

Bio Chem 2

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1 Enzymes

1.1 What is an Enzyme: Overview and Components

An Enzyme is a macromolecular biological catalysts that extraordinarily accelerate chemical reactions (10^6 fold). Enzymes possess a high degree of specificity. Enzymes are essential to the metabolism by conserving and transforming chemical energy as well as synthesizing biological macromolecules from simple precursors. Approximately 50% of drugs act by binding to enzymes.

Enzymes are either composed of either proteins or catalytically active RNA molecules. They can require additional components such as cofactors or helpers a.k.a. coenzymes.

1.1.1 Cofactors and Coenzymes

Cofactor: A cofactor is a chemical component, often an inorganic ion. **Coenzyme:** A Coenzyme is a complex organic or metallorganic molecules. Vitamins are precursors of coenzymes.

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^+	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

(a) Cofactors

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin (vitamin B_7)
Coenzyme A	Acyl groups	Pantothenic acid (vitamin B_5) and other compounds
5'-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:\text{H}^-$)	Nicotinic acid (niacin, vitamin B_3)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate (vitamin B_9)
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

(b) Coenzymes

Figure 1: Examples of Cofactors and Coenzymes

1.1.2 Enzymes Classification

Enzymes are named by adding the suffix "-ase" to the name of their substrate or activity. The more formal version is assigning each enzyme a four-part classification number and a systematic name. **Systematic name:** Includes its precise activity and the substrates it works with (e.g., hexokinase is ATP:glucose phosphotransferase). **Classification number:** Four number code, using again Hexokinase (2.7.1.1) as an example:

- Class name (2: Transferase)
- Subclass name (7: Phosphotransferase)
- The acceptor functional group (1: Phosphotransferase with hydroxyl group as acceptor)

-
- iv) The accepting molecule (1: D-Glucose as acceptor molecule)

Class number	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer
3	Hydrolases	Hydrolysis (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerasers	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds

Figure 2: Examples of Cofactors and Coenzymes

1.2 The Thermodynamics and an Enzymes Role

Enzymes reduce the Activation Energy for a given reaction, hence changing the rate of the reaction. They do not influence K_{eq} or ΔG

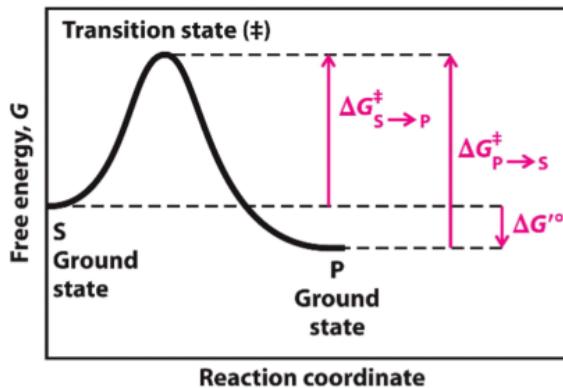


Figure 3: A reaction diagram

1.2.1 Transition State Theory

When plotting the progress of a reaction the Transition State is the very top of the hill. Note, that the **transition state ≠ Reaction Intermediate**. It should be understood as the moment in which the reaction is equally likely to progress towards substrate or product.

1.2.2 K_{eq}

The equilibrium constant K_{eq} , or K, is the reaction quotient at chemical equilibrium. K gives is calculated by:

$$K = \frac{[P]}{[S]} \quad (1)$$

An enzyme has no effect on K.

K'_{eq} is the K_{eq} at standard biochemical conditions (298K, pH = 7).

1.2.3 ΔG and ΔG^\ddagger

The activation energy (ΔG^\ddagger) is the difference between the ground state and the transition state.

ΔG° is the standard free energy under standard conditions ($T = 298K$, 1atm, 1M of each solute), while $\Delta G''$ is also at pH = 7.

1.2.4 The Relationship Between K_{eq} and ΔG

$$\Delta G^\circ = -RT \ln K'_{eq} \quad (2)$$

where, $R = 8.315 \frac{J}{mol \cdot K}$ and $T = 298K$.

Note that large K \leftrightarrow very negative $\Delta G''$

K'_{eq}	$\Delta G'' (\text{kJ/mol})$
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0.0
10^1	-5.7
10^2	-11.4
10^3	-17.1

Figure 4: Some examples showing the relationship between G and K

1.2.5 Return Rate

The rate of a reaction is determined by the concentration of the reactants and the rate constant k.

For a unimolecular reaction we have that

$$v = k[S] \quad (3)$$

in which the reaction only depends on [S] and k has units s^{-1} and the following formula:

$$k = \frac{k'T}{h} * e^{-\frac{\Delta G^\ddagger}{RT}} \quad (4)$$

where k' is the Boltzmann constant and h the Plank constant. This means that the lower ΔG^\ddagger the faster the reaction goes. Since, enzymes lower ΔG^\ddagger it raises k and voila reaction is now speedy Gonzo.

TABLE 6–5 Some Rate Enhancements Produced by Enzymes

Cyclophilin	10^5
Carbonic anhydrase	10^7
Triose phosphate isomerase	10^9
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

Figure 5: Some Rate enhancements Produced by Enzymes

1.3 Enzymes' Role in the Reaction

To piece it all together an enzyme does the following:

- Has no effect on equilibrium related things; it does not influence G or K;
- Lowers the energy of ΔG^\ddagger and with it the activation energy;
- Hence creating a larger k and with that speeding up the rate of the reaction.

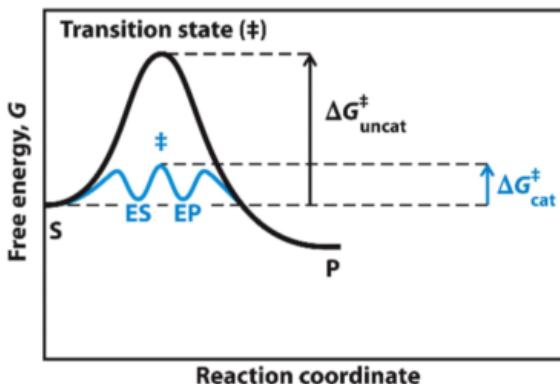


Figure 6: The Enzyme introduces a couple of new transition states (ES and EP), each of which is more stable than the original transition state

This speeding up happens due to three main things:

1.3.1 Catalytic Power and Mechanism

An enzyme has two ways to make a reaction faster:

- i) Providing a **lower-energy reaction path**.
- ii) Releasing energy through the **non-covalent binding** between the substrate and the enzyme. That energy is referred to as ΔG_B . This energy gain is a major driving force for the reaction to even happen

PSA: Your friendly reminder that the enzyme will remain unchanged when comparing beginning and end of reaction!

1.3.2 Active Site

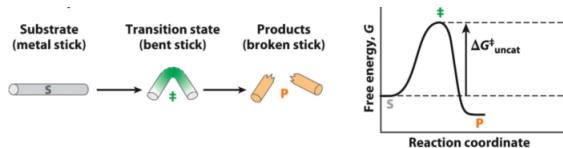
The active site is the place of the reaction. This site is lined with amino acids residue, which possess qualities to bind to the substrate and catalyze its transformation. Often times an enzymes will envelop a substrate separating it from the solution.

This binding is highly specific. This is where the lock and key principal comes into play. However it is slightly misleading as then the transition state would have to be more unfavorable than the substrate. Hence, it is more precise to see the **enzyme as very specific to the transition state** (which is till similar to the substrate).

1.3.3 Example for Transition State Complementarity and Rate Enhancement

Using the example of stickase we show the importance of the transition state complementarity, the fault of lock and key, and how that leads to rate enhancement.

No enzyme:



Enzyme which is complementary to the substrate:

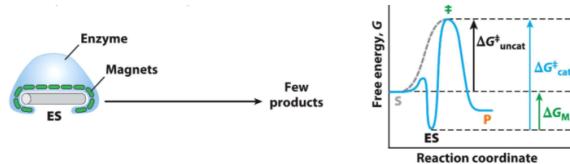


Figure 7: because the enzyme is complementary to the substrate it will stabilize the stick, making the ES state the most stable one and the required ΔG^\ddagger larger than without an enzyme.

Enzyme which is complementary to the transition state:

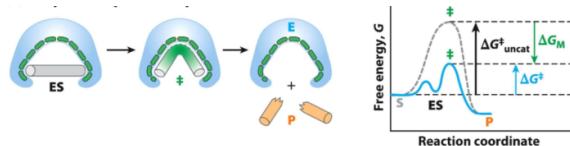


Figure 8: With the enzyme being complementary to the TS, the ES-state would still be more favorable, but not too favorable. More importantly the ΔG^\ddagger would be lowered as the TS is significantly more stable.

1.3.4 Binding and Specificity

There are numerous not favorable physical and thermodynamical factors which contribute to ΔG^\ddagger , which need to be overcome:

- freedom through entropy:** The motion of molecules which reduces the possibility of proximity and hence reaction;
- Solvation of the water shell**

-
- iii) **Distortion of substrates**
 - iv) **proper alignment** of the catalytic functional groups on the enzyme

All these factors are overcome by the binding energy, which is released once ES enter the transition state. The need for this binding energy further enhances specificity. Here is how exactly the binding energy comes to be and is favorable:

- i) **Entropy Reduction:** Through the binding of substrates to the enzyme, the freedom of motion of the substrates is significantly limited. This leads to the probability that they collide to react skyrocketing.
- ii) **Desolvation:** Due to the binding of enzyme and substrate water molecules are removed. While locally that means a slight decrease in entropy, overall it leads to an increase, making it energetically favorable.
- iii) **Substrate Distortion:** The binding energy we get later on formed through the transition state and enzyme help compensate for any initial distortion, especially electronic redistribution.
- iv) **Catalytic Group alignment:** As the substrate binds to the enzyme, the enzyme undergoes a so called induced fit, meaning it envelops the substrate in such a way that its functional groups can properly catalyze at the right position, as well as put the reaction sites of the substrates in the right position.

All these barriers and conditions to resolve them, make an enzyme very specific in which molecules it can catalyze. Those that work however, it is then able to create a huge rate enhancement.

1.4 Other Contributions to Enzyme Catalysis

Intermediates can often be very unstable, making them very unfavorable to get to (high ΔG^\ddagger). Here are some ways enzymatic complexes overcome this and hence enhance reaction rates:

- i) **Acid-Base Catalysis:** Some intermediates will be charged, which can lead to great instability. In order to stabilize them protonating/deprotonating can create a much more stable intermediate. Since water is rather weak, it will often be catalyzed with an amino acid residue which has acid or base properties.
- ii) **Covalent Catalysis:** An intermediate is formed in which a bond is formed between a substrate and the enzyme. This only happens, if the new pathway has lower ΔG^\ddagger . A further condition is the nucleophilic properties of the enzyme, which several amino acid residues and some cofactors possess. Of course they then proceed to go further reaction freeing them back up.
- iii) **Metal Ion Catalysis:** Metals can participate in catalysis in numerous ways. Ionic interactions can stabilize or orient charged reaction transition states. Its effects are similar to the enzyme-substrate binding energy of above. Metals can also mediate redox reaction through reversible changes in their oxidation state. Nearly a third of all enzymes require metal ions.

1.5 Enzyme Kinetics

We want to understand the role of enzyme mechanisms rate of a reaction. In particular how it changes in respect to changes in experimental parameters. This is called enzyme kinetics. The main factor affecting the rate is the substrate [S].

Studying the effects of [S] is pretty tough because it is constantly changing. Instead an easier approach is to study the initial velocity (V_0).

1.5.1 v_0

To find V_0 Because we take at the beginning of the reaction we can assume that the amount of product

Because at low substrate concentrations we can assume even lower product concentrations, we make the assumption that $[P]$ has no influence on the rate. This let's us conclude that the V_0 increases nearly linearly with an increase in $[S]$, at low $[S]$ concentrations:

$$[S]_t = [S]_0 - [P]_t V_0 = k[S]_0 \quad (5)$$

However, as $[S]$ increases the change in V_0 becomes smaller and smaller until we reach a point where it starts to plateau. That plateau it approaches is V_{max} .

The intuitive explanation: It's all about **Enzyme Saturation**; initially when the enzymes aren't saturated every new $[S]$ can immediately be turned into product, leading to the linear increase in V_0 . However, once the system starts to be saturated that effect diminishes, all the way until where the enzymes are fully saturated which leads us to approach V_{max} .

1.5.2 ES Complex and v_0



Looking at this equation, we can see that if the step from $[ES]$ to $[E] + [P]$ is rate limiting than that is what determines V_0 . So this gives us the equation:

$$V_0 = k_2[ES] \quad (7)$$

1.5.3 Michaelis-Menten - Derivation and Conclusions

The Michaelis-Menten equation:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Before getting into the derivation here are the core things to take away:

- i) **Steady state assumption:** We assume that V_0 reflects a condition where $[ES]$ is constant, that means that $[ES]$ produced = $[ES]$ breakdown. If we don't do this Michaelis-Menten falls apart, basically this is assuming that enzymes will immediately take up a new substrate when they leave the $[ES]$ complex.
- ii) $S \ll K_M \rightarrow V_0 = k[S]$
- iii) $S \gg K_M \rightarrow V_0 = V_{Max}$
- iv) $V_0 = \frac{V_{Max}}{2} \rightarrow K_M = [S]$

One important variation of the MM equation is the following:

Using the following:

$$V_{max} = k_{cat} \cdot [E]_T \quad (8)$$

we receive:

$$V_0 = \frac{k_{cat}[E]_T[S]}{K_m + [S]} \quad (9)$$

Now, the actual derivation:

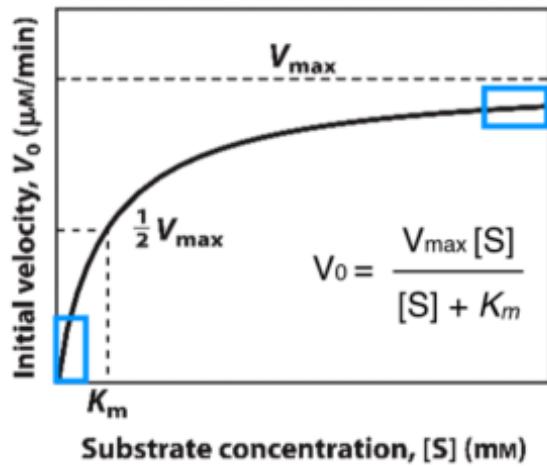


Figure 9: The blue box bottom left is when $S \ll K_M$, while the top right is when $S \gg K_M$



$$\text{Rate of formation of } [ES] = k_1([E]_{\text{tot}} - [ES])[S]$$

$$\text{Rate of breakdown of } [ES] = (k_{-1} + k_2)[ES]$$

$$k_1([E]_{\text{tot}} - [ES])[S] = (k_{-1} + k_2)[ES]$$

$$k_1[E]_{\text{tot}}[S] - k_1[ES][S] = (k_{-1} + k_2)[ES]$$

$$k_1[E]_{\text{tot}}[S] = (k_1[S] + k_{-1} + k_2)[ES]$$

$$[ES] = \frac{k_1[E]_{\text{tot}}[S]}{k_1[S] + k_{-1} + k_2}$$

$$\text{Define } K_m = \frac{k_{-1} + k_2}{k_1} \Rightarrow [ES] = \frac{[E]_{\text{tot}}[S]}{[S] + K_m}$$

$$V_0 = k_2[ES] = \frac{k_2[E]_{\text{tot}}[S]}{[S] + K_m}$$

$$V_{\text{max}} = k_2[E]_{\text{tot}} \Rightarrow V_0 = \frac{V_{\text{max}}[S]}{[S] + K_m}$$

1.5.4 Lineweaver-Burk

Due to the asymptotic behavior of V_0 , it's difficult to determine K_m and V_{max} from it. Hence we create a double-reciprocal plot, which is linear, called the Lineweaver-Burk equation:

$$\frac{1}{v} = \frac{K_m}{V_{\text{max}}} \cdot \frac{1}{[S]} + \frac{1}{V_{\text{max}}} \quad (10)$$

where:

- $m = \frac{K_m}{V_{\text{max}}}$ is the slope

- $b = \frac{1}{V_{\max}}$ is the y-intercept
- $c = -\frac{1}{K_m}$ is the x-intercept

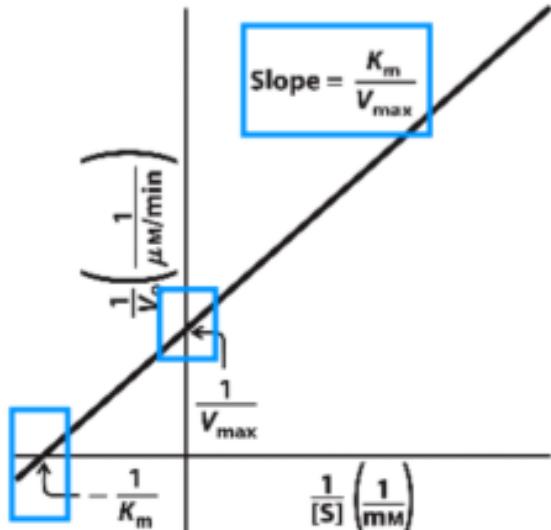


Figure 10: The blue boxes highlight the slope, y-intercept, and x-intercept of the Lineweaver-Burk equation. Those spots give are what make it so easy to find K_M and V_{\max}

Derivation of the Lineweaver-Burk equation:

We start with the Michaelis-Menten equation:

$$v = \frac{V_{\max}[S]}{K_m + [S]} \quad (11)$$

To linearize this, we take the reciprocal of both sides:

$$\frac{1}{v} = \frac{1}{\frac{V_{\max}[S]}{K_m + [S]}} \quad (12)$$

By inverting the fraction on the right-hand side:

$$\frac{1}{v} = \frac{K_m + [S]}{V_{\max}[S]} \quad (13)$$

Now split the numerator:

$$\frac{1}{v} = \frac{K_m}{V_{\max}[S]} + \frac{[S]}{V_{\max}[S]} \quad (14)$$

Which leaves us with the Lineweaver-Burk equation:

$$\frac{1}{v} = \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}} \quad (15)$$

1.5.5 K_m and k_{cat}

The meaning of K_m can vary greatly between different enzymes and even substrates.

One possible meaning for K_m can be the following: if $k_2 \ll k_{-1}$ then K_m essentially boils down to the following:

$$K_m = \frac{k_{-1}}{k_1} = K_D \quad (16)$$

So, for the case of $k_2 \ll k_{-1}$ K_m tells us the affinity of a certain enzyme for its substrate. Note, that this is however only true in this one special case!

k_{cat} is the rate constant at the rate-limiting step. This will often be k_2 , however that is not always the case (especially if we have more than two reaction steps).

1.5.6 Determining Enzyme Efficiency

The best way to determine the efficiency of an enzyme is by determining the following ratio:

$$\frac{k_{cat}}{K_M} \quad (17)$$

There is an upper limit to this ratio, set by the rate at which E and S can diffuse together. The diffusion-controlled limit is 10^8 to $10^9 M^{-1}s^{-1}$. Enzymes which have values close to this have achieved catalytic perfection.

PSA: this seems overkill from the lecture but there's a whole slide of it, so here is how we get the units by starting with the MM equation:

$$v = \frac{k_{cat}[E]_T[S]}{K_m + [S]} \quad (18)$$

When $[S] \ll K_m$, the equation simplifies to:

$$v \approx \frac{k_{cat}}{K_m} [E]_T [S] \quad (19)$$

This resembles a second-order rate law: first order in both enzyme and substrate concentrations.

Units:

- k_{cat} : has units of s^{-1} (per second)
- K_m : has units of M (molar, i.e., mol/L)

Thus, the specificity constant has units:

$$\frac{k_{cat}}{K_m} = \frac{1 s^{-1}}{1 M} = M^{-1} s^{-1} \quad (20)$$

1.5.7 V_{max}

V_{max} is the maximum initial velocity, and with that also the maximum velocity, that is attainable for a certain enzyme/substrate duo.

The equation for V_{max} is the following:

$$V_{max} = k_{cat} \cdot [E]_T \quad (21)$$

This equation comes from the fact we take the rate-limiting's step rate constant. Multiplying that with the enzyme concentration gives us the speed the enzymes can convert the substrate with at the choke point.

TABLE 6-6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β -Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

(a) K_m **TABLE 6-7** Turnover Number, k_{cat} , of Some Enzyme

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β -Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.5

(b) k_{cat} **TABLE 6-8** Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^3 to $10^5 \text{ M}^{-1}\text{s}^{-1}$)

Enzyme	Substrate	K_m (s^{-1})	K_m (M)	k_{cat}/K_m ($\text{M}^{-1}\text{s}^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^4	1.2×10^{-4}	8.3×10^7
	HCO_3^-	4×10^1	2.6×10^{-1}	1.5×10^7
Catalase	H_2O_2	4×10^1	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	3.7×10^4	2×10^{-4}	2.8×10^8
Fumarase	Fumarate	8×10^1	5×10^{-4}	1.6×10^8
	Malate	9×10^1	2.5×10^{-4}	3.6×10^7
β -Lactamase	Benzylpenicillin	2.0×10^4	2×10^{-5}	1×10^9

Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science*, p. 166, W. H. Freeman and Company, New York.

(c) limit

Figure 11: Examples of rate for K_m , k_{cat} , and their ratio determining the enzyme's efficiency.

1.6 Inhibition

Inhibitors are molecules that interfere with catalysis, decreasing or halting enzyme activity. They are critical for drug design, as nearly 50% of all pharmaceutical agents are enzyme inhibitors. Inhibitors are classified into two main types:

- Reversible Inhibitors: Interact transiently with the enzyme.
- Irreversible Inhibitors: Form a covalent bond or permanently alter the enzyme.

1.6.1 Reversible Inhibition

Reversible inhibitors can act by competitive, non-competitive and uncompetitive mechanisms. The inhibition constant K_I quantifies the strength of inhibition in blocking the activity of an enzyme. To take this into account in kinetics, the factor α and α' are used, where α is derived from K_I (binding to enzyme) and α' from K'_I (binding to enzyme-substrate complex).

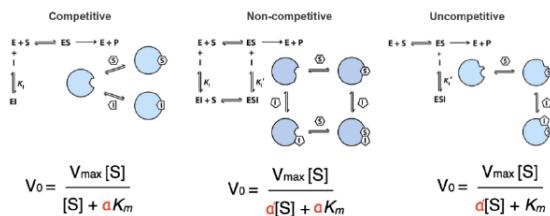


Figure 12: Types of Reversible Inhibition

- **Competitive Inhibitor:** The inhibitor competes with the substrate for the enzyme's active site. This increases K_m but does not affect V_{max} .

- **Non-competitive Inhibitor:** The inhibitor binds to a site other than the active site, reducing the concentration of the active enzyme-substrate complex. This decreases V_{max} , but does not affect K_m .
- **Uncompetitive Inhibitor:** The inhibitor binds only to the enzyme-substrate complex, reducing the concentration of the active enzyme-substrate complex. Both V_{max} and K_m decrease.



Figure 13: Impact of different Reversible Inhibition Types

1.6.2 Irreversible Inhibition

Irreversible inhibitors form covalent bonds with the enzyme, **permanently inactivating** it. Here are two types of irreversible inhibitors, their function, and usage:

- **Suicide Inhibition:** Also known as mechanism-based inactivators, these molecules are not reactive until they reach the active site, where they then go through the first steps of the reaction. At some point the inactivator becomes so reactive in a transition state it reacts irreversibly with the enzyme. Because these compounds are so highly specific, and passive otherwise, they often have little side effects.
- **Transition-State Analogs:** Molecules which are very similar to a transition-state. They bind significantly better to the enzyme and its active state than the substrate, making them an irreversible inhibitor.

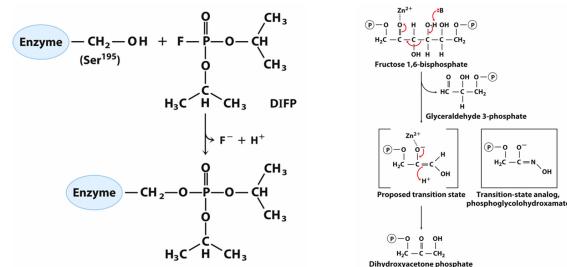


Figure 14: Mechanism of different types of inhibition; on the left is a suicide mechanism and on the right a transition-state analog.

2 Behind the Torch of Life

2.1 Bioenergetics and Thermodynamics

Bioenergetics is the quantitative study of energy transductions. Of particular interest is the second law of thermodynamics.

Definition 2.1 (The second law of thermodynamics). *The Second Law of Thermodynamics states that the total entropy of an isolated system can never decrease over time. Isolated systems spontaneously evolve toward thermodynamic equilibrium, the state with the maximum entropy.*

In a **chemical reaction** entropy increases when the products of the reaction are less complex and more disordered than its substrates. Therefore many biochemical reactions seem to contradict the second law as they "produce order"

To compensate the produced order by cells in their growth and division **free energy** is taken from the environment (organisms are not an isolated system) in the form of nutrients or solar light and exchanged for heat and entropy.

Definition 2.2 (Enthalpy, H). *Enthalpy (H) is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products. When a chemical reaction releases heat, it is said to be exothermic; the heat content of the products is less than that of the reactants and ΔH has, by convention, a negative value. Reacting systems that take up heat from their surroundings are endothermic and have positive values of ΔH .*

Definition 2.3 (Entropy, S). *Entropy is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.*

Definition 2.4 (Free energy (G)). *It represents the energy available to do biological work, such as muscle contraction, active transport, and biosynthesis. The change in Gibbs free energy (ΔG) for a reaction is given by:*

$$\Delta G = \Delta H - T\Delta S$$

In biochemistry, Gibbs free energy (G) determines whether a metabolic reaction "can occur spontaneously" (but may still be unlikely because of TS) in living systems. A process is favorable if it is **Exergonic Reaction**, $\Delta G < 0$.

An important property is that variations in delta G are **additive**:

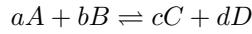
$$\Delta G_{\text{total}} = \Delta G_1 + \Delta G_2$$

This property lets us **make unfavorable reaction favorable when coupling them to a highly favorable reaction**. This is explored by various biological pathways.

Remark 2.5 (Standard transformed constants). Physical constants based on this biochemical standard state are called **standard transformed constants** and are written with a prime (such as $\Delta G'^{\circ}$ and K'_{eq}) to distinguish them from the untransformed constants used by chemists and physicists.

2.1.1 Equilibrium

By the second law of Thermodynamics, a reaction continues until equilibrium, the maximal entropy is reached. This is described by the **equilibrium constant** (K) that quantifies the ratio of **product over reactant** concentrations at equilibrium. It is defined for a general reaction:



$$K = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

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

Remark 2.6 (steady state). In biological process the equilibrium is practically never reached. Nevertheless the system reaches a steady state, where the the concentrations stays constant thanks to a net flow equal to zero.

2.1.1.1 Reaction Quotient

The **Reaction Quotient** is similar to the equilibrium constant, but it uses the actual, observed concentrations of reactants and products, rather than the equilibrium concentrations. Q is defined as:

$$Q = \frac{[C]_{{\text{obs}}}^c [D]_{{\text{obs}}}^d}{[A]_{{\text{obs}}}^a [B]_{{\text{obs}}}^b} \quad (22)$$

$$\Delta G = \Delta G^\circ + RT \ln Q \quad (23)$$

2.1.1.2 Mass Action Ratio (Q/K)

The **Mass Action Ratio** is the ratio of the reaction quotient (Q) to the equilibrium constant (K). The mass action ratio helps us understand **where a reaction is going**:

$$\Delta G = RT \ln \frac{Q}{K} \quad (24)$$

- If $Q/K < 1$, then $\Delta G < 0$, and the reaction will proceed in the forward direction to reach equilibrium.
- If $Q/K > 1$, then $\Delta G > 0$, and the reaction will proceed in the reverse direction to reach equilibrium.
- If $Q/K = 1$, then $\Delta G = 0$, and the reaction is at equilibrium.

2.1.1.3 Henderson-Hasselbach

Since in a biological context the environment is buffered at near-constant pH, the Henderson-Hasselbach equation is generally applicable to determine the ratio of the different protonation states of a compound.

$$\text{pH} = \text{pKa} + \log \left(\frac{[\text{A}^-]}{[\text{HA}]} \right)$$

2.2 Back to OCI

The reactions that do occur in cells represent a toolbox that evolution has used to construct metabolic pathways that circumvent the “impossible” reactions. Most of the reactions in living cells fall into one of five categories:

- reactions that make or break carbon–carbon bonds
- internal rearrangements, isomerizations, and eliminations
- free-radical reactions
- group transfers
- oxidation-reductions

Remark 2.7 (Covalent Bond). A covalent bond consists of a shared pair of electrons, and the bond can be broken in two general ways. In **homolytic cleavage**, each atom leaves the bond as a radical, carrying one unpaired electron. In **heterolytic cleavage** which is more common, one atom retains both bonding electrons.

Remark 2.8 (Nucleophiles and Electrophiles). Nucleophiles (functional groups rich in and capable of donating electrons) and electrophiles (electron-deficient functional groups that seek electrons).

2.2.0.1 Reactions that make or break carbon–carbon bonds

Heterolytic cleavage of C-C bonds yealds a carboanion and a carbocation. Conversely, the formation of a C-C bond involves the combination of a nucleophilic carboanion and an electrophilic carbocation. *Note, that carboanions and carbocations are generally so unstable that their formation as reaction intermediates can be energetically impossible even with the help of an enzyme.*

Therefore this reactions need assistance by functional groups containing electromotive atoms (O and N). This can alter the electronic structure of adjacent carbon atoms (**carbonyl-groups**, withdrawing electrons), stabilizing and facilitation the formation of carboanion and cation intermediates. *This can be further enhanced by the presence of metal ions such as Mg²⁺ for example.*

Aldol condensation is common way to creat C-C bonds, i.e. the aldolase reaction which converts six-carbon compounds to three-carbon compounds in glycolysis is an aldol condensation in reverse.

In a **Claisen condensation**, the carbanion is stabilized by the carbonyl of an adjacent thioester; an example is the synthesis of citrate in the citric acid cycle. Sometimes imine or certain cofactors play the role as the "electron-withdrawer".

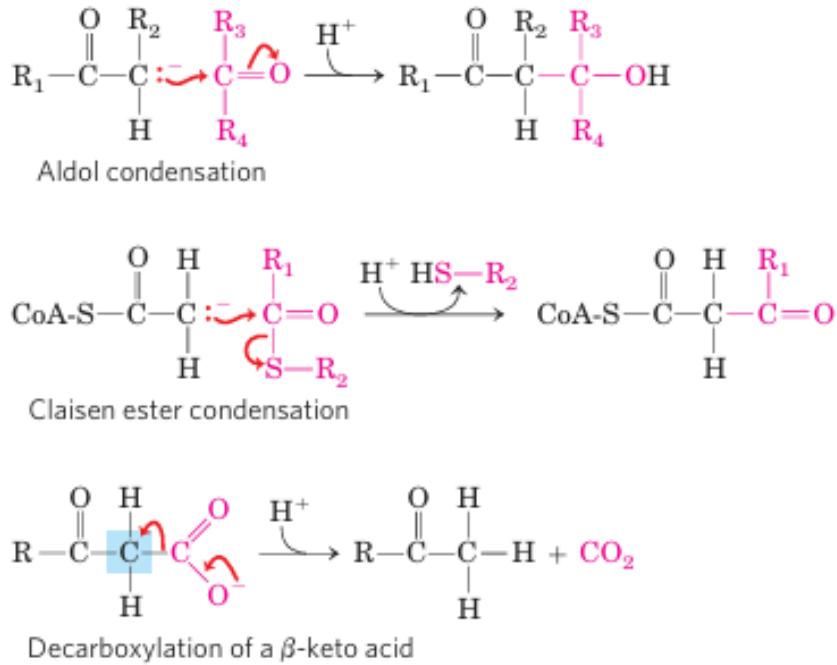


Figure 15: make or break carbon–carbon bonds

2.2.0.2 Internal rearrangements, Isomerizations, and Eliminations

In this type of reactions **electrons are redistributed altering the bonding framework** without changing the overall oxidation state of the molecule. For example different groups undergo oxidation-reduction leading to **cis-trans rearrangements** or shifting the **position of double bonds** , i.e. formation of fructose 6-phosphate from glucose 6-phosphate in glycolysis. Here C1 is reduced and C2 is oxidized.

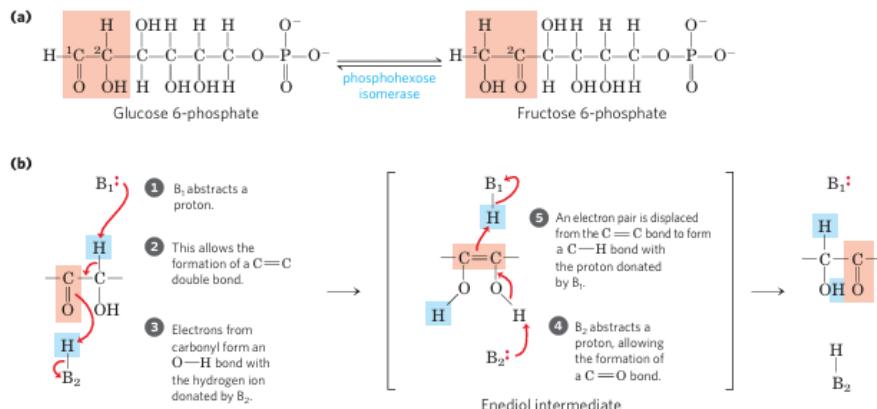


Figure 16: Isomerization and elimination reactions

An example for an **elimination** reaction is the loss of water from an alcohol resulting in a double C=C bond. *Similar reaction can result from eliminations in amines.*

2.2.0.3 Free-Radical Reactions

The homolytic cleavage of covalent bonds generate free radicals. These radicals can then trigger other reactions.

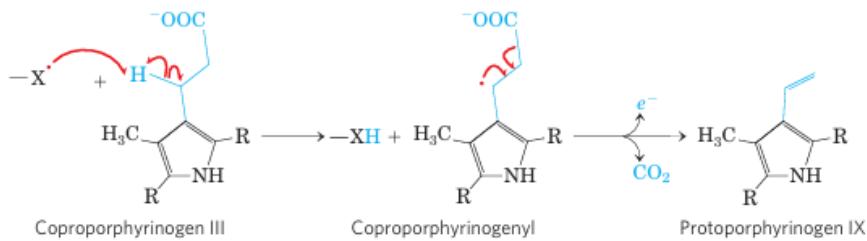


Figure 17: A free radical–initiated decarboxylation reaction

2.2.0.4 Group Transfer Reactions

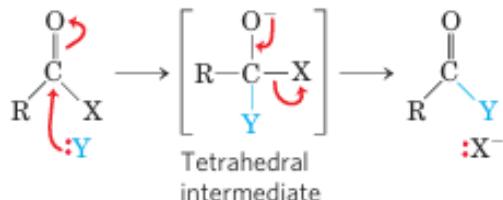
The transfer of acyl, glycosyl, and phosphoryl groups from one nucleophile to another is common in living cells. Acyl group transfer generally involves the addition of a nucleophile to the carbonyl carbon of an acyl group to form a tetrahedral intermediate.

A general idea in metabolism is to attach a good leaving group to a metabolic intermediate to trigger subsequent reactions. Since nucleophilic substitutions is made more favorable by the attachment of a phosphoryl group to an otherwise poor leaving group such as -OH.

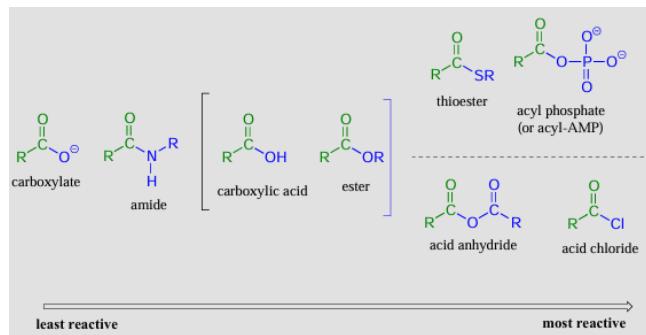
Remark 2.9 (Good leaving group). Recall that **weaker bases are better leaving groups**. One has to look how could the leaving group stabilize / balance the negative charge: **Inorganic orthophosphate** (the ionized form of H_3PO_4 at neutral pH, a mixture of H_2PO_4^- and HPO_4^{2-} , commonly abbreviated as P_i) and **inorganic pyrophosphate** ($\text{P}_2\text{O}_7^{4-}$, abbreviated as PP_i); **esters and anhydrides of phosphoric acid** and **thiols** are also good leaving groups.

2.2.0.5 Oxidation-Reduction Reactions

Carbon atoms can only exist in five oxidation states, depending on their binding partners. Note that **carbon is less electronegative than all atoms it is bound to, except hydrogen**. Thus all atoms that bind



(a) Nucleophilic substitution



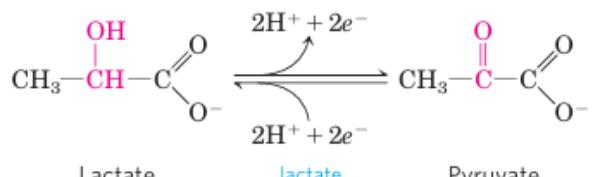
(b) Relative reactivity of carboxylic acid derivatives

Figure 18: Group Transfer Reactions

to carbon oxidize it except hydrogen and therefore removing a hydrogen and replacing that bond with any other atom (including carbon) is synonymous with oxydation. Recall that every oxidation must be linked to a reduction. Note that, **Oxydations generally release energy** (camp fires where wood is oxidized).

Often in biological oxidations, a compound loses two electrons and two hydrogens (2 hydrogen atoms), these reactions are called **dehydrogenations** catalyzed by **Dehydrogenase**.

Sometimes in biological oxidations a carbon becomes covalently bounded to an oxygen. The corresponding enzymes are called **Oxidase** and if the oxygen atom is derived from molecular oxygen they are called **Oxygenase**.



(a) The 5 oxidation levels of carbons

(b) lactate dehydrogenase

Figure 19: Oxidation-Reduction Reactions

2.3 Phosphoryl and ATP fun

In a phosphate transfer reaction, a phosphate group is transferred from a phosphate group donor molecule to a phosphate group acceptor molecule.

2.3.0.1 Phosphate groups

Recall some important properties of phosphorus from organic chemistry:

- Phosphates are **excellent leaving groups** in biological organic reactions, which can be seen for example in the hydrolysis of ATP.
- Phosphoric acid (H_3PO_4) is triprotic, meaning that it has three acidic protons available to donate with pK_a values of 1, 6.5, 13, respectively.
- Phosphorus can break the octet rule because it is in the third row of the periodic table and thus has **d orbitals** available for bonding.
- The phosphate group is really tetrahedral, the **negative charges are delocalized** over the non-bridging oxygens, and there is some degree of protonation at physiological pH (with the exception of the phosphate di-ester group.)

Phosphate transfer enzymes generally contain a **Mg²⁺ ion bound in the active site** in a position where it can interact with non-binding phosphate oxygens on the substrate. This magnesium ion pulls the electron density away from the phosphorus atom, making it more electrophilic.

A phosphate transfer reaction can be thought of as a S_N2 reaction at a carbon center. Recall that the phosphorus can form a "**5-bond transition state**".

2.3.0.2 The phosphate enzymes

Definition 2.10 (Kinases). *Kinase (from Greek *kinein*, "to move") is an enzyme that catalyzes the transfer of phosphate groups from high-energy donor molecules, such as ATP, to specific substrates, a process known as phosphorylation.*

Definition 2.11 (Phosphatases). *Phosphatase is an enzyme that removes phosphate groups from proteins or other molecules, a process known as dephosphorylation, which often regulates cellular activity.*

Remark 2.12 (Reactions catalyzed by kinases and phosphatases are not the reverse of one another). Kinases irreversible transfer phosphate groups from ATP (or sometimes other nucleoside triphosphates) to various organic acceptor compounds, while phosphatases transfer phosphate from organic compounds to water, releasing it as inorganic phosphate: this are hydrolysis reactions. Kinase reactions involve an inherently "uphill" step (phosphorylation of alcohols for example) being paid with an inherently "downhill" step (cleavage of an anhydride bond in ATP). Phosphatase reactions, on the other hand, are thermodynamically "downhill", and while they require an enzyme to speed them up, they do not involve "spending" energy the way kinases do.

2.3.0.3 ATP

ATP (Adenosine Triphosphate) is the the **energy currency** of the cell and links catabolism and anabolism. ATP is a high energy compound which can be seen when considering hydrolysis of ATP (highly exergonic), since:

- Hydrolysis relieves electrostatic repulsion between the negatively charged phosphates. One way to picture this is **a coil springing open, releasing potential energy**
- Inorganic phosphate can be stabilized by resonance hybrid.
- ADP-2 can ionize
- The products are better solvated than the reactants.

Note: that ATP-cleaving reaction are exothermic, but also have a high energy barrier, making it them very slow unless catalyzed by an enzyme. This helps us gain a tight control over the reactions in our metabolic pathways.

ATP provide energy by transferring its phosphate group and not by mere hydrolysis. Nevertheless often we say that a given reaction is coupled to ATP hydrolysis which provides the energy required for the reaction to happen. Note that **ATP hydrolysis per se only provides heat.**

In many reactions **ATP is used as a phosphate donor** to a substrate that, once phosphorylated, acquires an higher free energy.

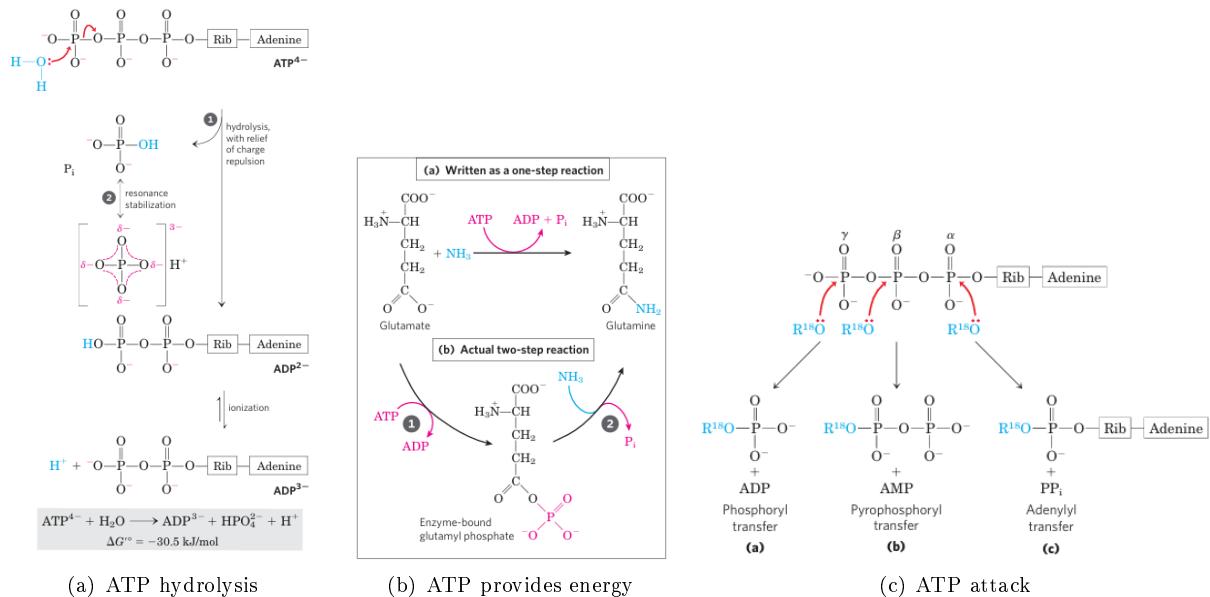
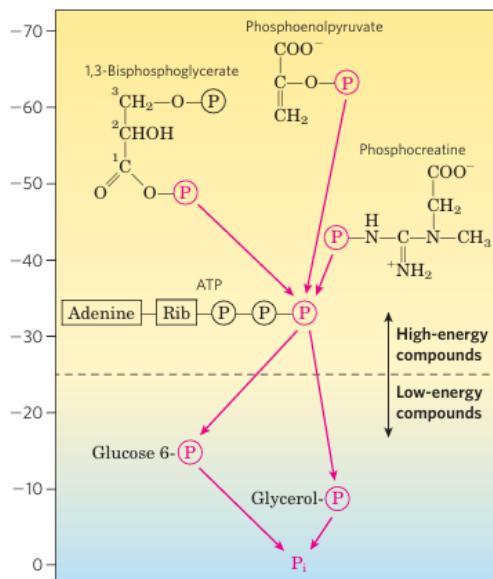


Figure 20: ATP

Note: To maintain its high group transfer potential, ATP concentration must be held far above the equilibrium concentration by energy-yielding reactions of catabolism.

Moreover, inorganic polyphosphate, present in all cells, may serve as a reservoir of phosphoryl groups with high group transfer potential.

To produce ATP we need higher energy compounds. Cells contain other metabolites with large, negative, free energies of hydrolysis, including phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine. These high-energy compounds, like ATP, have a high phosphoryl group transfer potential. Thioesters also have high free energies of hydrolysis.



(a) High energy phosphorylated compounds

	ΔG°	
	(kJ/mol)	(kcal/mol)
Phosphoenolpyruvate	-61.9	-14.8
1,3-Bisphosphoglycerate (→ 3-phosphoglycerate + P _i)	-49.3	-11.8
Phosphocreatine	-43.0	-10.3
ADP (→ AMP + P _i)	-32.8	-7.8
ATP (→ ADP + P _i)	-30.5	-7.3
ATP (→ AMP + PP _i)	-45.6	-10.9
AMP (→ adenosine + P _i)	-14.2	-3.4
PP _i (→ 2P _i)	-19.2	-4.0
Glucose 3-phosphate	-20.9	-5.0
Fructose 6-phosphate	-15.9	-3.8
Glucose 6-phosphate	-13.8	-3.3
Glycerol 3-phosphate	-9.2	-2.2
Acetyl-CoA	-31.4	-7.5

(b) Standard free energies of Hydrolysis

Figure 21: Hydrolysis of Phosphate compounds

Remark 2.13 (Arsenate Poisoning). A toxic condition caused by exposure to arsenate (AsO_4^{3-}), in which arsenate disrupts the cellular metabolism by **mimicking inorganic phosphate**. Arsenate can uncouple oxidative phosphorylation by substituting for inorganic phosphate oxidative pathways, ATP synthesis, leading to decreased ATP production and cellular toxicity.

For example, in the presence of arsenate, the product of glyceraldehyde 3-phosphate dehydrogenase is 1-arseno-3-phosphoglycerate, which nonenzymatically decomposes to 3-phosphoglycerate and arsenate; this substrate for the phosphoglycerate kinase is therefore bypassed, which leads in **no net glycolytic synthesis of ATP**.

2.4 Biological Oxidation-Reduction Reactions

Since we need high energy compounds to produce ATP. We have to ask us but how do we produce these Hi-NRG (NRG = energy)? **The flow of electrons can do it!**

Definition 2.14 (Electromotive force (emf)). Electrons flow from a reducing agent to an oxidizing agent due to their different electron affinities. This difference in affinities is called the electromotive force. Note that the reducing agent undergoes oxidation and the oxidizing agent undergoes reduction.

Living cells have an biological "circuit", with a relatively reduced compound such as glucose as the source of electrons. As glucose is enzymatically oxidized, the released electrons flow spontaneously through a series of electron-carrier intermediates to another chemical species, such as O₂. This **electron flow is exergonic**, because O₂ has a higher affinity for electrons than the electron-carrier intermediates. This is exploited by the **ATP synthase** in the inner mitochondrial membrane that uses the proton-motive force to do chemical work.

2.4.0.1 Dehydrogenation = Oxidation

Dehydrogenation corresponds to oxidation, since the carbon is less electronegative than all atoms it is bound to except hydrogen. Note that not all oxidation-reduction reactions involve carbon, i.e conversion from nitrogen to ammonia.

There are different ways that electrons can be transferred: Directly as electrons, as hydrogen atoms, as a hydrogen ion, or through direct combination with oxygen. Since all of these 4 types occur biologically, the term **Reducing Equivalent** is used.

Electronegativity series: O > N > S > C > H

Methane		8	Acetaldehyde (aldehyde)		3
Ethane (alkane)		7	Acetone (ketone)		2
Ethene (alkene)		6	Formic acid (carboxylic acid)		2
Ethanol (alcohol)		5	Carbon monoxide		2
Acetylene (alkyne)		5	Acetic acid (carboxylic acid)		1
Formaldehyde		4	Carbon dioxide		0

Figure 22: Oxidation levels of a carbon compound in the biosphere

2.5 Electron Carriers

There are a multitude of enzymes that catalyze oxidation reactions from a variety of substrates, but most electrons end up in a **small set of univale electron carriers**, such as NAD⁺, FAD, and Q (ubiquinone).

Definition 2.15 (Electron Carriers). *Electron Carrier are Molecules that can accept and donate electrons, facilitating the transfer of energy in redox reactions (e.g., NAD⁺, FAD).*

2.5.0.1 NADH and NADPH

These **watersoluble** coenzymes (**NAD⁺ (Nicotinamide Adenine Dinucleotide)** and **NADP⁺ (Nicotinamide Adenine Dinucleotide Phosphate)**) can undergo **reversible reduction of the necotinamide ring**, as a substrate undergoes oxidation (dehydrogenation) giving up **2 hydrogen atoms**.

- NAD⁺ and NADP⁺ take **2 electrons and 1 proton** while the second proton is released into solution.

In many cells and tissues, the ratio of NAD⁺ (oxidized) to NADH (reduced) is high, favoring hydride transfer from a substrate to NAD⁺ to form NADH. By contrast, NADPH is generally present at a higher concentration than NADP⁺, favoring hydride transfer from NADPH to a substrate.

This reflects the specialized metabolic roles of the two coenzymes: **NAD⁺ generally functions in oxidations—usually as part of a catabolic reaction; NADPH is the usual coenzyme in reductions—nearly always as part of an anabolic reaction.**

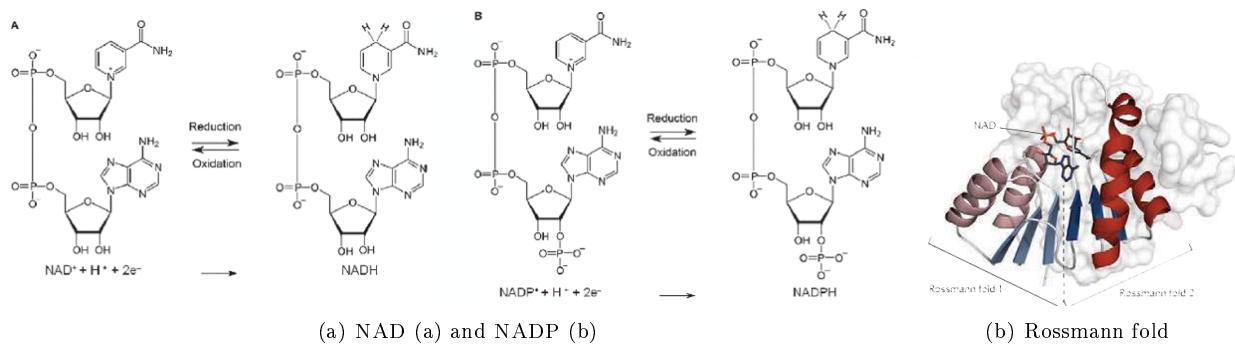


Figure 23: NADH and NADPH

Remark 2.16 (Dietary Deficiency of Niacin (Vitamin B3) cause Pellagra). NAD and NADP are derived from the Niacin (Vitamin B3), which is synthesized from tryptophan. Humans do generally not synthesize sufficient quantities of niacin, and this is especially so for individuals with diets low in tryptophan (diets based on maize for example). This leads to the disease pellagra.

But note since NAD⁺ is can oxidize many thousands of molecules of glucose, since it the reduction always can be reversed. It is not necessarily to consume a lot of the vitamin, in contrary to glucose for example.

