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Metabolic Biochemistry

Lecture Notes

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McGill

McGill Metabolic Biochemistry

Lectures (BIOC 311)

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Foreword

The generation of metabolic energy in higher organisms with an emphasis on its regulation at the molecular, cellular and organ level. Chemical concepts and mechanisms of enzymatic catalysis are also emphasized. Included: selected topics in carbohydrate, lipid and nitrogen metabolism; complex lipids and biological membranes; hormonal signal transduction. This notes include all of the lectures of Fall 2024.

This note includes a few terminologies in order to structurize the material. The following is the brief description of each terms:

1. Definition: An explanation on the meaning of a word
2. Observation: A further analysis of a word or idea through observing.
3. Method: Description of an experiment or usage of an idea in an experiment.
4. Concept: An important idea that's obtained through scientific testing
5. Notion: A less important idea (that could be realized after a concept)
6. Explanation: A more detailed analysis on a concept, notion or even definition. Note: a square marks the end of an explanation.
7. Example: A specific case used to demonstrate a general concept/notion
8. Mechanism of Action: A large stepwise explanation.

Prerequisites: BIOL 200, BIOL 201, PHGY 209 and 210.

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1.1 Introduction

Definition 1.1. **Metabolism** is the process through which living system acquire and utilize free energy they need to carry out various functions.

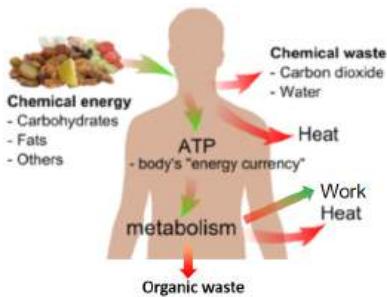


Figure 1.1: Food are made from molecules held together by chemical energy. Metabolism the process of breaking and extracting these chemical energy to produce heat, waste but most importantly, provide energy to do work.

Remark 1.1. *Our metabolism is an open system since we need a constant supplies of energy in because we're producing organic waste.*

Example 1.1.1. Some of the chemicals we take in like sugar, will release roughly -2850 kJ/mol when undergo metabolism. This energy released can be stored for later use. Same with fatty acid, which produce even higher amount of energy of -9781 kJ/mol . Interesting, the typical ATP that we view as the currency of energy of the cell releases only -30.5 kJ/mol .

Explanations. ATP releases so little because it's going to be used as the primary source on the spot and it's a **controlled** source of energy. Similar to how we don't use gold or diamond as currency but the Canadian Dollar.
□

Definition 1.2. **Catabolism (degradation)** is the process where nutrients and cell constituents are broken down to salvage their components and/or to generate free energy. **Anabolism (biosynthesis)** is the process where biomolecules are synthesized from simpler components

Concept 1.1 Metabolism is the sum of catabolism and anabolism

$$\text{Metabolism} = \text{Catabolism} + \text{Anabolism} \quad (1.1)$$

Notion 1.1 (Energy Balance) Metabolism must be balanced which means the energy intake must equal to the energy expenditure.

The **energy intake** is just food intake while **energy expenditure** is the sum of all physical/cellular activities along with the the SMR.

Definition 1.3. Standard (Basal) Metabolic Rate (SMR or BMR) is the metabolic rate of an organism not digesting food, at thermoneutrality, under resting and stress free conditions. \Rightarrow basic energy consumption at rest.

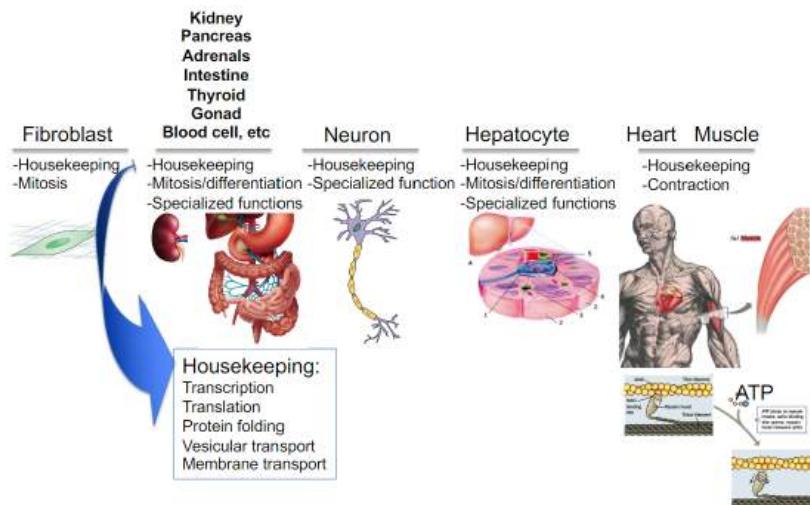


Figure 1.2: Different cells with different activities and SMR hence different ATP consumption. The **housekeeping aspect of each cells** can be thought of as the SMR.

Observation 1.1 A **negative energy balance** occurs when the energy intake < energy expenditure. This can happen because of **anorexia** (eating disorder) which lead to **cachexia** and then death.

Observation 1.2 On the contrary, A **positive energy balance** occurs when the energy intake > energy expenditure. This can leads to weight gain, obesity, **type 2 diabetes** and ultimately death.

Remark 1.2. *Obesity can be viewed as an epidemic in the US as the obesity rate increase by 26% within the last 15 years.*

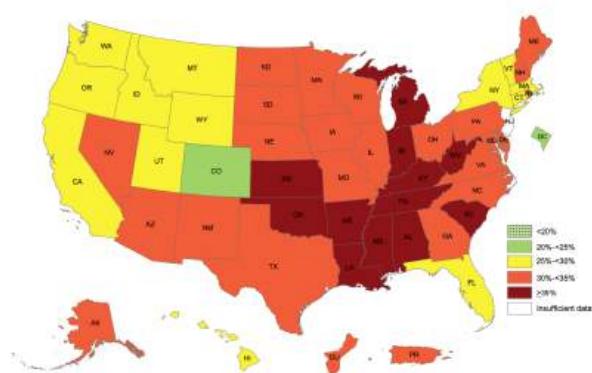


Figure 1.3: Prevalence of Self-Reported Obesity Among U.S. Adults by State and Territory, BRFSS, 2019

1.1.1 Principles of Metabolism

Remark 1.3. *All the metabolic pathway in your body is interconnected which means imbalance of 1 will effect the other.*

Definition 1.4. **Metabolic pathways** are series of consecutive enzymatic reactions that produce specific products. All the reactants, intermediates and products of the pathways is called **metabolites**.

There are 5 principles that governs the metabolic pathways and we'll look at each of them

Concept 1.2 (Irreversibility). Metabolic pathways are irreversible.

Explanations. The metabolic pathways are irreversible since it has steps that are **highly exergonic** which requires more energy initially input to go back thus making it irreversible. Having these irreversible steps is important as they act as a **regulation of directionality** of a pathway. □

Concept 1.3 (First Step). Every metabolic pathways has a first committed step.

Explanations. Almost every intermediate reactions in a pathway is at equilibrium however it requires that steps early on need to be irreversible since



Figure 1.4: Metabolic pathways are irreversible due to highly exergonic steps (arrows with red dots). These pathways also have to have a committed first step (from R to first I)

it can make the reactions more committed to producing the needed products. This have the same reasoning as concept 1.3., that is, conveying directionality. □

Concept 1.4 (Regulation). All metabolic pathways are regulated.

Explanations. Metabolic pathways needed to be regulated as it does not want to overproducing or underproducing any products. To have control on the metabolites flux, it needs to regulate the **rate-limiting steps**. □

Example 1.1.2. **Allotrophic regulation** is a way for a product to bind an earlier reactants to shut it down. You can also have covalent modification (enzymes: ON vs OFF), substrate cycles and even genetic control

Concept 1.5 (Differentiation). Anabolic and catabolic pathways must differ.

Explanations. Essentially, when 2 metabolites are interconvertible, the pathway from 1 to the other must different going in the opposite direction. This is because the body needs to regulate different metabolites based on metabolic needs e.g. running vs sitting would yield different metabolites production. □

Concept 1.6 (Localisation). In eukaryotic cell, pathways must occur in specific cellular locations.

Explanations. Pathways are localized in specific locations may be due to: different environment, reduce energy loss and even increase efficiency due to proximity. □

1.1.2 Reminder on Thermodynamics

Suppose the following reaction occurs



Then, the **change in Gibbs free energy (ΔG)** is given as

$$\Delta G = \Delta G^{\circ'} + RT \ln \frac{[C][D]}{[A][B]} \quad (1.2)$$

or

$$\Delta G = \Delta G^{\circ'} + RT \ln K_{eq} \quad (1.3)$$

Explanations. $\Delta G^{\circ'}$ is the indicator of the nature of the reaction i.e. it's a crude indication of the likelihood of a reaction being physiologically reversible.

- $\Delta G^{\circ'} > 0 \Rightarrow$ endergonic reaction \Rightarrow requires free energy input.
- $\Delta G^{\circ'} < 0 \Rightarrow$ exergonic reaction \Rightarrow produces energy.

While $\Delta G^{\circ'}$ is a crude indication, ΔG is the true indicator of directionality i.e. indication of the likelihood of its ir/reversibility *in vivo*.

- $\Delta G = 0 \Rightarrow$ equilibrium reaction \Rightarrow reversible *in vivo*.
- $\Delta G < 0 \Rightarrow$ energy-releasing reaction \Rightarrow irreversible *in vivo*.

□

Remark 1.4. In ideal condition when all reactants and products' concentration are equal, $\Delta G^{\circ'}$ becomes the true indicator which = ΔG .

Definition 1.5. A **reaction coupling** is the process where 2 (or more) reactions combined such that an spontaneous reaction is produced to drive a non-spontaneous one.

Example 1.1.3. Coupling an endergonic reaction to an exergonic ones can make the overall reaction favourable *in vivo*

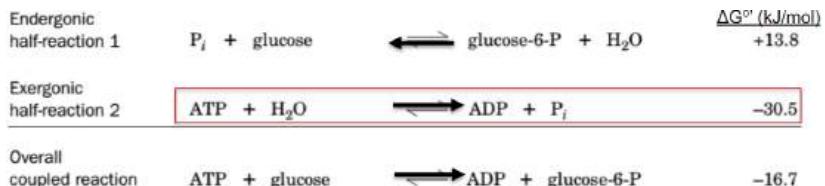


Figure 1.5: phosphorylation of glucose is an endergonic reaction which can be coupled ATP hydrolysis, an exergonic reaction to make the overall reaction possible.

1.2 Dietary Glucose

In today's lecture, we're going to discuss how glucose is transformed into energy.

Example 1.2.1. Supposed that you eat some rice, meat and vegetables for breakfast along with a cup of milk coffee.

In rice you'd find starch which is a polymer of glucose molecules (with branchings). In meat, you'd find glycogen which is also polymer of glucose + higher degree of branching. Lastly, in veggies, you'd find cellulose which is also a glucose polymer but is arranged in an alternative pattern of "up" and "down".

Remark 1.5. *We do not have the enzymes to digest cellulose. Cow have enzymes to break this down.*

(Continue from example 1.2.1) Supposed that you drink a cup of coffee with milk, you'd be consuming lactose and sucrose [both are forms of glucose] which will break down by *sucrase* and *lactase*.

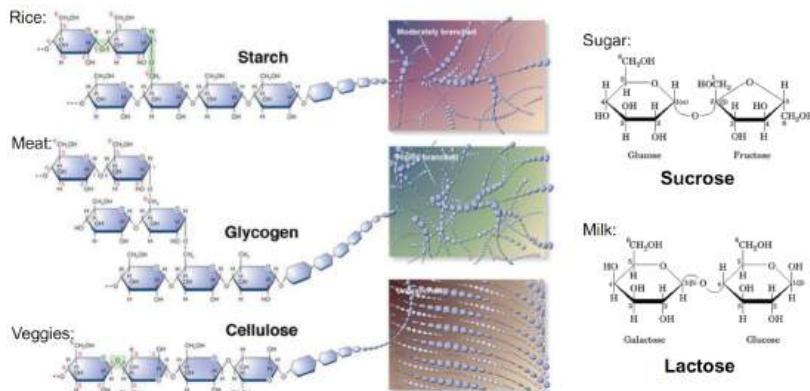


Figure 1.6: Different forms of glucose in food

Essentially, when we eat, we eat a lot of sugar. After consumption, these glucose need to be absorbed. This is done by the villi of the small intestine. The glucose can be taken up by the **Na⁺/glucose symport** into the brush border cell. Then it will exit the cell using the **glucose uniport**.

Explanations. The reason glucose needs a symport is because Na^+ is going down its concentration gradient so it can couple with glucose to enter the cell (secondary active transport). The usage of the uniport is because in the cell now has high [glucose] which can go down its gradient to capillaries. □

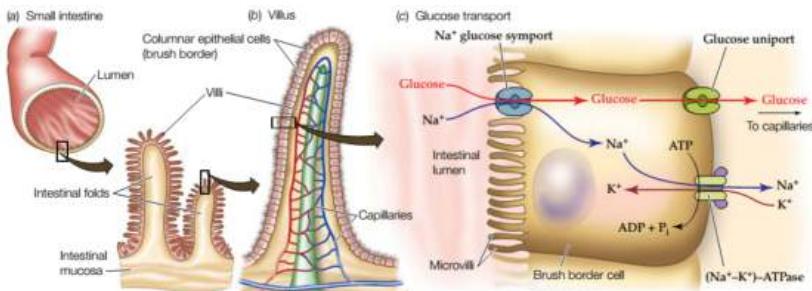


Figure 1.7: Absorption of glucose to the blood stream

This essentially highlight 2 types of transport **active and passive transport**. For the Na^+ /glucose symport, it's active transport since it uses the gradient of Na^+ to transport glucose (secondary active). For the glucose uniport, it's passive.

1.2.1 Blood to Pancrease: Insulin Response

Now, we have lots of glucose in our blood, this lead to the action of the pancreas. First of, in between meal, [glucose] is around $< 5.5\text{mM}$, the pancreatic β -cell is at rest. Here, little amount of glucose enter the cell and go through glycolysis and oxidative phosphorylation to produce some ATP. This [ATP] is now enough to close K^+ channel to enable membrane hyperpolarization.

After you have a meal, [glucose] increases this lead to an increase of glycolysis in pancreatic cell which increase [ATP]. $[\text{ATP}] \uparrow$ will block the K^+ channel leading to the opening of Ca^{2+} channel via membrane depolarization. Ca^{2+} enters the cell as signalling molecules to help insulin vesicles to fuse and release to the extracellular space.

Remark 1.6. *Insulin can decrease [glucose] in the blood stream.*

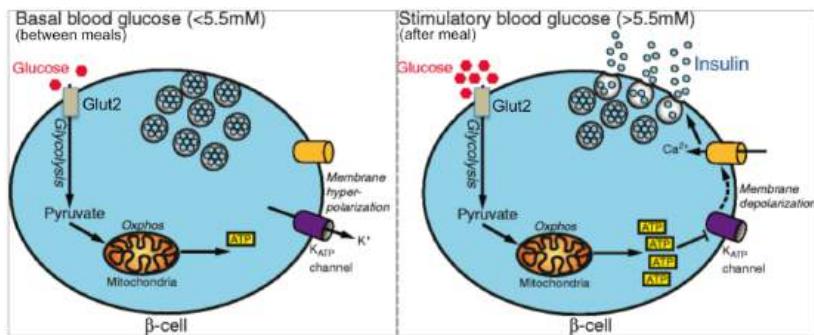


Figure 1.8: Pancreatic cell's activity between and after a meal

1.2.2 Uptake in Tissues

Now that insulin is in the bloodstream, glucose will be uptake by the cell. This is done via different transporters.

In the liver and all other cell types, **GLUT-2 transporter** will allow glucose to go down its gradient into the cell. The muscle and adipose tissues have extra transporter called **GLUT-4** to uptake even more glucose. Normally, GLUT-4 resides in the intracellular space but will be recruited to the surface when insulin binds to the **insulin receptor**.

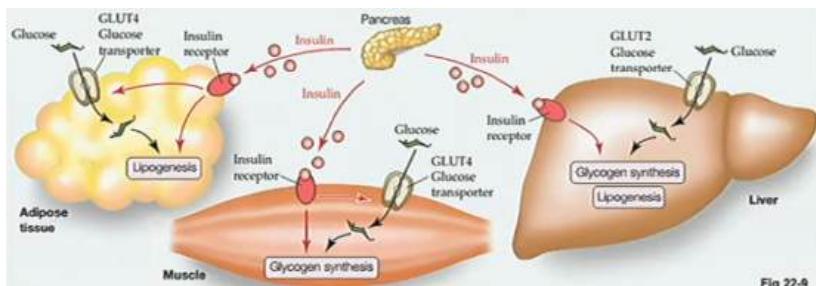


Fig 22-9

Figure 1.9: GLUT-2 and GLUT-4 transporter

Remark 1.7. *GLUT-2 is not insulin-responsive and only dependent on the [glucose]. This also means glucose move both way using this transporter depending on the concentration.*

Glucose Management

Liver is the place where glucose will be decided to stored or delivered to other tissue. The $[glucpse] = 5.5mM$ is maintained by the liver. If the level increase or decrease, glucose will be released or stored via the liver.

In the fed state, glucose is stored as glycogen in the liver. Meanwhile, in the fasting state, glycogen is broken down into glucose or you can make new glucose via **gluconeogenesis**.

For the uptake of glucose, tissues uses transporter. In fact, we have lots of glucose transporter and **why is that?** Well...because each transporters will tend to tissues' need to glucose (i.e. different tissues require different concentration).

Transporter	Major Sites of Expression	Characteristics
→ SGLUT 1	Intestinal mucosa, kidney tubules	Cotransports one molecule of glucose or galactose along with two sodium ions. Does not transport fructose.
GLUT-1	Brain, erythrocyte, endothelial cells, fetal tissues	Transports glucose (high affinity) and galactose, not fructose. Expressed in many cells.
→ GLUT-2	Liver, pancreatic beta cell, small intestine, kidney.	Transports glucose, galactose and fructose. A low affinity, high capacity glucose transporter; serves as a "glucose sensor" in <u>pancreatic beta cells</u> .
→ GLUT-3	Brain, placenta and testes	Transports glucose (high affinity) and galactose, not fructose. The primary glucose transporter for neurons.
→ GLUT-4	Skeletal and cardiac muscle, adipocytes	The <u>insulin-responsive glucose transporter</u> . High affinity for glucose.
GLUT-5	Small intestine, sperm	Transports fructose, but not glucose or galactose. Present also in brain, kidney, adipocytes and muscle.

Figure 1.10: Glucose transporters

1.2.3 Backward View of ATP Production

Normally, when we look at how ATP is produced, we look it from the reactants to products. In this lecture, we will look it on the opposite side.

During exercises, within the first seconds, we will use the abundant ATP stored in the muscle. When this stored ATP is depleted, we begin to use **creatine phosphate** to generate ATP using the enzyme **creatine kinase**. After this, our body will begin **anaerobic glycolysis** which is the breakdown of glucose to generate ATP. If the exercise continues for a longer period, we will begin aerobic glycogenesis. Lastly, if exercise duration last longer, glucose level in the body will be depleted hence our body will begin **aerobic lipolysis**, a process that breakdown fat to generate ATP.

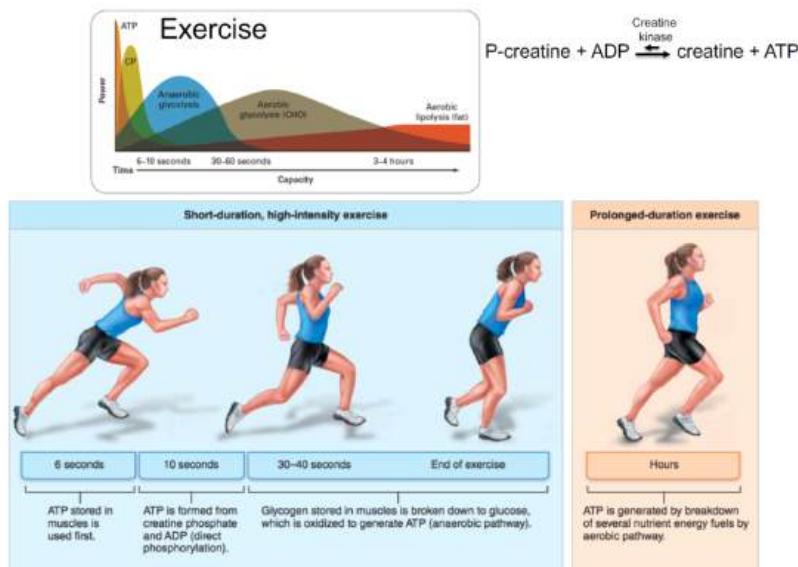


Figure 1.11: Different processes of generating ATP during exercise.

The production of ATP from glucose is through glycolysis which creates acetyl-CoA. Acetyl-CoA will go through the citric acid cycle to produce substrates used in oxidative phosphorylation to generate ATP.

What are these substrates and how does it work? Well...let's look at the oxidative phosphorylation. ATP is generated from ADP at complex V using H^+ gradient as the driving force. The H^+ gradient is generated by complex

I, III and IV by pumping H^+ in. In order for this to happen, there must be O_2 reaction to make water and e^- movement through the complexes. The H^+ and e^- is made from the breakdown of NADH and FADH₂ into NAD⁺ and FAD respectively.

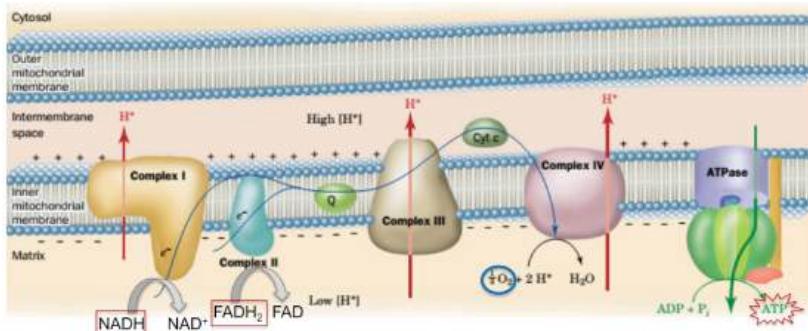


Figure 1.12: Oxidative phosphorylation.

How do you generate NADH and FADH₂? Well...we can use the citric acid (Kreb's) cycle to make it from the substrate acetyl-CoA.

