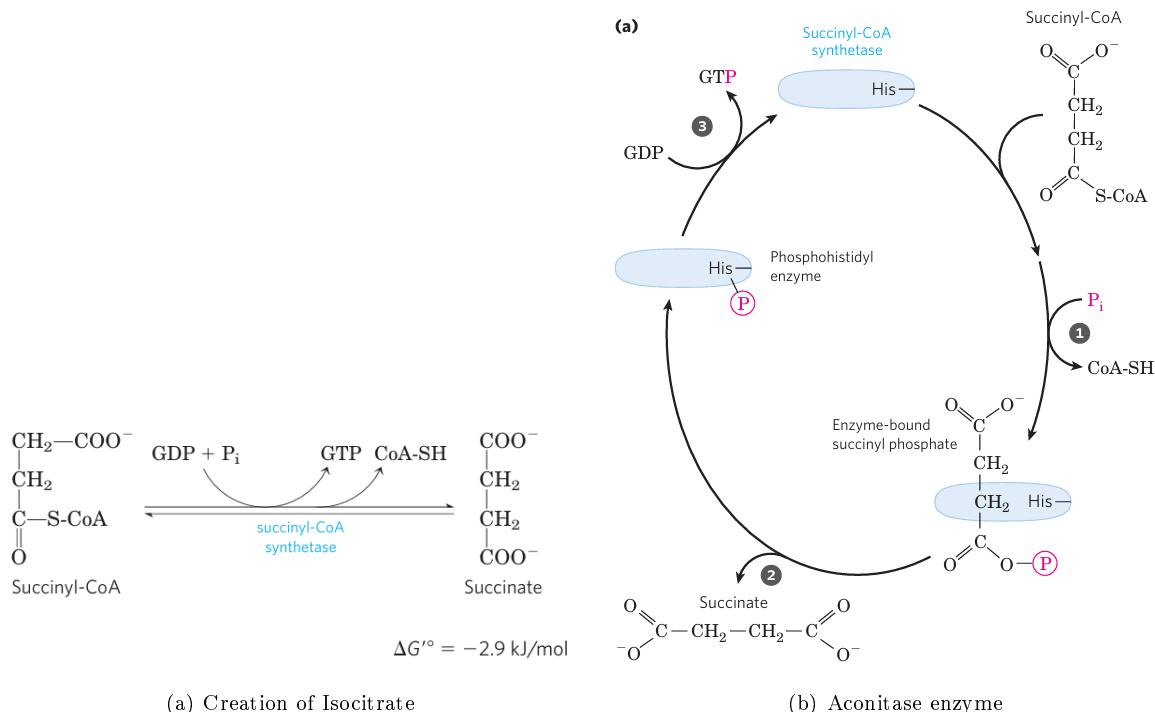


Figure 21: Conversion of Succinyl-CoA to Succinate

1.2.1.6 Step6: Formation of Fumerate

In step 6, succinate is oxidised to fumarate by succinate dehydrogenase. This is coupled to the reduction of FAD to FADH₂. See fig. 22(a)

Remark 1.12 (succinate dehydrogenase). Succinate dehydrogenase is bound to the inner mitochondrial membrane and its FADH₂ passes electrons to the electron transport chain.

1.2.1.7 Step7: Formation of Malate

Fumerase catalyses the hydration of fumerate into malate. Note that the water molecule attacks only at a specific site, thus only the L-isomer of malate is formed.

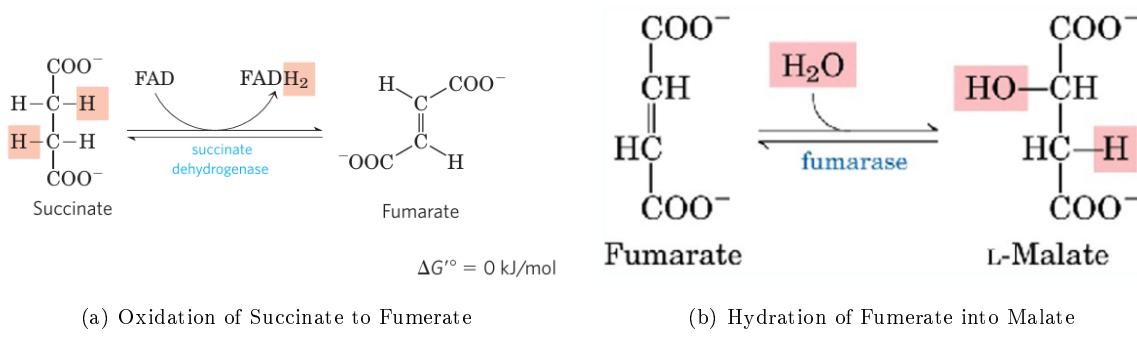


Figure 22: Step 6 and 7

1.2.1.8 Step8: Regeneration of Oxaloacetate

Finally, **malate dehydrogenase** regenerates oxaloacetate by oxidation of malate. This reaction is coupled with the reduction of NAD^+ to NADH . This process is highly **endergonic** and needs to be coupled with other exergonic steps.

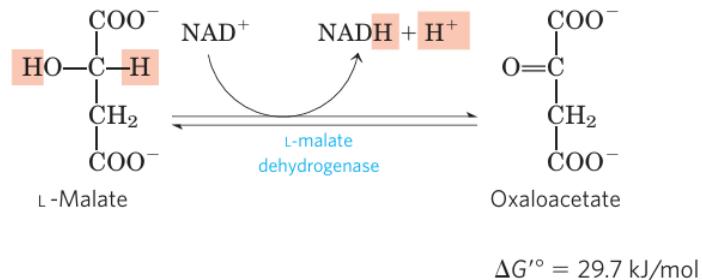


Figure 23: Oxidation of Malate to Oxaloacetate

1.3 Fatty Acid Oxidation

Adipose tissue cells (**adipocytes**) are specialised cells that store energy in the form of **triglycerides**. *Lipids are a form to long-time store energy, which will be used in moments with little glucose.* Upon demand triglycerides are hydrolysed to glycerol and fatty acids that are transported to target tissues.

Following this step **fatty acids** can be **activated (bound to CoA, see. fig. 26(a))** and transported to the mitochondrial matrix of cells. In the mitochondrial matrix they iteratively undergo a series of **4 reactions** that shorten the acyl chain by 2 carbons and release acetyl-CoA (that can feed in the TCA cycle)

This entire process is known as **fatty acid oxidation**.

Dietary fats are absorbed in the **small intestine**. **bile acids** are released and act as biological detergents, resuspending triglycerides into fine micelles. In this form triglycerides are accessible to water-soluble lipases in the intestine lumen.

The products of this lipases are then absorbed by the intestine mucosa and converted back to triglycerides. They are then transported through the bloodstream and finally stored in dedicated adipose cells that have the specialized organelles named **lipid droplets**.

Remark 1.13 (Triacylglycerols better than Polysaccharides). Triacylglycerols contain **more energy per gram than polysaccharides**. Moreover, they are unhydrated, thus the organism does not have to carry the

extra weight in form of water as with stored polysaccharides. Additionally in some animals, such as seals, fats stores under the skin serve as insulation against cold temperatures.