2.5.0.2 FAD and FMN

FAD (Flavin Adenine Dinucleotide) and FMN (Flavin Mononucleotide) are coenzymes that are used in oxidation-reduction reactions by **Flavoprotein**. They are **tightly bound to their enzymes**, in contrast to NAD and NADP. Moreover flavin nucleotides can also only carry one electron and one proton taking on the partially reduced form.

- FAD and FMN take **1 or 2 electrons and 1 or 2 protons** and remain tightly linked to the enzyme.

The ability to take on the partially reduced form is crucial for FAD and FMN, since they are **key elements in electron transport reactions** (e.g. **ETC Complex I and II**), where electrons are passed one at a time.

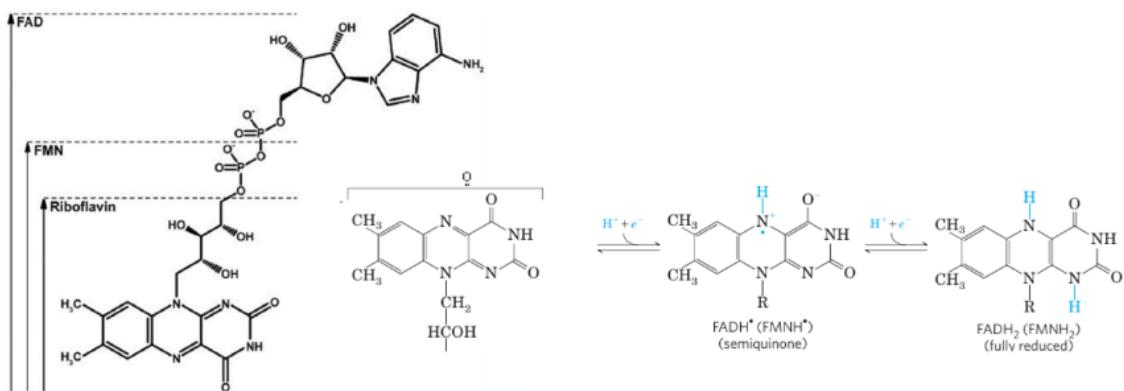


Figure 24: Flavin Nucleotides

These Flavin nucleotides are derived from the **vitamin riboflavin (B2)**

2.5.0.3 Ubiquinone, Q

Ubiquinone (Coenzyme Q) is a **lipid-soluble** electron carrier in the electron transport chain, transferring electrons between complex I/II and complex III. Ubiquinone exists in oxidized (Q), semiquinone (Q^-), and reduced (QH_2) forms.

- Coenzyme Q takes **1 electron or 2 electrons and 2 protons** and freely diffuses through the mitochondrial membrane.

3 Metabolism

Metabolism is the sum of all biochemical reactions in a living organism. The metabolism can be divided into two main pathways: catabolism and anabolism.

3.1 Catabolism \leftrightarrow Anabolism

Catabolism from greek meaning "breaking down" (Kata refers to down and bolë means to throw) is the process of breaking down complex molecules into simpler ones, **releasing/ producing energy in the form of ATP**. Examples include glycolysis, the Krebs cycle, and oxidative phosphorylation, which break down glucose and fatty acids to produce ATP. *Note catabolic pathways are mostly converging*

In contrast, **Anabolism** from Greek meaning "building up" (Ana means up or again) is the synthesis of complex molecules from simpler ones, **requiring energy**. This process is essential for growth, repair, and maintenance of cells. Examples include protein synthesis, DNA replication, and lipid biosynthesis. *Note anabolic pathways are mostly diverging*

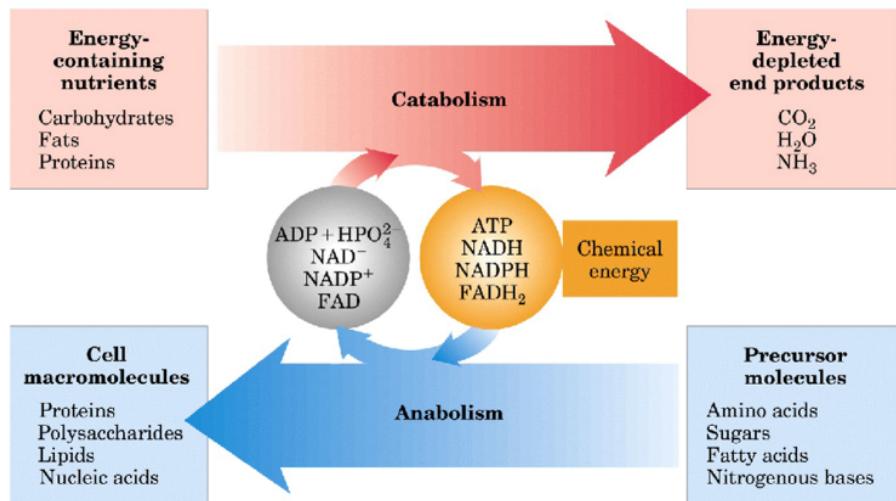


Figure 25: Catabolism \leftrightarrow Anabolism

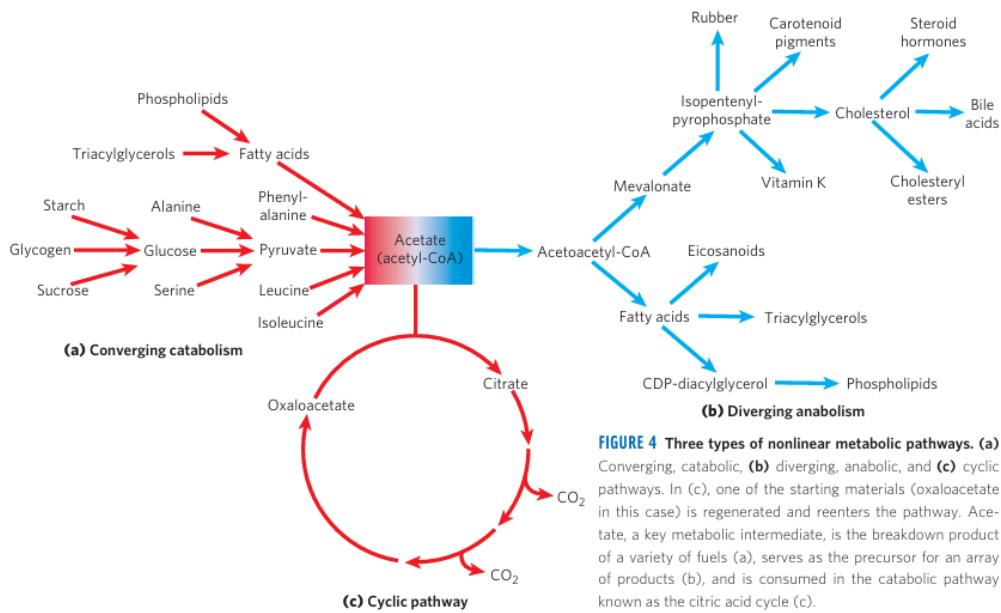


FIGURE 4 Three types of nonlinear metabolic pathways. **(a)** Converging, catabolic; **(b)** diverging, anabolic, and **(c)** cyclic pathways. In (c), one of the starting materials (oxaloacetate in this case) is regenerated and reenters the pathway. Acetate, a key metabolic intermediate, is the breakdown product of a variety of fuels (a), serves as the precursor for an array of products (b), and is consumed in the catabolic pathway known as the citric acid cycle (c).

Figure 26: Three types of nonlinear metabolic pathways

4 Catabolism

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

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

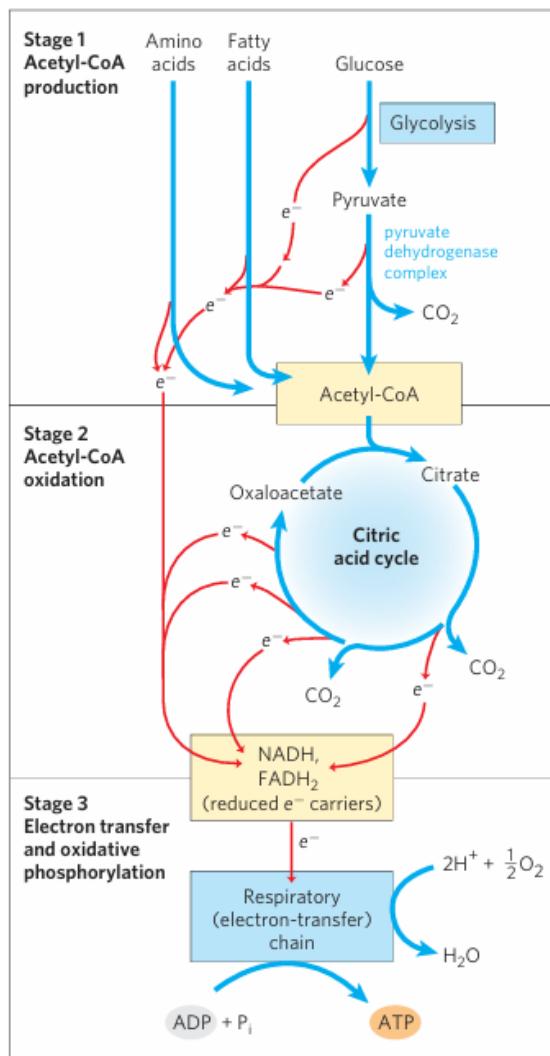


Figure 27: 3 stages of cellular respiration

4.1 Glycolysis

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

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

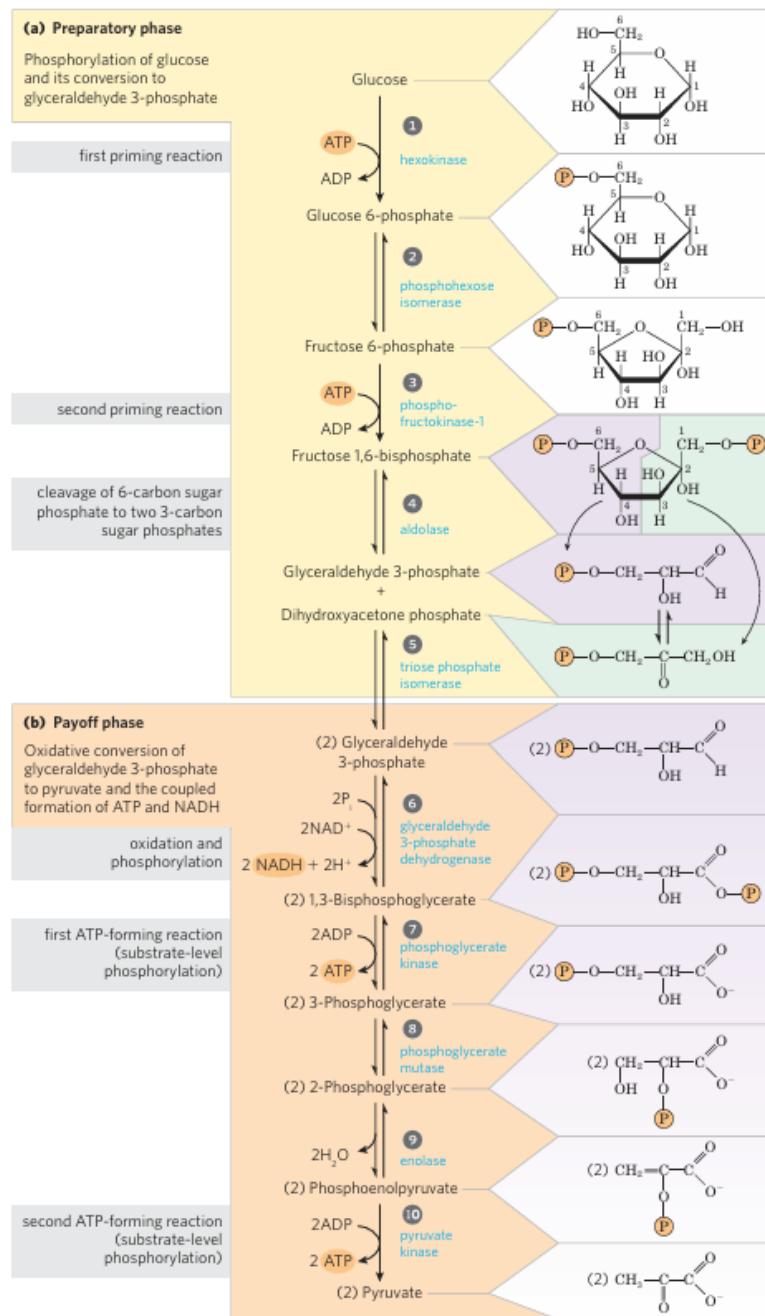


Figure 28: Glycolysis

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

4.1.0.1 Carbon labeling

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

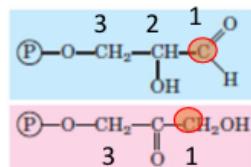
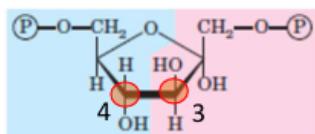


Figure 29: Carbon labeling

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

4.1.1 Stage 1, Preparation Phase

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

4.1.1.1 Step1: Phosphorylation of Glucose

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

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

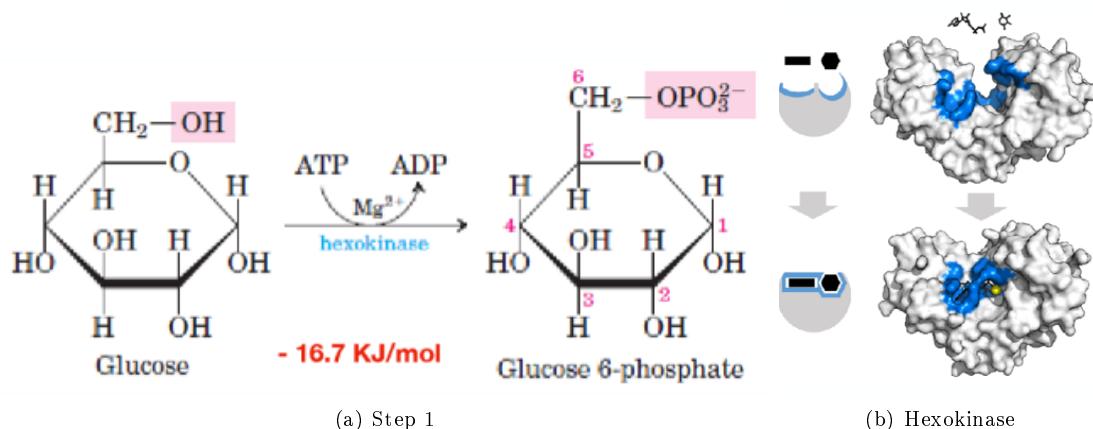


Figure 30: Phosphorylation of Glucose

Remark 4.1 (Hexokinase (HK)). Hexokinase is an enzyme that phosphorylates hexoses (like glucose) using ATP. Like most kinases it requires the presence of the cofactor Mg²⁺ in the active site.

The movement of Glucose into HK active site causes a conformational change whereby two HK lobes rotate by 12 degrees (10 \AA) creating an **induced fit**. This makes the **carbon 6 oriented towards ATP** and squeezes out water molecules. (see fig. 30(b))

4.1.1.2 Step2: Isomerization

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

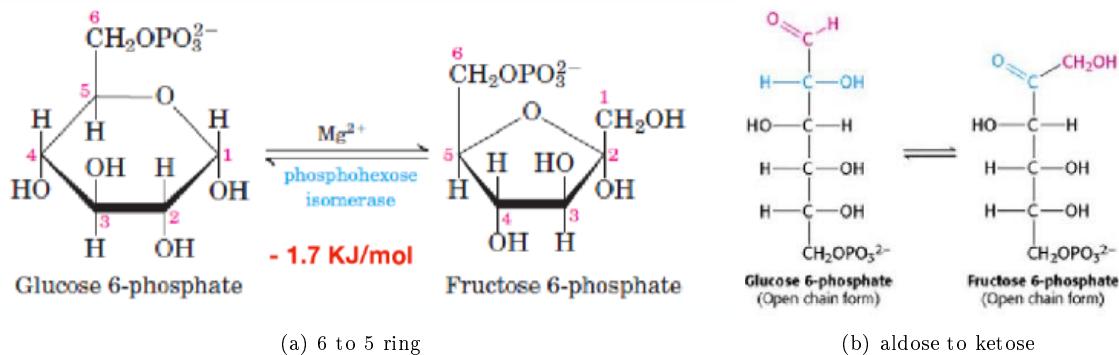


Figure 31: Isomerization

4.1.1.3 Step3: Second phosphorylation

The enzyme **phospho-fructo kinase-1 (PFK-1)** turns Fructose 6-phosphate into Fructose 1,6-biphosphate, completing the symmetry and making the compound even more reactive. This is again paid with the **investment of 1 ATP**. (see fig. 32(a))

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

4.1.1.4 Step4: Breakdown of Fructose 1,6-biphosphate

Aldolase catalyses the breakdown of Fructose 1,6-biphosphate into 2 different three-carbon molecules (**GA3P** and **DHAP**).

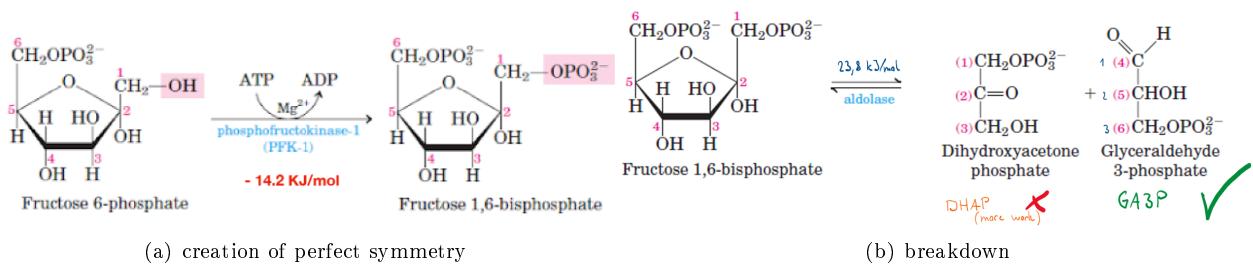


Figure 32: Step3 and Step4

GA3P feeds directly in the glycolytic pathway without any further change while DHAP needs to be first transformed. This is archived by Step5.

4.1.1.5 Step5: Isomerisation of DHAP to GA3P

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

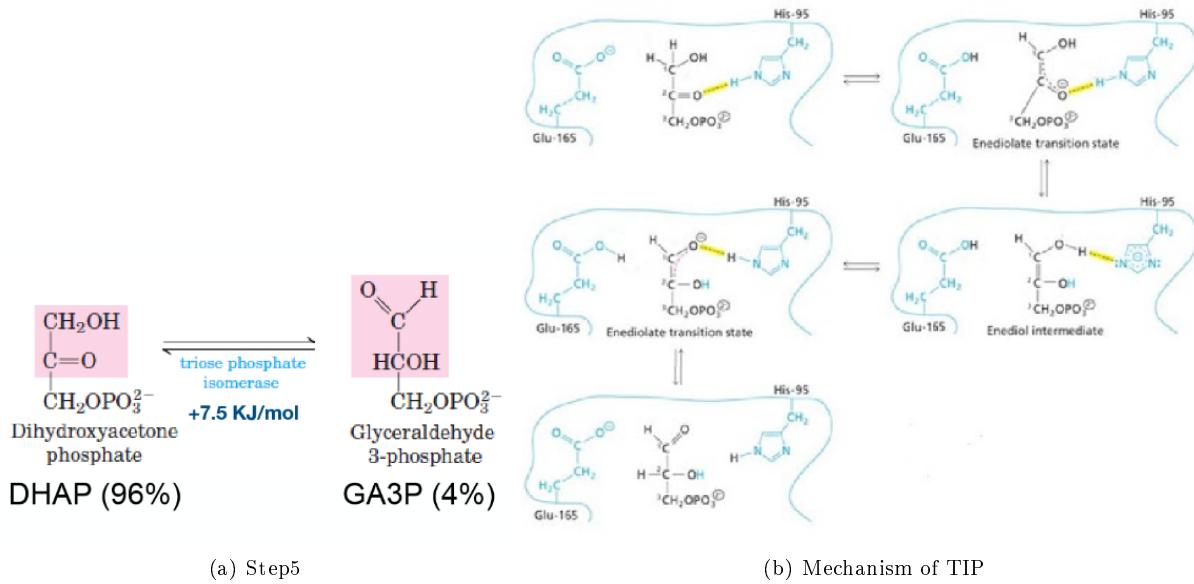


Figure 33: Isomerisation of DHAP to GA3P

Even though TIP increases the rate by 10 billion fold the equilibrium still lies on the unwanted side of DHAP (the **reaction is unfavorable, endergonic**). But since the reaction is coupled to an exergonic reaction that removes GA3P, thus via le chatelier **the reactions shifts to the side of the product GA3P**.

4.1.2 Stage 2, Payoff Phase

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

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

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

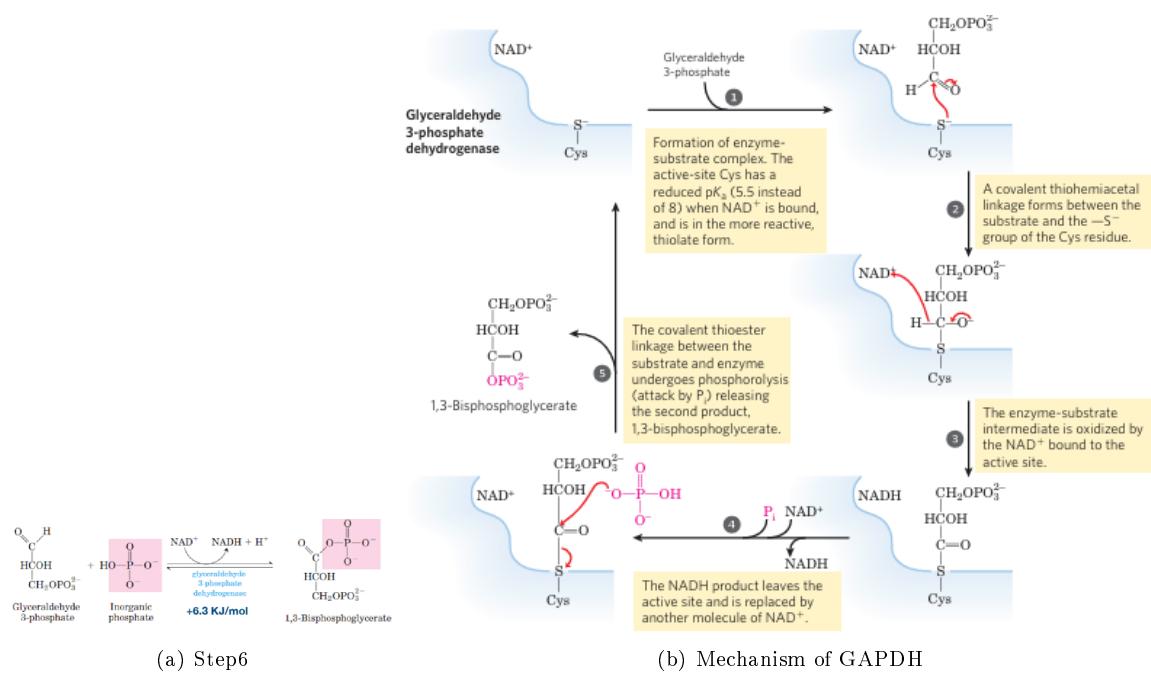


Figure 34: Conversion of GA3P to 1,3-BPG

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

Step7 is the **break-even point**. 1, 3-BPG is used as a phosphate doner to ADP. This reaction is catalyzed by **glycerophosphate kinase** and produces 3-Phosphoglycerate and ATP. (see fig. 35(a))

4.1.2.3 Step8: Conversion to 2-Phosphoglycerate

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

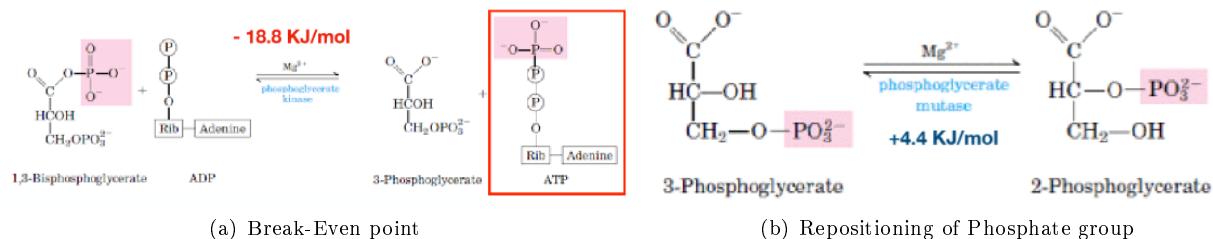


Figure 35: Step7 and Step8

4.1.2.4 Step9: Conversion to Phosphoenolpyruvate (PEP)

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

4.1.2.5 Step10: Conversion to Pyruvate

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

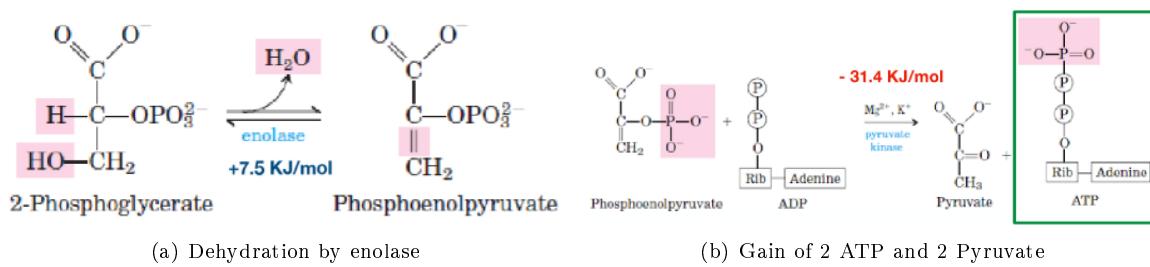


Figure 36: Step 9 and Step 10

4.1.3 The fates of Pyruvate

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

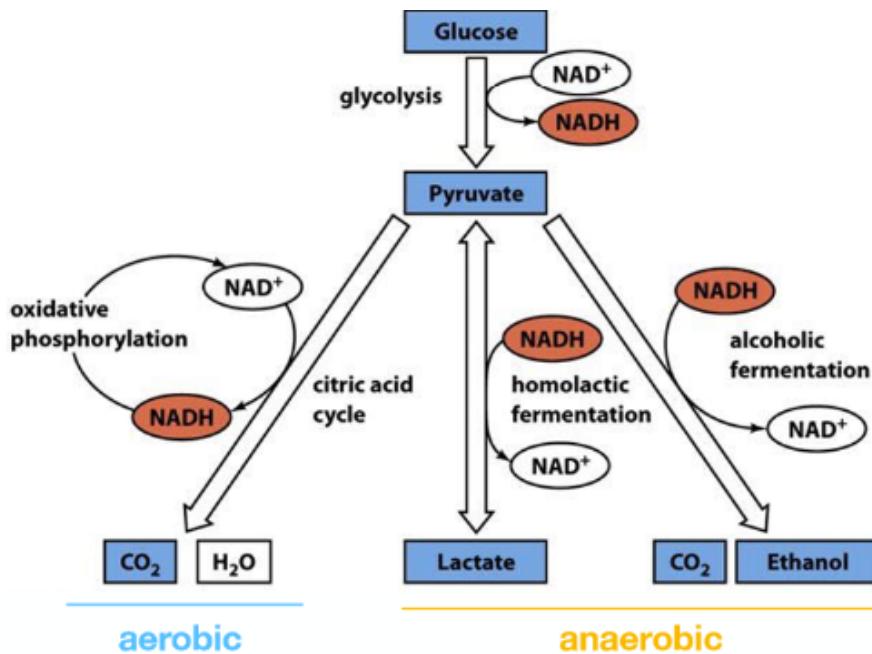


Figure 37: The fates of Pyruvate

Definition 4.2 (Facultative Anaerobic Organism). A Facultative Anaerobic Organism is able to produce ATP by anaerobic respiration if oxygen is present, but is also capable of switching to fermentation if oxygen is absent. For example *E.coli* or some muscle cells (temporarily in humans).

Remark 4.3 (Soy Sauce). Soy sauce is produced by fermenting a salted mixture of soy beans. Soybeans contain starch which will be broken down to glucose and then degraded via glycolysis to pyruvate. And the ferment in the absence of oxygen. However if oxygen were present pyruvate would be oxidized to acetyl-CoA entering the citric acid cycle. But some acetyl-CoA would get hydrolyzed to acetic acid (vinegar) which would result in a undesired strong vinegar taste.

4.1.3.1 Ethanol Fermentation

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

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

- Note, that the **C3 & C4 carbons of glucose will be cut away** in form of CO₂. (glucose -> 2 pyruvate)

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

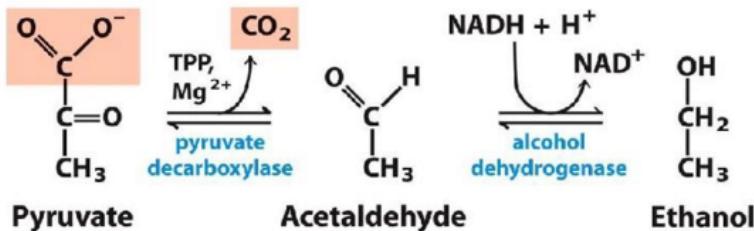


Figure 38: Ethanol Fermentation

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

4.1.3.2 Lactic Fermentation

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

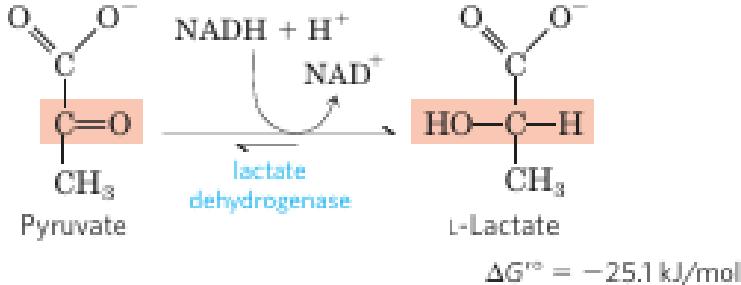


Figure 39: Lactic Fermentation

Remark 4.4 (Cancer, PET scan). Cancer cells often rely on aerobic glycolysis, known as the **Warburg effect**, where they preferentially use glycolysis followed by lactic acid fermentation, even in the presence of oxygen. This allows them to rapidly generate ATP and biosynthetic precursors for growth. Positron Emission Tomography (PET scans) exploit this metabolic shift by using **fluorodeoxyglucose (FDG)**, a radiolabeled glucose analog, that will not undergo full glycolysis (thus accumulate). Since cancer cells have a higher glucose uptake due to increased glycolysis, they accumulate FDG, which emits positrons detectable by **PET imaging**.

4.2 TCA cycle

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

Remark 4.5 (TCA cycle). The TCA cycle (TCA = tricarboxylic acid) is also called **Krebs cycle** or **citric acid cycle**

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

4.2.0.1 Pyruvate \Rightarrow Acetyl-CoA

Remark 4.6 (pyruvate translocase). Once produced in the cytosol, pyruvate migrates into the mitochondrial matrix through the action of pyruvate translocases that mediate the transport of pyruvate across mitochondrial membranes.

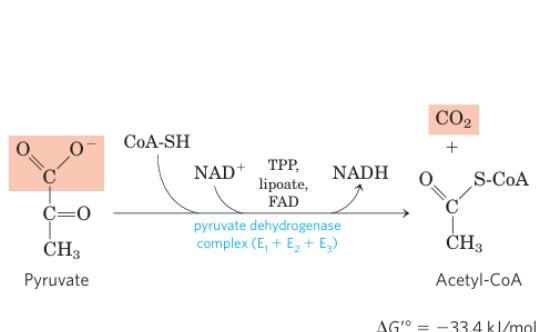
In the mitochondria pyruvate is converted to acetyl-CoA in order to enter the TCA cycle. This is done by the **pyruvate dehydrogenase complex** which catalyses an **oxidative decarboxylation**, an **irreversible** oxidation process in which the carbonyl group is removed from pyruvate as a molecule of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

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

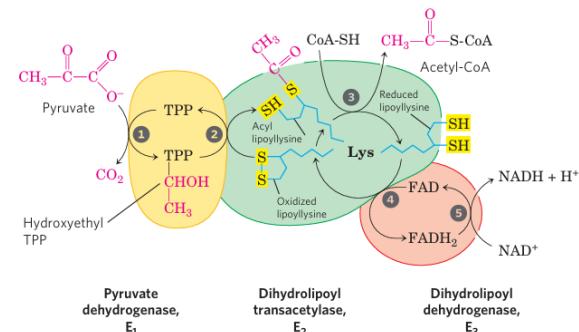
- E1 - Pyruvate dehydrogenase: Catalyses the redox-decarboxylation reaction.
- E2 - Dihydrolipoyl transacetylase: Catalyses the transfer of the acetyl group.
- E3 - Dihydrolipoyl dehydrogenase: Reforms the oxidised version of lipoamide.

Moreover 5 different co-enzymes are acting across the 5 different steps (see fig. 40(b)):

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



(a) Overall reaction



(b) PDH complex

Figure 40: Pyruvate + CoA-SH + NAD⁺ \Rightarrow Acetyl-CoA + CO₂ + NADH + H⁺

Remark 4.7 (pyruvate dehydrogenase complex (PDH complex or PDC)). PDH complex is a classic, much-studied example of a multi-enzyme complex in which a series of chemical intermediates remain bound to the enzyme molecules as a substrate is transformed into the final product. It has **3 important enzymes** and uses **5 different co-enzymes**, four derived from vitamins, participate in the reaction mechanism.

Moreover, the **PDH complex is the prototype** (evolutionarily) for two other important complexes: α -ketogutarate dehydrogenase, of the TCA cycle, and α -keto acid dehydrogenase, of the oxidative pathway of several amino acids.

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

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

- Acetyl-CoA and NADH, two products, **inhibit** (allosterically) **E2 and E3** respectively.

Under resting conditions, the energy charge of the cell is high (high acetyl-CoA, NADH, ATP). These molecules promote the activity of **PDC kinases (PDKs)** that phosphorylate and inactivate E1.

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

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

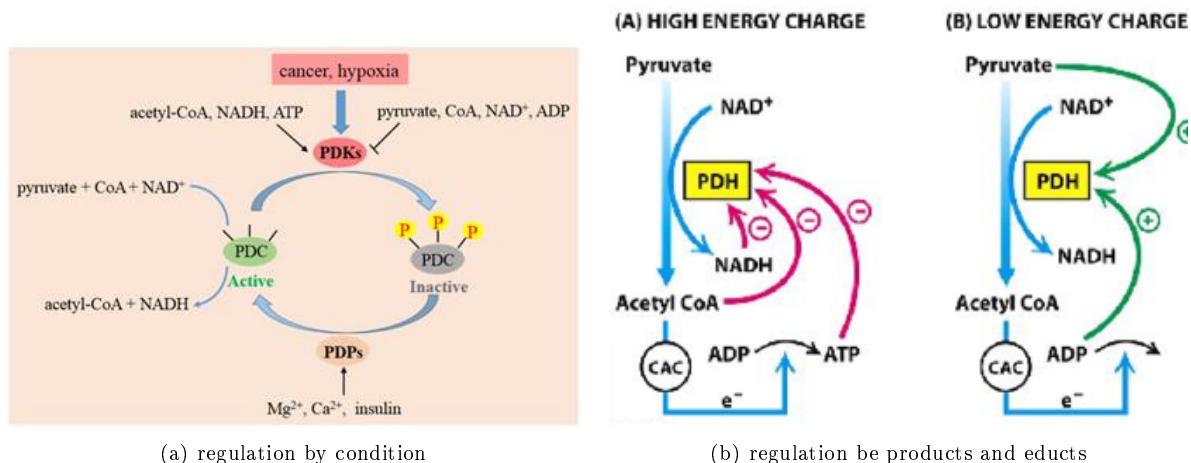


Figure 41: Regulation of PDC/PDH

4.2.1 TCA cycle steps

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

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

In the bigger picture, this is where the CO₂ is produced that we breathe out.

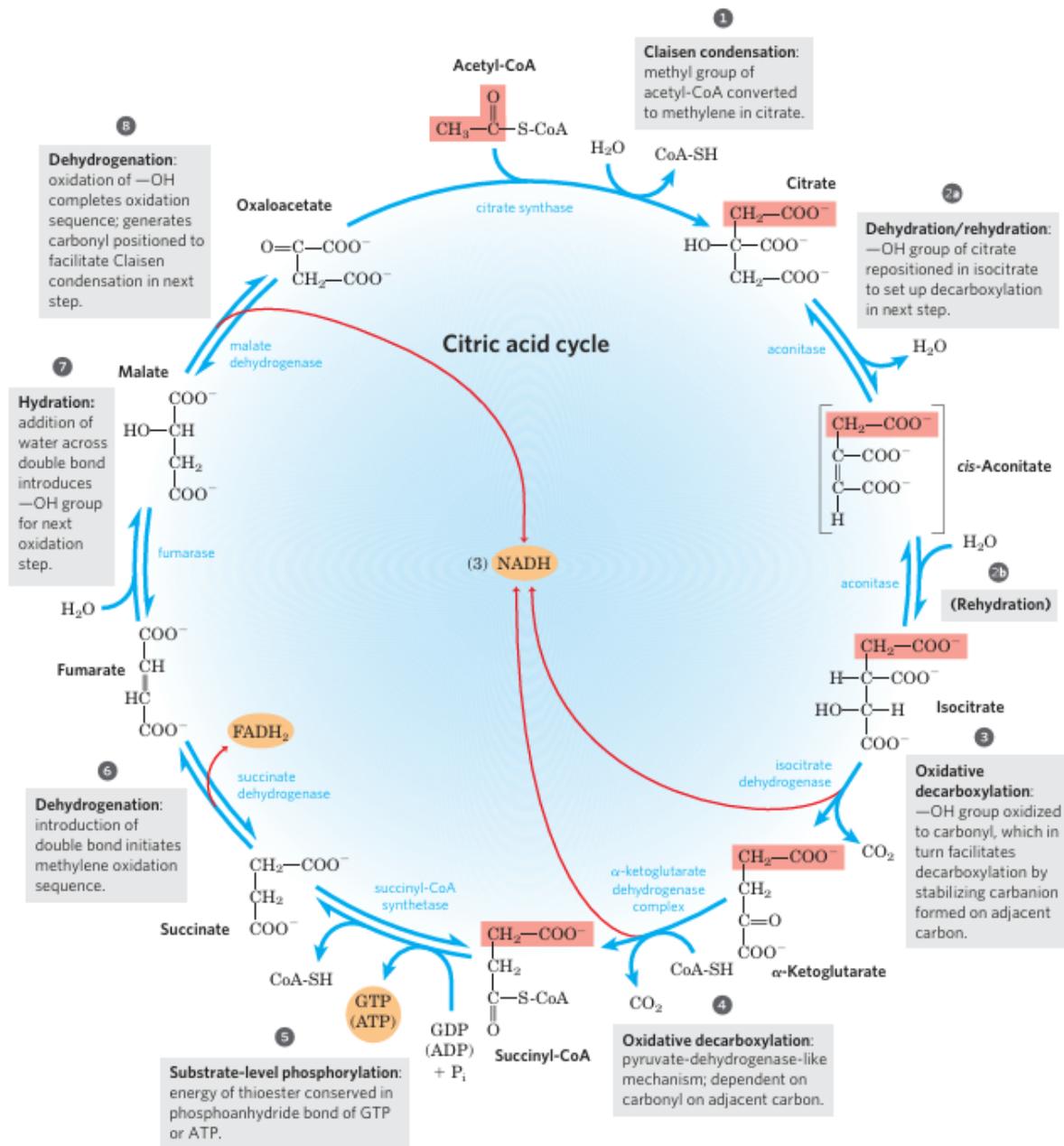


Figure 42: Overview of the TCA cycle: The carbons shaded in red are those derived from the acetate of acetyl-CoA in the first turn.

Remark 4.8 (What happens to 3-14C-pyruvate). The Resulting Acetyl-CoA will be labeled at C2 (methyl group). Therfore, at the end of the first cycle: Oxaloacetate will be labelled in C2 or C3 (because of symmetry in succinate). After two more cycles, half of the label will be released. (the label of C2 ends up at the second cycle at C2 or C3, while the lable on C3 ends up at C4, which is released in the following clycle) But il will take an infinite number of cycles to release all the label.

4.2.1.1 Step1: Formation of Citrate

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

Remark 4.9 (Citrate synthase). Step 1 is carried out by citrate synthase. Citrate synthase is a **dimer**. It first binds oxalacetate into its active site, which causes a conformational change from open to closed conformation. By doing so oxalacetate binding induces the formation of the acetyl-CoA binding site and shifts the catalytic residue into proper position.

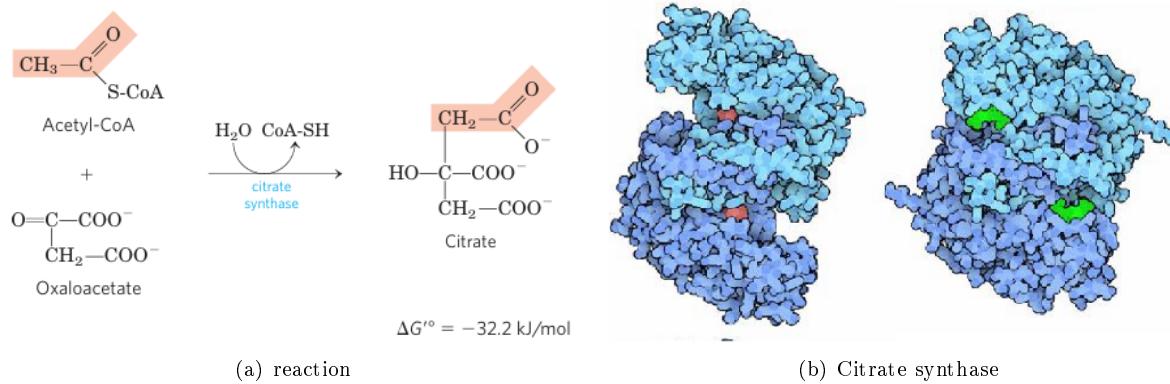


Figure 43: Formation of Citrate

4.2.1.2 Step2: Formation of Isocitrate

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

Remark 4.10 (Aconitase enzyme). Aconitase enzyme catalyzed this dehydration/hydration reaction. It contains a **Iron-Sulfur center**. 3 Cys residues of the enzyme bind 3 Fe atoms, the 4th is bound to one of the carboxyl groups of citrate and (non covalently) with a citrate hydroxyl group. **The Iron-Sulfur center acts in both substrate binding and catalysis.**

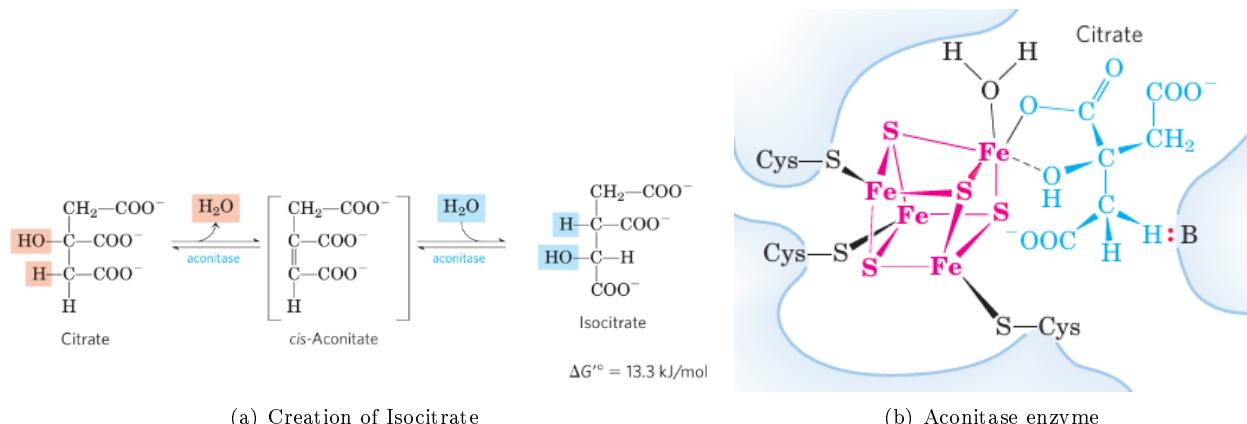


Figure 44: Formation of Isocitrate via cis-Aconitate

4.2.1.3 Step3: Decarboxylation of Isocitrate

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

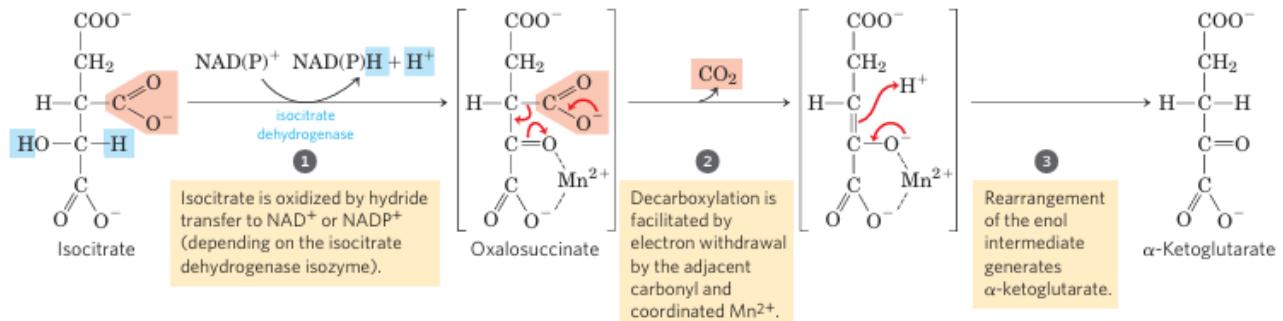


Figure 45: formation of α -ketoglutarate by isocitrate dehydrogenase

4.2.1.4 Step4 Decarboxylation of α -ketoglutarate

This is the **second oxidative decarboxylation reaction** in the TCA cycle. α -ketoglutarate is converted to succinyl-CoA with concomitant (at same time) production of **CO₂** and **NADH**. Note that the energy of the oxydation is conserved in the thioester bond.

Remark 4.11 (α -ketoglutarate dehydrogenase complex). α -ketoglutarate dehydrogenase complex is build very similar to the pyruvate dehydrogenase complex. Like PDC it has 3 has 3 important enzymes:

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

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

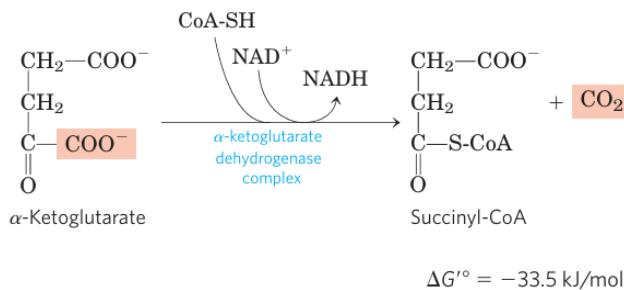


Figure 46: oxidation of α -Ketoglutarate to Succinyl-CoA and CO₂

4.2.1.5 Step5: Conversion of Succinyl-CoA to Succinate

The unstable and high energy thioester bond of succinyl-CoA is cleaved to release the CoA-SH unit. This releases free energy that is used to produce GTP from GDP. This reaction is catalyzed by **succinyl CoA synthetases**.

The reaction has 3 phases as illustrated by fig. 47(b):

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

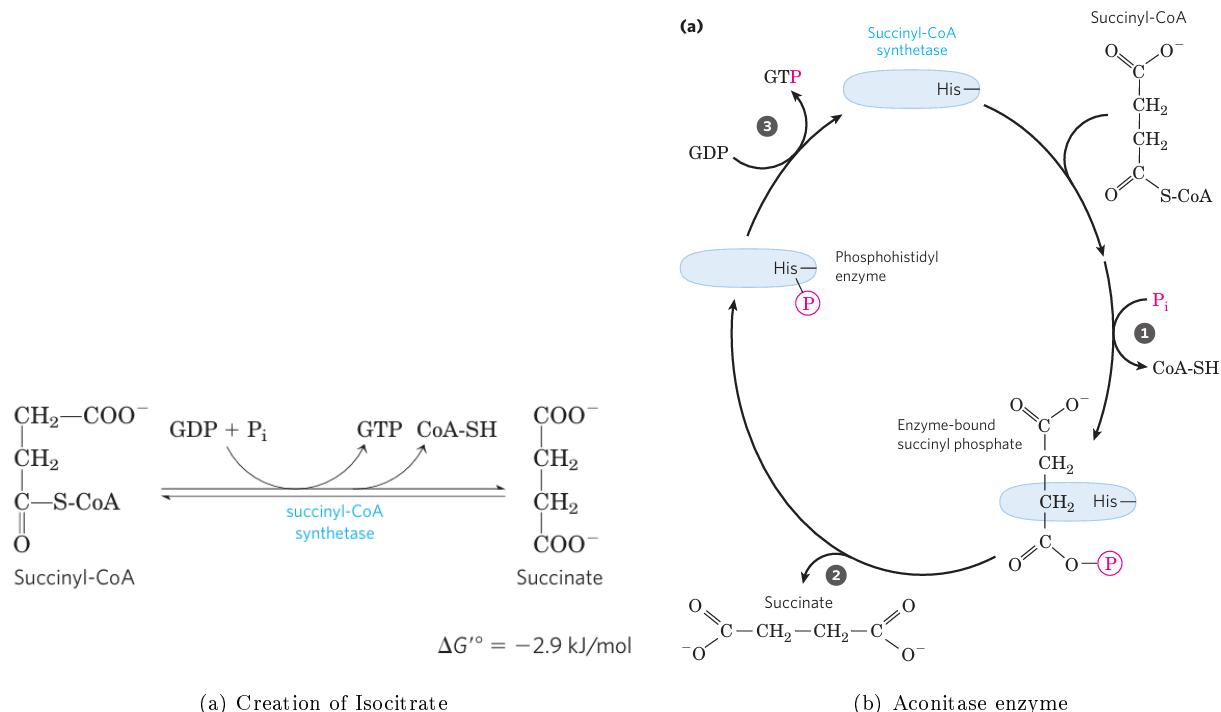


Figure 47: Conversion of Succinyl-CoA to Succinate

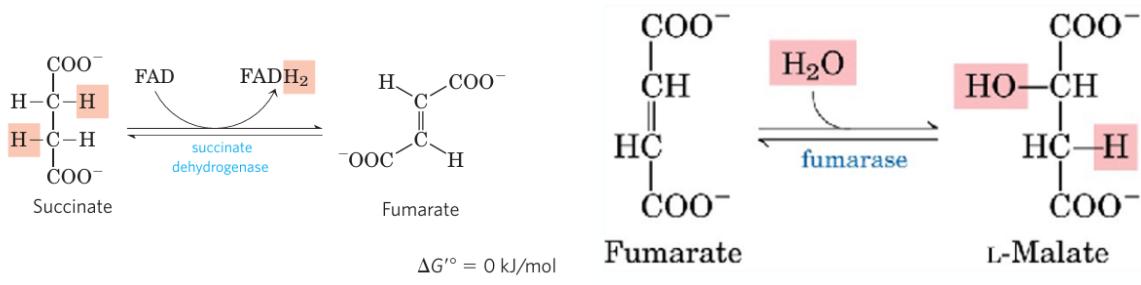
4.2.1.6 Step6: Formation of Fumerate

In step 6, succinate is oxidised to fumerate by succinate dehydrogenase. This is coupled to the reduction of FAD to FADH₂. See fig. 48(a)

Remark 4.12 (succinate dehydrogenase). Succinate dehydrogenase is bound to the inner mitochondrial membrane and its FADH₂ passes electrons to the electron transport chain.

