Biochemistry

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1 Reaction Kinetics

All reactions in the cell are in a steady state for most of the time, only deviating from this pseudo-equillibrium position whenever a quick in- or decrease of a certain metabolite is needed. For most biochemical reactions, $\Delta G^{\circ'} \approx 0$.

$$\Delta G = \Delta G^{\circ'} + RT\ln(Q) \qquad \Delta G^{\circ'} = -n \cdot F \cdot \Delta E_0^{'}$$

$$\Delta G^{\circ'} = -RT\ln(K_{eq}^{'}) \iff K_{eq}^{'} = e^{\left(\frac{-\Delta G^{\circ'}}{RT}\right)}$$

2 Major Metabolic Pathways

Glucose is the most commonly used sugar in the body (e.g. erythrocytes and brain cells, which rely nearly exclusively on glucose for their metabolic activity) and comes with a number of advantages: Most importantly, it is nearly 99% in its ring-conformer given it has only equatorial groups in it. Sugars that would be present in their aldose form would start reacting with other molecules through their aldehyde functional group.

2.1 Glycolysis

Glucose is passively transported into the cell through the **Glucose transporter** (**GluT**). Glycolysis takes place in the cytoplasm and is generally divided into two stages: an investment phase (-2 ATP) and a harvest phase (2 x +2 ATP).

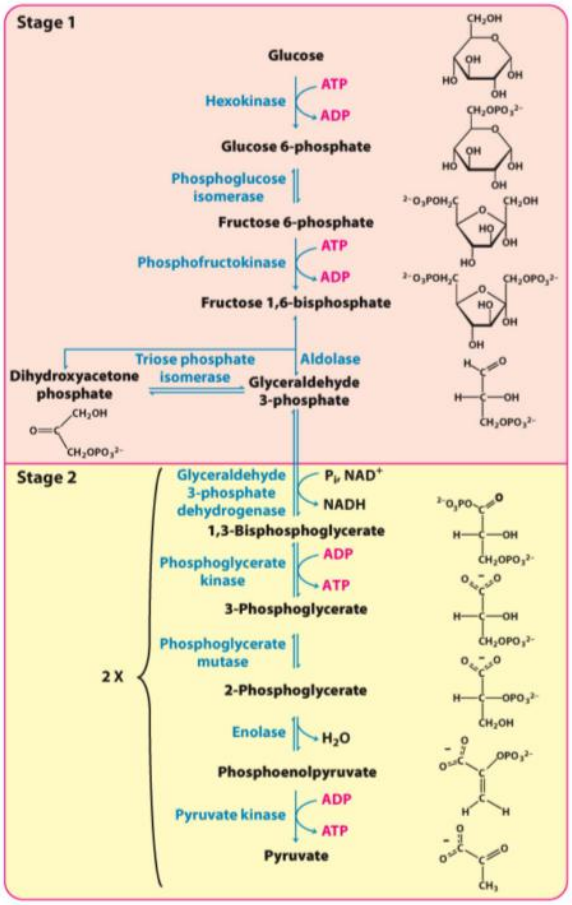

Figure 16.2
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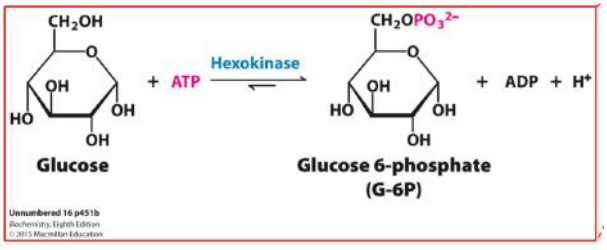
2.1.1 Phase 1: Hexokinase

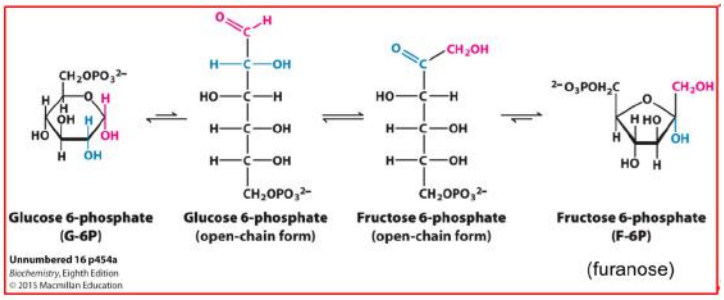
Glucose is phosphorylated to **Glucose 6-phosphate** (**G6P**) by **Hexokinase**. This prevents glucose from moving back through the glucose transporter and thus essentially traps it inside the cell. The P_i obviously needs to come from somwhere and is supplied by the hydrolysis of ATP (investment.)



The structure of hexokinase has two lobes around its active center, one of which will move upon binding of the substrate. The pocket that holds the substrate is very narrow and can only hold ring conformers with no axial positions, this makes it specific to glucose. Xylose for example (identical to glucose except for no -OH group at C5) would fit as well and in fact increases the activity of hexokinase substantially. The absence of the C5 -OH means the P_i is transferred to water accidentally instead, which is faster.

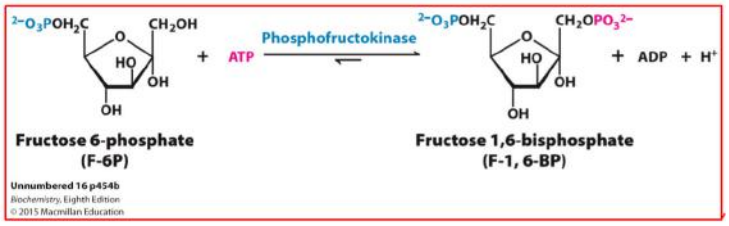
2.1.2 Phase 1: Phosphoglucose Isomerase

The small portion of open-chained G6P will then react with **Phosphoglucose isomerase** in order to move from the aldose to the ketose form. The aldehyde is reduced to 1° alcohol and first alcohol is oxidized to a ketone. The opne-chained **Fructose 6-phosphate** (**F6P**) will then also revert back quickly to its ring-conformer. Note that Fructose is a furanose (5-ring), whereas glucose was a pyranose (6-ring).



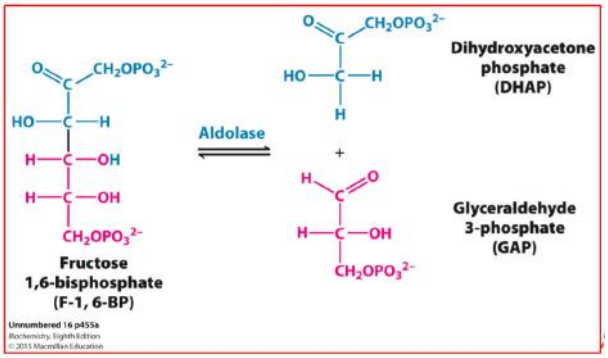
2.1.3 Phase 1: Phosphofructokinase

F-6P will then be phosphorylated exactly at the just formed 1° alcohol by **Phosphofructokinase**in order to form **Fructose 1,6-bisphosphate** (**F1,6BP**). Again this requires another equivalent of 1 ATP.

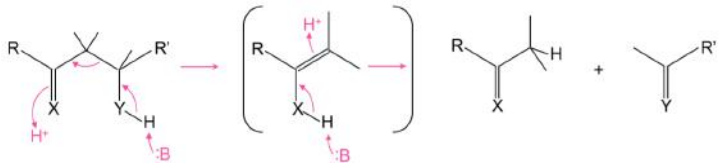


2.1.4 Phase 1: Aldolase

F1,6BP now has enough stored up energy to be cleaved into two by **Aldolase**. The two C3 fragments that form are Dihydroxyacetone phosphate (**DHAP**) and Glyceraldehyde 3-phosphate (**GAP**).



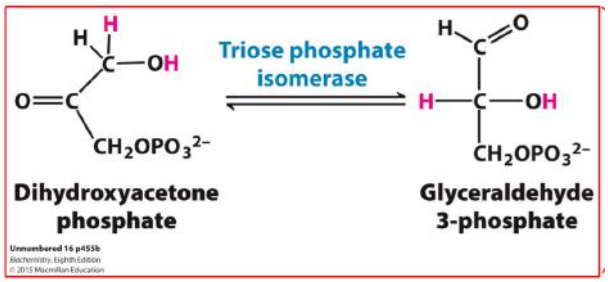
The breakage of the bond is possible due to the destabilizing nature of the β C=0 double-bond. This reaction is referred to as a retro-aldol cleavage:



The base that is necessary for this reaction mechnanism is provided in the form of a Lys AA by the enzyme. Lys can go on to form a Schiff base enabling the cleavage and can then simply be removed again by hydrolysis to also yield free DHAP.

2.1.5 Phase 1: Triose Phosphate Isomerase

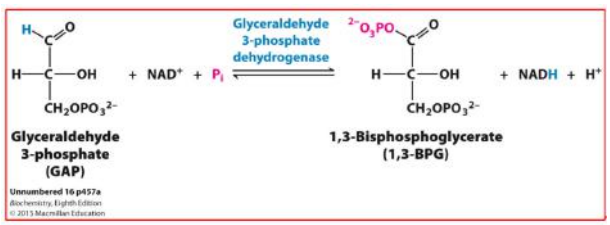
Only GAP will move on into stage 2 of the glycolysis. Thus, **Triose phosphate isomerase** is need to interconvert DHAP to GAP. At equillibrium the ratio is roughly 20 : 1 DHAP : GAP. This iosmerism is the reason why stage 2 can be run twice for each equivalent of glucose. Without it, glycolysis would not actually yield any energy.



The triose phosphate isomerase uses a Glu and a His at its active site: the Glu abstracts the acidic H at the β position to the carbonyl; the His donates an H to form the enediol intermediate, which will tautomerize quickly to the keto-version (i.e. GAP).

2.1.6 Phase 2: GAPDH

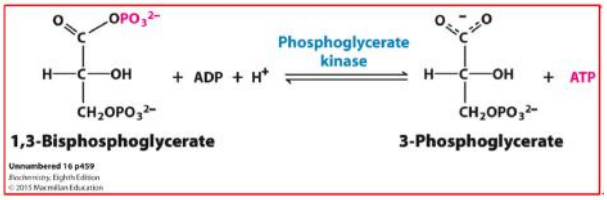
Phase 2 begins (somewhat surprisingly) with another phosphorylation: GAP reacts to **1,3-Bisphosphoglycerate** (**1,3BPG**) catalyzed by Glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**). Interestingly, this phosphorylation uses NAD^+ NOT ATP as additional substrate. This will become more important later on as the resulting NADH can be used in the respiratory chain, though under anaerobic conditions the cell must find other pathways in order to replenish NAD^+ (e.g. fermentation, lactation).



GAP to 1,3BPG conversion is based on two steps: First, GAP is oxidized to a carboxylic acid, this uses up NAD^+ as oxidative reagent. This first step is highly exergonic and powers the second, very endergonic step. Because second, the carboxylic acid reacts with P_i in order to form 1,3BPG. GAPDH catalyzses these reactions by no forming a carboxylic acid but rather a thioester intermediate. The thioester lacks the energetically favourauble resonance structures and is thus higher in energy. At the same time, this means that difference to the transition state is smaller, ergo lower activation energy. Furhtermore, sulfur is an excellent leaving group.

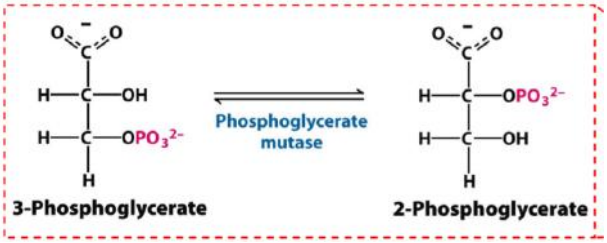
2.1.7 Phase 2: Phosphoglycerate Kinase

The energy-rich 1,3BPG can now finally be used to actually produce ATP. This reaction results in **3-Phosphoglycerate**. The phosphorylation of ADP is mediated by the enzyme **Phosphoglycerate kinase**. It is a substrate-level phosphorylation.



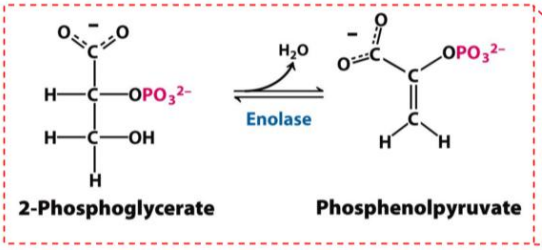
2.1.8 Phase 2: Phosphoglycerate Mutase

The phosphate group of the 3-Phosphoglycerate then has to move position, resulting in **2-Phosphoglycerate** mediated by the enzyme **Phosphoglycerate mutase**.



2.1.9 Phase 2: Enolase

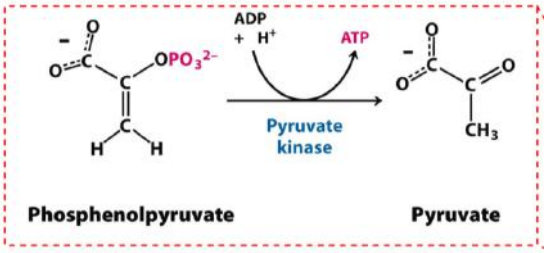
The 3-Phosphoglycerate is then processed by **Enolase** to **Phosphoenolpyruvate** (**PEP**[1]). PEP has such an enormous phosphorylation potential that it can very easily phosphorylate ADP to ATP.



[1]The 500 lb gorilla. In the sense of: an actual gorilla. There are actual tiny gorillas in your cells.

2.1.10 Phase 2: Pyruvate Kinase

PEP first gets rid off its highly energetic P_i-group, forming ATP and the enol-form of **Pyruvate** (we have now harvested our 2 ATP). . This reaction is mediated by **Pyruvate kinase.** Then by the keto-enol tautoterism, pyruvate quickly forms. This concludes the glycolytic pathway



2.1.11 Regulation

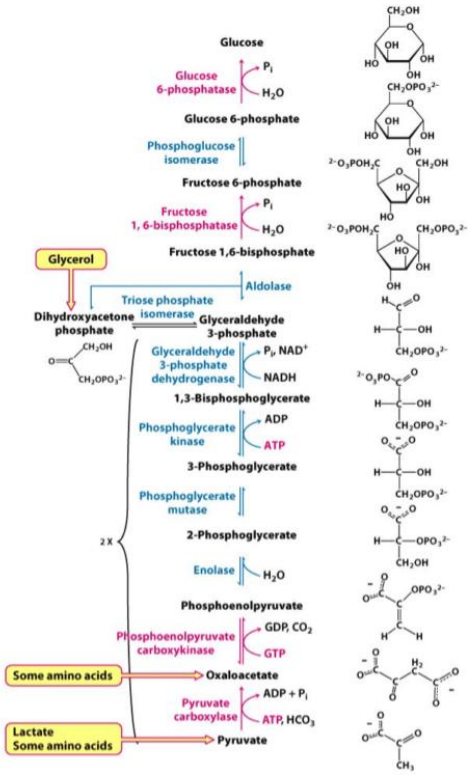
There are three major "waterfalls" in the glycolytic pathway: Glc → G6P, F6P → F16BP, PEP → Pyr.[2] Glycolysis is regulated at these points. In muscles, this happens for example by allosteric inhibition by ATP at the Phosphofructokinase (F6P → F16BP). This is a particularly good position to regulate glycolysis, because F6P is non-reactive and can be easily stored.

When energetic demands increase in the muscles, a feedforward stimulation between the Phosphofructokinase and the Pyruvate kinase (PEP → Pyruvate). This prevents a bottlenecks in glycolysis, which could result in the accumulation of reactive products in dangerous concentrations.

[2]Note that with the exception of 1,3BP → 3-Phosphoglycerate, these are exactly all the poins where ATP is either invested or harvested.

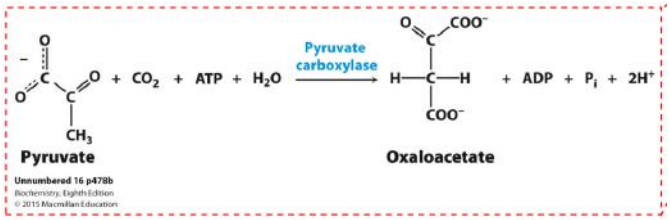
2.2 Gluconeogenesis

Gluconeogenesis synthesizes glucose from precursors such as lactate, AAs or glycerol and mostly takes place in the liver. It ensures that glucose levels are always constant (even during fasting, etc.) as brain and red blood cells rely on it for nutrition. In general, gluconeogenesis is just the reverse glycolysis though the 3 "waterfalls" must be circumvented. Note that gluconeogenesis and the TCA cycle use some of the same susbstrates, thus if the TCA cycle is very active in one direction, gluconeogenesis might not even be able to occur.



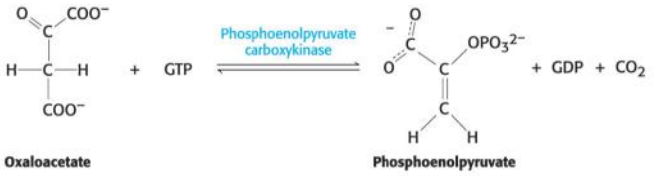
2.2.1 Bypass 1: PEP → Pyruvate

Gluconeogenesis begins in the mitochondrion, this has to do with the fact that the TCA cycle, which uses pyruvate, occurs there as well. Pyruvate + CO_2 can form **Oxaloacetate** if catalyzed by **Pyruvate decarboxylase**, this requires 1 ATP. The reaction further requires the cofactor biotin and needs to be positively regulated by allosteric binding of Acetyl CoA (ensuring the TCA cycle can still take place undisturbed).



Pyruvate carboxylase consists of four Biotin carboxyl carrier proteins (BCCP) and an ATP-Mg^{2+} active center. The cell now faces a transport problem, there are no Oxaloacetate transporters in the mitochondrion. Thus Oxaloacetate is first reduced to malate, then transported to the cytoplasm and finally oxidized again oxidized again to Oxaloacetate. This first uses up and then releases an NAD^+ equivalent.[3]

The Oxaloacetate must then react to PEP, this takes up one equivalent of GTP and releases CO_2, which naturally acts as the thermodynamic driving force of the reaction. This is mediated by **PEP carboxykinase**



2.2.2 Bypass 2: F16BP → F6P

The reverse reaction is mediated by Fructose 1,6-bisphosphatase and and thus becomes reversible.

2.2.3 Bypass 3: G6P to Gluc

The resulting G6P gets imported into the ER, where it is dephosphorylated to Gluc by Glucose 6-phosphatase. It then gets exported to the cytoplasm again, thus concluding gluconeogenesis.

2.2.4 Regulation

Glycolysis and glucoenogenesis are regulated in a way that secures that only one of the pathways is active at any moment. There are two main sites of regulation:

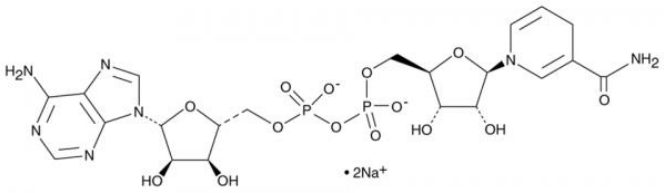
1. F1,6BP → F6P
2. PEP → pyruvate

If glucose is needed, gluconeogenesis is on, when ATP is needed, glycolysis is turned on.

2.3 NAD/H

Nicotinamide adenine dinucleotide (**NADH/H**)is a typical oxidation/reduction reagent in biology. All RedOx chemistry occurs at the nicotine group of NADH, the entire rest of the molecule is essentially just a handle for enzymes. Its more stable form is the NAD^+ due to a favourable resonance structure at the nicotine. Producing NADH thus costs energy.

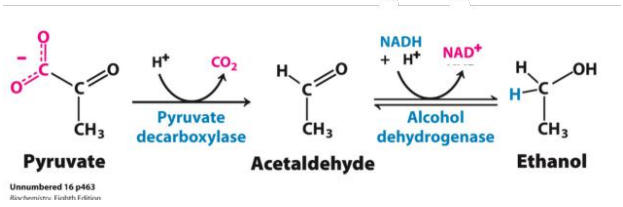
[3] Because both endosymbiont and the host had malate-oxaloacetate enzymes before endosymbiosis, it was not necessary for LECA to develop a mitochondrial malate transporter.



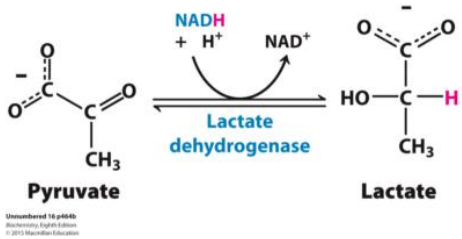
2.3.1 Regeneration of NAD^+

NAD^+ can either be replenished by taking pyruvate and oxidizing it completely to CO_2, which however can occur under aerobic conditions. This is essentially the TCA cycle. Under anaerobic conditions, pyruvate can form lactate or be fermented into ethanol. In both instances, NAD^+ is regained again.

For fermentation, pyruvate reacts to **acetaldehyde** using **Pyruvate decarboxylase**. The acetaldehyde then forms ethanol by using the **Alcohol dehydrogenase**. Alcohol dehydrogenase uses a Zn^{2+} active center. The reverse reaction (EtOH $\rightarrow$ acetaldehyde) also occurs in humans when we digest ethanol. Unfortunately, acetaldehyde is carcinogenic and decreases FA oxidation in the liver, contributing to fatty liver disease.



In highly active muscle cells that receive insufficient O_2 from the blood, pyruvate reacts to form **lactate** using the **Lactate dehydrogenase**. Lactate can only be metabolized slowly and thus the muscle cells will eventually become too acidic to function. An identical response occurs in highly proliferative tissue (incl. cancer) that require a lot of energy quickly and do not care so much for the more efficient total oxidation via TCA cycle and the respiratory chain. This is exploited diagnostically to screen for cancer by introducing a radioactively marked fluoroglucose.

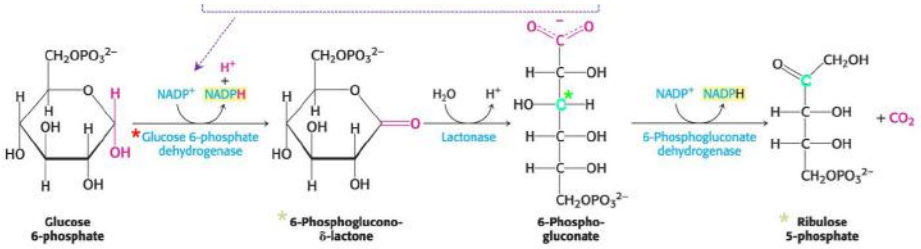


2.4 Pentose Phosphate Pathway

The **Pentose phosphate pathway** (PPP) is not a cycle but rather divided into two separate branches: one oxidative and one non-oxidative.