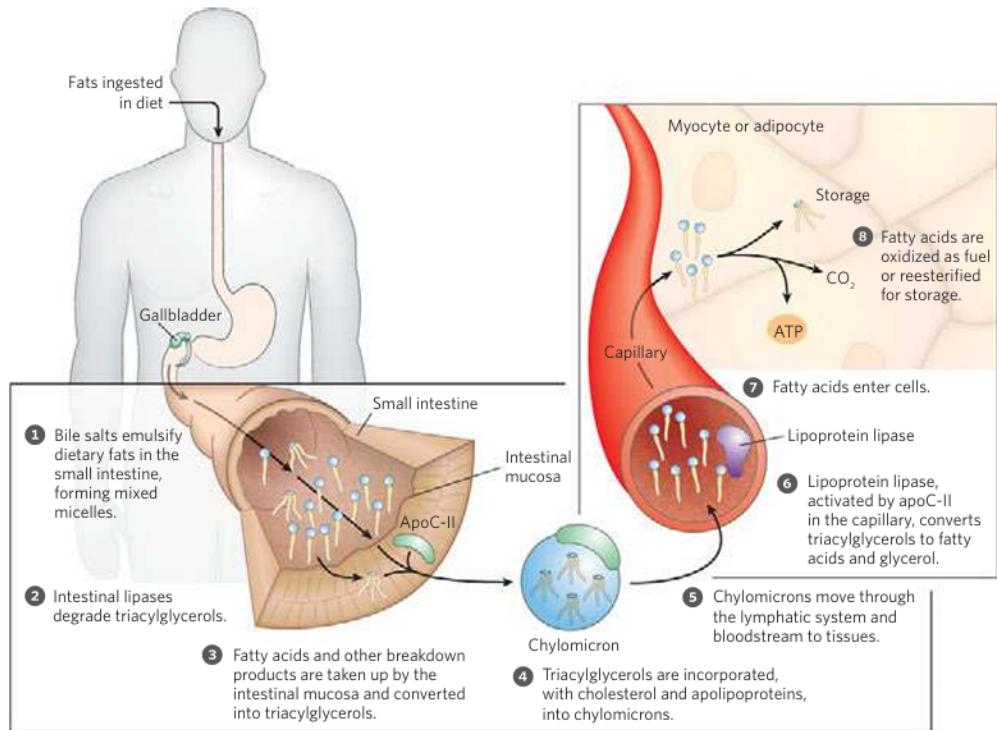
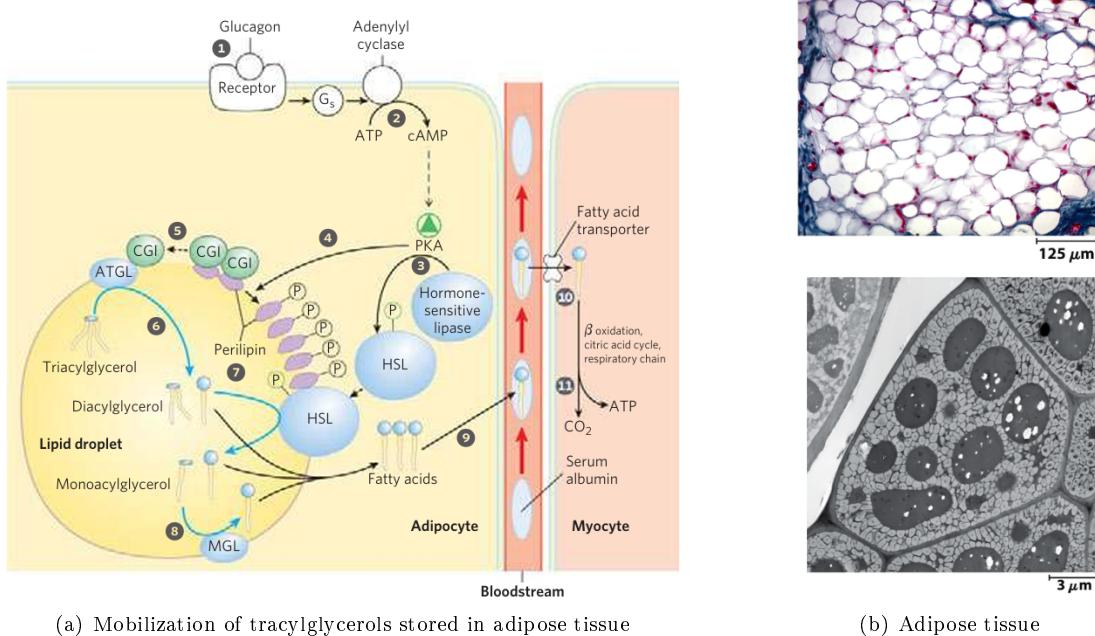


Figure 24: Processing of dietary lipids in vertebrates

When blood glucose levels are low, some hormones (**glucagon/adrenaline**) are released. They activated **adenylyl cyclase on the surface of adipocytes**. This leads to the production of **cAMP** which activates **protein kinase A (PKA)**, which triggers intracellular triglyceride lipase to produce fatty acids and glycerol. The products are released into the bloodstream, where they are transported with the serum protein **Albumin** to muscle cells (**myocytes**) where they are then oxidized for the production of ATP.



(a) Mobilization of triglycerols stored in adipose tissue

(b) Adipose tissue

Figure 25:

1.3.0.1 What about glycerol?

95% of the energy derived from triglycerides comes from the oxidation of the fatty acid chain, where **5% comes from glycerol**.

Glycerol, released by triglyceride lipases, is phosphorylated to glycerol 3-phosphate by **glycerol kinase** using 1 ATP. Then glycerol is oxidized by the enzyme glycerol 3-phosphate dehydrogenase to **DHAP**, thus entering the glycolytic pathway.

Remark 1.14 (How much ATP do we get from glycerol in the glycolytic pathway?). First we have to invest 1 ATP to produce DHAP entering the glycolytic pathway. We enter the payoff phase of glycolysis where we produce 2 ATP. Giving us a net of 1 ATP produced, additionally we still have the energy of 1 pyruvate and 2 NADH.

1.3.0.2 Transport into the Mitochondria

The transport into the mitochondria is the rate-limiting step in β -oxidation.

Fatty acid oxidation takes place in the mitochondrial matrix. Therefore fatty acids have to be transported into the mitochondria to be oxidized. For this purpose, they are again activated, linked to CoA. This is done by the enzyme **fatty acyl-CoA synthetases**

Fatty acyl-CoA synthetases have a **two phase mechanism** (see fig. 26(a)):

- i) The fatty acid made reactive by forming a complex with AMP, producing PPi and using ATP.
- ii) AMP is then exchanged with CoA-SH, in a nucleophilic attack, forming fatty acyl-CoA.

Note that PPi is immediately dissociated into phosphate molecules by **inorganic pyrophosphatase**.

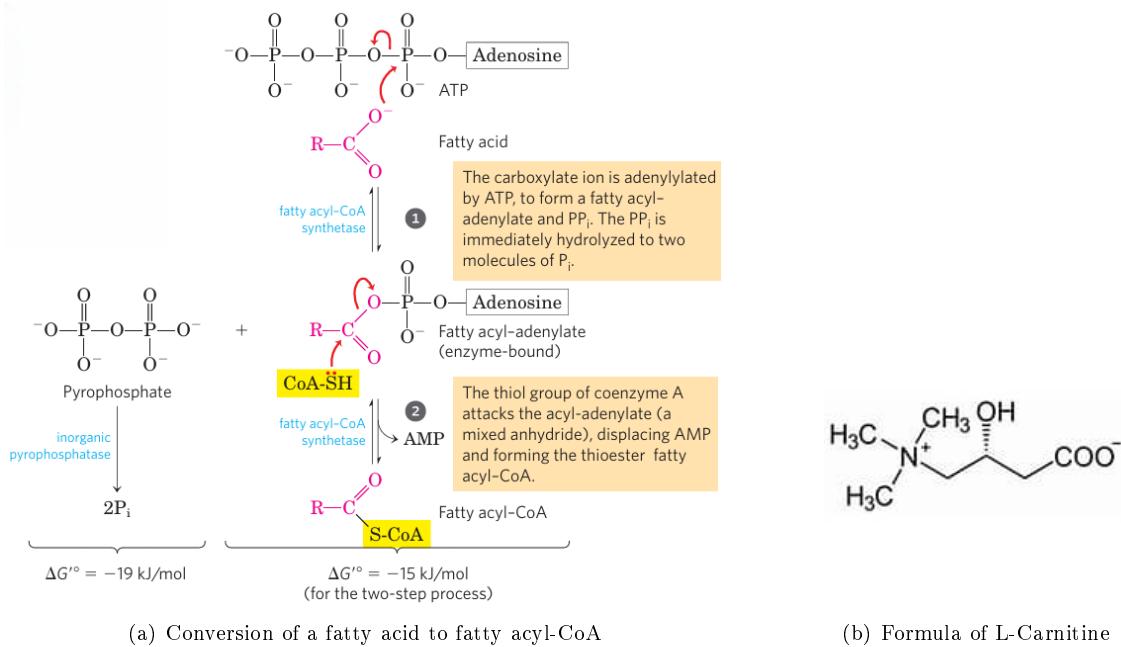


Figure 26:

Once formed in the cytosolic environment, fatty acyl-CoA crosses the mitochondrial membrane transiently exchanging its CoA with carnitine and exploiting the **acyl-carnitine transporter**.

- Carnitine acyltransferase I is located at the outer membrane and catalyzes the exchange of CoA with carnitine.
- Carnitine acyltransferase II is located in the inner membrane and exchanges the carnitine for CoA.

Definition 1.15 (Carnitine). Carnitine (see. fig 26(b)) is a quaternary ammonium compound. Carnitine forms a temporary conjugate with acyl groups (as acyl-carnitine), enabling them to cross the inner mitochondrial membrane via the carnitine shuttle system.

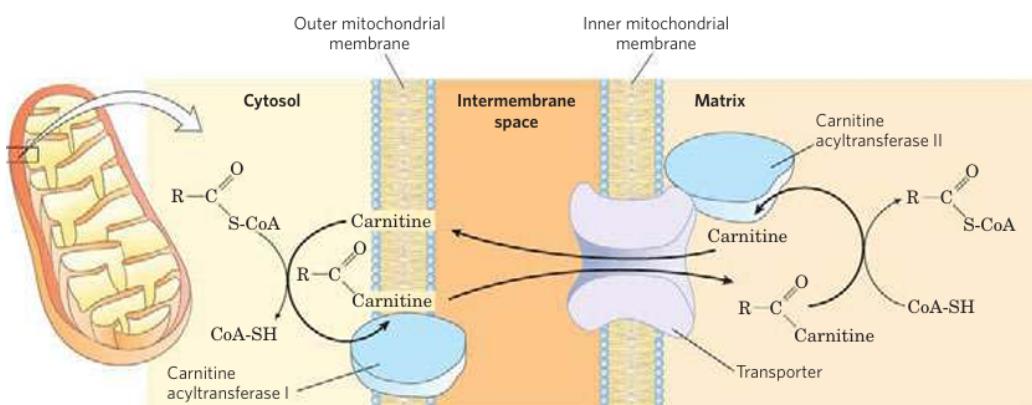


Figure 27: Fatty acid entry into mitochondria via acyl-carnitine/carnitine transporter

Remark 1.16 (Primary Carnitine deficiency). Primary carnitine deficiency is an autosomal recessive disorder affecting transport of carnitine. Since carnitine is essential for the transport of long-chain fatty acyl-CoA into the mitochondria, this disorder results in reduced fatty acid-derived energy production, leading to symptoms such as **fatigue**. Moreover, impaired import of fatty acids into mitochondria causes their accumulation in the cytoplasm (**lipotoxicity**), which can disrupt organ function. In the heart, this may result in cardiomyopathy.