4.2.1.7 Step7: Formation of Malate

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



(a) Oxidation of Succinate to Fumarate

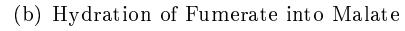


Figure 48: Step 6 and 7

4.2.1.8 Step8: Regeneration of Oxaloacetate

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

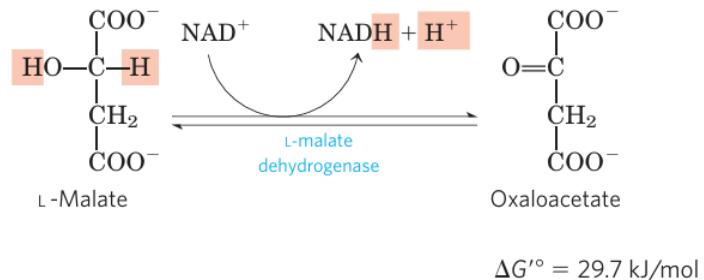


Figure 49: Oxidation of Malate to Oxaloacetate

4.3 Fatty Acid Oxidation

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

Following this step **fatty acids** can be **activated (bound to CoA, see. fig. 52(a))** and transported to the mitochondrial matrix of cells. In the mitochondrial matrix they iteratively undergo a series of **4 reactions** that shorten the acyl chain by 2 carbons and release acetyl-CoA (that can feed in the TCA cycle)

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

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

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

Remark 4.13 (Triacylglycerols better than Polysaccharides). Triacylglycerols contain **more energy per gram than polysaccharides**. Moreover, they are unhydrated, thus the organism does not have to carry the water along.

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

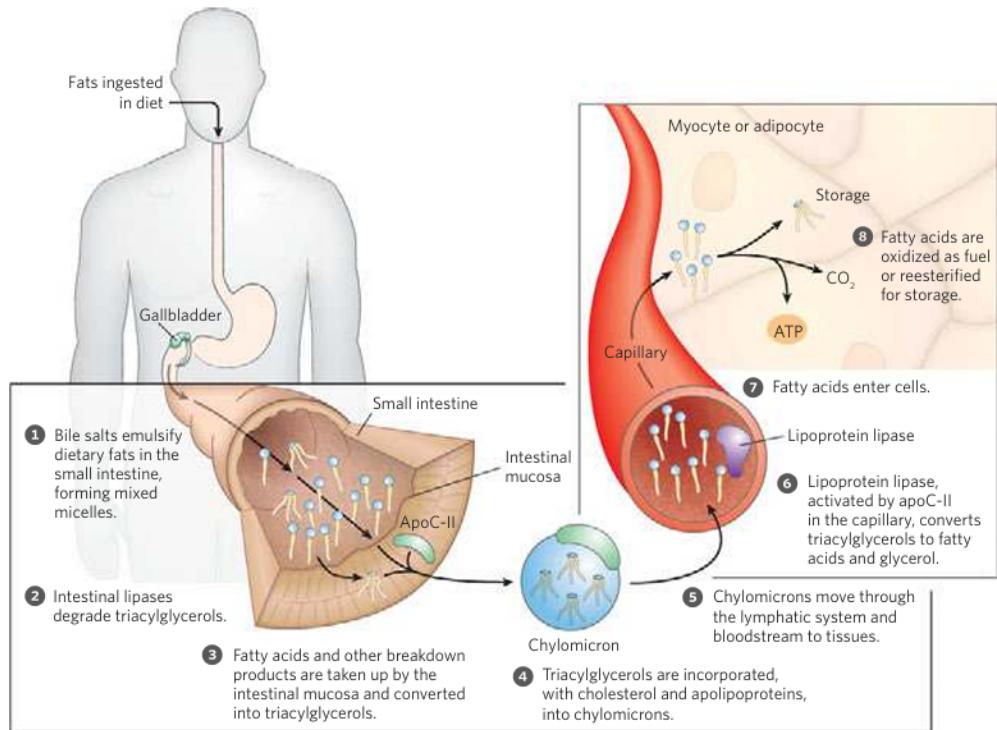
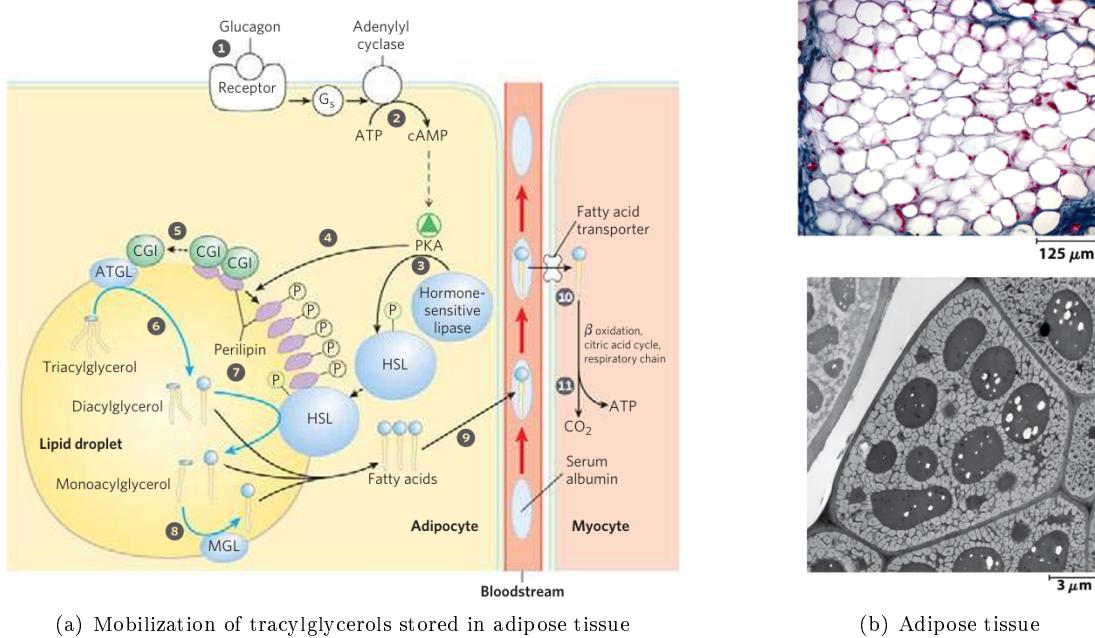


Figure 50: Processing of dietary lipids in vertebrates

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



(a) Mobilization of triglycerols stored in adipose tissue

(b) Adipose tissue

Figure 51:

4.3.0.1 What about glycerol?

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

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

Remark 4.14 (How much ATP do we get from glycerol in the glycolytic pathway?). First we have to invest 1 ATP to produce DHAP entering the glycolytic pathway. We enter the payoff phase of glycolysis where we produce 2 ATP. Giving us a net of 1 ATP produced, additionally we still have the energy of 1 pyruvate and 2 NADH.

4.3.0.2 Transport into the Mitochondria

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

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

Fatty acyl-CoA synthetases have a **two phase mechanism** (see fig. 52(a)):

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

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

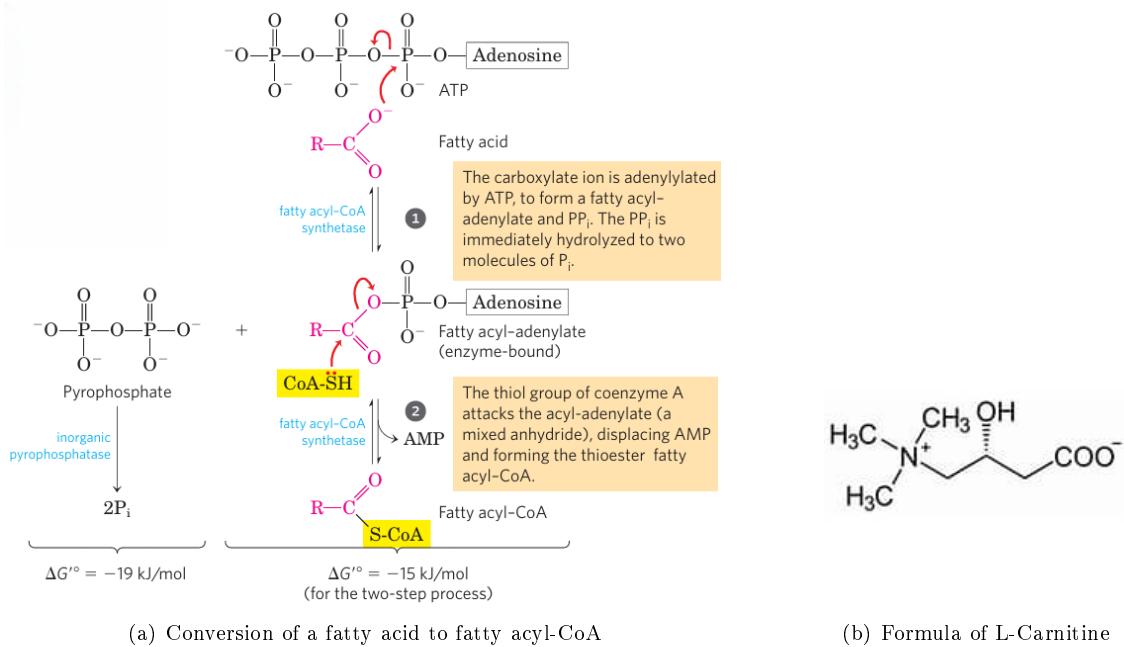


Figure 52:

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

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

Definition 4.15 (Carnitine). Carnitine (see. fig 52(b)) is a quaternary ammonium compound. Carnitine forms a temporary conjugate with acyl groups (as acyl-carnitine), enabling them to cross the inner mitochondrial membrane via the carnitine shuttle system.

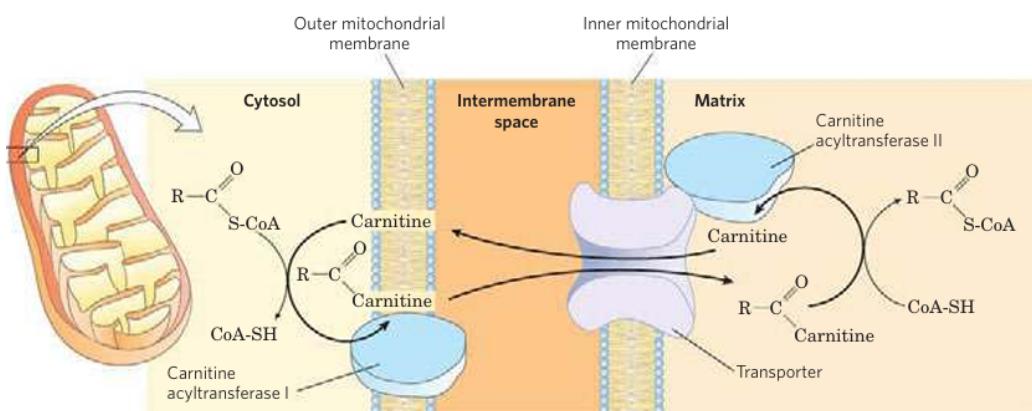


Figure 53: Fatty acid entry into mitochondria via acyl-carnitine/carnitine transporter

Remark 4.16 (Primary Carnitine deficiency). Primary carnitine deficiency is an autosomal recessive disorder affecting transport of carnitine. Since carnitine is essential for the transport of long-chain fatty acyl-CoA into the mitochondria, this disorder results in reduced fatty acid-derived energy production, leading to symptoms such as **fatigue**. Moreover, impaired import of fatty acids into mitochondria causes their accumulation in the cytoplasm (**lipotoxicity**), which can disrupt organ function. In the heart, this may result in cardiomyopathy.

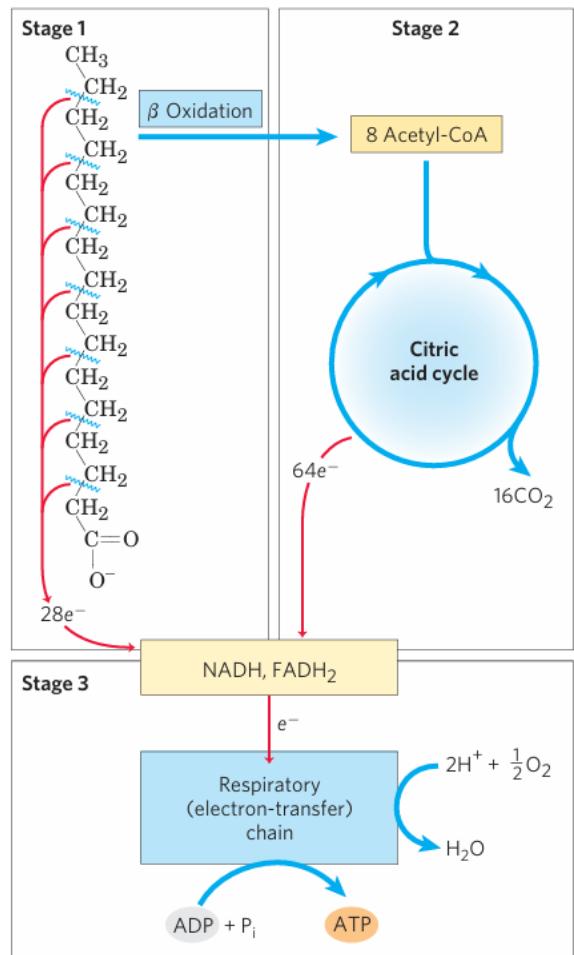
4.3.1 Beta oxidation

There are 3 stages in the oxidation of FAs (see fig. 54(a)). The **β -oxidation** is the first stage, where, in the mitochondrial matrix, fatty acetyl-CoA is progressively oxidised by an **iterative sequence of four reactions that produce acetyl-CoA**

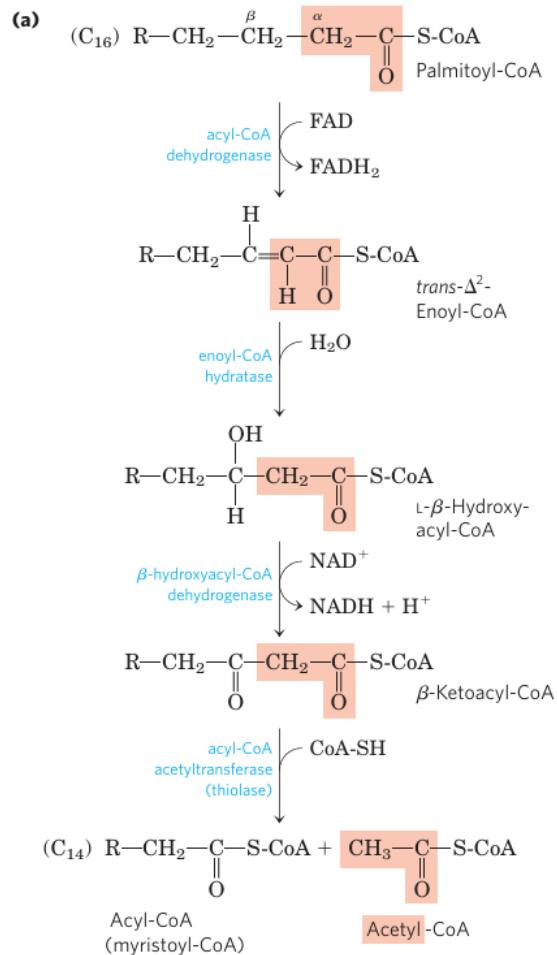
The acetyl-CoA units formed in stage 1 can then be feed in the TCA cycle (stage 2). Finally, the electrons subtracted in these oxidative reactions are used to produce ATP in the ETC.

There are 4 reactions involved in β -oxidation (see fig. 54(b)):

- i) Dehydrogenation produces a double bond between the α - and β -carbon (C2 and C3). This is catalyzed by **acyl-CoA dehydrogenase** (similar to succinate dehydrogenase from TCA cycle) and produces **1 FADH₂**.
- ii) Hydration, adding water to the α - β double bond. The hydroxy group is added to C3, producing β -Hydroxyacyl-CoA. This is done by **enoyl-CoA hydratase**
- iii) Dehydrogenation, oxidizing the alcohol group to a keton, produces β -Ketoacyl-CoA. This is done by **β -hydroxyacyl-CoA dehydrogenase**, whose action is homologous to that of malate dehydrogenase in the TCA cycle. The electrons are transferred to NAD⁺ creating **NADH**
- iv) Finally the chain is shortened by a thiolase which **detaches Acetyl-CoA** and creates a by 2 carbon shorter Acyl-CoA. This is catalyzed by **acyl-CoA acetyl transferase**.



(a) Stages of FA oxidation



(b) β -oxidation pathway

Figure 54: FA oxidation overview

Remark 4.17 (Unsaturated FAs). In the case of unsaturated fatty acids, two more enzymes are required (see fig. 55(a)):

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

Remark 4.18 (Odd-number FAs). Although the vast majority of FA are constituted by an even number of carbons. Odd-number FAs exist and need to be degraded in the same way, but at the **last iteration** of β -oxidation, they yield **propionyl-CoA** instead of acetyl-CoA.

The enzyme **propionyl-CoA carboxylase** is used to produce D-methylmalonyl-CoA, which can be converted through two further reactions to succinyl-CoA. **Succinyl-CoA** can then enter the TCA cycle. See fig. 55(b)

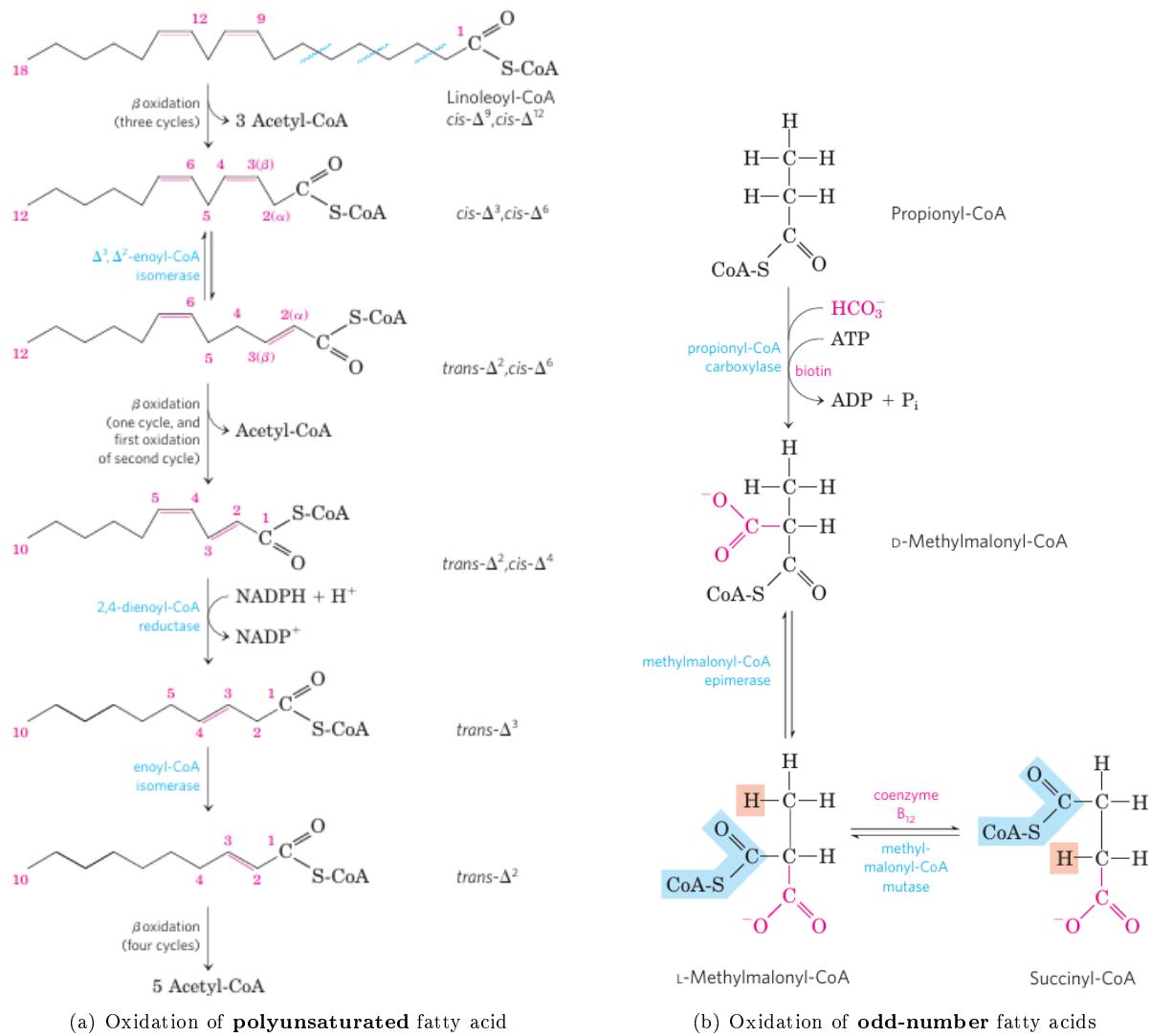


Figure 55: FA oxidation overview

4.3.1.1 Calculate amount of ATP from a FA of given length

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

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

Number of β -oxidation cycles:

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

Each β -oxidation cycle produces:

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

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

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

Activation of FA to Co-A costs 2 ATP.

Total ATP yield:

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

If we have an odd number of carbons:

Account for Propionyl-CoA \rightarrow Succinyl-CoA:

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

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

ATP Yield from a Polyunsaturated Fatty Acid:

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

Where:

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

4.4 Amino Acid Catabolism

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

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

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

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

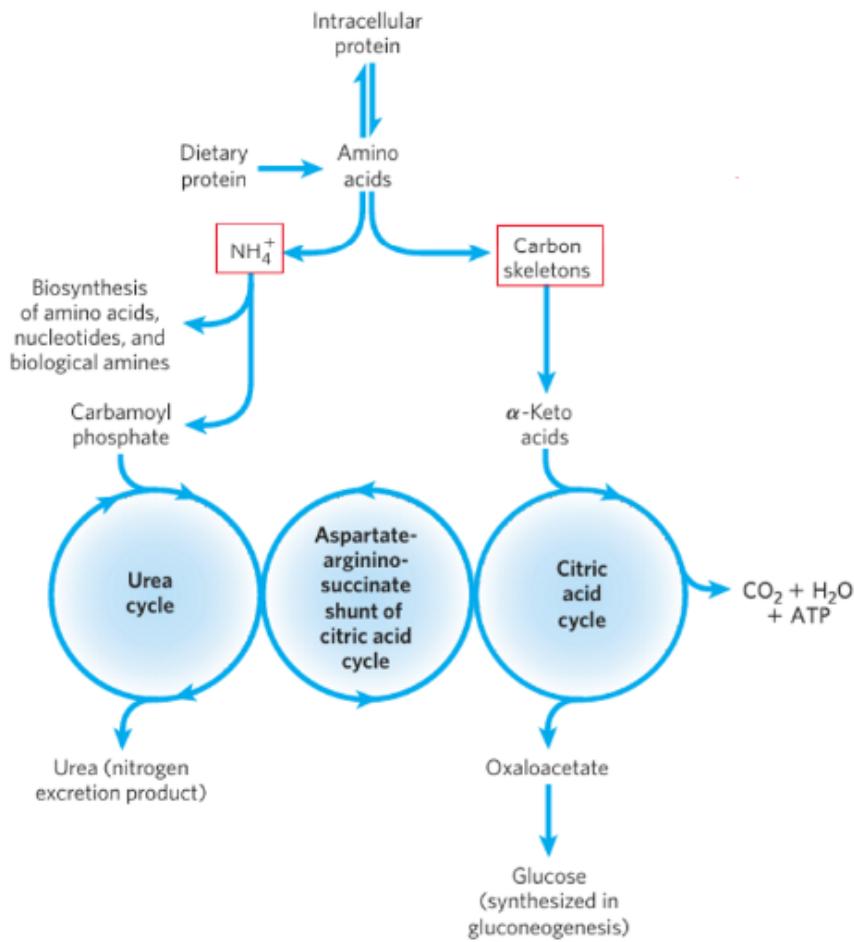


Figure 56: Overview of amino acid catabolism in mammals

4.4.1 AA oxidation and Urea production

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

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

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

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

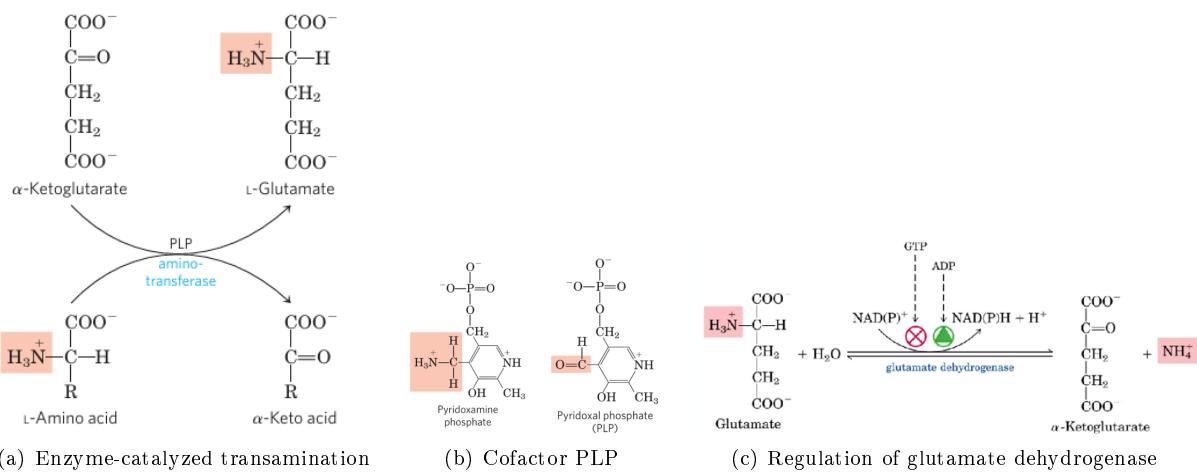


Figure 57: Amino acid oxidation and urea production

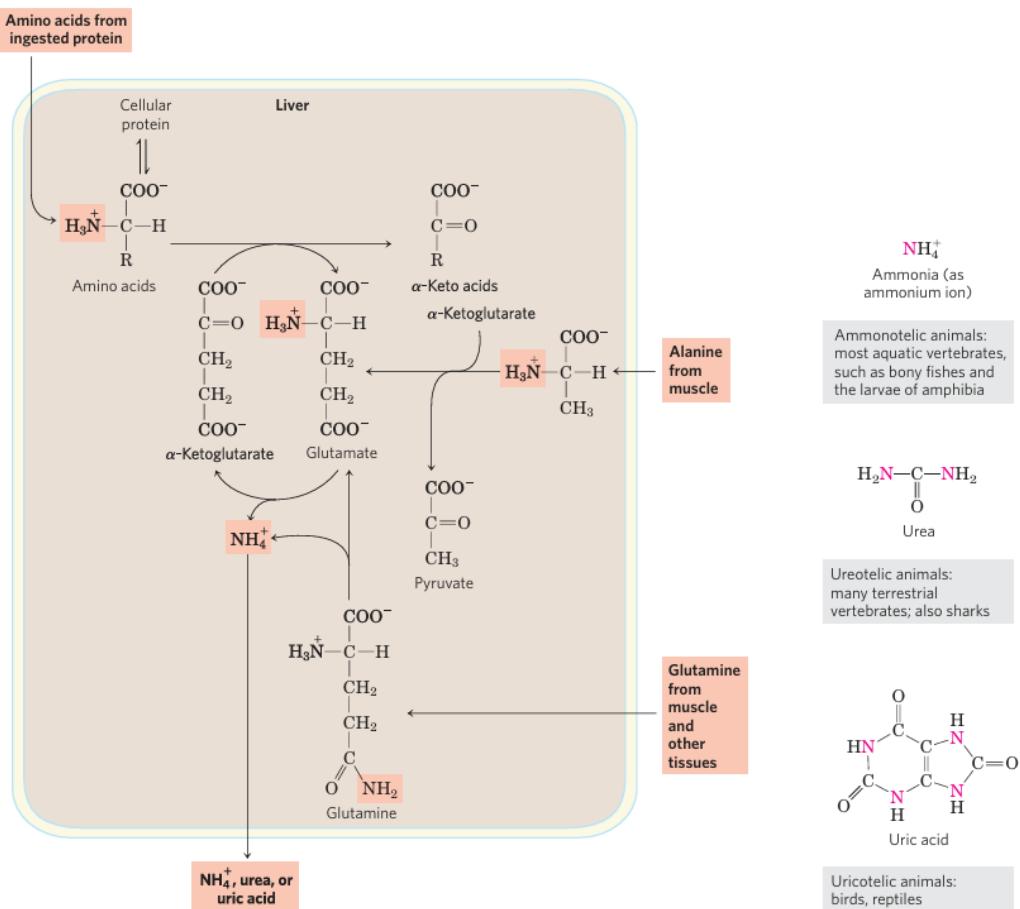


Figure 58: Amino group catabolism

4.4.1.1 Ammonia transport

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

In most tissues ammonia is therefore complexed to glutamate to produce glutamine. This is done by the enzyme **glutamine synthetase** and requires **ATP**. See fig. 59(a)

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

Once in the liver's mitochondria this is reversed by **glutaminase**. See fig. 59(a)

In the **muscle** and other tissues where amino acids are intensively used for the production of energy, the **amino group can be transferred to pyruvate** to form alanine. Which can be transported to the liver. See fig. 59(b)

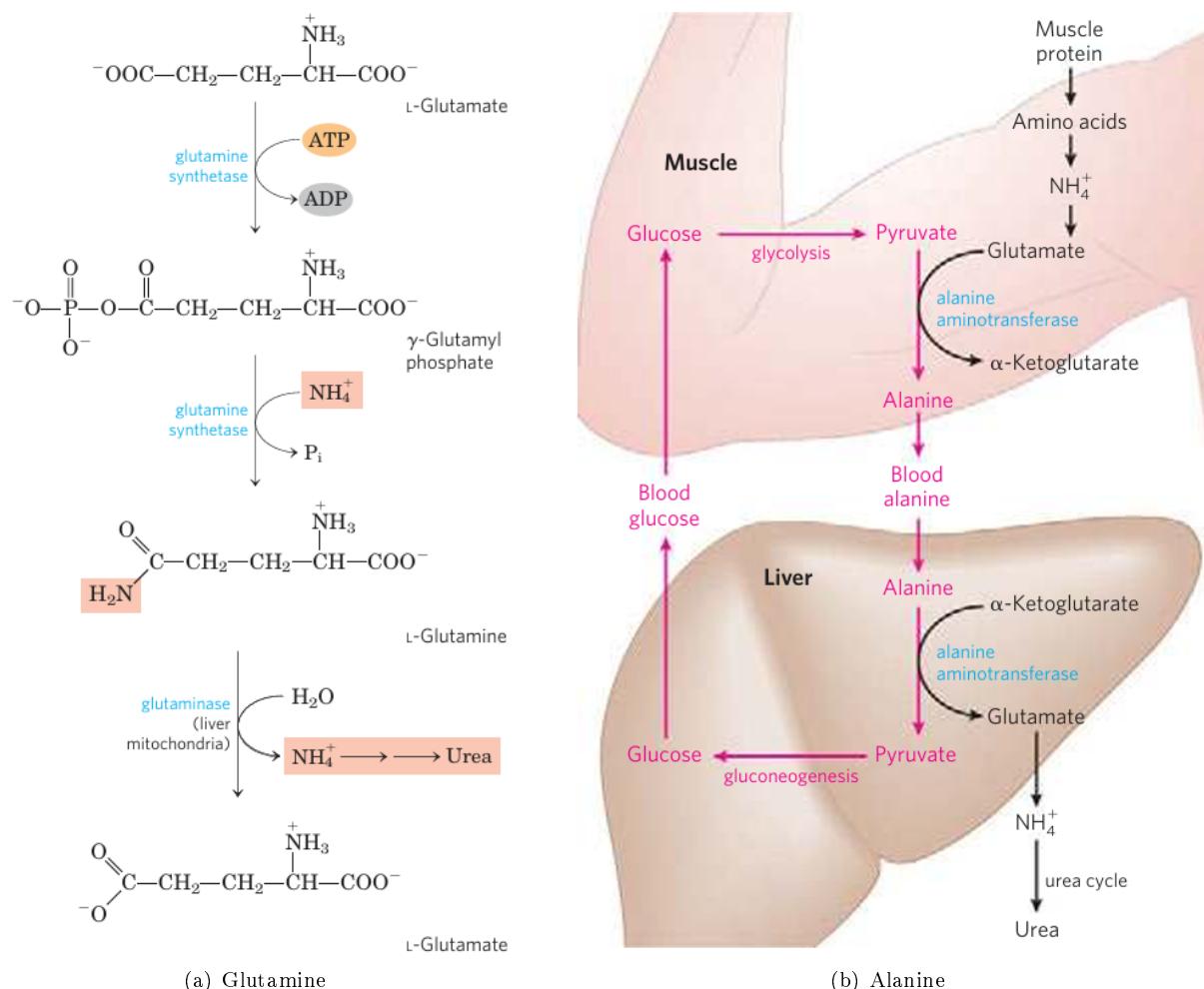


Figure 59: Transport of Ammonia

4.4.1.2 Urea production

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

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

4.4.1.3 Entry in TCA

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

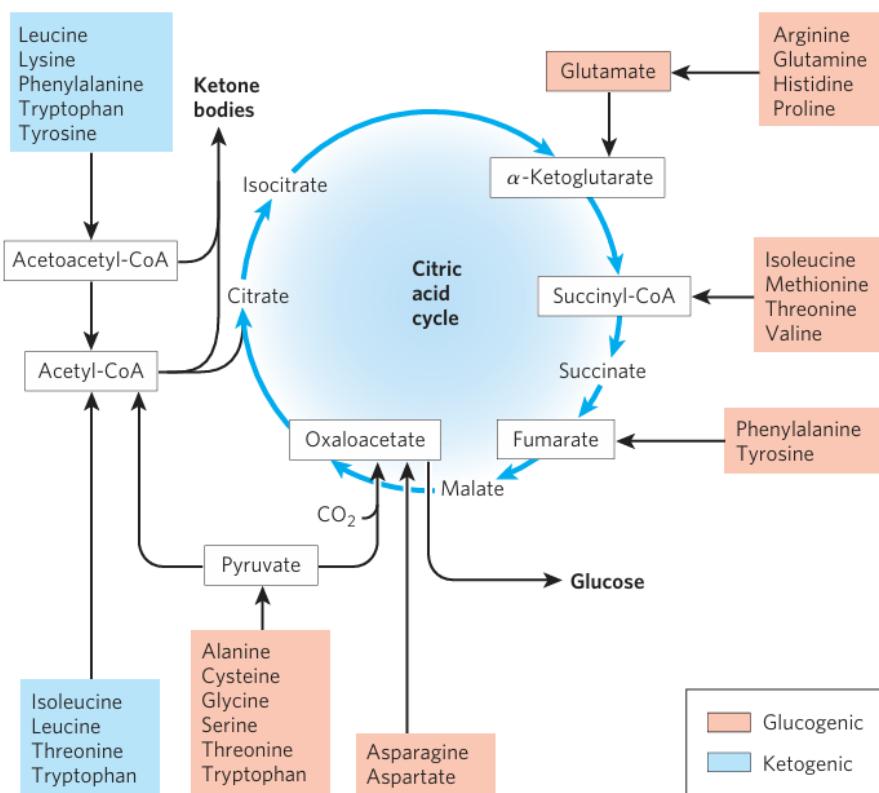


Figure 60: Glucose-alanine cycle

Remark 4.19 (Defects in amino acid metabolism). Several genetic defects have been identified in humans that impact the amino acid metabolism. These defects lead to the accumulation of neurotoxic intermediates resulting in intellectual disabilities.

4.5 Oxidative Phosphorylation

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

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

4.5.1 ECT

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

Note:

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

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

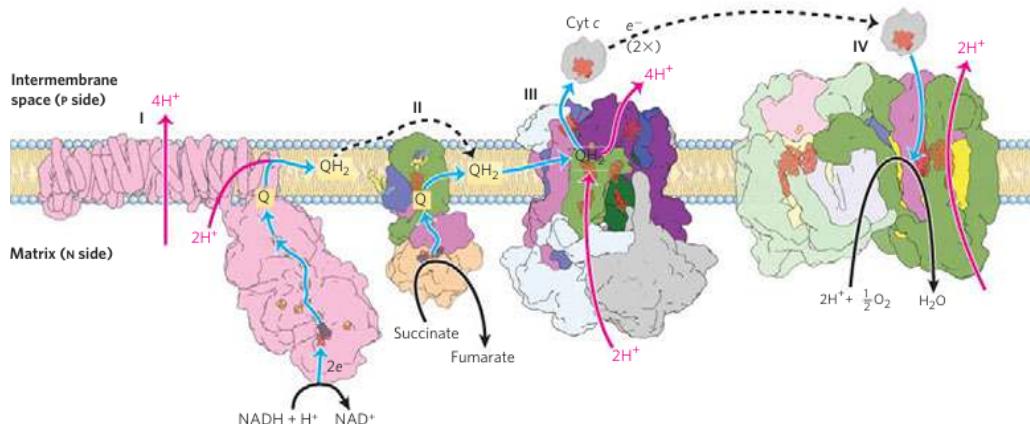


Figure 61: Overview of the ETC

Remark 4.20 (Coenzyme Q). Ubiquinone (Coenzyme Q), is a **hydrophobic electron carrier** that can dissolve in the inner mitochondrial membrane bilayer. It can accept 1 or 2 electrons thus being reduced to Semiquinone ($\cdot\text{QH}$) and Ubiquinol (QH_2) respectively. See fig. 62(a)

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

Remark 4.21 (Cytochrome C). A small **heme protein** located in the intermembrane space of mitochondria. See fig. 62(b)

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

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

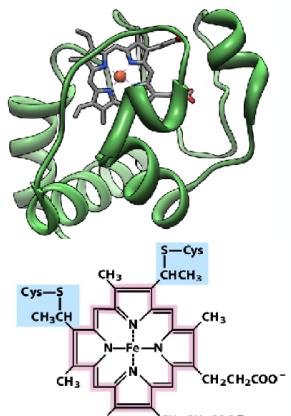
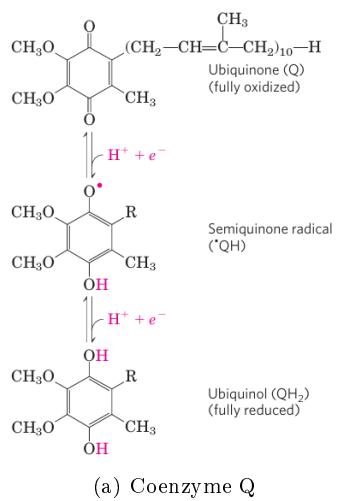


Figure 62: Electron carrier in the ETC

Definition 4.22 (Fe-S clusters). Iron-sulfur clusters occur in many biological systems, often as components of electron transfer proteins. The relevant redox couple in all Fe-S proteins is **Fe(II)/Fe(III)**. They may be more or less complex (see. fig. 4.5.1)

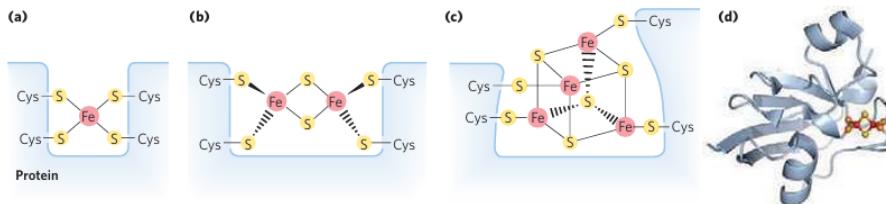


Figure 63: Iron-sulfur centers

4.5.1.1 Complex 1

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

Complex I receives high energy electrons from **NADH**. NADH donates electrons to a series to FMN, leading to the reduction of FMN to **FMNH₂**. FMNH₂ then donates the electrons to a series of **Fe-S clusters**. Finally two electrons are transferred to CoQ producing **CoQH₂**. See fig. 64(a)

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

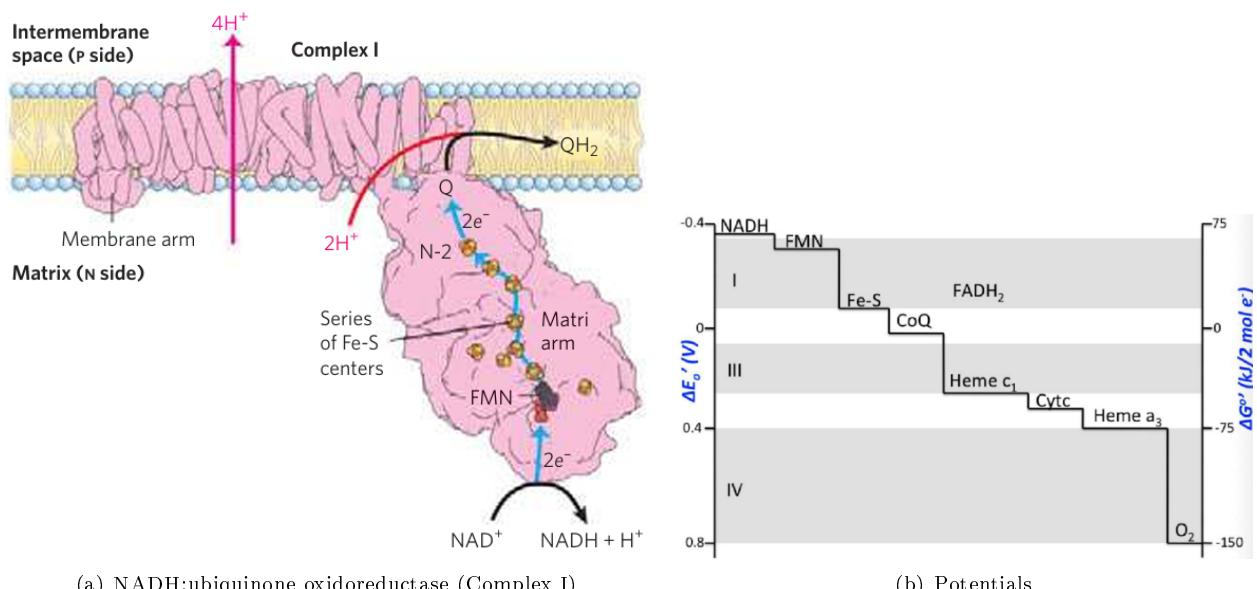


Figure 64:

4.5.1.2 Complex 2

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

The **FADH₂** is created in step 6 of the TCA cycle and stays tightly connected to the enzyme. FADH₂ transfers his **2 high energy electrons** to a series of **Fe-S clusters** that ultimately transfer the electrons to CoQ producing **CoQH₂**. See fig. 65.

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

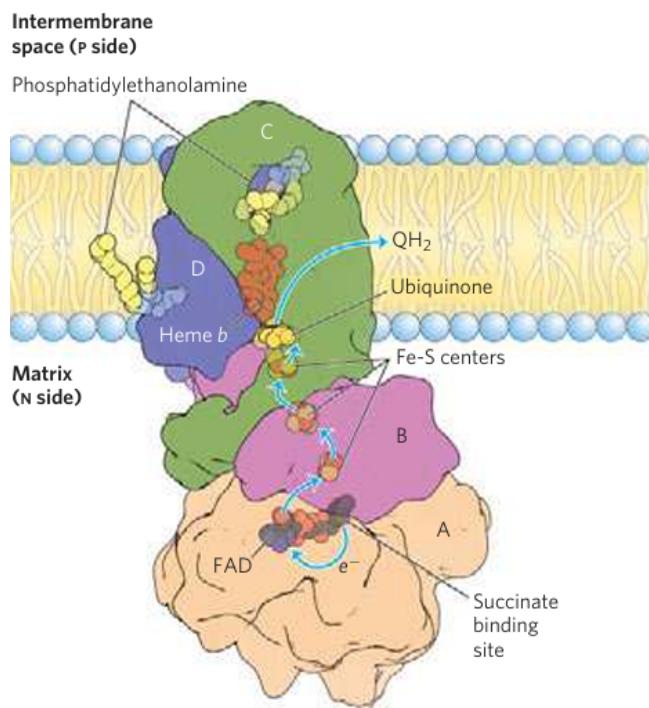


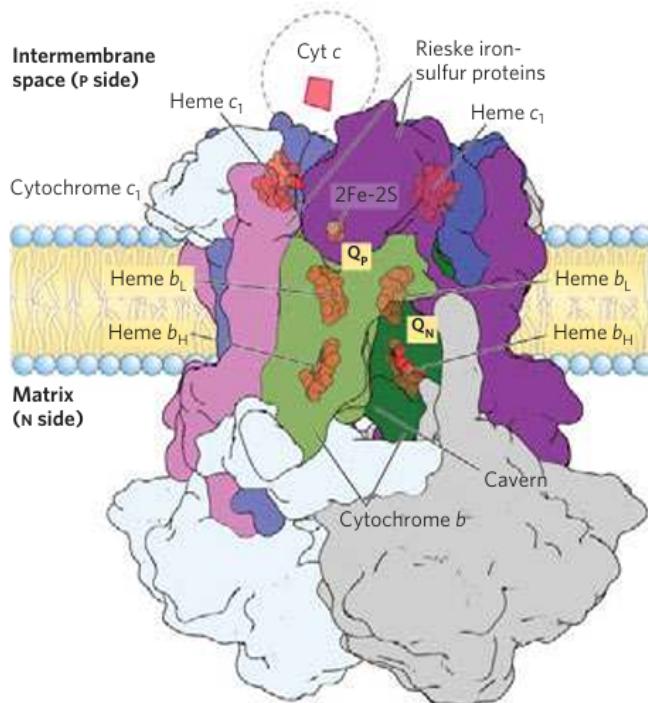
Figure 65: Complex II - succinate dehydrogenase

4.5.1.3 Complex 3

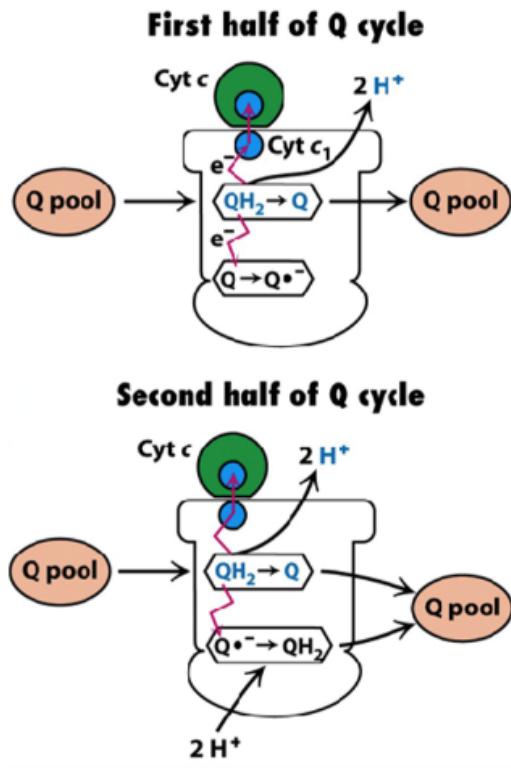
Complex III transfers electrons from **CoQH₂** to **Cytochrome c**. This is done through the Q cycle, which has two stages:

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

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



(a) Complex III - cytochrome bc_1



(b) The Q cycle

Figure 66: Complex III

4.5.1.4 Complex 4

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

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

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

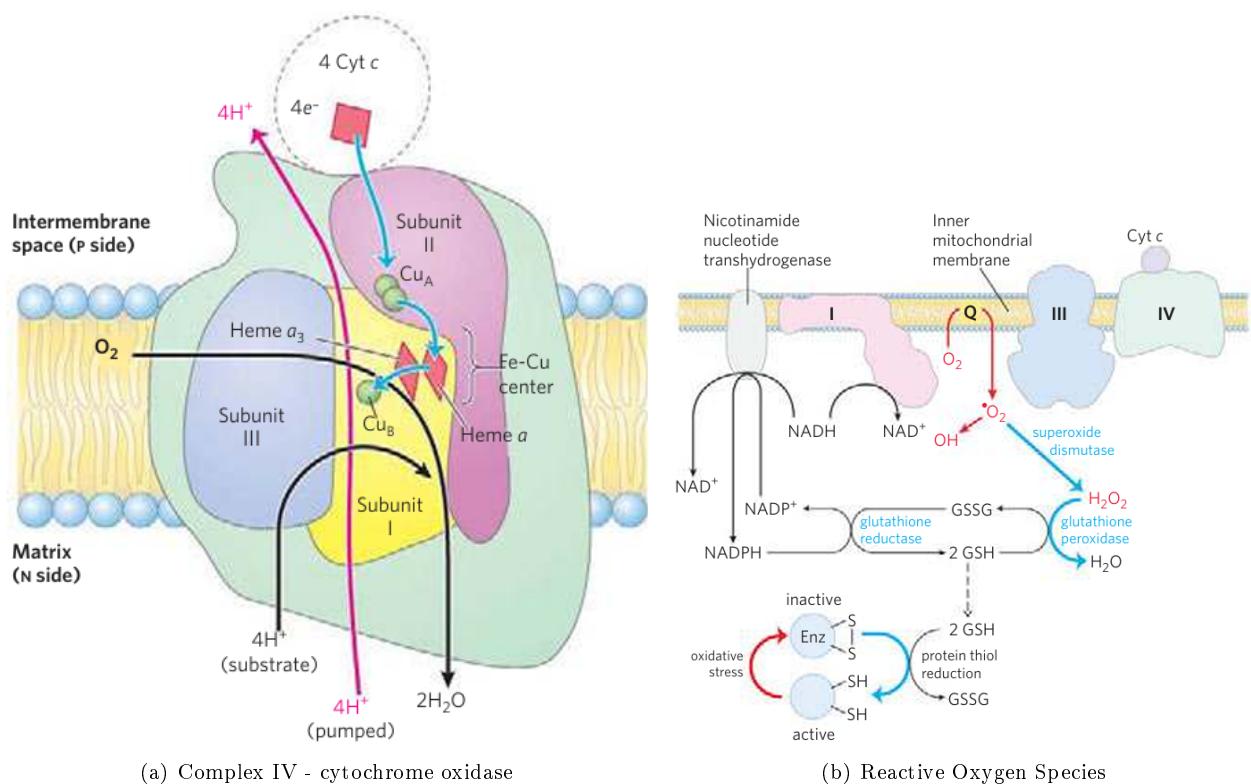


Figure 67: Complex IV and ROS

Remark 4.23 (Reactive Oxygen Species (ROS)). ROS are chemically reactive species containing oxygen. ROS are produced in a variety of biochemical reactions / organelles such as peroxisomes, **mitochondria**, etc. In the ETC about 0.1-2% of electrons are **prematurely transferred to oxygen**. Specific enzymes in our mitochondria are used to detoxify **superoxide radicals**, be converting them to **hydrogen peroxide** and then to **water**. See fig. 67(b)

4.5.2 ATP synthase - Complex V

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

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

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

Each unit has its purpose:

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

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

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

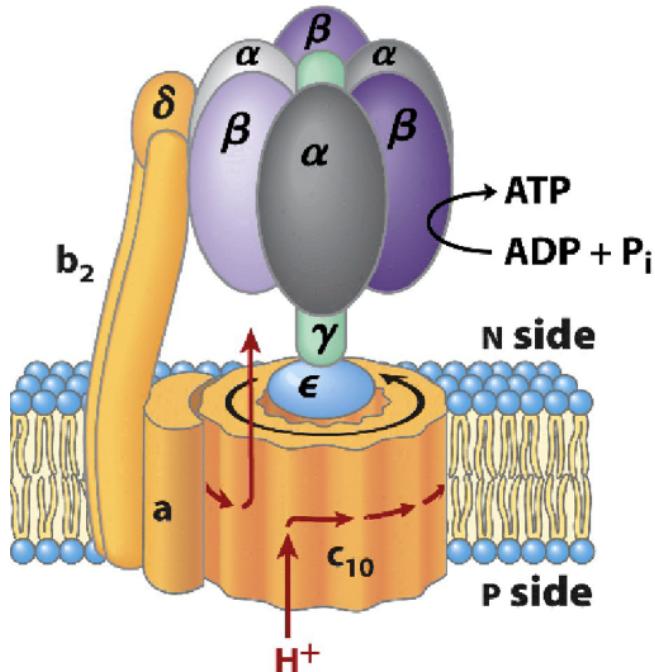


Figure 68: ATP synthase

Note in figure 69(b) N side = matrix and P side = intermembrane space.