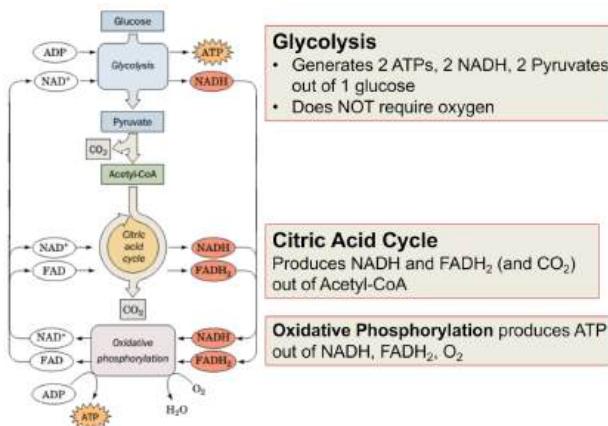


Figure 1.13: Complete summary of how ATP is generated from glucose

Where do you get acetyl-CoA? Well...we have glycolysis who end product is pyruvate to make acetyl-CoA and **this process does not require O_2 .**

Each of these processes will be regulated by different substrates. For oxidative phosphorylation, low [ATP] will be the activator while high [ATP] (+ low $[O_2]$ or $[NADH/FADH_2]$) is the inhibitor. For the citric acid cycle, high $[NADH]$ serves as the inhibitor. Lastly, for glycolysis, low $[ATP]$ serves as the activator while low $[NAD]$ and high $[ATP]$ serves as the inhibitor.

A Little Reminder About Glycolysis

Like we've said before, glycogenesis does not require O_2 however the rest of the process does require it. Under aerobic condition, the product of glycolysis, pyruvate, will undergo the citric acid cycle to generate NAD^+ from $NADH$.

Now supposed that we want to make NAD^+ to supply to glycolysis, **how could be do that?** Well...under anaerobic condition, in the muscle mostly, pyruvate will undergo **homolactic fermentation** to generate NAD^+ from $NADH$, and a byproduct of **lactate**.

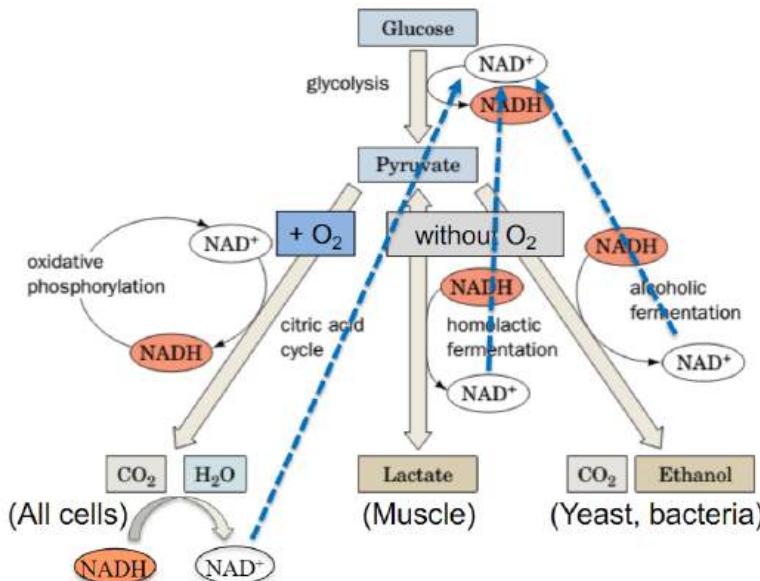


Figure 1.14: Generating NAD^+ via homolactic or alcoholic fermentation.

Homolactic fermentation is mostly for mammalian cells. For yeast and bacteria, they mostly experience **alcoholic fermentation** which will have the same generation of NAD^+ but byproduct of ethanol and CO_2 .

Remark 1.8. Any alcoholic beverages are made from alcoholic fermentation.

Concept 1.7 (Warburg Effect). It's found that majority of cancer cells use aerobic glycolysis in order to generate energy rather than other mechanism i.e. consume a large amount of glucose without using much oxygen

This is an important effect as we can develop scans such as **Fluoro-deoxy-glucose PET scan** to detect region of high metabolically active tumours.

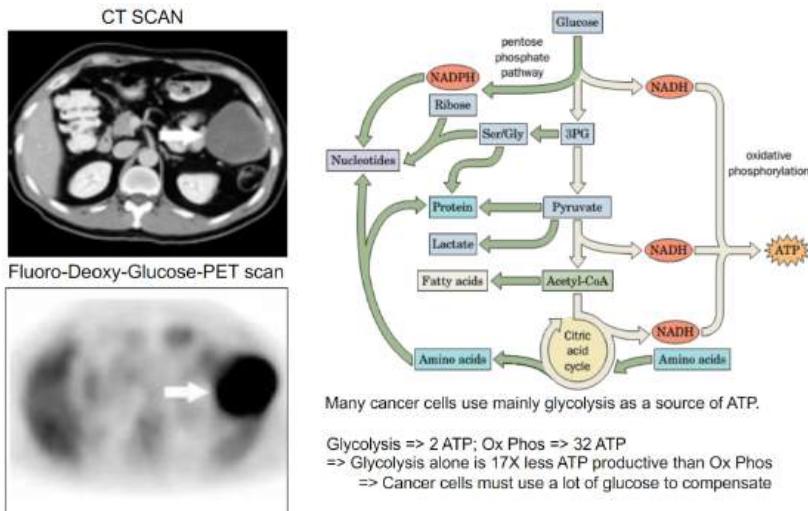


Figure 1.15: Warbur effect and new scan type.

1.3 Glycolysis

The whole pathway of glycolysis have rates-limiting steps based on the principle of metabolism.

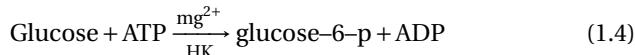
Definition 1.6. The **rate-limiting steps** are steps generating the most free energy in the pathway

Remark 1.9. Like we've discussed these steps are irreversible since the G is too large to overcome in-vivo.

in today's lecture, we'll be focusing on some of these rate limiting steps and their regulation.

1.3.1 Hexokinase vs Glucokinase

Definition 1.7. **Hexokinase (HK)** is the enzyme that catalyzes the first step to glycolysis as given below.



with $\Delta G = -27 \text{ kJ/mol}$ i.e. physiologically irreversible.

Mechanism of Action (HK's Phosphorylation): The phosphorylation of glucose will be coupled to the hydrolysis of ATP since this reaction does not happen *in vivo*. The phosphorylation will trap glucose which makes it hard to transport hence trapping it in the cytoplasm. This also creates a concentration gradient that drives more glucose in.

Interestingly, we actually have another enzyme that's similar to the hexokinase, that is, **glucokinase (GK)**. These 2 enzymes are present in the liver and now we're going to see the differences between them.

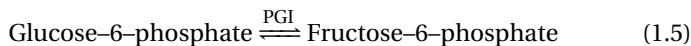
	Hexokinase	Glucokinase
Catalyzed reaction	$\text{Glucose} + \text{ATP} \rightarrow \text{Glucose-6-phosphate} + \text{ADP}$	$\text{Glucose} + \text{ATP} \rightarrow \text{Glucose-6-phosphate} + \text{ADP}$
Substrate specificity	Less Specific (Hexoses)	More specific for glucose
Tissue of prevalence	Everywhere	Liver and beta cells
Affinity Constant (K_m (mM))	Higher (low $K_m \sim 0.1 \text{ mM}$)	Lower (high $K_m \sim 5.0 \text{ mM}$)
Modulator	G6P	Glucokinase Regulatory Protein
Metabolic consequence	Because Hexokinase has a higher affinity for glucose, all cells will help themselves w/ glucose. The extra circulating glucose will then be converted to glycogen via glucokinase in the liver	

Figure 1.16: Hexokinase vs glucokinase.

Definition 1.8. **Glucokinase regulatory protein (GKRP)** is a protein that binds to GK when $[\text{glucose}] \downarrow$ and keeps it in the nucleus. It will unbind when $[\text{glucose}] \uparrow$.

1.3.2 Phosphoglucoisomerase

Definition 1.9. **Phosphoglucoisomerase (PGI)** is an enzyme that converts glucose-6-phosphate to fructose-6-phosphate in an equilibrium reaction.



Remark 1.10. This reaction is not rate limiting as it can go both ways and thus not a regulatory step.

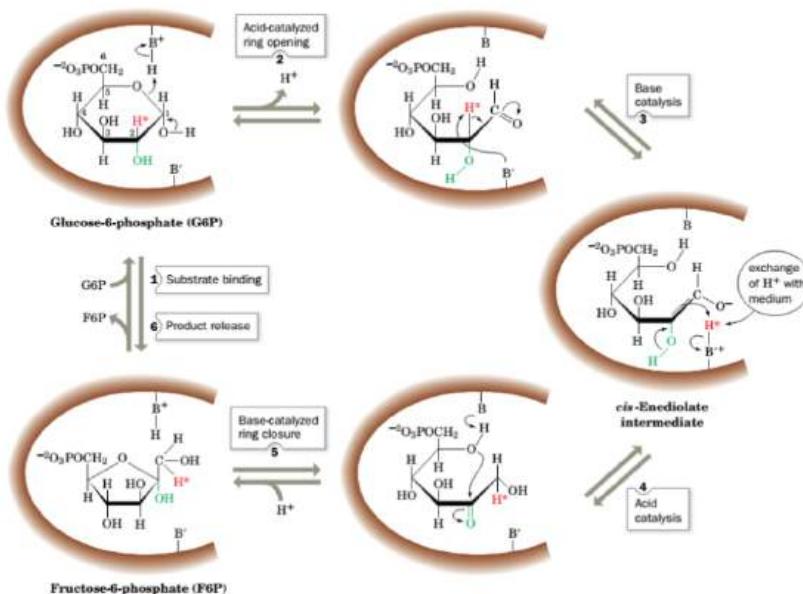


Figure 1.17: Mechanism of action of phosphoglucoisomerase.

1.3.3 Phosphofructokinase 1 vs 2

Definition 1.10. **Phosphofructokinase (PFK)** is an enzyme that catalyzes the rate-limiting step that commits cells to metabolize glucose (glycolysis). The reaction is given as



with $\Delta G = -26 \text{ kJ/mol}$, hence also physiologically irreversible.

Explanations. The reason this step is called the committing step is because the previous substrate of fructose-6-phosphate reversible turn back to glucose-6-phosphate which is a substrate for many reaction beside glucose e.g. conversion to glycogen. Fructose-1,6-bisphosphate on the other hand is a substrate that's specifically for glycolysis & its generation is irreversible hence it's a committing step. □

Because PFK1 is a rate-limiting step, it's highly regulated. Its regulators (inhibitors and activators) can be seen in the table below.

Enzyme	Activators	Inhibitors
HK	-	G6P
PFK	AMP ; F2,6P	ATP ; citrate
PK (muscle)	PEP ; FBP	ATP

Figure 1.18: Table of PFK regulator

Allosteric regulation of PFK1

First, we need to know that PFK1 exists in 2 states:

- **T-state:** Inactive state, forms PFK dimers
- **R-state:** Active state, forms PFK tetramers

In the abundance of ATP, it will bind to the PFK to yield the T state hence turning the PFK off. In the abundance of AMP, fructose-2,6-bisphosphate, it will turn on the PFK yielding the R state. See Figure 1.19.

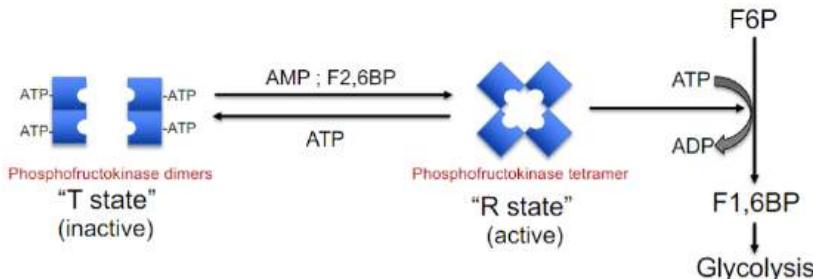


Figure 1.19: Regulation of PFK via ATP and AMP.

Therefore, we can see that ATP acts as an inhibitor while AMP acts as an inhibitor. Now, we can further ask ourselves, **is ATP or AMP more potent at modulating PFK?** i.e. **If present at same concentration, will it be ATP or AMP that control PFK?** Well...to answer this, we will perform an experiment.

Methods 1.1 Obtain the PFK enzyme and put it in a control environment with low [ATP] and a constant increasing fructose-6-p (F6P) level. Record and plot the [F6P] vs PFK activity. Now, add increase concentration of ATP of 1mM and plot the observed activity. Now, in the same batch, add in 0.1mM (1/10th of that of ATP) of AMP and plot the observed activity.

What you'd find is the following graph.

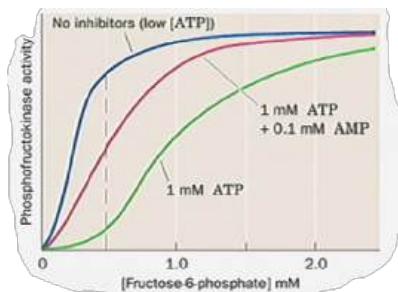


Figure 1.20: The blue curve indicate of the standard activity of PFK with no activator nor inhibitor. Upon adding 1mM ATP, the graph shift to the right (green) showing that ATP is inhibiting it. Now adding back only 1/10th of the concentration of AMP is enough to counter the activity of ATP hence it's a more potent inhibitor.

From the graph and the analysis, we can definitely say that **AMP is a much more potent regulator of PFK than ATP.**

Definition 1.11. **Adenylate kinase (ADK)** is an enzyme that re-generates ATP and AMP from 2 molecules of ADP.



Though it seems like ADK has nothing to do with PFK but we will see that it also helps with PFK's regulation. Let's see this through an example.

Example 1.3.1. Supposedly at rest, the ratio of ATP, ADP and AMP respectively is as follows, 100 : 20 : 2. When you perform an exercise, around 10% of the ATP will be hydrolyzed into ADP. This means you'll get an exercise ratio of 90 : 30 : 2. Now, ADK can come in and convert the 10 newly yielded ADP back to 5 ATP and 5 AMP which is. So now, the net ratio of ATP, ADP and AMP will be 95 : 20 : 7.

Compared to the original ratio, we can see a decrease of ATP by 5% but a

350% increase of AMP. When coupled this to PFK, we can see that ADK will help increasing AMP by large amount will massively activate the process of glycolysis.

Notion 1.2 ADK not only keeps ATP abundant during exercise but also generate AMP to activate PFK leading to glycolysis.

Substrate Regulation of PFK

We've mentioned above that aside from AMP, there's another substrate called **fructose-2,6-bisphosphate (F2,6P)** can also activate PFK. So, **where's this substrate comes from?** Well...it comes from PFK2

Definition 1.12. Phosphofructokinase 2 (PFK2) is an enzyme that synthesize F2,6P from fructose-6-phosphate in an equilibrium reaction. **fructose-2,6-bisphosphate (F2,6P)** serves as a potent activator of PFK but also an inhibitor of any **FBPase** (enzyme that breakdown fructose-1,6-bisphosphate).

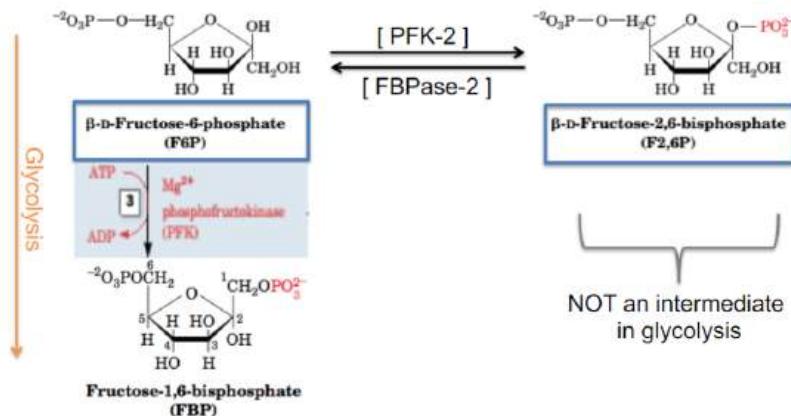


Figure 1.21: Synthesis of F2,6P from PFK2.

End of Lecture —

We'll go back to PFK2 and its comparison to PFK1 later. However, we want to show you that little enzyme mentioned in definition 1.12, namely, FBPase.

Definition 1.13. FBPase or fructose-1,6-biphosphatase is an enzyme that decompose FBP back to fructose-6-phosphate.

Basically, FBPase is performing the opposite task of PFK1.

Notion 1.3 At rest the rate of PFK1 catalysis of F6P is equal to that of FBPase's decomposition of FBP.

Explanations. We need the rate of degradation and synthesis of FBP to be equal since we don't really need to produce that much ATP at rest i.e. Imagine we bring FBP back to F6P, we'll be constantly producing ATP which can be bad. So by having an enzyme to reverse FBP, we can later revert it back to G6P for other purposes. □

Remark 1.11. This is not a perfect system as in order to have this duality, we're losing energy for no reason (PFK1 consume ATP).

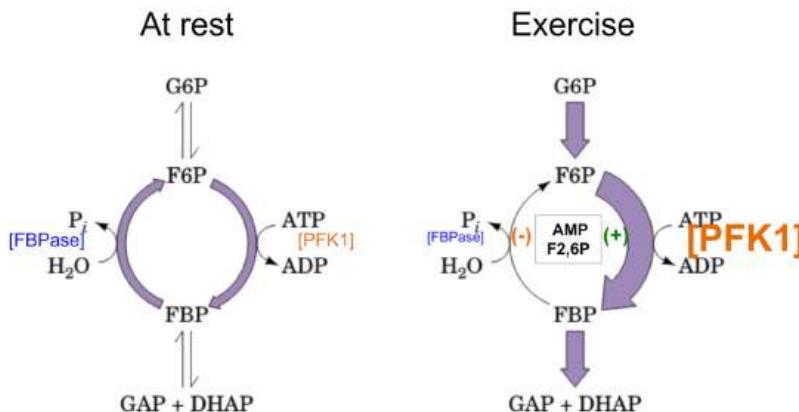


Figure 1.22: FBPase and PFK substrate cycle.

Nevertheless, during exercise, the pathway of FBPase is completely shut down or at least at a very minimal level, in order to favour that of PFK1 to produce FBP for glycolysis. The control of FBPase is the AMP and F2,6P which inhibits its activity.

Now, we'll return to PFK2. As we already said above, PFK2 will synthesize F2,6P from F6P in an equilibrium reaction. This product is not an intermediate of glycolysis but a regulator (for FBPase). With that being said, we're now going to discuss the differences between PFK1 and 2 (see Figure 1.23)

- A) How does the enzyme work?
 B) Catalyzed reaction?
 C) Role in glycolysis?
 D) Activity?
 E) Modulators?

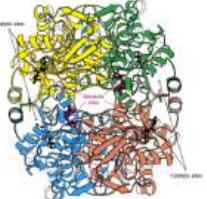
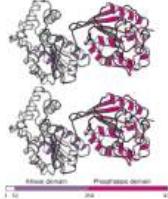
PFK-1	PFK-2
	
A) It works as a tetramer	It works as a dimer
B) $F6P + ATP \longrightarrow FBP + ADP$ (in the presence of Mg^{2+})	$F6P + ATP \longrightarrow F2,6P + ADP$
C) Creates the first committed step for glycolysis	Produce regulator/modulator for PFK1 and FBPase
D) Kinase	Kinase and phosphatase
E) Inhibitor(s): ATP, Citrate Activator(s): AMP, F2,6P	Inhibitor(s): Depending on the activities of activators Activator(s): Epinephrine, Glucagon

Figure 1.23: PFK1 and 2 comparison.

Covalent Modification Regulation of PFK2

Definition 1.14. An **isozyme** refers to the 1 of many different forms of an enzyme that will catalyze the same biochemical reaction but has different properties (i.e. regulated, activated differently, etc.)

PFK2 is an isozyme since the PFK2 present in the heart muscle tissue will differ from that in the liver:

Observation 1.3 (Liver Isozyme). Suppose that you are on fasting or performing an exercise for a prolonged period of time. What you'd find is the $[glucose] \downarrow$ this eventually will lead to $[glucagon] \uparrow \implies [cAMP] \uparrow$ which activate a mediator called **PKA**. This PKA can then phosphorylate PFK2, which in the case of liver, will deactivate it. When PFK2 is inactive, the activity of **FBPase-2** will be dominant which lead to $[F2,6P] \downarrow$. There's less F2,6P to inhibit FBPase thus it will favor the breaking down of FBP leading to **gluconeogenesis**.

Observation 1.4 (Heart Muscle Isozyme): Suppose that you're experiencing some stress that lead to heart beating harder. In this case, you'd find $[epinephrin] \uparrow \implies [cAMP] \uparrow$ which activate a mediator called **PKA**. This PKA

can then phosphorylate PFK2, which in the case of heart, will activate it. When PFK2 is active, the activity of FBPase–2 will be downsized which **leads to [F2,6P]↑.** There's now more F2,6P to inhibit FBPase thus it will favor the synthesis of FBP leading to **glycolysis.**

In both cases, the take home message is that [F2,6P] is going to modulate the direction of going toward glycolysis or toward gluconeogenesis.

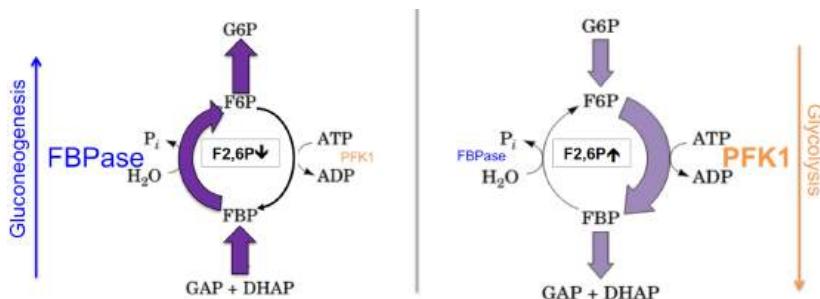


Figure 1.24: Gluconeogenesis vs glycolysis depending on [F2,6P].

1.3.4 Reversible Steps

Now we will look at some reversible steps (equilibrium reactions, $\Delta G \approx 0$) of glycolysis.