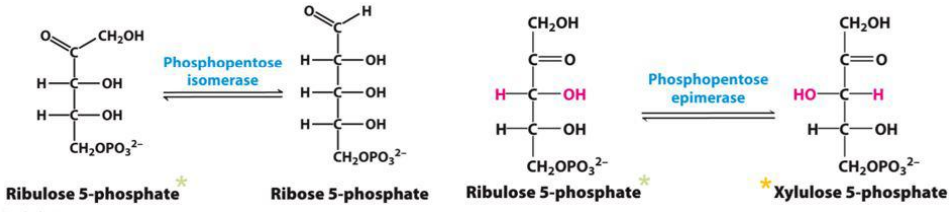
2.4.1 Oxidative Branch

The oxidative branch takes G6P and oxidizes the C1-OH to a ketone mediated by **G6P Dehydrogenase.** This is the rate-determining step and thus the site of regulation. The ring is then opened by a **lactonase** to form **6-Phospho gluconate**, which looses one CO_2 via **6-Phosphogluconate dehydrogenase** forming **ribulose-5-phosphate**.



2.4.2 Pentose-Phosphate Interconversions

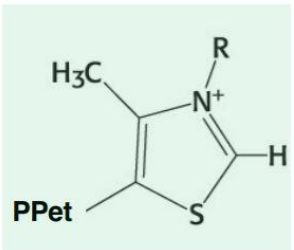
Ribulose-5-phosphate is in an equillibrium both with Ribose-5-phosphate (moving the C=0 up one position) by **Phosphopentose isomerase** as well as with Xylulose-5-phosphate via **Phosphopentose epimerase**.



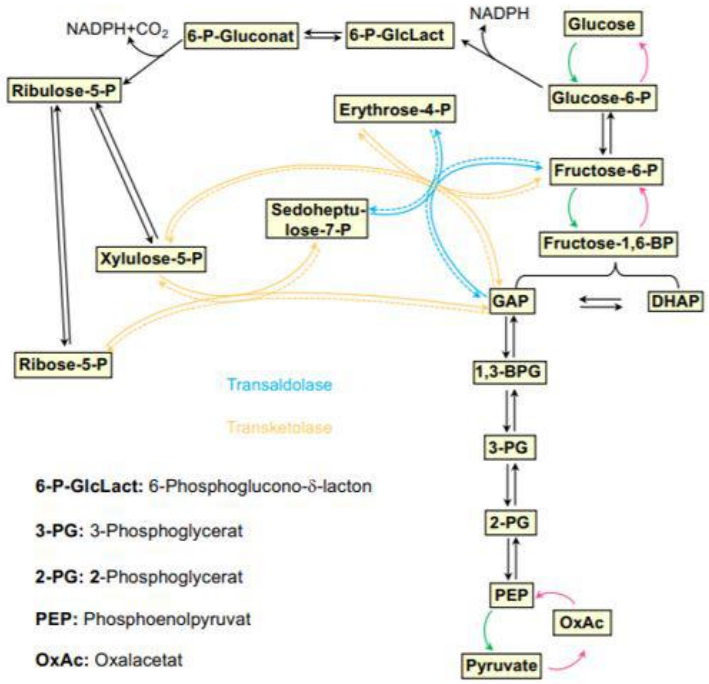
2.4.3 Non-Oxidative Branch

In the second branch of the PPP, **Glyceraldehyde-3-phosphate** (**GAP**) and Sedoheptulose are moved to **F6P** and erythrose mediated by **Transaldolase**. The reaction mechanism is fairly familiar: Lys forms a Schiffbase with the with the carbonyl, gets protonated and thus cleaves off one aldose leaving the enol. The enol is then attacked by a new aldose substrate that forms the ketose product again once the Schiff base is hydrolyzed away.

Erythrose and Xylulose can form **F6P** and **GAP** using **Transketolase**. The reaction mechanism involves the co-factor TPP (Thiaminpyrophosphate) that can form a carbene, which will react with the ketose first forming an aldose and enol product. The enol then attacks the new aldoese product again. Transketolase and Transaldolase are mechanistically very similar. In both cases, either through the Schiff base or TPP, the carbanionic intermediate needs to be stabilized.



Thiazole ring of TPP



2.4.4 Modes of the PPP

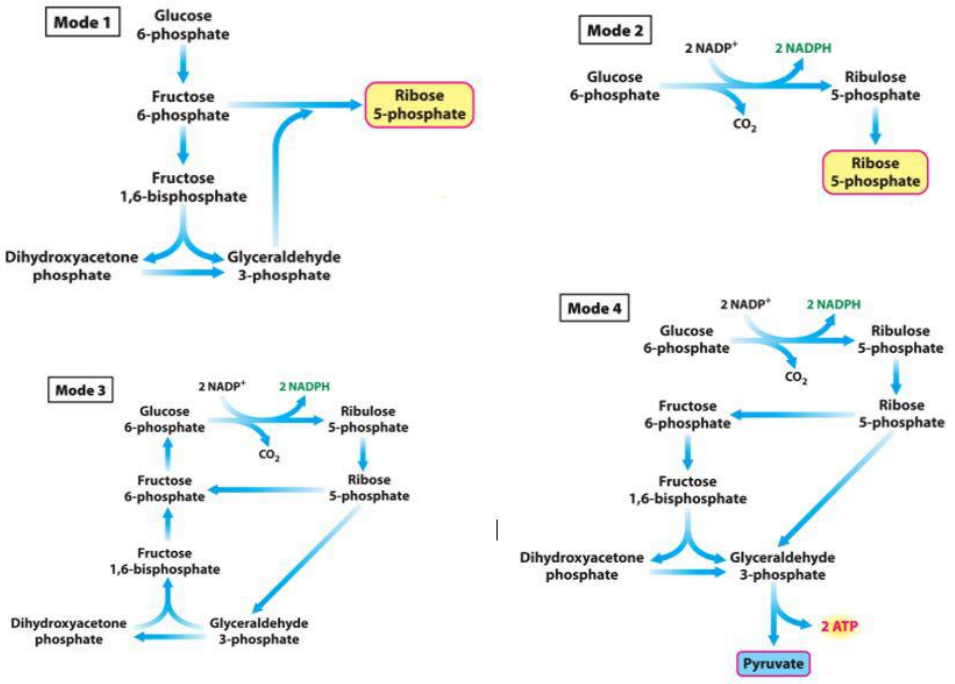
G6P can be destined for various compounds in the cell based on the current metabolic needs of the cell. Combining the PPP in a particular way with glycolysis/gluconeogenesis allows for optimal metabolic output of the cell. Four major such modes can be distinguished:

Mode 1: If **Ribose-5-phosphate** is needed, e.g. for nucleic acid synthesis, mode 1 will run glycolysis phase 1 in order to gain F6P and GAP, which together will form Ribose-5-phosphate using both transaldolase and transketolase. This is essentially jus the "reverse" non-oxidative branch of the PPP, preceded by phase 1 glycolysis in order to gain the necessary reactants.

Mode 2: An alternative to acquiring **Ribose-5-phosphate** is by moving through the oxidative branch first to Ribulose-5-phosphate and then to Ribose-5-phosphate. Unlike mode 1, mode 2 gives off 2 eq. of NADPH, which the cell somehow needs to make use of as well anabolitically. FA or cholesterol synthesis are examples, NADPH interestingly plays an important role in forming glutathione, a compound essential for protecting tissue from ROS.

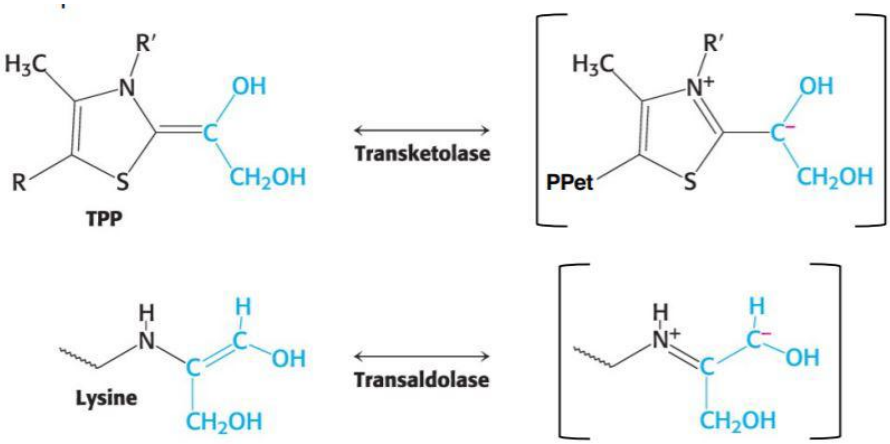
Mode 3: If NADPH is mainly needed instead of ATP or Ribose-5-phosphate, the cell will perform gluconeogenesis in order to maximize the G6P concentrations. The G6P will then move through the oxidative branch of the PPP, creating NADPH and Ribulose-5-phosphate. The non-oxidative branch will then go on forming F6P and GAP, which wil then be assimilated by gluconeogenesis again.

Mode 4: If both NADPH and ATP are needed, mode 3 gets modified so that some of the GAP still goes through phase 2 glycolysis (and potentially further towards TCA and respiration chain).



2.4.5 Catalytic Behaviour

In general the only two types of important catalytic steps in the PPP are based on stabilising the enol form of the aldose either via TPP or forming a Schiff base with Lys. In both instances the carbanionic form is also stabilized which makes them strong nucleophils.

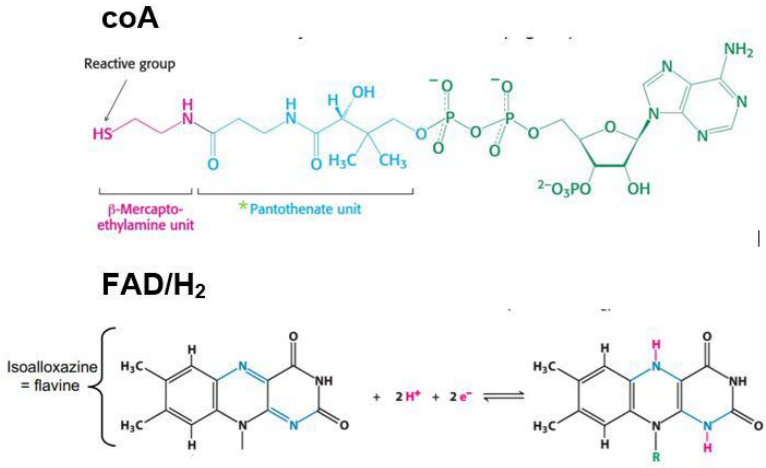


2.5 TCA Cycle

2.5.1 Pyruvate Dehydrogenase

Before the TCA cycle can begin, the pyruvate from gycolysis needs to be converted to **Acetyl CoA** by **Pyruvate dehydrogenase**. CoA is a cofactor that essentially consists of a very long handle and to the actually relevant -SH group. Besides CoA, FAD, TPP (Thiaminpyrophosphate) as well as a lipoamide with a disulfur bond are all necessary co-factors for Pyruvate dehydrogenase. Pyruvate dehydrogenase consists of three enzymes E1, E2 and E3.

1. **E1** (decarboxylase): TPP binds to pyruvate, releasing the ketone as CO_2.
2. **E2** (transacylase): The remaining TPP-2C fragment is oxidized and the C2 binds to the lipoamide, breaking the disulfur bond by attaching to one S via a thiolester and forming a thiol in the other.
3. CoA attaches and the lipoamide dissociates as a dithiol.
4. **E3** (dehydrogenase): The lipoamide then resorts to its original conformation by moving the two H^+ to FAD and reforming the disulfur bond. The FAD is then regained by reducing NAD^+ to NADH. [4]

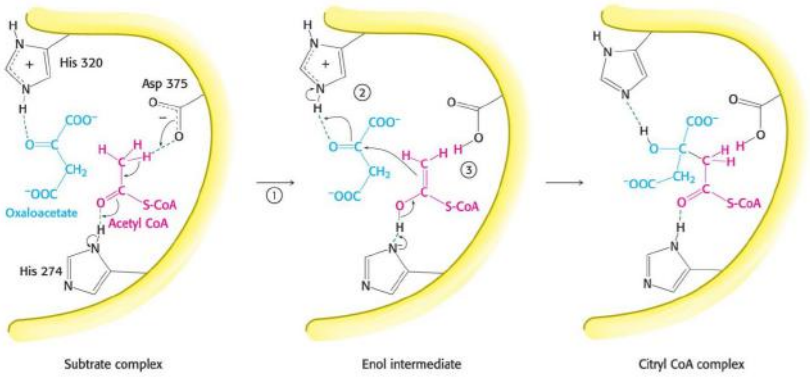


One major reason why arsenite can be so poisonous is because it inhibits the lipoamide from functioning properly.

2.5.2 Citrate Synthase

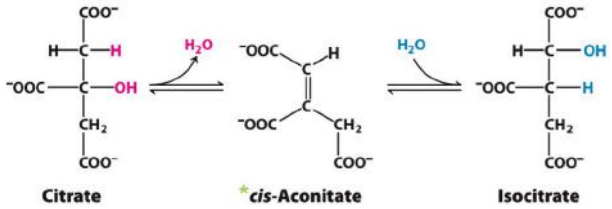
The entry point into the TCA happens when **Acetyl CoA** reacts with **Oxaloacetate** to form citrate mediated by **Citrate synthase**. Both the keto groups of OxAc and Acetyl CoA are bound by a His each, the methyl of the Acetyl CoA is further bound by an Asp. This forms he enol intermediate with the Acetyl CoA, the double bond will then attack the OxAc forming a citryl CoA complex that will yield the product under dissociation of the CoA. The enzyme makes sure that OxAc is bound before the Acetyl CoA so that no unwanted hydrolysis of either compound can occur.

[4] Note that this is only possible because $FADH_2$ is bent but will increase its redox-potential if flattened by an enzyme. Else $FADH_2$ would never be powerful enough to reduce NAD^+.



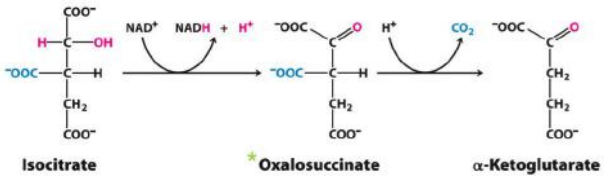
2.5.3 Aconitase

Aconitase lets Citrate react to **Isocitrate**.



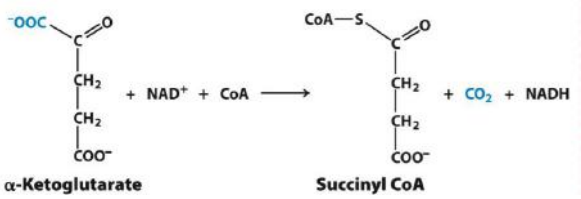
2.5.4 Isocitrate Dehydrogenase

Isocitrate Dehydrogenase lets Isocitrate react to **α-Ketoglutarate**.



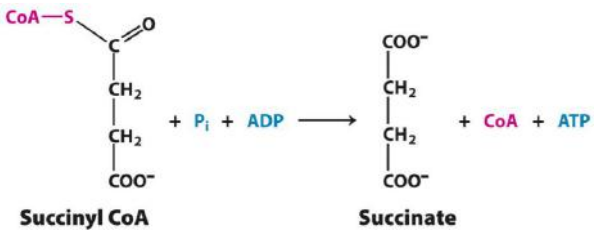
2.5.5 α-Ketoglutarate Dehydrogenase

α-Ketoglutarate Dehydrogenase uses α-Ketoglutarate + NAD^+ + CoA to form Succinyl CoA and NADH under loss of CO_2. The reaction mechanism is highly analogous to the Pyruvate dehydrogenase. Note that despite α-Ketoglutarat displaying a mirroring-plane, the chiral enzyme α-Ketoglutarate Dehydrogenase can distinguish the tow otherwise homotypic ends of the α-Ketoglutarate. This is no longer the case for Succinate, which additionally displays rotational symmetry unlike α-Ketoglutarate. This is the reason why labelling a particular position in the α-Ketoglutarate will always end up either in the CO_2 or in the Succinate but never both.

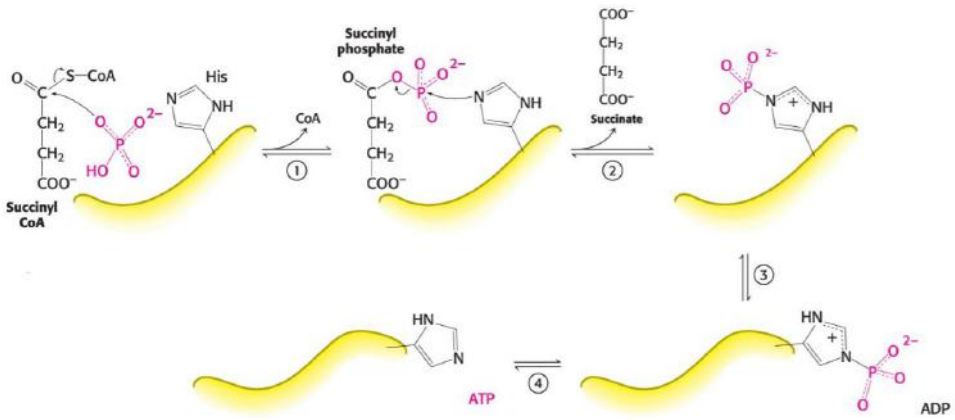


2.5.6 Succinyl-CoA Synthetase

Succinyl-CoA Synthetase finally cleaves the CoA forming **Succinate** and 1 eq. of ATP. This substrate-level phosphorylation is the only place where ATP is directly gained in the TCA cycle (but remember that for 1 eq. glucose one could theoretically undergo the TCA twice).

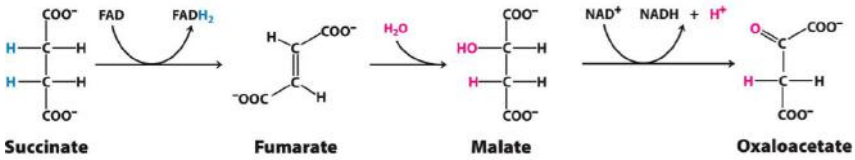


The reaction mechanism is highly analogous to GAPDH. The P_i attacks the Succinyl thus releasing the CoA, to which it is transiently bound until Succinate is released again and the P_i is transferred to a His, which will eventually phosphorylate ADP.



2.5.7 Return to Oxaloacetate

The last few oxidative steps are simply needed to replenish the Oxaloacetate used in the beginning, making the TCA an actual cycle rather than a linear metabolic process. Although no energ is directly gained from this part of the TCA cycle, the resulting $FADH_2$ and NADH can be used to gain energy in the respiration chain. The last stept involves the enzyme **Malate dehydrogenase** that despite a strongly positive $\Delta G'^{\circ}$ manages to force the reaction due to the constant removal of the products by further iterations of the TCA cycle (i.e. binding of the OxAc with Acetyl CoA).

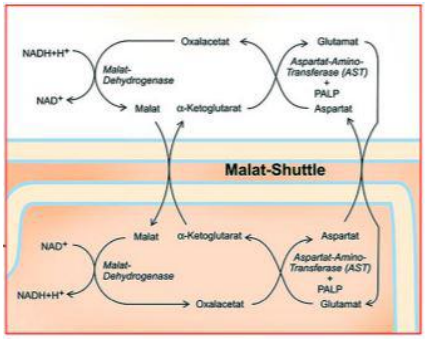


2.5.8 Regulation

There are three major points of regulation. ATP & NADH act as repressors while ADP induce the cycle.

1. Pyruvate → Acetyl CoA.
   (This step is further regulated by pyruvate/Acetyl CoA ratios.)
2. Isocitrate → α-Ketoglutarate
3. α-Ketoglutarate → Succinyl CoA.

2.5.9 Malate-Shuttle



There are many so-called "shuttles" that help bring products of the TCA cycle, essentially the key metabolic hub of the cell, across the mitochondrial membrane. A good example of this is the Malate-shuttle which actually exports both Malate as well as Aspartate, this is in order to first form Oxaloacetate in the cytoplasm and then reduce this to Malate via the Malate dehydrogenase. This releases NAD^+ in the cytoplasm. The Malate shuttle hence also shuttles NAD^+ across the membrane, as the NAD^+ is used in the mitochondrion to create Aspartate, which then also gets exported. This meant the cell did not have to come up with a *de novo* evolutionary solution to transport NAD/H across the mitochondrial membrane.

2.5.10 Glyoxylate Pathway

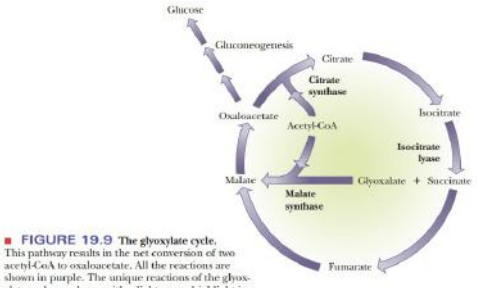
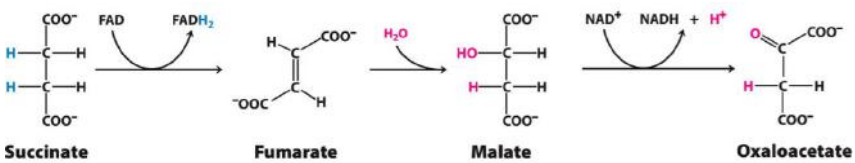


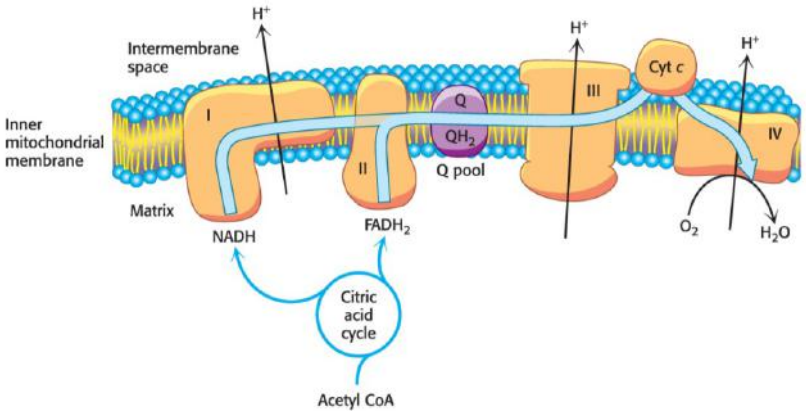
FIGURE 19.9 The glyoxylate cycle. This pathway results in the net conversion of two acetyl-CoA to oxaloacetate. All the reactions are shown in purple. The unique reactions of the glyoxylate cycle are shown with a light green highlight in the center of the circle.