Remark 4.24 (Why does C rotate?). First note that the **a subunit** spans the entire membrane. So first an **H⁺** enters the **a subunit** from the matrix and binds to the **aspartate residue in the closest c subunit**. Through the pressure of more **H⁺** coming into the **a subunit** the **c ring turns** until the **H⁺** finds the exit in **other half of the a subunit**. See fig. 69(a)

Remark 4.25 (How does the rotation produce ATP?). The alpha/beta hexamer produces the ATP. While the hexamer is kept in place the central stalk rotates, inducing conformational change in the oligomers.

There are 3 different states (stalk rotates always **rotates 120 degrees**) the alpha and beta units can be in (See fig. 69(b)):

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

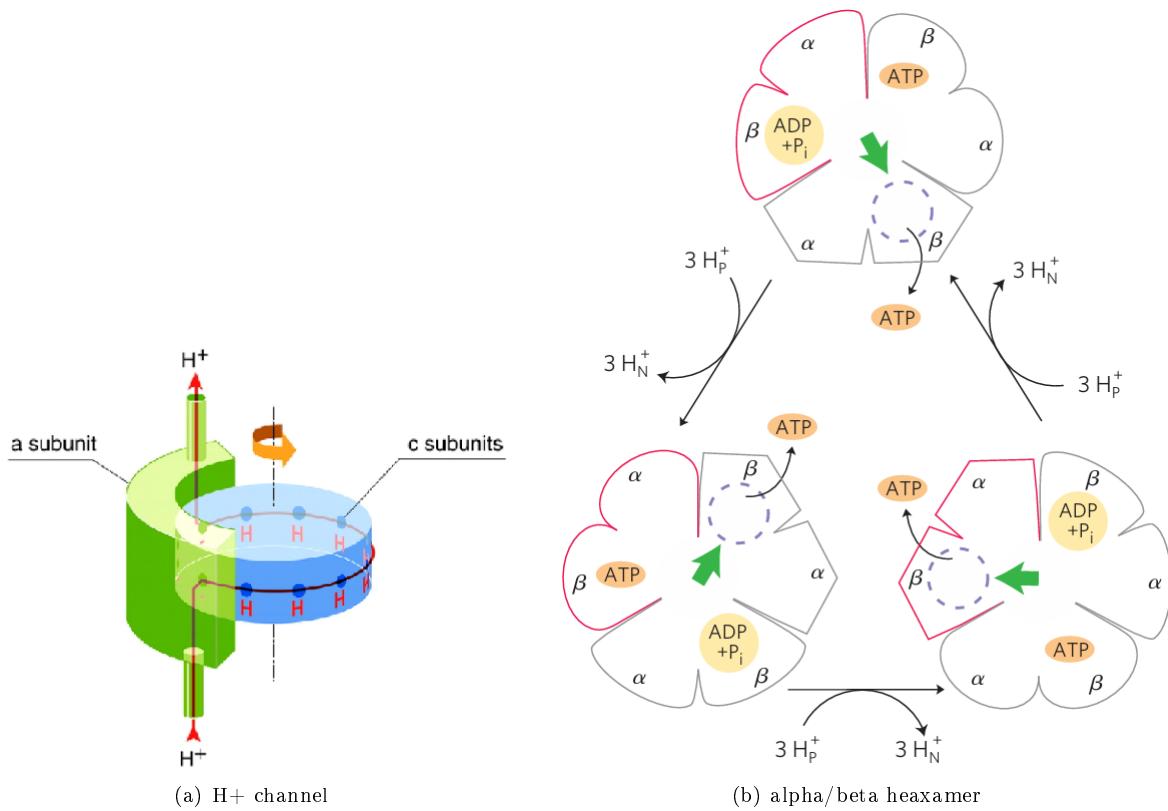
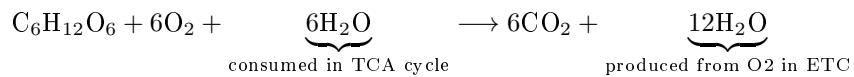


Figure 69: ATP production

4.6 Final calculation of aerobic respiration

Glucose Oxidation:



Glycolysis

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

Pyruvate decarboxylation (2x)

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

TCA Cycle (2x)

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

Oxidative phosphorylation

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

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

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

5 gluconeogenesis

Remark 5.1. General principles of anabolic pathways: Anabolic pathways synthesize complex cellular components from simple precursors **using chemical energy from ATP, NADH, or NADPH**. They are coupled to catabolic pathways in a dynamic steady state, where energy released from degradation is used for biosynthesis. **Although catabolic and anabolic pathways may share intermediates, they differ in at least one specific reaction, involving distinct enzymes.** Regulation occurs through these **pathway-specific enzymes**. Anabolic processes require a net input of energy greater than that produced by the corresponding catabolic reactions.

5.1 overview

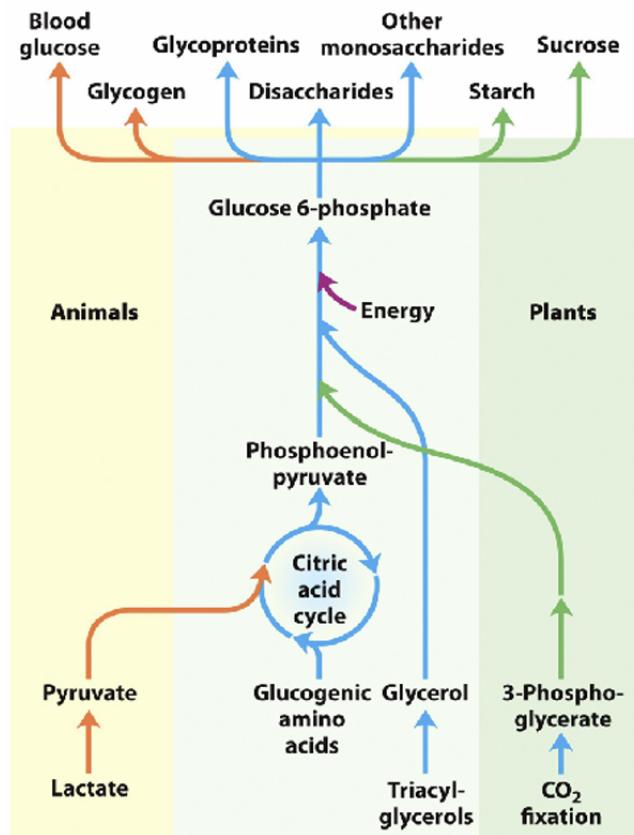


Figure 70: overview of the gluconeogenesis pathway

The body consumes on average around 160g of glucose per day and has storage for 210g. For cells whose sole energy source is glucose this poses a real problem. This is where Gluconeogenesis comes in. In its complete version gluconeogenesis occurs in the **liver and kidney cortex**. The main precursors are **pyruvate, lactate, glycerol and amino acids**.

5.1.0.1 total reaction



5.2 pathway steps

5.2.1 comparison to glycolysis

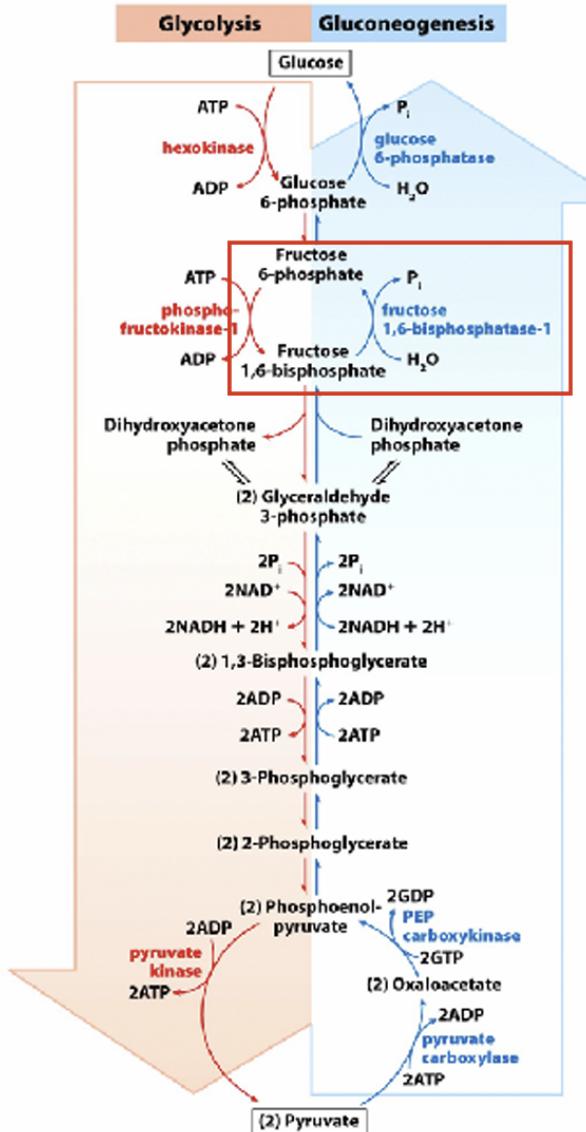


Figure 71: gluconeogenesis and glycolysis side by side

It differs from glycolysis in the 3 most exergonic reactions:

- 1. $\text{glucose} + \text{ATP} \rightarrow \text{Glucose} - 6 - \text{phosphate}$

- 3. fructose - 6 - phosphate + ATP \rightarrow fructose - 1,6 - biphosphate
- 10. phosphoenolpyruvate + ADP \rightarrow pyruvate + ATP

These reactions have different enzymes compared to glycolysis and are crucial for regulation. All other reactions are identical to glycolysis.

5.2.2 step 1 + 2: Pyruvate + ATP \rightarrow PEP

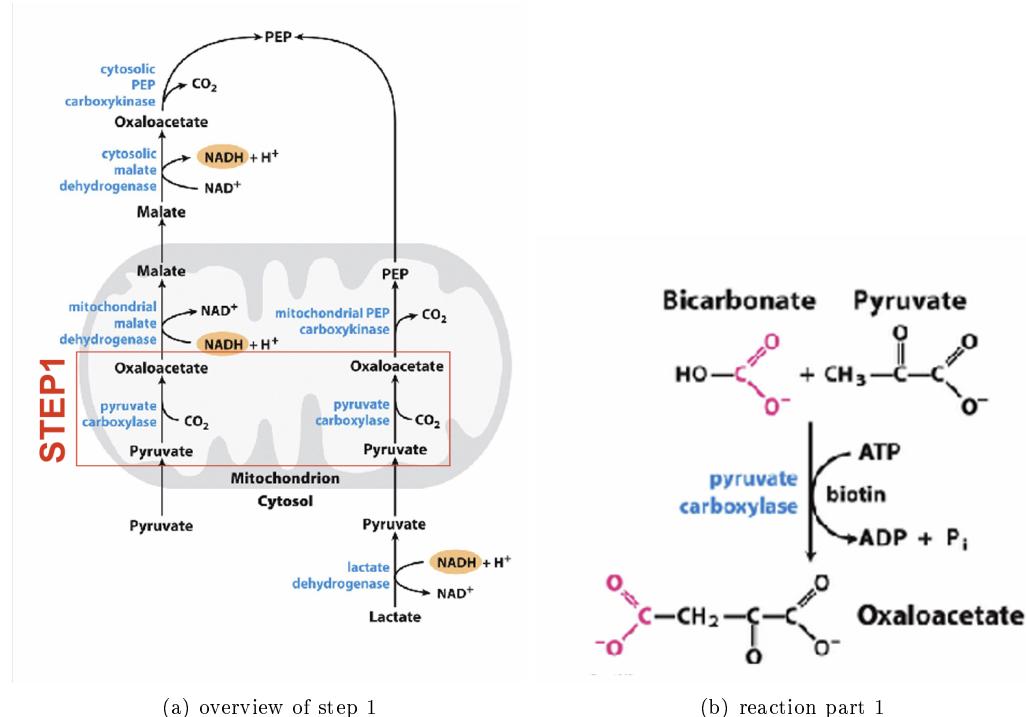


Figure 72: step 1 of gluconeogenesis

- i) Pyruvate makes it to the mitochondrial matrix.
- ii) In the mitochondria, pyruvate is carboxylated to oxaloacetate by **pyruvate carboxylase**.

- iii) Oxaloacetate is either transformed to PEP by mitochondrial **PEP carboxykinase** or converted to malate by **malate dehydrogenase**.
- iv) Malate and PEP are transported to the cytosol.
- v) Malate is reconverted into oxaloacetate by **cytosolic malate dehydrogenase**.
- vi) Oxaloacetate is transformed to PEP by **cytosolic PEP carboxykinase**.

Remark 5.2. Oxaloacetate can not be exported as such by mitochondria. This is why it is converted to malate dehydrogenase and then reconverted to oxaloacetate in the **cytosol producing an NADH/H⁺**, which will be needed in further steps. If lactate is the source this will generate NADH/H⁺ in the cytosol so can be processed directly to PEP by PEP carboxykinase

5.2.2.1 pyruvate carboxylase mechanism and endergonic - exergonic coupling

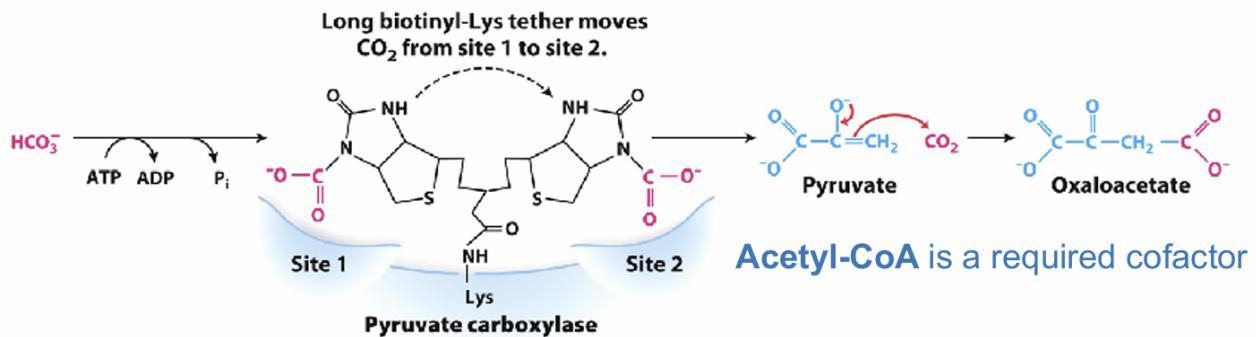


Figure 73: pyruvate carboxylase mechanism

This enzyme is a tetramer, each monomere has a **biotin-binding domain** that complexes with CO_2 and an **ATP binding domain**. The mechanism is as follows:

- i) **Phase 1:** ATP activates CO_2 , forming carboxyphosphate.
- ii) **Phase 2:** Phosphorylated CO_2 is attached to the biotin-enzyme with inorganic phosphate (Pi) release.
- iii) **Phase 3:** The CO_2 is transferred to pyruvate to form oxaloacetate.

Pyruvate carboxylase requires Acetyl-CoA as a cofactor

Remark 5.3. The highly endergonic phosphorylation that impeded the direct inverse of glycolysis is coupled to the highly exergonic decarboxylation, making this a net exergonic reaction.

5.2.3 step 8: fructose-1,6-biphosphate \rightarrow fructose-6-phosphate

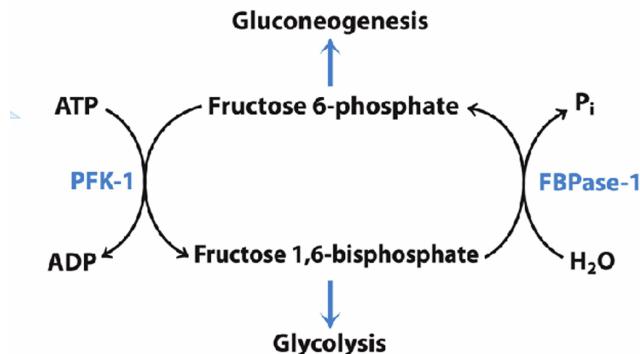


Figure 74: step 8 of gluconeogenesis

Note that step 8 is also not a direct reversal of glycolysis as the FBPase-1 hydrolyses a organic phosphate off instead of transferring it to ADP. **PFK-1 and FBPase-1 are highly regulated enzymes** See chapter on glucoenogenesis regulation.

5.2.4 step 10: glucose-6-phosphate \rightarrow glucose

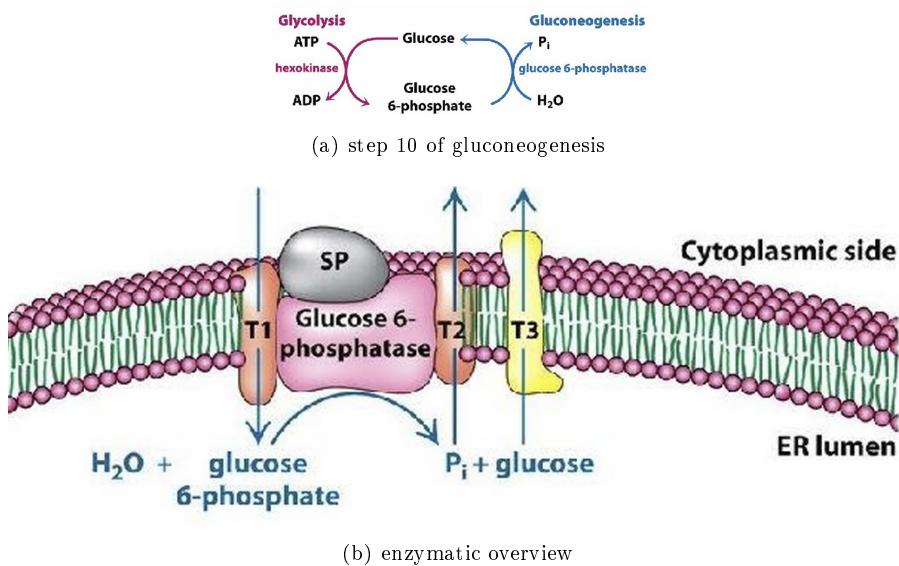


Figure 75: step 1 of gluconeogenesis

This is similar to step 8 however there is an interesting topological consideration:

- i) glucose-6-phosphate transporter (T1) (T1) imports glucose-6-phosphate (Glc(6)P) into the ER lumen.
- ii) glucose-6-phosphatase (Glc(6)Ptase) converts Glc(6)P to glucose and inorganic phosphate (Pi).
- iii) The reaction is stabilized by the calcium-binding protein stabilizing protein (SP) (stabilizing protein).

-
- iv) Pi is transported to the cytosol by the phosphate transporter (T2) (T2).
 - v) Glucose is transported to the cytosol by the glucose transporter (T3) (T3).

5.3 Regulation

Remark 5.4. In general low energy states (low ATP, high ADP and AMP) favor catabolic pathways such as glycolysis. While high energy states favor anabolic states such as gluconeogenesis. These processes are **regulated at cell autonomous** level, i.e each cell does whatever they want. But they are **also regulated at the organism level**. See chapter on metabolic integration for organism level regulation.

5.3.1 Acetyl-CoA activation

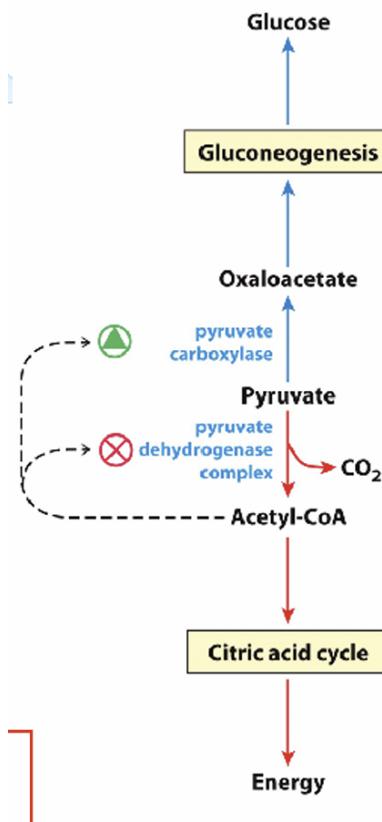


Figure 76: Acetyl-CoA inhibition

Acetyl CoA activates **pyruvate carboxylase** which is required to form oxaloacetate. This mechanism is useful as **when Acetyl-CoA accumulates, due to saturation of the TCA cycle it can be fed into gluconeogenesis**

5.3.2 PFK-1 / FBPase-1 regulation (autonomous level)

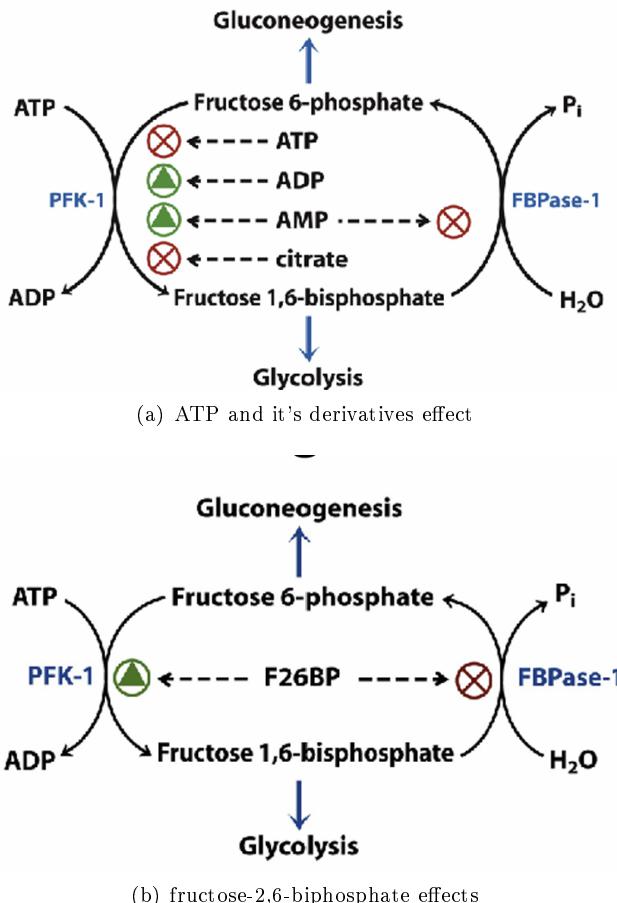


Figure 77: step 1 of gluconeogenesis

phosphofructokinase-1 (PFK-1) is **activated by ADP and AMP**, both molecules that are present under low energy conditions. on the otherhad it is **inhibited by ATP and citrate**, these are present in excess when the cell has a lot of energy.

fructose-16-bisphosphatase-1 (FBPase-1) is inhibited by AMP, which is a molecule present at low energy levels.

On top of this it **PFK-1 is activated by Fructose2,6-biphosphate while FBPase-1 is inactivated**

Remark 5.5. PFK-1 and PFK-2 are not the same thing. These are different enzymes with different regulations, same thing for the phsophatases!!

5.3.3 PFK-2 / FBPase-2 regulation on organism level (insulin and glucagon)

This is a bifunctional enzyme that has both a kinase domain (**PFK-2 domain**) and a phosphatase domain (**FBPase-2 domain**). This enzyme is responsible for organism level regulation of glycolysis gluconeogenesis.

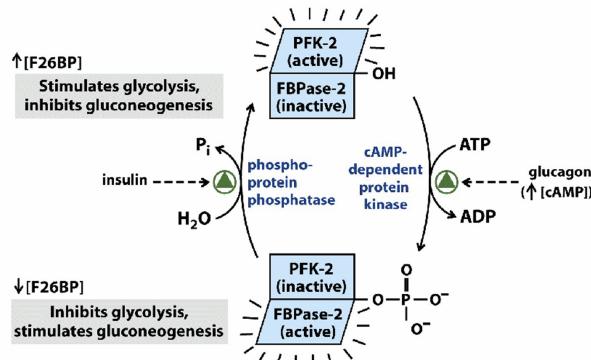


Figure 78: bifunctional enzyme domains integrate insulin and glucagon signals

This enzyme reacts to insulin and glucagon to either increase or decrease the concentration of **Fructose-2,6-biphosphate (F26BP)**

- **insulin activates PFK-2 domain** that increases F26BP concentrations this then activates PFK-1 that stimulate glycolysis and inhibit gluconeogenesis.
- **glucagon on the other hand activate FBPase-2 domain** that decreases F26BP concentrations that then inturn removes an inhibitor of gluconeogenesis stimulating it more.

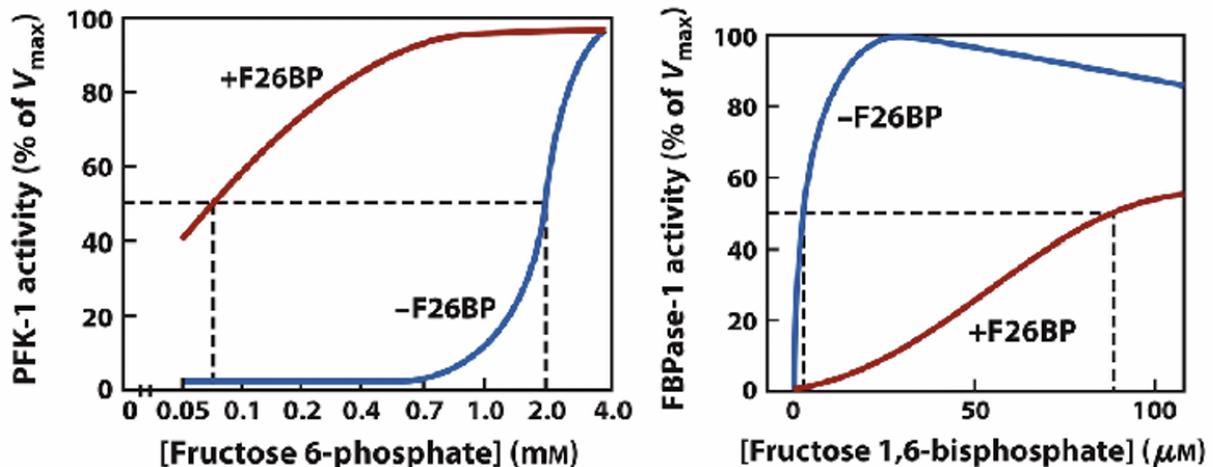


Figure 79: effect of F26BP on glycolysis and gluconeogenesis

This is seen when looking at relative V_{max} of PFK-1 and FBPase-1. PFK-1 is massively activated by the presence of F26BP while it has the opposite effect on FBPase-1 acting as an inhibitor

6 glycogen metabolism

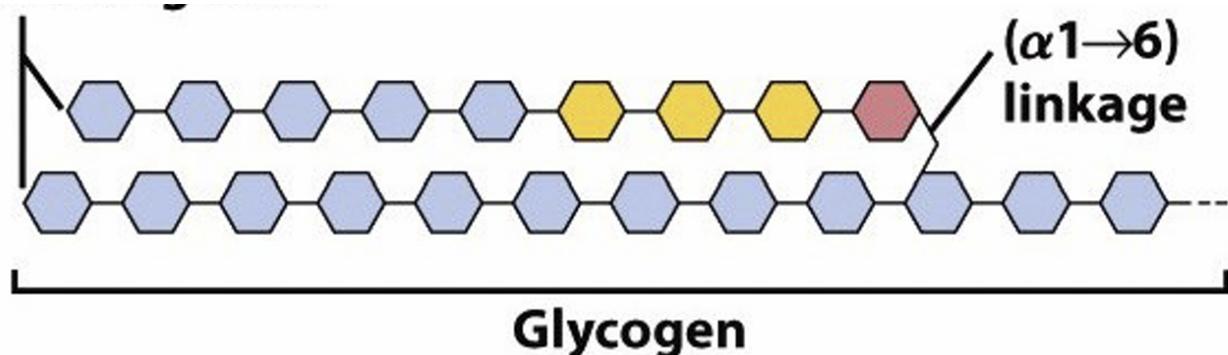


Figure 80: glycogen structure

Glycogen is a storage polymer that is used by liver cells to regulate the blood sugar level. It is a branched polymer consisting of two types of bonds

- $\alpha(1 \rightarrow 6)$ bonds: these are the bonds that form the branching points
- $\alpha(1 \rightarrow 4)$: These bonds are the ones that extend the polymer

6.1 glycogenesis

6.1.1 step 1: Glucose activation

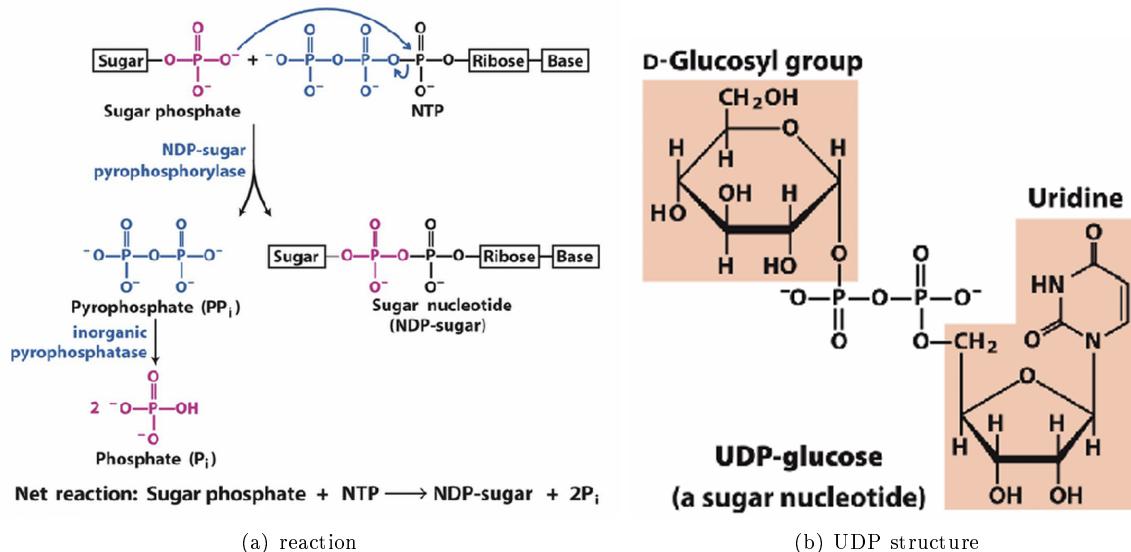


Figure 81: glucose activation

UDP-glucose pyrophosphorylase to transform a glucose 1-phosphate into a uridine diphosphate glucose (UDP-glucose). This reaction produces a pyrophosphate, which then is hydrolyzed by water to form two orthophosphate molecules. **This second step drives the reaction forward**

Remark 6.1. note that glucose-1-phosphate is not the glucose that arrives from glycolysis (that would be glucose-6-phosphate) It thus needs to be isomerized by **phosphoglucomutase**

6.1.2 step2: creating the primer

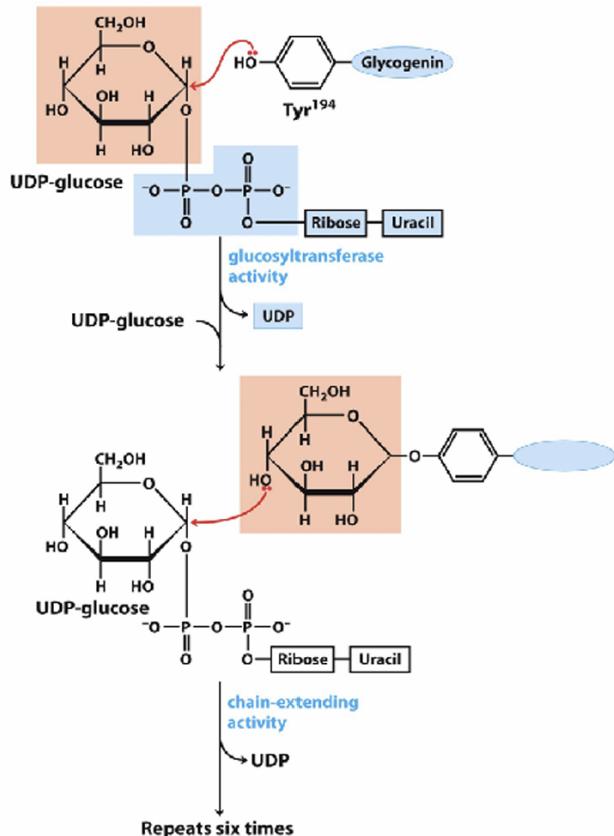


Figure 82: glycogenin autocatalyzes the addition of UDP onto itself

- Transfer of a glucose residue:** A glucose residue from UDP-glucose is transferred to a tyrosine residue of **glycogenin**. This reaction is catalyzed by glycogenin itself.
- Chain extension:** Glycogenin catalyzes the sequential addition of seven more glucose units, forming a short primer of $\alpha(1 \rightarrow 4)$ -linked glucose residues.

This leads to the formation of the **8-monomer-primer** that will subsequently be elongated.

6.1.3 step 3: elongation

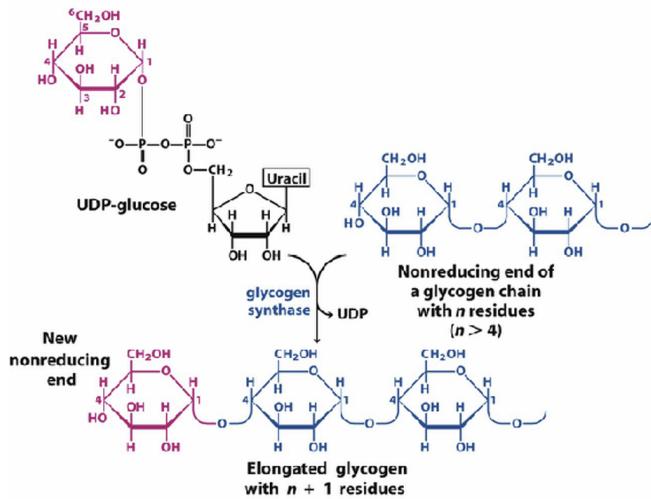


Figure 83: elongation of glycogen

glycogen synthase catalyzes the addition of further UDP-glucose molecules by catalyzing the formation of $\alpha(1 \rightarrow 4)$ glycosidic bonds

6.1.4 branching

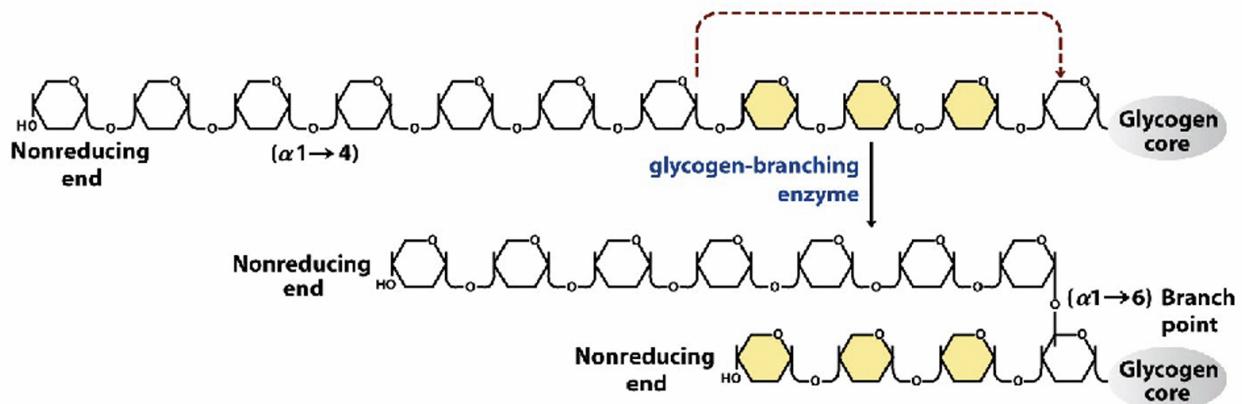


Figure 84: branching

Branching is a way to increase the solubility of glycogen as well as increase the number of non-reducing ends which will allow for faster breakdown. This is catalyzed by the formation of $\alpha(1 \rightarrow 6)$ glycosidic bonds by glycogen branching enzyme

Glycogen-branched enzyme catalyzes the transfer of a terminal fragment (6 or 7 residues long) from the nonreducing end of a branch (at least 11 residues long) to the C-6 hydroxyl group of a glucose residue on

the same chain or another chain creating a branch with an $\alpha(1 \rightarrow 6)$ linkage

6.2 glycogen breakdown

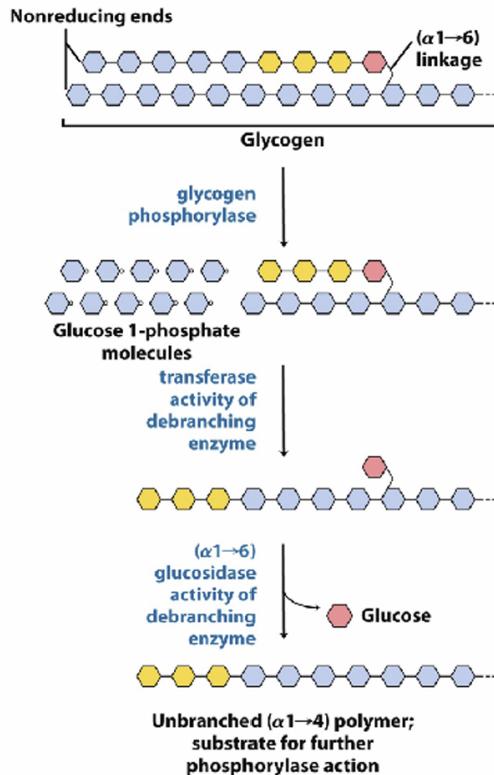


Figure 85: glycogen breakdown

- i) **Phosphorolysis:** The first step is catalyzed by glycogen phosphorylase, which uses orthophosphate (P_i) to cleave the glycosidic bond between a terminal glucose unit (with a free hydroxyl group) and the rest of the glycogen molecule. This reaction produces glucose-1-phosphate and a glycogen molecule shortened by one glucose residue.
- ii) **Branch point limitation:** Glycogen phosphorylase cannot cleave the $\alpha(1 \rightarrow 6)$ glycosidic bonds at the branching points. It stops cleaving when it is four glucose residues away from such a branch.
- iii) **Debranching enzymes:** A transferase enzyme shifts a block of three glucose residues from the branch to a nearby linear chain. Then, an $\alpha(1 \rightarrow 6)$ glucosidase hydrolyzes the remaining single glucose residue at the branch point, releasing a free glucose.
- iv) **Conversion to glucose-6-phosphate:** Finally, phosphoglucomutase converts the glucose-1-phosphate produced in the first step into glucose-6-phosphate, which can then enter glycolysis or other metabolic pathways.

6.3 glycogen regulation

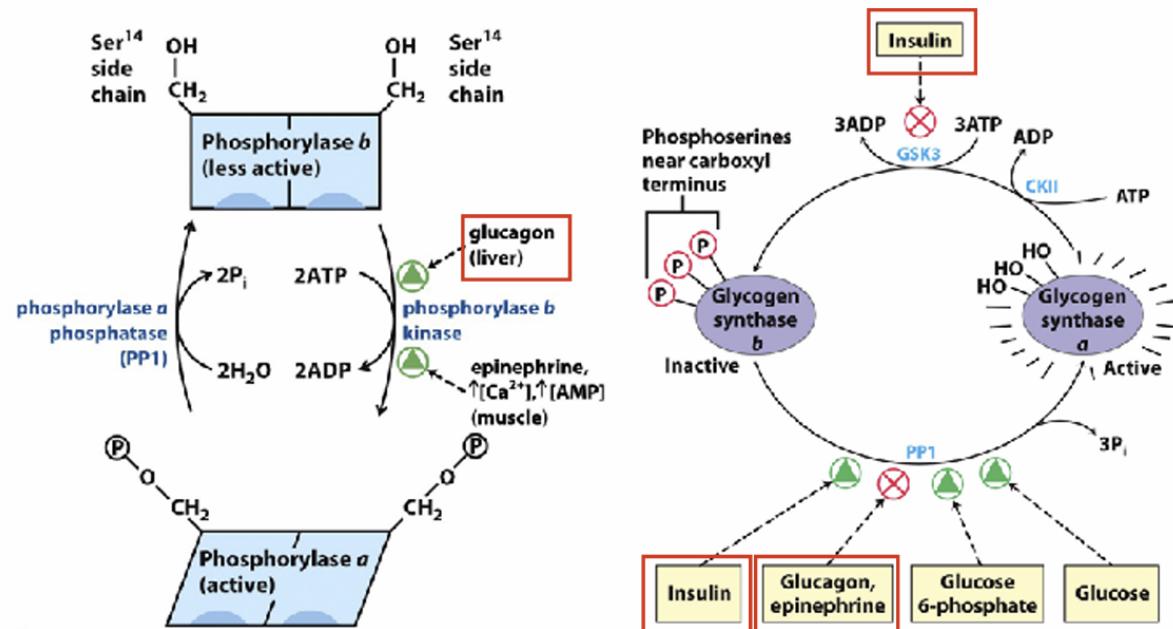


Figure 86: glycogen Regulation

Glycogen metabolism consists of two processes - glycogen synthesis and glycogen degradation. These two processes however do not take place the same moment in time. In fact, our body has a mechanism in place that regulates them in a reciprocal fashion - **when one process is on, the other process is off. Glycogen phosphorylase and glycogen synthase are reciprocally regulated**

7 lipid Sythesis

7.1 fatty acid sythesis

Fatty acid oxidation (β -oxidation) takes place in the **mitochondrial matrix**. However **fatty acid sythesis** takes place in the **cytosol**. This is an iterative reaction that **adds 2 carbons at a time** producing **palmitate**. However it isn't completely the reverse of β -oxidation). One key molecule that is unique to FA sythesis is **Malonyl-CoA**. its production via the **carboxylation of acetyl-CoA by acetyl co-A carboxylase** and is **the rate limiting step of Fatty acid Sythesis**. The main enzyme catalyzing this reaction is a mega complex called **Fatty Acid Synthase (FAS)**

7.1.1 maloney CoA

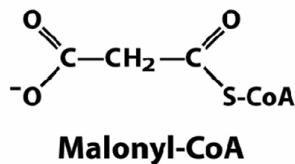


Figure 87: Maloney Co-A

Maloney Co-A is a **derivative of Acetyl-CoA** produced in the cytosol However acetyl-CoA is produced in the mitochondrial matrix so Acetyl-CoA needs to be transported to the cytosol:

7.1.1.1 transporting acetyl-CoA to cytosol

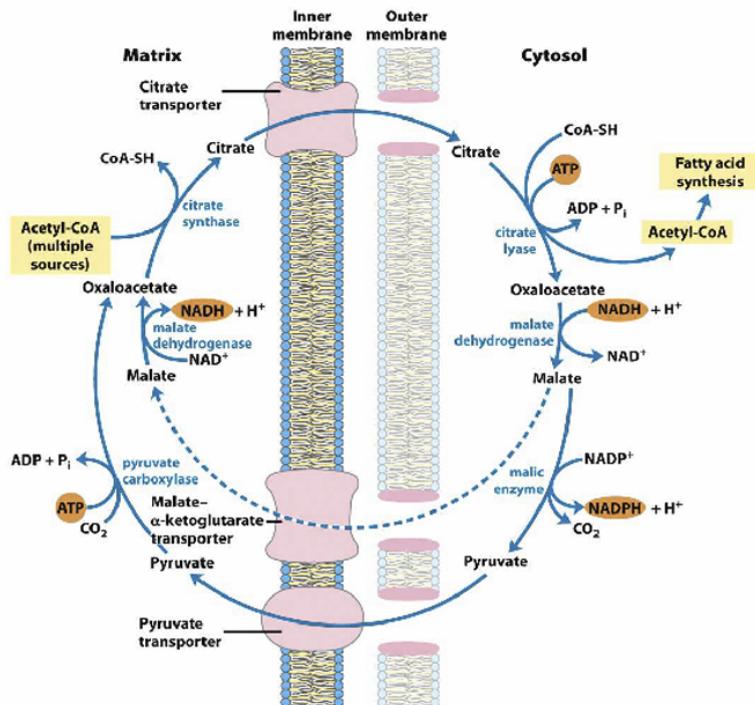


Figure 88: Acetyl Co-A needs to be transported to cytosol

- i) oxaloacetate and acteyl coA are turned to citrate by **Citrate Synthase**
- ii) citrate can be transported across the membrane via **TCA transporter (Citrate Transporter)**
- iii) citrate is turned to oxaloacetate and acetyl-CoA
- iv) oxaloacetate is converted to malate by **malate dehydrogenase** which in turn is turned to pyruvate by **Malic Enzyme (ME)**.

step 4 is **crucial for generating NADPH** which will be used for FA sythesis later!

7.1.1.2 producing Maloney Co-A

once acetyl-CoA has been transported into the cytosol maloney coA can be synthesized. This steps involves a carboxylation of acetyl-CoA by **Acetyl-CoA Carboxylase (ACC)** and is the **rate limiting step of Fatty acid synthesis**.

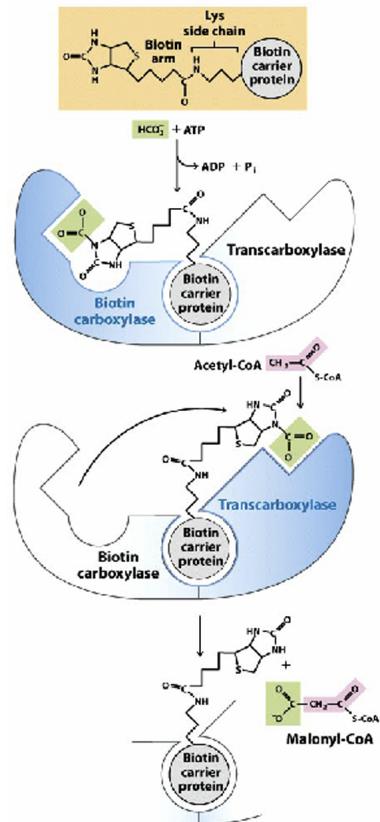


Figure 89: Maloney sythesis

A biotin carrier group which is part of Acetyl-CoA Carboxylase (ACC) is transiently carboxylated (with ATP consumtion) on a lysine - when in doubt lysine can do basically any PTM except phosphorylation .) - That then transfers the Co₂ to acetyl-CoA turning it to Maloney-CoA.

7.1.2 fatty acid sythase and it's domains overview

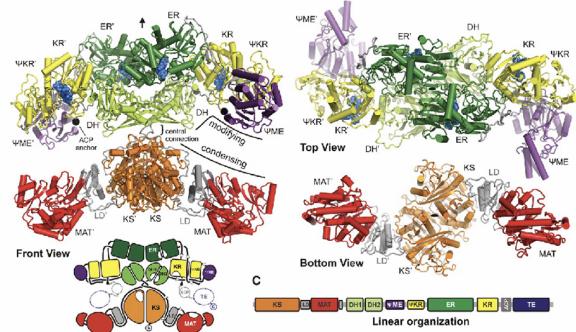


Figure 90: FA sythase structure and its domains

FAS is a huge multienzyme complex. The functional domains of FAS include:

- β -Ketoacyl Synthase (KS) domain
- Malonyl/Acetyltransferase (MAT) domain
- Acyl Carrier Protein (ACP) domain
- β -Ketoacyl Reductase (KR) domain
- β -hydroxyacyl-ACP dehydratase(DH)
- enoyl-ACP reductase (ER) domain
- Thioesterase (TE) domain

7.1.3 step by step mechanism

bilanz:



Figure 91: summary of what is used in palmitate synthesis

7.1.3.1 step 0- loading

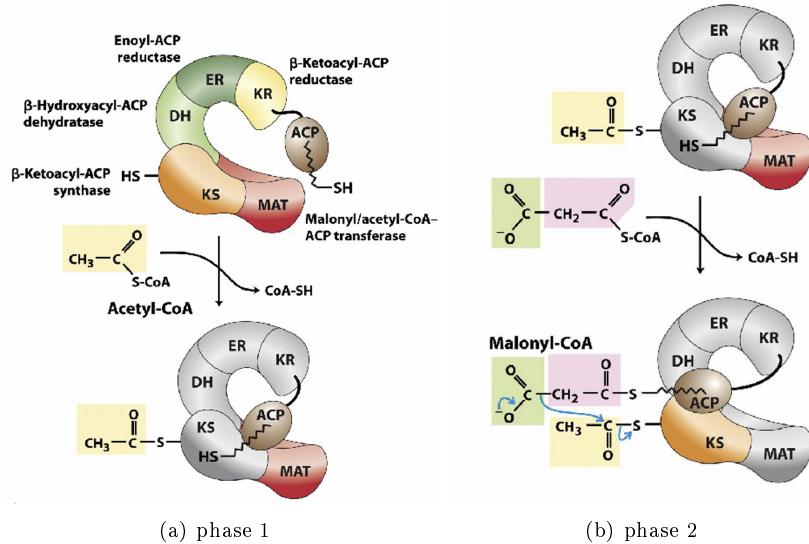


Figure 92: loading step of FA sythesis

- i) **phase 1:** acetyl-CoA molecule is condensed to a Cys residue in the **KS domain** domain. To do this it is **first loaded onto ACP domain then transferred to KS domain**. This is catalyzed by **MAT domain**
- ii) **phase 2:** Malonyl-CoA is added to **ACP domain**. where it is close to acetyl coA and ready to react.

7.1.3.2 step 1 - condensation

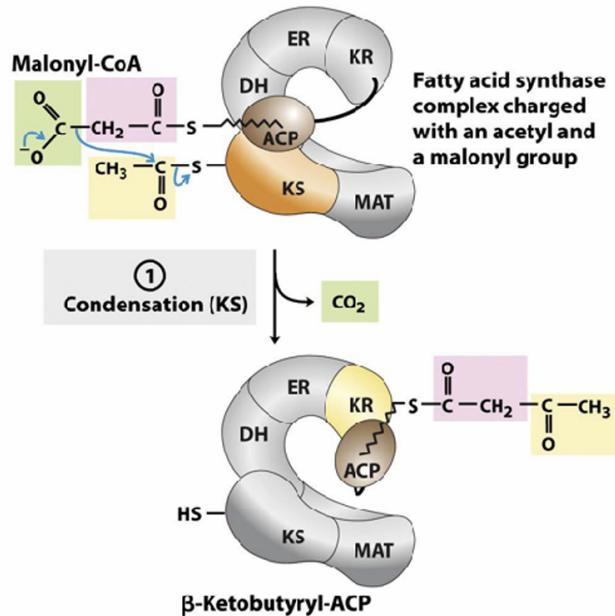


Figure 93: condensation step

In this step Malony and Acetyl are condensed to **acetoacetyl**, with the **release of Co2**. This reaction is catalyzed by **KS domain**. Facts to know:

- this is the energetically driving step of FA synthesis
- co2 release is the same one that was originally added by acetyl-coA carboxylase.
- this reaction is exergonic.
- reaction is similar to PEP-carboxykinase in gluconeogenesis.

7.1.3.3 step 2 - reduction

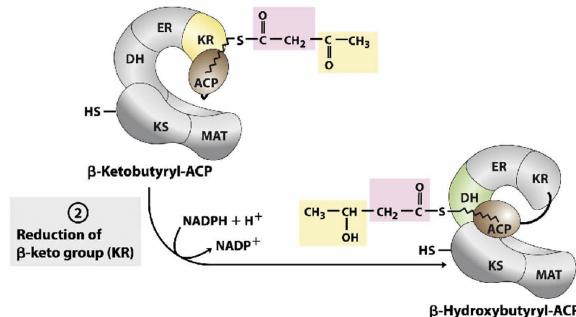


Figure 94: reduction step

In the second step (reduction) acetoacetyl-ACP is **reduced** to **β -hydroxybutyryl-CoA** by the **KR Domain** with oxidation of one NADPH molecule.