Definition 1.15. **Aldolase** is an enzyme that breaks down FBP into **dihydroxyacetone phosphate (DHAP)** and **glyceraldehyde-3-phosphate (GAP)** that is the following reaction.



Definition 1.16. **Triose phosphate isomerase (TIM)** is an enzyme that converts GAP to DHAP (v.v.) in an equilibrium reaction.

Observation 1.5 Normally $[\text{GAP}] < [\text{DHAP}]$ because glycolysis consumes GAP much more than DHAP; so by having TIM, when GAP is less, it will tip the balance toward GAP thus converting DHAP back to GAP.

Remark 1.12. *For every 1 glucose molecule, aldolase makes 2 GAP.*

This basically marks the first stage of glycolysis where we've spent 2 molecules of ATP. We'll be looking at the so call "payoff" stage where we're regenerating the lost ATP. In this case 1 GAP molecule will move through the second stage to make 2 ATP, but remember, 1 glucose will give 2 GAP hence it'll regenerate 4 ATP.

Definition 1.17. **GAP dehydrogenase (GAPDH)** is an enzyme that converts GAP into **1,3-bisphosphoglycerate (1,3-BPG)**, which is a high energy and unstable intermediate, and at the same time used the released H⁺ to generate NADH.

Remark 1.13. This reaction has a ΔG > 0

Now because 1,3-BPG is a high energy intermediate, it's best to couple it to another reactions for efficiency.

Definition 1.18. **Phosphoglycerate kinase (PGK)** is an enzyme that converts the high energy 1,3-BPG to **3-phosphoglycerate (3PG)** and also regenerate ATP from ADP.

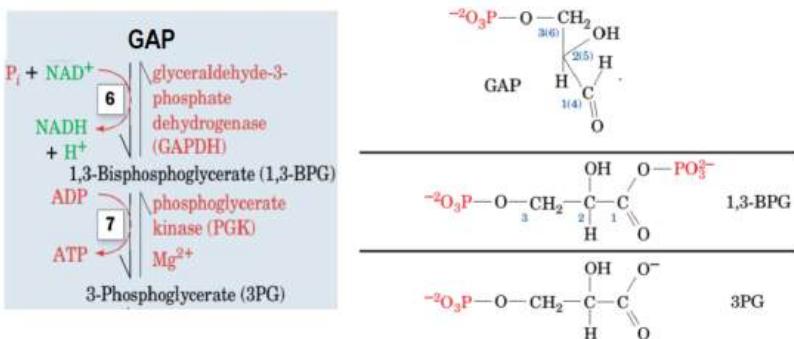


Figure 1.25: Equilibrium reactions converting GAP to 3PG.

Concept 1.8 (Substrate Channeling). A substrate/intermediate can be made and immediately be used by another enzyme to produce another compound.

Explanations. Substrate channeling can happen when 2 enzymes sit in close proximity with each other so that its reactions are coupled. Thus the product of 1 reaction can immediately be used as the substrate for the next.

In the case of GAPDH and PGK, it's good to have this channeling to prevent the accumulation of high energy 1,3-BPG. □

From the remark 1.12, we can deduce that from 1 molecule of glucose, 2 ATP and 2 NADH are generated through these 2 steps.

Definition 1.19. **Phosphoglycerate mutase (PGM)** is an enzyme that change the position of PO_3^- from 3PG to **2PG**.

Definition 1.20. **phosphoglycerate enolase (PGE)** is an enzyme that remove 1 H_2O from 2PG which yield **phosphoenolpyruvate (PEP)**, which is a high energy intermediate.

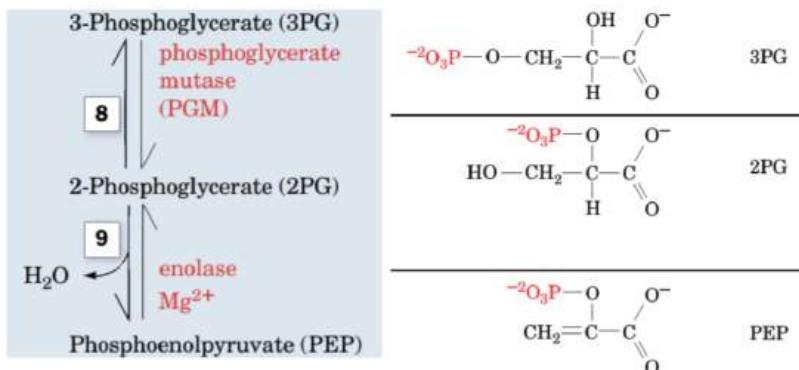


Figure 1.26: Equilibrium reactions converting 3PG to PEP.

1.3.5 Pyruvate Kinase

This is the last enzyme which means glycolysis is finished.

Definition 1.21. **Pyruvate kinase (PK)** is the last enzyme of glycolysis that turn PEP irreversibly to **pyruvate** while generating 2 ATP.

Observation 1.6 Because PK catalyzes an irreversible reaction, it will need some sort of regulation. Amazingly but not too surprisingly, its allosteric activator is FBP. ATP will be its inhibitor as well as Glucagon (hormonal inhibitor) i.e. when cells are having lots of energy or at rest post-meal, no need to produce more energy.

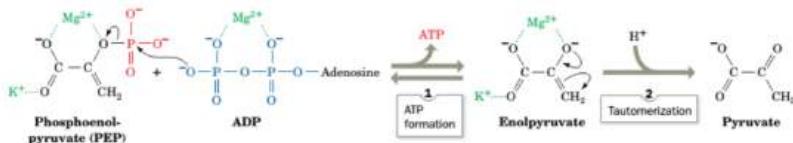


Figure 1.27: PK turning PEP to pyruvate and releasing ATP.

1.3.6 Summary

When looking at the final picture of glycolysis, we're mainly looking at the regulatory steps (irreversible steps) which includes hexokinase, phosphofructokinase 1 and pyruvate kinase:



In the end, we can also look at our net ATP balance in both glycolysis stage which yield $(2 + 2) - 2 = 2\text{ATP}$. However, this is only for "anaerobic glycolysis"; for "aerobic glycolysis", we have to consider the steps to oxidative phosphorylation which in total will have a net ATP yield of 32 i.e. **aerobic oxidative phosphorylation is 16× more efficient than anaerobic glycolysis.** Then why even bother doing anaerobic glycolysis? Well...because it's much faster than oxidative phosphorylation.

End of Lecture —

1.4 Other Carbohydrates

Obviously, it's not only glucose that can be metabolized by our body, we have the ability to metabolize others as well. In this lecture, we'll be looking at the metabolism of 3 different carbohydrate: **fructose, galactose and mannose.**

1.4.1 Fructose

When you eat a meal there will be some **fructose**, 5-membered ring carbohydrate, in your food. So fructose will go into your blood which goes into the liver.

Definition 1.22. **Fructokinase** is a liver enzyme that phosphorylate fructose into **fructose-1-phosphate (F1P)** which can isomerise into F1P open chain

Definition 1.23. F1P aldolase is an enzyme that converts the open chain F1P into **glyceraldehyde**.

From here, there are 2 potential pathways that glyceraldehyde can take

1. **Option 1:** Glyceraldehyde is converted directly into GAP.
2. **Option 2:** Glyceraldehyde is converted to 3 different intermediates (glycerol, glycerol-3-phosphate and dihydroxyacetone phosphate) before becoming GAP. See Figure 1.28.

The 2 options have no energetic advantages. The only difference is the pathway 2 will be favoured if fatty acid is needed since **glycerol-3-phosphate** is an essential substrate for its synthesis.

Remark 1.14. Unlike HK, fructokinase is not allosterically inhibited thus all of the metabolized fructose will end up in fat cell.

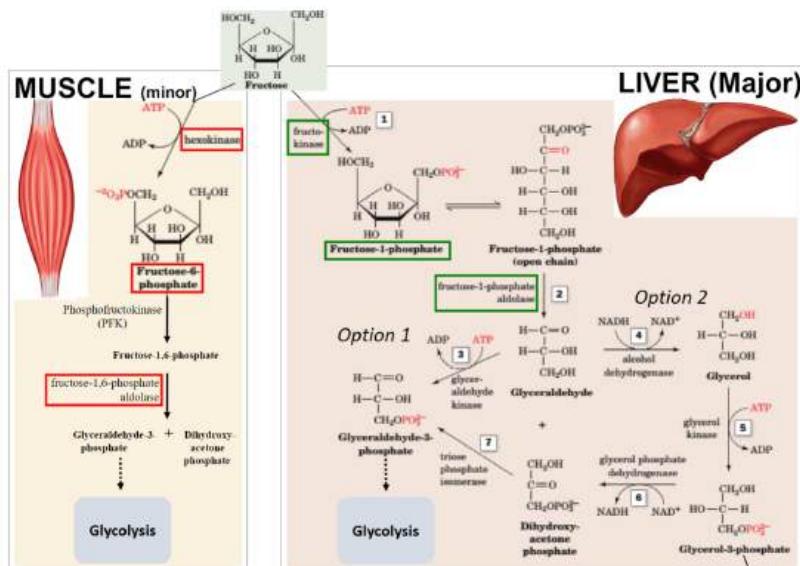


Figure 1.28: Catabolism of fructose in muscle vs liver

Fructose are mostly metabolised in the liver (95% of the time) which means its catabolism is rarely done but still is possible in other tissue.

Example 1.4.1. In the muscle, fructose can be phosphorylated by HK which can then be phosphorylated again by PFK1 to generate FBP. This substrate can be metabolized by FBP aldolase to become GAP, a substrate for glycolysis. See Figure 1.28.

Even though in both case, GAP will be generated but the point of entry into glycolysis is different.

- **Fructose Metabolism in Muscle:** Fructose will be metabolized first into F6P which is before a major regulatory point: PFK.
- **Fructose Metabolism in Liver:** Fructose will be metabolized to non-glycolysis intermediates then directly to GAP which is after the PFK regulatory point.

Observation 1.7 With the metabolism of fructose in the liver, it's the main contributor to **dyslipidemia** (irregular blood lipid level).

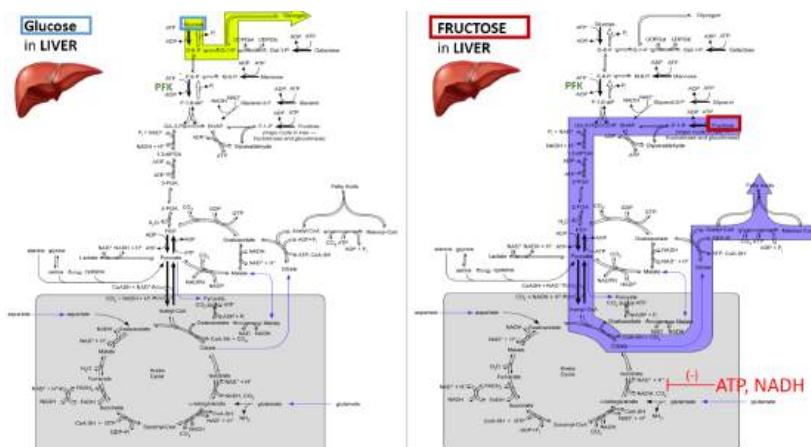


Figure 1.29: Glucose vs fructose metabolism in fed state

Glucose vs Fructose in A Fed State

In a fed state at the liver, it will store energy and deactivate PFK1.

- **Glucose:** Since PFK is deactivated and HK is saturated, GK will start the process and makes G6P to be stored as glycogen.

- **Fructose:** It will bypass PFK, enter glycolysis as GAP and enter TCA cycle. Here, it will produce **citrate** which can activate synthesis of fatty acids. So in the end, fructose will ended up as fatty acids due to unregulation. See Figure 1.29.

On the other hand, fructose will be regulated by PFK in all other cells except the liver.

1.4.2 Galactose

Definition 1.24. **Galactose** is 6-membered ring carbohydrate that is similar to glucose. To be more specific, it's the stereoisomer of glucose with different conformation at carbon 4.

Remark 1.15. *Galactose is the monomeric form of lactose (2 galactose together)*

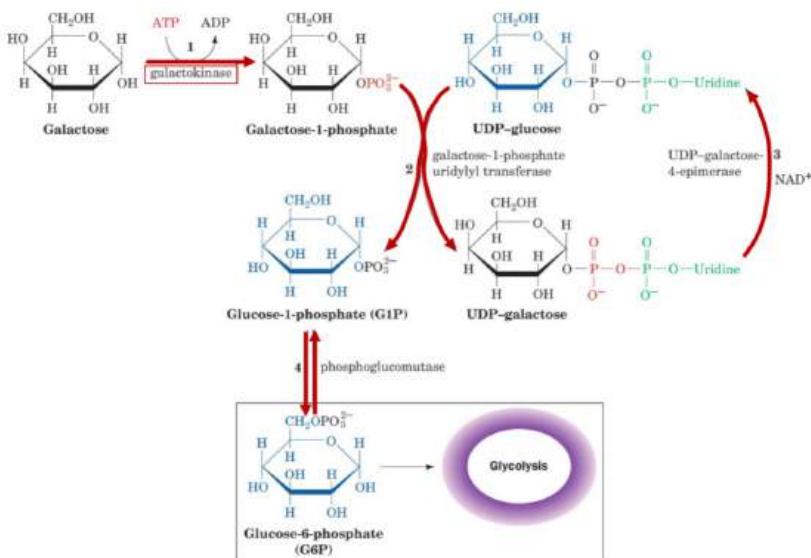


Figure 1.30: Pathway of galactose metabolism

Mechanism of Actions (Leloir Pathway): Galactose will enter the liver.

1. Galactose will first phosphorylated by **galactosekinase** using ATP.
2. It will then be attached to a UDP by **galactose-1-phosphate uridylyl transferase** making **UDP-galactose**.
3. It will then be used as substrate for **UDP-galactose-4-epimerase** to make **UDP-glucose**.
4. UDP-glucose can be transformed into **glucose-1-phosphate (G1P)** via galactose-1-phosphate uridylyl transferase. Lastly, G1P is converted back to G6P using **phosphoglucomutase**, to be used in glycolysis. See Figure 1.31

Observation 1.8 Galactosemia is a rare genetic disorder that disable a person from metabolizing galactose. There are 3 types of galactosemia: **type I**, dysfunctional uridyltransferase; **type II**, dysfunctional galactosekinase; and **type III**, dysfunctional epimerase.

So milk are essentially toxic for those with galactosemia because they cannot process it. A treatment for this is to avoid milk and galactose products.

Remark 1.16. *Some of the symptoms of said disease include cataracts, mental retardation, liver disease, etc.*

Observation 1.9 Interestingly, a galactosemic mother can produce milk. In the mammary gland, $PP_i \rightarrow 2P_i$ using inorganic phosphatase which release a huge amount of energy to produce UDP-glucose from G1P and UTP. UDP-glucose can then be converted back to UDP-galactose then galactose then lactose (milk).

1.4.3 Mannose

Definition 1.25. **Mannose** is another stereoisomer of glucose that are tend to be found in glycoproteins thus it presents in most meat.

Mechanism of Action (Mannose Metabolism):

1. Mannose will be phosphorylated by HK to make **mannose-6-phosphate**.

2. This will be used as substrate by **phosphomannose isomerase** to make F6P which is a substrate in glycolysis used by PFK.

1.4.4 Overall Metabolism of Carbohydrates

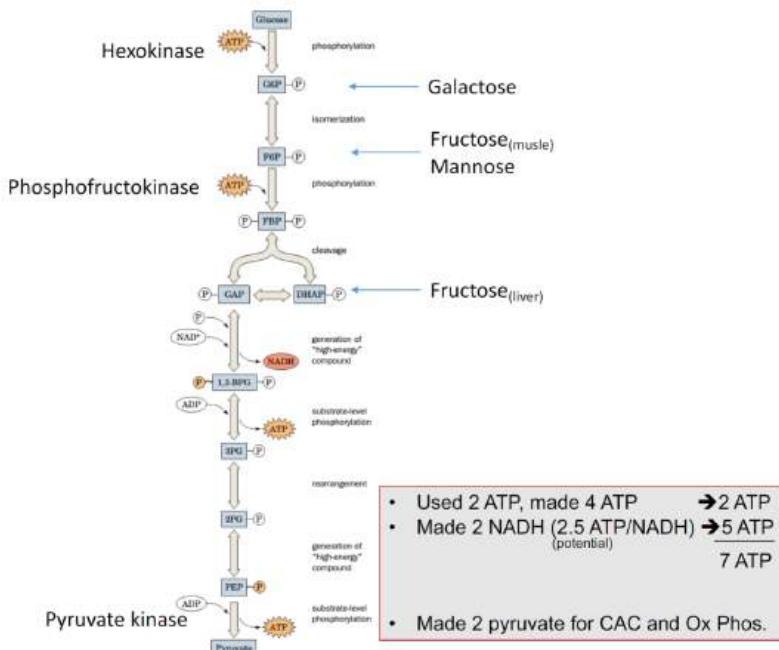


Figure 1.31: Glucose will follow the typical pathway of glycolysis. Meanwhile, fructose will enter as F6P in the muscle and as GAP in the liver. Galactose will enter as G6P and Mannose will enter as F6P. Either case, the net ATP production of 1 glucose is 2 ATP.

1.5 Pentose Phosphate Pathway

Remember how we said that hexokinase is not a rate-limiting step of glycolysis and the reason that G6P can be used in other pathway. In this section, we'll discover 1 of those pathways that is the **pentose phosphate pathway (PPP)**.

In the pentose phosphate pathway, ribose-5-phosphate is produced which can be used as substrate to make nucleic acids. It's basically a branch off from glycolysis.

In general, G6P will be converted into **ribulose-5-phosphate (Ru5P)** via a series of enzymes with the most important which is also the committing step: **G6P dehydrogenase (G6PDH)**. At the same time, we're also producing NADPH (we'll discuss its important later).

Ru5P can be further changes into **xylulose-5-phosphate (Xu5P)** and **ribose-5-phosphate (R5P)** of which Xu5P can be recycled back into F6P and GAP. R5P can be later be used to build nucleotides.

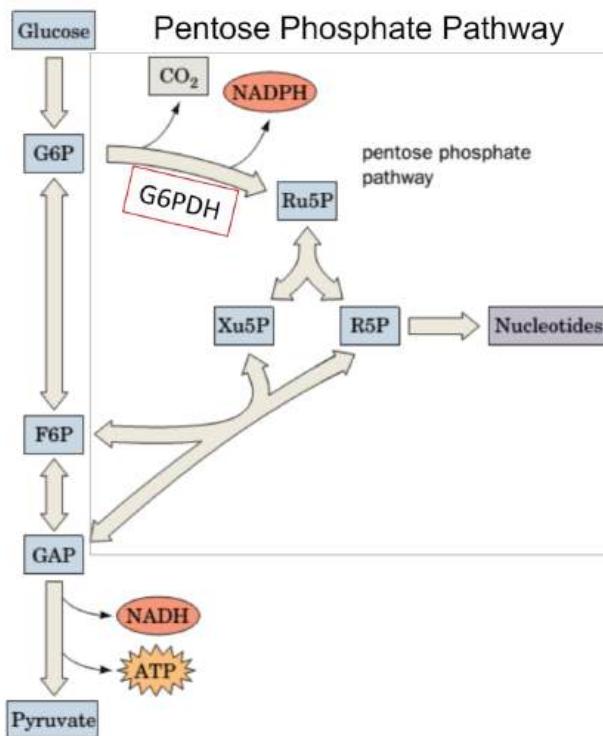


Figure 1.32: Pentose Phosphate Pathway.

Observation 1.10 PPP is mainly present in the liver, adipose tissue and red blood cell and are generally absent in muscle.

1.5.1 NADH vs NADPH

We need to note that NADH and NADPH is not the same molecule however, we do have the same root of **nicotinamide adenine dinucleotide (NAD^+)**. With NADH, we need to oxidize NAD^+ while with NADPH, we need to phosphorylate NAD^+ then oxidize it. **Majority of enzymes are either specific for NADH or NADPH.** The table below provide the differences between NADH and NADPH:

	NAD ⁺ /NADH	NADP ⁺ /NADPH
Generated from	Glycolysis, CAC	Pentose phosphate pathway
Metabolic uses	ATP production (Ox. Phos.) Oxidative reactions	Fatty acid & cholesterol biosynthesis Reduction reactions
[Cytosolic]	$[\text{NAD}^+]/[\text{NADH}] = 1000$	$[\text{NADP}^+]/[\text{NADPH}] = 0.01$

Explanations. We need to have high $[\text{NAD}^+]$ because glycolysis requires NAD^+ . Contrarily, the synthesis of cholesterol and fatty acids require a large amount of NADPH. See Figure 1.33. \square

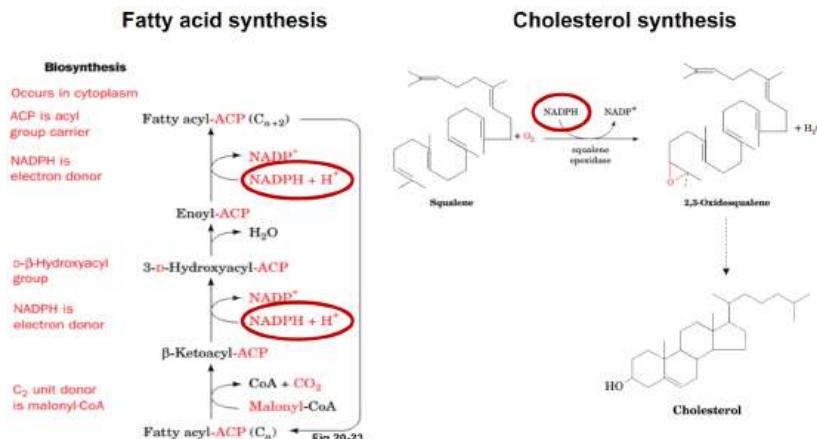
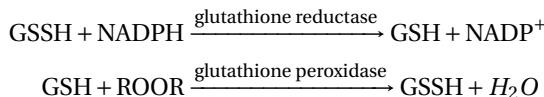


Figure 1.33: Biosynthesis of lipids using NADPH.

Do cancer cells needs the PPP? Well...yes and this this due to 2 things. 1, in order to keep growing and spread, it needs to replicate a lot which means it requires a lot of nucleotides to replicate its DNA and then divide. 2, in the same replication, it needs to produce lots of lipids to synthesize the phospholipid bilayer around it.