Fats are metabolized into Acetyl CoA and will thus enter the TCA cycle. The problem is that this leaves the existing Oxaloacetate reserves unaffected, meaning that the C from the FA cannot actually be used to build up C-matter. The 2 C from the Acetyl CoA get lost as CO_2 during the course of the TCA cycle. However, lots of energy can be created by going through the TCA . Gluconeogenesis will still not be possible, because no Oxaloacetate can be removed from the TCA cycle for the PPP. Some organisms have evolved a **glyoxylate cycle** that allows them to replenish the Oxaloacetate reservoirs and thus gluconeogenesis with Cs from FAs is actually possible. The glyoxylate cycle actually invests 2 Acetyl CoA and circumvents the decarboxylation step.



2.6 Respiratory Chain

The Electron Transport Chain (ETC) is responsible for the oxidative phosphorylation and occurs along the innermembrane of the mitochondrion. Principally, reducing agents from other metabolic processes (e.g. mainly glycolysis and TCA) are used to transfer e^- onto O_2 to form H_2O. Simultaneously, a proton gradient is established by Complex I, III and IV translocating H^+ from the matrix to the intermembrane space (IMS). This H^+-gradient can be used by ATP synthase to synthesize ATP. This has been demonstrated experimentally by implementing a bacteriorhodopsin and a ATP synthase in a simple vesicle, which was then able to perform ATP synthesis. There are four major complexes involved in the ETC among some other e^--shuttles, e.g. FeS-clusters, ubiquinone and Cyt c.



Complex I: **NADH-Q Oxidoreductase** takes NADH and moves the electrons onto an ubiquinone (Q). During the process 1 H^+ is also translocated to the IMS.

Complex II: **Succinate-Q Reductase** binds 1 $FADH_2$ and then oxidizes succinate to fumarate, also pushing the e^- onto a Q in the end. Complex II is the only complex of the four that does not contribute to the H^+-gradient.

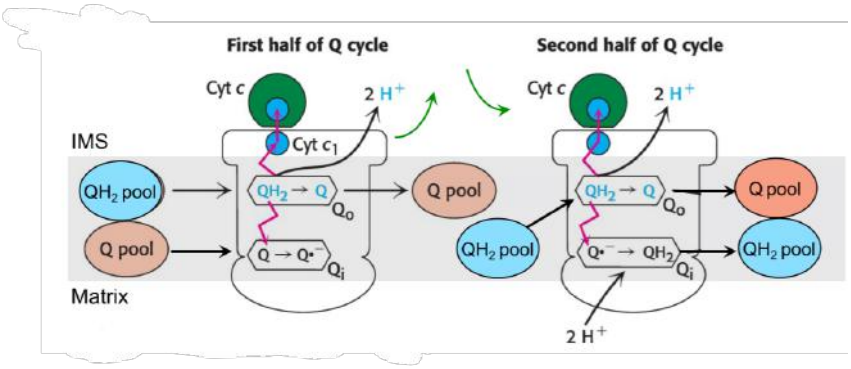
Complex III: **Q-Cytochrome c Oxidoreductase** takes a reduced QH_2 and transfers the e^- onto a Cytochrome c (which contains Fe). Note that for this reaction to work stochiometrically, 2 Cyt c need to be reduced. This also pushes 1 H^+ to the IMS.

Complex IV: **Cytochrome c Oxidase** finally takes the e^- from QH_2 and moves them onto O_2, also releasing 1 H^+ to the IMS and terminating the ETC.

Looking at the structure of these complexes, we can see that they contain themselves as "slide" for the e^- to move along made up out of FeS-clusters. Interestingly, the e^- manage to traverse distances they would not otherwise be able to in vacuo due to the AA side chains in sterical vicinity. The ETC therefore represents a very intricate system, evolutionarily fine-tuned to its purposes.

2.6.1 Coenzyme Q (Ubiquinone)

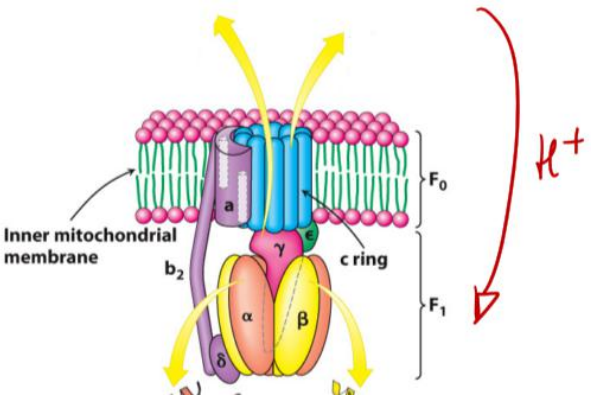
Coenyzme Q or ubiquinone is a very common e^--shuttle. It has a long, aliphatic and unsaturated tail and can take up two H^+ on its two ketones. Note that there is also a semiquinone state (next to oxidized Q_o and reduced QH_2) that actually has a radical structure and that can even further react to a $Q^{\cdot -}$ radical, also referred to as Q_i.



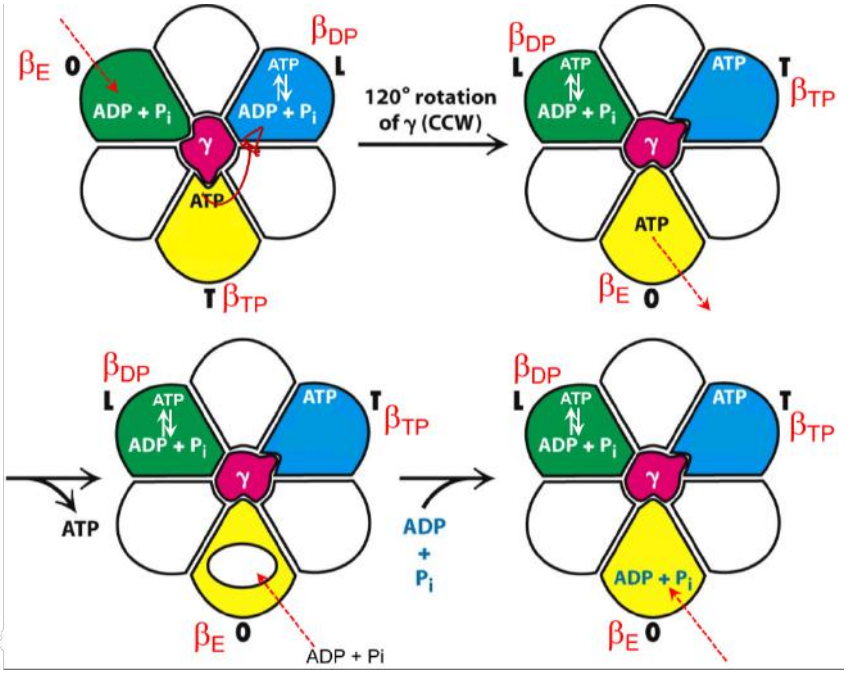
Q_i is formed during the so-called quinone cycle at complex III. Because complex III can only bind 1 Cyt c at a time and because each Cyt c can only take up 1 e^-, in the first half of the Q cycle one of the 2 e^- from the QH_2 will move onto the Cyt c and the other will take a Q_o and form Q_i. The 2 H^+ from the QH_2 are released to the IMS and contribute to the H^+-gradient. Then in the second half of the Q cycle, again 1 e^- moves onto the Cyt c and again 2 H^+ are released to the IMS, but now the additional e^- is used to reduced Q_i to QH_2 again, thus completing the Q cycle. This means that for a short period, dangerous radical species are present in complex III. This often leads to ROS that need to be dealt with by superoxide dismutase, catalse and glutathione.

2.7 ATP Synthase

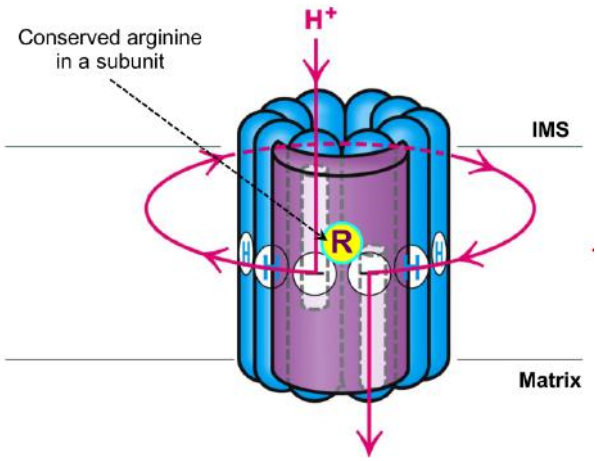
In the early times of describing the behavior of the ATP synthase a strange fact emerged: adding isotopically labelled H_2O even under non-energetic conditions led to some radioactive ATP, which however could not be dissociated from the enzyme. Apparently, ATP synthase could form 1 ATP without any H^+-gradient but was unable to dissociate it then.



The catalytic mechanism of ATP synthase is intimately linked with its structure: ATP synthase consists of a rotor and a stator. The rotor consists of a group of c subunits (ca. 10, depending on the strength of the H^+-gradient), as well as the γ-stalk and the occasional η-subunit. The stator consists of a long stalk that connects the $\alpha_3\beta_3$ hexamer and the rotor. The α and the β subunits are structurally nearly identical, but only the β actually are catalytically active. Note that for unknown reasons, all the α subunits still have 1 ATP bound (perhaps merely due to their structural similarity to the β-subunits).



The asymmetry of the γ-stalk is responsible for segregating the 3 β-subunits: The β_E is empty; the β_{DP} contains both ADP and P_i; the β_{TP} contains the newly formed ATP. The β_{TP} is compressed by a protrusion of the γ-stalk so tightly that not even H_2O will remain in the β_{TP}-subunit. The γ-stalk will not move before the P_i has been bound, but once it has moved, the ATP gets released.



The energy necessary for the γ-stalk rotation comes from the movement of H^+ back to the matrix, i.e. alongside the gradient. The c-unit has a band of negatively charged Asp in the middle of the otherwise highly hydrophic lipid bilayer. At the connection point of the stator, 1 H^+ from the IMS can connect to one subunit, rotate 360° and then dissociate towards the matrix. This will make the rotor rotate clockwise as seen from the IMS. The rotation does not occur in the opposite sense due to a positively charged Arg directly between the entry and exit point. If the Asp were to move anti-clockwise, i.e. towards the Arg, it would get trapped by a salt-bridge, forcing it to dissociate the H^+. The Arg thus acts like the cog on a wheel of fortune. Note in particular that though in yeast the rotor consists of a c_10 unit, many other numbers are possible. If the H^*-gradient is very small, more c units make this system slower but more efficient. Any number of c-subunits will always only create 3 ATP equivalents however.

In vivo, the ATP synthases are always found as dimers on the tips of the cristae, where the two stators are actually linked to each other. Most of the respiratory complexes are actually found in a so-called respiratory chain supercomplexes and not in the linear arrangement that the textbook would suggest.

2.7.1 Some Notes on a Metabolic Numerology

Often the number of 36 ATP is given for the number of ATP that can be gained out of 1 eq. of glucose. Though theoretically a maximal value, reality looks a lot different. The actual number is closer to about 30 ATP due to a number of reasons:

1. In order to synthesize ATP, ADP and P_i need to be present in the matrix. The import of P_i is a symport linked to import of H^+ to the matrix, i.e. along the gradient. This makes the import exergonic. Because ATP is then exported after synthesis to the IMS and then on through the anyway permeable outer membrane to the cytoplasm, net charge is lost at the matrix. This charge needs to be regained by the H^+-gradient, some of the H^+ hence are not "available" for ATP-synthesis.
2. The malate-aspartate shuttle also requires some H^+ in order to function in order to import Glutamate to the matrix.
3. The Glycerol-3-P shuttle actually for once increases the number of ATP artifically because it creates QH_2 during translocation. Some of the QH_2 at complex III thus might not actually from Gluc originally.
4. The number of c subunits also have an influence with regard to how many H^+ are bound for each cycle of the ATP synthase. This has an influence on how much energy is needed just to maintain the H^+-gradient in terms of free H^+.

3 Glycogen Metabolism

Glycogen is a branched polysaccharide of α-D-Glucoses connected via α-glycosidic bonds responsible for energy storage and thus is very similar in form and function to starch. These branched polymers of monosaccharides, i.e. glucose, can contain energy in a very dense form. Glycogen, by virtue of its branched structure, will spontaneously crystallize into glycogen grains at sufficient concentrations. The advantage of glycogen is not only its high energy-density but also that it allows for glucose level to remain static even when it is abundant, otherwise osmosis in the blood would break down and neurons might starve due to lack of glucose. Most glycogen can be found in muscles and in the liver, though it saves energy only for ca. 24h (in a marathon it can be depleted in 1.5h).

3.1 Metabolization

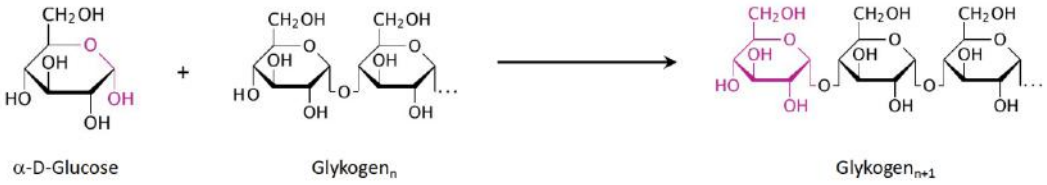
SGluT is the translocase that the intestinal epithelium uses to import glucose, it is then exported via a GluT in the interstitium, from whence it can diffuse into the *vena porta* and move into the liver. The liver imports the glucose via a GluT into the hepatocytes again, which surround the branched vene in so-called liver lobules. The liver exports glucose into the blood via GluT's

3.2 Amperometric Glucose Measurement

Amperometric glucose-sensors reduce glucose to gluconolacton, the number of e^- this requires are measured as a flow of e^-, i.e. current. The current thus indicates the number of glucose present.

3.3 Biosynthesis

Glycogen is synthesized by connecting Glucose units via α-1,4-glycosidic bonds using **Glycogen synthase**. A glycosidic bonds is simply formed by nucleophilic substitution at the chiral C, a S_N1 reaction that eliminates the -UDP unit on the just added glucose. Glucose-UDP is simply more nucleophilic than regular glucose and UDP is a superior entropic driving force than water. Note that glycogen synthase requires a chain-length of ≥ 4 in to be able to add new glucose.



The first few glucose are added by glycogenin itself, the enzyme that sits at the core of every glycogen grain. Glycogenin is a dimer, where one subunit autoglycosylates the other around 8 times, attaching glucose to a Tyr in either active site. Thus by virtue of being a dimer, it builds two glucose-chains in two directions simultaneously.

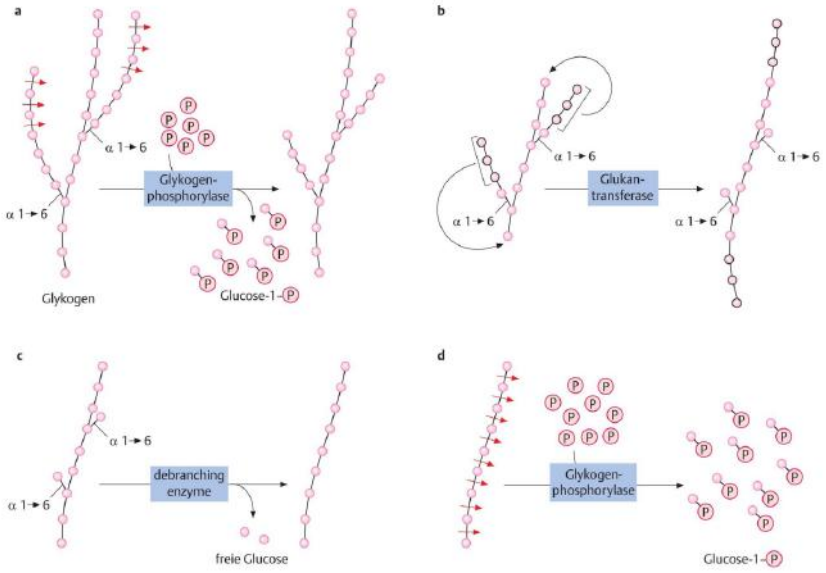
The **Branching enzyme** is then responsible for intermittently introducing α-1,6-glycosidic bonds resulting in divergent glucose-chains. Branching enzyme cleaves a α-1,4-bond and moves the cleaved section back a bit, where it attaches it via a α-1,6-bond again. There are a number of rules that branching enzyme seems to obey, ensuring that the branching is distant enough to another branching and that transferred chain is sufficiently long and not too close to the core either. Up to 12 layers of such branches can make up a single glycogen grain. Note that this branched structure results in many open ends, all possible sites for synthesis to continue or degradation to begin. Glycogen grains are relatively water-soluble.

3.3.1 UPD-Glucose Biosynthesis

UDP-Glucose is formed by reacting Glucose-6-phosphate from the glycolysis/gluconeogenesis pathway with UTP via a transferase.

3.4 Glycogenolysis

Glycogenolysis is a four-step reaction pathway that breaks down glycogen, releasing the glucose-1-phosphate. The branched structure of glycogen is perfect for commencing degradation at many sites simultaneously.



3.4.1 Glycogen Phosphorylase

Glucose units get removed via **Glycogen phosphorylase**, the enzyme that cleaves (most) α-1,4-glycosidic bonds. It cleaves the bond by introducing a phosphate group at the ether bond between the glucose using a PLP-cofactor, leaving a (n-1)-chain and Glucose-1-phosphate. PLP is a phosphate group attached to a N-heterocycle. It cannot cleave α-1,6-bonds nor α-1,4-bonds that are within 4 glucose units to a branching.

Glucose-1-phosphate is then modified to Glucose-6-phosphate via **Phosphoglucomutase**, which can then enter either glycolysis/gluconeogenesis or the PPP. Phosphoglucomutase just phosphorylates G1P again to form G1,6P, which will then dephosphorylate into G6P. No regioselective dephosphorylation involved!

Unfortunately phosphoglucomutase only exists in the liver. The liver furthermore has a membrane-bound **Glucose-6-phosphatase** that will convert G6P to glucose, which can then get exported via GluT's.

3.4.2 Glucan Transferase

After glycogen phosphorylase has removed all glucose up to 4 remaining before each branching, **Glucan transferase** takes 3 of these 4 glucose and adds them on the rest of the chain via α-1,4-bonds, where they can be then cleaved again using glycogen phosphorylase.

3.5 Debranching Enzyme

Debranching enzyme is the antagonist to branching enzyme and cleaves α-1,6-bonds, releasing glucose not glucose-1-phosphate as it uses H_2O not P_i as a nucleophile. The remaining glucose are released via glycogen phosphorylase.

3.6 Regulation

The main regulatory targets are glycogen synthase (biosynthesis) and glycogen phosphorylase (degradation). Regulation thus targets the two work-horses of both pathways by de/phosphorylating them if need as both enzymes will change their conformation after phosphorylation and thus behave switch-like.

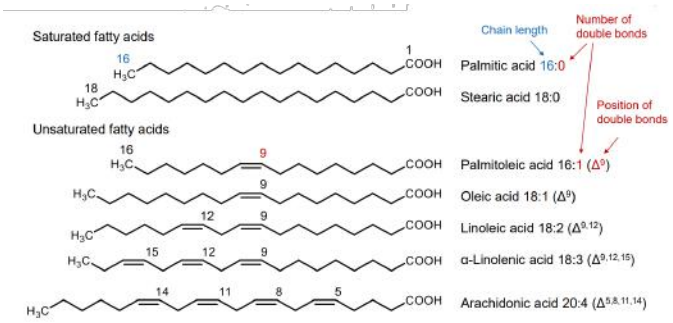
Glycogen phosphorylase needs to be phosphorylated to be active.
Glycogen synthase needs to be dephosphorylated to be active.

Glycogen phosphorylase will change from the inactive T-conformer to the active R-conformer. A second phosphorylation will actually change the activity of glycogen phosphorylase slightly as it is suddenly able to release glucose units not glucose-1-phosphate. This occurs in the liver between meals or during workout in the muscles.

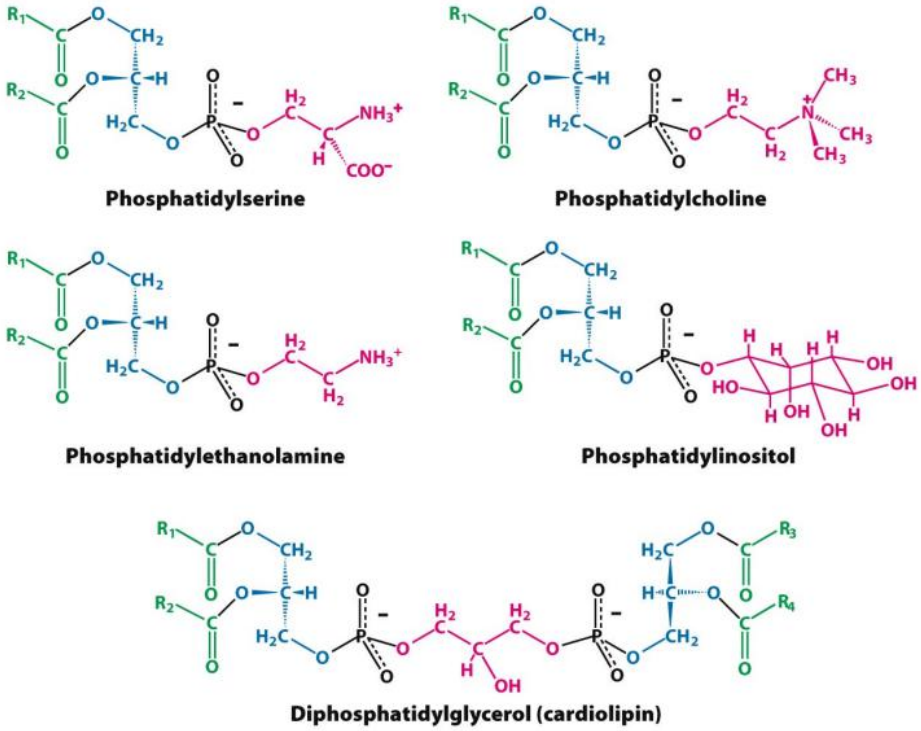
G-coupled receptor pathways also allow for endocrinological regulation via glucagon or adrenaline. If glucose levels are low, glucagon levels are high, which will upregulate glcyogen phosphoorylase activity for example. Allosteric interactions with substrates further add necessary negative feedback loops for both enzymes. Insulin would trigger the opposite response, resulting in more glycogen biosynthesis. Given that there ATP-sensitive K^+-channels in cells, abundant glucose and thus abundant ATP will actually alter the membrane potential, which triggers release of insulin. Insulin is stored in vesicles near the Golgi as a Zn-bound hexamer. Both hormone levels as well as glycogen activity are thus very time-dependent and oscillate, so to speak, between meals and throughout the day.