7.1.3.4 step 3 - dehydration

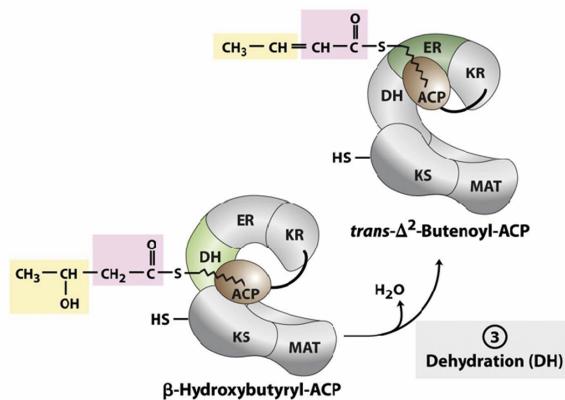


Figure 95: dehydration step

β -hydroxybutyryl-CoA loses a H_2O molecule to yield *trans*-D²-butenoyl-ACP in a reaction catalysed by the **DH domain**

7.1.3.5 step 4 - reduction

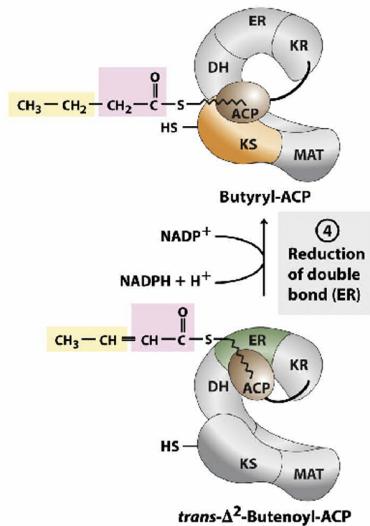


Figure 96: 2nd.reduction step

trans-D²-butenoyl-ACP is reduced to **butyryl-ACP** in a reaction catalyzed by the Enoyl Reductase domain where the double bond C2 / C3 is reduced and a molecule of NADPH is oxidized.

→ here we have elongated acetate-ACP (2C fatty acid) into butyrylACP (4C fatty acid)

7.1.3.6 step 5 - translocation

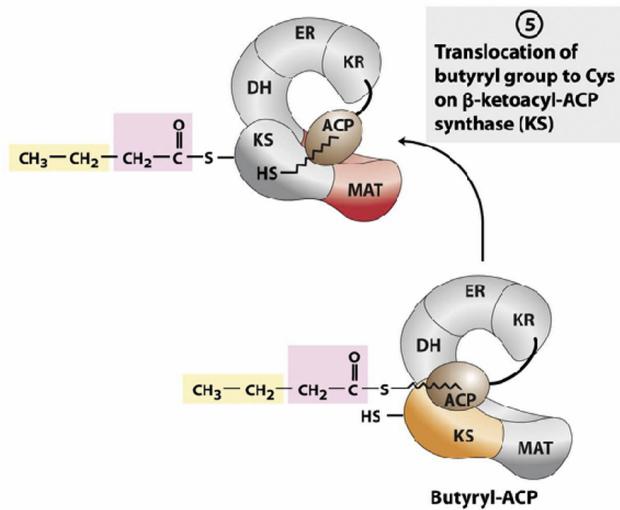


Figure 97: translocation of butyryl (or another FA) onto KS domain

To start a new cycle it needs to be transferred from the ACP to the Cys residue of the **KS domain**. This step is very similar to step one with one important difference. The **ACP domain only accepts maloney and acetyl so larger FA stay on the KS domain**

7.1.3.7 step 6 - reload a maloney

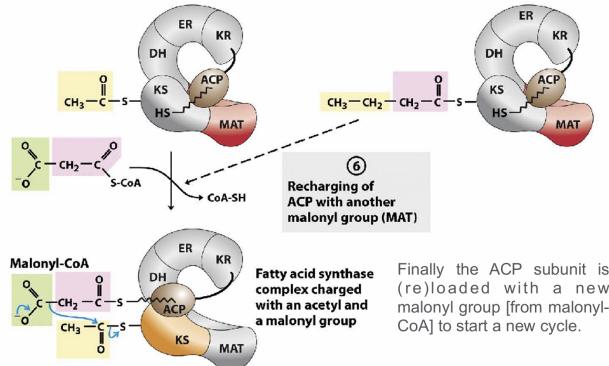


Figure 98: reloading a Maloney

This step is **identical to step one** except that there is no need to translocate Acetyl to KS domain. And now butyryl is in the place of acetyl.

7.1.4 useful for C-labelling shit

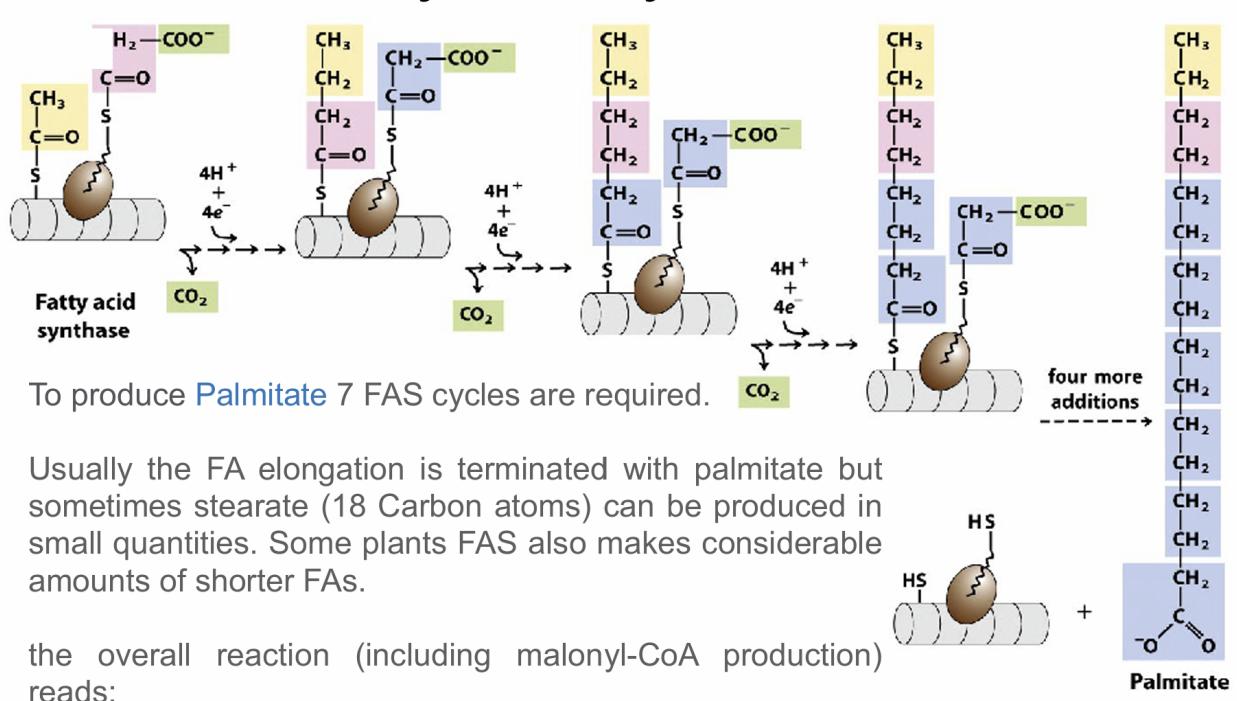


Figure 99: overview of palmitate carbons

7.1.5 palmitate as precursor and making other FA

Palmitate is the main FA produced via this pathway. It consists of 16C so will take **7 cycles to produce** ($7 \times 2 + 2C$ from acetyl). Longer Fatty acids can be produced by elongating palmitate via the same process. Double bonds can also be introduced to produce unsaturated fatty acids.

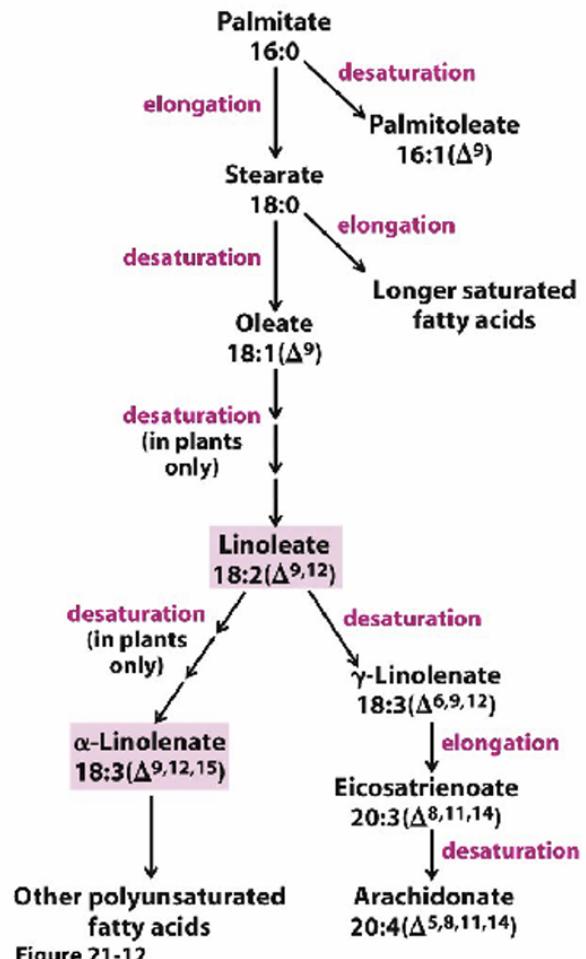


Figure 100: making other FA from plamitite as precursor

7.1.5.1 introducing Double bonds

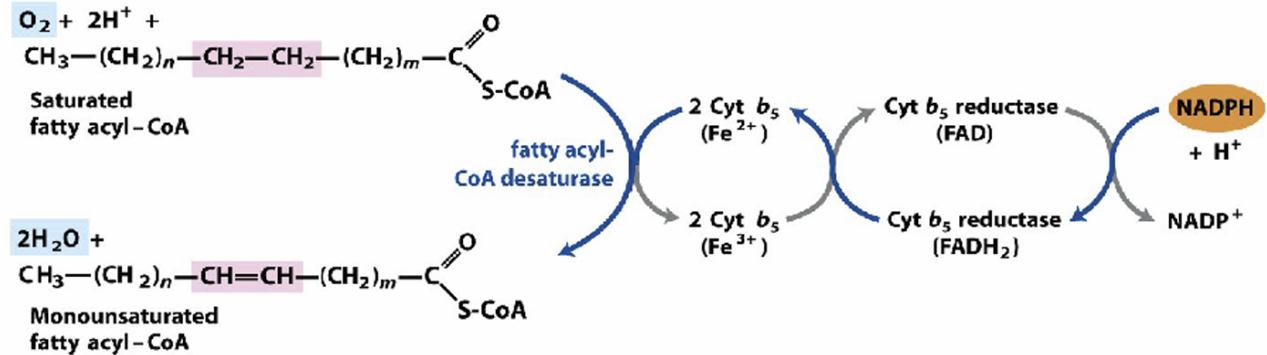


Figure 101: introducing double bonds

acyl co-As such as palmitate can be desaturated by **fatty acyl-CoA desaturase** Which transfers 2 electrons from NADPH (oxidation) to create the double bond. This takes place in the ER of liver cells. Not all unsaturated acids can be produced though. Mammals will only produce Δ^9 fatty acids, however some plants can produce $\Delta^{9,12}$ and $\Delta^{9,12,15}$ fatty acids. For mammals these are thus essential Fatty acids!

7.2 Phosphatidic acid synthesis

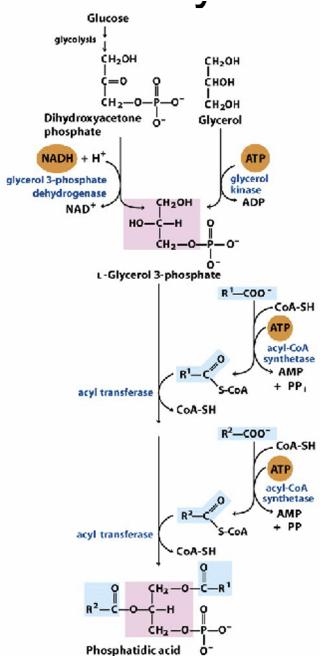


Figure 102: phosphatidic acid(PtAC) synthesis

phosphatidic acid (PA) is used for the synthesis of triglycerides (TAGs) and glycerophospholipids. therefore it is an important precursor for various lipids.

- i) generate **Glycerol-3-phosphate**
 - (a) **from glycolysis:** Dehydroxyacetonephosphate (**DHAP**) can be converted by **glycerol-3-phosphate dehydrogenase** into glycerol-3-phosphate.
 - (b) **Phosphorylation of glycerol:** kidney cells have **glycerol kinase** that can directly phosphorylate glycerol.
- ii) generate **Acyl-CoA:** This is similar to β -oxidation and uses **acyl-CoA synthase** to catalyze the reaction.
- iii) **acyl transferases** esterify acyl chains in positions C-1 and C-2 of the Glycerol-3-Phosphate to produce phosphatidic acid.

7.3 triglyceride synthesis

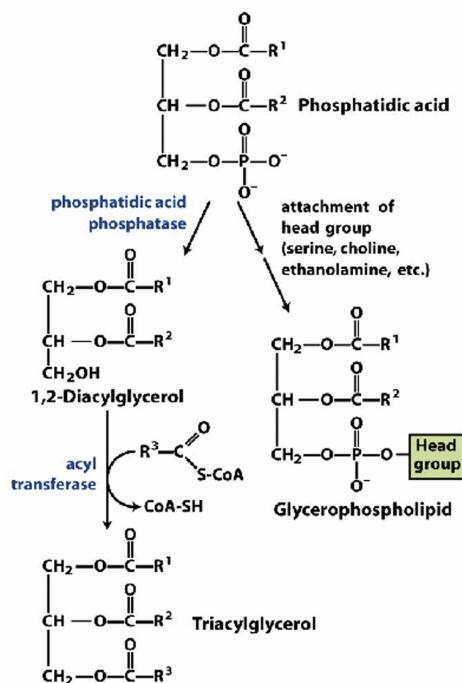


Figure 103: triglyceride synthesis

- i) phosphatidic acid is dephosphorylated to form diacylglycerol by **phosphatidic acid phosphatase (PAP)**
- ii) acyl transferases that catalyse the esterification of a third fatty acid to glycerol (in position C-3).

7.4 Glycerophospholipids synthesis

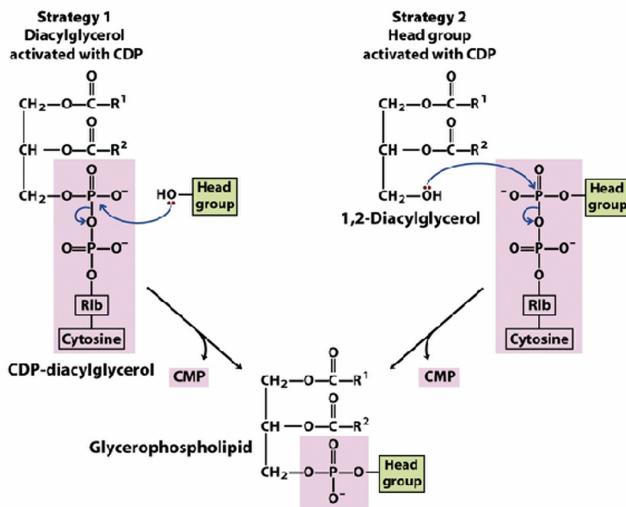


Figure 104: glycerophospholipid synthesis

There are two strategies for creating glycerophospholipids depending on which precursor is activated using cmp:

- strategy 1 - ptAc activation:** This consists in the activation of phosphatidic acid(ptAC) to form **CDP-diacylglycerol**.
- strategy 2 - head group activation:** Here instead of activating ptAc, the head group is activated using CMP.

In either case after the **condenstaion of the head group and the ptAC lead to release of CMP**

7.4.1 bacterial glycerophospholipid synthesis

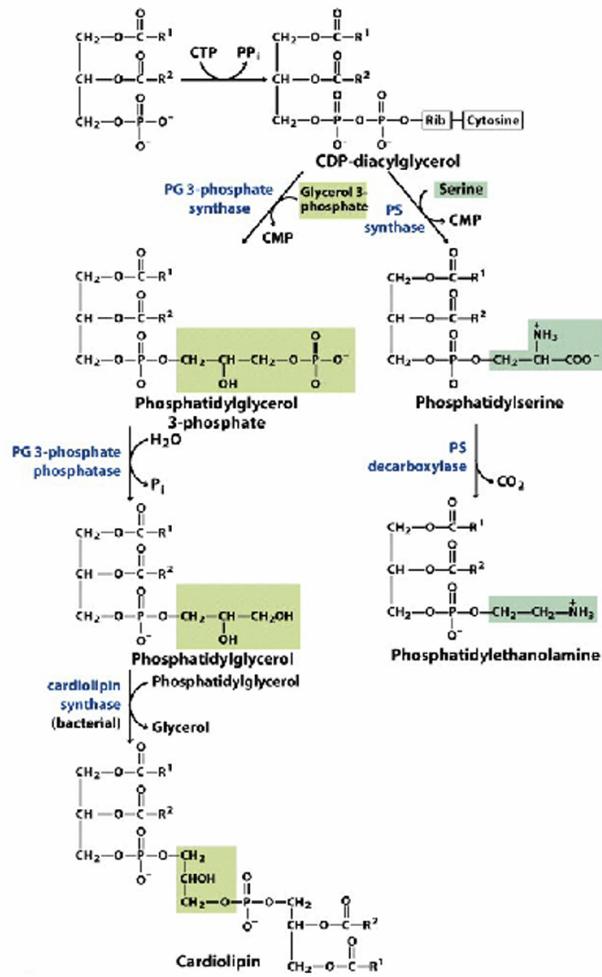
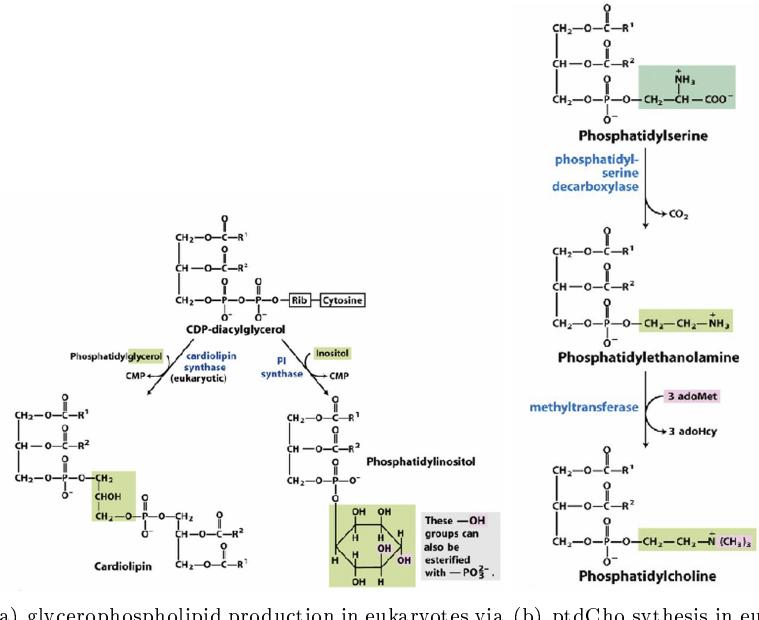


Figure 105: bacteria can make most glycerophospholipids via strategy 1

In E. coli almost all glycerophospholipids can be obtained using strategy 1

- phosphatidylSer (ptdSer): strategy 1
- phosphatidylglycerol-3-phosphate (ptdGly(3)p): strategy 1
- phosphatidylethanolamine (PtdEtn): decarboxylation of ptdSer
- PtdGly: dephosphorylation of ptdGly(3)p
- cardiolipin: condensation of two ptdGly molecules

7.4.2 eucaryotic glycerophospholipid synthesis



(a) glycerophospholipid production in eukaryotes via strategy 1
 (b) ptdCho sythesis in eukaryotes

Figure 106: loading step of FA sythesis

- **PtdIns:** strategy 1 using **phosphatidylinositol synthase**
- **PtdGly:** dephosphorylation of ptdGly(3)p
- **cardiolipin:** CDP-diacylglycerol is used to produce cardiolipin similar to E. coli but only one ptdGly is used.
- **ptdSer:** strategy 1 (for yeast, not for mammals) or head group exchange between PtdSer and PtdEtn (mammals do it like this instead)
- **phosphatidylcholine (ptdCho):** addition of three methyl groups to the PtdEtn amine group operated by a methyltransferase. (this is for yeast. for mammals see section 1.4.2.1)
- (for mammals) **amine containing glycerophospholipids** (ptdCho, ptdEtn): strategy 2

7.4.2.1 phosphatidylinositol synthesis

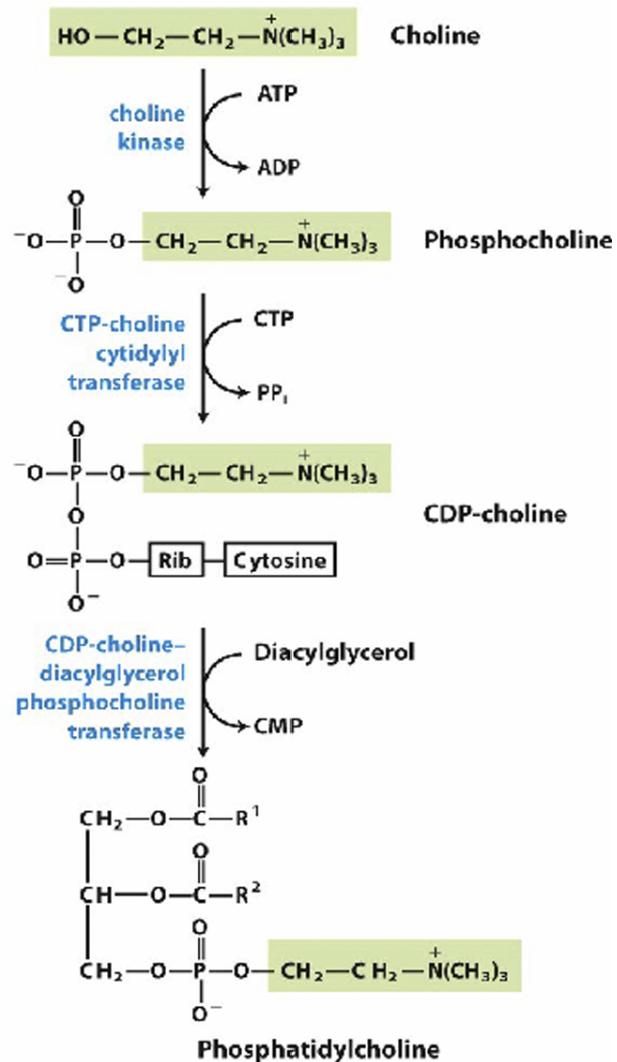


Figure 107: ptdCho synthesis in mammals

mammals synthesis ptCho via strategy 2 as follows:

- choline is phosphorylated by **choline kinase** to phosphocholine
- phosphocholine is complexed to CDP to form CDP-choline by **CTP-choline cytidylyltransferase**
- CDP-choline is condensed to diacylglycerol to produce PtdCho by the action of **CDP-choline diacylglycerol phosphocholine transferase**

7.5 Cholesterol synthesis

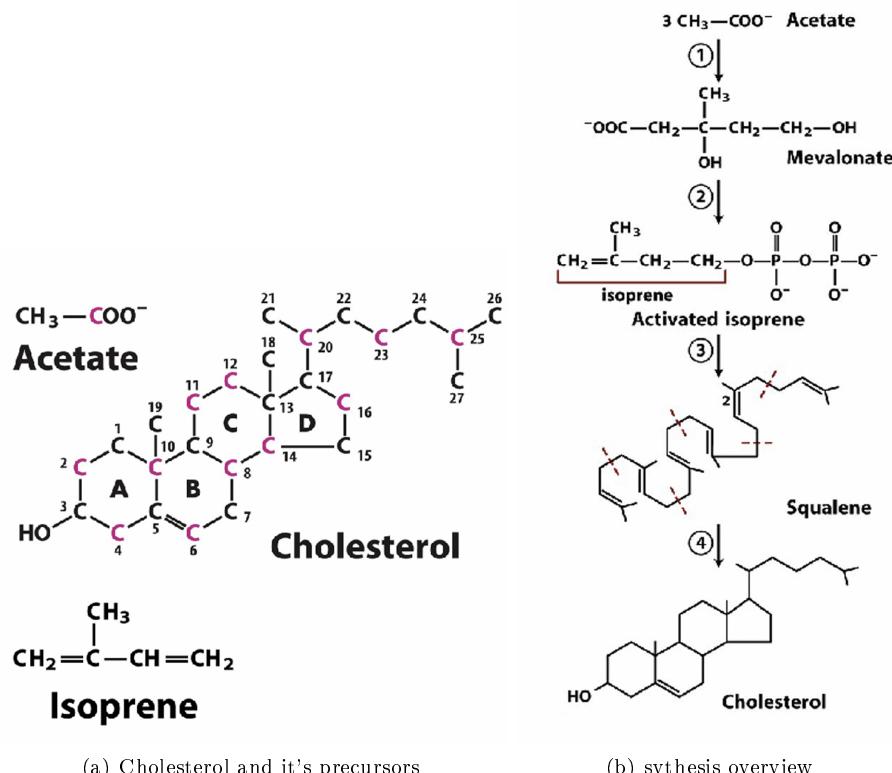


Figure 108: cholesterol synthesis

Cholesterol is formed in a 4 step pathway:

- 3 Acetyl groups are condensed to form **mevalonate**
- Mevalonate is converted into **activated isoprene**
- 6 isoprene units are polymerised into **squalene**
- squalene is converted to cholesterol.

7.5.1 step 1 - melvalonate synthesis

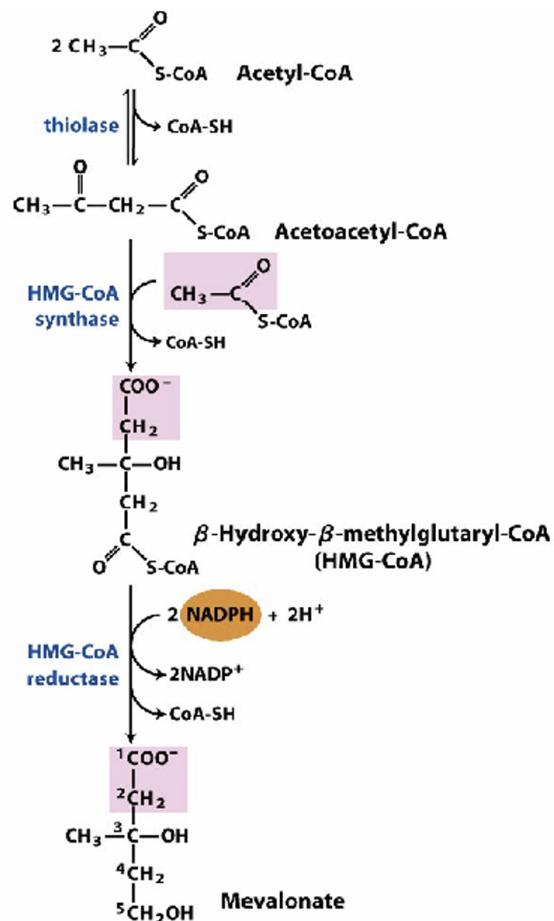


Figure 109: mevalonate synthesis

- i) Two acetyl-CoA molecules are condensed to form **acetoacetyl-CoA** by **thiolase**.
 - ii) **acetoacetyl-CoA** is further condensed with another acetyl-CoA molecule to produce β -hydroxy- β -methylglutaryl-CoA (**HMG-CoA**) by **HMG-CoA synthase**.
 - iii) β -hydroxy- β -methylglutaryl-CoA is reduced to *mevalonate* by **HMG-CoA reductase**.

7.5.2 step 2 - isoprene synthesis

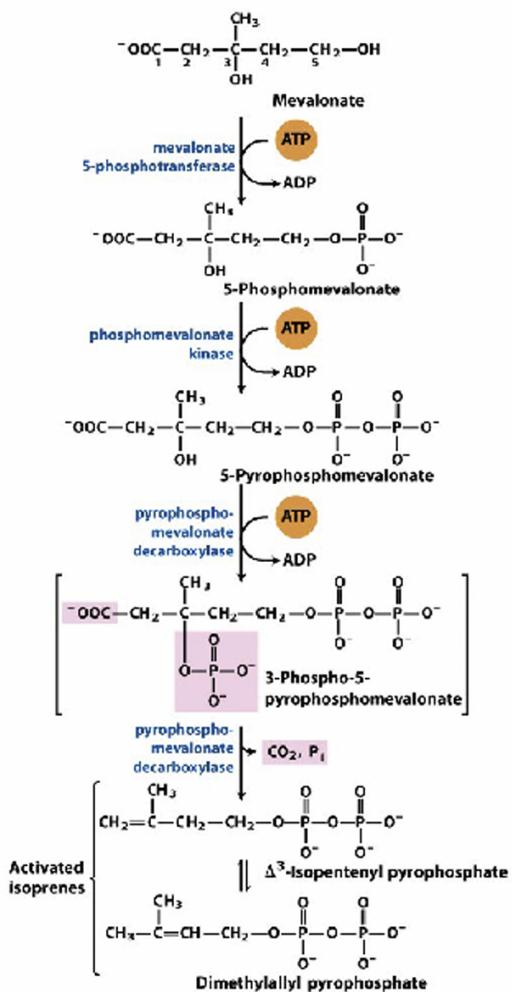


Figure 110: isoprene synthesis

- i) Mevalonate is phosphorylated by **mevalonate 5-phosphotransferase** to form **5-phosphomevalonate**.
- ii) 5-phosphomevalonate is phosphorylated by **phosphomevalonate kinase** to produce **5-pyrophosphomevalonate**.
- iii) 5-pyrophosphomevalonate is phosphorylated to **3-phospho-5-pyrophosphomevalonate** by **pyrophosphomevalonate decarboxylase**.
- iv) The same enzyme, **pyrophosphomevalonate decarboxylase**, catalyzes the decarboxylation of 3-phospho-5-pyrophosphomevalonate to produce activated isoprene units (Isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate.)

7.5.3 step 3 - squalene synthesis

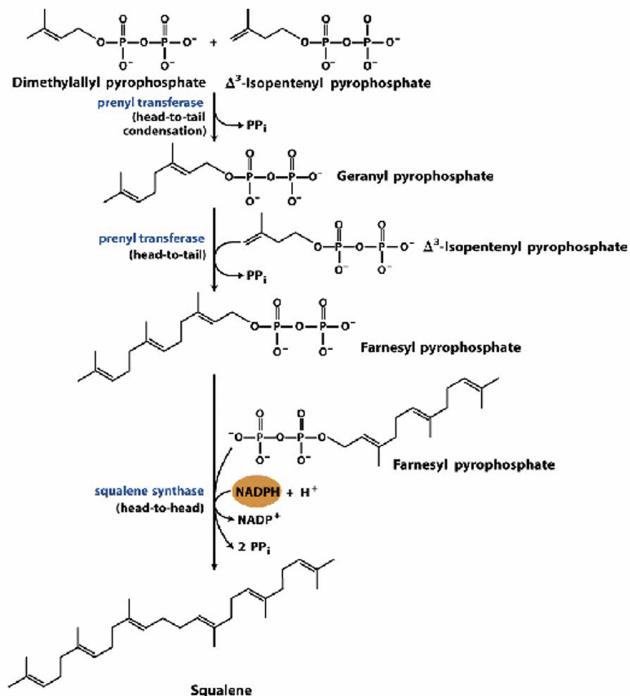


Figure 111: squalene synthesis

- isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (isoprene units) undergo a **head-to-tail** condensation to form **geranyl pyrophosphate (GPP)**, catalyzed by the enzyme **prenyl transferase**.
- Geranyl pyrophosphate and isopentenyl pyrophosphate undergo another head-to-tail condensation to form **farnesyl pyrophosphate (FPP)**, also catalyzed by **prenyl transferase**.
- Two farnesyl pyrophosphate molecules are condensed **head-to-head** to form **squalene**, catalyzed by **squalene synthase**.

7.5.4 step 4 - ring closure

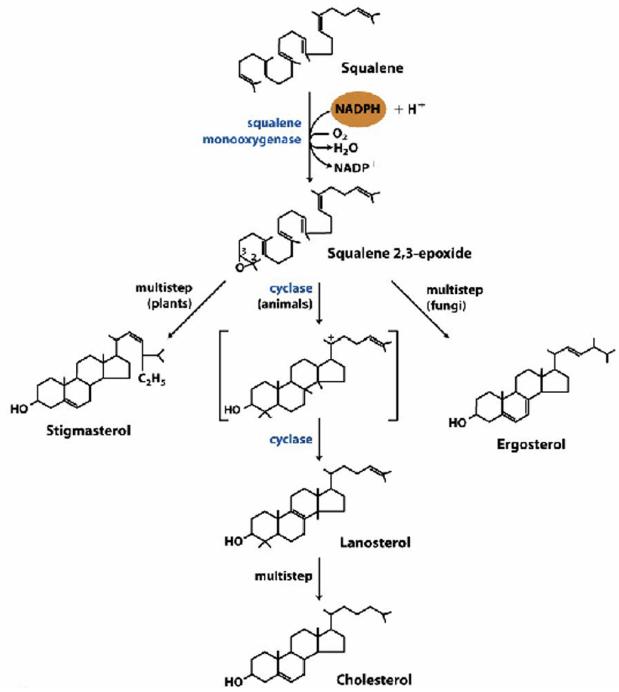


Figure 112: ring closure and final step of cholesterol synthesis

- i) squalene is oxygenated by **squalene monooxygenase** to form **squalene 2,3-epoxide**.
- ii) Cyclization reactions then convert squalene 2,3-epoxide into **lanosterol**, which, through a complex series of about 20 reactions, is ultimately converted into cholesterol.

7.6 sphingolipid synthesis

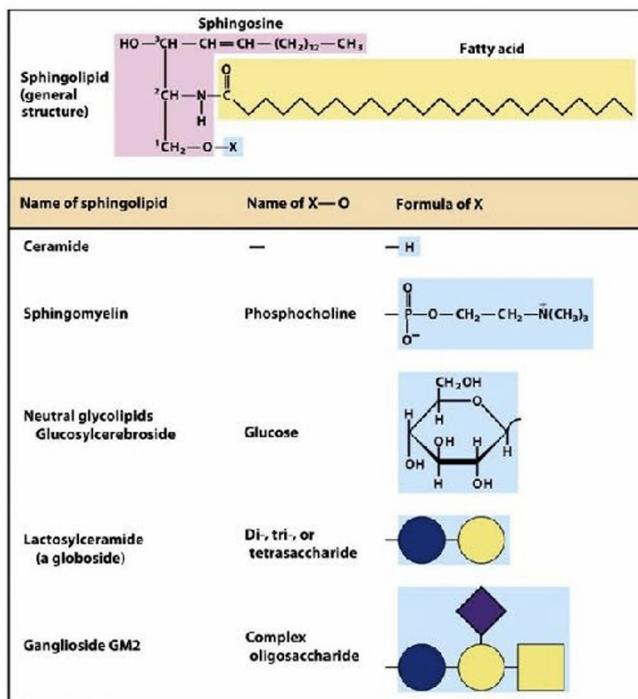


Figure 113: sphingolipid structural overview

sphingolipids all contain **ceramide** backbone and a variable head group. An example of such head group is phosphocholine which produces **sphingomyelin, the most abundant sphingolipid in mammals**. Ceramide can be **glycosylated to produce various glycosphingolipids**

7.6.1 ceramide synthesis

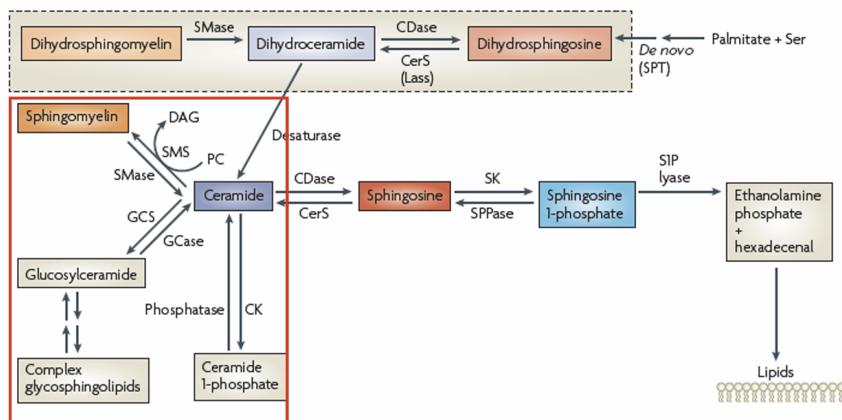


Figure 114: overview of de novo(grey box) and salvage pathway(red box)

Ceramide being the precursor of many sphingolipids can be created in two ways, the **salvage pathway** and the **de novo pathway**.

7.6.1.1 de novo pathway

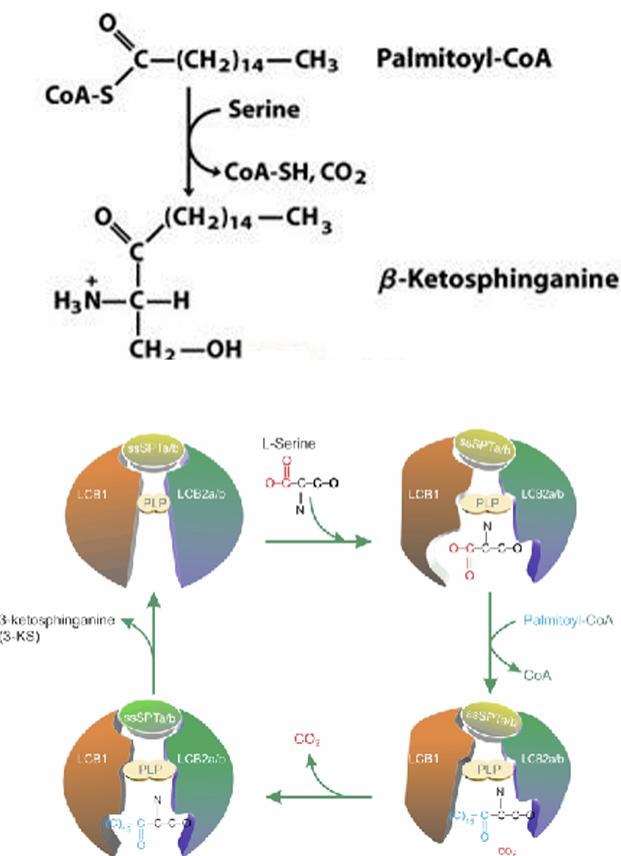


Figure 115: condensation step

step1 - condensation serine and a palmitoyl-CoA molecule are condensed by **serine-palmitoyltransferase (SPT)** to form β -ketosphinganine with the **release of a CO₂ molecule**.

- i) SPT uses pyridoxal 5'-phosphate (PLP) as a cofactor.
- ii) PLP initially binds to an active-site lysine residue.
- iii) L-serine displaces the lysine, forming a serine-PLP intermediate.
- iv) The serine-PLP intermediate attacks palmitoyl-CoA.
- v) Following decarboxylation, β -ketosphingosine is released.
- vi) PLP is regenerated in its catalytically active form.

In **eukaryotes**, SPT is a heterodimer localized to the endoplasmic reticulum with The active site faces the cytosol. \rightarrow this is the rate limiting step

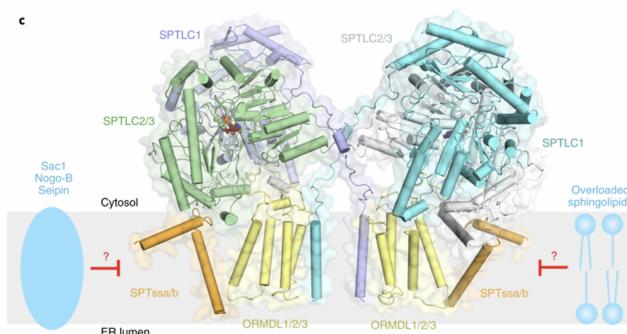


Figure 116: serine-palmitoyltransferase (SPT)

The SPT enzyme is tightly regulated. Among other things it processes an **ORMDL1/2/3** domain. These are responsible for **sensing the amount of ceramide** and create a **negative feedback loop**.

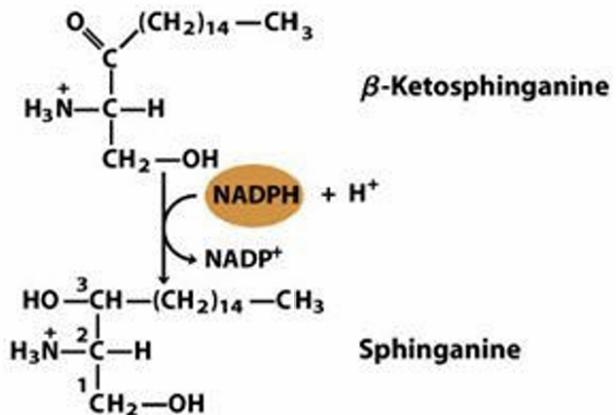


Figure 117: reduction step

step2 - reduction β -ketosphingosine is reduced to sphinganine by **KDSR (β -ketosphinganine reductase)** with consumption of an NADPH molecule. KDSR is an ER membrane protein and has its active site oriented towards the cytoplasmic leaflet.

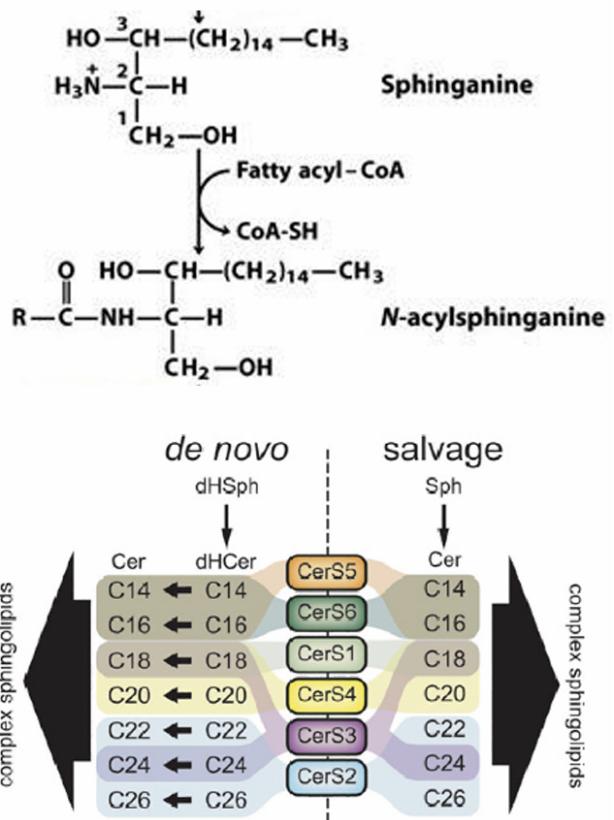


Figure 118: condensation step

step3 - condensation sphinganine is condensed with a **fatty acyl-CoA** to produce **N-acylsphinganine** with **release of a free CoA** molecule. This reaction is catalyzed by ceramide synthase. There are 6 ceramide synthases (CerS1-6). Each with individual substrates:

- CerS2 prefers very long-chain acyl-CoAs (C22–C24–C26).
 - CerS4 prefers C20 acyl-CoAs.
 - CerS5 prefers shorter chains (C14–C16–C18).

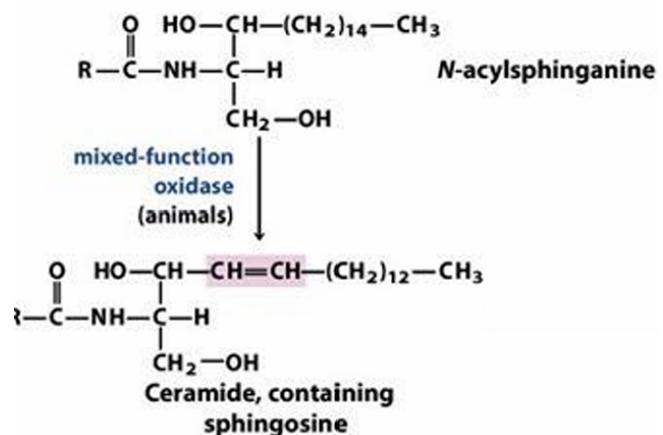


Figure 119: desaturation step

step4 - desaturation N-acylsphinganine (dihydroceramide) is desaturated to ceramide by **Dihydroceramide Desaturase (DES)**. DES requires the **O₂** and **NADPH** as cofactors to add a **4,5-trans-double** to N-acylsphinganine.

7.6.1.2 salvage pathway

Sphingolipid (general structure)		
<chem>CCOC(=O)N(C)C=C(O)C(=O)R</chem>	<chem>HO-C(=O)R</chem>	<chem>CH2-O-X</chem>
Name of sphingolipid	Name of X—O	Formulas of X
Ceramide	—	— H
Sphingomyelin	Phosphocholine	<chem>CCOC(=O)[P](=O)([O-])O-CH2-CH2-N+(CH3)3</chem>
Neutral glycolipids Glucosylceramide	Glucose	<chem>OC[C@H]1OC(O)C[C@H](O)[C@H](O)[C@H](O)[C@H]1O</chem>
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

Figure 120: sphingolipid structural overview

The salvage pathway involves the production of ceramide through the cleavage of the head group from other sphingolipids.

7.6.2 sphingomyelin synthesis

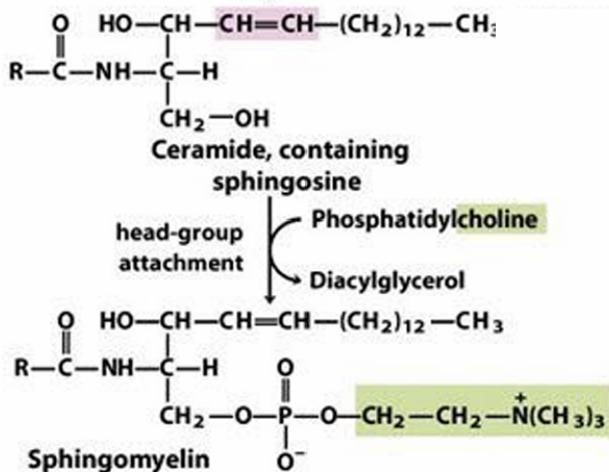


Figure 121: sphingomyelin synthesis

ceramide can be converted into sphingomyelin by the **Sphingomyelin Synthase (SMS)**. This is a **multispan membrane protein located in the trans golgi lumen** that transfers **phosphocholine onto ceramide**. This has **diacylglycerol as a byproduct**

7.6.2.1 ceramide transport to golgi via cert1

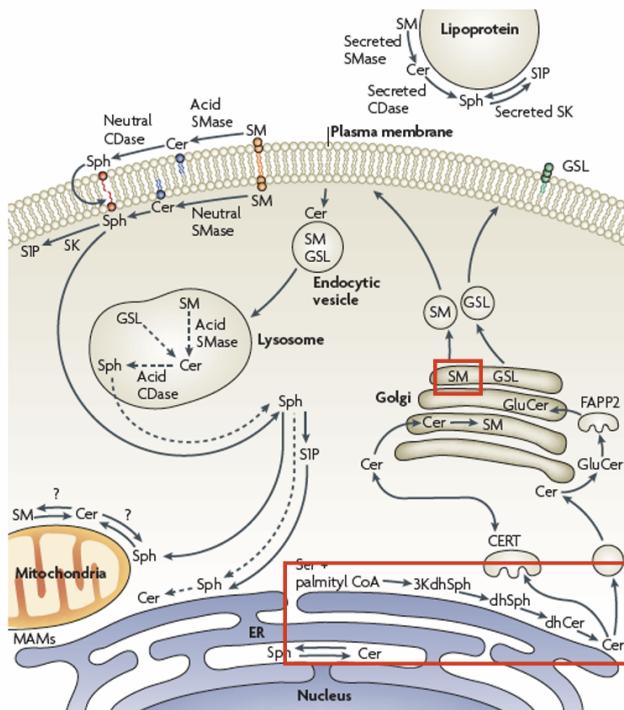


Figure 122: transporting ceramide to trans golgi lumen

Ceramide is synthesized in the ER. However the active site of SMS is located in the Lumen of the trans golgi compartment. This means that cermaide needs to be transported to the SMS active site. Since it can't diffuse through the cytosolic enviroment as this is highly unfavorable it needs to be activly transported from ER to the lumen trans golgi compartment by CERT1 (Ceramide Transfer Protein 1)

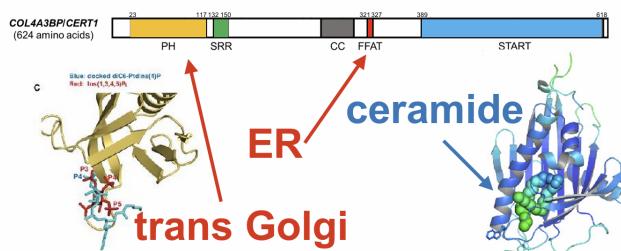


Figure 123: cert1 structure and domains

cert1 is a cytosolic protein that has an N-terminal Pleckstrin Homology (PH) Domain that will bind to PtdIns(4)P on golgi membrane. It also has a FFAT Motif that will bind to ER membrane protein VAP (Vesicle-associated membrane protein-associated protein). A C-terminal START Domain allows cert to bind to cermide so it can be transported to Golgi lumen. CERT1 operates ceramide transfer at ER-trans Golgi-Membrane Contact Sites (MCS)

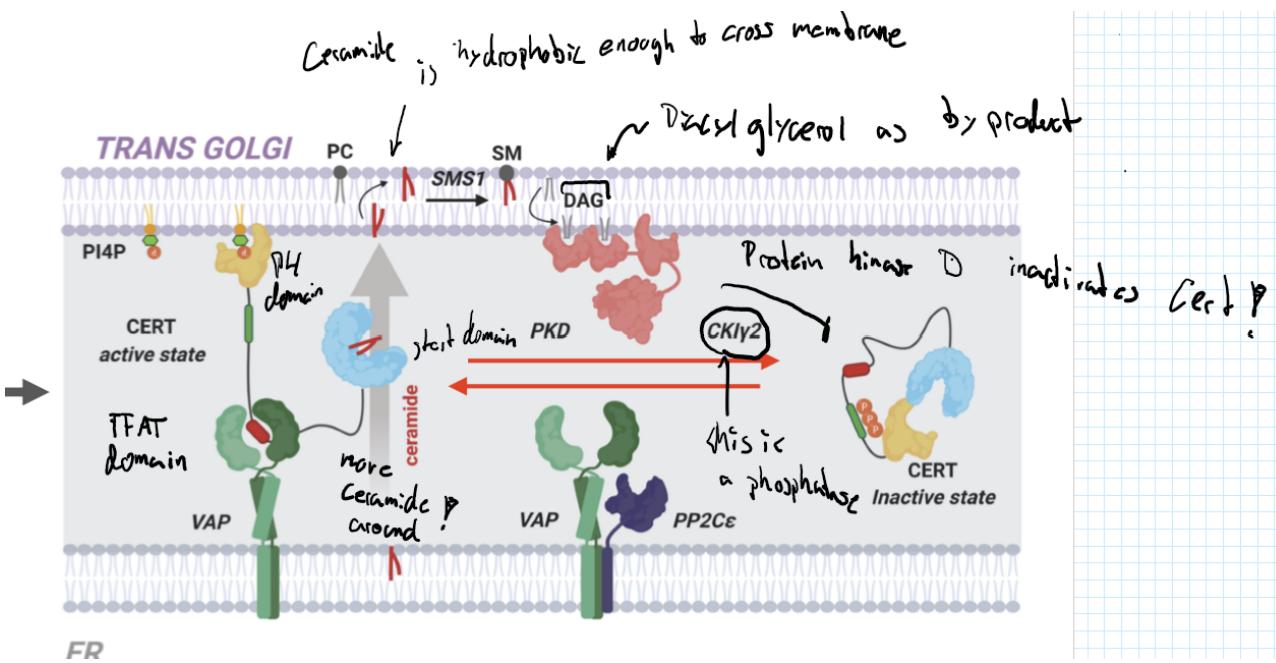


Figure 124: cert1 and it's negative feedback loop

(there is a normal image to this aswell but in the lecture he spoke a lot about it and I feel like the anoted version might help. LMK :)) Cert is tightly regulated and has a negative feedback loop, where if there is too much ceramide it will be phosphorylated by PKD which will inactivate it. CKLy2 will dephosphorylate it and inactivate it once again if there is too little ceramide in the golgi. Since cert is involved in regulating sphingomyelin synthesis if mutated this will cause many **intellectual disabilities** (more than those caused by writing this shit at 2AM in a room that smells like a years supply of weed) as **sphingomyelin is a key component of the myelinsheaths**

7.6.3 glucosylceramide (cerebroside) synthesis

Ceramide can also be converted into **glucosylceramide synthesis** (GlcCer) also known as **Cerebroside** by **GlcCer Synthase (GCS)**. Cerebroside synthesis happens on the **cytosolic leaflet of the cis-golgi compartment**. This is a key difference to that of sphingomyelin. There is **no need for specialized transporter** like Cert1 in the case of sphingomyelin production. Here simple vesicles will do the trick! (see fig. 122). Cert1 will actually force the production of sphingomyelin by delivering directly to the trans golgi compartment. After GlcCer has been produced it will be **translocated to the trans-golgi compartment** by FaPP2 (Fatty Acid Phosphatase 2).