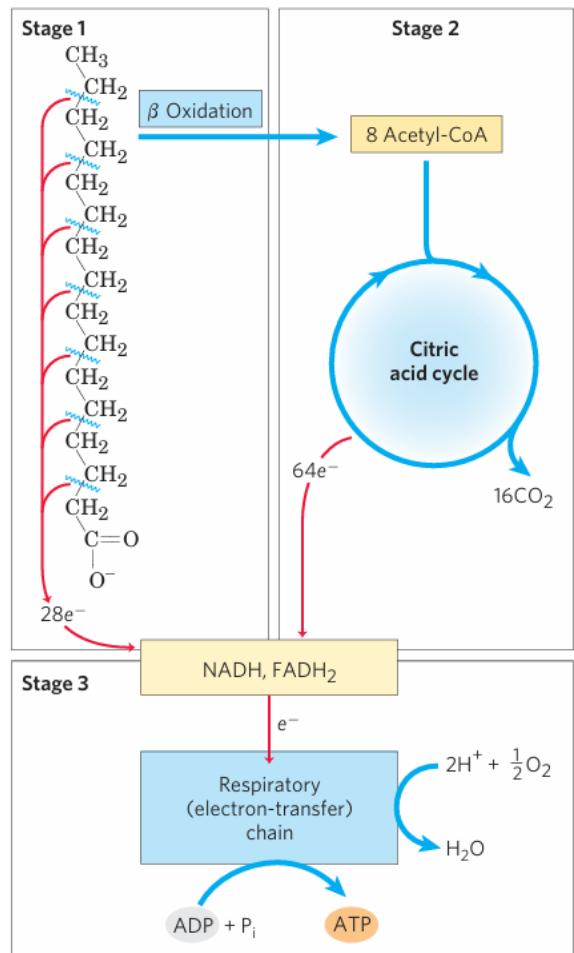
1.3.1 Beta oxidation

There are 3 stages in the oxidation of FAs (see fig. 28(a)). The β -oxidation is the first stage, where, in the mitochondrial matrix, fatty acetyl-CoA is progressively oxidised by an iterative sequence of four reactions that produce acetic units in the form of acetyl-CoA

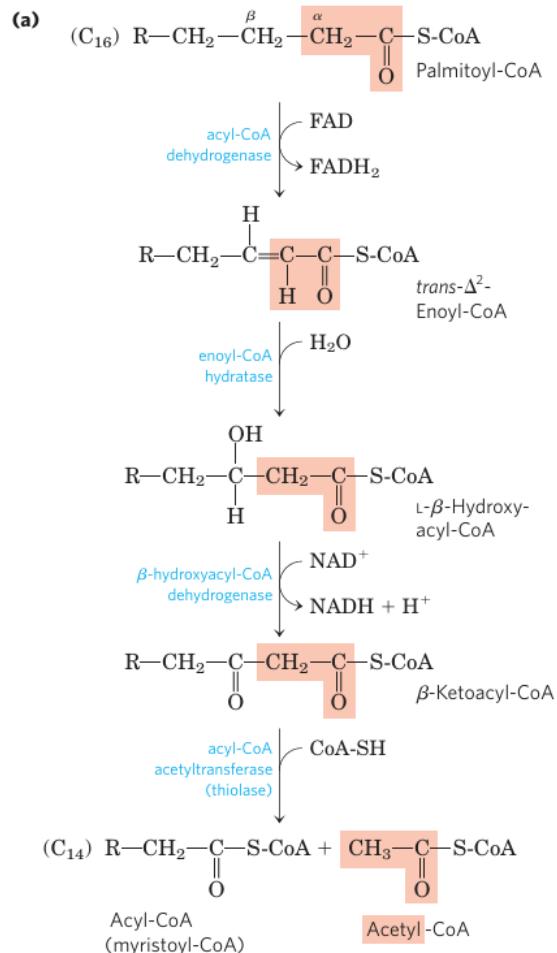
The acetyl-CoA units formed in stage 1 can then be feed in the TAC cycle (stage 2). Finally, the electrons subtracted in these oxidative reactions are used to produce ATP in the ETC.

There are 4 reactions involved in β -oxidation (see fig. 28(b)):

- i) Dehydrogenation produces a double bond between the α - and β -carbon (C2 and C3). This is catalyzed by **acyl-CoA dehydrogenase** (similar to succinate dehydrogenase from TCA cycle) and produces **2 FADH₂**.
- ii) Hydration, adding water to the α - β double bond. The hydroxy group is added to C3, producing β -Hydroxyacyl-CoA. This is done by **enoyl-CoA hydratase**
- iii) Dehydrogenation, oxidizing the alcohol group to a keton, produces β -Ketoacyl-CoA. This is done by **β -hydroxyacyl-CoA dehydrogenase**, whose action is homologous to that of malate dehydrogenase in the TCA cycle. The electrons are transferred to NAD⁺ creating **NADH**
- iv) Finally the can is shortened by a thiolase which **detaches Acetyl-CoA** and creates a by 2 carbon shorter Acyl-CoA. This is catalyzed by **acyl-CoA acetyl transferase**.



(a) Stages of FA oxidation



(b) β -oxidation pathway

Figure 28: FA oxidation overview

Remark 1.17 (Unsaturated FAs). In the case of unsaturated fatty acids, two more enzymes are required (see fig. 29(a)):

- **Enoyl-CoA isomerase** that converts the **cis-isomer into a trans-isomer** or **shift the double bond** to the right position (C₂=C₃), which can be used by enoyl hydratase. (Obviously only for FA containing cis double bonds)
- For **polyunsaturated FAs (2)** **2,4-dienoyl-CoA reductase** has to be used. It reduces 2 double bonds to one, **consuming an NADPH**. Following this step, the **enoyl-CoA isomerase** can act on the substrate and make it degradable by β -oxidation.

Remark 1.18 (Odd-number FAs). Although the vast majority of FA are constituted by an even number of carbons. Odd-number FAs exist and need to be degraded in the same way, but at the **last iteration** of β -oxidation, they yield **propionyl-CoA** instead of acetyl-CoA.

The enzyme **propionyl-CoA carboxylase** is used to produce D-methylmalonyl-CoA, which can be converted through two further reactions to succinyl-CoA. **Succinyl-CoA** can then enter the TCA cycle. See fig. 29(b)

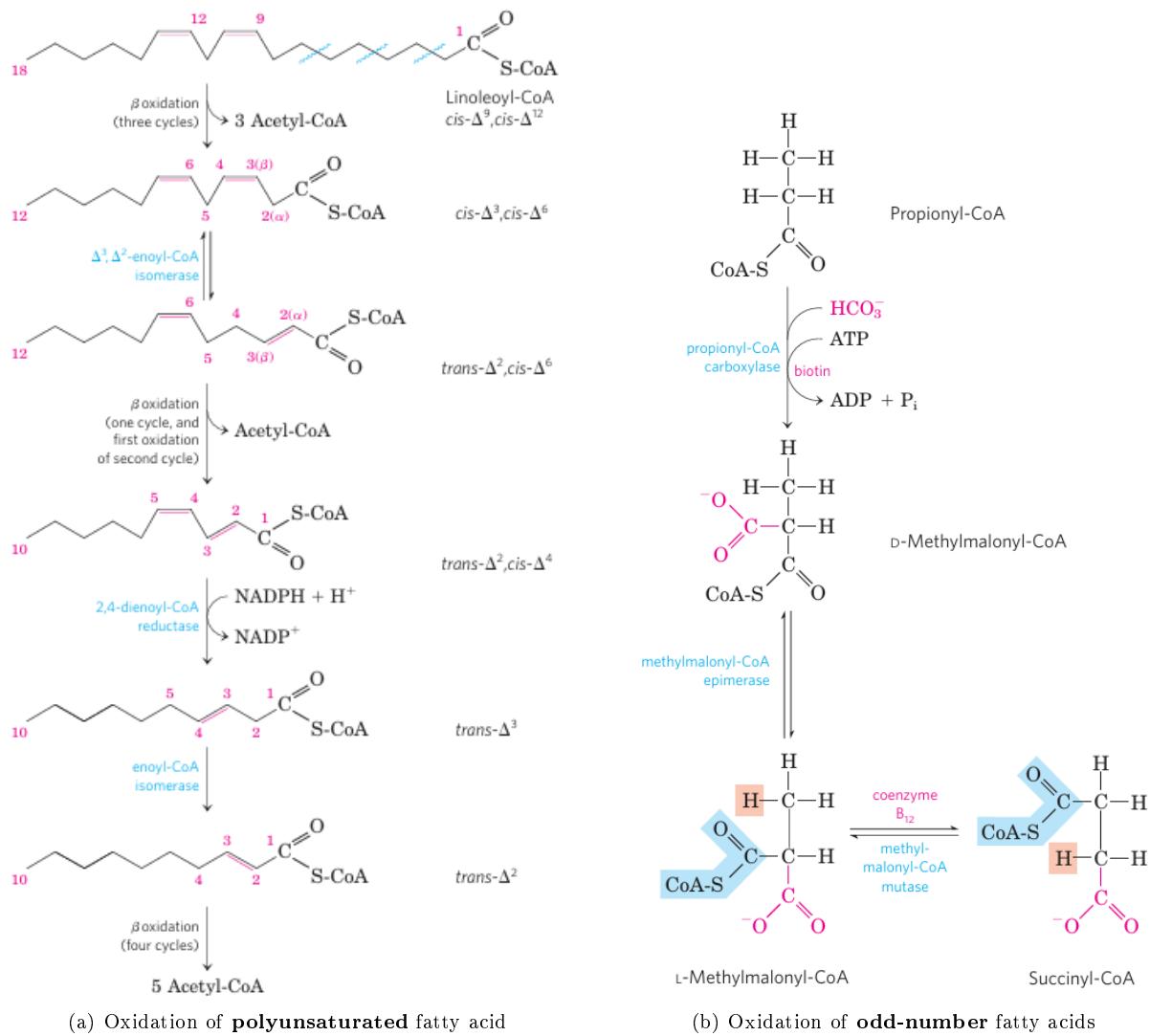


Figure 29: FA oxidation overview

1.3.1.1 Calculate amount of ATP from a FA of given length

This section assumes the fucked up numbers given in ETC for NADH and FADH₂, sucks!