Observation 1.11 NADPH is also used as a substrate along with **glutathione disulfide (GSSH)** for **glutathione reductase** to synthesize glutathione (GSH). GSH along with naturally produced peroxides will be turned into GSSH and water again by **glutathione peroxidase**. The main purpose for these reaction is to minimize the production of peroxide that can lead to **hemolysis of red blood cells**.



1.5.2 Oxidative Steps

We won't be going through the non-oxidative steps as the oxidative ones are much more significant since they're the irreversible steps.

Mechanism of Action (Oxidative Reactions of PPP):

1. G6P is turned into **6-phosphoglucono- δ -lactone** by **G6P dehydrogenase** while also producing NADPH.
2. The product will be turned into **6-phosphogluconate** via **6-phosphogluconolactonase**.
3. Finally, it will be turned into Ru5P using **6-phosphogluconate dehydrogenase**.

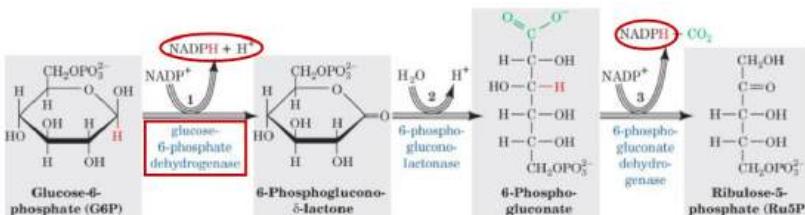


Figure 1.34: oxidative reactions of PPP

Note that for this course, you do not have to memorize these steps. The main takeaways are:

1. Producing 2 NADPH from 1 G6P.

2. G6PDH reaction is irreversible, specific for NADP⁺ and strongly inhibited by NADPH.

1.5.3 Non-oxidative Steps

As we've said before, we will not be focusing on these steps has it's just mainly a recycling steps. The main importances to know are:

- I. Ru5P will split into R5P and Xu5P.
- II. R5P is of major important as it's used in nucleotides biosynthesis.
- III. Xu5P will undergo series of intermediate reaction to be recycled back as either F6P or GAP, which will all be used in glycolysis.

1.5.4 Regulation

For the regulation of PPP, we will consider 3 situations where cells require:

- **Both NADPH and Nucleotides:** NADPH and R5P will be produced normally. Any excess byproducts will be recycled back to F6P and GAP.
- **Only NADPH:** NADPH and R5P will be produced. Everything will be recycled back to glycolysis beside NADPH.
- **Only Nucleotides:** No reactions from PPP directly. F6P and GAP will be used in a reversed synthesis of R5P.

Case Studies

In this little subsection, we will look at some medical cases that involve these pathways/enzymes.

Observation 1.12 A patient suffers from **fructosemia** will not be able to metabolize fructose which lead to a build up of it in blood i.e. fructose intolerance. If the patient has a loss-of-function mutation for some enzymes, it's most likely to be **fructokinase** and **F1P aldolase**.

Observation 1.13 We've seen before that consumption of fructose while in a fed state will lead to a build up of fatty acid because accumulation of

its activator: citrate and its inhibitors: ATP. However, supposed you're running a marathon while consuming fructose. In this case, we're depleting ATP which forces the fructose to complete the TCA cycle to produce more NADH for oxidative phosphorylation.

Observation 1.14 A 23 y.o. male came into clinic with diffused abdominal pain and vomiting for 2 days. He has yellowish discoloration of sclera and skin and experience acute breathlessness for 4h. Fever was recorded at 38°C, pulse of 124 BPM, and urine as red/brownish in colour.

He then was admitted to a blood test with a result of Hb level of 5.4mg/dL and bilirubin of 17.08mg/dL. No malarial parasite and dengue was found. It was also determined that he has a decreased in RBC count, and has sepsis with hemolytic jaundice.

Treatment: **plasmapheresis** is used to remove any unknown causative agent of hemolysis. Then he was given 4 units of leukocyte-reduced packed RBCs.

Explanations. **The patient has G6PDH deficiency with hemolysis.** This is because low G6PDH will lead to low NADPH thus giving less substrate for glutathione reductase to produce glutathione to uptake peroxide (along with glutathione peroxidase) that can cause hemolysis. □

1.6 Gluconeogenesis

Definition 1.26. **Gluconeogenesis** is the biosynthesis of new glucose molecules from other sources.

Explanations. Gluconeogenesis mainly occurs in the liver (and rarely in the kidneys). It occurs because when glucose and glycogen storage is depleted and the liver wants to maintain a [glucose] of 5.5mM. The main substrates it uses are lactate, pyruvate, intermediates of TCA cycle(starting point at **oxaloacetate**) and mainly amino acids as backbone. □

Remark 1.17. *Gluconeogenesis do not use substrate from fatty acid however it will use the ATP given from fatty acid catabolism.*

For gluconeogenesis, we're basically going the opposite direction of glycolysis and we'll see that this will pose a problem later on. We'll first look at the generation of **phosphoenolpyruvate (PEP)**

Remark 1.18. Some of the reaction will use GTP which is basically a conversion from ATP \Rightarrow GTP↓ then that also means ATP↓.

Mechanism of Action (PEP Synthesis):

1. Pyruvate is carboxylated by **pyruvate carboxylase** to make oxaloacetate, a high energy intermediate.
2. high energy Oxaloacetate will provide energy to **PEP carboxykinase** to synthesize a reaction to make PEP.

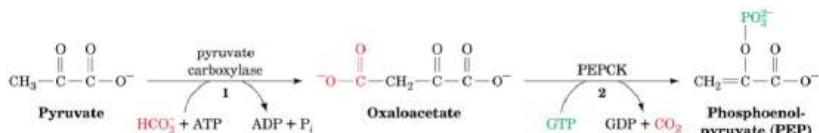


Figure 1.35: Synthesis of PEP from pyruvate

Now with PEP, we will work backward from all of the reversible step of glycolysis until we reach FBP where we encounter a problem. The problem here is that PFK1 catalyzes an irreversible reaction which means it cannot FBP cannot go back to F6P.

Nevertheless, we had another enzyme that will be highly activated when we're fasting/(F2,6P level is down) and that is: FBPase (recheck lecture 3). FBPase will synthesize F6P from FBP.

Remark 1.19. Please remember that FBPase only presents IN THE LIVER!

Finally, F6P can isomerize to G6P. Here, we cannot use hexokinase since it's an irreversible step so we'll use another enzyme called **glucose-6-phosphatase (G6Pase)**. In the end, G6Pase turns G6P back to glucose and so we're done with gluconeogenesis. See figure 1.36 for full summary.

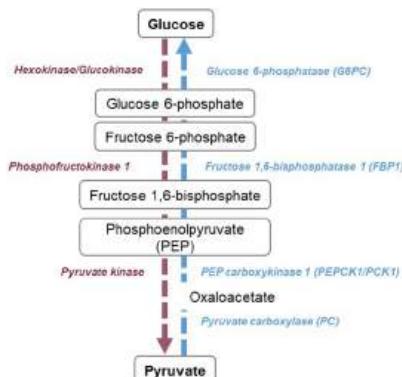


Figure 1.36: Total Summary of Gluconeogenesis and Glycolysis.

1.7 Cori Cycle

Definition 1.27. **Cori cycle** is a pathway used in our body when anaerobic glycolysis is occurring and overproducing lactate.

Explanations. During anaerobic glycolysis, pyruvate will immediately be used in homolactic fermentation to produce lots of lactate while consume NADH (to give off NAD⁺). Note that for NAD⁺ level, it's not a problem since it will be used back in the above step of glycolysis but not lactate. This is where Cori cycle begin. □

Definition 1.28. **Lactate dehydrogenase** is an enzyme found in both the muscle and liver that can convert pyruvate and NADH to lactate and NAD⁺, respectively, and vice versa.

During exercise, pyruvate and NADH are being converted into lactate and NAD⁺ when the cells are absence of O₂.

Remark 1.20. *Cancer cells performs lots of anaerobic glycolysis which means there will be a build up of lactate in a cancer patient.*

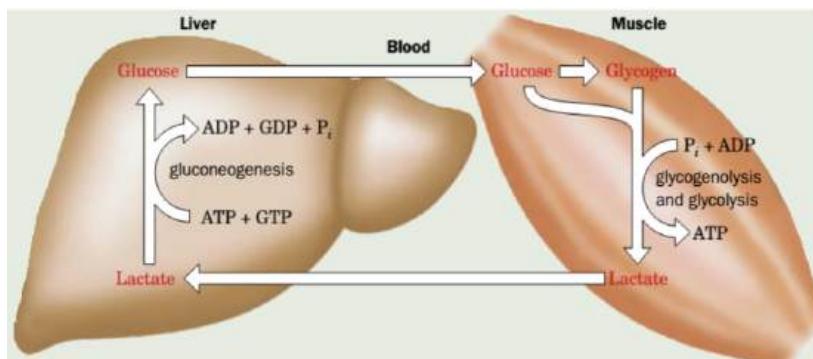


Figure 1.37: Cori cycle illustration.

Mechanism of Action (Cori Cycle): During exercise, lactate produced from the muscle will be excreted and move into the liver.

1. In the liver, the opposite reaction will happen where tons of lactate are converted back into pyruvate.
2. Pyruvate will be used as a source of carbon for gluconeogenesis.

3. The newly synthesized glucose will be delivered to the muscle through the bloodstream.
4. This glucose (and stored glycogen) will be used in anaerobic glycolysis to produce lactate. Then the cycle repeats (see figure 1.37).

1.8 Integration

In this section, we'll just be integrating whatever we're learning to the physiological state our body is at:

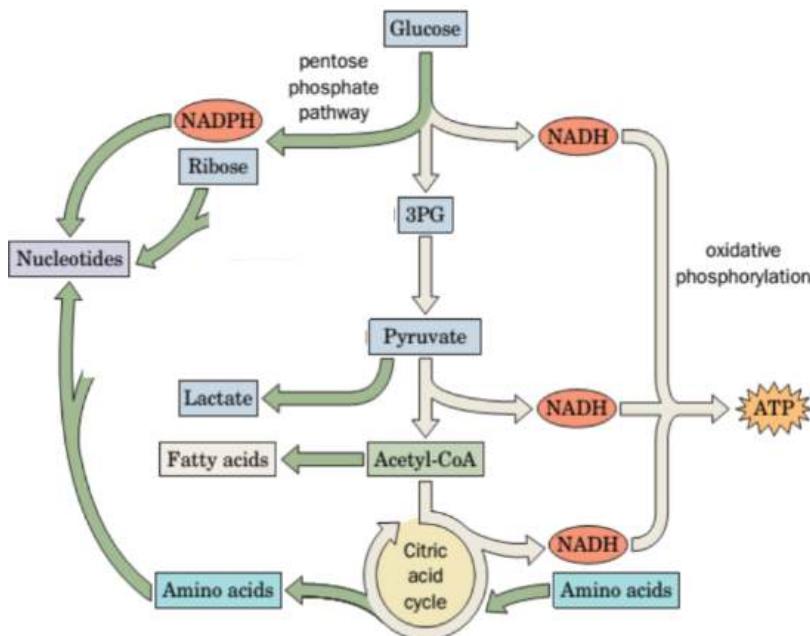


Figure 1.38: Glucose metabolism

1. In the Presence of oxygen: The typical pathway of glycolysis is turned on to produce a little ATP, NADH and acetyl-CoA that will be used in TCA cycle to produce more NADH. All of these will then culminate to oxidative phosphorylation.

2. In the Abscence of oxygen: Pyruvate produced from the body will go through homolactic fermentation which produce NAD⁺ that can be used again for glycolysis but also produces byproduct of lactate (can be recycled via Cori cycle).
3. In Cell Division: Glucose will be consumed in PPP to produced nucleotides while all other byproducts will be recycled back.
4. In Low Blood Sugar: The liver needs to supply its own synthesize glucose the blood stream. Here, it uses pyruvate as the main substrate for gluconeogenesis.

End of Lecture —

2.1 Glycogen I

Definition 2.1. **glycogen** is a linear glucose polymer that is highly branching.

Essentially, glycogen is a storage form of glucose. Looking at the following EM image of a liver cell. What you'd notice are lots of these purple dots and those are **glycogen granules** each of which contains of upward of 50000 units of glucose.

why do cells store glucose like this? Well...there're many reasons but the most important is *compactness* and *structural* i.e. when packing glucose close together in granules like this, it's much easier to regulate its synthesis and degradation. Another thing about glycogen is that it does not change the osmotic pressure because 1 molecule of glycogen is equivalent to 1 free molecule of glucose monomers (glycogen would be better since it packs more without changing the pressure).

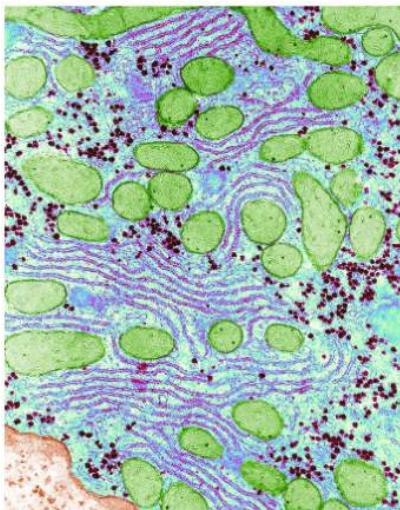


Figure 2.1: glycogen granules.

Observation 2.1 Glycogen are mainly stored in either the muscle or liver but the way they uses them can differ.

- **Muscle:** The muscle will use up its own glycogen storage to provide ATP to only itself. It cannot give breakdown glycogen and give to

other tissue because it does not have G6Pase. Store only 2% of glycogen by weight

- **Liver:** The liver can store glycogen and degrade it to G6P. It has G6Pase which will dephosphorylate G6P making glucose again. This glucose, synthesized in the ER lumen, will be transported out to the bloodstream. Store 10% of glycogen by weight

Explanations. The reason we need to remove the phosphate from G6P is because there are no transporter for phosphorylated products of any kind. Another thing for not having a transporter for G6P because if we do, almost all of the glucose will leave the cell. □

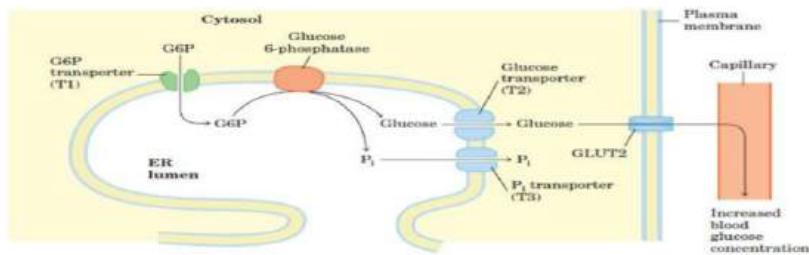


Figure 2.2: Hydrolysis of G6P by G6Pase in the ER lumen

Observation 2.2 Looking at the location of the G6Pase, we can see that it's a transmembrane protein with 1 of its catalytic side facing the ER lumen why the other non-catalytic faces the intracellular space (see Figure 2.2). The reason we have this is because of compartmentalization. Cells needs to compartmentalize these reactions in order to control productions of certain substrates.

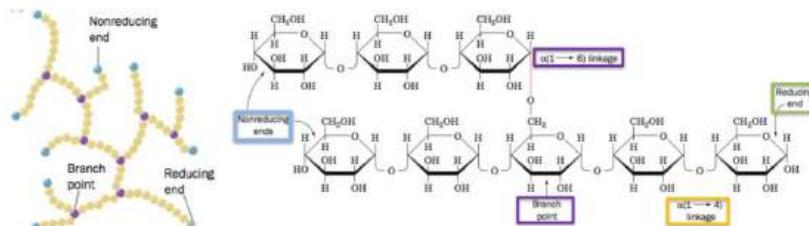


Figure 2.3: A closer look at glycogen structure

Observation 2.3 Looking at glycogen more closely, we'll figure out some interesting structure. First, these glucose molecules are attached on carbon 1 and 4 between each other which is called the **$\alpha 1 - 4$ linkage**. Every 6-8 of glucose monomers, it will start branch out at the **branch point** where the linkage is now called **$\alpha 1 - 6$ linkage** (linking between carbon 1 and 6).

All glycogen molecule has only 1 **reducing end** which is the origin of polymerization. On the other hand, it has lots of **nonreducing ends** where glucose can be removed or added back in.

Remark 2.1. At the branch point, linkage is $\alpha 1 - 6$ however, as the branch polymerize, it will be the $\alpha 1 - 4$ linkage.

Now, we will briefly go through the process of glycogen degradation and synthesis. In later subsections, we will dive more into the detail of each steps

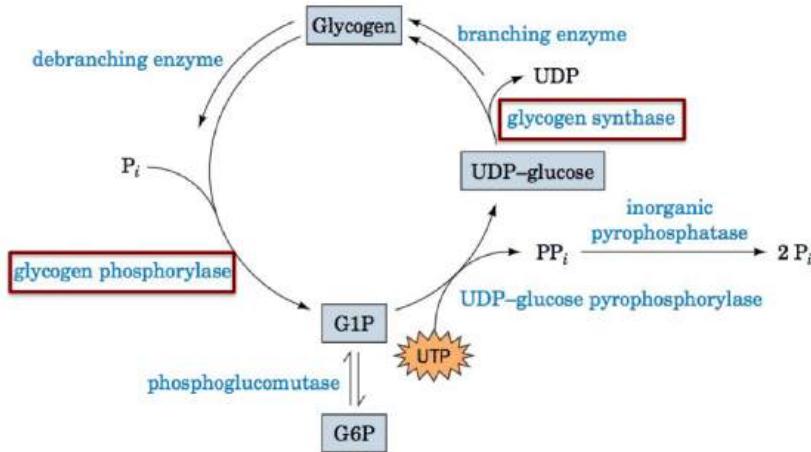


Figure 2.4: Glycogen synthesis and degradation

Mechanism of Action (Glycogen Synthesis and Degradation):

Starting from glycogen degradation

1. Glycogen will be broken down by **debranching enzyme**
2. It will then be phosphorylated by **glycogen phosphorylase** to produce **glucose-1-phosphate (G1P)**.

3. G1P can then isomerize to G6P to enter glycolysis and vice versa using **phosphoglucomutase**. This will be the end of degradation and beginning of glycogen synthesis.
4. **UDP-glucose phosphorylase** will use UTP to phosphorylate G1P again to make **UDP-glucose** while releasing by products of **pyrophosphate** (PP_i). PP_i can be converted back to 2 phosphate ion using **inorganic pyrophosphatase**.
5. UDP-glucose can then be dephosphorylated by **glycogen synthase** and then attached together into glycogen molecules using **branching enzymes**.

Explanations. The reason we have to attach another phosphate group onto the G1P because phosphorylated glucose are very stable so adding a second one will destabilize it and make it what to get rid of both the phosphate. □

2.1.1 Glycogen Synthesis

Within the synthesis of glycogen, there will be 3 major steps: 1, synthesizing the UDP-glucose; 2, the UDP-glucose will then be attached to the exists branch; 3, or we will create a new branching point.

Synthesis of UDP-glucose

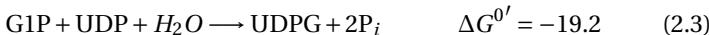
Already, starting from glucose we will phosphorylate it first using hexokinase to form G6P that can be isomerized into G1P via phosphoglucomutase. Now, looking at G1P that will be phosphorylated again using UDP-glucose phosphorylase,



What you'd notice is the change in free energy is near 0 which means this reaction can goes both ways. However, because you have a fast-acting inorganic pyrophosphatase that catalyzes the following reaction



which means the amount of product is less than the amount of reactant in reaction (2.1) which would be it more favourable to the right. You can see this by adding (2.1) and (2.2) together to get



So, by coupling these 2 reactions, you get an overall irreversible reaction.

Elongation via Glucose Synthase

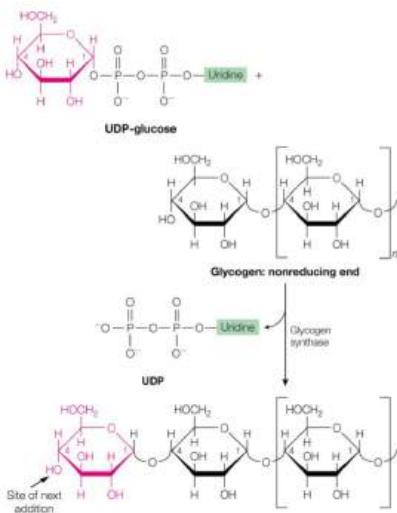
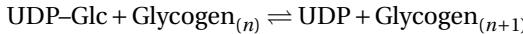


Figure 2.5: Elongation of glycogen reaction.

The elongation reaction using glucose synthase is irreversible as its $\Delta G^{\circ'} = -13.4 \text{ kJ/mol} < 0$.



Not only could we synthesize from an existing glycogen, we can also start a new glycogen molecule. In order to do this, we need another molecule since glycogen synthase cannot link 2 glucose residue together, it can only extend an exists $\alpha 1 - 4$ bond.

Definition 2.2. **glycogenin** is an enzyme that is crucial for the synthesis of new glycogen molecule. Each glycogen molecule will have only 1 glycogenin that sits near the reducing end.

Elongation of glycogen catalyzed via **glycogen synthase**. UDP-glucose will be dephosphorylated by glycogen synthase and attach the glucose to the exists branch of glycogen molecule. This lead to the elongation of the glycogen by 1 glucose unit.

Observation 2.4 When this reaction happens, UDP will be released which can be converted back to UTP via **nucleoside diphosphate kinase** using the following reaction:



Mechanism of Action (Glycogenin Synthesis): We now have the UDP-glucose formation.

1. UDP-glucose will be dephosphorylated by the glycogenin and attach the glucose residue to the glycogenin's tyrosine residue.
2. Glycogenin can then extend the this glycogen chain up to 8-12 residues. This will form a *primer*.
3. When the primer is formed, glycogen synthase's action will takeover.