7.6.3.1 transporting from cis to trans golgi via FaPP2

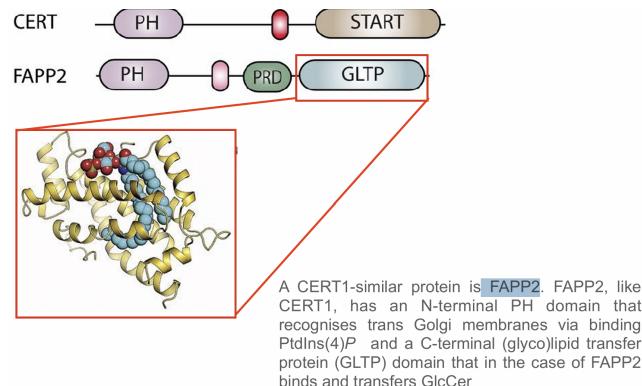


Figure 125: FAPP2 domains

this transporter also has a Pleckstrin Homology (PH) Domain to recognize the golgi via **PtdIns(4)P** binding which is located in the golgi membrane on the cytosolic leaflet side. It has a GLTP (Glycosphingolipid Transfer Protein) which analogously to the START domain of Cert1 will be used to bind cerebroside (GlcCer). **FAPP2 translocation to trans golgi compartment will actually seal the fate of the sphingolipid and what it can be turned into:**

- FAPP2 → will be processed to **Globoside production**
- if it gets to trans golgi on its own → **ganglioside production**

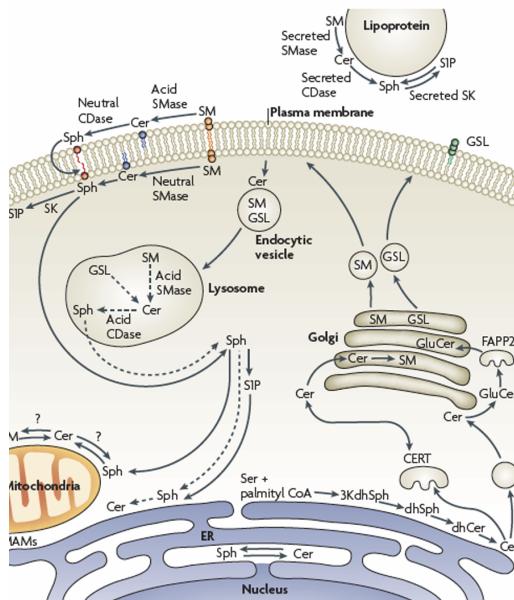


Figure 126: overview of transport pathways

8 Phosphoinositides

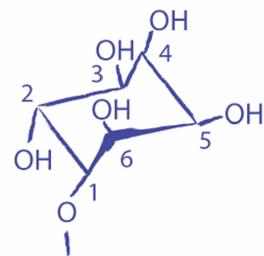


Figure 127: inositol

These are derived via phosphorylation of phosphatiyld inositol (ptdIn) that are produced via **phosphatidylinositol synthase** (see section on glycerophospholipid synthesis)

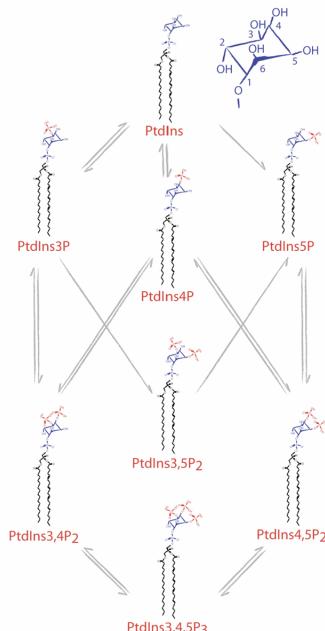


Figure 128: the 7 phosphoinositides (PI)

phosphorylation occurs in three, four and five hydroxyl However, the two and six hydroxyl groups are typically not phosphorylated due to steric hindrance. There are about 50 phosphoinositide kinases (PIKs and phosphatases that are present in practically all cell compartments.

8.1 phosphatidylinositol transfer proteins (PITPs)

Phosphatidylinositol Transfer Proteins (PITPs) play a crucial role in transporting Phosphatidylinositols through the cytosol. These transporters can actually **transport monomers of PtdIns or PtdCho between membrane compartments**. This distribution means that ptdIn is on the **cytosolic leaflet of various organelles**

8.2 regionalisation

- PtdIns(3)P
- PtdIns(4)P
- PtdIns(3,5)P₂
- PtdIns(4,5)P₂
- PtdIns(3,4,5)P₃

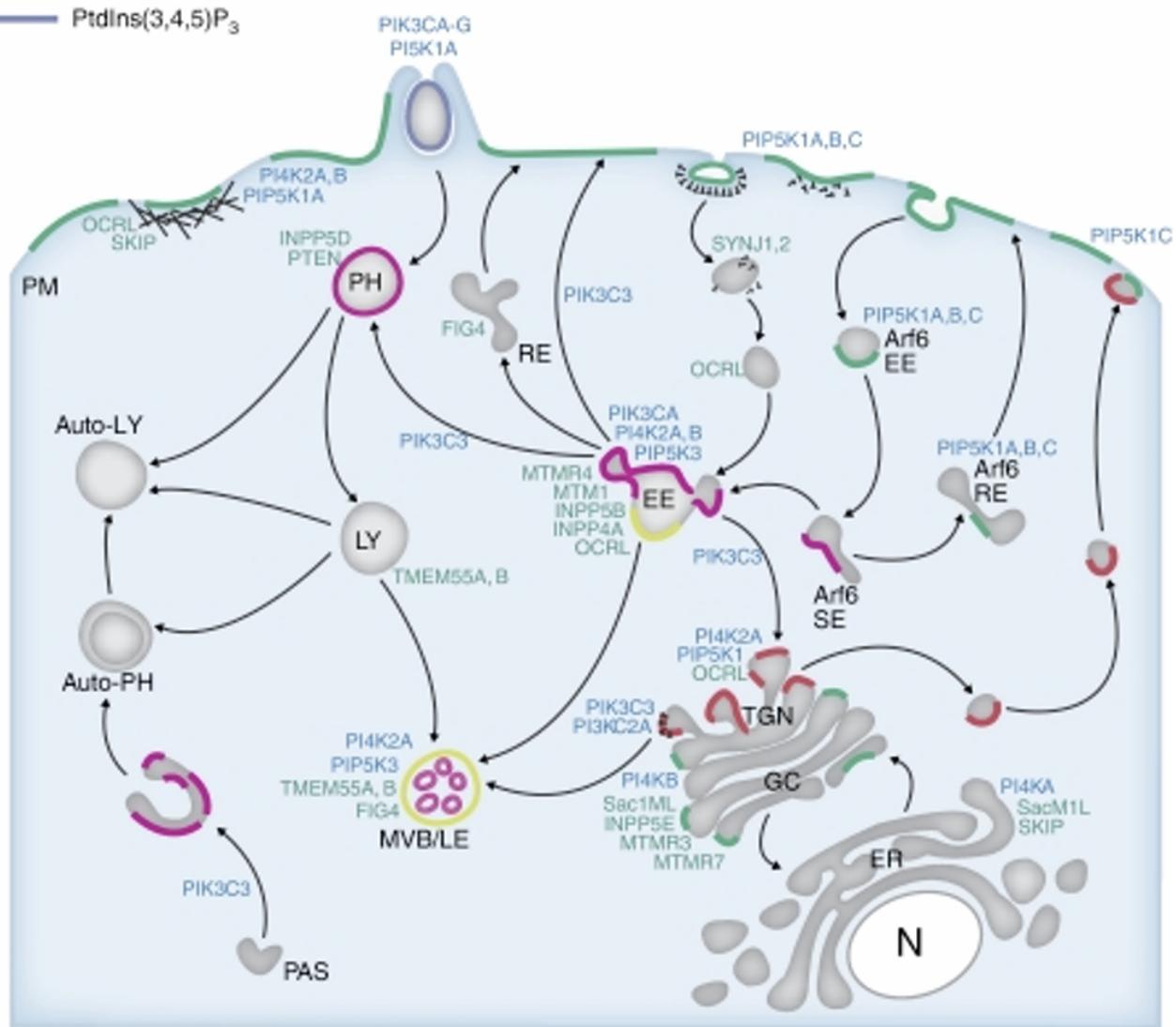


Figure 129: different phosphorylations in different compartments

PtdIns are not distributed evenly, in fact it is quite the opposite where different phosphoinositides are located in different cellular compartments. This is due to the fact that there are different PI-kinases in the different cellular compartments. **The dynamic interplay between PtdIns transport and processing leads to lipid compartmentalisation.**

8.3 recognizing membrane compartments

Various domains recognize phosphoinositides, in fact they are **specific to the phosphorylation status of PI!**. These domains include Pleckstrin Homology (PH) Domain, FYVE domain, gelsolin homology domain, SH2 domain, PTB domain. Here is an overview of a few more domains:

Domain	Typical size (amino acids)	Structure	Preferred target*	Membrane insertion?	Ca ²⁺ required?	Dimerization required?
C1	~50	Zn ²⁺ finger	DAG, phorbol esters	Yes	No	No
PKC C2 [‡]	~130	β-sandwich	PtdSer (and others)	Yes	Yes	No
PH	~125	β-sandwich	Phosphoinositides, quite diverse, some highly specific	Some reported ²⁰	No	Some examples
FYVE	60–70	Zn ²⁺ finger	PtdIns3P	Yes	No	Most cases
PX	~130	α+β structure	PtdIns3P (a few bind other phosphoinositides)	Yes	No	Most cases
PROPPIN	~500	β-propeller	PtdIns(3,5)P ₂ (PtdIns3P also in some cases)	Unknown	No	No
Gla	~45	α-helical (requires Ca ²⁺ to fold)	PtdSer	Yes	Yes	No
Annexin	~310	α-helical array	Acidic phospholipids	Unknown	Yes	No
Discoidin C2 [‡]	~160	β-sandwich	PtdSer (specific)	Yes	No	No
ENTH	~150	α-helical solenoid	PtdIns(4,5)P ₂ (some promiscuity)	Yes	No	No
ANTH	~280	α-helical solenoid	Phosphoinositides, relatively little specificity	No	No	Yes

Figure 130: table of domains that recognize PIs

typically these domains have low affinities for their binding partner. This means that they will stumble along the cytosol probing the various membrane compartments and becoming enriched in the areas where there are more of its substrate. On top of this there is avidity effect, where many of these domains recognize other things as well and might just by chance find Phosphoinositides. This is called **coincident detection**. PIs are therefore crucial for localisation of various proteins to the different organelles. **The encode the organelle's identity**

8.3.1 PtdIns(4)P example of lipid transport coordinator

PtdIns(4)P is the most abundant of the monophosphorylated phosphoinositides. It is produced by ptdIn phosphorylation by **PI4Ks** which are active in the golgi. PtdIns(4)P are dephosphorylated by **Sac1** which is active in the ER.

→ more PtdIns(4)P in trans golgi than ER

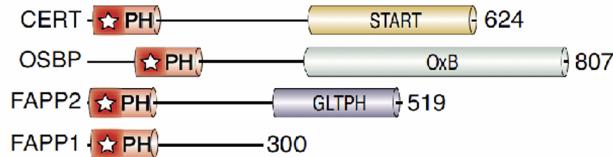


Figure 131: domains that recognize PtdIns(4)P on trans golgi

Various domains such as CERT1 (Ceramide Transfer Protein 1), FaPP2 (Fatty Acid Phosphatase 2) or OSBP will transport ceramide to the trans golgi compartment.

→ PtdIns(4)P determines the lipid composition of the post Golgi compartments.

8.3.1.1 OSBP1

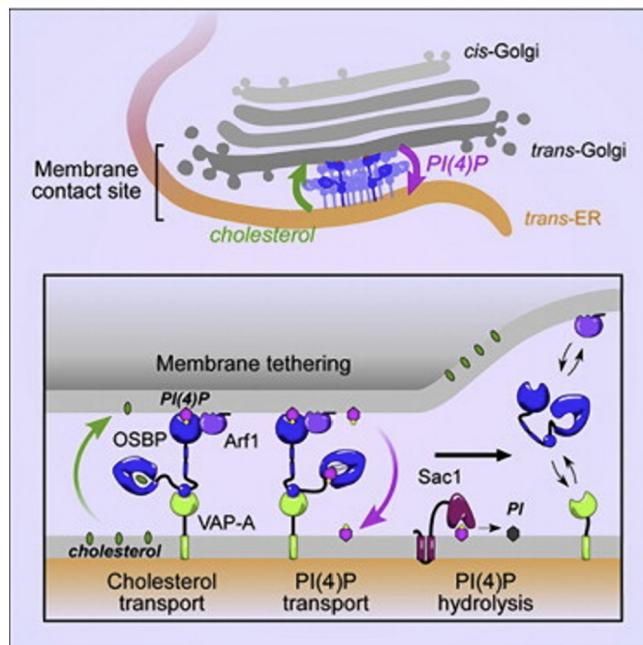


Figure 132: OSBP1

This is an interesting case for transporters as ontop of transporting **cholesterol to trans golgi compartment** it wil also transport **PtdIns(4)P back to ER** where it will be dephosphorylated by **Sac1**. This is a sort of **negative feed back loop** as OSBP1 consumes its recruiting factor [i.e., PtdIns(4)P] while pumping cholesterol to the trans-Golgi

8.4 PTEN and oncology relevance

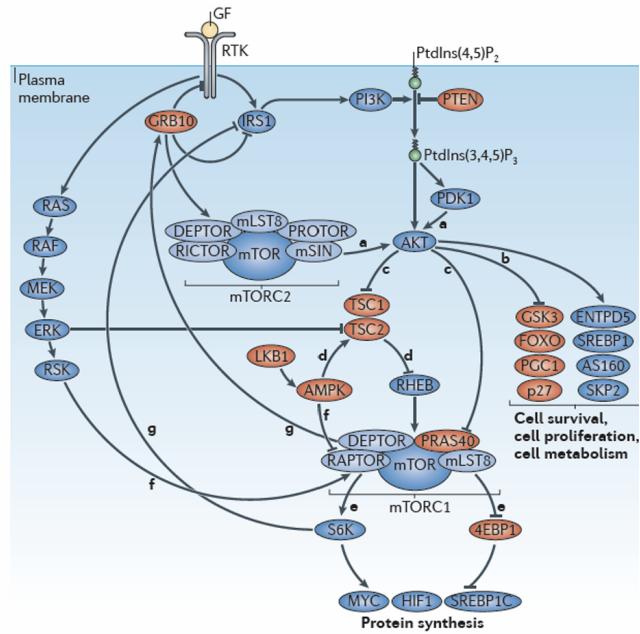


Figure 133: PTEN is a tumor suppressor gene

PTEN is one of the most commonly lost tumor suppressor genes. It is a **phosphoinositide phosphatase**, which will reduce the effects of **PI3k**. If lost it will lead to uncontrolled growth.

9 Amino Acids in Biosynthesis

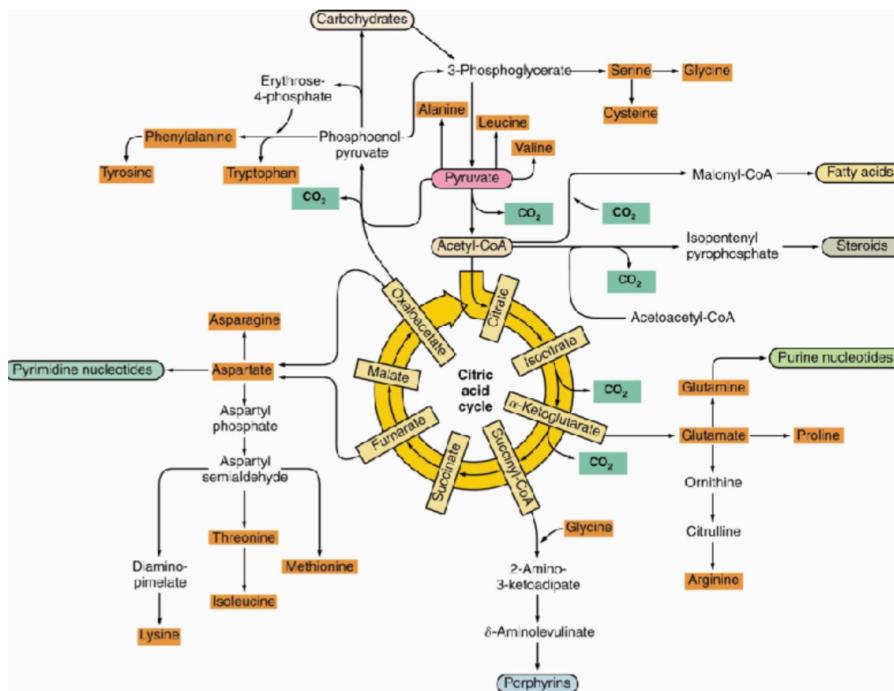


Figure 134: This image shows an overview of many of the products stemming from the citric Acid Cycle. This ranges from amino acids, to nucleic acids, porphyrines, and more.

9.1 The actual synthesis of Amino Acids

Amino acids are produced from intermediates of glycolysis of the TCA cycle or of the pentose phosphate pathway. Nitrogen is transaminated onto these substrates from glutamine or glutamate

9.1.1 Prelude: Incorporating Nitrogen - Glutamate and Glutamine

Nitrogen is required in amino acids. However, we humans can't get it from the atmosphere. That means we rely bacteria and archaea to fix N₂ from the atmosphere to produce ammonia. That ammonia then enters the cell metabolism and is incorporated into **Glutamate** and **Glutamine**.

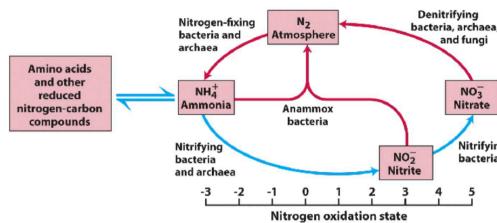


Figure 136: Shows the nitrogen cycle, which happens in bacteria and archaea. Humans can absorb ammonia.

The production of glutamate in bacteria follows the production of glutamine. Here's what that looks like:

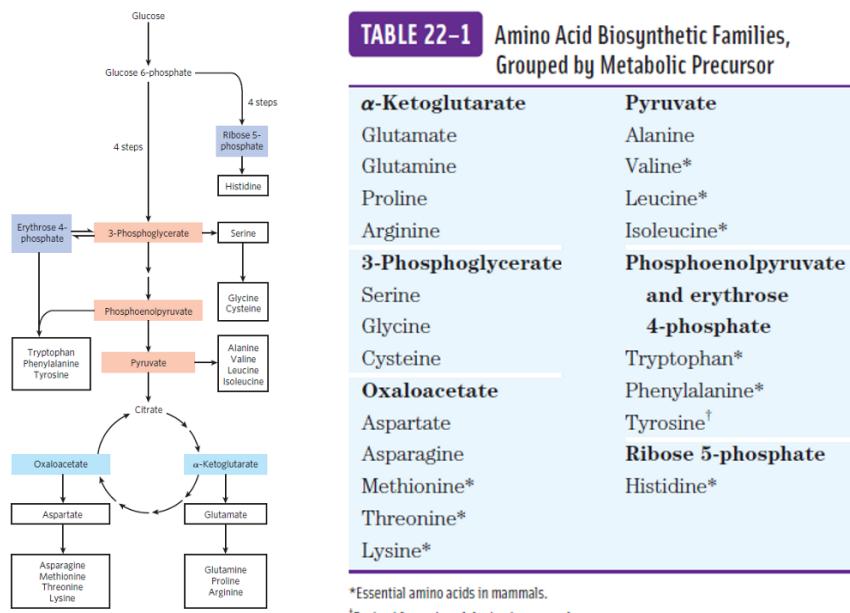


TABLE 22-1 Amino Acid Biosynthetic Families, Grouped by Metabolic Precursor

α-Ketoglutarate	Pyruvate
Glutamate	Alanine
Glutamine	Valine*
Proline	Leucine*
Arginine	Isoleucine*
3-Phosphoglycerate	Phosphoenolpyruvate and erythrose 4-phosphate
Serine	Tryptophan*
Glycine	Phenylalanine*
Cysteine	Tyrosine†
Oxaloacetate	Ribose 5-phosphate
Aspartate	Histidine*
Asparagine	
Methionine*	
Threonine*	
Lysine*	

*Essential amino acids in mammals.

†Derived from phenylalanine in mammals.

Figure 135: the image on the left shows in which part of glycolysis we derive the amino-acids from. The table on the right shows the precursor for each amino acid.

- i) Glutamine production (both in humans and bacteria): $\text{Glutamate} + \text{NH}_4^+ \rightarrow \text{glutamine}$, through the enzyme **Glutamine synthase** using a 1 ATP.
- ii) Glutamate production (in bacteria): $\alpha\text{-ketoglutarate} + \text{glutamine} \rightarrow 2 \text{ glutamate}$, through the enzyme **Glutamate synthase** using both an NADPH and an ATP. This reaction is a **transamination** and how bacteria produce more glutamate.

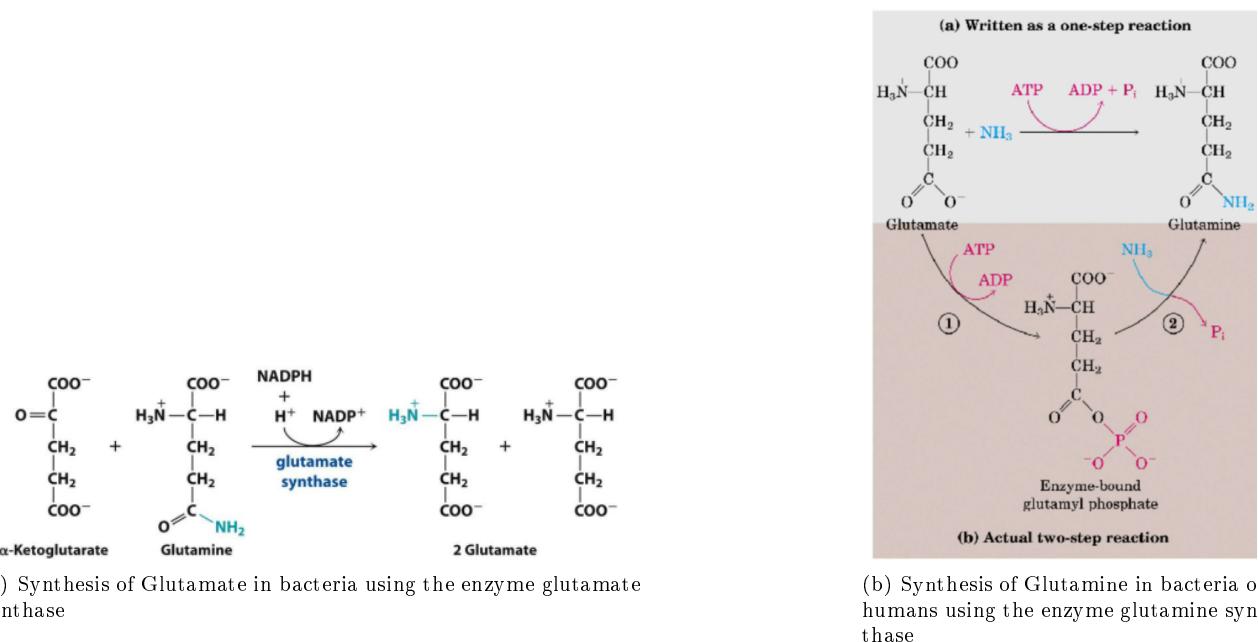


Figure 137: Shows the synthesis of both glutamine and glutamate

Glutamate and glutamine are then used to transfer NH₃ to a variety of different products, producing aminated molecules; these are called **transamination** reactions.

Glutamine amidotransferase is a common enzyme for transaminating glutamine. How the transamination happens:

- This enzyme is constituted by two domains, one that binds glutamine, the other binds the acceptor substrate.
- A Cys residue in the Glutamine-binding domain breaks the acidic bond and forms a glutamyl-enzyme intermediate
- NH₃ travels to the NH₃-acceptor domain
- There an activated substrate (usually activated by ATP) is aminated and released.

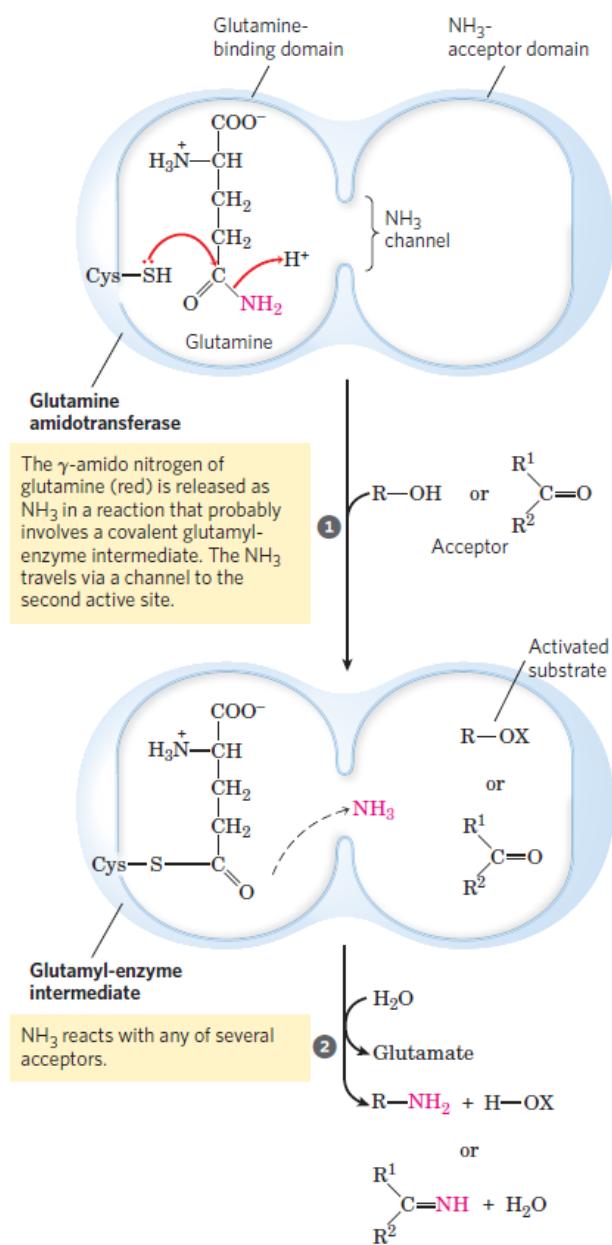


Figure 138: The enzyme glutamine amidotransferase's mechanism.

9.1.2 Proline and Arginine

Proline is a cyclic derivative of glutamate. Here is its synthesis:

- Glutamate is phosphorylated
- Glutamyl-P is dephosphorylated and reduced
- Glutamate semialdehyde undergoes spontaneous cyclisation.

iv) Pyrroline-5-carboxylate is reduced to Proline.

Arginine is synthesized in a similar pathway:

i) Glutamate is first acylated.

ii) [Proline – equiv.] Acetylglutamate is phosphorylated.

iii) [Proline – equiv.] Acetylglutamate-P is then reduced.

iv) The acylation impedes the cyclisation. Instead through further transamination and de-acylation Ornithine is produced.

v) Ornithine is then converted to Arginine in the urea cycle (seen in lecture on Lipid biosynthesis).

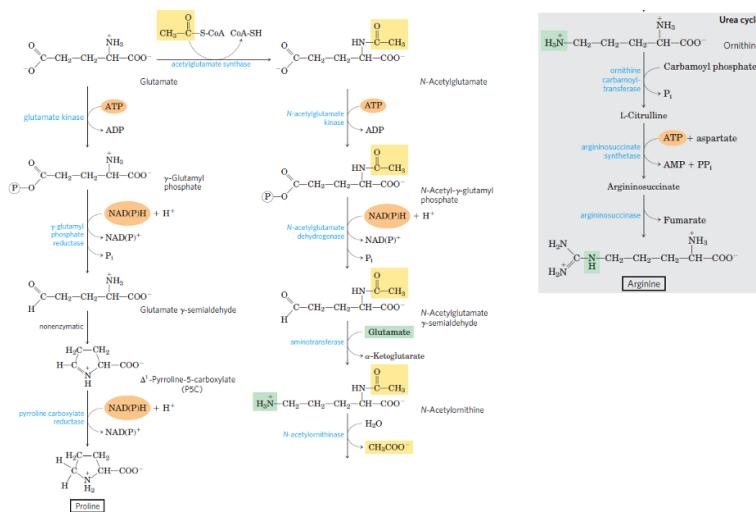


Figure 139: Shows the pathway for the production of both Proline and Arginine, stemming from glutamate.

9.1.3 Serine, Glycine, and Cysteine

Serine is formed from an intermediate of glycolysis: 3-phosphoglycerate. Cysteine and Glycine on the other hand are derivatives of Serine.

First the synthesis of Serine:

- Oxidation of 3-phosphoglycerate
- Transamination of 3-phosphoypyruvate
- Dephosphorylation of 3-phosphoserine

To continue to **Glycine**, the enzyme **serine hydroxymethyltransferase** removes a carbon atom from glycine. For this it uses tetrahydrofolate, as well as PLP (activated vitamin B6) as a cofactor.

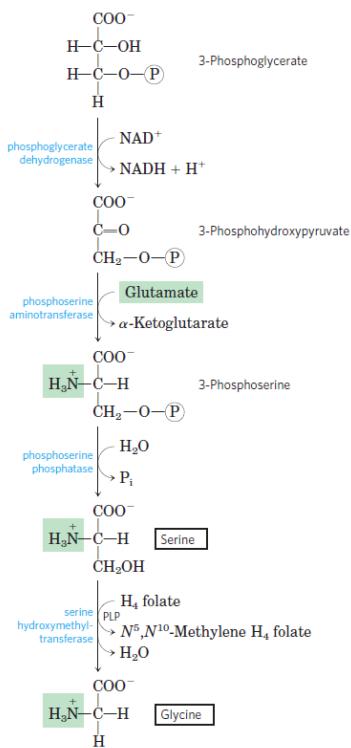


Figure 140: The synthesis of Glycine and Serine. Glycine is a derivative of Serine.

In mammals **Cysteine** is formed from **Serine** and **Methionine**. Here's how:

- Through a series of reactions Methionine becomes homocysteine.
- Homocysteine is condensed to bond with Serine to form cystathioneine.
- Cystathioneine is hydrolysed with a loss of NH4+ to form cysteine and **alpha-ketobutyrate**.

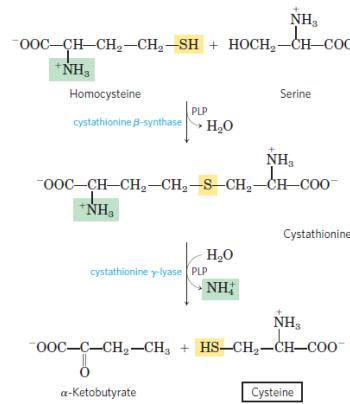


Figure 141: The synthesis of Cysteine from Serine and Methionine.

9.1.4 Aspartate, Asparagine, and Alanine

Asparagine and Alanine are produced mainly in the liver. So, if you have too high concentrations of the two, something is probably off in your liver.

Aspartate, Asparagine, and Alanine are produced the following way:

- Aspartate: Transamination of oxaloacetate, catalyzed by **Aspartate transaminase (AST)**.
- Asparagine: Amidation of aspartate by glutamine, catalyzed by **Asparagine Synthase** using an ATP into AMP.
- Alanine: Transamination of pyruvate, catalyzed by **Alanine transaminase (ALT)**.

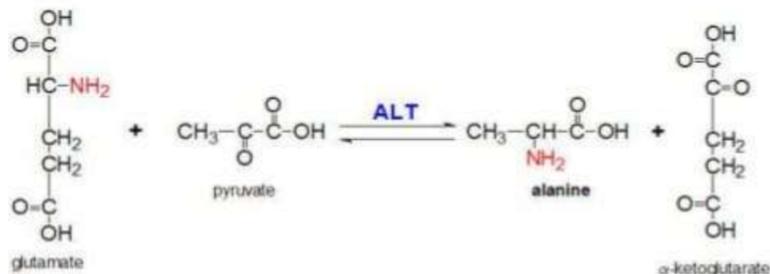
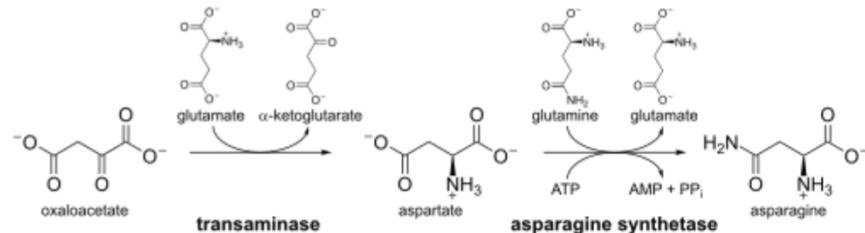
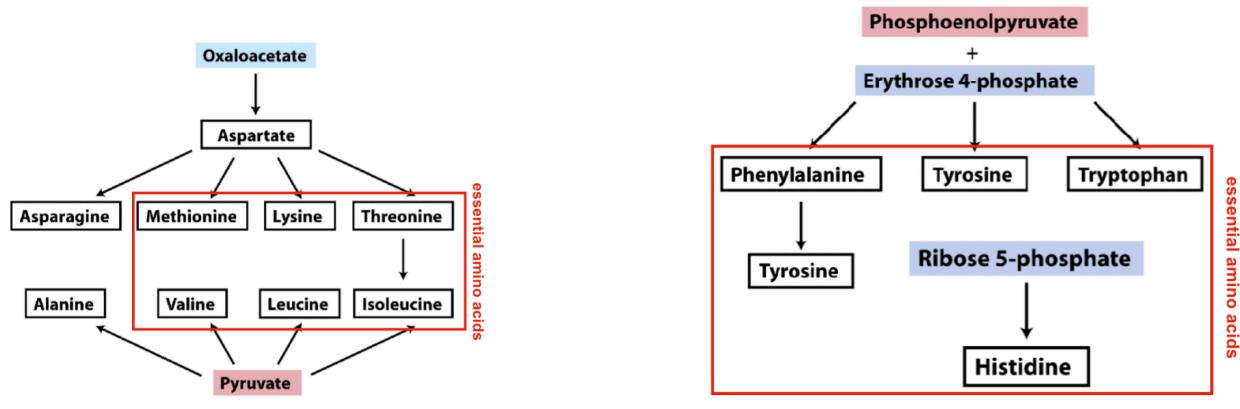


Figure 142: The synthesis of Aspartate (to become Aspartic Acid), Asparagine, and Alanine.

9.1.5 Essential Amino Acids

Now, that leaves us with the **essential amino acids**, which are those amino acids the human body can't synthesize. Instead we consume food to get to them. This even though they will often have precursors which would be in our body. Here is a quick overview:



(a) Essential amino acids with the precursors Oxaloacetate or pyruvate.

(b) Essential amino acids with the precursors Ribose 5-phosphate, Phosphoenolpyruvate and erythrose 4-phosphate.

Figure 143:

9.2 Amino Acids derived Biomolecules

Amino acids are not only the precursors for proteins. They are also precursors for lipid production, neurotransmitters, porphyrins, and hormones.

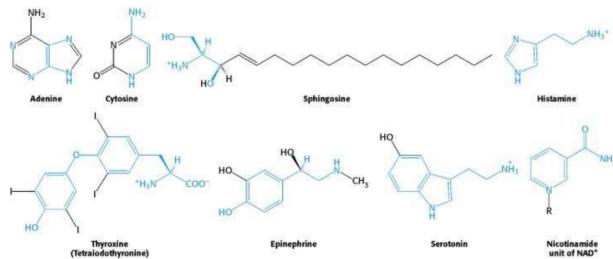


Figure 144: Some examples of amino acids being precursors for other biomolecules.

9.2.1 Porphyrins

Porphyrins are a group of heterocyclic compounds, that absorb strongly in the visible region of the EM spectrum. Metal complexes derived from porphyrins occur naturally. One very prominent example of a porphyrin is heme, which makes blood cells red, and is a cofactor of hemoglobin.

Glycine is the main precursor for the synthesis of porphyrins. Glutamate is an alternative source. Here's how:

- Glycine (mammals) reacts with succinyl-CoA to form α -amino-ketodipate. This is then decarboxylated to **d-aminolevulinate**.
- Glutamate (plants and bacteria): Through the reduction of Glutaminyl-tRNA, followed by the isomerization of glutamate semialdehyde, we also end up with d-aminolevulinate.

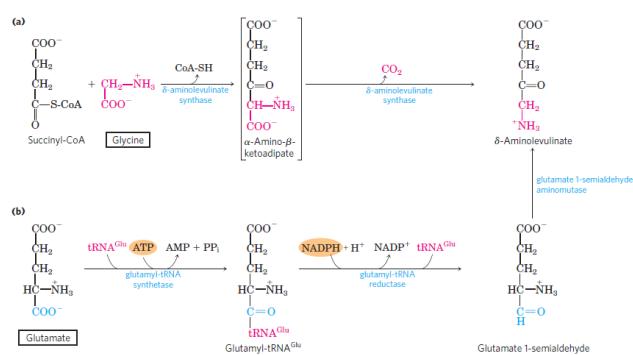


Figure 145: The biosynthesis of delta-aminolevulinate. mammals use glycine, while plants use glutamate.

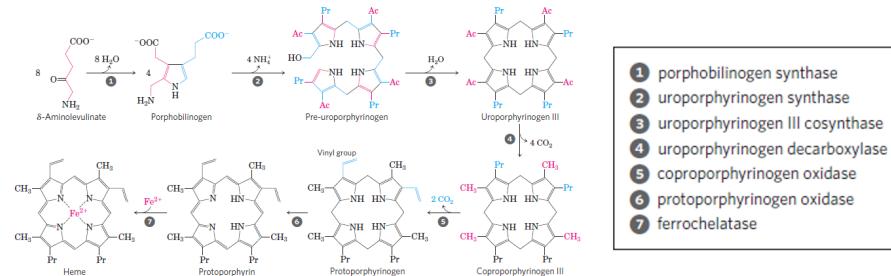


Figure 146: Biosynthesis of heme from delta-aminolevulinate.

Remark 9.1 (Porphyria). **Porphyria** is a group of diseases which stems from the intermediates of porphyrin build up, negatively affecting the skin or nervous system. This is due to defects in the genes encoding the enzymes of the pathway. The nervous system porphyrias are also called acute porphyria as the symptoms are rapid in onset and last a short time. Cutaneous porphyria includes the skin symptoms, e.g., through a sensitivity to sunlight, but usually don't include the nervous system.

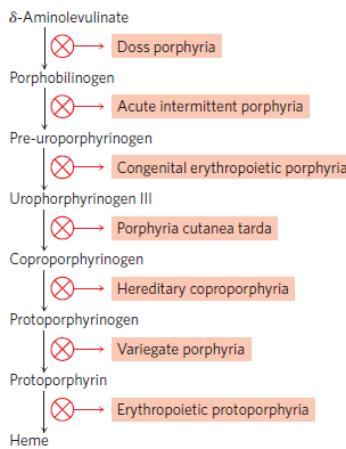


Figure 147: This image shows the types of diseases that stem from a certain enzyme defect. Accordingly the symptoms will vary, as the built up molecule will be different.

Remark 9.2 (Degradation and excretion of heme). **Heme** from senescent erythrocytes are the origin for degraded hemes. It can also be degraded coming from other cell types. Senescent erythrocytes are degraded in the spleen.

- i) Here, Heme is first converted to **bilirubin** in a two-step enzymatic process which employs biliverdin as an intermediate.
- ii) These steps result in oxidation and opening of the heme ring. Bilirubin is then excreted into the plasma.
- iii) Within hepatocytes, one or two molecules of glucuronic acid are attached to bilirubin, generating bilirubin momo/di-glucuronide.
- iv) These are excreted into bile canaliculi from where they are secreted into the duodenum as part of bile.

9.2.2 Phosphocreatine

Phosphocreatine (Pcr), a.k.a. creatine phosphate (CP a.k.a. Pcr) is a phosphorylated creatine molecule that serves as a rapidly mobilisable reserve of high-energy phosphates in skeletal muscle, myocard and the brain. This allows it to recycle ATP.

Creatine: the direct precursor of Phosphocreatine is produced from glycine and arginine with participation of Methionine as donor of a methyl group.

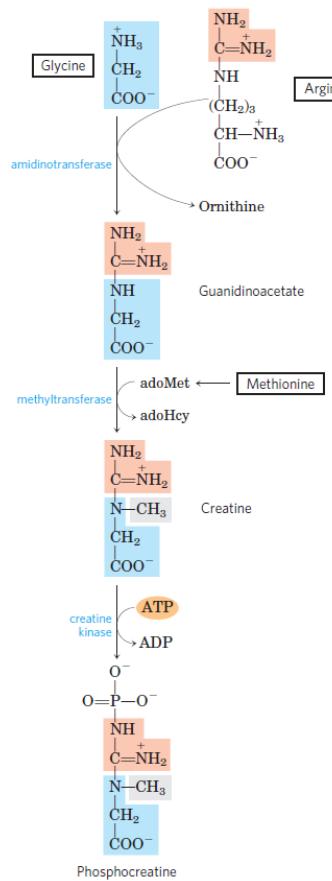


Figure 148: Biosynthesis of Creatine and phosphocreatine.

9.2.3 Glutathione

Glutathione is an antioxidant capable of preventing damage to cellular components caused by reactive oxygen species. It is a γ -peptide linkage between the carboxy group of glutamate side chain and cysteine. The carboxy group of cysteine is attached through a regular peptide bond to glycine.

The GSH biosynthesis involves two ATP-dependent steps:

- i) **gamma-glutamylcysteine** is synthesized from L-glutamate and cysteine, by the enzyme **glutamate-cysteine ligase (GCL)**.
- ii) glycine is added to the C-terminal of γ -glutamylcysteine. This condensation is catalyzed by **Glutathione synthetase**.

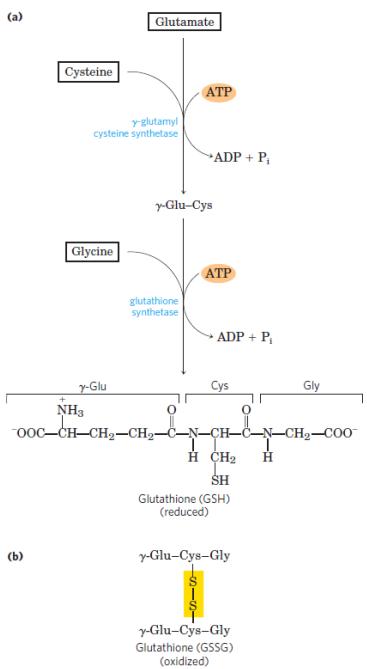


Figure 149: The top picture shows the biosynthesis of GSH. The bottom picture shows the oxidized form of glutathione.

9.2.4 Biogenic Amines

Biogenic amines are organic bases, which have a low molecular weight, which are produced in many different cells (e.g., adrenaline in adrenal medulla or histamine in mast cells and liver). Many biogenic amines are **neurotransmitter** (e.g., acetylcholine, serotonin, histamine, epinephrine, and dopamine). They can also be agonists or dedicated receptors.

Biogenic amines are produced by modification (mostly decarboxylations and hydroxylations) of different amino acids:

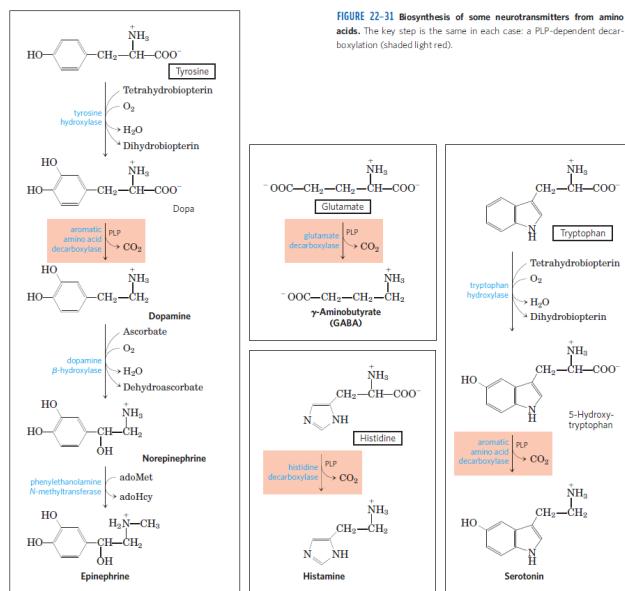


Figure 150: Biosynthesis of some neurotransmitters from amino acids.

9.2.5 Polyamines

The biosynthesis of **Polyamines** is highly regulated, nevertheless the function of polyamines is only partly understood. In their cationic ammonium form, they bind to DNA and are compounds that are found at regularly spaced intervals. They have also been found to act as promoters of programmed ribosomal frameshifting in translation.

Spermidine and spermine are synthesized starting from ornithine. Ornithine itself is obtained from arginine in the urea cycle.

- Spermidine synthesis: from putrescine, using an aminopropyl group from a decarboxylated S-adenosyl-L-methionine (SAM), which is catalyzed by spermidine synthase.
- Spermine synthesis: from the reaction of spermidine with SAM, which is catalyzed by spermine synthase.

10 Nucleic Acids in Biosynthesis

10.1 Biosynthesis of Nucleic Acids

Nucleotides are molecules consisting of a nucleoside and a phosphate group. They are precursors for:

- **DNA and RNA**
- energy molecules such as **ATP, GTP, CTP**, and **uridine triphosphate (UTP)**
- second messengers such as **cAMP** and **cGMP**
- key enzyme cofactors such as **CoA, FAD, NAD⁺, and NADP⁺**

Nucleotides contain either a **purine** or a **pyrimidine** base.

10.1.1 Biosynthesis of Purines

Purine is a heterocyclic aromatic compound that consists of a pyrimidine and fused to an imidazole ring.

The pathway of Purine production:

- i) It starts with the formation of 5-Phosphoribosyl pyrophosphate (PRPP) from 5-phosphoribose, which is formed in the pentose phosphate pathway.
- ii) PRPP's pyrophosphate is displaced by an amide from a glutamine.
- iii) Next, a glycine is incorporated.
- iv) A carbon unit from folic acid coenzyme N_{10} -formyl-THF is added.
- v) A second amide is transferred from a glutamine to the first carbon of the glycine unit.
- vi) The ring is closed.
- vii) Carboxylation of the second carbon of the glycine unit is concomitantly added. This new carbon is modified by the addition of a third amide.
- viii) Finally a second carbon unit from formyl-THF is added to the nitrogen group and the ring covalently closed to form the common purine precursor **inosine monophosphate (IMP)**.

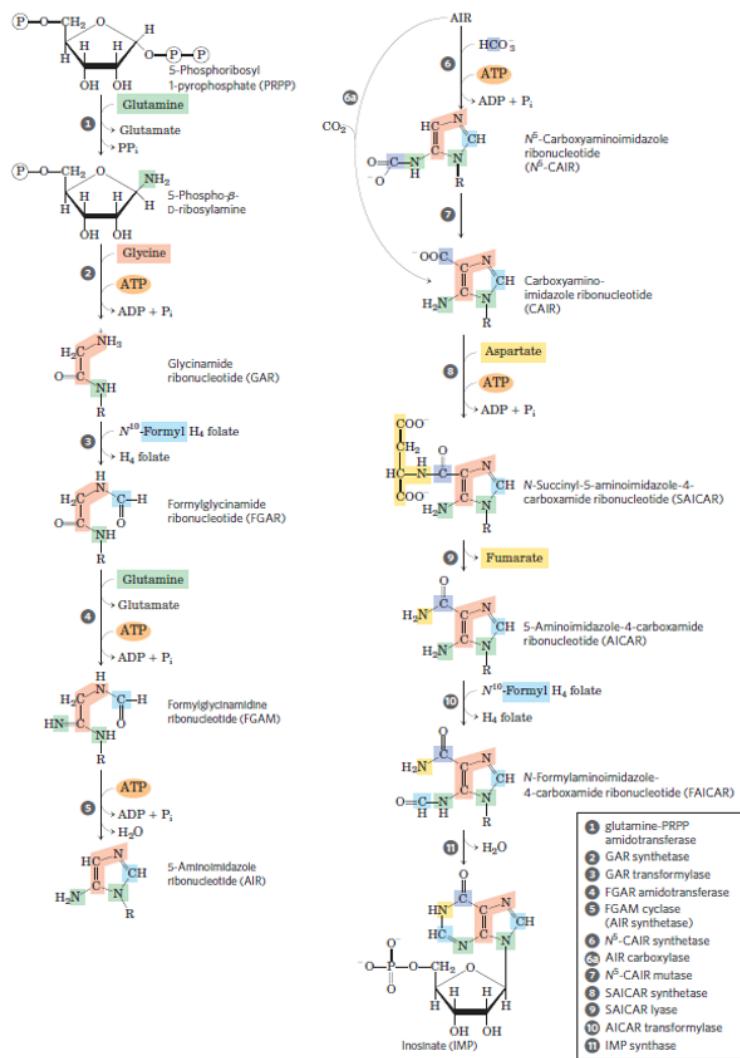


Figure 151: De novo synthesis of purine nucleotides: construction of the purine ring of IMP. On the bottom right one can see all the involved enzymes.

Creating the individual nucleic acids:

- IMP is converted to **adenosine monophosphate (AMP)** in two steps:
 - i) GTP hydrolysis fuels the addition of Aspartate to IMP, through the substitution of a carbonyl oxyxygen for a nitrogen forming the intermediate adenylosuccinate by adenylosuccinate synthase.
 - ii) Fumarate is then cleaved off forming AMP, catalyzed by **adenylosuccinate lyase/adenylosuccinate lyase**.
- IMP is converted to **guanosine monophosphate** by:
 - i) the oxidation of IMP forming xanthylate. NAD⁺ is the electron acceptor.
 - ii) An amino group is inserted at the C₂, which is fuelled by ATP hydrolysis. +

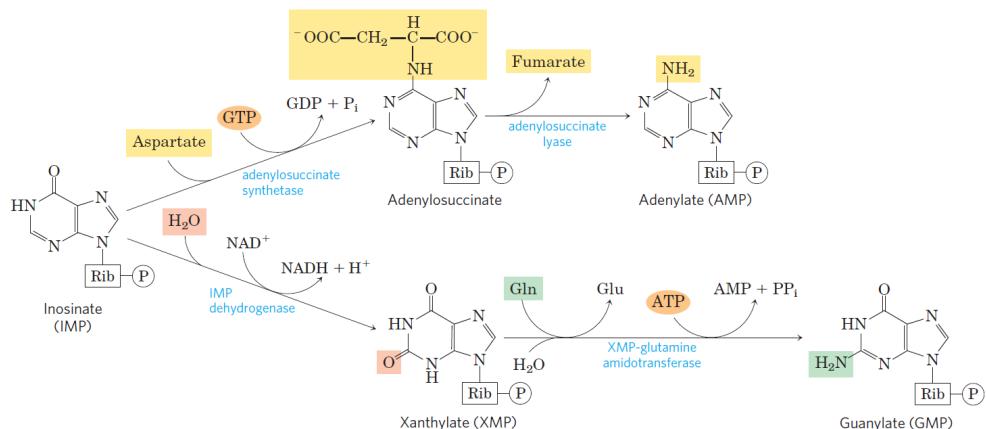


Figure 152: Biosynthesis of AMP and GMP from IMP.