Assuming the FA is fully saturated and consists of n carbons (even number):

Number of β -oxidation cycles:

$$\frac{n}{2} - 1$$

Each β -oxidation cycle produces:

- 1 NADH \rightarrow 3 ATP
- 1 FADH₂ \rightarrow 2 ATP
- 1 Acetyl-CoA (last cycle gives 2) \rightarrow 12 ATP via TCA

Each acetyl-CoA entering the citric acid (TCA) cycle generates:

- 3 NADH $\rightarrow 3 \times 3 = 9$ ATP
- 1 FADH₂ $\rightarrow 1 \times 2 = 2$ ATP
- 1 GTP (equivalent to ATP) $\rightarrow 1$ ATP

Activation of FA to Co-A costs 2 ATP.

Total ATP yield:

$$ATP_{\text{total}} = \left(\frac{n}{2} - 1\right) \cdot (3 + 2) + \frac{n}{2} \cdot 12 - 2$$

If we have an odd number of carbons:

Account for Propionyl-CoA \rightarrow Succinyl-CoA:

- 1 NADH $\rightarrow 3$ ATP
- 1 FADH₂ $\rightarrow 2$ ATP
- 1 GTP $\rightarrow 1$ ATP

$$ATP_{\text{total}} = \left[\frac{n}{2}\right] \cdot 5 + \left[\frac{n}{2}\right] \cdot 12 + 6 - 2$$

ATP Yield from a Polyunsaturated Fatty Acid:

$$ATP_{\text{total}} = \left(\frac{n}{2} - 1\right) \cdot 5 + \left(\frac{n}{2} \cdot 12\right) - (p \cdot 2) - (q \cdot 3) - 2$$

Where:

- p : Number of double bond "islands"; each skips FADH₂ production (-2 ATP).
- q : Sum of (number of connected double bonds in one island - 1); each costs 1 NADPH (-3 ATP).

1.4 Amino Acid Catabolism

Amino acids that are derived from the degradation of proteins are the third class of biomolecules (after carbohydrates and fatty acids) that significantly contribute to the cellular energy metabolism.

In animals amino acids are oxidised in three different metabolic conditions:

- During protein turnover some amino acids can be oxidized, if they are not required for the synthesis of other proteins.
- In a protein rich diet, food-derived amino acids can exceed the needs for protein biosynthesis. Note there is no way to store amino acids.
- In starvation when carbohydrates are not available. AA from endogenous (produced within the body) proteins are used as an energy source.

Amino acids are split into a carbon skeleton and an amino group (which contains nitrogen). The carbon skeleton can be used for energy production or biosynthesis. The amino group, however, is converted into ammonia (NH₃), which is toxic to our cells.

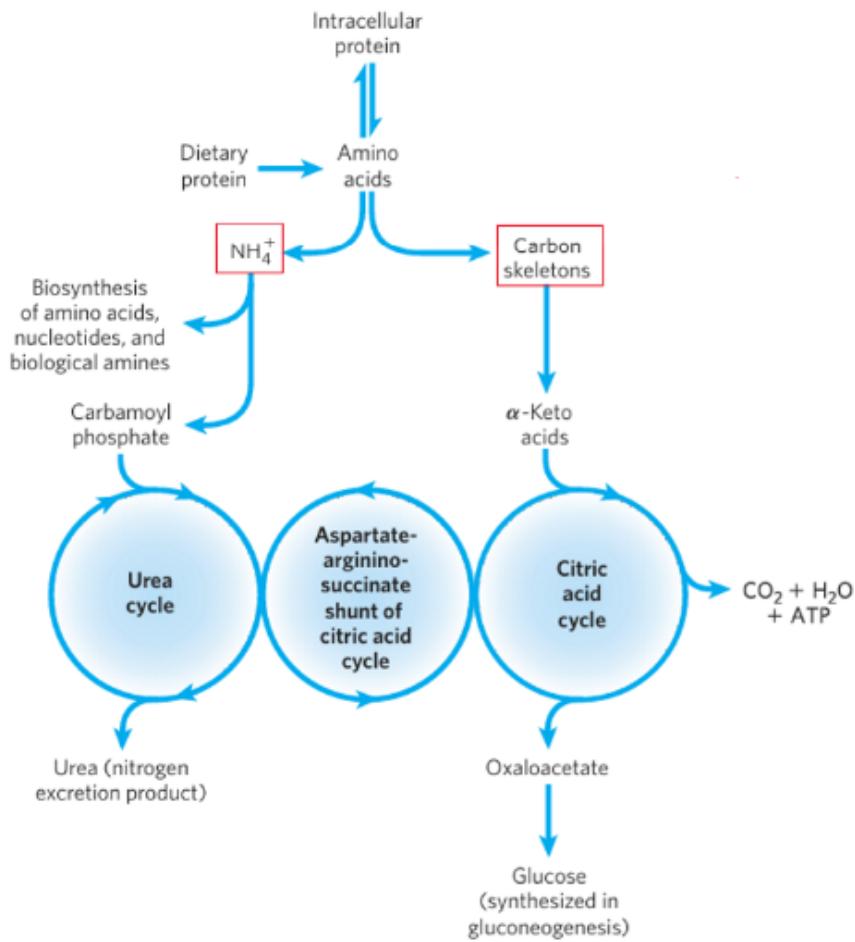


Figure 30: Overview of amino acid catabolism in mammals

1.4.1 AA oxidation and Urea production

The first step is the detachment of the α amino group. This is catalyzed by **amino transferase** that transfers the amino group to the α -ketoglutarate to form a α -keto acid.

The amino transferase requires the **cofactor pyridoxal phosphate (PLP)**, a derivative of Vitamine B6. PLP acts as a **transitory transporter of amino groups**.

One formed in the cytoplasm, glutamate is transported in the mitochondria where it undergoes **oxidative deamination** catalyzed by **glutamate dehydrogenase** that oxidizes glutamate back to α -ketoglutarate and creates NH_4^+ . This reaction is coupled to the reduction of NAD(P)^+ to $\text{NAD(P)}\text{H}$.

Glutamate dehydrogenase is tightly controlled by the energy charge. GTP (indicating high energy charge) acts as an inhibitor and ADP (indicating low energy charge) acts as a simulator.