4 Fatty Acids

Fatty acids (FA) are carbonic acids with long carbon chains that can either be saturated or unsaturated. Most FAs have an even number of carbons. Many FAs are incorporated into triglycerides, where three FAs form ester bonds to a glycerol unit. Variation of this include a glycerol with two FA and a phosphate group, a so-called phospholipid.



FA play a major role in storing and releasing energy, storage occurs in their triglyceride form, commonly referred to as "fat". FAs are synthesized and decomposed in C2 units.

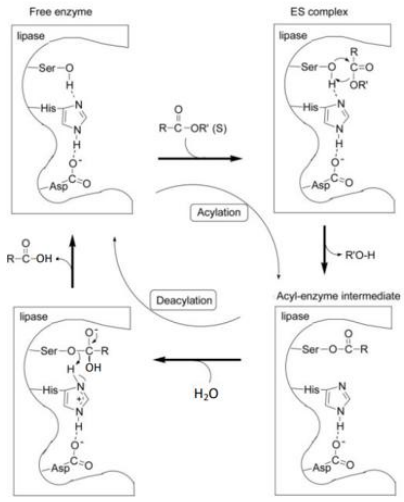


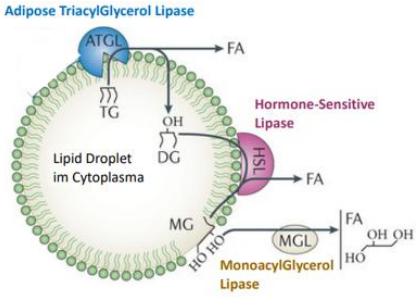
4.1 Metabolization

Fat that we consume is first emulgated by gallic acids in the small intestine, where lipases from the pancreases begin to digest the triglycerides into glycerol and FAs. The FAs are resorbed through the epithelium, where they get reassembled into triglycerides and stored in so-called chylomicrons for further transport in the blood or the lymph.

4.2 Lipolysis

Fat-reservoirs in adipocytes are released by glucagon and adrenaline signalling through a G-protein coupled pathway. Logically, there are three distinct lipases required to break down a triglyceride: one for each FA. Two lipases act on the membrane of the lipid-droplet of the adipocyte itself, while the last lipase acts on the then released monoacylglycerol.

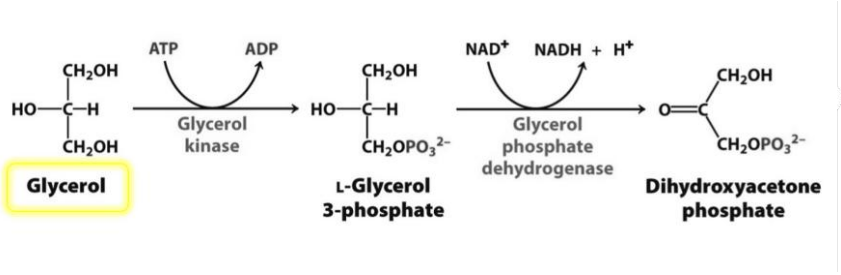




Lipases are catalysts for the nucleophilic substitution of the ester-bonds with water. A Ser-His-Asp acts as the catalytic center, analogous to the Ser-proteases. The FA attaches to the Ser and the His acts as a base. The His is made more basic by H-interactions with the adjacent Asp.

4.2.1 Further use: Gluconeogenesis

Lipolysis alone produces free FAs and glycerol units, which without further biosynthesis would be wholesomely unusable. Hepatocytes (unlike adipocytes) however possess two enzymes called **glycerol kinase** and **glycerol phosphate dehydrogenase** that can form DHAP from glycerol. DHAP + GA3P are of course part of the gluconeogenetic pathway and can therefore be used to restore glucose levels. The reverse glycolytic pathway is possible as well of course, resulting in pyruvate that can then be



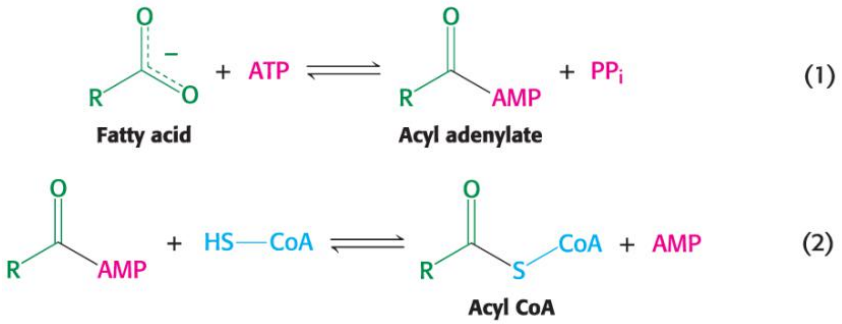
4.3 FA-Transport

Extracellular: FAs are highly hydrophobic and thus need carrier-proteins to be transported through the aqueous blood. Serumalbumin is the most abundant protein in the blood system. Each serumalbumin can carry up two 7 FAs.

Intracellular: FABP (Fatty Acid Binding Protein) is very abundant in the cytosol and is also responsible for transporting FAs.

4.3.1 Transport in Cytoplasm

Once a FA enters the cytoplasm it is immediately refunctionalized into a thioester. Thioesters are more reactive than esters due to the reduced mesomeric involvement of the keto group with the S. More concretely, the FA first reacts with ATP to form an activated intermediate with a bound AMP, which then reacts with the thiol end (i.e. the Acetyl CoA group) of Coenzyme A. This releases the AMP, making the reaction irreversible. For the oxidative pathway, all the Acetyl Co-A comes from Coenzyme A.



4.3.2 Translocation into Mitochondrium

FA-catabolism occurs within the mitochondrium, which requires translocation. The outer membrane is permeable to the activated Acyl CoA-FAs.

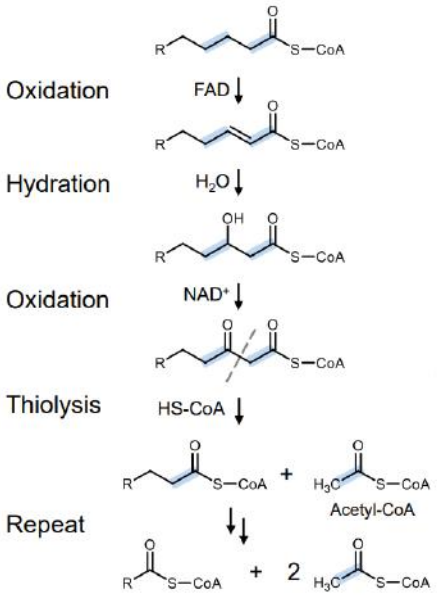
Transport across the inner membrane requires the condensation of the Acetyl CoA-FA with Carnitine. This forms Acyl carnitine and releases free Acyl CoA. This reaction is mediated by a transferase called **Carnitinacyl transferase** I in the outer membrane, a translocase in the inner membrane specific to the Acyl carnitine then translocates the FA. Once in the matrix, the reaction is reversed by Carnitinacyl transferase II, where the Acyl CoA can then be β-oxidized, see below.

4.4 β-Oxidation of FAs

β-Oxidation occurs through a series of repeated catabolic steps that relase a C2 unit in each iteration.

4.4.1 Oxidation I

FAD is used as co-substrate in order to oxidize the C_α-C_β to a double bond via the enzyme **Acyl-CoA-dehydrogenase**.



4.4.2 Hydration

The enzyme **Enoyl-CoA-Hydratase** lets H_2O react stereospecifically with the double bond in a Michael addition.

4.4.3 Oxidation II

The just introduced hydroxy group is oxidized to a ketone by **Hydroxyacyl-CoA-Dehydrogenase** using NAD^+ as co-substrate.

4.4.4 Thiolysis

Through the enzyme **thiolase** a new Acetyl-CoA unit can be introduced, releasing the C2-unit, i.e. the Acetyl-CoA. This is an example of a retro-Claisen condensation, where Acetyl-CoA acts as the nucleophile. → A new iteration can now begin, this type of cyclic reaction mechanism is sometimes referred to as a "spiral".

4.4.5 Energy Balance

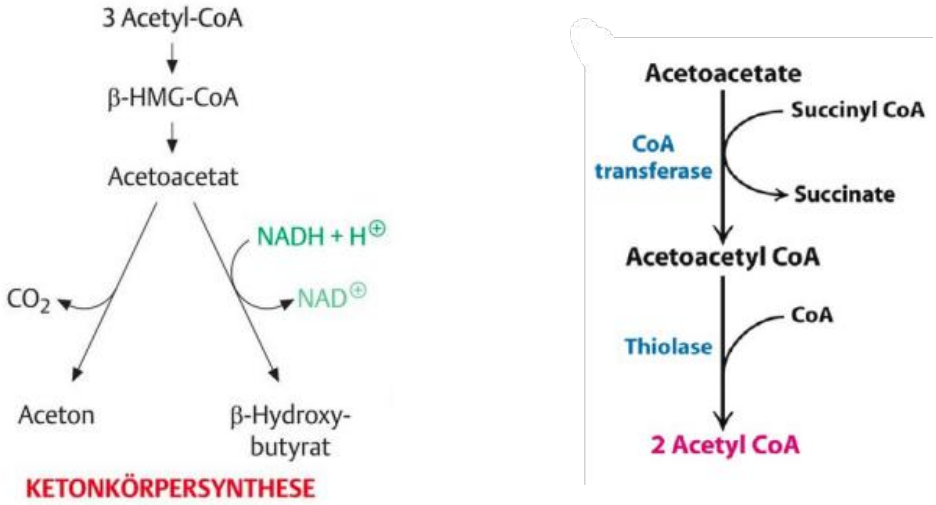
Energy is released in the β-oxidation both through the resulting $FADH_2$ and NADH, which will go into the respiratory chain, and the Acetyl CoA units, that go into the TCA cycle. Note that if the FA is odd-numbered one C3-unit, called propionyl CoA, gets also formed, which can also be used to release energy.

Note also that in mammals, very long FAs are often first catabolized into smaller subunits in the peroxisomes. The reaction mechanism is essentially identical to the β-oxidation, except that in the first oxidation, the $FADH_2$ transfers its H onto O_2 forming hydrogen peroxide H_2O_2, that can be dealt with by catalase.

4.5 Ketogenesis

In case of serious nutrient deprivation, gluconeogenesis is very active in the liver as neurons, erythrozytes and some other cells can only use glucose as energy. Gluconeogenesis begins with pyruvate, which is then lacking for pyruvate carboxylase to form new oxaloacetate from. If oxaloacetate is missing, the Acetyl CoA units that are released through β-oxidation just accumulate.

Three Acetyl-CoA equivalents can react via two Claisen-condensations to form acetoacetate. Acetoacetate can then reduced to D-3-Hydroxybutarate, which also releases 1 equivalent of NAD^+ for the TCA. Other ketones that D-3-Hydroxybutarate exist. Ketone-body synthesis is typically triggered by high glucagon levels.



The acetoacetate will then react with succinyl CoA to form acetoacetyl CoA using a CoA transferase. The acetoacetyl CoA and an additional CoA unit react via a thiolase to form two units of acetyl CoA, which can be used directly for the TCA cycle.

Note that the acetoacetate can sometimes spontaneously decarboxylate into acetone, which is released pulminarily. Because glucagon is typically very high in diabetes (type I) patients, they sometimes even present with a characteristic acetone breath. Both myo- and adipocytes can only take up glucose through an insulin-dependent translocase. In both types of diabetes, this translocase is not activated and a hunger-response is triggered. The muscle will release AAs into the blood whereas the fat tissue will begin lipolysis leading to ketone body synthesis.

4.6 Lipoprotein-Particles

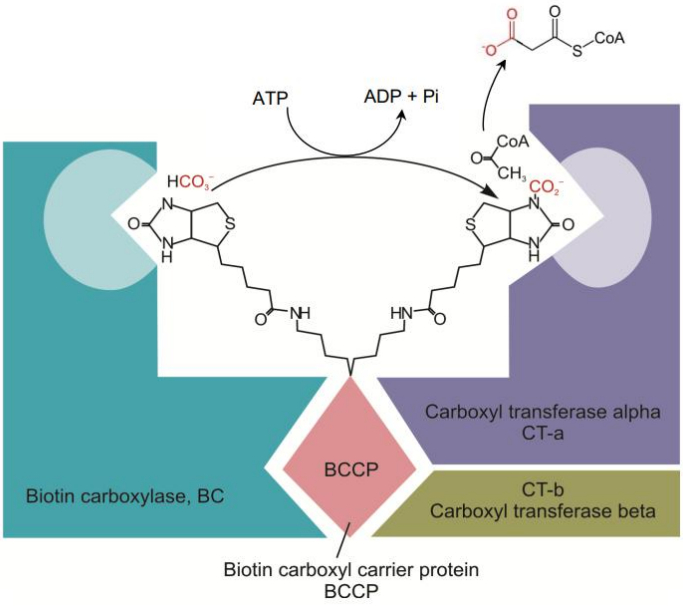
FAs as well as cholesterol are mostly stored and transported in lipoprotein-particles, collections of various lipids that are surrounded by a phospholipid bilayer. Lipoprotein-particles contain apoproteins in order to package the lipids with variable lipid-density. The lowest-density lipoprotein-particles are the chylomicrons that transport the triglycerides after they have been resorbed at the intestinal epithelium. Some of the low-density lipoproteins are artherogen, meaning they will deposit as plaques in the arteries causing atherosklerosis. Not to be confused with arteriosklerosis, which is caused by inelasticity of the lumen epithelium due to ageing in absence of plaques.

4.7 FA-Biosynthesis

FA-Biosynthesis functions symmetrically to β-oxidation, with three major differences: Firstly, the Acetyl-CoA now comes from Acyl Carrier Protein (ACP). ACP is a large protein that has an Acetyl-CoA unit and most of the handle of Coenzyme A attached to it post-translationally. Secondly, whereas β-oxidation used NAD/H, FA-biosynthesis uses NADPH, so the two pathways can be distinguished during regulation.

4.7.1 Malonyl-CoA Biosynthesis

Furthermore, Biosynthesis cannot begin with 2 eq. of Acetyl-CoA but requires 1 Acetyl-CoA and 1 Malonyl-CoA. After that it elongates exclusively with Malonyl-CoA units. This requires the additional step of creating Malonyl-CoA from 1 Acetyl-CoA via **Acetyl-CoA-Carboxylase**. Acetyl-CoA-carboxylase actually consists of two catalytic subunits and a biotin carboxyl carrier unit to which a biotin unit is attached. The biotin first needs to be carboxylated using up 1 HCO_3^- and 1 ATP at the Biotin carboxylase subunit. The biotin arm then swings over to the Carboxyl transferase subunit, where the COO^- unit is transferred onto an Acetyl-CoA forming Malonyl-CoA.



Biotin has a very strong interaction with the avidin family of proteins. Avidin was actually first discovered in egg-white, where it likely plays an anti-microbial role. It was quickly named after its incredible avidity to bind biotin (avid+(biot)in). Avidin has 4 biotin binding sites and is now widely used in experimental biology.

4.7.2 ACP-attachment

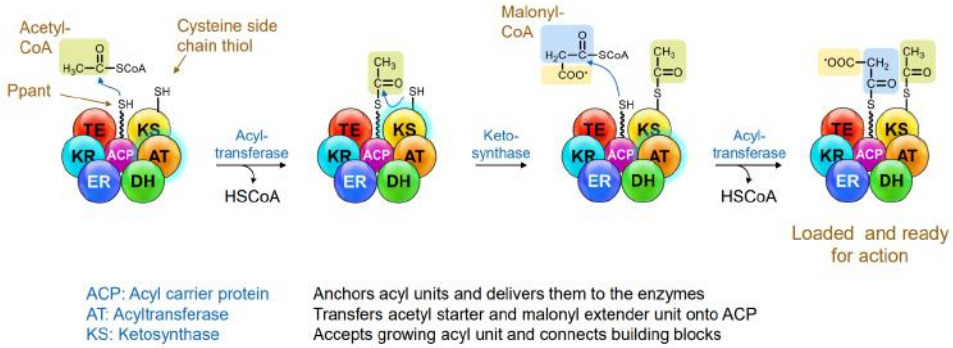
Both the Acetyl-CoA and the Malonyl-CoA will have there CoA units replaced with an ACP-bond. The ACP is essentially a large protein with an Acetyl-CoA at its tip and will still activate the keto-groups on Acetyl and Malonyl just like CoA but is an additional requirement for FA-biosynthesis. In FA-biosynthesis, both malonyl and acetyl will attach to the same ACP but at different times.

The *apo*-ACP first reacts with Coenzyme A to form the *holo*-ACP, whose thiol group will then react (just like Coenzyme A would) with the Acetyl CoA, forming the thioester that is referred to as Acetyl-ACP.

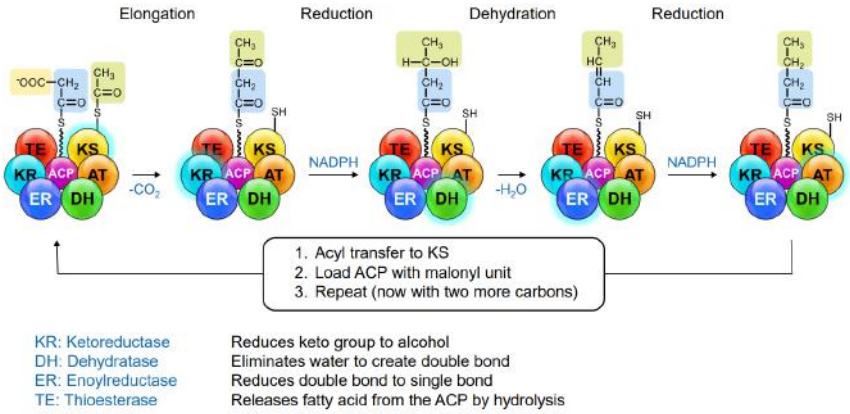
4.7.3 Fatty Acid Synthase (FAS)

There are two types of **Fatty Acid Synthases** (FAS): In opisthokonts, all subunits are assembled into one mega-enzyme (type I); in bacteria and plants, the individual units are in steric proximity but independent and modular (type II).

1. The ACP sits in the middle of the complex and is then charged with an Acetyl-CoA unit. The CoA is cleaved off.
2. The **Ketosynthase** (KS) will then take over the Acetyl. The ACP is now free to attach to the Malonyl-CoA. The CoA is cleaved off again.

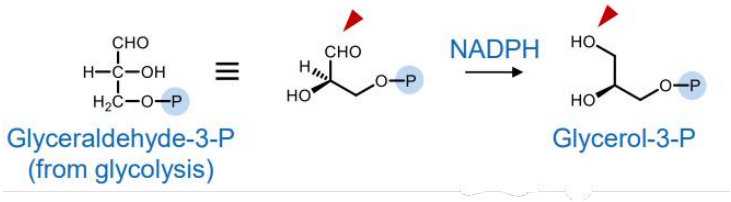


3. The Acetyl on the KS will then attach to the Malonyl, which decarboxylates, is now just like an acetyl unit. The CO_2 acts as an additional driving force however.
4. The Acetyl at position -1 then has its ketone reduced to an alcohol using NADPH via **Ketoreductase** (KR).
5. Dehydration of the chain now results in a double-bond between the acetyl units at -1 and 0 via **Dehydratase** (DH).
6. The double bond is then reduced, again using NADPH via **Enoylreductase** (ER).
7. The chain is then transferred back onto the KS by **Acetyl transferase** (AT).
8. A new Malonyl-CoA can attach to the ACP and the cycle starts anew.
9. Termination: If AT does not return the chain to KS, the chain can be cut by **Thioesterase** (TE).



4.7.4 Glycerol Biosynthesis

Most FAs are incorporated into triglycerides or phospholipids. Both require glycerol. But where does it come from? Glycerol is the product of the reduction GA3P from glycolysis/gluconeogenesis.



4.7.5 FA-Modification

It would be quite inefficient if all FAs that we consume would first need to be fully β-oxidized and then reincorporated biosynthetically. Rather some FAs that are taken up are either used directly or, if only minor modifications are needed, altered enzymatically without any metabolism involved.

Elongases are a class of enzymes that can elongate existing FAs and are FAS-like enzymes.

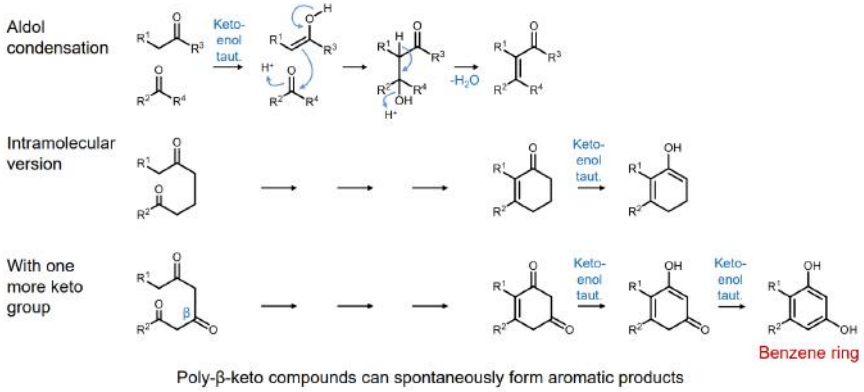
Desaturases are a class of Fe-containing enzymes that can create a double-bond. Oddly, mammalians never bothered to evolve (or more likely maintain) desaturases for enzymes beyond the first 10. Some essential FAs however are unsaturated at these unreachable positions meaning we are dependent on plants and fish, who do have these particular desaturases, to obtain them.