10.1.2 Biosynthesis of Pyrimidines

Pyrimidine is an aromatic heterocyclic organic compound.

Here is the pathway:

- i) It starts with the formation of **carbamoyl phosphate** from glutamine and CO_2 .
- ii) **Aspartate carbamoyltransferase** catalyzes a condensation reaction between aspartate and carbamoyl phosphate to form **carbamoyl aspartic acid**.
- iii) This is cyclized into **4,5-dihydroorotate acid** by **dihydroorotase**.
- iv) which is then converted to orotate by **dihydroorotate oxidase**.
- v) Orotate is covalently linked with a phosphorylated ribosyl unit.
- vi) Orotidylate is decarboxylated to form **uridine monophosphate (UMP)**.
- vii) UMP is phosphorylated by two **kinases** to form **uridine triphosphate (UTP)** via two sequential reactions with ATP.
- viii) **CTP** subsequently formed by the amination of UTP by the **CTP synthetase**, where glutamine is the NH₃ donor and is fueled by ATP hydrolysis.

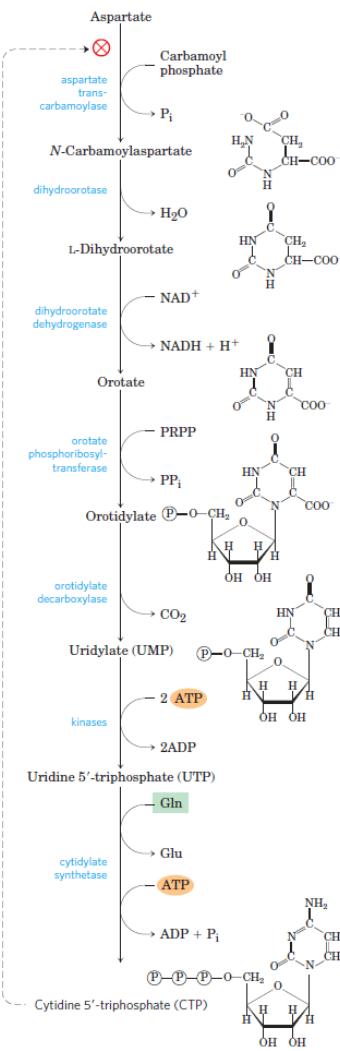


Figure 153: De novo synthesis of pyrimidine nucleotides. Biosynthesis of UTP and CTP from orotidylate.

10.1.3 Reduction from Ribonucleotides to Deoxyribonucleotides

The formation of ribonucleotides to deoxyribonucleotides is done through the removal of the 2'-hydroxyl group on the ribose ring of the nucleoside diphosphate. The enzyme **ribonucleotide reductase (RNR)**.

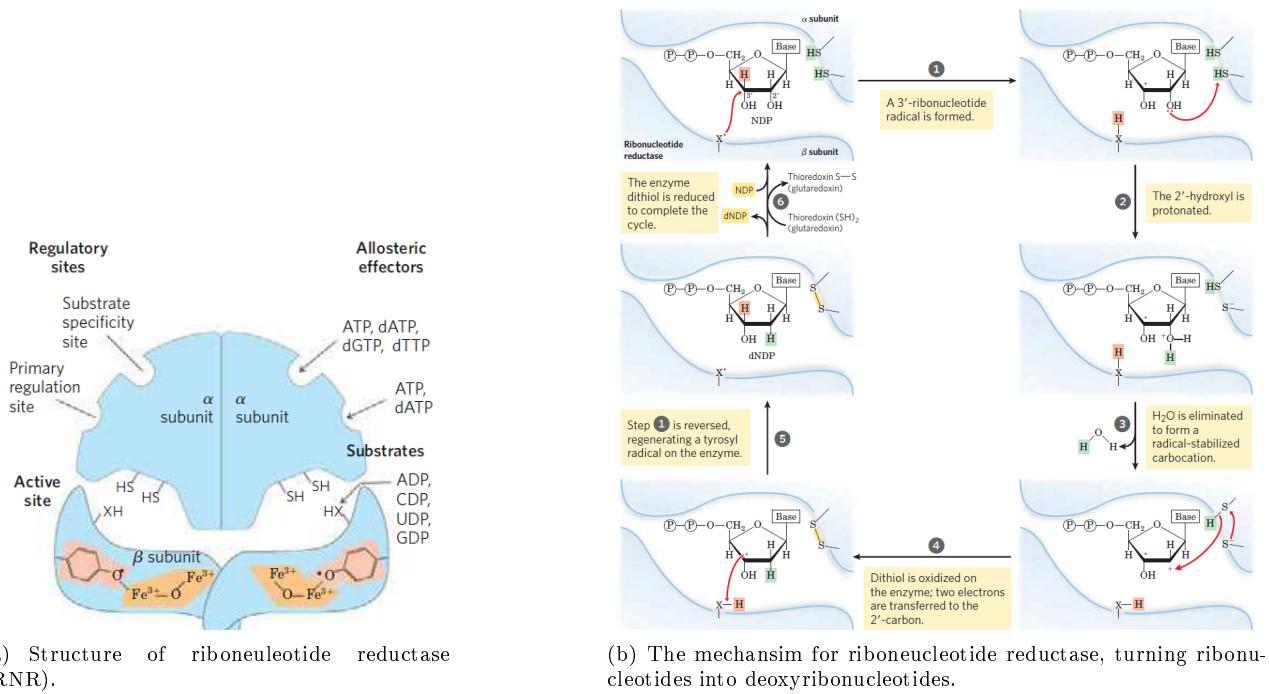


Figure 154: The structure and mechanism of RNR.

Remark 10.1 (Regeneration of Ribonucleotide reductase (RNR)). In order for RNR to catalyze the next reduction it has to be regenerated. This means the disulfide bond has to be broken into two sulfide groups. For this we have an electron chain which has two possible pathways:

- i) NAPDH
- ii) GSSG (oxidized state)
- iii) Glutaredoxin
- iv) RNR

Or:

- i) NAPDH
- ii) FAD (oxidized state)
- iii) Thioredoxin
- iv) RNR

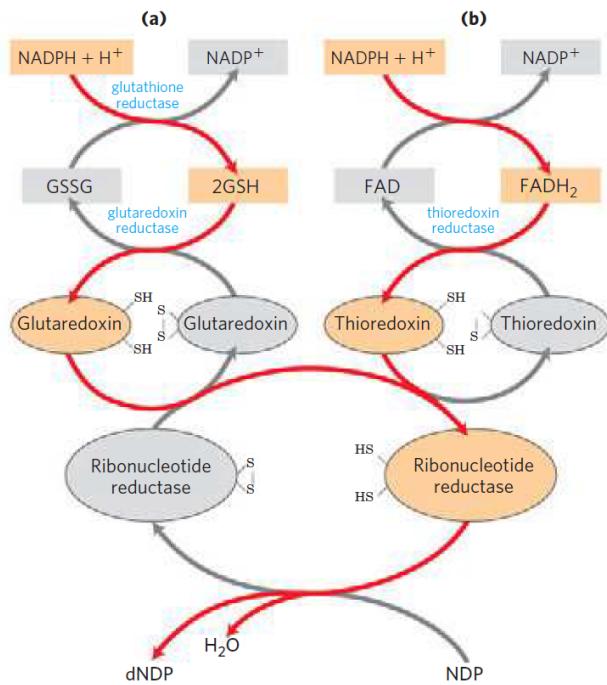


Figure 155: Shows the regeneration of RNR. Red arrows indicate electron movement, orange molecules are the reduced molecule, grey the oxidized. At the very bottom is the reduction of ribonucleotides to deoxyribonucleotides.

10.1.4 Biosynthesis of Thymidylate

Thymidylate (dTMP) is a component of DNA. It is synthesized de novo from deoxyuridylate (dUMP) and methylenetetrahydrofolate by **thymidylate synthase (TYMS)**. Dihydrofolate is a by-product.

dTMP is produced in the nuclear lamina, which is where DNA replication happens. DNA can't tell the difference between dUMP and dTMP. So, if there is a lack of dTMP uracil can be misintegrated into the DNA leading to point mutations if not repaired properly.

Remark 10.2 (Consequences of dTMP lacking). The lack of dTMP can have some consequences, due to the DNA being messed up:

- Can cause neural tube defects, megaloblastic anemia, and immune system problems.
- Pregnant women are more likely to have a **folate** deficiency, which is why it is often supplemented.
- Drugs that block **TYMS** or **dihydrofolate reductase** (DHFR, the folate regenerator) slow down cell division and are used to treat cancer.

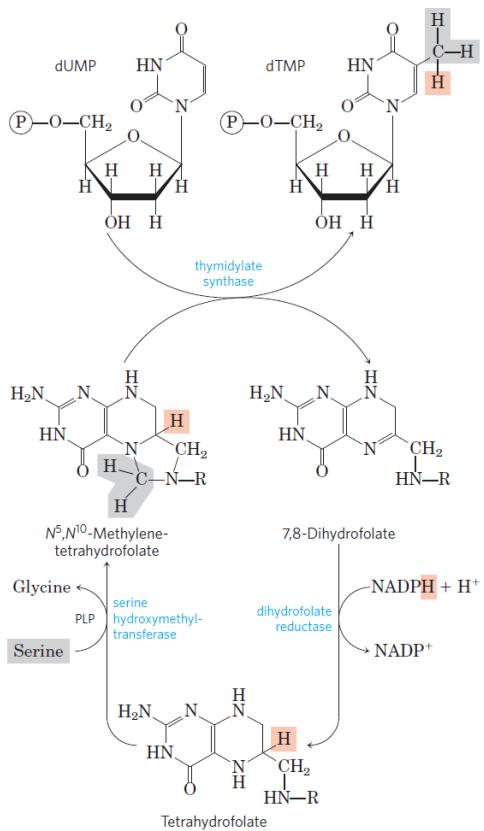


Figure 156: The conversion of dUMP to dTMP by TYMS and DHFR.

10.2 Disposal of Nucleic Acids

10.2.1 Purines Disposal

Purines are degraded to **uric acid**, here's how:

- i) Phosphate is hydrolyzed by **5'-nucleotidase**.
- ii) **Adenosine** is deaminated to **inosine**.
- iii) Inosine loses the ribose to form **hypoxanthine**
- iv) Through two oxidative reactions that is converted to uric acid.

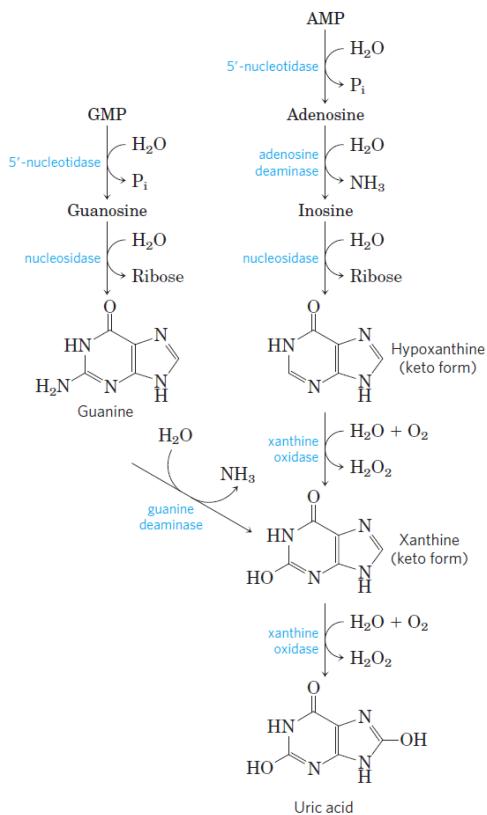


Figure 157: Catabolism of purine nucleotides.

Remark 10.3 (Degradation Purine). GMP follows a similar pathway, just that it has one deamination less in its degradative pathway.

10.2.2 Pyrimidines Disposal

Pyrimidines are ultimately catabolized to CO_2 and H_2O , and **urea**.

Let's start with Cytosine:

- Cytosine gets broken down into Uracil.
- Uracil gets further broken down N-carbamoyl- β -alanine.
- That gets broken down to β -Alanine, by **beta-ureidopropionase**, with CO_2 and ammonia as by-products

Thymine's catabolism:

- Thymine is broken down into **beta-aminoisobutyrate**.
- This is further broken down into **methylmalonyl semialdehyde**(intermediate of valine catabolism).
- Which is then converted into **succinyl-CoA**, which then enters the citric acid cycle.

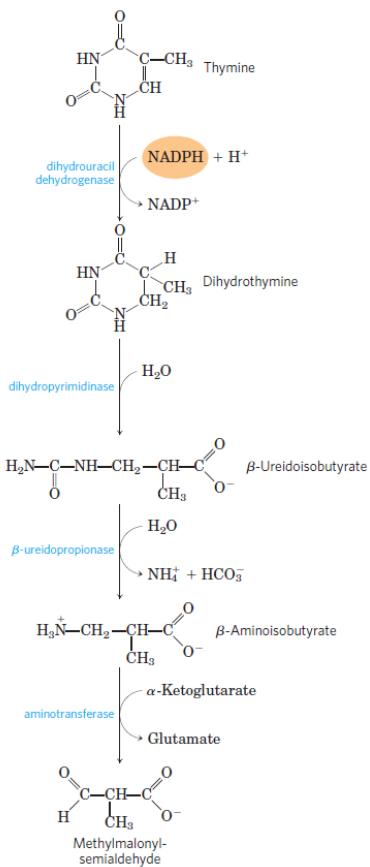


Figure 158: The catabolism of pyrimidines.

Remark 10.4 (Consequences of Genetic Defects: Severe combined immune deficiency). Genetic defects in the catabolism lead to diseases in humans. Defects in the enzyme adenosine deaminase leads to **severe combined immune deficiency (ADA-SCID)**. Why immunodeficiency? Because the defect results in an accumulation of deoxyadenosine, which in turn leads to a buildup of dATP in all cells. This in turn inhibits ribonucleotide reductase, preventing DNA synthesis. This means cells can't divide. T and B cells, with their high mitotic rate are very susceptible to this condition.

10.2.3 Salvage Pathways

Instead of catabolising the nucleotides there are also **salvage pathways** which recover bases and nucleosides that are formed in the degradation of RNA and DNA.

For Pyrimidines:

- uridine monophosphate (UMP) regeneration:
 - i) Uridine phosphorylase or pyrimidine-nucleoside phosphorylase adds ribose 1-phosphate to the free base uracil forming uridine.
 - ii) Uridine-cytidine kinase can then phosphorylate this nucleoside into UMP.
- TMP regeneration:
 - i) Thymidine phosphorylase or pyrimidine-nucleoside phosphorylase adds 2-deoxy- α -D-ribose 1-phosphate to thymine, forming Thymidine.

ii) **Thymidine kinase** can then phosphorylate this compound into **TMP**.

- **CMP** and **dCMP** regeneration has multiple options:

- Salvage it along the uracil pathway, through **cytidine deaminase**, which converts them **uridine** and **deoxyuridine**, respectively
- **Uridine-cytidine kinase** can phosphorylate them into **CMP** or **dCMP**.

For Purines: **Phosphoribosyltransferases** add phosphoribosyl pyrophosphate to bases, creating the nucleoside monophosphate (adenosine monophosphate (AMP), GMP). There are two types of phosphoribosyl-transferases:

- i) **adenine phosphoribosyltransferases (APRT)**,
- ii) **hypoxanthine-guanine phosphoribosyltransferases (HGPRT)**.

10.3 Chemotherapics Targeting Nucleotide Metabolism

Cancer cells usually grow at faster rates than normal cells. As a consequence they have a higher need for nucleotides (for their DNA replication and RNA transcription), meaning they are more susceptible to the inhibition of nucleotide synthesis. For example some commonly used anti cancer drugs inhibit thymidylate synthesis.

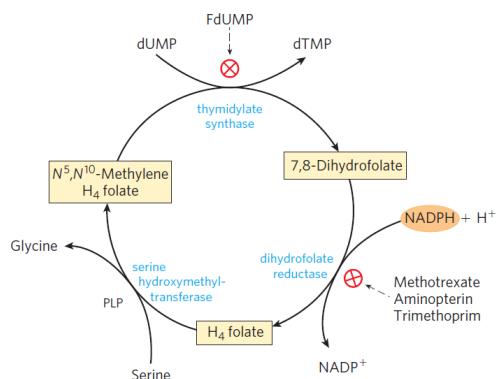


Figure 159: Thymidylate synthesis and folate metabolism as targets of chemotherapy.

11 integration of metabolism

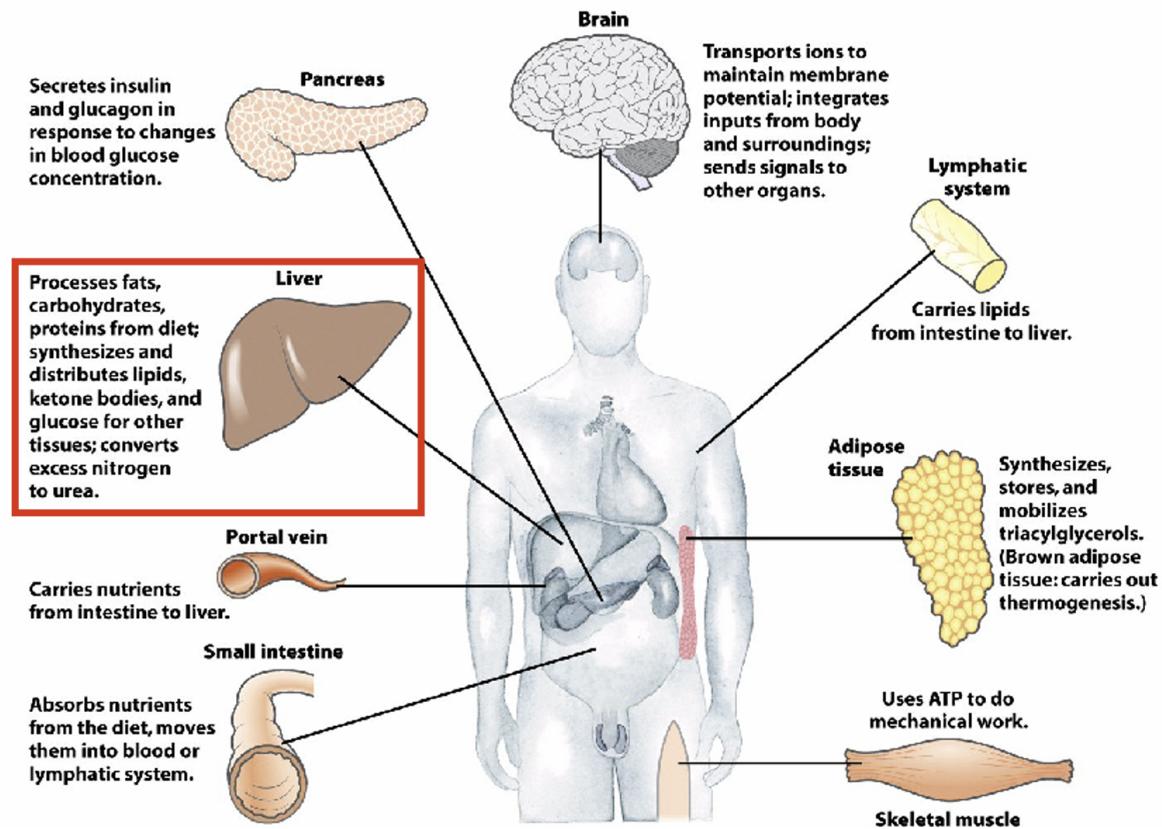


Figure 160: division of labour of metabolism

Not all cells are equally metabolically active. In fact some cells have totally different tasks in the metabolic pathways. (not much else here)

11.1 The liver

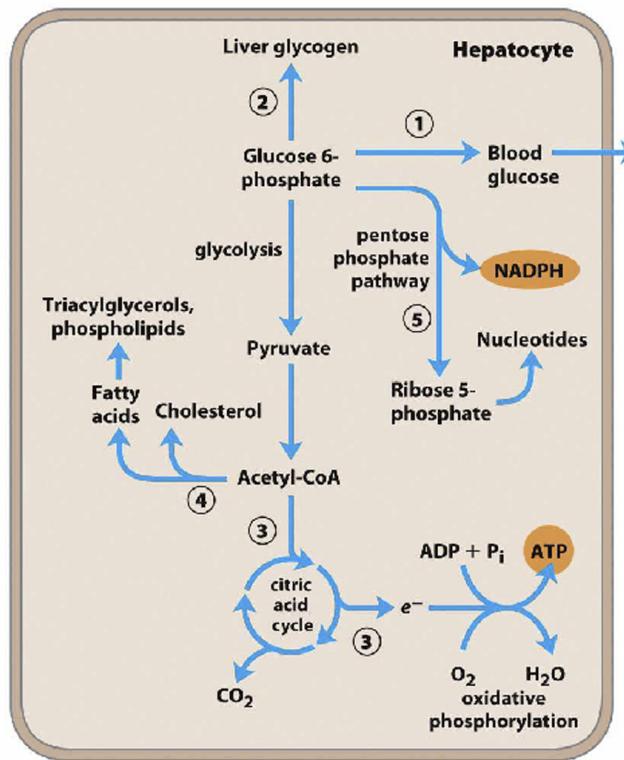


Figure 161: Liver has various options to process Glucose 6 phosphate

11.1.1 glucose 6 phosphate processing

When we eat sugar it is imported into the liver and converted to glucose 6 phosphate. The liver has many different things it **can do with glucose-6-phosphate**:

- convert it back to glucose and export it into the blood stream (this is a unique ability of the liver cells)
- make glycogen
- use it to make ATP via glycolysis TCA cylce and oxidative phophorylation
- make Acetyl-CoA after glycolysis to then make Fatty acids, triglycerols and Fatty acids.
- feed it to the pentose pathway (used for dNTP sythesis)

11.1.1.1 the pentose pathway

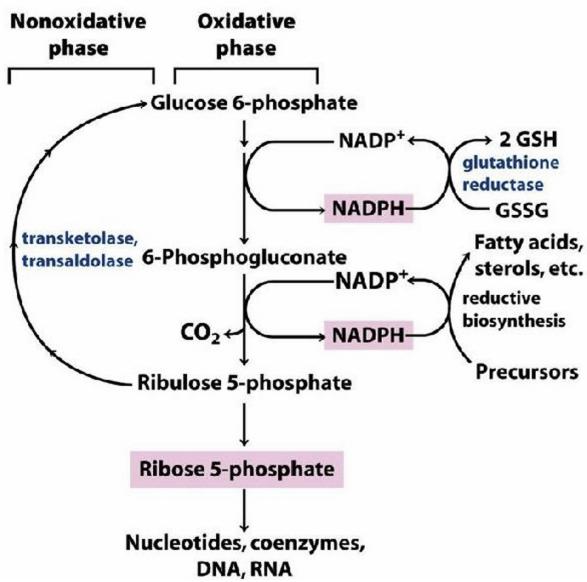


Figure 162: pentose pathway

This is a very short summary since we didn't cover this in detail but this pathway is used to **produce nucleotides**. This pathway is **highly active in cancer cells**, which is a reason for the Warburg effect. (cancer cells prefer glycolysis because they tend to have less oxygen and need more nucleotides to fuel their expansive growth)

11.1.2 amino acid processing

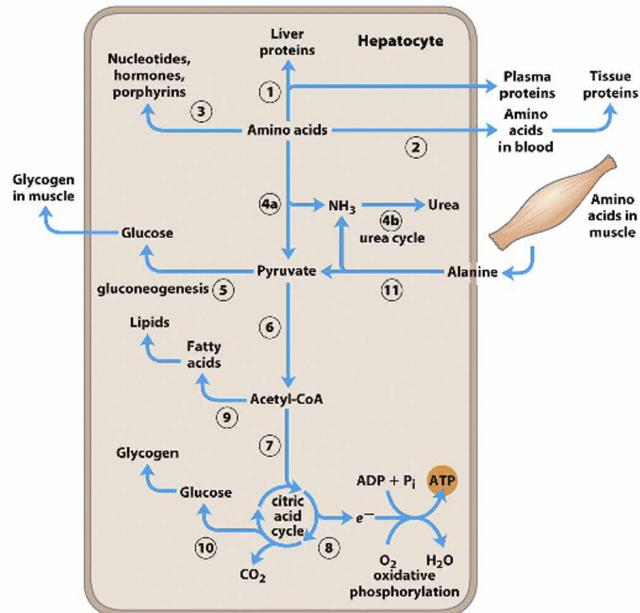


Figure 163: amino acid processing in the liver

The liver is also the organ that is responsible for **processing amino acids**

- used to make liver proteins
- mobilized to blood stream to go somewhere else
- produce nucleotides hormones and porphyrines
- deanimated. amine group is turned to urea via the urea pathway
- rerouted to lipid or glucose metabolism (not favored)
- used to produce ATP (not favored)

11.1.3 Lipid processing

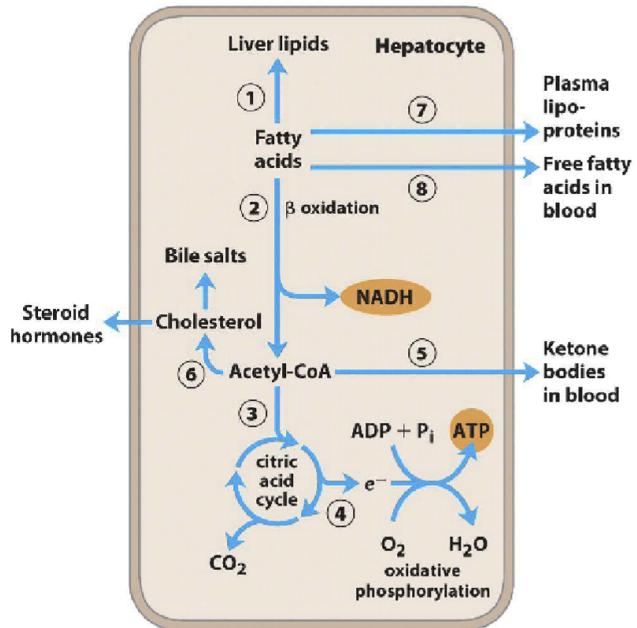


Figure 164: lipid processing in the liver

the liver has many possibilities to process liver cells:

- used to produce lipids that the liver itself needs
- use β -oxidation to produce Acetyl-CoA:
 - feed Acetyl-CoA into TCA cycle to produce ATP
 - use it to **make keton bodies**
 - use it to make sterols like **cholesterol**
- Fatty acids can be mobilised through the blood stream either as phospholipids/ triglycerides bound to lipoproteins or as free fatty acids bound to albumin

11.1.3.1 keton bodies

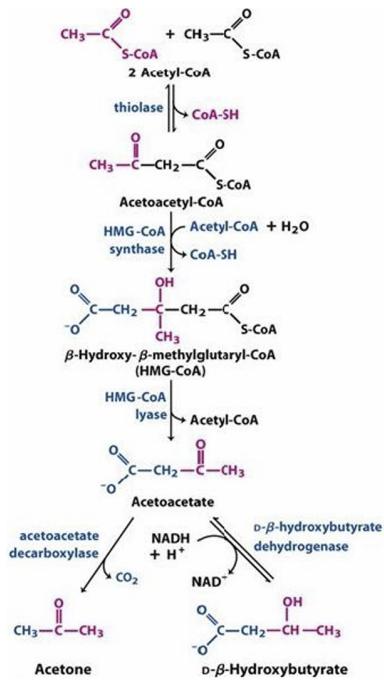


Figure 165: keton bodies production

keton bodies are produced during starvations carbohydrate restrictive diets, prolonged intense exercise, alcoholism, or in untreated (or inadequately treated) type 1 diabetes mellitus. . These are readily transported to the body and converted to acetyl-CoA which can be fed into the TCA cycle to produce ATP. In the brain they can be used to make long fatty acid chains.

11.2 The muscles

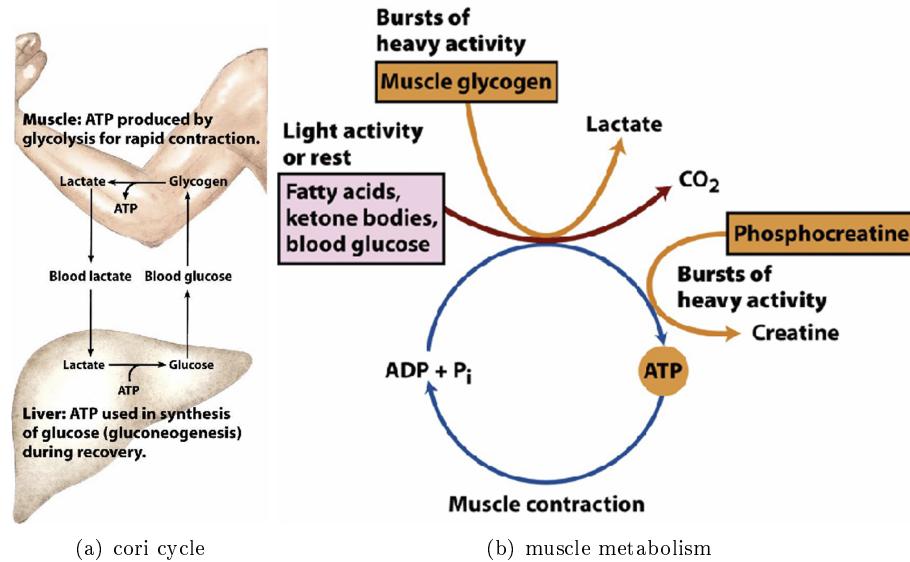


Figure 166: muscle metabolism

The muscle cells consume around **50% of the bodies ATP** at rest (**during exercise it's 90%**). Muscle cells use different energy sources depending on the activity level due to issues with replenishment:

- under resting conditions: use glucose, keton bodies and fatty acids
- during heavy prolonged exercise: use glycogen storages and make ATP via **lactic fermentation**
- for heavy short bursts of energy the muscles can use phosphocreatine.

The **Cori cycle** is at the heart of why you're muscles heart like shit after sport. The liver produces glucose that reaches the muscles, these then use it for ATP and produce lactate, which is transported to the liver to be broken down.

11.3 The brain

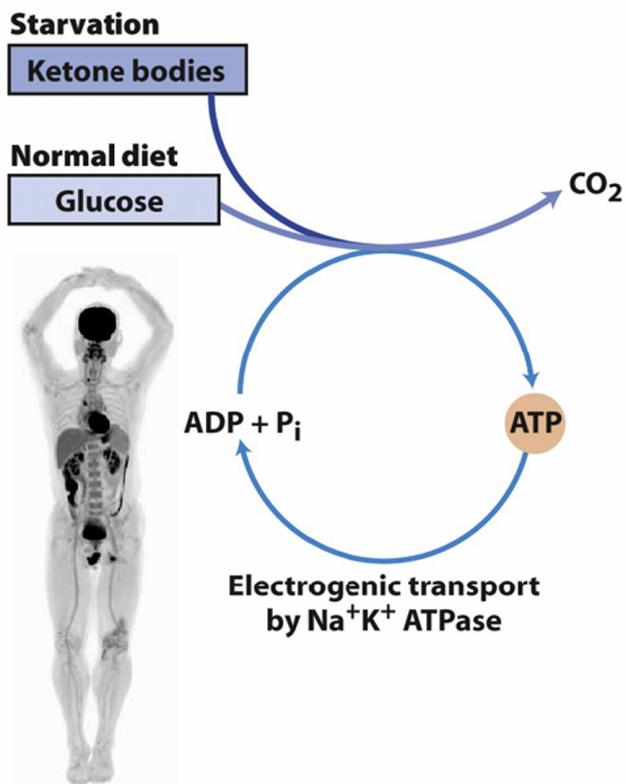


Figure 167: the brain consumes an insane amount of glucose

The brain is responsible for 20% of the total oxygen consumption, due to its high energy demand. This is because it almost exclusively uses glucose and ketone bodies for ATP production. It has very little glycogen storage so is highly dependent on the blood glucose levels being high enough (so eat bread my friends..)

11.4 Adipose tissue

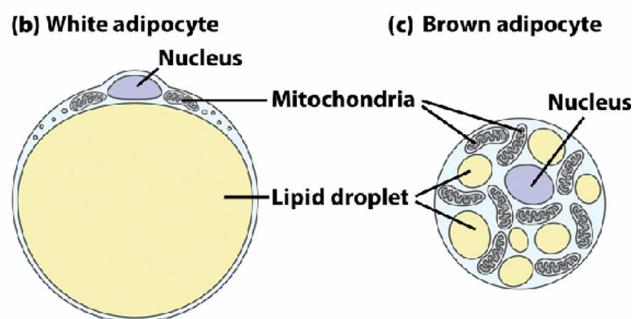


Figure 168: two types of Adipose tissue

Adipocytes are cells that are responsible for storing Fatty acids for energy use as well as thermal insulation of the body. There are **two types white adipocytes whose main role is to store fatty acids and brown adipocytes which generate heat**. Free floating fatty acids need to be transported around the blood stream by lipoproteins. Once they reach the adipose tissue they are cleaved from the lipoprotein by **lipoprotein lipase (LPL)**

11.5 the Pancreas

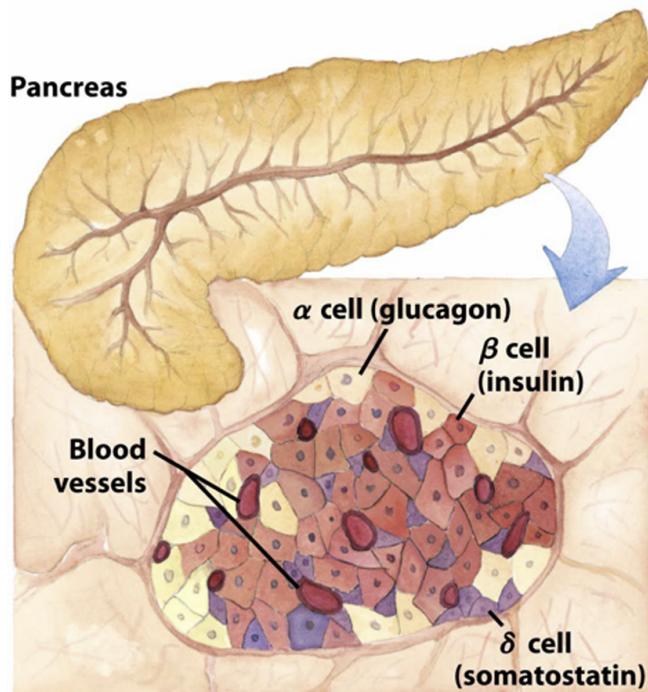


Figure 169: pancreas and langerhans islets

The pancreas is a super important organ for **blood sugar level regulation**. In the pancreas there are **Langerhans islets** which consist of α -cells, β -cells, and δ -cells. These are well connected with the cardiovascular system to deliver their hormones everywhere. These islets secrete insulin and glucagon: α -cells produce glucagon and β -cells insulin.

11.6 the blood

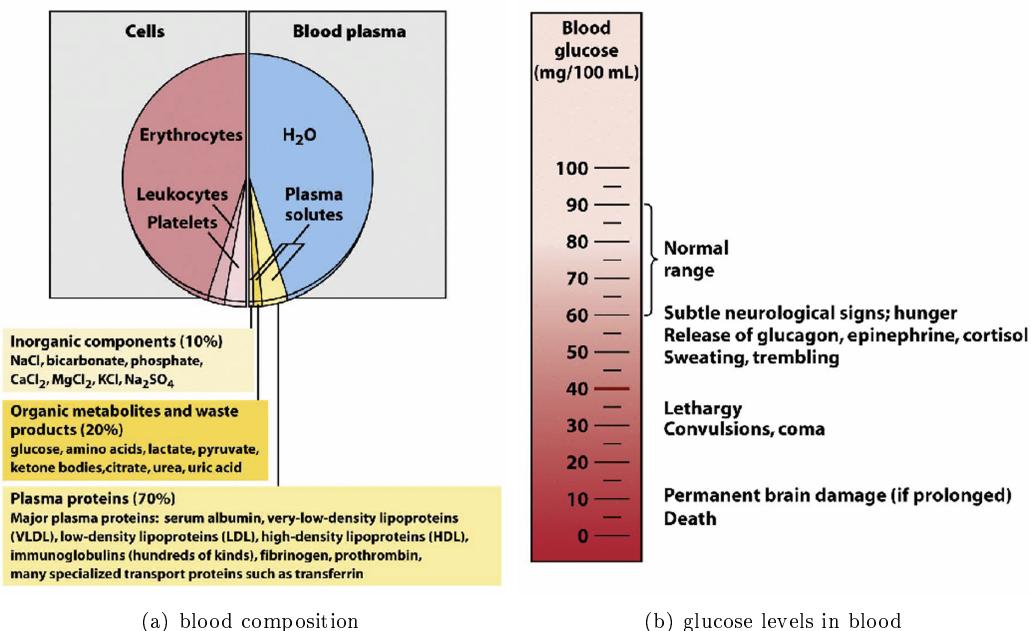


Figure 170: the blood overview

The blood is responsible for **transporting everything throughout the body**. In the context of metabolism it's main role is to **distribute glucose** to all the cells in the body, as well as **transport hormones** so that the cells can respond accordingly.

When there isn't enough sugar in the blood (**below 2.2 mM or 40 mg/dL**) the body is in a state of **hypoglycemia**. This can have various not so good consequences and if prolonged will lead to permanent brain damage or even death.

11.7 neuro endocrine system

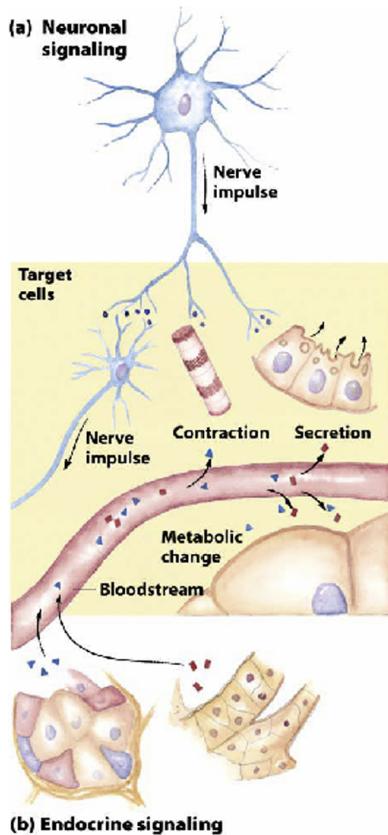


Figure 171: Enter Caption

Neurons act locally via neurotransmitters, which can only act at very small distances and generally have very specific responses. Hormones on the other hand travel long distances and trigger can trigger diverse responses in different organs. Through neuroendocrine integration, **neuroendocrine cells convert neuronal input into hormonal output to coordinate body functions.**

11.7.1 types of hormones

- **Peptide hormones:** Made of a chain of amino acids, ranging from just 3 to hundreds.
- **Amino acid hormones:** Derived from amino acids, most commonly tyrosine.
- **Eicosanoid hormones:** Derived from lipids such as arachidonic acid.
- **Steroid hormones:** Derived from cholesterol.

11.7.2 signal transduction

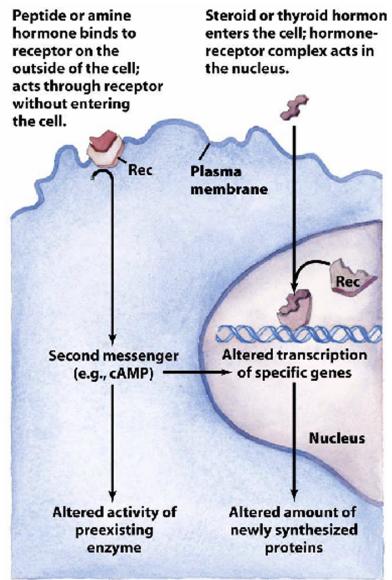


Figure 172: signal transduction

Hormones act by binding to **receptor proteins**, triggering a **signal transduction pathway** that leads to **gene transcription** or faster responses like **phosphorylation of effector proteins** and **membrane trafficking**.

Effector proteins are molecules that carry out the cellular response to a signal, such as enzymes or ion channels. They are often regulated by phosphorylation. This type of signal transduction is much faster than via transcription factors.

11.7.3 the endocrine system's main players and heirarchy

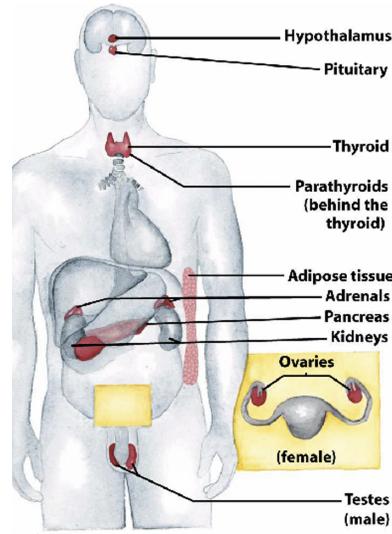


Figure 173: main organs in endocrine system

- **hypothalamus**: Produces hormones that target the anterior pituitary and sends neuronal signals to the posterior pituitary in response to nervous system stimuli.
- **anterior pituitary**: Produces hormones that target downstream endocrine organs including the adrenal cortex, thyroid, testes, and ovaries in response to signals from the hypothalamus.
- **posterior pituitary**: Contains axons of neurons whose cell bodies reside in the hypothalamus. These neurons secrete oxytocin and vasopressin.
 - **oxytocin** targets smooth muscle in the uterus and mammary glands to induce labor and lactation.
 - **vasopressin** regulates blood pressure.

These organs are usually organized in a hierarchy with the hypothalamus calling the shots and telling the rest of the organs what to do.

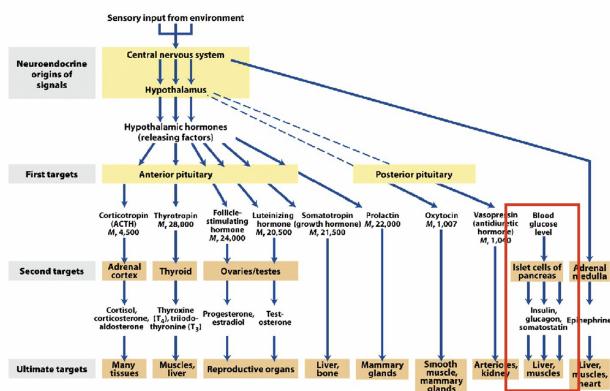


Figure 174: hierarchy of the neuroendocrine system

11.8 blood glucose regulation

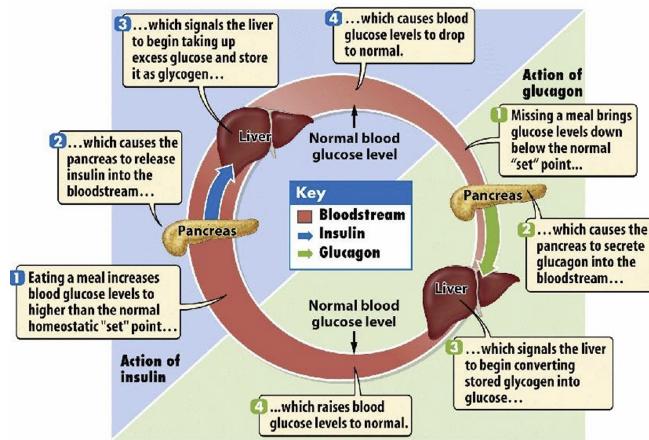


Figure 175: blood sugar regulation

Blood sugar regulation happens primarily via two enzymes **insulin and glucagon**.

- insulin: insulin is used to lower the blood sugar level
- glucagon: is used to raise the blood sugar level.

11.8.1 beta cells

11.8.1.1 insulin release mechanism

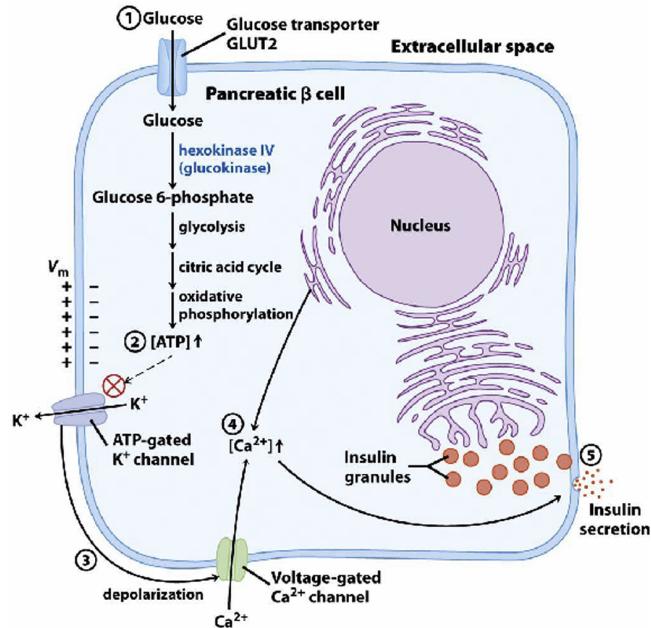


Figure 176: insulin function

glucose enters β -cells via **glut2 glucose transporter**. This will then be used to **create ATP**. this is how the cells measure the glucose transportation in the blood. when there is too much glucose in blood:

- i) high ATP concentration inhibits ATP-gated K^+ channels
- ii) This will cause the membrane to depolarize, which will **activate a Ca^{2+} voltage gated channels**.
- iii) the **influx of calcium** into the cell from the outside aswell as the ER, will cause the fusion of insulin granules to fuse with the membrane and be released via the **SNARE complex**

11.8.1.2 β -cells K-ATP channels

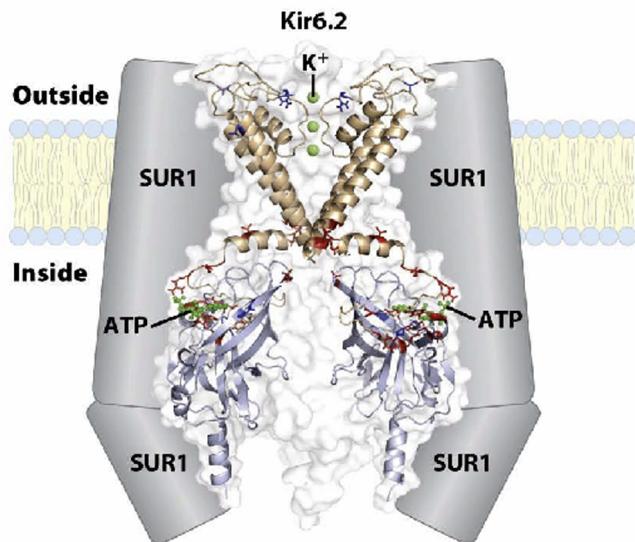


Figure 177: KATP channels

K_{ATP} channel are regulated by the receptor sulphonylurea (SUR1), of which it has 4. It has **4 pore forming** K₊ channels. ATP inhibits KATP channels at the **Kir6.2 subunit**. Some **anti-diabetic sulphonylurea drugs** work by interacting with SUR1 through an interaction with SUR1, to stimulate insulin secretion.

11.8.1.3 SNARE vesicle fusion

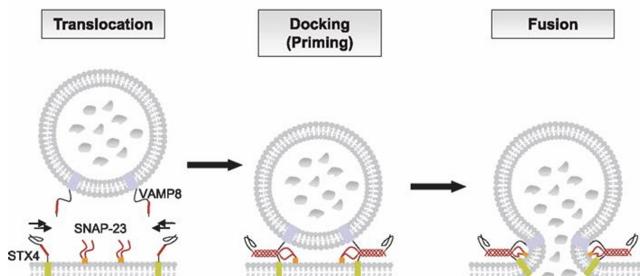


Figure 178: SNARE vesicle fusion

The type of snare protein on the granules is **Synaptobrevin2**, and the membrane bound SNARE **Syntaxin1A** in a complex with **SNAP-25**. **Synaptotagmin** is used to control insulin granule fusion through the concentration of Ca^{2+} . Upon exposure to Calcium this protein will bind the **Syntaxin1A-Synaptobrevin2** pair

11.8.1.4 insulin production

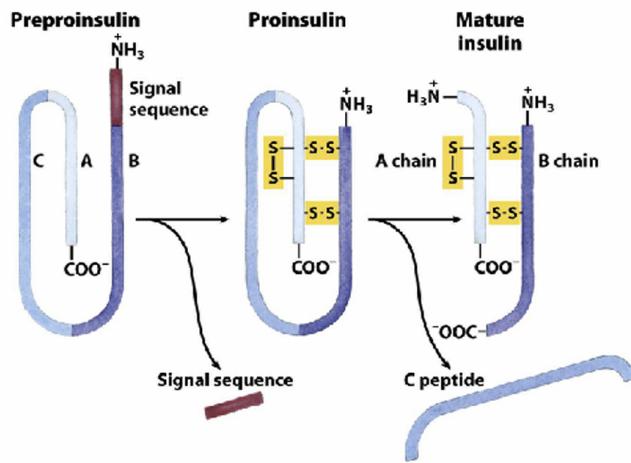


Figure 179: insulin processing

Preproinsulin has 3 chains A, B and C and an ER localization signal. chains **A and B** are linked by disulfide bonds, while chain **C** is cleaved off this is done by prohormone convertases (PC1 and PC2), as well as the exoprotease Carboxypeptidase E

11.8.1.5 insulin response pathway in other cells

(the professor didn't talk much about this but I'll add it in just in case)

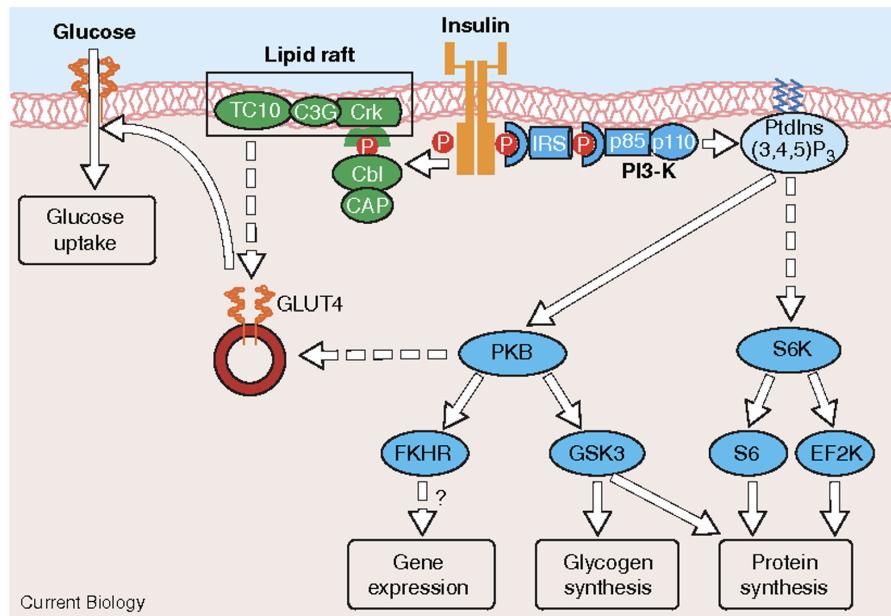


Figure 180: insulin pathway

Insulin pathway is a classical example of **RTK receptor signaling**

11.8.1.6 effects of insulin table

TABLE 23–3	Effects of Insulin on Blood Glucose: Uptake of Glucose by Cells and Storage as Triacylglycerols and Glycogen
Metabolic effect	Target enzyme
↑ Glucose uptake (muscle, adipose)	↑ Glucose transporter (GLUT4)
↑ Glucose uptake (liver)	↑ Glucokinase (increased expression)
↑ Glycogen synthesis (liver, muscle)	↑ Glycogen synthase
↓ Glycogen breakdown (liver, muscle)	↓ Glycogen phosphorylase
↑ Glycolysis, acetyl-CoA production (liver, muscle)	↑ PFK-1 (by ↑ PFK-2) ↑ Pyruvate dehydrogenase complex
↑ Fatty acid synthesis (liver)	↑ Acetyl-CoA carboxylase
↑ Triacylglycerol synthesis (adipose tissue)	↑ Lipoprotein lipase

Figure 181: table of insulin effects

11.8.2 alpha cells

11.8.2.1 release mechanism

Like insulin α -cells sense the glucose concentration via the concentration of produced ATP.