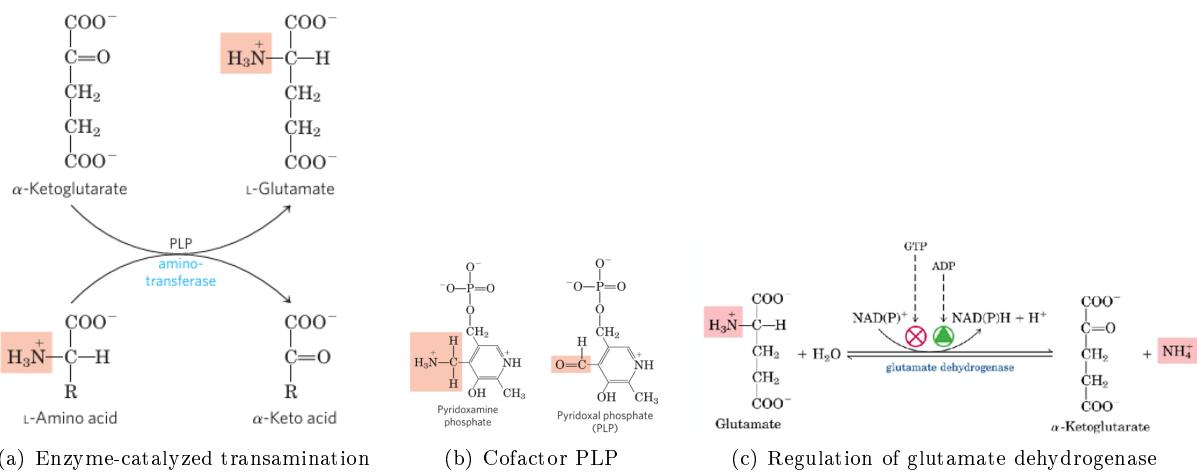


Figure 31: Amino acid oxidation and urea production

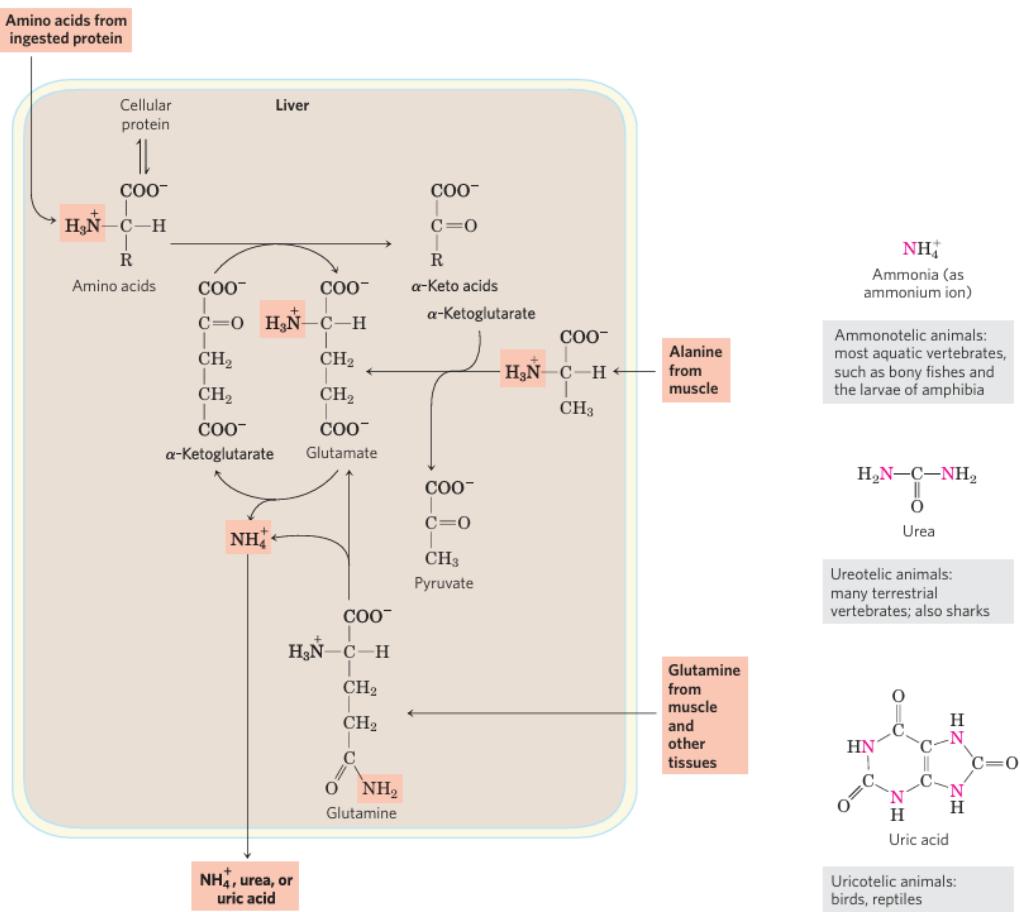


Figure 32: Amino group catabolism

1.4.1.1 Ammonia transport

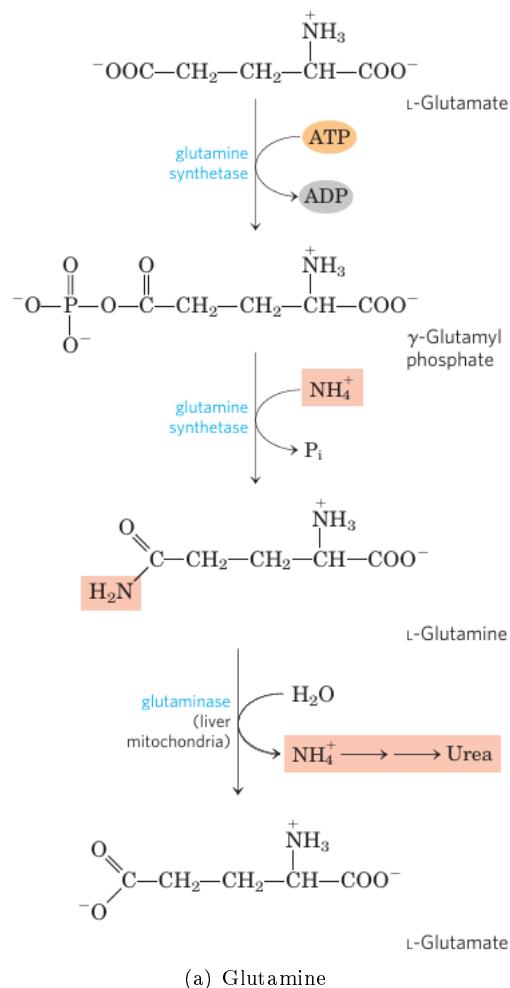
Ammonia is toxic for most tissues. Thus, in order to be transported to the liver where it is converted into urea, it needs to be incorporated into a non-toxic compound.

In most tissues ammonia is therefore complexed to glutamate to produce glutamine. This is done by the enzyme **glutamine synthetase** and requires **ATP**. See fig. 33(a)

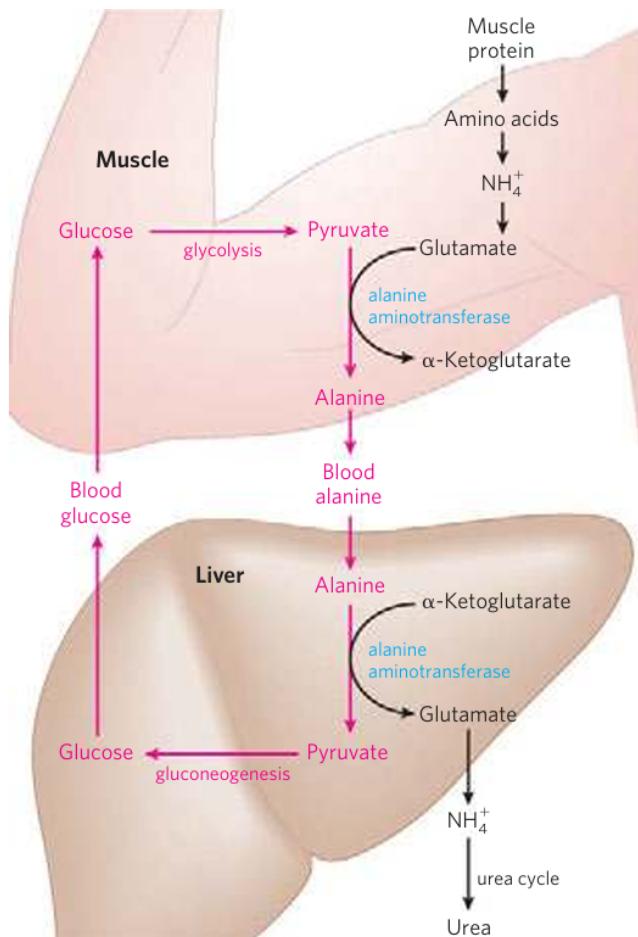
Note this a perfect example how ATP hydrolysis can foster otherwise unfavorable reactions.

Once in the liver mitochondria this is reversed by **glutaminase**.

In the **muscle** and other tissues where amino acids are intensively used for the production of energy, the **amino group can be transferred to pyruvate** to form alanine. Which can be transported to the liver. See fig. 33(b)



(a) Glutamine



(b) Alanine

Figure 33: Transport of Ammonia

1.4.1.2 Urea production

Once transported to liver mitochondria converted to urea in a serie of 4 reactions (**the Urea cycle**). The first happens in the mitochondrial matrix while the remaining three happen in the cytosol.

- i) Citrulline is formed from Ornithine by the addition of a carbamoyl group. **Carbamoyl** carries the ammonia from glutamine. The construction of Carbomyl used 2 ATP.
- ii) Argininosuccinate is formed by condensation of citrulline and asparate. This uses ATP.
- iii) Argininosuccinate is decomposed in **fumarate** (that feeds in the TCA cycle) and arginine.
- iv) Arginine is decomposed into urea and ornithine by **arginase**

1.4.1.3 Entry in TCA

As there are 20 amino acids there are 20 catabolic ways for those amino acids.

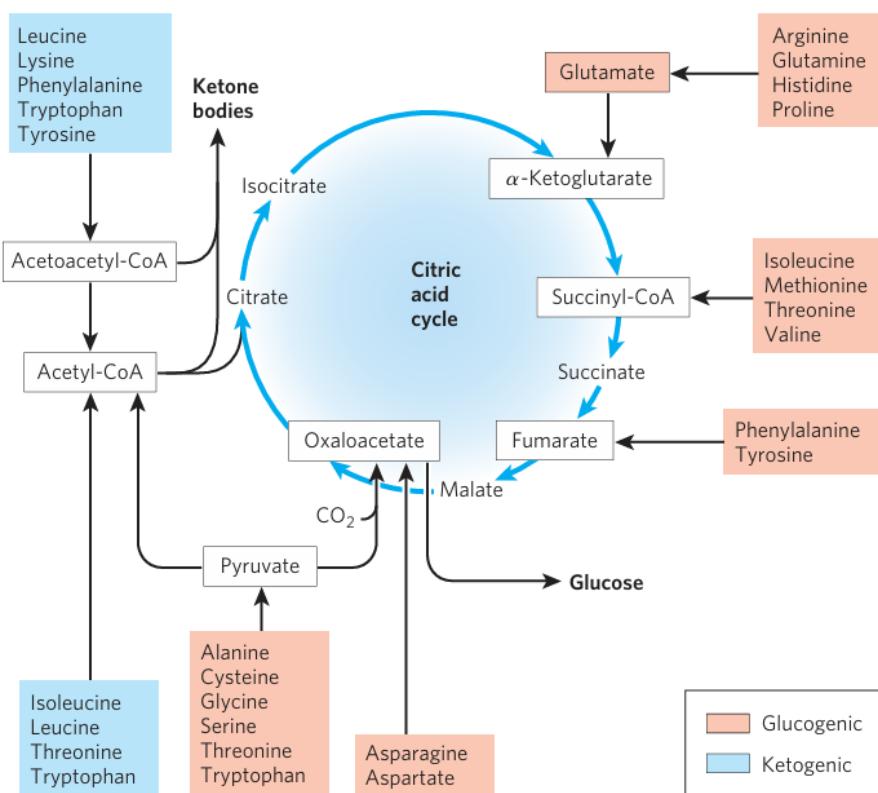


Figure 34: Glucose-alanine cycle

Remark 1.19 (Defects in amino acid metabolism). Several genetic defects have been identified in humans that impact the amino acid metabolism. These defects lead to the accumulation of neurotoxic intermediates resulting in intellectual disabilities.