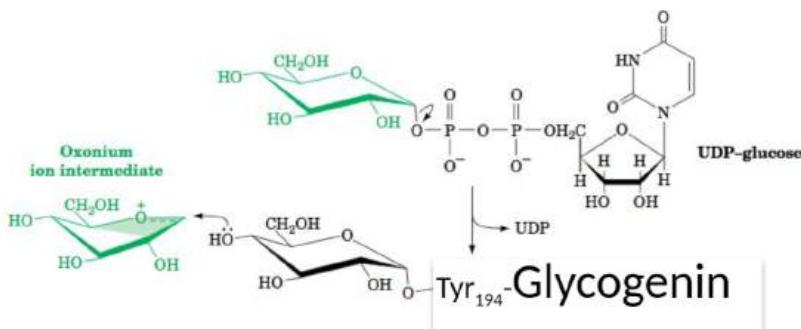


Figure 2.6: GLycogenin mechanism of action

Synthesize New Branching Point

Now, we can synthesize a new branch from an existing chain using branching enzyme.

Definition 2.3. A **branching enzyme (amylo-(1,4→1,6)-transglycosylase)** is an enzyme that remove a 7 glycosyl residues from a around 11 chain glucose residue and transfer it to a glucose carbon 6 that it at least 4 residue away from another branching point.

So now, we will go through the full glycogen synthesis once again. Starting from a single glucose molecule, it will be attached to a glycogenin that will be extend to around 8 to 12 residues. Then, glycogen synthase can take over and further extend the chain. Then, branching enzyme will come and cut 7 glycosyl residues to attach it to a glucose's C6. From here, glycogen synthase can then further extend the existing branch and the original chain which can be further branched by branching enzymes, so on and so fourth. See Figure 2.7.

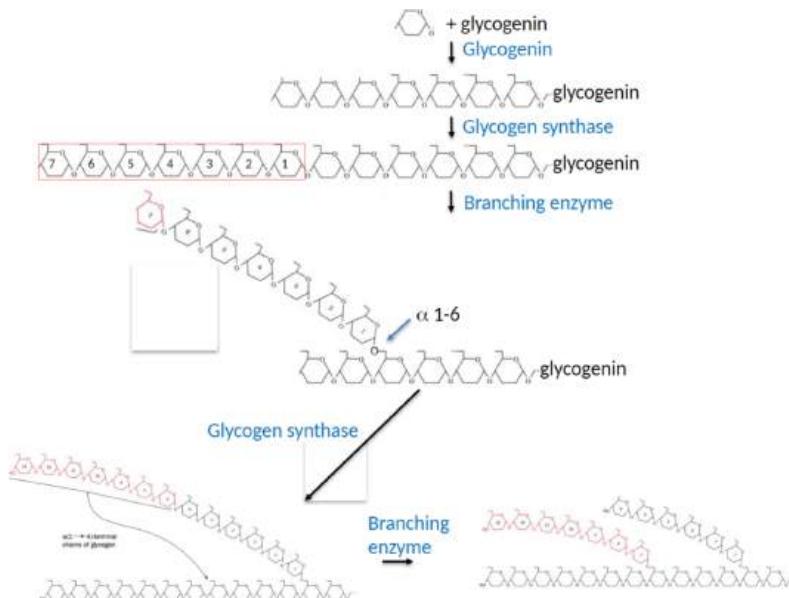


Figure 2.7: Summary of glycogen synthesis

Remark 2.2. The act of attaching 1 glucose to an exists branch glycogen will require 2 ATP.

2.1.2 Glycogen Degradation

For the breaking down of glycogen, there are 3 main processes we'll be looking at: 1, breaking a glucose from glycogen to generate G1P; 2, breaking the branch of a glycogen to then degrade more; 3, converting G1P to G6P.

When it comes to detach a glucose from its $\alpha 1 - 4$ linkage with another glucose, there are 2 ways to do it:

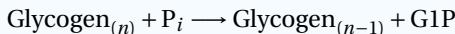
1. **Hydrolysis:** Performing hydrolysis with said bond will yield a glucose molecule in the end.
2. **Phosphorolysis:** Performing phosphorolysis on said bond will yield a phosphorylated glucose molecule at carbon 1 i.e. G1P.

Evidently, phosphorolysis would be preferable because we want to control our glucose efflux by phosphorylating it.

Phosphorolysis of Glycogen to G1P

We will now look at the mechanism of generating G1P from glycogen.

Mechanism of Action (Phosphorolysis of Glycogen): Phosphorolysis will begin and can only occur at the non-reducing end. Phosphorolysis is mediated by **glycogen phosphorylase** that breaks the non-reducing end's $\alpha 1 - 4$ linkage from the chain. This will release 1 G1P from the chain. It has the following reaction



where $\Delta G^\circ' = 3.1 \text{ kJ/mol}$.

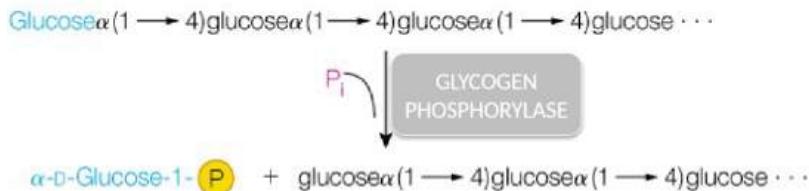


Figure 2.8: Glycogen phosphorylase reaction.

Observation 2.5 Though this reaction will have a $\Delta G^\circ' = 3.1 > 0$ which means that it's unfavourable under normal condition (endergonic). However, the $[\text{P}_i]$ is significantly larger than that of $[\text{G1P}]$ which means the reaction will push all the way to the right hence it can happen and is exergonic *in-vivo*.

Remark 2.3. *Glycogen phosphorylase can only synthesize G1P from glucose at least 4 residues away from a branch point.*

Debranching of Glycogen

Now, looking at remark 2.3, glycogen phosphorylase will stop working as it reaches near a branching point. This is because the conformation and

linkage at the branching point is different from a typical linear chain. To get around this, we will be using 2 kinds of enzymes.

Definition 2.4. **$\alpha(1-4)$ glucosyltransferase** is an enzyme that transfer 3 glucosyl residue from a branch to an existing linear chain. **$\alpha(1-6)$ glucosidase** is an enzyme that hydrolyze the sole glucose residue at the branching point to produce glucose.

So with these 2 enzyme, we can look at how debranching works.

Mechanism of Action (Glycogen Debranching): We begin with glycogen phosphorylase reach near a branching point and stop.

1. $\alpha(1-4)$ glucosyltransferase will come in and begin to remove 3 glucose residues and attach it back to the linear chain until there's only 1 glucose residue left at the branch point.
2. $\alpha(1-6)$ glucosidase will come in a hydrolyze the glucose residue at the branch point to yield 1 glucose molecule.
3. Now the chain is debranched, glycogen phosphorylase can come back and continue to generate G1P.

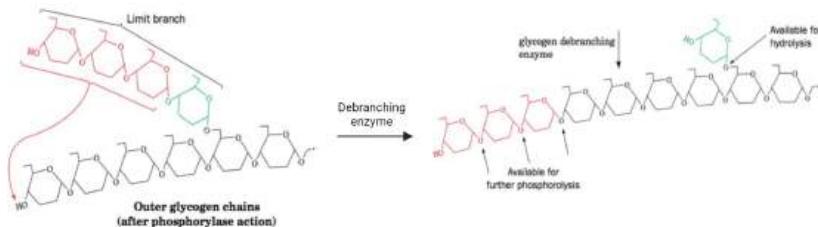


Figure 2.9: Glycogen debranching.

You can essentially see that because of branching, not all the glucose will be converted to G1P. In fact, **only 92% of glycogen is converted to G1P** **will the rest are converted directly to glucose.**

Conversion G1P to G6P

Definition 2.5. **Phosphoglucomutase** is an enzyme that convert G1P back to G6P and vice versa.

Explanations. Phosphoglucomutase has serine residue on its structure that is phosphorylated. As the enzyme gets close to G1P, it will donate its

phosphate group to the carbon 6 position. After losing a phosphate group, it will retake one from the carbon 1 position. In total, this will yield G6P. □

Efficiency of Glycogen Storage

Now, we can check how efficient at glycogen when it comes to storing energy. We'll just be looking at the reaction of 1 molecule of glucose.

First, The amount of energy it takes to store 1 glucose residue is around 2 ATP while breaking it down takes 33 ATP. The reason we got 33 is because of the typical glycolysis of glucose and 1 extra ATP for the fact that it's already in the form of G6P. In the end, net ATP for glycogen would get $33 - 2 = 31$ ATP.

Now, supposed that 1 glucose begin to move through glycolysis and oxidative phosphorylation. We'll see that the total energy produce from glucose is 32 ATP. With all of that information, we can calculate the efficiency of glycogen storage as

$$\text{Efficiency of glycogen storage} = \frac{31}{32} \times 100 = \boxed{97\%}$$

This also means that the storage of glucose in the form of glycogen is quite efficient.

2.2 Glycogen II

In today's lecture we will look at some enzyme cascade and more on the enzymes that participate in glycogen synthesis and degradation.

2.2.1 Enzyme Cascades

Definition 2.6. A **cascade reaction** is a combination of different reactions in a sequential manner hence a cascade. Therefore, an **enzyme cascade** is simply a sequences of reaction via enzymatic activities.

When it comes to there enzymatic activities, there can be many ways to modify it but in today's lecture, we'll be look at covalent modification and allosteric regulation.

Observation 2.6 In covalent modification, you're forming strong molecular bond between 2 molecules (e.g. an enzyme and another substrate). When this bond occur, you'll get a change in conformation of the main molecule or activity.

On the other hand, non-covalent modification are formation of weak bonds between molecules. This type of modification are typically seen in allosteric regulator.

When it comes to the covalent modification of a protein, it can either be reversible or irreversible (the name implies the meaning).

Example 2.2.1. The phosphorylation, carry out by *kinase*, of glycogen phosphorylase is reversible because of a molecule called **phosphatase** that can remove that phosphate group.

Meanwhile, the activation of insulin from pro-insulin, using protease, is irreversible since the covalent bond along with any irrelevant are permanently destroyed.

Definition 2.7. A **monocyclic enzyme cascade** is an enzyme cascade with only 1 level/cycle of control.

Example 2.2.2.

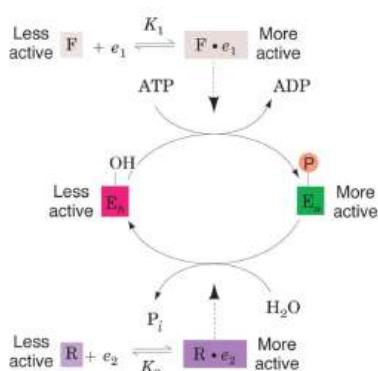


Figure 2.10: monocyclic enzyme cascade

R to carry its function, it needs to be activated by another allosteric modulator *e₂*. See Figure 2.10.

We will focus on this specific enzyme *E*. What we'll see that the enzyme can cycle between being active *E_a* and inactive *E_b*. In order to be activated *E* will be phosphorylated by another enzyme *F*. *F* can only phosphorylate *E* if it's active and for it to be active, it requires an allosteric regulator *e₁* to be presence at high enough concentration.

Contrarily, there's another there's an enzyme *R* that can deactivate *E* by dephosphorylate it. In order for

Observation 2.7 As you can see from example 2.2.2., The main point of this cascade is to perform a covalent modification on the enzyme E using the activities of other enzymes (F or R).

Definition 2.8. A **bicyclic enzyme cascade** is an enzyme cascade with only 2 level/cycle of control.¹

Example 2.2.3.

An enzyme E can be activated through phosphorylation that's mediated by another active enzyme F_2 . Additionally, This F_2 enzyme can only activated if there exists another enzyme F_1 to phosphorylate it. In order F_1 to be phosphorylated, it must be active via the action of an allosteric regulator e_1 . You can realise the same for the inhibitor of E . See Figure 2.11.

Observation 2.8

So, essentially, in example 2.2.3., you're trying to perform covalent modification on F in order to perform another covalent modification on the main enzyme E .

Remark 2.4. All of these are simply signal transduction cascade which is used to amplify an original signal in a cell.

2.2.2 Regulation of Glycogen Metabolism

Regarding to the regulation of glycogen metabolism (both synthesis and degradation), it's controlled by 2 regulatory mechanism

1. Allosteric regulation of glycogen phosphorylase and synthase.
2. Covalent modification of by cascade phosphorylation.

The entire regulatory cascade pathway of glycogen synthesis and degradation can be seen in Figure 2.12, which we will go through each of the enzyme and substrates involved in said cascade.

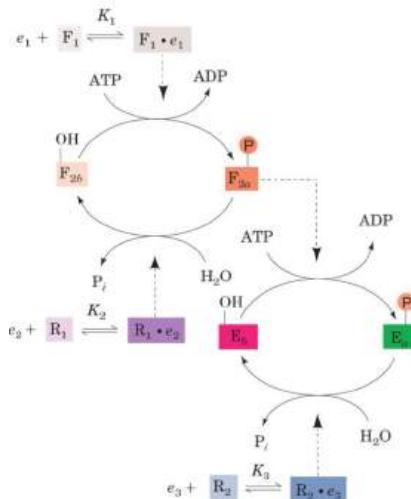


Figure 2.11: Bicyclic enzyme cascade

¹Obviously, we can extend this to however many level/cycle of control.

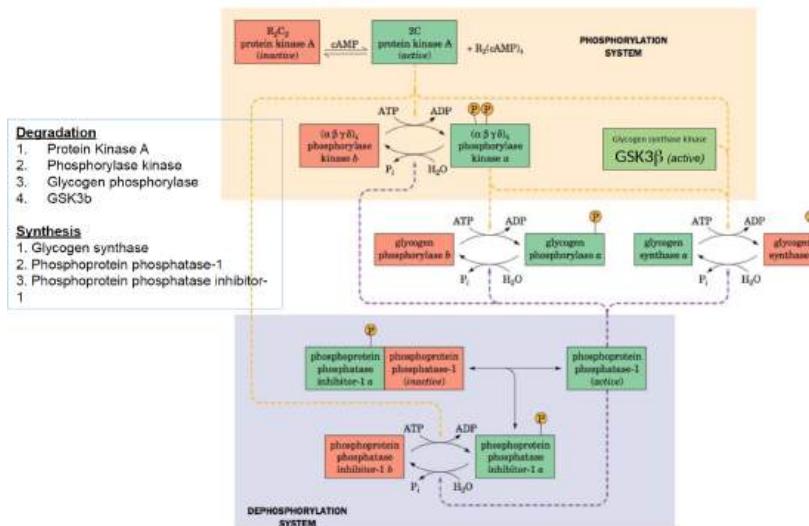


Figure 2.12: Complete regulatory pathway of glycogen metabolism

Glycogen phosphorylase

Before talking about glycogen phosphorylase, we need to note that **most enzymatic** are not simply on or off, it can be modulated/fine tune in between.

Even when this is the case, glycogen phosphorylase (and most enzymes) can adopt 2 conformations: **T (tense)** and **R (relaxed)** that will correlate to it being inactive and active respectively. The 2 conformation is controlled via allosteric regulators while its activity level is controlled via covalent modification.

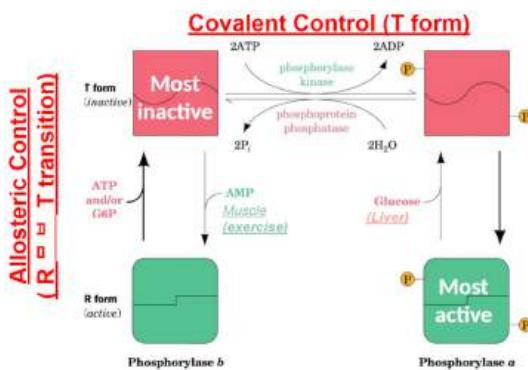


Figure 2.13: Regulation of glycogen phosphorylase.

Observation 2.9 When the body is at rest, allosteric regulators such as ATP and G6P will be present, thus glycogen phosphorylase will adopt the T conformation and will be in its most inactive. When its most inactive T conformation is phosphorylated by a kinase, its inactivity will decrease by a slight bit then finally change its conformation to R at the maximum active state.

Why don't we go from most inactive to active then most active? Well... because the kinetics is much quicker to phosphorylated something than changing a metabolite concentration. For sure, the opposite can happen but its much faster to have the covalent modification than allosteric regulation.

Remark 2.5. Because covalent modification is faster, the proportion of active glycogen phosphorylase is determined by the rate of said modification (under most physiological condition).

Phosphorylase Kinase

Definition 2.9. Phosphorylase kinase is an enzyme that can phosphorylate other substrates. Its main substrates include **glycogen phosphorylase** and **glycogen synthase**.

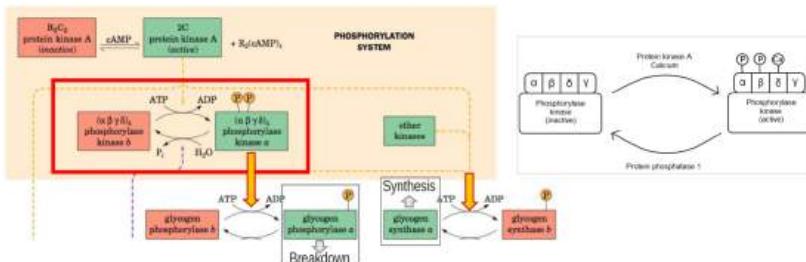


Figure 2.14: Phosphorylase kinase forms and regulation.

Observation 2.10 It's a 16 individual protein but is combined together as a 4 subunits complex. The α and β subunits are for regulation. γ is the site of catalysis (where it does the phosphorylation) and δ site confers to calcium sensitivity (it's also called the **calmodulin protein** (Ca^{2+} -modulating protein)).

It can adopt 2 forms: **phosphorylase kinase-b**, which is the inactive form even if $[Ca^{2+}] \uparrow$; and **phosphorylase kinase-a**, which is the active form even if $[Ca^{2+}] \downarrow$

Under the phosphorylase kinase-b form, it can be activated through phosphorylation by protein kinase. Once activated, it can then phosphorylate the glycogen phosphorylase to activate it or deactivate glycogen synthase.

Conversely, its phosphorylase kinase-a form can be deactivated through dephosphorylation of phosphoprotein phosphatase 1.

Remark 2.6. This enzyme can also be modulated through **hormonal inputs** (e.g. cAMP) or **$[Ca^{2+}]$ inputs**.

Protein Kinase A

So now we're the upper most regulators of glycogen phosphorylase and any other enzymes involved.

Definition 2.10. **Protein kinase A** is the upper-most regulators for glycogen metabolism. Its main job is to phosphorylate multiple substrates.

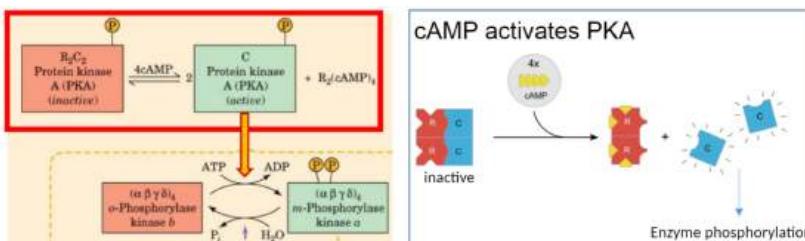


Figure 2.15: PKA structure and activities.

Observation 2.11 PKA is a *heterotetramer* meaning that it has 4 different subunits. Even so, 2 of those subunits have the catalytic sites (R) and 2 are regulatory sites (C). When PKA is inactive, the C subunits and R subunits are bound together in to a heterotetramer. When **cAMP** is presence, it will **bind to the R subunits and dissociate the C subunits**. Once the C subunits are dissociate, it's now active and can begin to phosphorylate its substrate accordingly. N.B. this activation is via allosteric regulation where the regulator molecule **cAMP**.

Remark 2.7. *The intracellular [cAMP] can determine the amount of active PKA.*

Now, the released C subunits will mainly affect the following enzymes

1. **Phosphorylase kinase:** PKA can phosphorylate this enzyme to activate it thus lead to glycogen degradation.
2. **Glycogen synthase:** PKA can phosphorylate it to deactivate it leading to glycogen degradation.
3. **Phosphoprotein Phosphatase 1:** PKA can phosphorylate it to activate it leading to glycogen synthesis.

Glycogen Synthase

Definition 2.11. **Glycogen synthase** is an enzyme that can build up glycogen using UDP-glucose.

Observation 2.12

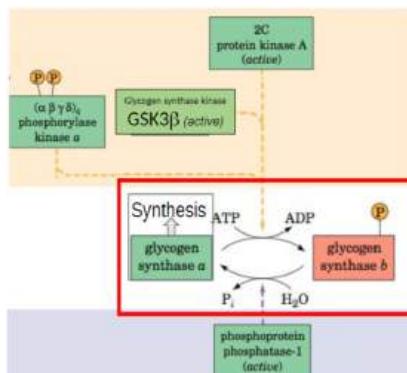


Figure 2.16: Glycogen synthase regulation

Contrarily, in its b form, the enzyme is phosphorylated which is when phosphorylase kinase, PKA, and GSK3 β are active. Here, you would find phosphoprotein phosphatase to be inactivated (such as in the presence of epinephrine).

Explanations. When the degradation enzymes are activated, the glycogen synthase must be deactivated. The reason we have to shut it down is simple which is to conserve energy □

Glycogen synthase takes on 2 form, active (a) and inactive (b). In its a form, the enzyme is dephosphorylated which is when phosphoprotein phosphatase 1 is activated, phosphorylase kinase is inactivated and [cAMP] \downarrow which also lead to another enzyme called **glycogen synthase kinase (GSK3 β)** is inactive (such as in the presence of insulin).

Remark 2.8. Nevertheless, there are cell that do these on purpose to produce heat.

What we've just uncovered was simply covalent modification. When it comes to allosteric regulation, only the b form is affected. This effect is via G6P which can facilitate its dephosphorylation.

Remark 2.9. We can see that glycogen synthase activity depends on the fraction of the enzyme in the unmodified form.

Phosphoprotein Phosphatase

All of the above are mostly enzymes for regulating glycogen degradation. Meanwhile for its synthesis, there's only 1 enzyme.

Definition 2.12. Phosphoprotein Phosphatase (PP1) is the only enzyme that regulate glycogen synthesis by removing the phosphate group from glycogen synthase (to activate it).

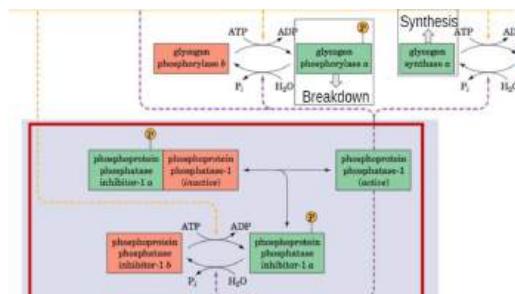


Figure 2.17: PP1 regulation.