4.8 Polyketides

There are two major types of polyketides: aromatic and reduced (or complex) polyketides. Aromatik polyketides have a polyaromatic six-ring structure. Reduced polyketides have very intricate structures typically based on a macrolactone.

4.8.1 Polyketide Biosynthesis

Polyketides are biosynthesized by the same enzymes that would biosynthesize FAs, with the only difference that the keto-group of the adducts are not reduced. This can even happen intramolecularly, where three Acetyl-CoA can form an aromatic benzene derivative.



Polyketide biosynthesis reflects the fundamentally modular structure of FA-biosynthesis: Depending on which enzymes are turned off, products of great chemical variety can result. One can even predict the product from the particular combination of enzymes used.

In actual polyketide biosynthesis these partial FAS are fused to form long chains of enzymatic machinery that can connect heterogenous C2-units to the chain, resulting in the complex and varied structure of the polyketide. Through this variable assembly line like synthesis, complicated molecules such as the antiobiotic erythromycin, which binds to bacterial ribosomes and inhibits translocation of tRNA, can be synthesized *in toto*. Some peptides actually use so-called nonribosomal peptide synthetases (NRPS) in order to produce functionally varied polypeptides. Penicillin is produced in this way, for example.

Using these enzymatic machines facilitates regulation but also increases efficiency as the substrate cannot diffuse away. As we have just seen, it is also a major boon to evolution, where the number of possible combinations of these different enzymes is nearly endless.

5 Terpenes

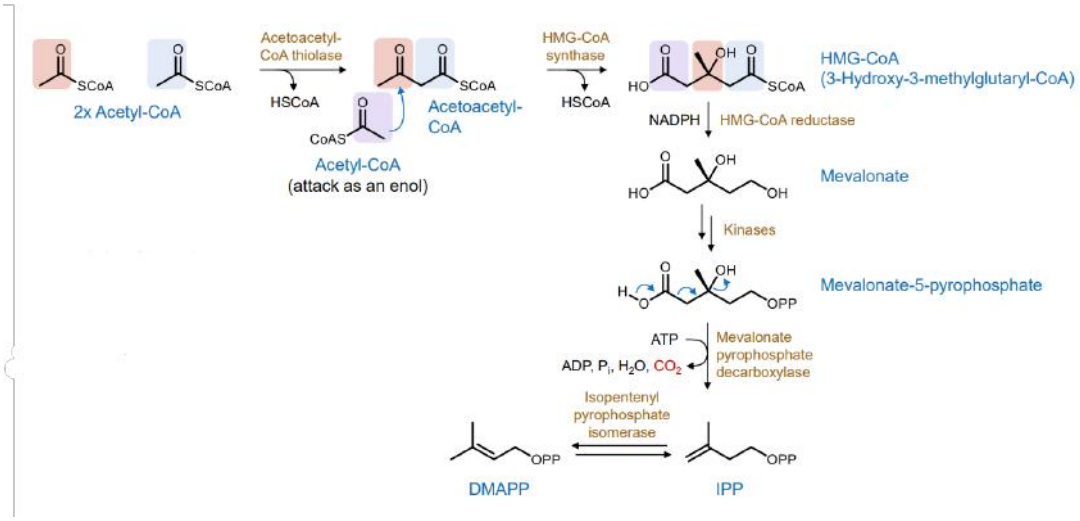
Terpenese are molecules that consist of repeated isoprene units. Note that there are exceptions, where methyls can sometimes be positioned unexpectedly and that isoprene is in fact not the biosynthetic precursor to terpenes. Terpens are named for the number of 2-Isoprene units: a sesqui-terpene therefore contains 3 isoprene units.

Some parts of the respiratory chain are terpenes, e.g. ubiquinone (coenzyme Q). Proteins often have long terpene chains attached, e.g. Farnesyl. Farnseyl is attached to proteins via **Farnseyl transferase** and then acts as the lipophilic membrane-anchor for this protein, Ras proteins use Farnesyl for example. Retinal is a terpene, so is β-Carotene the precurosor of vitamin A, which in turn is the precursor for retinal. Vitamin A is excessively stored in the livers of polar animals, having lead to hypervitaminosis in some early polar expeditions. Whereas bacteria have FA-lipids, archaea (and thus eukaryotes with their archaea-derived outer membrane) have terpene lipids. Rubber and latex are also both terpenes.

5.1 Biosynthesis

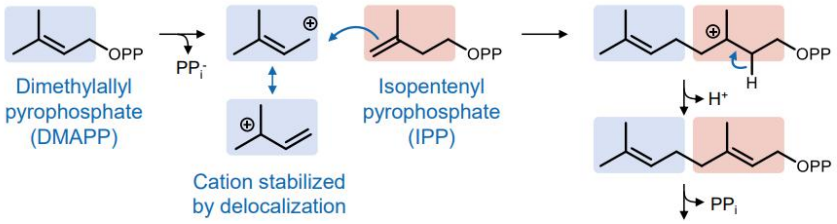
5.1.1 Precursor Biosynthesis

Three Acetyl-CoA units condense and get modified to form mevalanote via **HMG-CoA-synthase** (an important regulatory check-point) and **HMG-CoA reductase** (a target of many cholesterol-reducing drugs). Mevalonate then gets phosphorylated and forms isopentenyl pyrophosphate (IPP) via Mevalonate pyrophosphate carboxylase. IPP and Dimethylallyl pyrophosphate (DMAPP) are interchanged via **Isopentenyl pyrophosphate isomerase**.

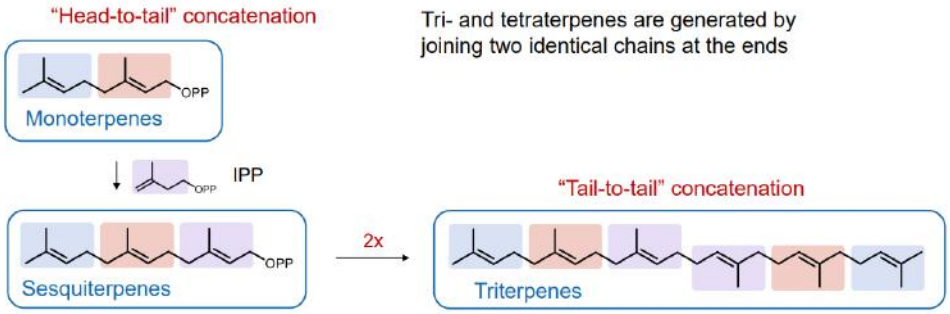


5.1.2 Chain-Elongation

DMAPP and IPP concatenate to begin the chain. IPP then successively binds to the chain to form long chains of isoprene units. These can then cyclize in many cases.



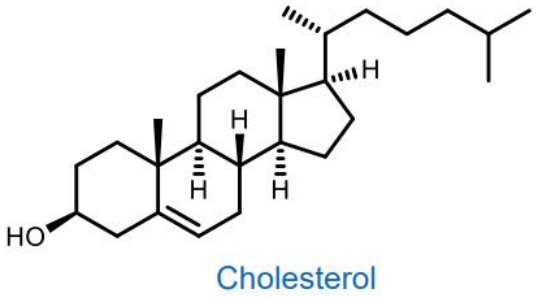
This type of concatenation is referred to as "head-to-tail", where a new IPP-unit is introduced at every iteration. Alternatively, "tail-to-tail" concatenation occurs when two existing chains connect. This will end the chain-elongation. The example shown is of squalene, a common terpen that is secreted on human skin.



5.1.3 Cyclization

The actual determinant of terpene diversity occurs at the cyclization step mediated by a wide class of cyclases. The exact structure of the cyclases determines which double bonds cyclize.

5.2 Cholesterol



Cholesterol

Cholesterol is part of the steroids class and is also a terpene-derived metabolite. Cholesterol sits in the lipid bilayer of membranes and disrupting the hydrophilic chains, thus controlling the permeability and fluidity of the membrane.

The precursor to cholesterol is the terpen squalene. Squalene first undergoes epoxidation by squalene epoxydase using 1 eq. of NADPH. This activated intermediate acts as substrate for **Oxidosqualene cyclase**, that lets a series of double-bonds cyclize, forming large parts of cholesterol ready-made notwithstanding some chemical modifications. It is one of the most complex reactions mediated by only one enzyme known today. The precise position of Phe, which π-interact with double-bonds, in the active center regulate selectivity for very particular double-bonds.

6 Protein and Amino Acid Metabolism

Though proteins do not represent a traditional energy reservoir, they can, under extreme conditions, be used to power the metabolism.

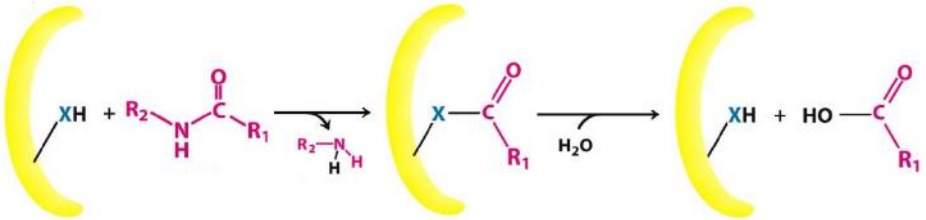
6.1 Extracellular Degradation and Metabolization

Proteins are degraded mostly in the stomach by peptidases and other proteolytic enzymes. The proteins are first denaturated in the acidic chyme. The same acidic environment also activates the secrete pepsinogen from the pancreas to pepsin (an aspartyl protease). Some peptidases are outer-membrane-located at the epithelium. AAs and small tri- or dipeptides are actively transported from the lumen into the epithelium and on to the blood.

The released AAs are predominantly taken up by the liver, where most protein biosynthesis occurs. Some AAs are broken down into their underlying C-skeletons, the N is disposed through urea. These C-skeletons can be used for gluconeogenesis, FA synthesis or even cellular respiration. This secondary metabolization mostly occurs in the liver as well but also in the muscles. The transamination required to remove the N occurs exclusively in the muscles, as the liver lacks the necessary enzymes.

6.2 Enzymatic Catalysis of Proteolysis

Peptide bonds are famously strong and thus do not break spontaneously. Catalysis in enzymes is based on first nucleophilically attacking the carbonyl, thus releasing the amino-group and typically forming some kind of (thio)ester, and subsequently releasing the attached carbonyl via hydrolysis.



The water molecule for the hydrolysis is typically directed either via hydrogen-bonds (as is the case for all **Aspartyl proteases**) or via metal cations (as with **Metalloproteases**).

6.3 Intracellular Degradation

Proteins need to be degraded intracellularly as well, e.g. due to age or because a certain regulatory protein needs to be reduced. As proteins in the cell are still folded, unlike in the chyme, this poses a particular challenge.

Most intracellular proteases have a channel-architecture, where an ATPase-ring at the entrance is actually responsible to unfold the protein before proteolysis inside the channel. This uses up energy.

6.3.1 Proteasome

Proteolysis by the proteasome is intracellularly triggered by ubiquitination. An enzyme cascade including three enzymes (E1, E2, E3) is responsible for attaching the ubiquitin to the protein. Typically a poly-ubiquitin chain is attached to a protein meant for degradation. K48-linked polyUb-chains are the mains signal that get bound by the proteasome via Rpn13 and Rpn10 subunits.

The proteasome itself consists of a regulatory and a catalytic subunit and is structured as a channel. It recognizes the poly-ubiquitin chain at the beginning of the channel and deubiquitinates the protein. The proteasome degrades the protein into oligopeptides.

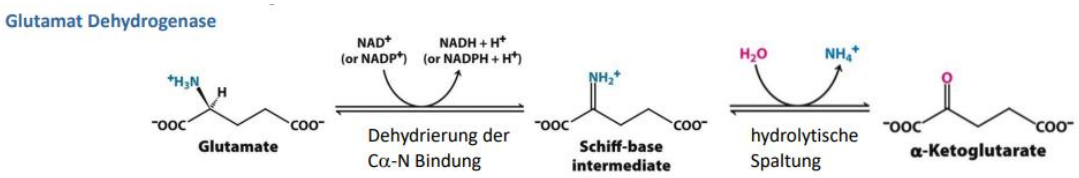
6.4 Amino Acid Degradation[5]

The first step in AA-degradation is to remove the amino group. Either through deamination, where the amino group is released as NH_3, or through transamination, where a ketoacid accepts the amino group, thus forming a new AA.

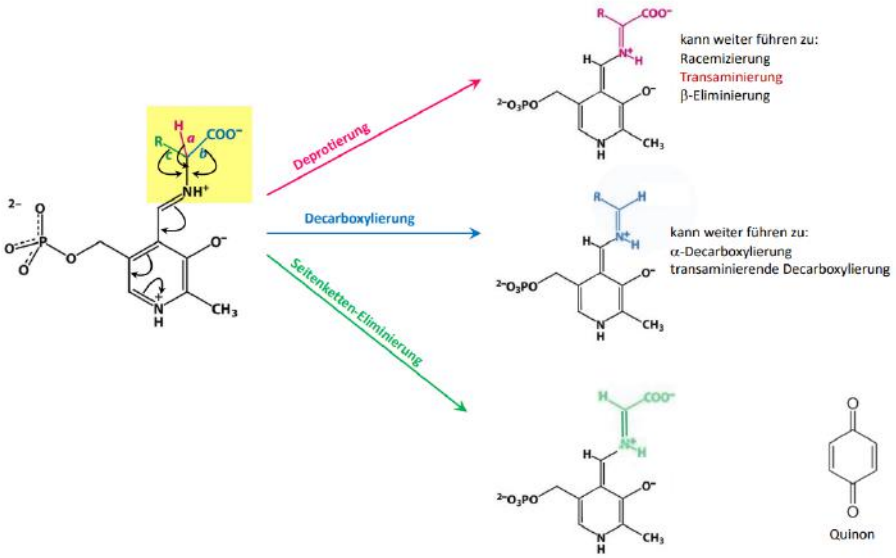
6.4.1 Step 1: Deamination

Oxidative deamination: Only works for glutamate, forming α-ketoglutarate. Through oxidation, using NAD^+ as reducing agent, a Schiff base is formed, which then gets hydrolytically cleaved.

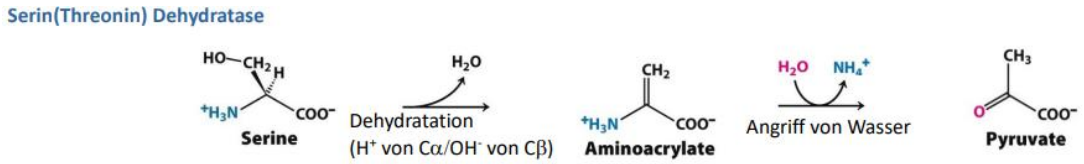
[5]Note that for AA-degradation as well as - biosynthesis, only an exemplary overview of AA metabolism is provided, excluding some more complicated individual AAs.



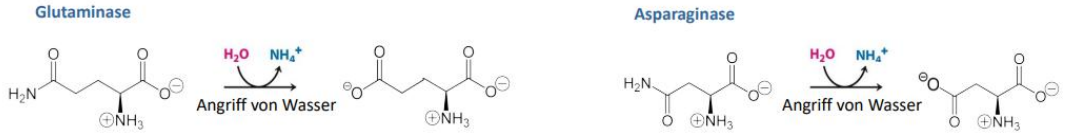
Eliminative deamination: First activate the AA by the attachment of PLP, an electron-sink, which forms a Schiff base through its aldehyde and the NH_3 of the AA. The PLP activates all three bonds for cleavage, which allows for three distinct possible products. The bond that is orthogonal to the aromatic plane of the PLP will get cleaved.



Deprotonation, β-elimination and subsequent hydrolysis will lead to deamination in certain AAs.



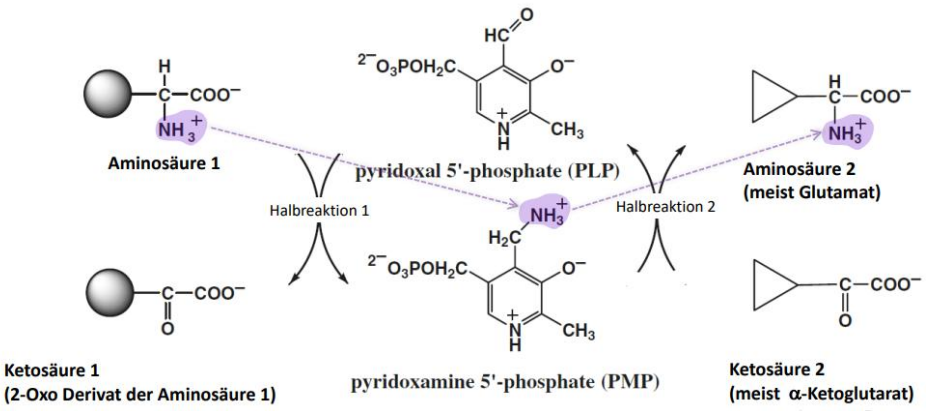
Hydrolytic deamination: It is responsible for deamination in the side-chains, for glutamin or asparagin for example. Here the amino group is simply released by the nucleophilic attack of water.



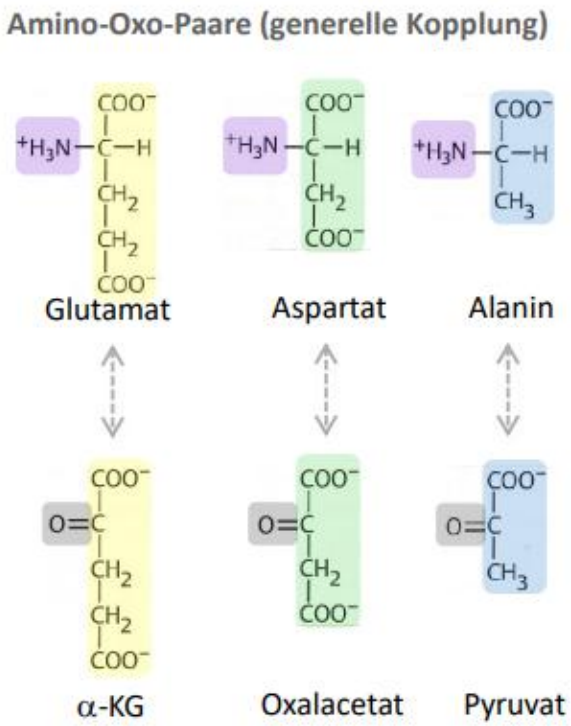
6.4.2 Step 1': Transamination

Amino groups can also be transferred to ketoacids, thus forming a new AA, which often is more metabolically efficient, given that most metabolic activity is focused on AA biosynthesis anyway. Note that only myocytes contain the necessary transaminases for transamination.

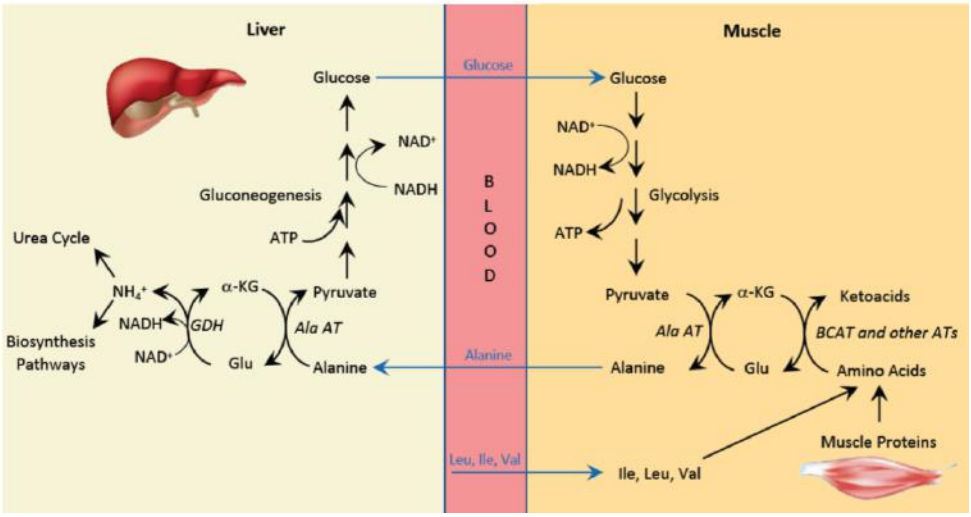
The amino group is first transferred onto PLP, thus forming a keto acid, which then transfers it onto the original keto acid, which then forms an amino acid.



The most common pair is α-ketoglutarate and glutamate, but others exist as well. Deamination of the glutamate can regenerate the α-ketoglutarate, which is also of course part of the TCA cycle.

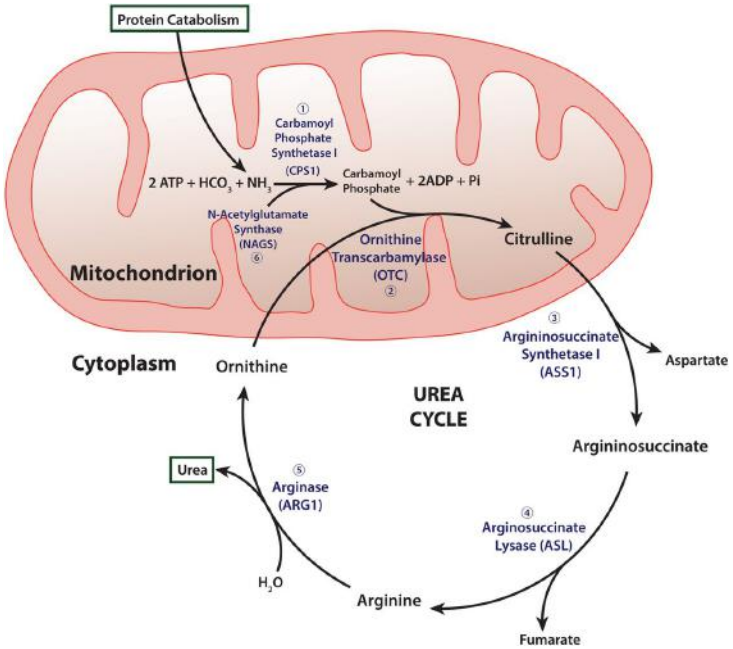


Note that these transamination reactions play a crucial role in the Cahill cycle. Where glucose is degraded into Alanine in the muscles, using pyruvate as substrate for transaminases. The alanine then moves into the liver, where it is incorporated, again via pyruvate, into glucose. The Cahill cycle is a vital part of the metabolite exchange between liver and muscles.