1.5 Oxidative Phosphorylation

The process of aerobic respiration is completed in the **mitochondria** by oxidative phosphorylation. The NADH molecules formed in glycolysis as well as the NADH and FADH₂ molecules formed in TCA cycle, in β-oxidation and in the amino oxidation are used to produce ATP along the **electron transport chain (ETC)**.

- A NADH molecule in the mitochondrial matrix yields 3 ATP.
- A cytosolic NADH or FADH₂ yields 2 ATP

1.5.1 ECT

The electron transport chain (ETC) is a series of protein complexes that reside in the **inner mitochondrial membrane**. The ETC transports electrons, creating an electrical current that is coupled to the **pumping of H⁺ ions from the mitochondrial matrix to the inter-membrane mitochondrial space**.

Note:

- The outer mitochondrial membrane is permeable to the majority of small molecules and anions (**not** cations [H⁺]) to the presence of mitochondrial porins.
- The inner mitochondrial membrane lacks porins and is thus impermeable to ions and small metabolites that require specialised transporters to leave the mitochondrial matrix.

The ETC consists of 4 protein complexes that accept the electrons from NADH and FADH₂ and move the electrons along the inner mitochondrial membrane onto **the finally acceptor oxygen**. This produces the protein gradient which is exploited by ATP synthase to produce ATP.

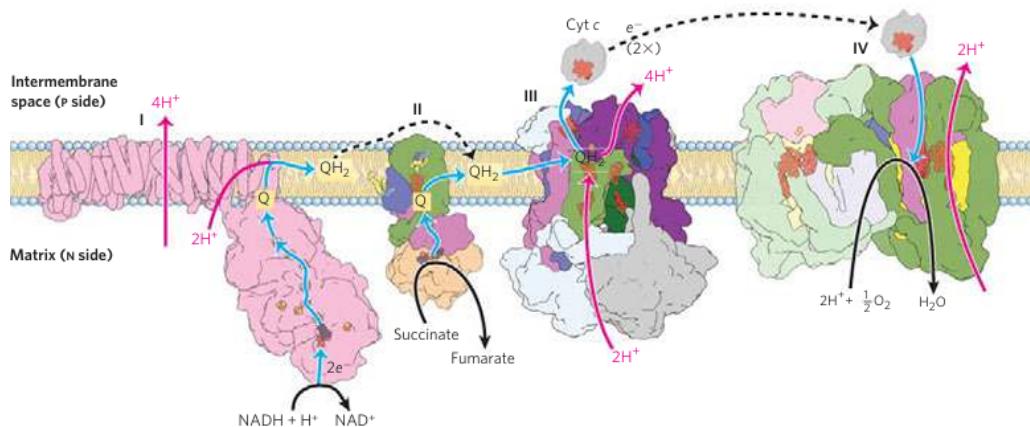


Figure 35: Overview of the ETC

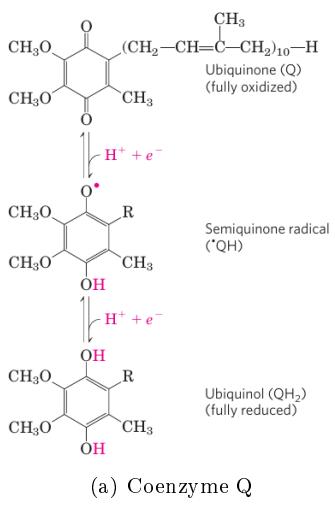
Remark 1.20 (Coenzyme Q). Ubiquinone (Coenzyme Q), is a **hydrophobic electron carrier** that can dissolve in the inner mitochondrial membrane bilayer. It can accept 1 or 2 electrons thus being reduced to Semiquinone (*QH) and Ubiquinol (QH₂) respectively. See fig. 36(a)

It serves as a transporter from **Complex I to II and II to III**.

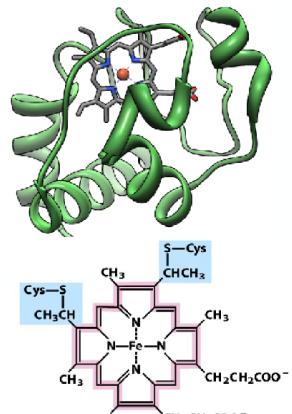
Remark 1.21 (Cytochrome C). A small **heme protein** located in the intermembrane space of mitochondria. See fig. 36(b)

Cytochrome C is highly **water-soluble**, and is able to undergo **oxidation and reduction** as its iron atom converts between the ferrous(2+) and ferric(3+) forms. (it does *not* bind oxygen)

It plays a key role in the electron transport chain by transferring electrons between **Complex III** (cytochrome bc₁ complex) and **Complex IV** (cytochrome c oxidase)



(a) Coenzyme Q



(b) Cytochrome C

Figure 36: Electron carrier in the ETC

Definition 1.22 (Fe-S clusters). Iron-sulfur clusters occur in many biological systems, often as components of electron transfer proteins. The relevant redox couple in all Fe-S proteins is Fe(II)/Fe(III). They may be more or less complex (see. fig. 1.5.1)

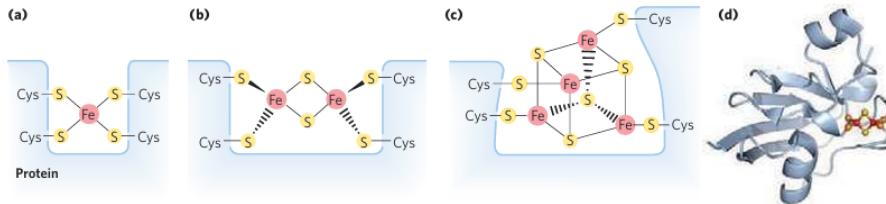


Figure 37: Iron-sulfur centers

1.5.1.1 Complex 1

Complex I (NADH dehydrogenase or NADH oxidoreductase), is a very large protein complex consisting of 46 polypeptides. It has a L shape. One arm lies within the inner membrane while the vertical component lies in the matrix.

Complex I receives high energy electrons from **NADH**. NADH donates electrons to a series of FMN, leading to the reduction of FMN to **FMNH₂**. FMNH₂ then donates the electrons to a series of **Fe-S clusters**. Finally two electrons are transferred to CoQ producing **CoQH₂**. See fig. 38(a)

As the electrons move along the series of Fe-S clusters, the complex uses the electrical power to pump a total of **4H⁺** into the intermembrane space.

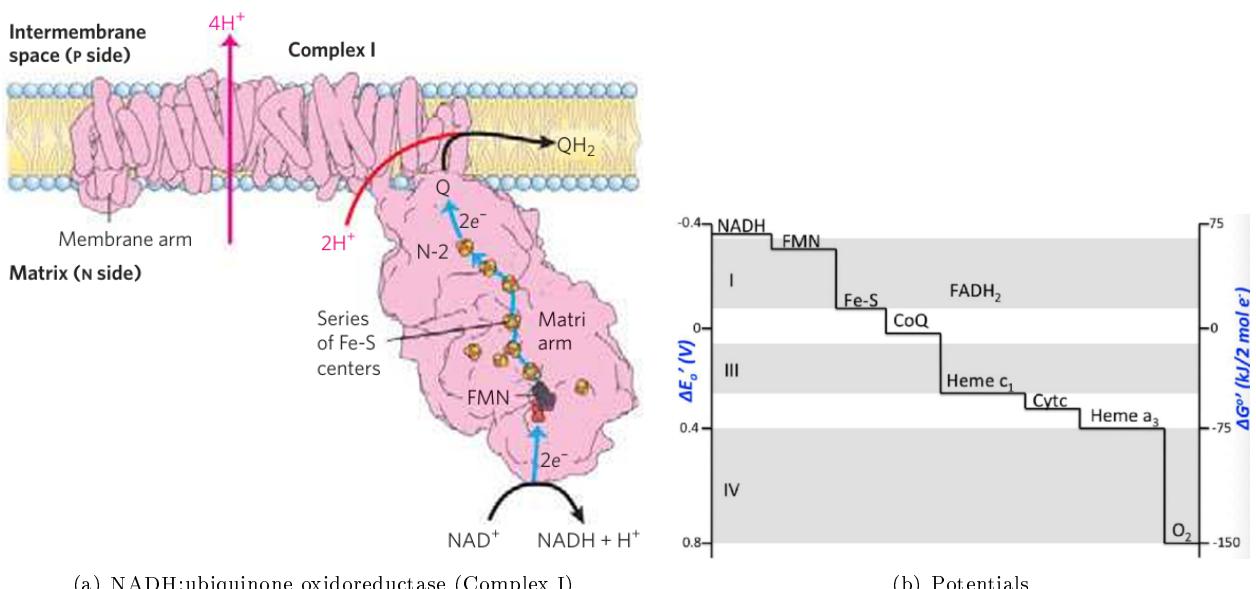


Figure 38:

1.5.1.2 Complex 2

Complex II contains the succinate dehydrogenase used in to TCA cycle to form fumerate from succinate (**step 6**). Note complex II is **not** a proton pump.