Observation 2.13 PP1 like other has an active form (a) and an inactive form (b). In its a form, PP1 is phosphorylated by PKA. Active PP1 can inhibit glycogen degradation and promote synthesis by dephosphorylate: glycogen phosphorylase, phosphorylase synthase, glycogen synthase and even its own inhibitor **PP1-inhibitor**.

Under its b form, PP1 is dephosphorylated by itself. Typically, the b form is found to be attached to PP1-inhibitor, which can be phosphorylated (by PKA) to increase its chance to bind to PP1 and thereby inhibit PP1.

Mechanism of Action (Muscle PP1c Activity): We will begin with the body in a fed state.

1. PP1 catalytic site (PP1c) can be found attached to glycogen molecule via an intermediate regulatory protein subunit G_M . This is the least active state.
2. When insulin is present (fed state), insulin-stimulated protein kinase is activated and phosphorylate the G_M subunit.
3. The phosphorylated will lead to the most active state of the PP1c therefore increase synthesis of glycogen.

Now, we'll look at what happen when the body is in a fasting state.

1. PP1c is in the same conformation as before.
2. Epinephrine↑ will lead to [cAMP]↑ which then activate PKA that can phosphorylate the fed state G_M once again.
3. When G_M is phosphorylated twice, PP1c will be in its inactive form and dissociate from G_M . This lead to an increase of glycogen degradation. See Figure 2.18.

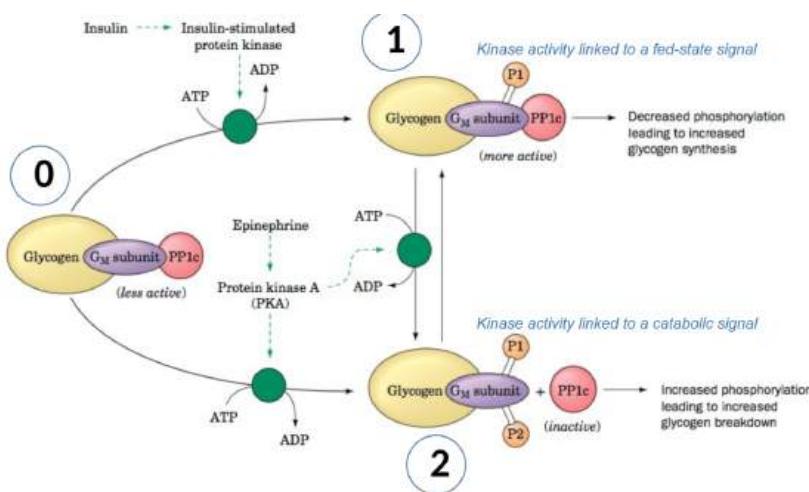


Figure 2.18: Muscle PP1c activity regulation.

2.3 Glycogen III

In this lecture we will look at the regulation of glycogen metabolism in the liver and muscle.

2.3.1 Hormonal Signalling

To begin with, let's look at some receptors that plays an important role in this regulation along with their mechanism of action.

Insulin Receptor

Definition 2.13. **Insulin receptor** is a type of tyrosine receptor kinase that can be activated with the binding of insulin.

Mechanism of Action (Insulin Receptor): Under non-insulin condition, the insulin receptor will appear as 2 separate monomer.

1. As insulin come and bind to it, the 2 monomers dimerize which lead to its **auto-phosphorylation**.
2. This phosphorylation open a binding site for intracellular **insulin receptor substrate (IRS)** to come and be phosphorylated.
3. Phosphorylated IRS can go through a signalling cascade that lead to the activation of protein kinases which (from previous lecture) will turn on glycogen synthase.

Observation 2.14 Although we've already said this from previous lecture, just remember that activation of protein kinase will lead to **inhibition of the inhibitor of glycogen synthase**.

Gas-coupled receptor

Definition 2.14. **Gas-coupled receptor** is a subunit of GPCRs that can turn on an important cellular machinery: adenyalaate cyclase.²

Mechanism of Action (Gas pathway): When is not bound by a ligand, GPCR is in its in active form, the α subunit is bounded to GDP along with the β and γ subunit (forming a heterotrimer).

²There are around 400 GPCR just for smell and 370+ for other purposes

1. Upon binding of a ligand, GPCRs is activated and the $\text{G}\alpha$ subunits will bind to GTP instead of GDP.
2. Once bound by GTP, $\text{G}\alpha$ will dissociate from the β and γ subunits.
3. GTP-bound $\text{G}\alpha$ can travel to **adenylate cyclase** and activate it which lead to the generation of cAMP.
4. cAMP can then activates PKA which can trigger a cascade of cellular response (specifically for glycogen).

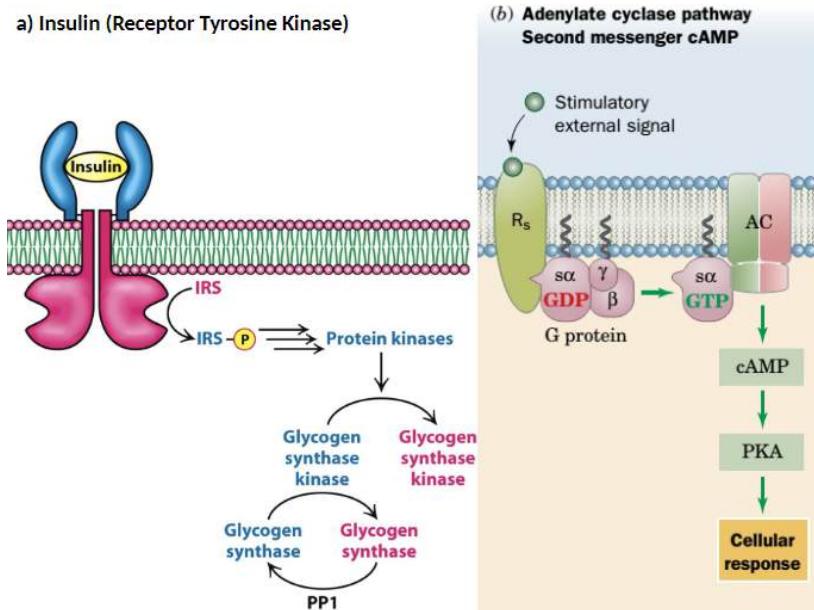


Figure 2.19: Insulin receptor and $\text{G}\alpha s$ -coupled receptor

Definition 2.15. **$\text{G}\alpha q$ -coupled receptor** is similar to $\text{G}\alpha s$ with the only different that it activates another type of machinery.

Mechanism of Action ($\text{G}\alpha q$ pathway): Inside the cell, when the GPCR is off, the α subunit is bounded to to GDP along with β and γ subunit (forming a heterotrimer).

1-2. The first 2 steps are similar to that of $\text{G}\alpha s$. **3.** GTP-bound $\text{G}\alpha q$

can go and activate **phospholipase C (PLC)**.

3. PLC can then cleave PIP₂ into its membrane-bound component: **DAG** and free moving component: **IP₃**.

4a. IP₃ can bind to the **endoplasmic reticulum (ER)** receptors which trigger the release of Ca²⁺ \Rightarrow Ca²⁺ from ER enter the intracellular space.

5a. Once inside the intracellular space, Ca^{2+} can go and activates signalling pathways whose enzymes has a calmodulin subunit.

4b. At the same time, DAG can bind to **protein kinase C (PKC)** and along with Ca^{2+} , it will be activated.

5b. Upon activation, PKC will phosphorylate proteins that ultimately lead to cellular response.

Remark 2.10. Essentially, hormone acts on every part of the regulation of glycogen synthesis/degradation.

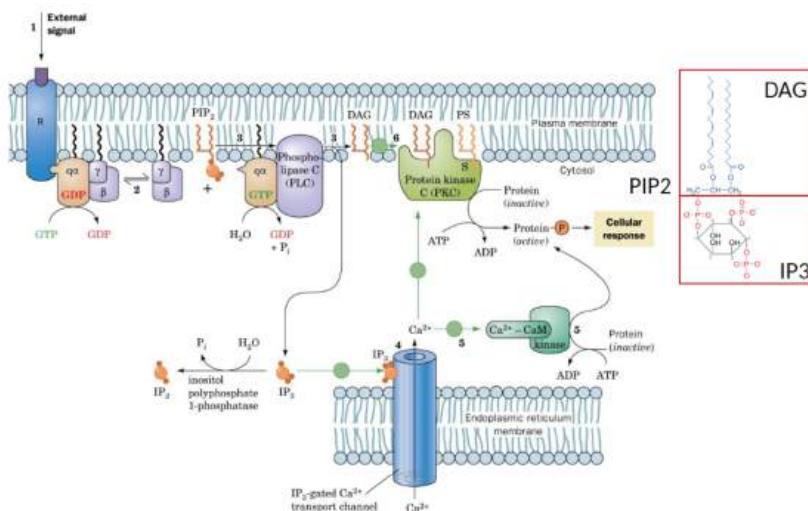


Figure 2.20: G_{αq} coupled receptor.

2.3.2 Physiological Regulation

Now, we will look at how these regulation are induced depending on the physiological condition the body is in. For this, we will look at 2 types of tissues: **muscle and liver**, with 2 different physiological conditions of either: in fed-state or fasting-state.

Remark 2.11. *Glucagon receptors are unique in the liver cell (won't present in muscle cell).*

N.B. 2 important GPCRs we'll be looking at are β - and α -adrenergic receptors. It must be understood correctly that not all GPCRs are adrenergic (only 9 subtypes!). The β -adrenergic will follow the same pathway as that of $G_{\alpha s}$ while α -adrenergic will follow that of $G_{\alpha q}$.

Remark 2.12. *Epinephrine (EPI) is the ligand for both receptors however α -adrenergic receptor has higher affinity for EPI than β .*

Regulation in the Muscle

Mechanism of Action (Muscle): Let's begin with the body in the **fed-state**.

1. The pancreas will release insulin to the bloodstream that will come to muscle tissues and bind to its insulin receptor.
- 2a. Upon binding, insulin receptor is activated which triggers a signalling pathway that leads to glycogen synthesis.
- 2b. At the same time, insulin receptor also activates GLUT4 to transport glucose into the cell to be used as building block for glycogen.

Suppose that the body is in a **fasting-state** or even exercising.

1. The adrenal gland will release EPI that can bind to β -adrenergic receptors which lead to the beginning of the $G_{\alpha s}$ pathway thus cAMP are made.
2. When the muscle contracts, **action potential (AP)** will propagate along the muscle cell membrane then down the **T-tubules**.
3. At the T-tubules lies the **sarcoplasmic reticulum (SR)** of which upon receiving AP will begin to release its stored Ca^{2+} into the intracellular space.
4. Now, the released Ca^{2+} can help with muscle contraction or aid cAMP that lead to glycogen degradation and ultimately glycolysis.

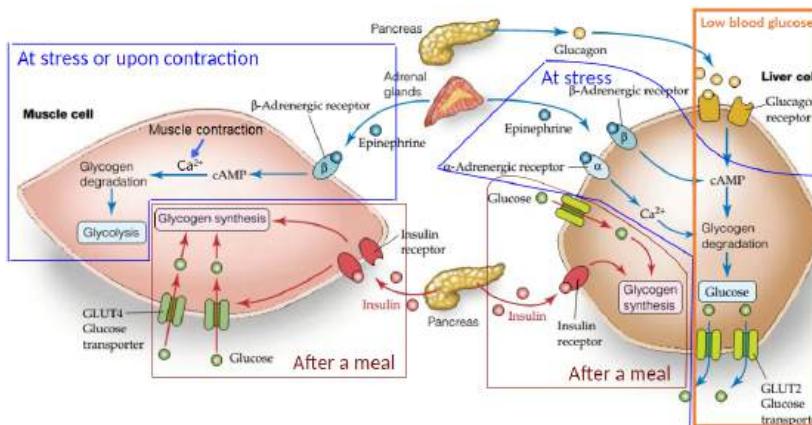


Figure 2.21: Hormonal control of glycogen metabolism depending on physical condition.

Regulation in the Liver

For the liver, we actually no longer have only a dichotomous regulation between fed- and fasting-state, we will have another regulation when blood glucose level drop. This is self-evident as the liver the the main organ that control the glucose level.

Mechanism of Action (Liver): Let's begin with the body being in the **fed-state**. For this, it follows the exact same mechanism as muscle with a slight difference that it also have GLUT2 transport that is **not insulin dependent**.

Suppose that the body is in a **fasting-state** or even exercising.

1. Released EPI will come and bind to α and β -adrenergic receptors which then trigger the released of Ca^{2+} and cAMP respectively.
2. These 2 components will lead to glycogen degradation back to glucose.
3. Now, there are high [glucose] \Rightarrow it will be transported out of the cell via the GLUT2.

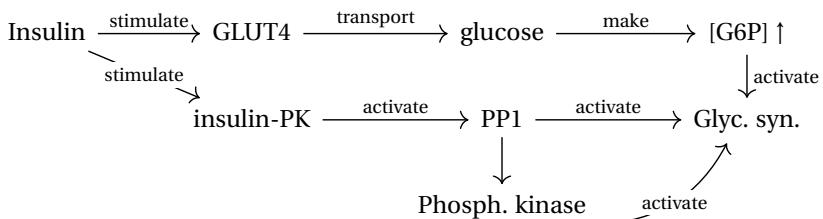
Observation 2.15 Another situation can arise that we've briefly mentioned is low blood glucose. In this case, the pancreas releases **glucagon** which

can bind to the liver's **glucagon receptor** which lead to the release of cAMP. Once cAMP is released, glycogen degradation begin, glucose is made and transported out.

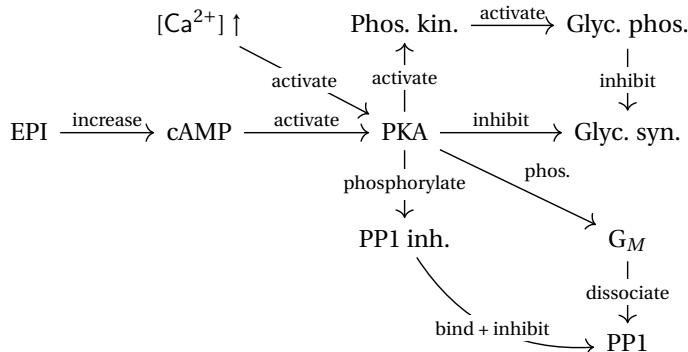
Remark 2.13. *Glucagon receptors is uniquely present on liver cells.*

N.B. We do not mention what happens during glycogen synthesis nor degradation because it was previously talked about. Nevertheless, we can give a brief scheme for it:

Synthesis:



Degradation:



Remark 2.14. *The degradation process for liver will be slightly different as you'd also consider from α and glucagon as well.*

2.3.3 Glycogen Storage Disease

Glycogen storage disease is a class of disease that linked to mutation in enzymes that are essential for glycogen breakdown. There are around 10 of these disease however we will only look at 3 of them: type I, V and VI.

Type	Enzyme Deficiency	Tissue	Common Name	Glycogen Structure
I	Glucose-6-phosphatase	Liver	von Gierke's disease	Normal
II	α -1,4-Glucosidase	All lysosomes	Pompe's disease	Normal
III	Amylo-1,6-glucosidase (debranching enzyme)	All organs	Cori's disease	Outer chains missing or very short
IV	Amylo-(1,4 \rightarrow 1,6)-transglycosylase (branching enzyme)	Liver, probably all organs	Andersen's disease	Very long unbranched chains
V	Glycogen phosphorylase	Muscle	McArdle's disease	Normal
VI	Glycogen phosphorylase	Liver	Hers' disease	Normal
VII	Phosphofructokinase	Muscle	Tarui's disease	Normal
VIII	Phosphorylase kinase	Liver	X-Linked phosphorylase kinase deficiency	Normal
IX	Phosphorylase kinase	All tissues		Normal
0	Glycogen synthase	Liver		Normal, deficient in quality

Figure 2.22: Glycogen storage diseases

Definition 2.16. Type I or **von Gierke's disease** is a disease characterized by the deficiency of G6Pase in the liver.

Explanations. G6Pase is an essentially enzyme that catalyzes the final step to release glucose into the bloodstream. In this particular disease, there's will be an accumulation of normally structured glycogen but is unable to release it in the form of glucose when EPI and glucagon is present. □

Observation 2.16 Some of the common symptoms include massive liver enlargement, hypoglycemia, and cells failure to thrive. Because of a large accumulation of glycogen, evidently the liver will be enlarged. This enlargement is due to inability to release it as glucose hence drop in blood glucose. Because glucose is low, cell cannot grow properly as there is no energy to do so.

Treatment: Reroute the portal vein so the any excess glucose coming in will not end up in the liver (this is an extreme way).

Definition 2.17. Type V or **McArdle's disease** is a disease characterized by the deficiency of glycogen phosphorylase in the muscle.

Explanations. Glycogen phosphorylase is important to catalyze the breakdown of glycogen to G1P. If there's a deficiency in this enzyme, there won't be enough fuel for glycolysis to keep up with metabolic demand. □

Observation 2.17 A common symptom from this disease is painful cramps during exercises.

Interestingly, when looking at the concentration of ADP in patient suffering from McArdle's disease during exercise, we see a sharp increase in its value, **why is that?** Well...in order to turn ADP back to ATP, you need substrates to do so but because you cannot breakdown glycogen, substrate level decreases and thus ADP level increases.

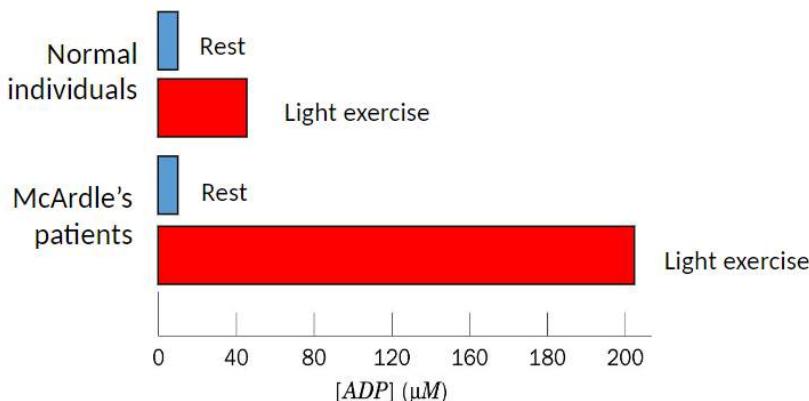


Figure 2.23: ADP level of McArdle's disease vs normal patient.

Definition 2.18. Type VI or **Hers' disease** is a disease characterized by the deficiency of glycogen phosphorylase in the liver

Explanations. This is similar to type V but now it's in the liver i.e. inability to breakdown glycogen □

Observation 2.18 The most common symptom is hypoglycemia since no glycogen can be broken down to yield glucose to release back to the blood-stream.

2.3.4 Cori Cycle Again

we're talking about the cori cycle again but the main idea is that there's this sort of intercommunication between organs of different places and Cori cycle is most famous example.

In the muscle, when glycogen is being used up, it will produce pyruvate at the end of glycolysis. In normal condition, pyruvate can be further oxidized in the mitochondria using O_2 as an electron acceptor. Now, suppose

that the mitochondria is backed up **what can the body do?** Well...the build up of pyruvate will also build up the electron pool which we can "dump" it onto lactate to be transported out of the cell.

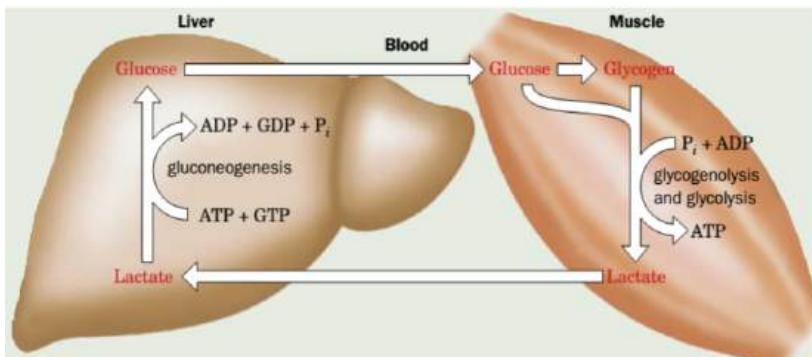


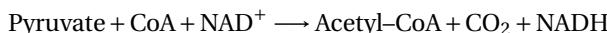
Figure 2.24: Cori Cycle

Amazingly, the liver has some mechanism to use up those lactate and convert it back to glucose via gluconeogenesis. This glucose will then be released back to the bloodstream and uptake by muscle.

Remark 2.15. *Basically, muscle and liver are participating in a metabolic cycle.*

2.4 Pyruvate Dehydrogenase Complex

Definition 2.19. **Pyruvate dehydrogenase complex (PDC)** is an enzyme that can convert pyruvate and CoA into acetyl-CoA, more specifically, the following reaction:



Observation 2.19 When glycolysis finally produced pyruvate, there are 2 decisions to make: 1) convert it to lactate or 2) convert it to acetyl-CoA to be used in the citric acid cycle. Suppose we want to convert it to acetyl-CoA. In said instance, **pyruvate will get transported into the mitochondria where PDC is located.**

Remark 2.16. The transporter protein is not PDC but H^+ /pyruvate symport.

Observation 2.20

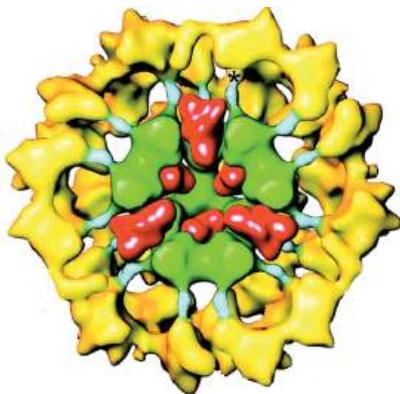


Figure 2.25: PDC structure.

size the necessary substrate.

Example 2.4.1. To make CoA, you need vitamin B₅ as a cofactor while for NAD⁺, it will be vitamin B₃.

PDC is a very large enzyme of around 9.5MDa (to put into reference, pyruvate is only around 88Da). Within this complex it consists a lot of proteins and their copies. The proteins we will focus mostly in this course are: pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E1) and dihydrolipoyl dehydrogenase (E1). (each of these proteins has multiple copies that also form its own complex).