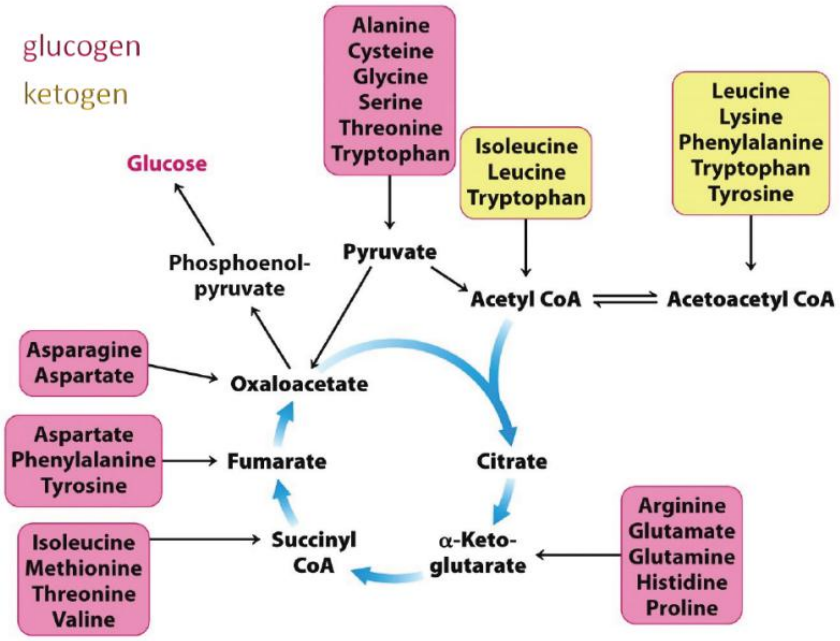
6.4.3 Step 2: Inserting Nitrogen in Urea

Because NH_4 can react with α-ketoglutarate, it hinders regular metabolism and is thus a potent cytotoxin. This is the reason why NH_3 are incorporated into urea, a carbonic acid diamide. The N is incorporated into urea in a metabolic cycle between the mitochondrial matrix and the cytoplasm and that includes ornithin, citrullin and arginin(osuccinate) as intermediates.

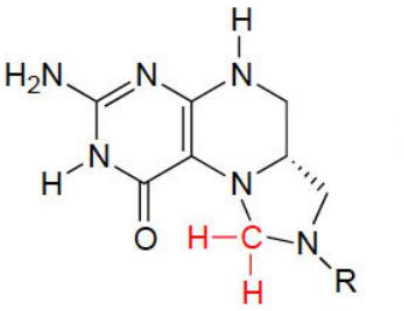


6.4.4 Step 3: Recycling the Carbon-Skeleton

In general, AAs are either of gluco- or ketogenic type, depending on which pathway their C-skeletons end up in. Ketogenic AAs produce Acetyl CoA or Acetocacetyl CoA and are thus ideal for ketone body biosynthesis; glucogenic AAs (the majority) produce pyruvate, α-ketoglutarate, fumarate or succinyl CoA of the intermediates of the TCA cycle



Either pyruvate dehydrogenase or in the case of AAs with branched residues an enzyme called **Branched-Chain Ketoacid Dehydrogenase** (BKCD) are responsible for breaking down the C-skeleton into acetyl CoA. Both use TPP and a tetrahydrofuran as part of their catalytic cycle, see also section 2.5.1. BKCD is mechanistically analogous to the pyruvate dehydrogenase, it also consists of three subenzymes E1, E2 and E3, and will form some type of acyl CoA, depending on the exact residue present.



Glycine: In the case of glycine, the one remaining C (remember, the other left as CO_2), is actually transfered to tetrahydrofolate (THF) through an additional enzyme at the oxidation. THF is essentially a 1C-storage system that can carry the C in different oxidation states (typically CH_2). The C can then get transferred onto serine, from whence (with 2 C after decarboxylation), acetyl CoA can actually be formed.

C3-AAs: All C3 AAs end up as **pyruvate**. Ser via the PLP-mechanism, i.e. eliminative deamination, section 6.4.1. Ala via its transamination, section 6.4.2, where Ala goes to pyruvates and α-ketoglutarate to glutatamate.

C4-AAs: All C4-AAs will form **oxaloacetate**. Asn will form Asp through hydrolytic deamination, see section 6.4.1. Asp will form oxaloacetate through its transamination, where α-ketoglutarate goes to glutatamate.

C5-AAs: All C5-AAs form **α-ketoglutarate**. Gln will form Glu through hydrolytic deamination, see section 6.4.1. Glu will then form α-ketoglutarate through oxidative deamination, see section 6.4.1.

Branched-chain AAs: Leucin is catabolized to acetyl CoA and acetoacetate and is thus a purely ketogenic AA.

Aromatic AAs: Phenylalanin can easily react to tyrosine. Tyrosine will form fumarate and acetoacetate.

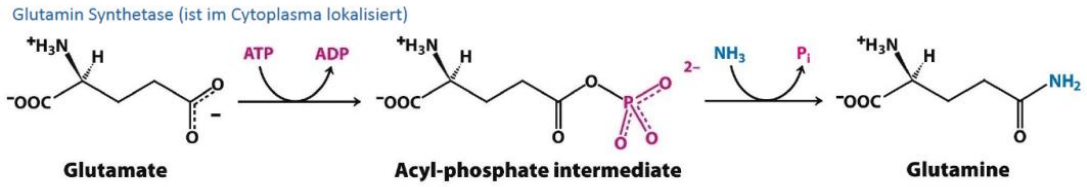
6.5 Amino Acid Biosynthesis

Humans cannot anabolize all AAs, notably those with more complicated structures must be taken up through our diet, the so-called essential AAs. Some AAs can be anabolized from essential AAs and are sometimes referred to as semi-essential AAs.

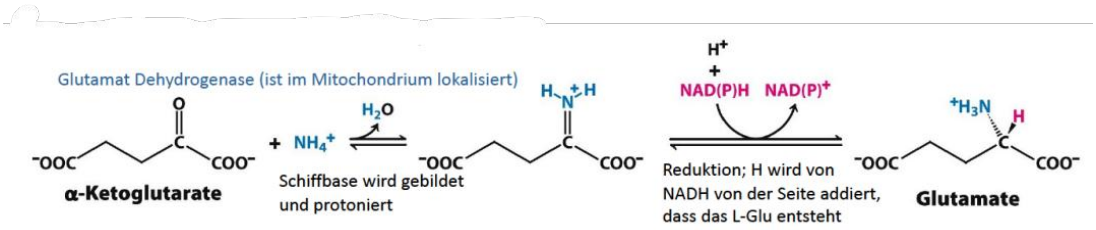
6.5.1 Nitrogen Insertion

Ala and Gln are the two AAs that are mainly responsible for N-transport in the body. For this reason, they make up about 50% of all free AAs in the blood plasma.

Glu to Gln: Using **Glutamine synthetase** in the cytoplasm, glutamate can be primed by ATP and will then accept a NH_3-unit to form glutamine.



α-Ketoglutarate to Glu Using **Glutamate dehydrogenase** in the mitochondrial matrix, α-ketoglutarate can form a Schiff-base with NH_4 and H_2O, which can then be reduced to glutamate using NADPH.



The subsequent N-insertion into other AAs often occurs through transaminations, see section 6.4.2, reacting glutamate to α-ketoglutarate.

6.5.2 C-Skeleton Biosynthesis

The reverse logic to the AA-degradation applies for AA-biosynthesis: The products of the catabolism, pyruvate, α-ketoglutarate, oxaloacetate, that are present as precursors and intermediates of the TCA anyway can be used for AA-biosynthesis.

C3-AAs: Pyruvate can get transaminated into Ala.

C4-AAs: Oxaloacetate gets transaminated to Asp. Asp can react with one ATP to form an acyl-adenylate intermediate that will actually accept a NH_3-unit to form Asn.

C5-AAs: α-Ketoglutarate can react to glutamate, see section 6.5.1. Glutamate can then react to glutamine, also see section 6.5.1.

Aromatic AAs: Phenylalanine needs to be taken up through our diet, but tyrosine can then be subsequently derived from it. Tyr is also a major precursor for all catecholamines, e.g. epinephrine.

Phenylketonuria, the most common inheritable metabolic disease, results from an inability to interconvert Phe and Tyr and can lead to major anatomical and cognitive symptoms. Babies are nowadays screened immediately after birth as a rigorous diet can let the diesease remain asymptomatic for life.

6.6 Transport

AAs have a highly complex transport system, with typically highly specialized transporters and related interconversion reactions on both sides of the membrane. This can also include intermediates, as is the case with the malate shuttle, wherein α-ketoglutarate and glutamate as well as aspartate and oxaloacetate are involved. Remember that glutamate and aspartate are "related" via their shared transamination reaction, see section 2.5.9.

7 Enzymatic Kinetics

7.1 Reaction Laws and Rate Constants

7.1.1 1. Order

$$[A] = [A]_0 \cdot e^{-k \cdot t} \qquad t_{1/2} = \frac{\ln 2}{k} \qquad v_0 = k \cdot [A]_0$$

In the case of consecutive first order reactions, there is a characteristic lag phase if k_i and k_{i+1} are within two orders of magnitude. Only if the second reaction is rate-determining will large concentrations of the intermediate be present (bottleneck), else it will react away too quickly.

7.1.2 2. Order

$$v_0 = k \cdot [A]_0 \cdot [B]_0 \qquad \text{falls } [A] = [B]: \quad [A] = \frac{1}{kt + \frac{1}{[A]_0}} \qquad t_{1/2} = \frac{1}{k \cdot [A]_0}$$

$$A + A \rightarrow A_2 \qquad t_{1/2} = \frac{1}{2 \cdot k \cdot [A]_0}$$

7.1.3 Pseudo-first Order

$$\text{falls } \; [A] >> [B] \qquad [B] = [B]_0 \cdot e^{-k_{pseudo} \cdot t} \qquad t_{1/2} = \frac{\ln 2}{k_{pseudo}}$$

Enyzmatic **rate constants** span a range between 10^3 to $10^7 \mathrm{M}^{-1}\,\mathrm{s}^{-1}$. The theoretical limit is determined by the **maximum diffusion rate** in cells at $10^{10}\mathrm{M}^{-1}\,\mathrm{s}^{-1}$. Rate constants at these orders of magnitude are determined via stopped-flow mixing techniques, where the rapid addition through a syringe nearly instantaneously starts the measurement and stops the mixing.

If an enzyme is fully saturated with substrate (i. e. $[S] >> [E]$), the rate constant will remain constant (unlike a pseudo-first order k_{pseudo} which would increase with larger $[S]$). The alcohol dehydrogenase is such an example.

7.2 Reversible Reactions: Two-State Equilibrium

If an equilibrium is perturbed, a tendency of the system to move back into equilibrium at rate constant k_{obs} is observed, where both forward and backward reactions contribute.

$$\frac{[A]}{[B]} = \frac{k_1}{k_{-1}} = K \qquad k_{obs} = k_1 + k_{-1} \qquad t_{1/2} = \frac{\ln 2}{k_{obs}}$$

Gibbs-Equation: Rule of thumb: a change of $5.7\ \mathrm{kJ\,mol}^{-1}$ changes the $\Delta G^{0'}$ by factor 10. Another useful application is in the determination of ΔG of protein folding, where the equilibrium is perturbed with a denaturant, the fraction of folded to unfolded measured, from which ΔG can be determined and then extrapolated.

$$\Delta G^{0'} = -R \cdot T \cdot \ln \frac{[B]}{[A]} = -R \cdot T \cdot \ln \frac{k_1}{k_{-1}}$$

Arrhenius law: Reactions with a high activation energy will be highly temperature-dependent, those with low activation energies tend to be more temperature-independent.

$$k = A \cdot \exp\left(-\frac{E_A}{RT}\right)$$

Uncatalyzed, biological reactions tend to be very slow as they usually have very high activation energies. Nonetheless, performing these reactions at high temperatures allows the determination of their rate constants through extrapolation. The efficiency of an enzyme is determined by the **acceleration factor** $a = \frac{k_{cat}}{k_{uncat}}$. Enzymes accelerate any reaction equally in both directions and thus will not change the position of the equilibrium.

7.3 Non-Covalent Bond-Equilibria

The smaller the **dissociation constan**t K_{Diss} of a complex, the higher its affinity.

$$P + L \rightarrow PL \qquad K_{Diss} = \frac{k_{off}}{k_{on}}$$

In case of low affinity, i.e. excess of L $[L] >> [P]$, the **occupation** y of the protein becomes:

$$\frac{K_{Diss}}{[L]_0} = \frac{[P]}{[PL]} \qquad y = \frac{[L]_{tot}}{K_{Diss} + [L]_{tot}} = \frac{[PL]}{[P] + [PL]}$$

Note that the differences in K_{Diss} are nearly exclusively due to large variations in k_{off}. A typical K_{Diss} values is between 10^{-3} and 10^{-6}M[6]. Because the rate of formation of a complex is mostly regulated by diffusion and the probability of proper interaction upon contact (hence any reaction will be slower than expected by pure diffusion), which remain constant across enzymes. Diffusion across *E.coli* takes about 10 ms and across a HeLa cell roughly 10 s. k_{off} however is due to the specific interaction of the substrate with the binding site of the enzyme. Some electrostatically driven complexes will very quickly form an encounter complex PL*, which then slowly forms PL. Only the second, concentration-independent reaction is rate-determining and no concentration-dependency is experimentally observed at sufficiently large $[S]$ and $[P]$. k_{off} values can be measured using a competition experiment, where two similar (A and A') but distinguishable substrates are monitored. The exchange rate of A for A' will follow first order reaction kinetics. The highest k_{off} values are measured at high denaturant concentrations from which a zero-denaturant rate constant is extrapolated.

Henderson-Hasselbalch:

$$\frac{[AH]}{[A^-]} = \frac{[H^+]}{K_S} = 10^{pK_S - pH}$$

7.3.1 Cooperative Binding

In complex with multiple binding sites, binding at one might be entirely independent of the binding state of the others. More often, positive cooperativity leads to higher affinity in the unbound sites if the other sites are already bound. The binding at one site must thus somehow change the quarternary structure of the enzyme. Maximal positive cooperativity will lead to a protein that is either entirely bound or entirely unbound. For a maximally cooperative complex with n binding sites:

$$y = \frac{[L]_{tot}^n}{K_{50}^n + [L]_{tot}^n}$$

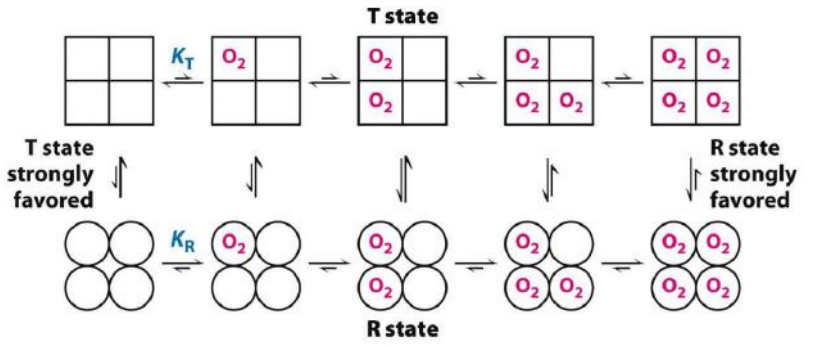
The y-function will also now have sigmoid shape, unlike for the case of $n = 1$ which is hyperbolic. Hemoglobin is an example of a cooperatively binding protein with $n = 4$ heme (oxygen-binding) sites, which are made up of two α and two β subunits. Its Hill-coefficient (the number of hypothetical binding sites if the complex were maximally cooperative) however is only 2.8. Hemoglobin is thus not maximally cooperative. Upon binding with oxygen, he oxyhemoglobin will rotate the heterodimer $\alpha_1\beta_1$ by 15° relative to $\alpha_2\beta_2$. This conformational change in the quarternary structure will lead to higher affinity towards oxygen (from low affinity T-conformer to the high-affinity R-conformer). The actual O_2 binding site is situated at the Fe inside a (proto)porphyrin ring, which in deoxyhemoglobin is actually slightly below the ring and by binding will move into the ring, which pulls a side-chain attached to it inducing a quaternary structural change in the entire hemoglobin protein.

The structurally similar, myoglobin is essentially equivalent to one subunit of heme though it has a higher affinity to O_2 and is thus not cooperative at all (it has a higher affinity however). Its hyperbolic curve (vs. the sigmoidal curve of the cooperative hemoglobin) is the very reason why O_2 can be efficiently exchanged between the blood and the muscles. The steepest part of the y-curve of hemoglobin is (not incidentally) exactly between the highest and lowest physiological p_{O_2} (20-40 torr).

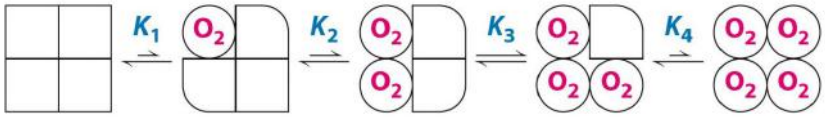
There are two competing models to describe the kinetics of hemoglobin:

Concerted model: All four subunits are always either T or R. If all are in T_4 complete dissociation is favored; if all are in R_4 complete association is favored.

[6] An exception would be avidin-biotin complexes with 10^{-15} MK_{Diss}



Sequential Model: Only the subunits in direct contact change conformation. A sate of roughly 3 O_2 is favored even if in this state the fourth unbound must also be in R.



Neither model fully explains the observations: A 3 x O_2 bound hemoglobin only ever exits in full R_4 (–¿ concerted). But 1 x O_2 exists in T-state but with thrice the affinity for O_2 (–¿ sequential). Inside erythrocytes, hemoglobin actually has an even lower affinity due to the presence of 2,3-Bisphosphoglycerate (2,3-BPG). 2,3-BPG binds to T-state of hemoglobin and lowers O_2 affinity.

Affinity is of course also influenced by the Bohr effect, which stipulates that at low pH (and/or high CO_2) O_2-affinity will be lower. CO_2 can thus either influence the equilibrium by it dissolving as carbonic acid or via direct attachment to N-terminal amino groups of the hemoglobin to form carbamates. Those carbamates will stabilize the low-affinity T-state via Coulomb interactions. Sickle cell hemoglobin are due to a single mutation that leads hemoglobin to oligomerize, hence loosing their functionality.

7.4 Michaelis-Menten Kinetics

As enzymes leave and enter the reaction unchanged they can undergo a great number of reaction cycles while retaining an approximately constant concentration $[\dot{E}]_{tot} = 0$. This requires excess of substrate ($[S] >> [E]$).

$$v_0 = [ES] \cdot k_{cat} \qquad v_{max} = [E]_{tot} \cdot k_{cat} \quad [\mathrm{M\,s^{-1}}]$$

In the short time-window in which the initial rate v_0 is determined, the substrate concentration is assumed to be constant $[\dot{S}]_{tot} = 0$. A so-called steady-state assumption. In biological systems, the substrate will be in a dynamic equilibrium anyway, making this assumption applicable for arbitrary time t.

$$v_0 = v_{max} \cdot \frac{[S]_0}{[S]_0 + K_M} = [E]_{tot} \cdot k_{cat} \cdot \frac{[S]_0}{[S]_0 + K_M} \quad [\mathrm{M\,s^{-1}}]$$

The **Michealis constant** K_M defines the substrate concentration at which the reaction will operate at half-maximal rate:

$$K_M = \frac{k_{-1} + k_{cat}}{k_1} \quad [\mathrm{M}] \qquad k_{cat} = \frac{v_{max}}{[E]_{tot}} \quad [\mathrm{s}^{-1}]$$

Interestingly, most substrates are typically found to operate at concentrations $c \approx K_M$. Most enzymes will thus function at roughly half-maximal rate: $v = \frac{1}{2} v_{max}$. An alternative to steady-state measurements are pre-steady-state experiments, where those early catalytic steps are analyzed that need to occurr before the steady-state (i.e. a constant v_0) is reached. Enzymes are placed at equal concentrations to the substrate ($[S] \approx [E]$). This is where stopped-flow set-ups are commonly used.

The pre-steady-state analysis of chymotrypsin for example revealed that the enzyme first attaches to the carbonyl of the peptide bond (forming an acyl), which makes it more reactive towards hydrolysis, after which the enzyme will dissociate again, releasing the cleaved peptide. This second dissociation step via hydrolysis is the rate-determining step.

The efficiency of an enzyme is high

- if small $[S]$ will lead to high activity, i.e. K_M is small;
- if $k_{cat} = \frac{v_{max}}{[E]_{tot}}$ is high.

The **specificity constant** $\kappa = \frac{k_{cat}}{K_M}$ $[\mathrm{M}^{-1}\,\mathrm{s}^{-1}]$ is often used to combine these two metrics.

7.5 Cofactors

Co-factors are a big part of the functional variety of enzymes and are defined as an strongly bound exogenous part of the enzyme that is essential for its catalytic activity. If it is bound covalently it is referred to as a prosthetic group. There are organic and inorganic cofactors. apoenzyme + cofactor = holoenzyme.

There are six classes of enzymes:

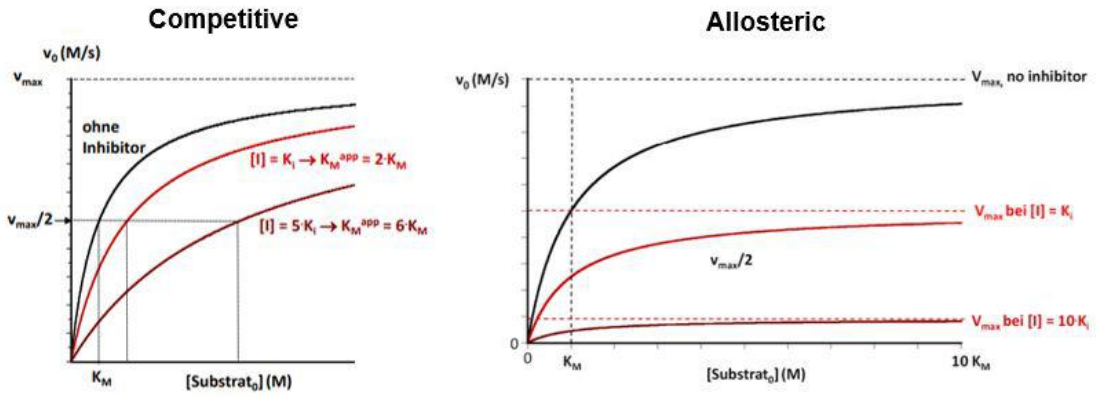
1. Oxidoreductase
2. Transferase
3. Hydrolase
4. Lyase
5. Isomerase
6. Ligase

7.6 Enzyme Inhibition

Irreversible inhibitors will covalently modify the active site of the enzyme; reversible inhibitors will only transiently bind to any part of the enzyme. Metabolic regulation is nearly always through reversible inhibition often through feedback-inhibition, where the product will inhibit its own enzyme. The **inhibition constant** K_i is defined:

$$K_i = \frac{[E] \cdot [I]}{[EI]} \quad [M]$$

- Competetive Inhibition: Inhibitor competes with substrate for binding site, typically inhibitor will be structurally very homologous to substrate. It will seem as if K_M has increased, as a higher $[S]$ is required for half-maximal rate.
- Uncompetitive Inhibition: I will only bind ES and then prevent dissociation.
- Allosteric Inhibition: Inhibitor binds to some part of enzyme, changing its quarternary structure so the enzyme can no longer efficiently operate. The K_M values remain unchanged but the v_{max} values will decrease.