The **FADH₂** is created in step 6 of the TCA cycle and stays tightly connected to the enzyme. FADH₂ transfers his **2 high energy electrons** to a series of **Fe-S clusters** that ultimately transfer the electrons to CoQ producing **CoQH₂**. See fig. 39.

Note, Hemme b group does not appear to be part of the electron transporting pathway.

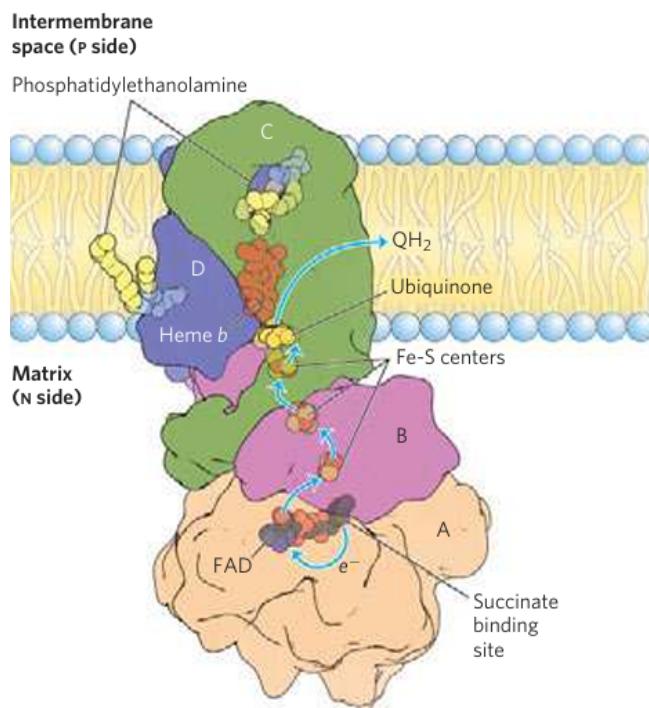


Figure 39: Complex II - succinate dehydrogenase

1.5.1.3 Complex 3

Complex III transfers electrons from **CoQH₂** to **Cytochrome c**. This is done through the Q cycle, which has two stages:

- i) In the first half cycle **CoQH₂** binds to the complex III and transfers **one electron** to the **Rieske center**. From there it is transferred to **Cyt c₁** and then to **Cyt c**. This reaction **pumps 2 H⁺**. The **second electron** is transferred from **Cyt b** to an other CoQ producing ***CoQH**.
- ii) In the second half cycle is practically a repetition of the first, but the **second electron** is transferred **to the *CoQH** produced in the first half cycle. The resulting QH₂ is released back into the Q pool.

This leads to the net equation of: **QH₂ + 2 cyt c₁ (oxidized) + 2H⁺ => Q + 2 cyt c₁ (reduced) + 4H⁺**. Therefore **pumping 4H⁺** and the reduced cyt c is transferred to the complex IV.

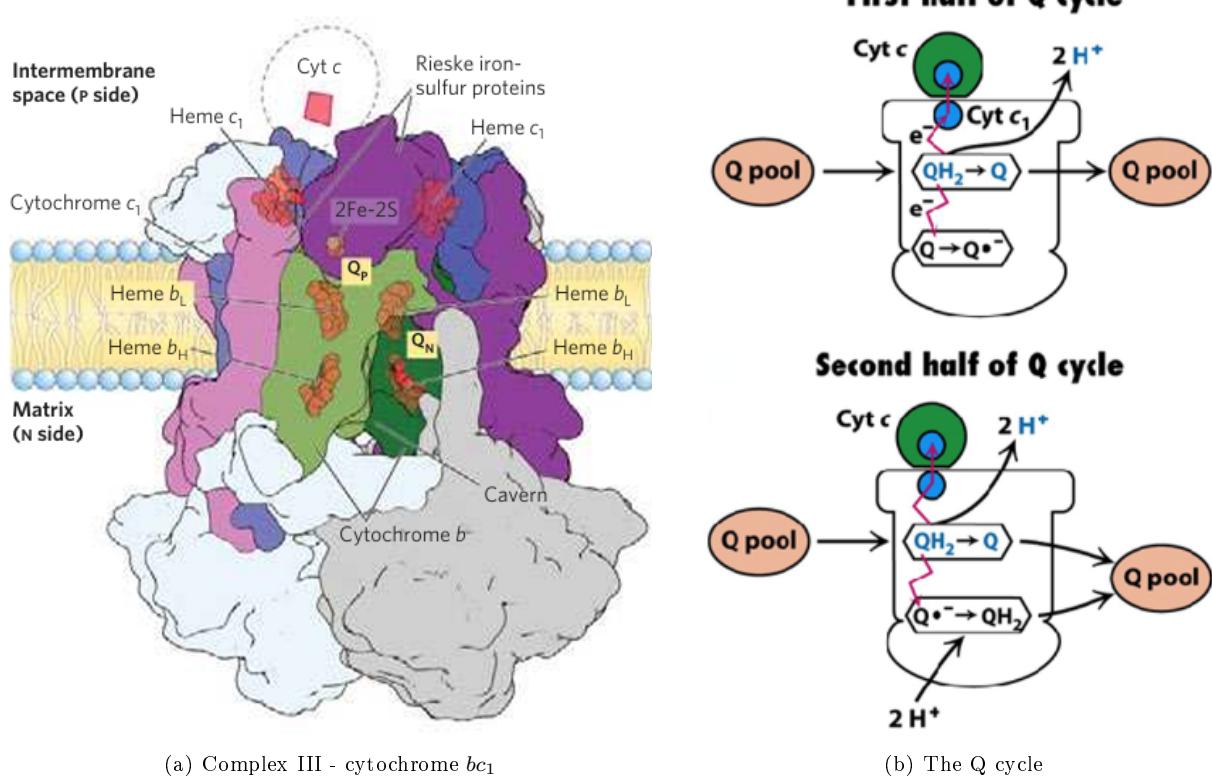


Figure 40: Electron carrier in the ETC

1.5.1.4 Complex 4

Complex IV transfers the electrons received from complex III via **cytochrome c to oxygen**. Complex IV pumps 4H^+ using 4 Cyt c. This happens in 4 steps:

- Two reduced Cyt c give off 2 electrons. One goes to Hemme a_3 and the other to Cu_B
- once Hemme a_3 and Cu_B are reduced, 1 O_2 molecule can bind and abstract 2 electrons thus forming a peroxide bridge.
- Two additional Cyt c are reduced transferring their electrons to 2H^+ (from the matrix). They then break the peroxide bridge and form Hemme $a_3\text{-OH}$ and $\text{Cu}_B\text{-OH}$
- Two additional protons from the matrix oxidize Hemme a_3 and Cu_B to their original state producing 2 molecules of water.

This **pumps 4H^+** in the intermembrane space. Sometimes also referred as 2H^+ , in this case it used 2 Cyt and 0.5 O_2 .