Not only that, PDC requires many cofactor in order to synthe-

2.4.1 PDC Reaction

Now, we will focus on the reaction that the 3 proteins E1 to E3 catalyzes. One thing to note is that these proteins/reactions sit in close proximity to each other.

Mechanism of Action (E1 and E2 Reaction): To begin with, pyruvate is decarboxylated which is the committed step to making acetyl-CoA.

1. A molecule called **TPP**, which is a cofactor attached to E1, will be transferred to the decarboxylated pyruvate to form **hydroxyethyl-TPP**.

2. E2 then oxidizes the hydroxyethyl-TPP and attaches its **lipoamide** to it to form **acetyl-dihydrolipoamide**. It also removes TPP to be attached back to E1. Because of the oxidation, $2e^-$

are removed which reduce the S–S of the lipoyl group to SH on the E2. This thioester bond is high in energy.

3. The acetyl–dihydrolipoamide will undergo a transesterification reaction to CoA which lead to the creation of **acetyl–CoA** and release **dihydrolipoamide**.

Remark 2.17. *The compound acetyl–CoA is a very high energy compound and is the main entry to citric acid cycle.*

Now, from the above, we can see that E2 will regenerate TPP which lead reset E1 back to its active state. Now, we will look at the reaction of E3 that reset E2 (but also itself).

Mechanism of Action (E3 reaction): E2 has performed its reaction which now has its lipoamide converted to dihydrolipoamide.

1. FAD–bound to E3 will be reduced while the dihydrolipoamide will be oxidized which will make lipoamide (reset E2) and FADH₂ respectively.
2. The FADH₂–bound E3 can then be oxidized along with the reduction of NAD⁺ to make FAD (reset E3), NADH and a free H⁺.

So in the end for all 3 reactions, you will get an acetyl–CoA for citric acid cycle and 1 NADH for oxidative phosphorylation.

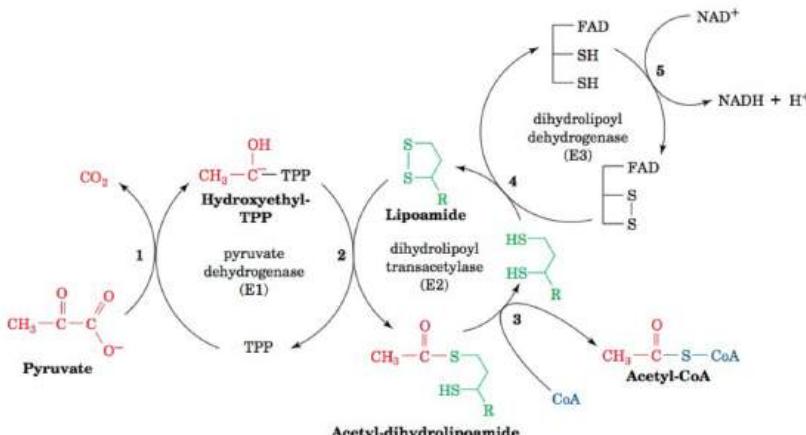


Figure 2.26: E1 to E3 reactions.

Question: What are the advantages of having this multienzyme complexes?

Answer. First, having everything in a complex will reduced the distance between reaction which increases efficiency. Not only that, it allows some regulation of these reactions.

2.4.2 PDC Regulation

It is important to have the PDC pathway regulated as it is the only pathway in mammals that can produce acetyl-CoA from pyruvate. When it comes to its regulation, it mainly involves 2 levels:allosteric inhibition and covalent modification.

Allosteric inhibition via Acetyl-CoA and NADH

When PDC is producing too much acetyl-CoA and NADH, it will use these products as its own inhibitors.

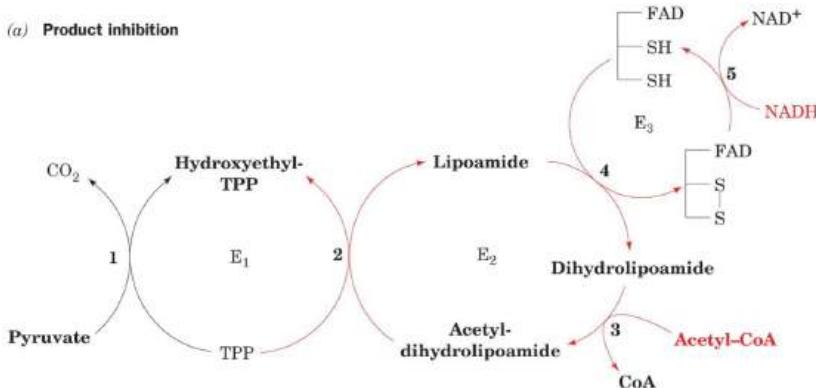


Figure 2.27: Product inhibition of PDC.

Mechanism of Action (Inhibition of PDC): When there's an accumulation of acetyl-coA and NADH, it will begin to acts like inhibitors.

1. $[\text{Acetyl-CoA}] \text{ and } [\text{NADH}] \uparrow \implies$ Reactions of E₂ and E₃ will run backwards.
2. E₂ is in its acetylated form and cannot proceed to breaking TPP

from the hydroxyethyl-TPP.

- 3. E3 is reduced which cannot regenerate the S-S in E2.

So basically, this kind of inhibition happens when there's a high level of acetyl-CoA and NADH hence prevents pyruvate consumption.

Covalent Modification of E1

The other kind of regulation of PDC is via phosphorylation and dephosphorylation of E1.

Remark 2.18. *This phosphorylation and dephosphorylation can only happen in eukaryotic PDC.*

- **Phosphorylation:** When acetyl-CoA and NADH level is high, not only they can act as an allosteric inhibitor, they can also be activators of **pyruvate dehydrogenase kinase (PDK)**. When PDK is activated, it will phosphorylate E1 which deactivate it and hence shut down PDC.

There are many inhibitors of PDK includes: **pyruvate, ADP, Ca^{2+} and K^+ .**

- **Dephosphorylation:** When Ca^{2+} and Mg^{2+} is high, they act as activator for **pyruvate dehydrogenase phosphatase (PDP)**. Activated PDP will dephosphorylate E1 which activate it and hence PDC is activated.

(b) Covalent modification

Activators

Mg^{2+}

Ca^{2+}

P_i

pyruvate
dehydrogenase
phosphatase

H_2O

E1-OH (active)

ATP

pyruvate
dehydrogenase
kinase

ADP

Activators

Acetyl-CoA

NADH

Inhibitors

Pyruvate

ADP

Ca^{2+} (high Mg^{2+})

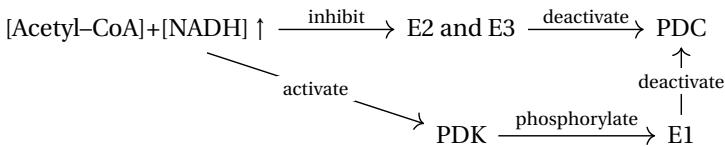
K^+

Figure 2.28: Covalent Modification of PDC.

Observation 2.21 What's interesting is in cancer cells. Like we've known from before, cancer cells have altered metabolism where they enjoyed anaerobic glycolysis much more than aerobic (eventual build up of pyruvate will convert to lactate). This is because its PDC's activity is inhibited by PDK. You can actually see this through a FDG-PET scan using radioactive glucose (safe).

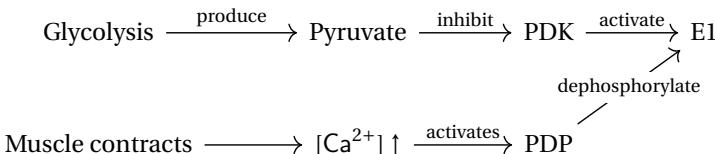
We can now summarize regulation of PDC when we exercise and when we're at rest in the following diagram:

At rest:



Overall, this will stop production of excess ATP.

During exercise:



Overall, this will increase the production of ATP.

End of Lecture —

2.5 Citric Acid Cycle

2.5.1 Introduction

In today's lecture, we will look at the citric acid cycle, along with its reactions and regulation.

Definition 2.20. **Citric acid cycle (CAC)** is a series of 8 enzymatic reactions that uses oxaloacetate and acetyl-CoA to make CO_2 , NADH and FADH_2 . In the end, it will also regenerate oxaloacetate to begin a new cycle.³

³The CAC was discovered by Hans Krebs (in 1937) hence it's also called Krebs cycle. Another name for CAC is tricarboxylic acid (TCA) cycle

CAC is the core mechanism in aerobic metabolism though it does not consume O_2 directly.

Remark 2.19. *Keep in mind that 1FAHD₂ worths 2.5ATP while 1NADH worths 1.5ATP*

Definition 2.21. **Amphibolic** is a property of a pathway that can be both anabolic or catabolic.

Observation 2.22 CAC is amphibolic which means it can either break things down or build things up. Now, here comes the confusing part:

1. For anabolism: With respect to the body, yes, CAC is building up stuffs; however, with respect to CAC itself, it's actually breaking down its substrates to do anabolism.
2. For catabolism: Similarly, with respect to the body, CAC is breaking things down; however, with respect to the CAC, it's building back new substrates.

To avoid said confusion, we'll give a different term. During anabolism, with respect to the CAC, we say it's doing **cataplerotic reactions**. On the other hand, during catabolism, with respect to the CAC we say it's doing **anaplerotic reactions**.

Example 2.5.1. In order to build fatty acid, you'd need an anabolic reaction. For CAC, when it builds the fatty acid, it will perform a cataplerotic reaction.

Observation 2.23 CAC is neither aerobic nor anaerobic pathway but can also generate some energy in the form of GTP, NADH and FADH₂. For the CAC, the first reaction is conversion of acetyl-CoA citrate while the last reaction is conversion of oxaloacetate back to acetyl-CoA.

The entire pathway involves 8 different enzymes. 3 of these enzymes has $\Delta G < 0 \implies$ the reaction is irreversible which includes: **citrate synthase, isocitrate dehydrogenase and α -ketoglutarate dehydrogenase**. These 3 enzymes are thus the ones that give directionality to CAC. We also note that the first 5 steps of CAC is considered as high-energy (gives off NADH, FADH₂ and GTP) while the last 2 are recycling steps.

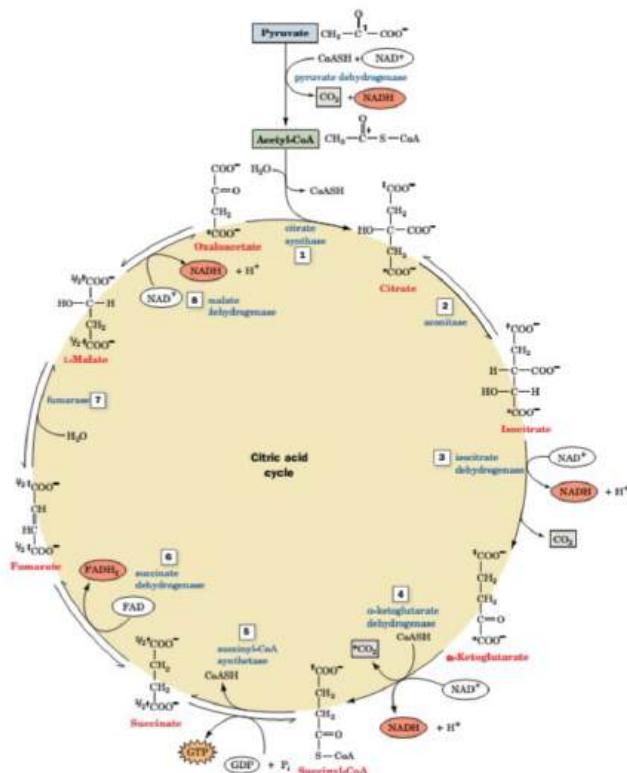
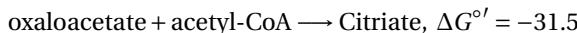


Figure 2.29: Complete citric acid cycle.

2.5.2 High-Energy Steps

Citrate synthase

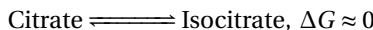
The overall reaction of citrate synthase follows



This is the only step in the entire CAC where new carbon bonds are formed. This step is irreversible.

Aconitase

The overall reaction of aconitase follows



In this reaction, citrate is first dehydrated to form *cis*-aconitate and then rehydrated to form isocitrate.

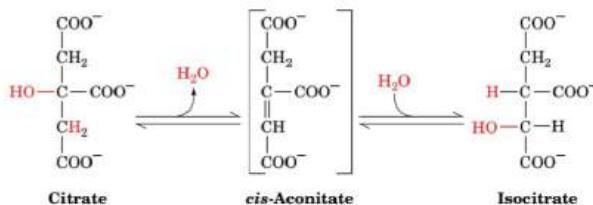


Figure 2.30: Citrate to isocitrate .

Isocitrate Dehydrogenase

The overall reaction of isocitrate dehydrogenase follows



First, isocitrate is deprotonated in order to form NADH. The deprotonated form, oxalosuccinate, will then be decarboxylated to form another intermediate that can be reprotonated to form α -ketoglutarate. This reaction is irreversible.

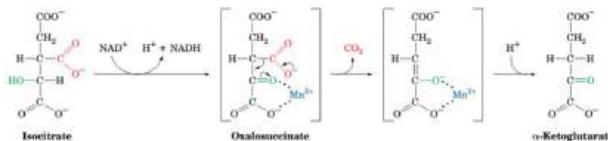
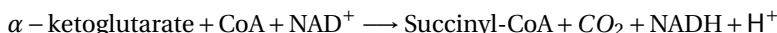


Figure 2.31: Conversion of isocitrate into α -ketoglutarate.

Ketoglutarate Dehydrogenase

The overall reaction of ketoglutarate dehydrogenase follows



where $\Delta G = -33$. The enzymes of this reaction is similar to that of pyruvate dehydrogenase, that is, it's a multienzyme complex made up of E1, 2 and 3 subunits. In fact, its E3 are the exact same with pyruvate dehydrogenase. What's important to this reaction is the attachment of CoA that lead to a high energy intermediate that can be coupled to the next reaction. This reaction is irreversible.

Succinyl-CoA Synthase

The reaction of succinyl-CoA synthase follows



Notice that the reaction of breaking succinyl-CoA to succinate will release some energy ($\Delta G = -32.5$) which can be coupled to the generation of GTP synthesis ($\Delta = +30.5$). Hence, the overall reaction is approximately 0 and thus reversible.

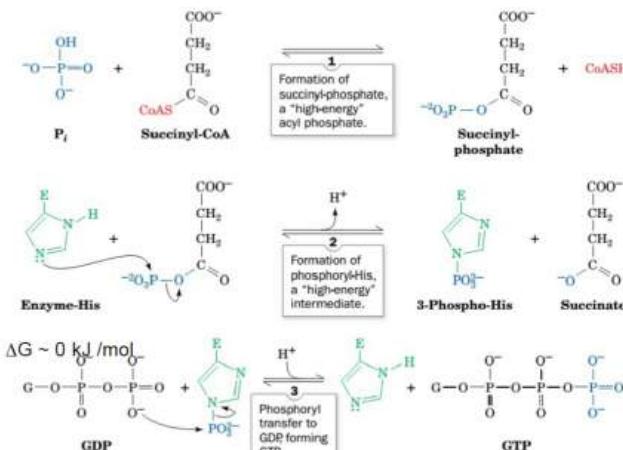


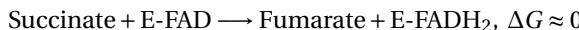
Figure 2.32: Succinyl-CoA to succinate.

Up until this point in the CAC, 1 GTP and 2 NADH is made.

2.5.3 Recycling Steps

Succinate Dehydrogenase

The reaction of succinate dehydrogenase follows



In this reaction, succinate is oxidized by succinate dehydrogenase to form fumarate while the FAD attached to the enzyme is reduced to FADH₂.

Remark 2.20. *Succinate dehydrogenase is the same enzyme in complex II of oxidative phosphorylation.*

Because of this, succinate dehydrogenase-FADH₂ can restore its own e⁻ into the electron transport chain.

Fumarase

The overall reaction of fumerase follows



First fumarate is hydroxylated to form a carbanion transition intermediate which can then be protonated into malate.

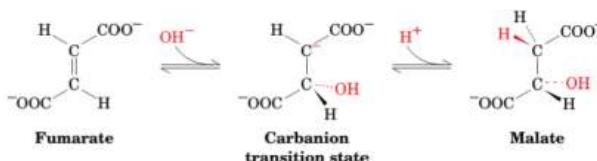


Figure 2.33: Fumarate to malate.

Malate Dehydrogenase

The overall reaction of malate dehydrogenase follows



One thing to note is that theoretically, this reaction is actually not favourable to make oxaloacetate since $\Delta G^\circ > 0$. However, in reality, the [oxaloacetate] is so low that it pushes that reaction forward. Not only that, oxaloacetate can couple to higher exergonic reaction of citrate synthase \Rightarrow the overall CAC will be driven forward.

2.5.4 Energy Totality

So now, let's look back at how much energy we've produced so far using 1 glucose molecule.

Observation 2.24 Glycolysis and pyruvate. In this step, you've produced a net of 2ATP and 2NADH (which is equivalent of 5ATP). Now, conversion of 1 pyruvate to acetyl-CoA will generate 1 NADH (or 2.5ATP). However, it must be noted that 1 molecule of glucose produced 2 pyruvate \Rightarrow 5ATP \Rightarrow total of $2 + 5 + 5 = 12\text{ATP}$.

Observation 2.25 CAC. In this cycle with only 1 acetyl-CoA, we've produced 3NADH (or 7.5ATP), 1 FADH₂ (or 1.5ATP) and 1GTP (or 1ATP). Now, 1 pyruvate produces 1 acetyl-CoA \Rightarrow 2 pyruvate produce 2 acetyl-CoA \Rightarrow CAC made 6NADH (or 15ATP), 2 FADH₂ (or 3ATP) and 2GTP (or 2ATP) \Rightarrow a total of 20ATP.

So in the end, we've fully generate 32ATP with 1 molecule of glucose.

2.5.5 CAC Regulation

When it comes to the regulation of CAC, we'll be mainly focusing on controlling the irreversible steps (for obvious reason). The mechanism for this regulation includes lots of allosteric inhibition and activation, and even competitive inhibition. In fact, **CAC is actually regulated by feedback mechanisms that coordinate its production of NADH and energy usage.**

Citrate Synthase

First, citrate synthase is regulated by [oxaloacetate] and [acetyl-CoA] which are its own substrates. It also have product inhibition via citrate i.e. a build up of citrate will lead back to its own inhibition. In this particular case, it will act as a competitive inhibitor of oxaloacetate for the binding site on the enzyme.

You can also have competitive feedback with succinyl-CoA later on that competes with acetyl-CoA. It's a competitive feedback because it's not a product directly from the citrate synthase reaction. Lastly, it has an allosteric inhibitor of NADH and activator of ADP.

Isocitrate Dehydrogenase

Because isocitrate dehydrogenase is the first step in CAC to produce NADH, it will be evident that NADH will act as its product inhibitor. When it comes to allosteric activator, it has 2 and those are ADP and Ca^{2+} . When it's inhibited and turned off, the [isocitrate] $\uparrow \Rightarrow [\text{citrate}] \uparrow \Rightarrow$ it exits into the cytoplasm. Here, it can do lots of things like inhibiting PFK \Rightarrow inhibiting glycolysis. Or it can activates acetyl-CoA carboxylase to activate fatty acid synthesis.

Remark 2.21 (citrate). \uparrow is usually indicative of high energy charge.

If citrate can leave mitochondria and inhibit glycolysis, there's another "crosstalk" between pyruvate and CAC where pyruvate is carboxylated to produced more oxaloacetate thus driving the CAC forward.

α -Ketoglutarate Dehydrogenase

For α -ketoglutarate dehydrogenase, its product inhibitors are NADH and succinyl-CoA. While its allosteric activator is Ca^{2+} .

More Regulation and Summary

To look back at other enzymes we've talked about, we see that PFK will be inhibited in the presence of citrate, pyruvate dehydrogenase will be inhibited by acetyl-CoA and NADH, and pyruvate carboxylase will be activated by acetyl-CoA.

In total, it's NADH that plays a central role in regulation here. When the cell is requiring energy, CAC will increase its flux to the max. Otherwise, its intermediates will be used for some other reactions.

When energy charge is high, there's an efflux of citrate to the cytosol and this will stimulate the biosynthesis of fatty acid while inhibiting glycolysis.

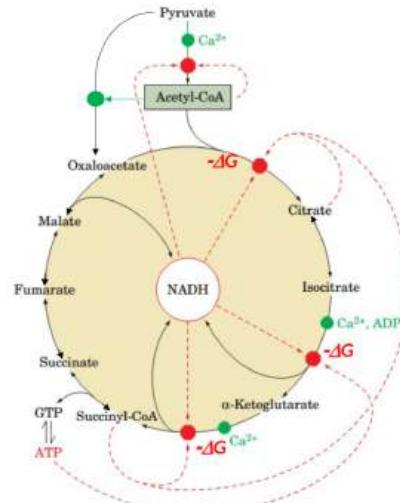


Figure 2.34: Regulation of CAC.

Lecture 11: September 25th, 2024.

2.6 Anaplerotic and Cataplerotic Reactions of CAC

Like we've mentioned from previous lecture, CAC is amphibolic i.e. it can perform both anabolism and catabolism of certain biomolecules. To be more specific, CAC can perform cataplerotic and anaplerotic reaction which can deplete or replenish the CAC intermediates respectively.

Observation 2.26 Notice that when you remove something from the CAC, it will be replenish back and the major route to do this is through pyruvate carboxylase. This enzymes can turn pyruvate back to oxaloacetate.