7.6.1 Allosteric Enzymes

Alosteric enzymes are oligomeric enzymes with multiple, cooperative active sites. Their rate-constants display a sigmoidal shape. An example is the asparate transcarbamolyase (ATCase), which is feedback-inhibited by its product CTP. ATCase is a hetero-oligomeric enzyme with both active and regulatory subunits. It can exist in the less-active T and more active R state, all six active sites are either all R or all T. This extreme cooperativity leads to a very steep dependence on substrate concentrations of the reaction rate. The equilibrium between R/T can be shifted by the inhibitor PALA.

7.6.2 Covalent and Coulombic Interactions

Coulomb interactions within proteins will both stabilize the protein and the bond itself. Acidic residues in a stable bond will actually become more acidic and basic residues will become more basic, because reverting to their uncharged states has become energetically more unfavored. This can result in dramatic shifts in p_{K_A}-values of 6 units. The impact of pH on the stability of proteins are quantized by measuring the change in their free energy $\Delta\Delta G$.

Disulfide bond formation cannot occur spontaneously as it requires an oxidation agent. Forming disulfide bonds is actually very useful during protein folding, as forming a disulfide will entropically destabilize the the unfolded state. This "artificially" reduces ΔG, leading to more folded proteins in equilibrium.

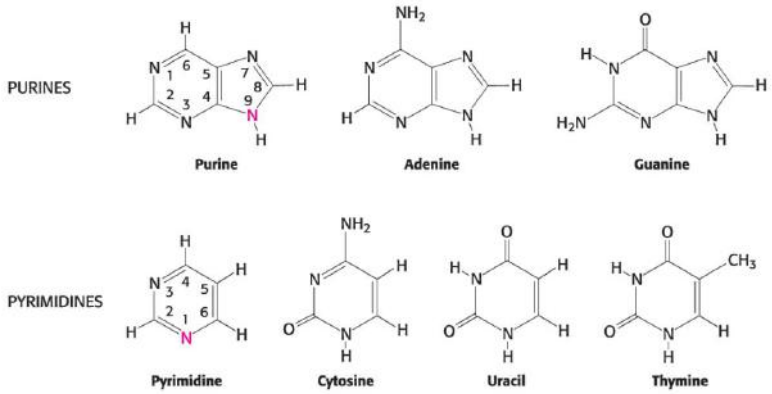
SS-bond formation is always rate-limiting and occurs through a generally pH-dependent S_N2-type reaction. It is typical for secretory proteins that need to be structurally particularly rigid. The SS-bond formation occurs in the oxidizing cellular compartments ER (or periplasm for bacteria). A typica oxidizing partner is glutathione (which itself then dimerizes through a SS-bond with another glutathione during the RedOx). Too many SS-bonds can however slow folding down again, because the number of erroneous combinations between two thiols grows exponentially. Dithiol isomerases help to quickly cover

all possible combinations. Genetic defects can sometimes lead to those secretory proteins trying to fold in the cytoplasm, which leads to aggregation (inclusion bodies) inside the cell.

8 Transcription and Translation

DNA and RNA are polymers of nucleotides. A Nucleotide consists of a sugar (ribose for RNA; deoxyribose for DNA), a phosphate and a base (either purine or pyrimidine). (Deoxy)Ribose is a hetero-five-ring with either two or three hydroxyl groups bound to the ring and one hydroxyl group a the ring-external 5' end. A nucleoside consists of just a base + a sugar. There are two purine bases: adenine (A) and guanine (G); and three pyrimidine bases: thymine (T), cytosine (C) and uracil (U). Note that uridine in RNA and thymidine in DNA fulfil identical roles but only occur in one or the other. The base is always connected at a N to the C-1' of the ribose in a glycosidic bond. The ribose forms the backbone by forming phosphodiester bonds between nucleotides once at the 3' and once at the 5' hydroxygroup. Free nucleotides have a triphosphate group bound to them, two phosphates get cleaved during formation of the phosphodiester bond and thus bound nucleotides only have a monophosphate group. Both forms are typically referred to as nucleotides, but deactivated nucleotides have the abbreviations (deoxy)_*nucleotide*_triphosphate (d)NTP: (d)ATP, (d)GTP, (d)CTP, UTP and TTP.

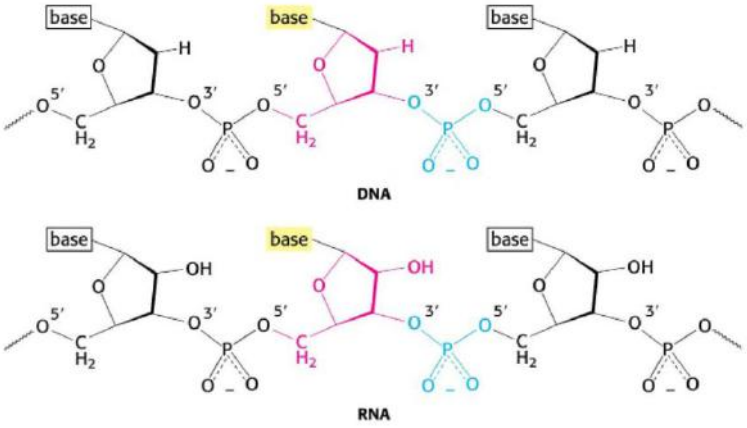
	Nucleoside	Nucleotide
RNA	Adenosine	Adenylate
	Guanosine	Guanylate
	Cytidine	Citydylate
	Uridine	Uridylate
DNA	Deoxyadenosine	Deoxyadenylate
	Deoxyguanosine	Deoxyguanylate
	Deoxycytidine	Deoxycytidylate
	Thymidine	Thymidylate



Base sequences are always written from the 5' to 3' end in accordance with the direction of synthesis. For example: "ACG" indicates that the 5'-OH is on A and the 3'-OH is on G. Nucleotides are always added at the 3'-OH end, i.e. at G. A purine and a pyrimidine each form a complementary base pair (bp) of nucleotides that bind through H-bonds: G-C and A-T/U. G-C forms three H-bonds, while A-T/U only two; though both are considered reasonably stable interactions. While RNA typically occurs in single-stranded form, DNA is always found as a double-strand (ds), i.e. two polymers of nucleotides bound

together at the bases in so-called Watson-Crick (WC) base pairs. Note that each individual strand of dsDNA contains all necessary information to recreate the other strand just by replicating the WC base pairs. RNA will sometimes form so-called hairpins, where a single-strand binds to itself or distant base-pairings occur mediated by ions (e.g. Mg^{2+}).

The sugar-backbone of DNA + the H-bonds between the bases leads to a double-helical structure of DNA. The double-helix is further stabilized by stacking of the bases inside of the double-strand. Stacking is favoured through the hydrophobicity of the AAs in an aqueous cell as well as simple van der Waals forces. Each base is stacked with a distance of 3.4 Å. The double-helix itself repeats every 34 Å, i.e. every 10.4 bases, and is roughly 20 Åwide. Due to a slight angle in the base pairs, dsDNA displays a more exposed major and a less exposed minor groove.

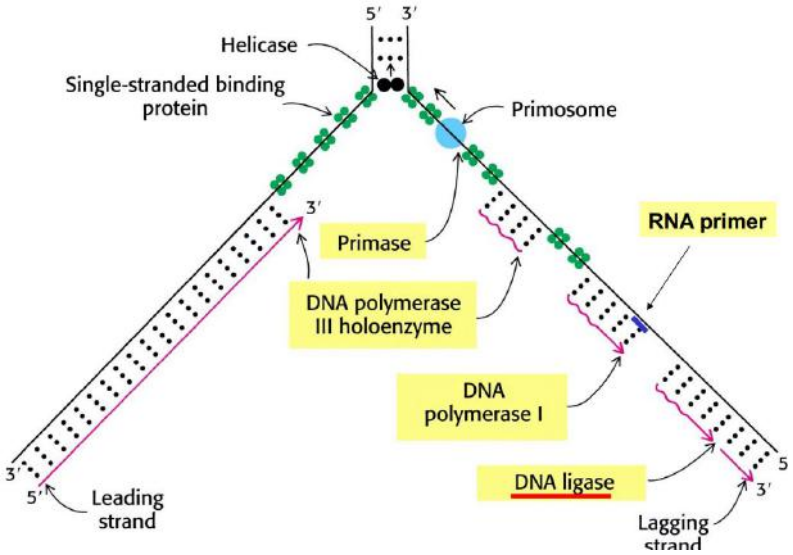


There are two main forms of double-stranded DNA: A and B form. Both are right-handed double helices. Natural DNA is in the B-form, where the bases are (nearly) perpendicular to the backbone. dsRNA and RNA-DNA hybrids often adopt the A form, where the bases are tilted relative to the backbone. The A form is wider but shorter than the B form. DNA under dehydrated conditions will also be in the B form. The angle of the bases relative to the backbone is a result of different pucker conformations of the ribose sugar. A rare, left-handed Z form with poorly defined structure can also be found.

There are major differences between eukaryotes and prokaryotes in all parts of the DNA-to-protein cycle. Already, the human genome consists of 6 billion bp on 23 linear chromosomes with 30'000 origins of replication. The E.coli genome is 4.6 million bp long but with only 1 circular chromosome and 1 origin of replication ("OriC"). Replication at this 1 origin occurs in both directions of the circle , which doubles the speed of replication.

8.1 Replication: DNA → DNA

DNA is replicated semi-conservatively: the original dsDNA gets separated and each single strand is used as a template to replicate the dsDNA. The two resulting dsDNA thus are each 50% original and 50% copy. In the lab, separation of the DNA and rebinding of DNA are referred to as "melting" and "annealing". The enzymes necessary for DNA-replication are clumped together in a superenzyme called "replisome". Note that topoisomerases are not part of the **replisome**. Replication reads the DNA from 3' to 5', but extends the nascent strand from 5' to 3' as the DNA polymerase always requires a free 3'-OH group, see 8.1.6.

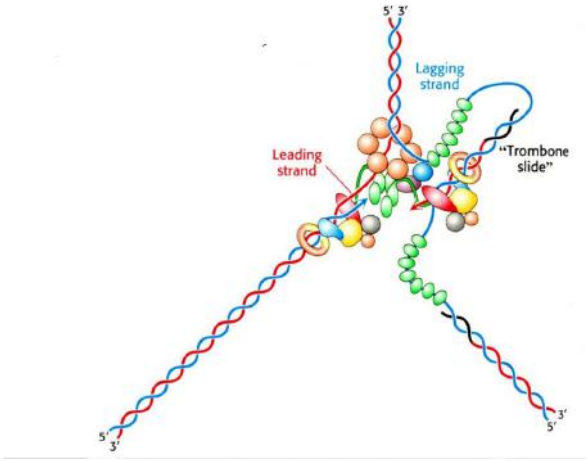


8.1.1 Initiation of Replication

In E.coli, the OriCis a locus in the genome with unusual features that induce replication. OriC displays five DnaA-binding sites, a protein that necessary for initiation. The five bound and an additional DnaA will form a hexamer that then brings in DnaB, the helicase, that gets wrapped around the sDNA with the help of DnaC (similar to clamp loading). Eukaryotes use a similar system though the proteins are named differently, e.g. Orc complexes.

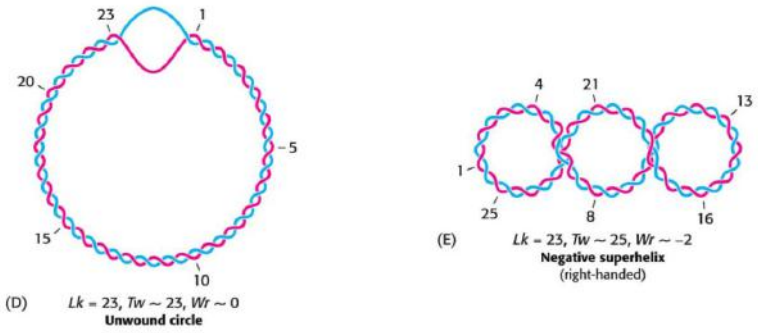
8.1.2 Lagging vs Leading Strand: The Trombone Model

Because synthesis of DNA can only occur 5'→3', the template strand that allows for synthesis in this direction gets replicated continuously and is referred to as the leading strand. The lagging strand however, will fold out so as to allow for 5'→3' synthesis for a certain time, after which the fold becomes too long, contracts and then elongates again. This is reminiscent of a trombone slide.



8.1.3 Topoisomerases: Supercoiling

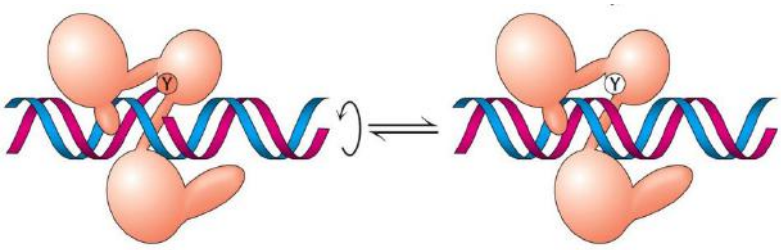
Most DNA is found in a so-called "supercoiled" state, where torsional stress on the DNA leads the DNA to fold itself into "knots". This occurrs both in circular prokaryotic DNA, as well as in linear eukaryotic DNA, as the latter forms oops via histone-interactions. Supercoiling is induced by strand-separation: Let us consider a 25-turn long circular B DNA. If we separate this DNA for the length of 2 turns, the circular has two options: either it can retain 23-turns of B DNA and 2 turns of energetically disfavored, unwound DNA or it supercoils with still 23-turns of B DNA but no unwound DNA. For topological reasons, the DNA will always undergo negative (i.e right-handed) supercoiling during unwinding. As we will shortly, reverting this negative supercoiling is an integral part of replicating and transcribring DNA. Some DNA however is also positively supercoiled though it is nearly impossible for the cell to undo this, thus positively supercoiled DNA is not typically transcribed or replicated.



Topologically, supercoiling is described by the linking number (Lk). Positive supercoiling increases the Lk, negative supercoiling reduces it. Molecules of the same composition that differ in Lk are dubbed "topoisomers". Topoisomers can only be interconverted by cutting and rejoining one or both strands of DNA, this is done by enzymes called **topoisomerases**. Many topoisomerases are great targets for antibiotics because they differ between prokaryotes and eukaryotes. Even the same topoisomer can differ in the numbers of twists (Tw), essentially the number of turns in a B DNA, and the number of writhes (Wr), essentially the number of coils in the DNA, whereas

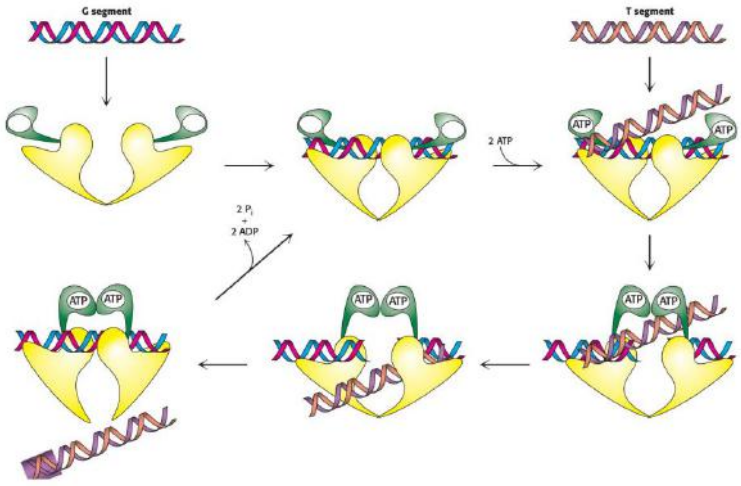
$$Lk = Tw + Wr$$

Type I Topoisomerase cleaves only one strand of DNA to undo supercoiling. As there is energy stored in the coiling, uncoiling occurs passively. It does this by using the hydroxyl group of a Tyr in its active center to cleave the phosphodiester bond in the backbone of one DNA strand. The freed DNA-strand will then rotate until some of the strain from the supercoiling is relieved. This happens in a controlled manner, decelerated by the enzyme. The backbone is then put together again.



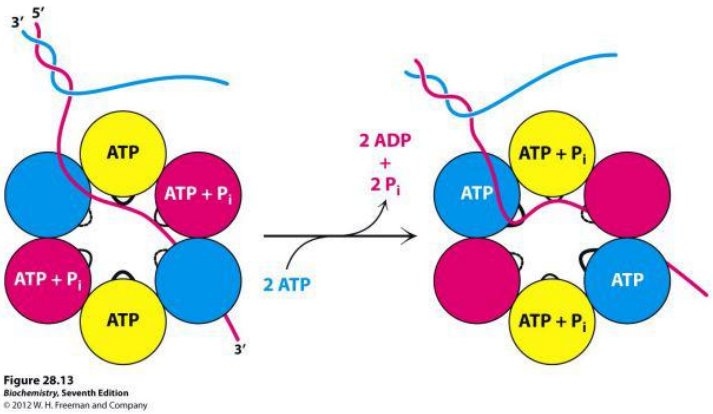
Type II Topoisomerase (DNA Gyrase) cleaves both strands of DNA to introduce more negative supercoiling, which requires two ATP per iteration. Topoisomerase II consists of two monomers (a gate

and an ATP-binding region), each with a Tyr to bind DNA. The enzyme uses two DNA double helices, the first helix binds spontaneously to the gate of the enzyme, i.e. the gate or G-segment. The second double helix, the transported or T-segment, then binds to the ATP-activated second monomer of the enzyme. The T-segment is now above the G-segment. The G-segment then gets cut, the T-segment lead below it, forming the knot. The G-segment is now religated, which releases the T-DNA. The 2 ATP eq. are then released and the cycle can restart.



8.1.4 Helicases: Strand-Separation

The first part of the actual replisome is an enzyme called **helicase**, responsible for separating the dsDNA. Helicase is a ring-shaped hexamer that requires ATP for strand-separation: Each monomer has a loop for DNA-binding directed towards the center of the ring and each can hold 1 ATP. There are three states a monomer can be in and the two monomers opposite each other are always in the same state: ATP-bound, ADP-bound or empty.



The 5'→3'-strand (template to the lagging strand) will bind to one ATP and one ADP monomer. The 2 ATP-monomers then hydrolyze to become ADP-monomers and the two empty-monomers become ATP-monomers. This process is tightly coordinated by conformational changes in the monomers. The strand is thus moved down by one nucleotide through the motion of the DNA-binding loops due to the conformational changes in the monomers.

8.1.5 Primase: Primer Addition

Primases add short complementary RNA sequences called "primers" to the single-strand, they are thus RNA polymerases. The addition of new dNTP by DNA polymerases can only begin with already existing free 3'-OH groups. RNA-polymerases however can add NTP at will though they are more inaccurate. Fortunately, the resulting RNA-DNA hybrid is weakly bound and is later removed by a more accurate DNA polymerase to form a completely copied dsDNA. In eukaryotes, primase-activity comes from DNA polymerase α, which still adds a RNA primer however.

8.1.6 DNA Polymerases: DNA Replication

DNA polymerases do the actual copying of the template by adding new dNTP on the replicated strand. DNA polymerases are relativley slow but accurate. Replication in general is much slower than transcription, as errors during replication would get implemented into the actual DNA of the off-spring, which could lead to inviability. DNA polymerases have a hand-like shape with fingers, a palm and a thumb.

DNA polymerase require triphosphate nucleosides (dNTP) and Mg^{2+}ions to work. The phosphodiester bond between sugars is formed in a concerted manner, the negatively charged transient pentacoordinated complex is stabilized by Mg^{2+}. Mg^{2+}also stabilizes the orthophosphate leaving group. DNA polymerases only extend 5'→3' and often have exonuclease sites, where wrong WC base pairs are removed.

Whether a WC base pair has succesfully formed is checked by two mechanisms: (i.) some AAs from the enzyme bind to the minor-groove to check whether the base-pair has the correct length (not the case for purine-purine or pyrimidine-pyrimidine); (ii.) after dNTP-incorporation the fingers fold done to the thumb, which narrows the exit so only a proper WC base pair will fit through. There are 3 main types of DNA polymerases in prokaryotes.

DNA Polymerase I removes the primers and immediately fills in the gap on the lagging strand between the Okazaki fragments. Very slow (10 nuc/s), with two exonuclease sites both in 5'→3' and 3'→5' direction.

DNA Polymerase II is mostly responsible for proof-reading due to its exonuclease site.

DNA Polymerase III is the work-horse of DNA-replication with quick (1000 nuc/s) replication and some proofreading. Synthesizes the leading strand and the Okazaki fragments between the primers on the lagging strand.

DNA Polymerase β is responsible for eukaryotic DNA repair and the role of DNA polymerase III is taken over by **DNA polymerase** δ in eukaryotes.

8.1.7 Ligases: Connecting Okazaki Fragments

The Okazaki fragments themselves derive from DNA pol III, the primers get removed and filled by DNA pol I. The connection between these discontinuous DNA segments get connected by a **DNA ligase**. It uses a Lys in its active site and engulfs the entire nicked[7] DNA.

8.1.8 Clamp Loader: Increasing DNA Pol Processivity

DNA polymerases need to be fast, accurate and processive, meaning they can synthesize many nucleotides in succession. This means they should not dissociate from the template-strand before they have reached

[7] A "nick" is a break in only one strand of dsDNA.

the end. The dissociation is prevented by using a clamp, a ring around the template strand attached to the DNA pol, that essentially chains the DNA pol to the strand. The enzyme **clamp loader** loads the clamp on the strand using 1 ATP. One clamp is used for the entire leading strand. In the case of the lagging strand, the clamp is released and a new one added each contraction of the trombone.