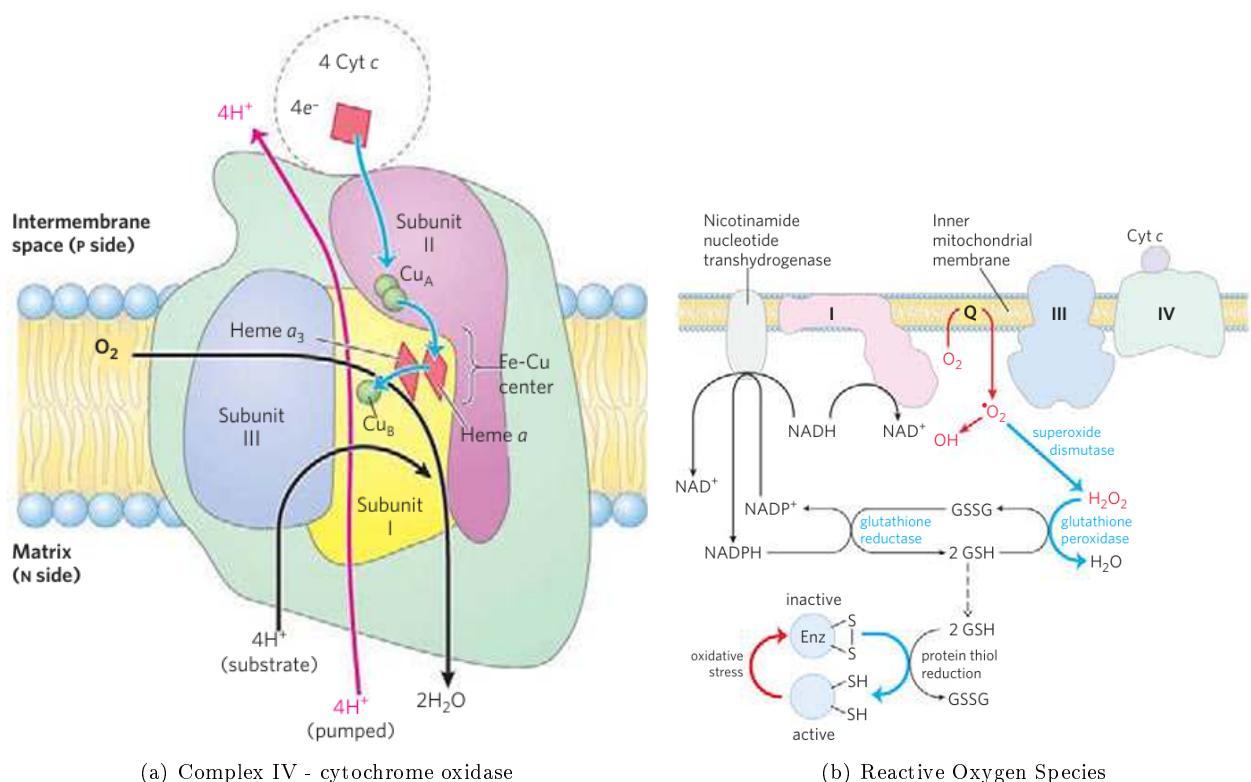


Figure 41: Complex IV and ROS

Remark 1.23 (Reactive Oxygen Species (ROS)). ROS are chemically reactive species containing oxygen. ROS are produced in a variety of biochemical reactions / organelles such as peroxisomes, **mitochondria**, etc. In the ETC about 0.1-2% of electrons are **prematurely transferred to oxygen**. Specific enzymes in our mitochondria are used to detoxify **superoxide radicals**, by converting them to **hydrogen peroxide** and then to **water**. See fig. 41(b)

1.5.2 ATP synthase - Complex V

The ATP synthase generates ATP molecules and uses the proton motive force due to the H^+ gradient to release ATP into the mitochondria.

The structure consists of two major regions, F0 and F1:

- **F0** is **hydrophobic** and lies within the inner membrane. It consists of **10-14 c subunits** organised in a ring; a single **a subunit** and **2 b subunits**.
- **F1** is the **catalytic region**. It lies in the matrix of the mitochondria and it is constituted by five polypeptides: **3 alpha, 3 beta, gamma, delta, and epsilon**.

Each unit has its purpose:

- The **alpha and beta** subunits form an **hexameric hetero-oligomer** having a central cavity.
- The **gamma and epsilon** subunits form what is called the **central stalk** that runs through the cavity of the beta/alpha hexamer.
- The **delta** subunit keeps the alpha/beta hexamer from rotating.

- The **c ring** and the **a subunit** works as a proton channel. The c ring also rotates because of the proton flow. This also rotates the central stalk.

Note that the *a*, *b*, *delta*, alpha, and beta subunits do not rotate.

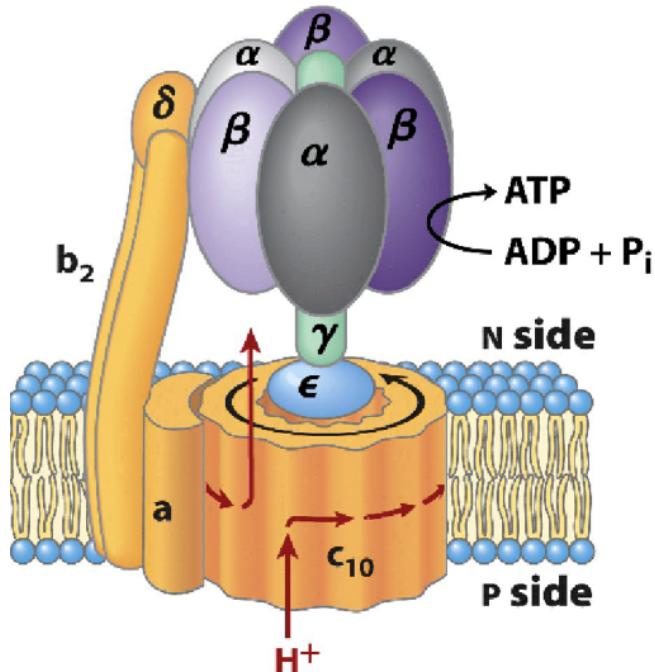


Figure 42: ATP synthase

Note in figure 43(b) N side = matrix and P side = intermembrane space.

Remark 1.24 (Why does C rotate?). First note that the **a subunit** spans the entire membrane. So first an H^+ enters the **a subunit** from the matrix and binds to the **aspartate residue in the closest c subunit**. Through the pressure of more H^+ coming into the **a subunit** the **c ring turns** until the H^+ finds the exit in **other half of the a subunit**. See fig. 43(a)

Remark 1.25 (How does the rotation produce ATP?). The alpha/beta hexamer produces the ATP. While the hexamer is kept in place the central stalk rotates, inducing conformational change in the oligomers.

There are 3 different states (stalk rotates always **rotates 120 degrees**) the alpha and beta units can be in (See fig. 43(b)):

- Tense T:** ADP and Pi are brought close so that they can be combined into ATP.
- Open O:** The formed ATP is released and a new ADP-Pi set can bind.
- Loos L:** The bound ADP and Pi become trapped and can not leave.

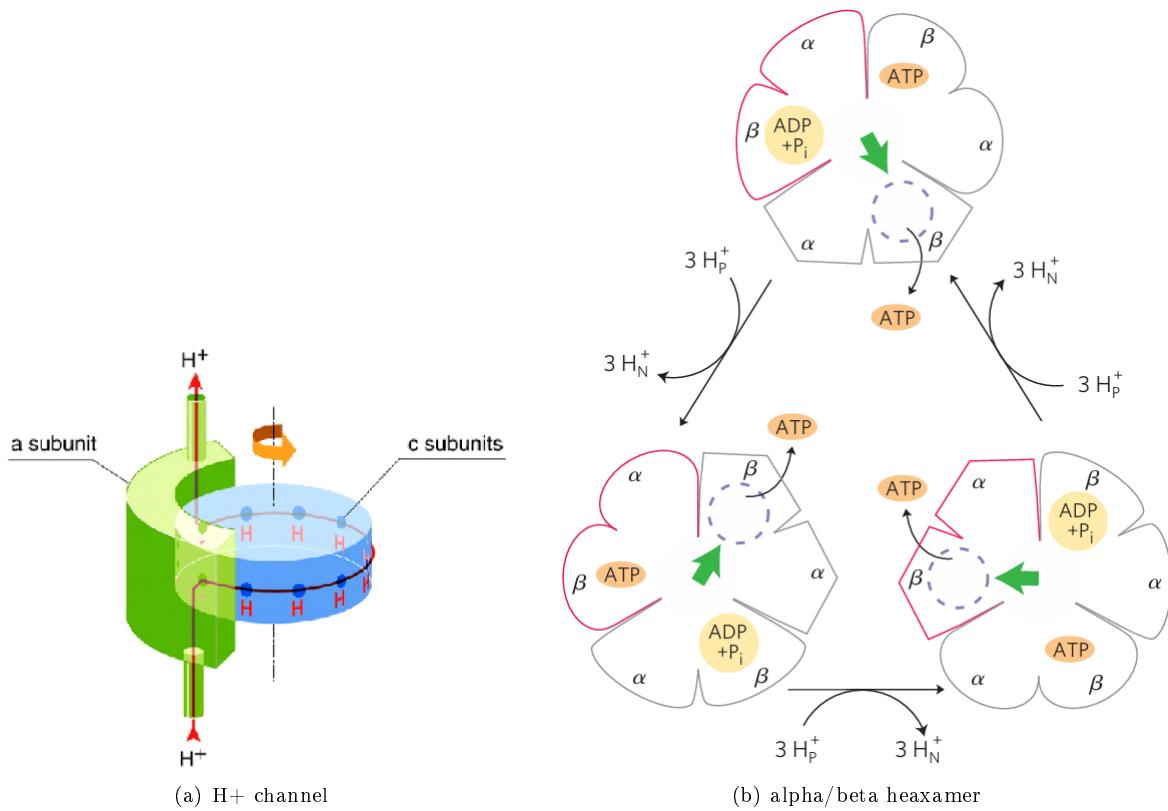


Figure 43: ATP production

1.6 Final calculation of aerobic respiration

Glycolysis

Glocose breaks down into 2 pyruvate and produces 2 NADH and 2ATP. *It does not require oxygen.*

Pyruvate decarboxylation (2x)

Pyruvate (in the presence of oxygen) is transported into the mitochondrial matrix and transformed into acetyl-CoA, producing 2NADH and one CO₂.

TCA Cycle (2x)

Acetyl-CoA produces 2 Co₂, 3 NADH and 1 FADH₂ and 1 GTP.

Oxidative phosphorylation

NADH and FADH₂ are used to produce ATP. A NADH molecule produced in the mitochondria matrix (TCA cycle and Pyruvate decarboxylation) it yields 3 ATP while cytosolic NADH or FADH₂ yeald 2 ATP.

This gives us a net total of 36 ATP = 6 (glycolysis) + 6 (pyruvate decarboxylation) + 24 (TCA) produced by 1 glucose molecule.

These numbers are totally fucked (NADH = 2.5 ATP, FADH = 1.5 and citosolic NADH depending on shuttle.) but they are in the slides. So just stick to them.