- i) high ATP concentration inhibits ATP-gated K⁺ channels
- ii) This will cause the membrane to depolarize, which will **activate a ca2+ voltage gated channels**.
- iii) the **influx of calcium** into the cell from the outside aswell as the ER, will cause the fusion of insulin granues to fuse with the membrane and be released via the **SNARE complex** However in α -cells this happens at much lower glucose concentrations. and if the ATP concetration too much depolarization will cause the closure of the ca2+ voltage gated channels

11.8.2.2 glucagon response pathway in other cells

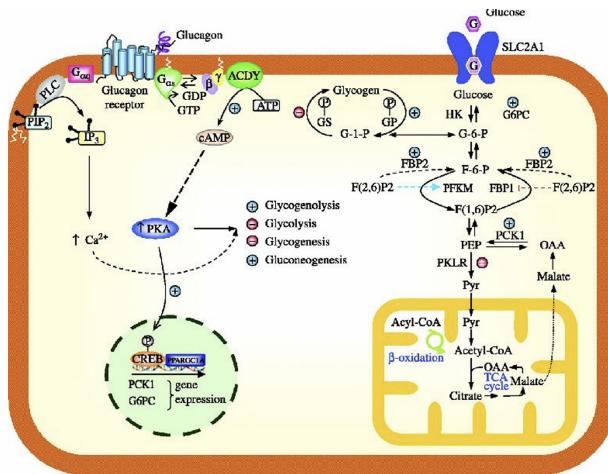


Figure 182: Glucagon pathway

Glucagon pathway works over a GPCR

11.8.2.3 effect of glucagon

TABLE 23–4 Effects of Glucagon on Blood Glucose: Production and Release of Glucose by the Liver

Metabolic effect	Effect on glucose metabolism	Target enzyme
↑ Glycogen breakdown (liver)	Glycogen → glucose	↑ Glycogen phosphorylase
↓ Glycogen synthesis (liver)	Less glucose stored as glycogen	↓ Glycogen synthase
↓ Glycolysis (liver)	Less glucose used as fuel in liver	↓ PFK-1
↑ Gluconeogenesis (liver)	Amino acids Glycerol Oxaloacetate } → glucose	↑ FBPase-2 ↓ Pyruvate kinase ↑ PEP carboxykinase
↑ Fatty acid mobilization (adipose tissue)	Less glucose used as fuel by liver, muscle	↑ Hormone-sensitive lipase ↑ PKA (perilipin—P)
↑ Ketogenesis	Provides alternative to glucose as energy source for brain	↓ Acetyl-CoA carboxylase

Figure 183: table of glucagon effects

11.8.3 diabetes

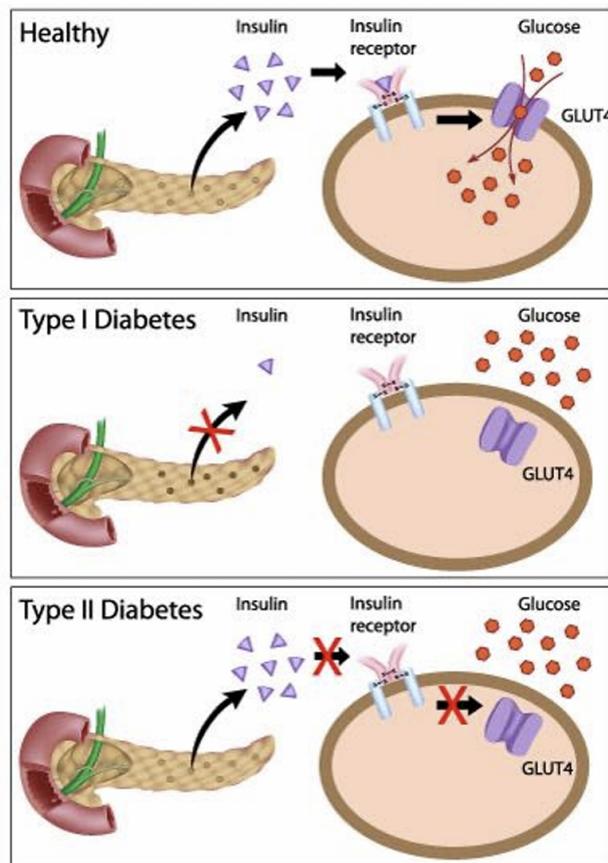


Figure 184: diabetes types

There are two types of diabetes:

- **Type1 diabetes:** This is an autoimmune disease where the insulin producing β -cells are destroyed.
- **Type2 diabetes:** This involves a desensitization of the bodies cells to insulin due to a constatly high level of insulin. Thus loosing their ability to respond to it.

Glossary

K_{eq} The equilibrium constant of a reaction, given by the ratio

$$\frac{[P]}{[S]}$$

. Not affected by enzymes:contentReference[oaicite:8]index=8.. 2, 3, I

ΔG^\ddagger The activation free energy, the difference in energy between the ground state and the transition state:contentReference[oaicite:9]index=9.. 3, I

ΔG The change in free energy during a reaction. ΔG refers to standard conditions, and $\Delta G'$ includes biochemical conditions (pH = 7):contentReference[oaicite:9]index=9.. 3, I

β -Ketoacyl Reductase domain (KR) Reduces β -ketoacyl intermediates to β -hydroxyacyl derivatives using NADPH. 74, 77

β -Ketoacyl Synthase domain (KS) Catalyzes the condensation of acyl and malonyl groups to form β -ketoacyl intermediates. 74–76, 78

β -hydroxyacyl-ACP dehydratase(DH) Dehydrates β -hydroxyacyl intermediates to form enoyl derivatives. 74, 77

v_0 The initial velocity of an enzyme-catalyzed reaction, measured at the very beginning before product accumulates. At low [S], v_0 increases almost linearly with [S]:contentReference[oaicite:4]index=4.. 7, I

β -hydroxybutyryl-CoA A coenzyme A (CoA) thioester of β -hydroxybutyric acid. It is an intermediate in FA synthesis. 77

trans-D²-butenoyl-ACP intermediate of FA synthesis. 77

4,5-dihydroorotic acid An intermediate in pyrimidine biosynthesis formed by the cyclization of carbamoyl aspartic acid via dihydroorotase.. 121

5'-nucleotidase An enzyme that hydrolyzes phosphate from nucleotides like AMP during purine degradation.. 125

acetoacetyl A functional group derived from acetoacetic acid. It is intermediate of FA synthesis. 76, 88

Acetyl-CoA A central metabolic intermediate formed from the breakdown of carbohydrates, fats, and proteins. It delivers the acetyl group to the citric acid cycle (Krebs cycle) for energy production and also acts as an allosteric cofactor that activates pyruvate carboxylase. Acetyl-CoA is an allosteric inhibitor of pyruvate dehydrogenase complex, while it activates pyruvate carboxylase. sort=acetylcoa . 62

Acetyl-CoA Carboxylase (ACC) The rate-limiting enzyme in fatty acid synthesis. It catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, using biotin as a cofactor. ACC is regulated by phosphorylation, allosteric effectors (e.g., citrate activation, palmitoyl-CoA inhibition), and hormonal signals such as insulin and glucagon. 73

Acid-Base Catalysis A catalytic mechanism in which amino acid residues act as proton donors or acceptors to stabilize charged intermediates, increasing reaction rates when water alone is insufficient:contentReference[oaicite:0]index=6

Activation Energy The energy barrier (

$$\Delta G^\ddagger$$

) between the ground state and the transition state that must be overcome for a reaction to proceed:contentReference[oaicite:5]index=5.. 2

Active Site The region of the enzyme where substrate binding and catalysis occur, often through specific amino acid residues:contentReference[oaicite:13]index=13.. 5, I

Acyl Carrier Protein domain (ACP) A flexible domain that shuttles the growing fatty acid chain between catalytic sites during synthesis.. 74, 75

acyl transferases A class of enzymes that catalyze the transfer of acyl groups from one molecule to another. They play essential roles in lipid biosynthesis.. 82

acyl-CoA synthase An enzyme that activates free fatty acids by catalyzing their conversion into fatty acyl-CoA using ATP and coenzyme A. This activation is required for fatty acid metabolism, including β -oxidation and lipid biosynthesis.. 82

adenine phosphoribosyltransferases (APRT) A biochemical term relevant to amino acid or nucleotide metabolism: adenine phosphoribosyltransferases (APRT).. 128

Adenosine A nucleoside formed from adenine and ribose, a breakdown product of AMP that is deaminated to inosine.. 125

adenosine monophosphate (AMP) A biochemical term relevant to amino acid or nucleotide metabolism: adenosine monophosphate (AMP).. 120, 128

adenylosuccinate lyase A biochemical term relevant to amino acid or nucleotide metabolism: adenylosuccinate lyase.. 120

Alanine Formed by transamination of pyruvate, catalyzed by alanine transaminase, and involved in nitrogen transport.. 112, 126

Alanine transaminase (ALT) An enzyme that transfers an amino group from glutamate to pyruvate to form alanine, also a liver marker.. 112

alpha-ketobutyrate A by-product in the synthesis of cysteine from homocysteine and serine, ultimately entering the TCA cycle.. 111

Anabolism Metabolic pathways that build complex molecules from simpler ones, requiring an input of energy.. 23

anterior pituitary An endocrine gland that produces hormones in response to nervous stimuli, targeting downstream organs such as the adrenal cortex, thyroid, testes, and ovaries. 141

Arginine A urea cycle amino acid synthesized from glutamate via a pathway involving acylation, reduction, and conversion through ornithine.. 110

Arsenate Poisoning A toxic condition caused by exposure to arsenate (AsO_4^{3-}), which disrupts cellular metabolism by mimicking phosphate. Arsenate can uncouple oxidative phosphorylation by substituting for inorganic phosphate glycolysis or ATP synthesis, leading to decreased ATP production and cellular toxicity. Symptoms include nausea, vomiting, neurological disturbances, and multi-organ failure in severe cases.. 20

Asparagine Synthesized from aspartate via amidation by asparagine synthase using glutamine as NH₃ donor and ATP.. 112

Asparagine Synthase An enzyme that catalyzes the ATP-dependent conversion of aspartate and glutamine into asparagine.. 112

Aspartate Formed from oxaloacetate by transamination, serves as a precursor for nucleotides and participates in the urea cycle.. 112

-
- Aspartate carbamoyltransferase** An enzyme that catalyzes the condensation of aspartate with carbamoyl phosphate in pyrimidine biosynthesis.. 121
- Aspartate transaminase (AST)** An enzyme that transfers an amino group to oxaloacetate forming aspartate, used as a liver function marker.. 112
- ATP** Adenosine triphosphate, the primary energy currency of the cell used to drive many biosynthetic reactions.. 118
- ATP (Adenosine Triphosphate)** The primary energy currency of the cell, used to power many cellular processes. Its hydrolysis releases heat but not energy, while the transfer of phosphate leads to a higher state in free energy of the substrate.. 18
- beta-aminoisobutyrate** A biochemical term relevant to amino acid or nucleotide metabolism: β -aminoisobutyrate.. 126
- beta-ureidopropionase** A biochemical term relevant to amino acid or nucleotide metabolism: β -ureidopropionase.. 126
- bile acids** amphipathic molecules derived from cholesterol that aid in the digestion and absorption of dietary fats by emulsifying lipids and facilitating micelle formation. They are synthesized in the liver, stored in the gallbladder, and released into the small intestine. Bile acids are also involved in cholesterol excretion and undergo enterohepatic circulation. 39
- bilirubin** A yellow compound that occurs in the normal catabolic pathway that breaks down heme in red blood cells. It is excreted in bile and urine, and elevated levels may indicate liver dysfunction or disease. 115
- Binding** The specific interaction between enzyme and substrate, often involving multiple weak noncovalent interactions to drive catalysis:contentReference[oaicite:16]index=16.. 5, I
- Biogenic amines** Low molecular weight amines derived from amino acids, acting as neurotransmitters or hormones.. 117
- butyryl-ACP** A saturated four-carbon acyl carrier protein (ACP) thioester intermediate in fatty acid biosynthesis.. 77
- C-terminal START Domain** A lipid-binding domain located at the C-terminus of certain proteins, including ceramide transport proteins (CERT). The START (StAR-related lipid transfer) domain facilitates the selective binding and transfer of lipids such as ceramide between membranes, contributing to non-vesicular lipid transport and membrane homeostasis.. 98
- cAMP** Cyclic AMP, a second messenger derived from ATP involved in signal transduction pathways.. 118
- carbamoyl aspartic acid** A biochemical term relevant to amino acid or nucleotide metabolism: carbamoyl aspartic acid.. 121
- carbamoyl phosphate** A biochemical term relevant to amino acid or nucleotide metabolism: carbamoyl phosphate.. 121
- Carboxypeptidase E** An exoprotease that removes C-terminal basic amino acids from peptide precursors after initial cleavage by prohormone convertases such as PC1 and PC2, completing the maturation of many peptide hormones. 145
- Carnitine** ammonium compound that plays a crucial role in the transport of long-chain fatty acids into the mitochondrial matrix for β -oxidation. Carnitine enabling them to cross the inner mitochondrial membrane via the carnitine shuttle system. 42

Catabolism Metabolic pathways that break down complex molecules into simpler ones, releasing energy.. 23

Catalytic Power The ability of enzymes to lower the activation energy and provide alternate reaction pathways, enhancing the reaction rate:contentReference[oaicite:12]index=12.. 4, I

CDP-choline diacylglycerol phosphocholine transferase An enzyme that catalyzes the final step in the CDP-choline pathway, transferring the phosphocholine group from CDP-choline to diacylglycerol to form phosphatidylcholine. 86

ceramide synthase An enzyme that catalyzes the N-acylation of sphinganine or sphingosine with a fatty acyl-CoA to produce ceramide, a central intermediate in sphingolipid metabolism. It is localized to the endoplasmic reticulum. 95

Cerebroside A type of glycosphingolipid consisting of a ceramide backbone (sphingosine + fatty acid) attached to a single sugar moiety, typically glucose or galactose. Cerebrosides are important components of cell membranes, particularly in the nervous system, where they contribute to myelin formation and cell-cell communication. In the brain, the most common cerebroside is galactocerebroside, found predominantly in myelin. 99

CERT1 (Ceramide Transfer Protein 1) A lipid transfer protein that transports ceramide from the endoplasmic reticulum (ER) to the Golgi apparatus, where it is used for the synthesis of sphingomyelin. CERT1 plays a crucial role in sphingolipid metabolism and membrane organization. . 98, 104

cGMP Cyclic GMP, a signaling molecule and second messenger involved in cellular responses to hormones.. 118

Chemotherapeutics Drugs that target rapidly dividing cells, often by inhibiting nucleotide biosynthesis (e.g., thymidylate synthesis).. 128, IV

cholesterol A vital sterol molecule in animal cells, serving as a structural component of membranes and a precursor for steroid hormones and bile acids. 91

choline kinase An enzyme that catalyzes the phosphorylation of choline to form phosphocholine, a key step in the biosynthesis of phosphatidylcholine, an essential phospholipid in cell membranes. 86

Citrate Synthase A key enzyme of the citric acid (TCA) cycle that catalyzes the condensation of acetyl-CoA and oxaloacetate to form citrate. This reaction initiates the TCA cycle and also provides citrate for export to the cytosol, where it can be used for fatty acid synthesis after conversion back to acetyl-CoA. 72

TCA transporter (Citrate Transporter) A mitochondrial membrane transporter that exports citrate from the mitochondrial matrix to the cytosol in exchange for malate. This shuttle is essential for transferring acetyl-CoA equivalents into the cytosol, where citrate is cleaved by ATP-citrate lyase to regenerate acetyl-CoA for fatty acid synthesis. 72

Classification number A four-part code assigned to enzymes based on the type of reaction they catalyze (e.g., 2.7.1.1 for hexokinase):contentReference[oaicite:4]index=4.. 1

CoA Coenzyme A, a cofactor involved in acyl group transfer reactions, derived in part from nucleotide structures.. 118

Coenzyme A complex organic or metallorganic molecule required for enzyme function, often derived from vitamins. Acts as a transient carrier of specific functional groups:contentReference[oaicite:2]index=2.. 1

Cofactor An inorganic chemical component required for enzyme activity. Often metal ions such as Fe^{2+} , Mg^{2+} , or Zn^{2+} :contentReference[oaicite:1]index=1.. 1

-
- Competitive Inhibitor** A molecule that competes with the substrate for binding at the active site. It increases K_m but does not affect v_{max} :contentReference[oaicite:2]index=2.. 11
- Cori cycle** A metabolic pathway in which lactate produced by anaerobic glycolysis in the muscles is transported to the liver, converted to glucose via gluconeogenesis, and then sent back to the muscles to be used as an energy source. 135
- Covalent Catalysis** A mechanism where the enzyme forms a transient covalent bond with the substrate, creating an alternative reaction pathway with lower activation energy. This requires nucleophilic groups on the enzyme:contentReference[oaicite:1]index=1.. 6
- Creatine** A compound derived from glycine, arginine, and methionine, converted into phosphocreatine for ATP regeneration.. 115
- CTP** Cytidine triphosphate, synthesized from UTP and used in RNA synthesis and lipid metabolism.. 118, 121
- CTP synthetase** An enzyme that converts UTP to CTP using glutamine as the nitrogen donor and ATP for energy.. 121
- CTP–choline cytidylyltransferase** A regulatory enzyme in the CDP-choline pathway that catalyzes the conversion of phosphocholine and CTP to CDP-choline, a precursor for phosphatidylcholine synthesis. 86
- Cysteine** An amino acid synthesized from serine and homocysteine via cystathione, involved in antioxidant synthesis (e.g., glutathione).. 111
- cytidine deaminase** A biochemical term relevant to amino acid or nucleotide metabolism: cytidine deaminase.. 128
- d-aminolevulinate** A biochemical term relevant to amino acid or nucleotide metabolism: d-aminolevulinate.. 113
- Dehydrogenase** An enzyme that catalyzes the removal of two hydrogen atoms from a substrate, typically transferring them to an electron acceptor such as NAD⁺ or FAD. Dehydrogenases play a crucial role in metabolic pathways like glycolysis and the citric acid cycle.. 17
- deoxyuridine** A biochemical term relevant to amino acid or nucleotide metabolism: deoxyuridine.. 128
- Dihydroceramide Desaturase (DES)** An enzyme that catalyzes the introduction of a double bond into dihydroceramides, converting them into ceramides. This reaction is crucial in the biosynthesis of sphingolipids, which are important components of cell membranes, playing key roles in cell signaling, structure, and function.. 96
- dihydrofolate reductase** An enzyme that reduces dihydrofolate (DHF) to tetrahydrofolate (THF), a form required for the synthesis of purines, thymidylic acid, and certain amino acids. It plays a critical role in DNA synthesis and cell replication, and is a target of drugs like methotrexate. 124
- dihydroorotase** A biochemical term relevant to amino acid or nucleotide metabolism: dihydroorotase.. 121
- dihydroorotate oxidase** A biochemical term relevant to amino acid or nucleotide metabolism: dihydroorotate oxidase.. 121
- dimethylallyl pyrophosphate (DMAPP)** An isomer of IPP and another activated isoprene unit that participates in head-to-tail condensations during isoprenoid biosynthesis. 90
- DNA** Deoxyribonucleic acid, the carrier of genetic information built from deoxyribonucleotides.. 118

-
- Electron Carrier** Molecules that can accept and donate electrons, facilitating the transfer of energy in redox reactions (e.g., NAD⁺, FAD).. 21
- Enoyl Reductase domain (ER)** Reduces enoyl intermediates to saturated acyl chains using NADPH. 74, 77
- Enthalpy (H)** A thermodynamic quantity representing the total heat content of a system. In biochemistry, enthalpy changes (ΔH) are associated with bond formation and breaking, influencing biochemical reactions and energy transfer.. 13
- Entropy** A measure of the disorder or randomness of a system. The second law of thermodynamics states that the total entropy of an isolated system tends to increase over time.. 13
- Enzyme** A macromolecular biological catalyst that significantly accelerates chemical reactions, often with high specificity. They can be proteins or catalytically active RNA molecules:contentReference[oaicite:0]index=0.. 1
- Enzyme Efficiency** Measured by the specificity constant $\frac{k_{cat}}{K_m}$, which reflects both substrate binding and catalytic turnover. The theoretical upper limit is 10^8 to $10^9 \text{ M}^{-1}\text{s}^{-1}$:contentReference[oaicite:9]index=9.. 10, I
- Enzyme Kinetics** The study of the rate of enzyme-catalyzed reactions and how they change in response to experimental parameters like substrate concentration:contentReference[oaicite:3]index=3.. 6, I
- Enzyme Saturation** The condition where increasing substrate concentration no longer increases the reaction rate because all active sites are occupied, resulting in a plateau at v_{max} :contentReference[oaicite:5]index=5.. 7
- essential amino acids** Amino acids that cannot be synthesized by the human body and must be obtained from the diet.. 112
- Exergonic Reaction** A chemical reaction that releases energy (has a negative ΔG) and is therefore favorable. 13
- Facultative Anaerobic Organism** A organism that is able to produce ATP by anaerobic respiration if oxygen is present, but is also capable of switching to fermentation if oxygen is absent. 31
- FAD** Flavin adenine dinucleotide, a redox cofactor involved in many metabolic reactions.. 118
- FAD (Flavin Adenine Dinucleotide)** A redox-active coenzyme associated with various enzymes, particularly in the electron transport chain and fatty acid oxidation. FAD is reduced to FADH₂, which donates electrons to the respiratory chain.. 22
- FaPP2 (Fatty Acid Phosphatase 2)** An enzyme involved in the hydrolysis of phospholipids and sphingolipids, specifically catalyzing the dephosphorylation of fatty acid phosphates. FaPP2 plays a role in lipid metabolism by regulating the levels of fatty acid phosphates, which are important intermediates in various signaling pathways.. 99, 104
- farnesyl pyrophosphate (FPP)** A 15-carbon isoprenoid intermediate formed by the head-to-tail condensation of GPP and IPP; precursor for squalene and other isoprenoids. 90
- Fatty Acid Synthase** A multifunctional enzyme complex that catalyzes the de novo synthesis of long-chain saturated fatty acids from acetyl-CoA and malonyl-CoA. 71, 74
- fatty acyl-CoA desaturase** An enzyme that catalyzes the insertion of a cis double bond into a saturated fatty acyl-CoA molecule, converting it into an unsaturated fatty acid.. 81

fructose-16-bisphosphatase-1 (FBPase-1) A key enzyme in gluconeogenesis that catalyzes the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate, bypassing the irreversible step of phosphofructokinase-1 (PFK-1) in glycolysis; inhibited by fructose-2,6-bisphosphate and AMP. 65

FBPase-2 domain The phosphatase domain of the bifunctional enzyme PFK-2/FBPase-2. It hydrolyzes fructose-2,6-bisphosphate back to fructose-6-phosphate, reducing glycolytic flux and promoting gluconeogenesis. Its activity is stimulated by glucagon signaling. 65

FFAT Motif A short linear amino acid sequence (two phenylalanines in an acidic tract) found in certain proteins that bind to the VAP (vesicle-associated membrane protein-associated protein) family on the endoplasmic reticulum. The FFAT motif mediates protein targeting to membrane contact sites, particularly between the ER and other organelles.. 98

Flavoprotein A protein that contains a flavin coenzyme, such as FAD or FMN, as a prosthetic group. 22

FMN (Flavin Mononucleotide) A coenzyme derived from riboflavin (vitamin B2) that acts as a prosthetic group in various oxidoreductases, including NADH dehydrogenase in the electron transport chain. FMN is involved in redox reactions, cycling between oxidized and reduced states.. 22

folate A biochemical term relevant to amino acid or nucleotide metabolism: folate.. 124

FYVE domain A protein domain that binds phosphatidylinositol 3-phosphate and is involved in membrane trafficking and signal transduction. 103

gamma-glutamylcysteine A biochemical term relevant to amino acid or nucleotide metabolism: γ -glutamylcysteine.. 116

gelsolin homology domain A domain found in gelsolin and related proteins, involved in actin filament severing, capping, and nucleation. It recognizes phosphoinositides. 103

geranyl pyrophosphate (GPP) A 10-carbon isoprenoid intermediate formed by the head-to-tail condensation of IPP and DMAPP. 90

GlcCer Synthase (GCS) An enzyme responsible for the synthesis of glucosylceramide (GlcCer) from ceramide and UDP-glucose. GlcCer is an important glycosphingolipid involved in cell signaling, membrane structure, and interactions. GCS plays a pivotal role in the synthesis of complex sphingolipids and is essential for the proper function of various cellular processes, including cell growth and differentiation.. 99

GLTP (Glycosphingolipid Transfer Protein) A protein that facilitates the non-vesicular transfer of glycosphingolipids between cellular membranes. It is used to transfer cerebroside to the luminal leaflet of the Golgi. 100

glucose-6-phosphatase (Glc(6)Ptase) An ER-membrane-bound enzyme that hydrolyzes glucose-6-phosphate into glucose and inorganic phosphate (Pi). 63

glucose-6-phosphate transporter (T1) A transport protein that shuttles glucose-6-phosphate (Glc(6)P) from the cytosol into the lumen of the endoplasmic reticulum (ER). 63

glucose transporter (T3) A transporter that moves glucose from the ER lumen to the cytosol following its generation from glucose-6-phosphate. 64

Glutathione An antioxidant tripeptide composed of glutamate, cysteine, and glycine, protecting cells from oxidative damage.. 116

Glutamate An amino acid that acts as a nitrogen donor in biosynthesis and is produced in bacteria from α -ketoglutarate and glutamine via glutamate synthase.. 106

-
- Glutamate synthase** An enzyme found in bacteria that converts α -ketoglutarate and glutamine into two molecules of glutamate.. 107
- glutamate-cysteine ligase (GCL)** A biochemical term relevant to amino acid or nucleotide metabolism: glutamate-cysteine ligase (GCL).. 116
- Glutamine** A nitrogen-carrying amino acid formed from glutamate and NH₄⁺ by glutamine synthase; serves as a nitrogen donor in many biosynthetic reactions.. 106
- Glutamine amidotransferase** An enzyme with two domains that transfers NH₃ from glutamine to other molecules, using a glutamyl-enzyme intermediate and ATP-activated acceptors.. 108
- Glutamine synthase** An enzyme that catalyzes the ATP-dependent conversion of glutamate and ammonium into glutamine.. 107
- Glutathione synthetase** A biochemical term relevant to amino acid or nucleotide metabolism: Glutathione synthetase.. 116
- glycerol kinase** An enzyme that catalyzes the phosphorylation of glycerol to glycerol-3-phosphate using ATP. Used in the FA synthesis pathway. 82
- glycerol-3-phosphate dehydrogenase** An enzyme that catalyzes the reversible redox conversion between dihydroxyacetone phosphate (DHAP) and glycerol-3-phosphate.. 82
- Glycine** A derivative of serine, synthesized by removing a carbon via serine hydroxymethyltransferase using tetrahydrofolate and PLP as cofactors.. 110
- glycogen branching enzyme** An enzyme that introduces $\alpha(1 \rightarrow 6)$ glycosidic branches into the linear glycogen chain, increasing its solubility and accessibility for rapid synthesis and degradation. 69
- glycogenin** A self-glucosylating glycosyltransferase enzyme that initiates glycogen synthesis by catalyzing the attachment of glucose residues to a specific tyrosine residue on itself, forming the primer required for glycogen synthase to elongate the glycogen chain. 68
- glycogen synthase** The key enzyme responsible for elongating the glycogen chain by adding glucose residues from UDP-glucose to the non-reducing ends of an existing primer via $\alpha(1 \rightarrow 4)$ glycosidic bonds. 69
- GTP** Guanosine triphosphate, a nucleotide used as an energy source and a precursor for RNA.. 118
- guanosine monophosphate** A biochemical term relevant to amino acid or nucleotide metabolism: guanosine monophosphate.. 120
- Heme** A biochemical term relevant to amino acid or nucleotide metabolism: Heme.. 115
- Hexokinase** An enzyme that catalyzes the phosphorylation of glucose to glucose-6-phosphate, the first step in glycolysis.. 27
- HMG-CoA synthase** An enzyme that catalyzes the condensation of acetoacetyl-CoA with acetyl-CoA to form β -hydroxy- β -methylglutaryl-CoA (HMG-CoA), a key intermediate in the mevalonate pathway. 88
- hypoglycemia** A condition characterized by abnormally low levels of blood glucose, which can lead to symptoms such as shakiness, confusion, sweating, and in severe cases, loss of consciousness. 138
- hypothalamus** A brain region that produces hormones targeting the anterior pituitary and sends neuronal signals to the posterior pituitary in response to nervous stimuli. 141
- hypoxanthine** A biochemical term relevant to amino acid or nucleotide metabolism: hypoxanthine.. 125

hypoxanthine-guanine phosphoribosyltransferases (HGPRT) A biochemical term relevant to amino acid or nucleotide metabolism: hypoxanthine-guanine phosphoribosyltransferases (HGPRT).. 128

Inhibition The decrease or complete loss of enzyme activity due to the binding of a molecule (an inhibitor) that interferes with catalysis. Inhibition is fundamental to drug action and metabolic regulation:contentReference[oaicite:0]index=11, I

inosine A biochemical term relevant to amino acid or nucleotide metabolism: inosine.. 125

inosine monophosphate (IMP) A biochemical term relevant to amino acid or nucleotide metabolism: inosine monophosphate (IMP).. 119

Irreversible Inhibition Inhibition where the inhibitor covalently binds or permanently inactivates the enzyme, preventing further catalytic activity:contentReference[oaicite:5]index=5.. 12, I

isopentenyl pyrophosphate (IPP) An activated isoprene unit and key intermediate in the biosynthesis of terpenoids and sterols via the mevalonate pathway. 90

isoprene A volatile hydrocarbon molecule that serves as a basic building block for the synthesis of terpenes and sterols, including cholesterol and other isoprenoid compounds. 87

K_{ATP} channel An ATP-sensitive potassium channel that links cellular metabolism to electrical activity by opening or closing in response to intracellular ATP levels. These channels are important in tissues such as pancreatic beta cells, cardiac muscle, and neurons. 144

KDSR (β -ketosphinganine reductase) An enzyme that catalyzes the NADPH-dependent reduction of β -ketosphingosine to sphinganine during sphingolipid biosynthesis. It is an ER membrane protein with its active site facing the cytosol. 94

Kinase An enzyme that catalyzes the transfer of phosphate groups from high-energy donor molecules, such as ATP, to specific substrates, a process known as phosphorylation.. 18

kinases A biochemical term relevant to amino acid or nucleotide metabolism: kinases.. 121

lanosterol The first sterol intermediate formed from squalene 2,3-epoxide during cholesterol biosynthesis in animals. 91

Lineweaver-Burk A double reciprocal plot of enzyme kinetics: $\frac{1}{v_0} = \frac{K_m}{v_{max}} \cdot \frac{1}{[S]} + \frac{1}{v_{max}}$, used to linearize the Michaelis-Menten equation for easier interpretation:contentReference[oaicite:8]index=8.. 8, I

lipoprotein lipase (LPL) An enzyme that hydrolyzes triglycerides in lipoproteins, such as chylomicrons and very low-density lipoproteins (VLDL), into free fatty acids and glycerol for uptake by tissues such as muscle and adipose tissue. 137

lipoyllysine a covalent complex of lipoic acid attached via an amide bond to the ϵ -amino group of a lysine residue. It serves as a swinging arm in multi-enzyme complexes like the pyruvate dehydrogenase complex, transferring reaction intermediates between active sites. It has **two thiol groups** that can undergo reversible oxidation to a disulfid bond.. 33

malate dehydrogenase An enzyme that catalyzes the reversible conversion between malate and oxaloacetate, present in both the mitochondria and cytosol. 62, 72

Malic Enzyme (ME) An enzyme that catalyzes the oxidative decarboxylation of malate to pyruvate, producing NADPH in the cytosol. This NADPH is essential for the reductive steps of fatty acid synthesis. Malic enzyme links the citrate-malate shuttle with the cell's need for reducing power. 72

Malonyl/Acetyltransferase domain (MAT) Transfers malonyl and acetyl groups to the acyl carrier protein (ACP), initiating fatty acid synthesis. 74, 75

Malonyl-CoA A key intermediate in fatty acid synthesis, formed by the carboxylation of acetyl-CoA via the enzyme acetyl-CoA carboxylase. Malonyl-CoA serves as the two-carbon donor in chain elongation steps catalyzed by fatty acid synthase (FAS). 71

Membrane Contact Sites (MCS) Specialized regions where the membranes of two organelles are closely apposed, typically within 10–30 nm, without fusing. MCSs facilitate direct inter-organelle communication, allowing the exchange of lipids, ions, and signaling molecules. They play key roles in cellular homeostasis, lipid metabolism, and organelle dynamics.. 98

Metal Ion Catalysis Catalysis involving metal ions that can stabilize negative charges on reaction intermediates, help substrate orientation, or mediate redox reactions through reversible changes in oxidation state:contentReference[oaicite:2]index=2.. 6

Methionine An essential amino acid that donates a methyl group in the biosynthesis of creatine and also forms homocysteine in cysteine synthesis.. 111

methylmalonyl semialdehyde A biochemical term relevant to amino acid or nucleotide metabolism: methylmalonyl semialdehyde.. 126

mevalonate A key intermediate in the biosynthesis of sterols, isoprenoids, and other terpenoids, formed from acetoacetyl-CoA through a series of enzymatic reactions in the mevalonate pathway. 88

mevalonate 5-phototransferase An enzyme that catalyzes the phosphorylation of mevalonate to 5-phosphomevalonate, using ATP as the phosphate donor. 89

Michaelis-Menten A kinetic model describing enzyme activity: $v_0 = \frac{v_{max}[S]}{K_m+[S]}$, where K_m is the substrate concentration at half-maximal velocity:contentReference[oaicite:7]index=7.. 7, I

NAD⁺ (Nicotinamide Adenine Dinucleotide) A coenzyme involved in redox reactions, serving as an electron carrier in cellular respiration. NAD⁺ is reduced to NADH, which donates electrons to the electron transport chain for ATP production.. 21

NAD+ Nicotinamide adenine dinucleotide, a coenzyme involved in redox reactions.. 118

NADP⁺ (Nicotinamide Adenine Dinucleotide Phosphate) A phosphorylated form of NAD⁺ that functions as an electron carrier, primarily in anabolic pathways such as fatty acid and nucleotide biosynthesis. NADP⁺ is reduced to NADPH, which provides reducing power for biosynthetic reactions.. 21

NADP+ A phosphorylated form of NAD+ used in anabolic redox reactions.. 118

neurotransmitter A biochemical term relevant to amino acid or nucleotide metabolism: neurotransmitter.. 117

Niacin (Vitamin B3) A water-soluble B vitamin essential for energy metabolism, DNA repair, and cell signaling. Niacin is a precursor to the coenzymes NAD⁺ and NADP⁺, which are crucial for redox reactions in cellular respiration and biosynthetic pathways. 22

Non-competitive Inhibitor Binds to a site other than the active site and reduces the effective concentration of active enzyme. It decreases v_{max} without changing K_m :contentReference[oaicite:3]index=3.. 12

Nucleotides A biochemical term relevant to amino acid or nucleotide metabolism: Nucleotides.. 118

ORMDL1/2/3 A family of ER-resident membrane proteins that negatively regulate serine-palmitoyltransferase (SPT) activity, contributing to sphingolipid homeostasis. 94

OSBP Oxysterol-binding protein, a lipid transfer protein that shuttles cholesterol and phosphatidylinositol 4-phosphate (PI4P) between organelle membranes, particularly the endoplasmic reticulum and Golgi apparatus.. 104

Oxidase An enzyme that catalyzes oxidation reactions, using molecular oxygen (O_2) as the electron acceptor without incorporating it into the substrate. Oxidases are involved in various biological oxidation processes, including those in the electron transport chain.. 17

Oxygenase An enzyme that catalyzes the incorporation of oxygen atoms from molecular oxygen (O_2) into a substrate. Oxygenases are classified into monooxygenases and dioxygenases, which incorporate one or two oxygen atoms, respectively, and are essential in metabolic pathways like drug metabolism and biosynthesis.. 17

oxytocin A hormone secreted by the posterior pituitary that induces uterine contractions during labor and milk ejection during lactation. 141

PC1 and PC2 Prohormone convertases 1 and 2; endoproteases that cleave prohormone precursors at specific dibasic residues, enabling the production of mature, biologically active peptide hormones in neuroendocrine cells. 145

PEP carboxykinase An enzyme that catalyzes the conversion of oxaloacetate to phosphoenolpyruvate (PEP); exists in both mitochondrial and cytosolic forms and is important in gluconeogenesis. 62

phosphofructokinase-1 (PFK-1) A key regulatory enzyme in glycolysis that catalyzes the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate using ATP; activated by AMP and fructose-2,6-bisphosphate, and inhibited by ATP and citrate. 65

PFK-2 domain The kinase domain of the bifunctional enzyme PFK-2/FBPase-2. It catalyzes the phosphorylation of fructose-6-phosphate to fructose-2,6-bisphosphate, which activates PFK-1 and stimulates glycolysis. Its activity is enhanced by insulin signaling. 65

Phosphatase An enzyme that removes phosphate groups from proteins or other molecules, a process known as dephosphorylation, which often regulates cellular activity.. 18

phosphate transporter (T2) A transporter that exports inorganic phosphate (Pi) from the ER lumen to the cytosol after glucose-6-phosphate hydrolysis. 64

phosphatidic acid (PA) A key intermediate in lipid metabolism, composed of a glycerol backbone with two fatty acid chains and a phosphate group; precursor for both triacylglycerol and phospholipid synthesis. 82

phosphatidic acid phosphatase (PAP) An enzyme that catalyzes the dephosphorylation of phosphatidic acid to form diacylglycerol (DAG), a key step in lipid biosynthesis. 82

phosphatidylinositol synthase An enzyme responsible for catalyzing the synthesis of phosphatidylinositol, a phospholipid involved in cellular signaling and membrane structure.. 85, 101

Phosphatidylinositol Transfer Proteins (PITPs) A family of lipid transfer proteins that mediate the transfer of phosphatidylinositol (PI) and other phospholipids between membrane compartments. PITPs are crucial for phosphoinositide signaling, membrane trafficking, and lipid homeostasis. They play essential roles in processes such as vesicle formation, cell signaling, and membrane identity.. 101

Phosphocreatine (Pcr) A biochemical term relevant to amino acid or nucleotide metabolism: Phosphocreatine (Pcr).. 115

phosphoglucomutase An enzyme that catalyzes the reversible conversion of glucose-1-phosphate to glucose-6-phosphate, a key step in both glycogen synthesis and glycogenolysis. 68

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- phosphomevalonate kinase** An enzyme that phosphorylates 5-phosphomevalonate to form 5-pyrophosphomevalonate in the mevalonate pathway.. 89
- Phosphoribosyltransferases** A biochemical term relevant to amino acid or nucleotide metabolism: Phosphoribosyltransferases.. 128
- PI4Ks** Phosphatidylinositol 4-kinases, a family of enzymes that phosphorylate phosphatidylinositol at the D-4 position of the inositol ring to produce phosphatidylinositol 4-phosphate (PI4P), a key lipid signaling molecule involved in membrane trafficking and signaling.. 103
- Pleckstrin Homology (PH) Domain** A protein domain of approximately 100 amino acids found in many proteins involved in intracellular signaling. The PH domain binds to phosphoinositides in membranes, facilitating the localization of proteins to specific membrane compartments. It plays key roles in signal transduction and membrane trafficking.. 98, 100, 103
- PLP** Pyridoxal phosphate, an active form of vitamin B6 and a coenzyme involved in amino acid metabolism, including transamination and decarboxylation.. 110
- Polyamines** Cationic molecules derived from ornithine involved in DNA binding and translational regulation.. 118
- Porphyria** A group of diseases caused by defective enzymes in the heme biosynthetic pathway, leading to accumulation of porphyrin intermediates.. 114
- Porphyrins** Heterocyclic macrocycles that coordinate metal ions; precursors to heme.. 113
- posterior pituitary** A part of the pituitary gland containing axons of hypothalamic neurons that secrete oxytocin and vasopressin. 141
- prenyl transferase** An enzyme that catalyzes head-to-tail condensations of activated isoprene units (e.g., IPP and DMAPP) to form larger isoprenoid intermediates like GPP and FPP. 90
- Proline** A cyclic derivative of glutamate formed through phosphorylation, reduction, and spontaneous cyclization of glutamate semialdehyde.. 109
- PTB domain** Phosphotyrosine-binding domain, a protein domain that recognizes and binds to phosphotyrosine-containing motifs, typically in receptor tyrosine kinases. It recognizes phosphoinositides. 103
- Purine** A nitrogenous base composed of fused imidazole and pyrimidine rings; a building block for DNA and RNA.. 119
- pyridoxal phosphate (PLP)** A cofactor derived from vitamin B₆, essential for amino acid metabolism. PLP is involved in transamination, decarboxylation, and deamination reactions. 47
- Pyrimidine** A six-membered nitrogen-containing ring found in cytosine, uracil, and thymine.. 121
- pyrimidine-nucleoside phosphorylase** A biochemical term relevant to amino acid or nucleotide metabolism: pyrimidine-nucleoside phosphorylase.. 127
- pyrophosphomevalonate decarboxylase** A bifunctional enzyme that catalyzes both the phosphorylation of 5-pyrophosphomevalonate to 3-phospho-5-pyrophosphomevalonate and its decarboxylation to yield activated isoprene units. 89
- Pyruvate** A three-carbon molecule that is the end product of glycolysis.. 31
- pyruvate carboxylase** A mitochondrial enzyme that catalyzes the carboxylation of pyruvate to form oxaloacetate, playing a key role in gluconeogenesis and anaplerotic reactions. 61, 64

pyruvate dehydrogenase complex (PDH complex or PDC) a multi-enzyme complex that catalyzes the conversion of pyruvate into acetyl-CoA, linking glycolysis to the citric acid cycle. 34

pyruvate translocase a transport protein located in the inner mitochondrial membrane that facilitates the import of pyruvate from the cytosol into the mitochondrial matrix for further metabolic processing. 33

Rate Enhancement The increase in reaction speed due to the lowering of the activation energy by enzyme catalysis:contentReference[oaicite:15]index=15.. 5, I

Reaction Intermediate A short-lived, chemically distinct species that occurs during the transformation of reactants to products, such as enzyme-substrate (ES) complexes:contentReference[oaicite:7]index=7.. 2

Reducing Equivalent A unit of reducing power in biochemical redox reactions, referring to the transfer of one electron (or its equivalent as a hydrogen atom or hydride ion). Reducing equivalents are carried by molecules such as NADH, NADPH, and FADH₂, playing a crucial role in cellular respiration and biosynthetic pathways.. 21

Return Rate The rate of the reaction based on substrate concentration and a rate constant

$$k$$

, where

$$v = k[S]$$

:contentReference[oaicite:11]index=11.. 3, I

Reversible Inhibition Inhibition where the inhibitor binds non-covalently and can dissociate from the enzyme. Includes competitive, non-competitive, and uncompetitive mechanisms:contentReference[oaicite:1]index=1.. 11, I

ribonucleotide reductase (RNR) A biochemical term relevant to amino acid or nucleotide metabolism: ribonucleotide reductase (RNR).. 122

RNA Ribonucleic acid, a nucleic acid involved in gene expression, composed of ribonucleotides.. 118

Sac1 A conserved phosphoinositide phosphatase that dephosphorylates phosphatidylinositol 4-phosphate (PI4P), playing a key role in lipid homeostasis and membrane trafficking. 103, 104

salvage pathways A biochemical term relevant to amino acid or nucleotide metabolism: salvage pathways.. 127

Second Law of Thermodynamics States that the total entropy of an isolated system can never decrease over time.. 12

Serine An amino acid derived from 3-phosphoglycerate (glycolysis intermediate) via oxidation, transamination, and dephosphorylation steps.. 110, 111

serine hydroxymethyltransferase An enzyme that converts serine to glycine by removing a carbon, requiring tetrahydrofolate and PLP as cofactors.. 110

serine-palmitoyltransferase (SPT) An enzyme that catalyzes the first and rate-limiting step in sphingolipid biosynthesis, condensing serine and palmitoyl-CoA to form 3-ketosphinganine. 93

severe combined immune deficiency (ADA-SCID) A biochemical term relevant to amino acid or nucleotide metabolism: severe combined immune deficiency (ADA-SCID).. 127

SH2 domain Src Homology 2 domain, a protein domain that binds specifically to phosphorylated tyrosine residues and is critical in signal transduction pathways. It recognizes phosphoinositides. 103

SNARE complex A protein complex that mediates vesicle fusion by bringing membranes into close proximity, crucial for intracellular trafficking and neurotransmitter release. SNARE stands for Soluble N-ethylmaleimide-sensitive factor Attachment protein REceptor. 143, 146

Specificity The enzyme's ability to select exact substrates and catalyze a specific reaction, influenced by binding energy and structural complementarity:contentReference[oaicite:17]index=17.. 5, I

Sphingomyelin Synthase (SMS) An enzyme that catalyzes the conversion of ceramide and phosphatidyl-choline into sphingomyelin and diacylglycerol. This reaction is a key step in sphingolipid metabolism.. 97

squalene A triterpenoid compound formed from the condensation of two molecules of farnesyl pyrophosphate (FPP), which is an intermediate in the mevalonate pathway, and serves as a precursor for sterols such as cholesterol. 87, 90, 91

squalene 2,3-epoxide An oxygenated intermediate formed from squalene by squalene monooxygenase; undergoes cyclization to form lanosterol. 91

squalene monooxygenase An enzyme that catalyzes the first oxygenation step in sterol biosynthesis by converting squalene to squalene 2,3-epoxide. 91

squalene synthase An enzyme that catalyzes the head-to-head condensation of two farnesyl pyrophosphate molecules to produce squalene, a key precursor to sterols. 90

stabilizing protein (SP) A calcium-binding protein that stabilizes the glucose-6-phosphatase complex in the ER lumen. 63

succinate dehydrogenase an enzyme that catalyzes the oxidation of succinate to fumarate in the citric acid cycle (step 6). It is also part of Complex II in the electron transport chain, linking the TCA cycle with oxidative phosphorylation by transferring electrons from FADH₂ to ubiquinone (coenzyme Q). 38

succinyl-CoA A biochemical term relevant to amino acid or nucleotide metabolism: succinyl-CoA.. 126

Suicide Inhibition A special type of irreversible inhibition where the enzyme converts the inhibitor into a reactive intermediate that covalently modifies and inactivates the enzyme:contentReference[oaicite:6]index=6.. 12

SUR Sulfonylurea Receptor, a regulatory subunit of the K_{ATP} channel that binds sulfonylurea drugs and modulates channel activity. There are different isoforms such as SUR1 and SUR2, which influence channel properties in different tissues. 144

Synaptobrevin2 Also known as VAMP2 (Vesicle-Associated Membrane Protein 2), a vesicular SNARE protein critical for membrane fusion during exocytosis. 144

Synaptotagmin A calcium-sensing protein that binds calcium ions and regulates SNARE-mediated membrane fusion, especially in neurons and endocrine cells. 144

Syntaxin1A A membrane-bound SNARE protein located on the plasma membrane that forms a complex with SNAP-25 and Synaptobrevin2/VAMP2 to mediate vesicle fusion. 144

Systematic name A precise name given to an enzyme that indicates the substrates and type of reaction it catalyzes (e.g., ATP:glucose phosphotransferase for hexokinase):contentReference[oaicite:3]index=3.. 1

Thioesterase domain (TE)	Hydrolyzes and releases the final fatty acid product from the acyl carrier protein.
74	
thiolase	An enzyme that catalyzes the condensation or cleavage of acetyl-CoA molecules, playing a key role in cholesterol synthesis.
88	
Thymidine	A nucleoside component of DNA formed from thymine and deoxyribose, used in salvage pathways..
127	
Thymidine kinase	An enzyme that phosphorylates thymidine to TMP in nucleotide salvage pathways..
128	
Thymidine phosphorylase	An enzyme that salvages thymine by converting it to thymidine using sugar phosphates..
127	
Thymidylate (dTMP)	A nucleotide synthesized from dUMP by thymidylate synthase; essential for DNA replication..
124	
thymidylate synthase (TYMS)	An essential enzyme that catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), a key step in DNA synthesis and repair. It is a target for certain anticancer drugs.
124	
transamination	A biochemical process where an amino group is transferred from one molecule (usually glutamate or glutamine) to another, forming new amino acids..
108	
Transition State	The highest-energy configuration along the reaction coordinate. It is the point at which the system is equally likely to proceed toward products or return to reactants. It is not a stable intermediate and is denoted as <code>:contentReference[oaicite:0]index=0..2</code>
Transition State Complementarity	The enzyme binds more tightly to the transition state than to the substrate, thereby lowering the activation energy and enhancing catalysis: <code>:contentReference[oaicite:14]index=14..5, I</code>
Transition State Theory	A model describing the top of the energy hill in a reaction coordinate where reactants are equally likely to proceed to products or return to reactants. Not to be confused with reaction intermediates: <code>:contentReference[oaicite:6]index=6..2, I</code>
Transition-State Analogs	Stable molecules that resemble the transition state of a substrate and bind tightly to the enzyme, acting as potent inhibitors by exploiting transition state complementarity: <code>:contentReference[oaicite:7]index=12</code>
Ubiquinone (Coenzyme Q)	A lipid-soluble electron carrier in the electron transport chain, transferring electrons between complex I/II and complex III. Ubiquinone exists in oxidized (Q), semiquinone (Q^-), and reduced (QH_2) forms..
23, 51	
UDP-glucose pyrophosphorylase	An enzyme that catalyzes the formation of UDP-glucose from glucose-1-phosphate and UTP. UDP-glucose is a key precursor for glycogen and polysaccharide biosynthesis in many organisms.
67	
Uncompetitive Inhibitor	Binds only to the enzyme-substrate complex, preventing product formation. It decreases both v_{max} and K_m :
<code>:contentReference[oaicite:4]index=4..12</code>	
uric acid	A biochemical term relevant to amino acid or nucleotide metabolism: uric acid..
125	
uridine	A biochemical term relevant to amino acid or nucleotide metabolism: uridine..
127, 128	
uridine monophosphate (UMP)	A biochemical term relevant to amino acid or nucleotide metabolism: uridine monophosphate (UMP)..
121, 127	

Uridine phosphorylase An enzyme that adds ribose-1-phosphate to uracil in pyrimidine salvage.. 127

uridine triphosphate (UTP) A biochemical term relevant to amino acid or nucleotide metabolism: uridine triphosphate (UTP).. 118, 121

Uridine-cytidine kinase An enzyme that phosphorylates uridine and cytidine in salvage pathways.. 127, 128

VAP (Vesicle-associated membrane protein-associated protein) A family of ER-resident membrane proteins that serve as scaffolds for the recruitment of cytosolic proteins containing FFAT motifs. VAPs play a central role in organizing membrane contact sites and facilitating lipid transfer and signaling between the ER and other organelles.. 98

vasopressin A hormone released by the posterior pituitary that helps regulate blood pressure. 141