8.1.9 Exonuclease: Proofreading

Many DNA pol have an exonuclease site that can remove wrong base pairs, if the copied strand folds down into the exonuclease part of the DNA pol. This requires dissociation of the tip of the copied strand, which will only happen if its is merelye weakly attached, i.e. in the case of a mismath base pair. This reduces the error rate of DNA pol to 1 in 1 Million bp.

8.1.10 Termination of Replication (E.coli)

In E.coli, once the two helicases have gone through half the circular chromosome, they run into the 3'-end of already newly synthesized DNA. They dissociate a short piece of this new DNA and then dissociate. Those 3'-flaps are then removed and DNA pol I fills the gaps and terminates replication. Termination can sometimes lead to positively coiled knots in the DNA called "catenanes". Thus, termination requires decatenation by topoisomerase II. There are genetic "weirs" which allow the helicase to pass in one direction but not in the other, so every helicase can only move through half of the circle. So replication by just one replication fork is impossible.

8.1.11 Telomeres & Telomerase

Chromosomes are protected at their ends by telomeres. The replication of telomeres is ensuerd by the enyzme **telomerase**, a reverse transcriptase that uses its own built-in RNA-template to extend telomere ends. Telomerase will thus only extend one strand (copying the its RNA into the DNA), DNA pol can then copy the telomere into its double-stranded form. Shortening of telomeres is associated with diseases as well as ageing.

8.2 Transcription: DNA → mRNA

DNA is first transcribed into messenger RNA (mRNA), which subsequently translated into AAs. The advantage of using messenger boils down to this: ssRNA is easier to read than dsDNA; the short mRNA can be copied many times, if many proteins are needed at once; mRNA can be further modified (processing, only in eukaryotes). Transcription can also occur quicker than replication, given that any error made will only be found in the transient mRNA and not be permanently stored in the DNA. The template DNA strand is called the antisense, its complementary sequence the sense strand. Transcription reads the DNA from 3' to 5' and writes RNA from 5' to 3'.

8.2.1 Initiation of Transcription

Initiation is the slowest step in transcription. Unlike replication, trancription can start *de novo* as RNA polymerases do not require primers.

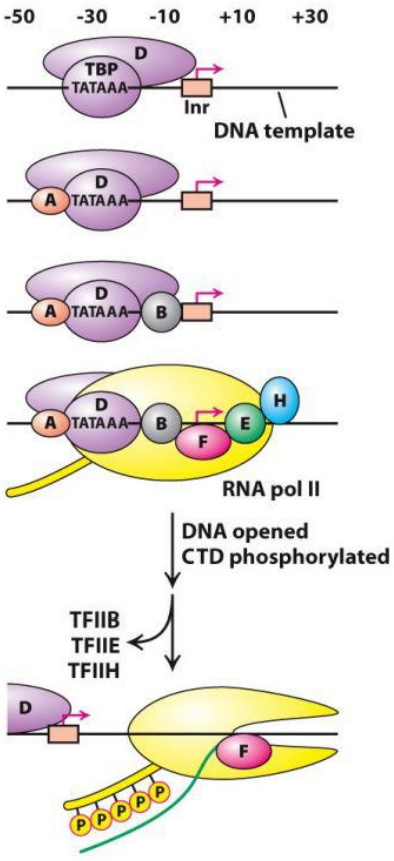
Prokaryotes: Before RNA polymerase can become active, the σ-subunit needs to bind. The σ-subunit's structure has two DNA-binding sites with a distance that corresponds to roughly 15 bp. The DNA-

template has a -10[8] and a -35 (more or less) conserved sequence that get recognized by the σ-subunit. After a few nucleotides, the σ-subunit dissociates. Prokaryotes have many different σ, which allow them to fine-tune which genes are currenlty supposed to be expressed.

Eukaryotes: Eukaryotic promoters are very diverse and act in heterogenous ways to activate transcription. Two conserved sequences are still necessary: the CAAT-box (-75) and the TATA (-35) box. Before RNA pol II resonsible for mRNA transcription can bind, the preinitation complex must be formed from transcription factors (TF), which are in fact subunits of RNA pol II.

1. TATA-binding protein (TBP) binds to TATA-box and induces a kink in the DNA. TBP is also a subunit of RNA pol II.
2. TFIID, another subunit of RNA pol II, will bind.
3. A number of additional subunits bind: TFIIB, TFIIF, TFIIE and TFIIH.
4. All TF dissociate, excpect TFIID and TFIIF.
5. RNA pol II gets phosphorylated and begins elongation. TFIID stays behind.

The interaction between TF and RNA pol II can ofte noccur across many 1000's of bp through a class of proteins called "mediators". These act as bridges between the RNA pol and to TF bound to some part of the DNA. Because eukaryotic DNA is also very tightly packed on the level of chromatin, further co-activators (e.g. histone acetyltransferases) are used to remodel the chromatin before transcription can begin.



8.2.2 Elongation & RNA Polymerases

RNA polymerases cannot only begin elongation without needing primases, they can also unwind the DNA autonomously by providing good ssDNA-interaction sites within them. A RNA pol bound to DNA is referred to as a transcription bubble. The actual transcription is mechanistically very similar to DNA pol, e.g. also using two Mg^{2+}.

Prokaryotes: RNA pol apoenzyme consists of a pentamer (2 α, 2 β and a ω subunit) that form the holoenzyme upon binding the σ-subunit. A RNA pol can elongate (add nucleotide), translocate (move to next position) and even backtrack if an error has been made. Note that the RNA pol will always backtrack by two positions. The part of an DNA segment between initiation and termination is called a "transcriptional unit". One transcriptional unit can sometimes contain multiple genes, which all "work together" and are thus referred to as an operon. Operons result in so-called polycistronic mRNA, with multiple genes each having a start- and a stop-codon, see translation 8.3, i.e. an "open reading frame".

[8] The first copied position in template is +1 and the position before is -1.

Eukaryotes: Eukaryotic and prokaryotic RNA pol are structurally and functionally very similar. Eukaryotes however distinguish three types:

DNA Polymerase I	rRNA + snoRNA
DNA Polymerase II	mRNA
DNA Polymerase III	tRNA

8.2.3 Termination of Transcription

Prokaryotes: Termination in prokaryotes happens through two mechanisms: Either a region of mRNA is transcribed that will spontaneously fold into a hairpin followed by a series of U. The U-A is the weakest bp and thus the mRNA will easily dissociate from the DNA. Alternatively, a protein called ρ can induce termination. ρ is a helicase that moves up the nascent mRNA chain towards the transcriptional bubble after it recognized a termination signal on the mRNA. Once it reaches the RNA pol, it forces its dissociation from the DNA.

Eukaryotes: Eukaryotes do not have terminator sequences *per se* but display "poladynelation signal sequences". Once these signals get transcribed, endonucleases recognize the signal sequence and cleave the mRNA thus releasing the transcribed mRNA. Note that the RNA pol II actually continous transcribing for another several hundred nucleotides before it dissociates. Some fungal toxins attack RNA pol II.

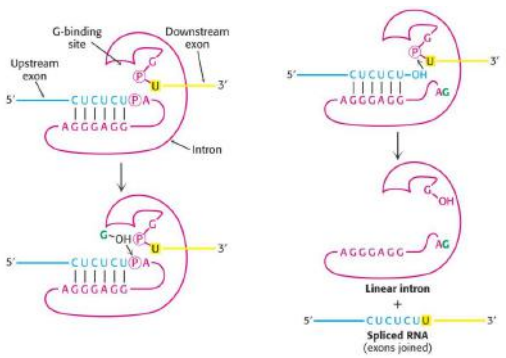
8.2.4 RNA-Processing

Prokaryotes: mRNA undergoes no processing. Indeed, transcription and translation are often happening simultaneously in prokaryotes. Only ribosomal and transfer RNA (rRNA and tRNA, see translation 8.3) get cleaved and modified a bit. All tRNA for example need the same 5'-end in E.coli, so it is added after transcription. Occasionally, even the nucleotides themselves get modified by removing N-hetoroatoms in the bases or methylation.

Eukaryotes: RNA pol II has a phosphorylated carboxyl-terminal domain (CTD) which is initiates all three types of eukaryotic mRNA processing: 5'-capping, polyadenylation at 3'-end and splicing (i.e. removing introns). Preprocessing is particularly imporant in eukaryotes for two reasons: (i.) transcription occurs in the nucleus, translation occurs in the ribosomes of the rough ER, mRNA thus has to travel for long distances; (ii.) eukaryotes have non-coding introns that need to be removed by splicing. rRNA and tRNA also get processed through cleavage.

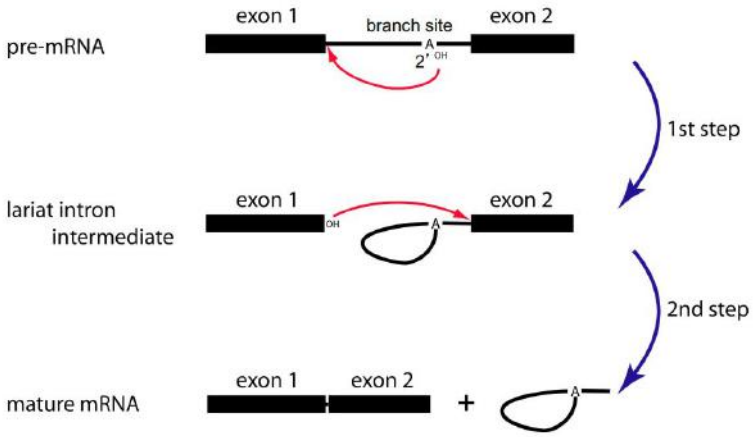
- 5'-processing: A modified guanine nucleotide is added to the 5'-OH of the mRNA.
- 3' Poly-A tail: Directed by the polyadenylation signal sequence the **poly-A polymerase** adds an AAAAAA-sequence to the 3'-end of the mRNA.
- Splicing: Either the intron portion of the mRNA folds spontaneously and then gets released by using a GTP. Or the spliceosome recognizes conserved sequences at the 5'- and 3'-end of the intron and catalyzes splicing.

During self splicing, a GTP will bind to the 5' end of the intron with a hydroxy group, the GTP rest is left on an A (always an A) on the intron. The intron will not dissociate however due to WC bp at the 5' end. The 5'-OH group then attacks the 3' end of the intron relasing the spliced mRNA as



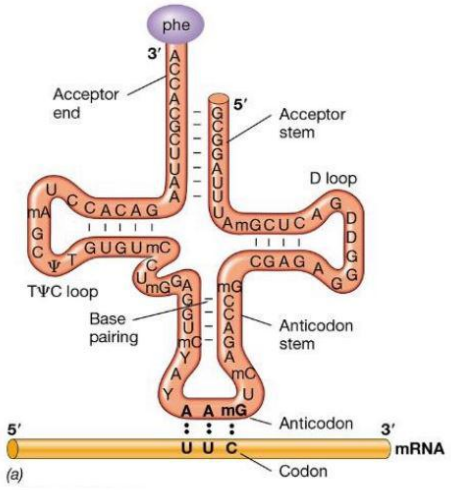
well as the intron. Every intron thus expresses a guanosine binding site for the GTP (or equivalent substrate) to bind.

The spliceosome consists of proteins that have small nucleolar RNA (snRNA) attached and are thus called snRNP. There are six types: U1-6 snRNP. U1 recognizes the 5'-end and U2 the 3'-end through their snRNA, the spliceosome then assembles. Just as in self splicing, a -OH group attaches to the 5'-end, releasing that part of the intron. The 5'-OH will then attack the 3'-end re-forming the phosphodiester backbone and releasing the intron. In essence, amounting to a two-step transesterification. In the spontaneous case, the -OH comes from the GTP. In the spliceosome a 2'-OH from an adenine fulfils this role, leading to an intron that is folded onto itself, a so-called "lariat".

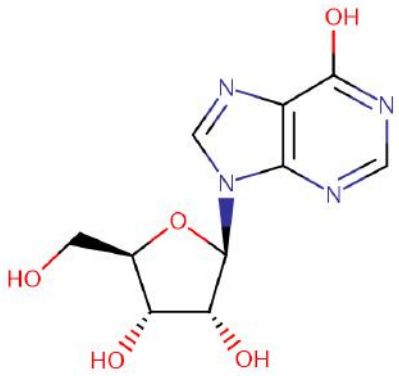


8.3 Translation: mRNA → AA

A codon refers to a three-nucleotide sequence, each codon encodes one amino acid or a STOP. The genetic code is thus degenerate $4^3 = 64$ for only 21 AAs. The cell uses tRNA to transport AA and to read the codon. A tRNA is a ssRNA that is partially folded on itself by internal bp and that consists of a TΨC loop, a D loop an anticodon portion, i.e. three nucleotides that will pair selectively to the codon the tRNA is specific for. The tRNA thus connect the genetic code to the AA-chain. Three codons encode a STOP, so a total of 61 different codons need to be recognized by tRNA.



The cell however only expressed around 31 tRNAs, which is much more efficient given the high degeneracy of the codons. While two codon-anticodon bp are very strong, the third bp at the 3' end, the so-called "wobble position", allows one tRNA to bind imperfectly to many codons. Often the third position of tRNA make use of inosine, which can bp to C, U and A by slightly moving out of the plane. This movement is only structurally allowed by the tRNA on the third position. Note that membrane proteins need to be translated differently than periplasmic or secretory proteins because those hydrophobic proteins cannot be folded and translated in aqueous cytoplasm. This necessitates translation immediately into the membrane, see 8.3.5.



8.3.1 Aminoacyl-tRNA Synthetase

The enzyme **aminoacyl-tRNA synthetase** is repsonsible for loading the tRNA with their respective AA. Every tRNA has a CCA sequence at its 3' end, this is where aminoacyl-tRNA synthetase will transfer the AA to. This requires a two-step proces: (i.) charging the AA with ATP to form AA-AMP; (ii.) transfering the AA-AMP to tRNA to form AA-tRNA. There are two classes of aminoacyl synthetases: type I charge the tRNA at the 2'-OH group of the A; type II charge the tRNA at the 3'-OH group of the A. The two positions are in equilibrium however, so this distinction is irrelevant downstream. There is *one* aminoacyl-tRNA synthetase for each AA, i.e. 21, and each recognises only selectively the tRNA that all recognise codons encoding the same AA. The tRNA can be recognized with the help of certain conserved identity regions.

8.3.2 Ribosome

The ribosome is an enzyme responsible for the actual translation of mRNA. It mostly consists of rRNA, with some mostly structural proteins incorporated, and therefore is often referred to as a rybozyme. We thus again see RNA capable of catalyzing its own reactions, which has been considered strong evidence for the RNA-world hypothesis.

The ribosome consists of a large and a small ribosomal subunit (30S and 50S[9]), which binds the mRNA like two hands coming together. The few proteins incorporated in the ribosome show very distinct modifications (e.g. extreme charge polarization) and must have closely evolved with the ribosome. The ribosome is made up of 2/3 RNA and has 3 bindings sites for tRNA: A, P and E. First, the tRNA attaches to the codon on which the ribosome sits at the Aminoacyl (A) position. Then the AA of the tRNA attaches to the existing AA-chain, the tRNA move to the Peptide (P) position. This repeats and the tRNA is now at the Exit (E) position and will dissociate. The tRNA interacts with the P- and A-loop of the ribosome during binding.

The assembled ribosome displays a tunnel from the active site to the exterior, throuhg which the nascent peptide chain is continuously pushed. The tunnel is wide enough to accommodate water, which allows for simultaneous translation and folding of the protein. Many antiobiotics, e.g. erythromycin, function by essentially plugging this tunnel. Ribosomes in many bacteria form polyribosomes, where multiple ribosomes are simultaneously translating a single mRNA. This allows many copies of a protein to be made in relatively little time without requiring any further transcription. Though in bacteria, transcription and translation typically occur simultaneously, mediated by so-called Nus factors.

[9] Note that Svedberg units [S] refer to the sedimentation time for a given particle and is thus not additive. The holoenzyme for ribosome is 70S.

Small (30S) ribosomal subunit: The 30S subunit mediates the interaction between mRNA codons and tRNA anticodons. It also recognizes the Shine-Dalgarno (SD) sequence. A conserved RNA sequence on the mRNA that gets recognized by the 30S ribosome, else it will not bind to the mRNA.

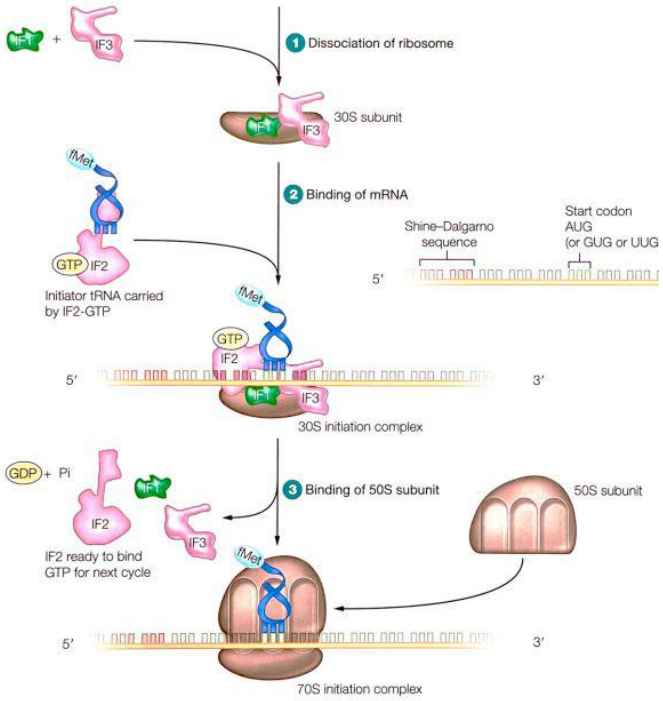
Large (50S) ribosomal subunit: The 50S subunit catalyzes peptide bond formation and binds IF, EF and RF. The actual peptidyl transferase, the elongation of the peptide chain between A and P position, is catalyzed by RNA in the 50S subunit.

RNA tertiary interactions (structural motifs) are very important for the structure and functionality of the ribosome. The A-minor (a RNA-RNA interaction) and Kink-turn motif are probably among the most important motifs. A-minor motifs can for example lead to (RNA)-helix-helix packing within in the ribosome. A-minor motifs are responsible for tRNA binding at the active site of the 50S subunit. A Kink-turn motif (RNA-protein interaction) will also from two angled double helices from the RNA with an exposed base that can interact with proteins. Mg^{2+}(as well as Na^{+}) also play a pivotal role in maintaining the structure of the ribosome. e.g. by binding GTP or within the peptidyl transferase part of the 50S subunit.

8.3.3 Initiation of Translation

IF-1, IF-2 and IF-3 are all necessary prokaryotic initiation factors for translation. IF-1 and IF-3 bring in the small (30S) subunit, while IF-2 activates the bound 30S subunit with one GTP. The GTP is hydrolyzed and the large (50S) subunit will also bind. The N-terminal side of the protein gets synthesized first, which is also the first part out of the tunnel.

Prokaryotes then make use of a dedicated initiator tRNA formylmethionyl-tRNA (fMet-tRNA). The formylation prevents self-cleavage of the carbonyl from the first peptide and the amine of the second peptide. fMet-tRNA is encoded by the AUG codon, the conserved START codon for all translation. If AUG is detected during ongoing translation, regular Met is incorporated however. The fMet is eventually removed from the protein post-translationally.



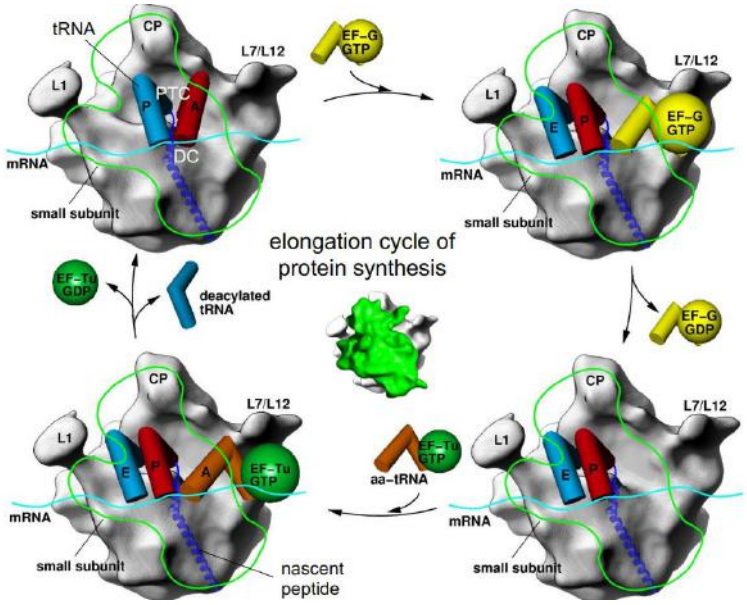
8.3.4 Elongation of Translation

Elongation in prokaryotes depends on three elongation factors: EF-Tu, EF-Ts, EF-G. EF-Tu is responsible for the binding of AA-tRNA to GTP. EF-Ts frees EF-Tu, so it can catalyze the next iteration. EF-G is responsible for moving the tRNA from the A to the P position.

1. tRNA binds to the A-position.
2. EF-G-GTP binds to the A-position, pushing the AA-tRNA to move to the P position by incorporating the AA into the nascent peptide chain.
3. EF-Tu-GTP delivers a tRNA to the A position, also pushing the tRNA in the E position out of the ribosome.
4. The cycle begins anew.

8.3.5 Termination of Translation

Translation is terminated when a STOP codon is recognized by either RF-1 or RF-2, which will then bind in the A position. RF-1/2 coordinate a water molecule that will hydrolyze the peptide chain. RF-3-GTP binds in the vicinity helping with the release and using up a GTP equivalent. The ribosome subsequently dissociates into the 50S and 30S subunits.



Protein secretion mostly relies on the Sec system: unfolded proteins to be exported are recognized post-translationally in the periplasm by SecA. Proteins destined for membrane-insertion are recognized by SRP co-translationally. In both cases a 15-20 AA long sequence at the N-terminus (which, remember, is the first out of the tunnel) gets recognized by the label. These labelled polypeptides are then moved to their final destination by translocases.

SRP actually works by recognizig the signal sequence, then halting the translation until the ribosome has moved to the target membrane. SRP then dissociates and translation is resumed until the protein is pushed into the membrane by the sheer force of the peptide-elongation. The signal sequence then is cleaved off, which is true also for SecA. Both SRP and SecA are conserved among all domains of life.