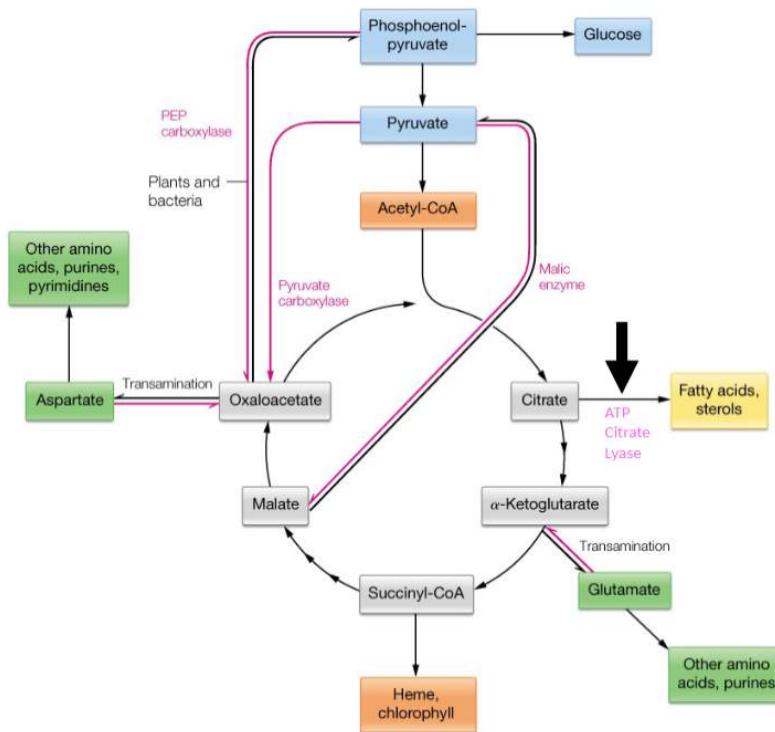


Figure 2.35: Cataplerotic and anaplerotic reactions of CAC.

Remark 2.22. We will be referencing on figure 2.35 a lot so just to note, black arrows are for anabolic pathways (cataplerosis for CAC) while pink arrows are for catabolic pathways (anaplerosis for CAC)

2.6.1 Cataplerotic Reactions

For cataplerotic reactions of CAC, the body is performing anabolism which can be glucose, fatty acid or amino acid biosynthesis.

Example 2.6.1. Let's look at glucose biosynthesis. We begin with oxaloacetate which is transported out of mitochondria by converting into **malate** or **aspartate**. Once transported out, it can then be reverted back to oxaloacetate. It can then be decarboxylated into phosphoenolpyruvate and enter gluconeogenesis.

Example 2.6.2. For fatty acid biosynthesis, we need acetyl-CoA which cannot exit the mitochondria. So CAC uses citrate instead to be first transported out to the cytosol and then it can be converted back to acetyl-CoA using **ATP citrate lyase**. From then, fatty acid biosynthesis can begin using the newly made acetyl-CoA.

Example 2.6.3. For amino acid biosynthesis, there are 2 **transamination** reaction that can be done on the CAC intermediates. It's called transamination because you're **converting a CAC intermediate that have chemical backbone of amino acids (beside having the amines)**.

In this case, we have oxaloacetate that can be transaminated into aspartate which can then form other amino acids. Then, you also have α -ketoglutarate which can be transaminated into glutamate to make other amino acids as well.

Remark 2.23. When there's an excess of amino acids post-meal, there will be excess nitrogen (from amine group) which will be get rid of in urea cycle.
4

There's also another transamination pair such as that of pyruvate and alanine.

2.6.2 Anaplerotic Reactions

We will now look at the anaplerotic reactions where we will replenish CAC intermediates. There are 3 main reactions for this replenishing: **pyruvate carboxylase**, **pyruvate dehydrogenase** and **transaminase**.

Example 2.6.4. To replenish oxaloacetate, CAC and use pyruvate directly using the enzyme **pyruvate carboxylase**. To replenish malate, you have

⁴This is because the amino acids will be broken down to oxaloacetate or α -ketoglutarate while releasing the amine groups under excess condition.

malic enzymes can that convert pyruvate and NADPH back to malate. Note: The reverse reaction of malic is essentially for fatty acid biosynthesis as it makes NADPH.

Example 2.6.5. For the transamination, we've already talked about in the anaplerotic reactions (it can go back and forth depending on the need of the body). What you can see is that the structure of these CAC intermediates are very close to that of amino acids, specifically glutamate + α -ketoglutarate, and aspartate + oxaloacetate \Rightarrow transamination is possible.

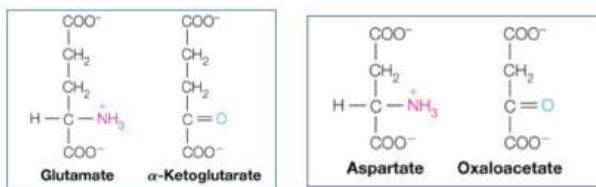


Figure 2.36: Transamination pair.

Remark 2.24. *Glutamate to α -ketoglutarate is an important transamination in the body.*

Explanations. The reason that glutamate is because we have an enzyme called **glutamate dehydrogenase** can convert irreversibly glutamate into α -ketoglutarate using water and releasing NADH at the same time as well. One of a by-product of this reaction is ammonia which can be eliminated in the urea cycle.⁵ □

Also, this enzyme can be allosterically activated by ADP while inhibited by ATP.

2.7 Oxidative Phosphorylation I

In today's lecture, we will look at oxidative phosphorylation but first, we will look at the organelle that is the main site of action for this: the mitochondria.

⁵Theoretically, you can have the reverse reaction but you'd need a really toxic level of ammonia to do so.

2.7.1 Introduction to the Mitochondria

Observation 2.27 Here, we see a mitochondrion with its double membrane with the inner one have folds and crevices called **cisternae**. Interestingly, it's at these cisternae is where the machinery of the oxidative phosphorylation lies and does it function. The interior of the inner membrane is called the **matrix** while the space between the 2 membranes is called **intermembrane sapce**.

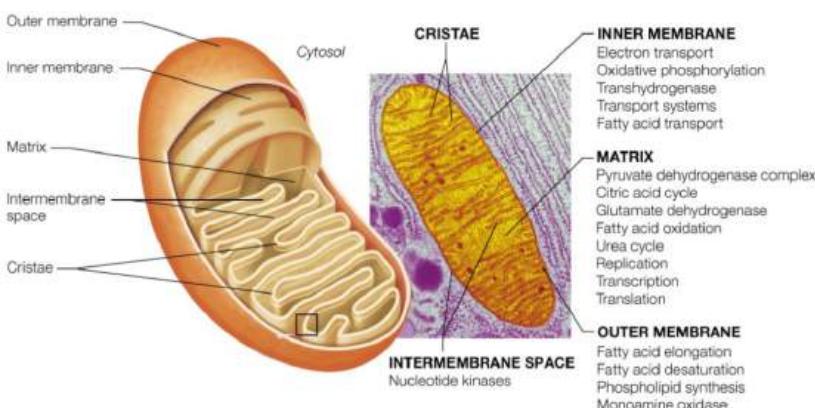


Figure 2.37: Mitochondrion structure.

Definition 2.22. The **electron transport chain (ETC)** is a series of different proteins complexes (complex I-IV) that perform oxidative phosphorylation.

Observation 2.28 Now, let's look at the ETC in general. It begins with NADH oxidized by complex I into NAD^+ i.e. NADH donates its $2e^-$ to complex I which will travel through a series of redox centers. N.B. **These redox center will have higher reduction potential as you go down the chain with oxygen being the highest**⁶ When the e^- flows through the complex I, 4H^+ will be pumped out of the matrix by complex I. Similarly, for complex III, it's 4H^+ and for complex IV, it's 2H^+ .

⁶Higher reduction potential means the center wants to accept more electrons than the previous.

So in the end, you'd get a total of 10H^+ for $2e^-$ (that will end up in making 1 water molecule) \Rightarrow 10H^+ are stoichiometrically equivalent to 10 or $2e^-$.

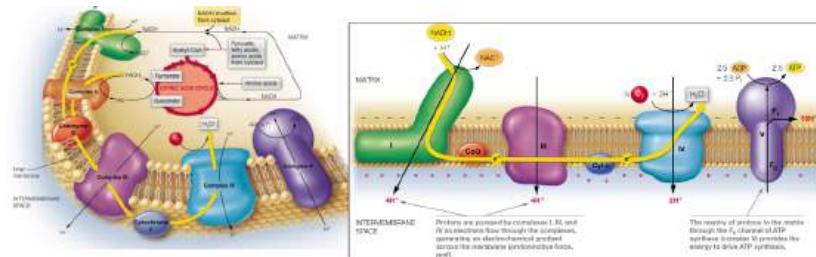


Figure 2.38: The ETC.

The purpose of this entire ETC is to create an **electrochemical gradient** i.e. there's a chemical gradient with more H^+ outside of the matrix than inside + more positive in the outside than inside.

Definition 2.23. This electrochemical gradient will create a potential between the intermembrane space and matrix called the **proton motive force (PMF)** which will be used to drive ATP synthase.

Remark 2.25. *ATP synthase will make 3 ATP molecule for every full rotation (360°).*

Observation 2.29 It was measured that the electrical potential is around 150-200mV which is barely nothing. Nevertheless, if we were to consider the size of a mitochondrion in question, we'd realize the actual number it experiences is around 300 million V/m (comparable to a lightning bolt).

2.7.2 The Electron Transport Chain

Observation 2.30 One thing you can see from the ETC that we've said is that there's lots of redox center that would be increasingly higher in reduction potential. Starting with complex I and II, they have **flavoproteins** which is a class of protein that have FAD in their structure \Rightarrow can accept electron. To be specific complex I has **flavin mononucleotide** while complex II has **FAD** (remember that complex II is also known as the succinate dehydrogenase).

The next thing you can find are these **iron-sulfur clusters (Fe-S)** that's present on complex I-III. Next are **cytochromes**, that have heme groups (which have irons) are mainly found from complex II-IV.

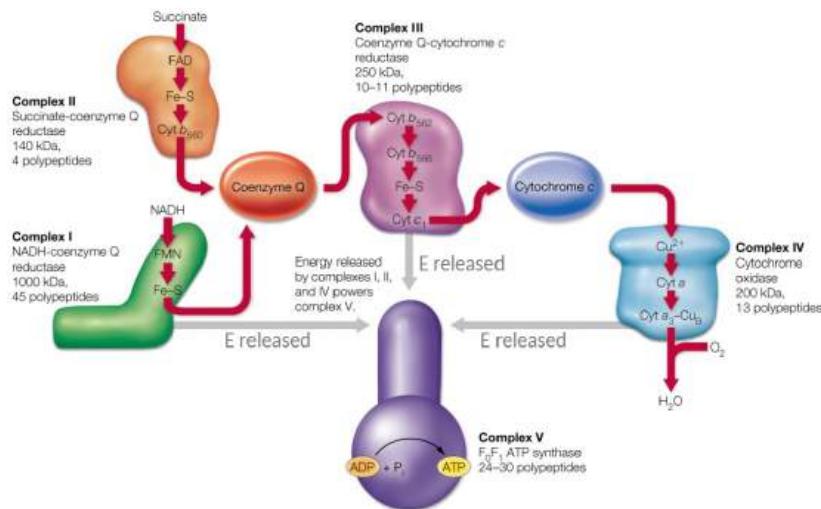


Figure 2.39: The ETC in detail.

Concept 2.1 The standard reduction potential can change depending on the local microenvironment of a particular protein.

Observation 2.31 e^- will flow through both complex I and II independently of which both will unload them in the **coenzyme Q (CoQ)** (Q pool) that can then feed to complex III. It's important to remember that unreduced form of CoQ is also known as **ubiquinone**, when it's fully reduced it's called **ubiquinol** and at half reduction - **semiquinone**.

Going from complex III to IV, there's another protein called **cytochrome C** that can mediate/shuttle the e^- flow. Interestingly, complex IV is also known as the **cytochrome C oxidase** because it oxidizes cytochrome C of which the donated e^- will be moved to oxygen to make H₂O.

As e^- being flow through complex I, III and IV, energy will be released in the form of pumping H⁺ out that will drive synthesis.

2.7.3 Complex I to IV

We will now look more in-depth at the 4 complexes (structure and function-wise) that's essential for oxidative phosphorylation.

Complex I

Observation 2.32 First, when looking at complex I, it has a hydrophobic complex, that sits parallel to the inner membrane, and a hydrophilic arm that extend inside the matrix.⁷ This complex is made from 44 different proteins that are located on 2 genomes: 7 of the 44 are that of mitochondrial DNA while the rest are from the cell nucleus. This means complex I synthesis requires a fairly good communication between the nucleus and the mitochondria.

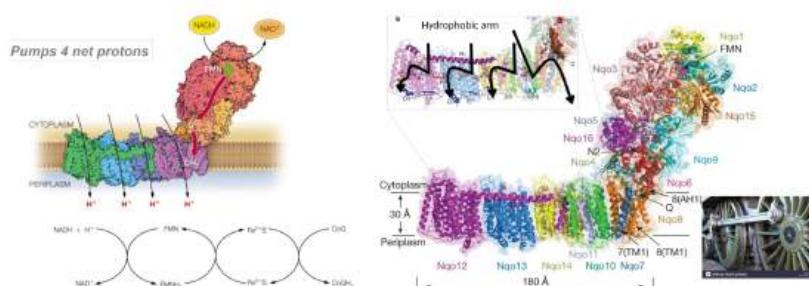


Figure 2.40: Illustration and crystal structure of complex I

Observation 2.33 So you start first in the FMN redox center that slowly where NADH will donate its e^- . Then, it will move through Fe-S and then into CoQ (ubiquinone). At CoQ, it will be fully reduced to ubiquinol.

Another thing you can see when looking at the above illustration, that from the subunit Nuo12, it has a long arm extended parallel to the hydrophobic complex. We can think of this arm as the *coupling rod* on a train i.e. when it moves, a series of H^+ channel open; and when it moves again, these channels close.

Remark 2.26. These H^+ channels evolved from H^+ transporters.

⁷The illustration was that of a bacterial which is why it's called the cytoplasm instead of matrix, and periplasm instead of intermembrane space.

Complex II

Observation 2.34

Unlike other complexes, complex II do not transport H^+ but will feed to the Q pool. Structurally, it has a transmembrane section and a hydrophilic head, that is composed of 2 subunits and extend into the matrix.

With its extension to the matrix via the hydrophilic head, it can capture the e^- coming from $FADH_2$ being oxidized to FAD^+ . This electron will travel from FAD to Fe-S, CoQ and Heme. In all cases, all of these e^- will be dumped into the Q pool.

Observation 2.35 Now, before moving on to the next complex, we must mention that there are 2 ways to donate even more electrons to CoQ for the Q pool.

1. In Matrix: There are lots of dehydrogenase with an FAD center that can capture e^- once the enzymes does its reactions. Then, this FAD will relay these e^- to **electron transferring flavoprotein (ETF)**, then **ETF-QO** which finally ended in CoQ.
2. In Intermembrane Space: Suppose that you want to transport some cytosolic NADH into the matrix for usage...well you can't since there's no such transporter. Now, another way to do this is to convert it into G3P that can move through the membrane

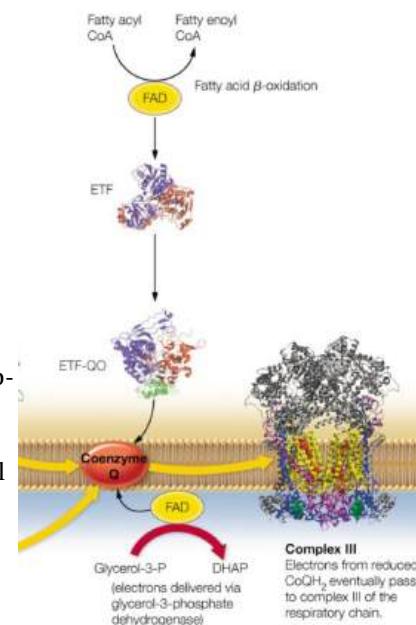
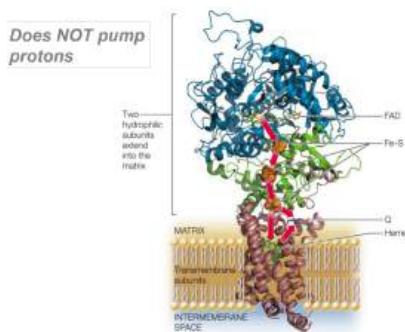


Figure 2.41: Complex II crystal structure (top) and 2 ways to add e^- to CoQ.

easily. At the intermembrane space, G3P can be oxidized by **G3P hydrogenase** (that has an FAD center) into DHAP. This dehydrogenase can then donate its e^- to CoQ.

Complex III

Observation 2.36 So now, complex III can transport $4H^+$ similar to how complex I is. Its main e^- source is coming from **ubiquinol, NOT ubiquinone**. This is because the ubiquinol was completely reduced by the donation of e^- earlier by complex I and II.

An important aspect that's part of complex III is the Q cycle which involves the Q pool that we've been mentioning. The reason that the Q cycle is necessary is because only $1e^-$ is utilized in complex IV to make the water molecule \Rightarrow you have $1e^-$ to go somewhere (Q cycle solves this).

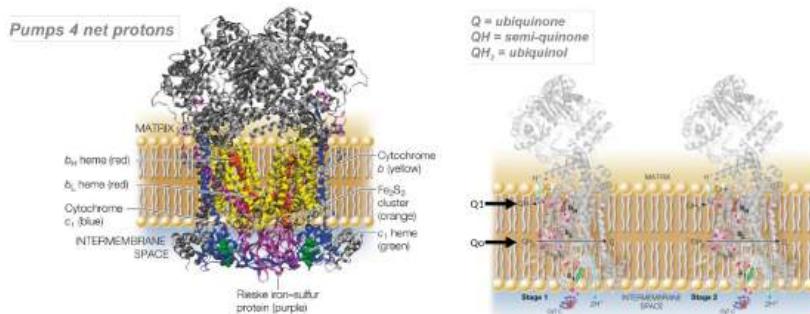


Figure 2.42: Complex III and mechanism of Q cycle.

Mechanism of Action (Q cycle): In the Q cycle, there are 2 stages and it takes place at 2 sites: Q_1 and Q_O .

1. (Stage 1) Starting at the Q_O site, ubiquinol comes and binds to complex III where it's fully oxidized into ubiquinone. 1 of its e^- will move down to cytochrome C (to complex IV) and the other will be used to reduce ubiquinone into semiquinone in the Q_1 site.
2. The semiquinone^a will enter stage 2 in the Q_1 site where it will be reduced again with $1e^-$ to make ubiquinol. They got this $1e^-$ from another fully oxidized ubiquinol again in its respective Q_O site (the other 1 will move to cytochrome C).

3. In the end, 2 cytochrome molecule is reduced and now each is carrying $1e^-$ 1.

^aThis semiquinone doesn't necessarily need to be the same as the one in step 1.

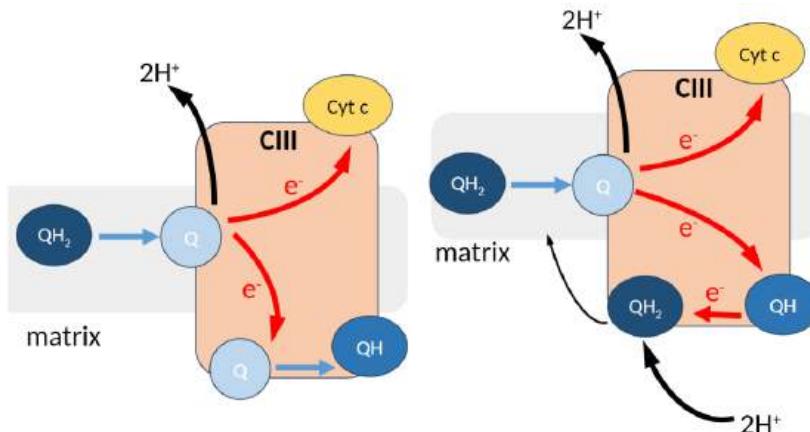


Figure 2.43: Simplified version of Q cycle.

Complex IV

There's not much to discuss with complex IV as it's like complex I and III with a modification that it transports only $2H^+$ and can catalyze the reaction of making water molecule.

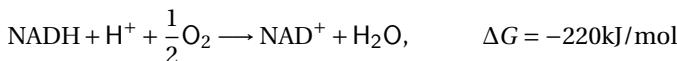
Respirasome

It's a common misleading idea that these complexes form a chain (hence the name of ETC) but they're actually arranged into a massive supercomplex called the **respirasome**. This makes sense because we've explained before that when pathways and enzymes sit closer, its efficiency increases.

ΔG of NADH to Oxygen

Now, it's evident that there's a series of reactions one after the other that lead to the synthesis of a water molecule and ATP. If we add all of them up, you'd

get the following reaction:



We can calculate this ΔG by using the difference in standard reduction of NADH to be -0.32 V and for O_2 , which is $+0.82 \text{ V}$. To be specific, we will add up all of the energy produced as electron flow through the system.

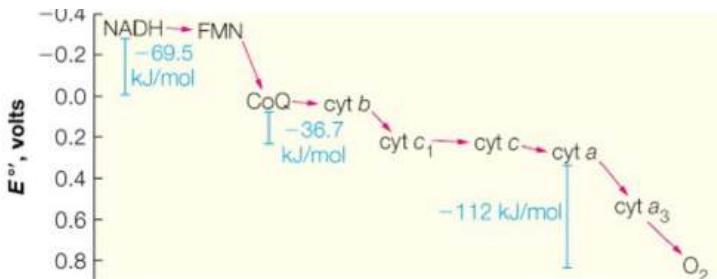


Figure 2.44: Standard reduction of the entire ETC.

Observation 2.37 We know that the energy needed to produce 1ATP is roughly 50 kJ/mol . We know that 1NADH produce around 2.5ATP thus going from 1NADH to ATP will need around 125 kJ/mol of energy of which the above reaction can most definitely provide.

Question: Why do we need so much redox reactions?

Answer: Well, it's because the synthesis of H_2O is a combustion reaction and releases lots of energy. So instead of dumping all of this energy at once of which the cell can barely harvest, they make a series of redox reactions that slowly extract the energy in the form of e^- and H^+ movement.

2.7.4 Shuttles of NADH

We will now discuss the methods of shuttling NADH of which there's 2 ways: **G3P and malate/aspartate shuttle**.

G3P shuttle

First, you reduce DHAP by NADH to form G3P that can then travel through the outer membrane of the mitochondria. In the intermembrane space, it will be captured by G3P dehydrogenase that extract the e^- to put on FAD

forming FADH₂ to Q pool then complex III, while reproducing DHAP again. The DHAP will move out from the outer membrane into the cytosol.

Remark 2.27. This process will produce less ATP because it bypass complex I which will not pump H⁺ \Rightarrow lower pmf.⁸

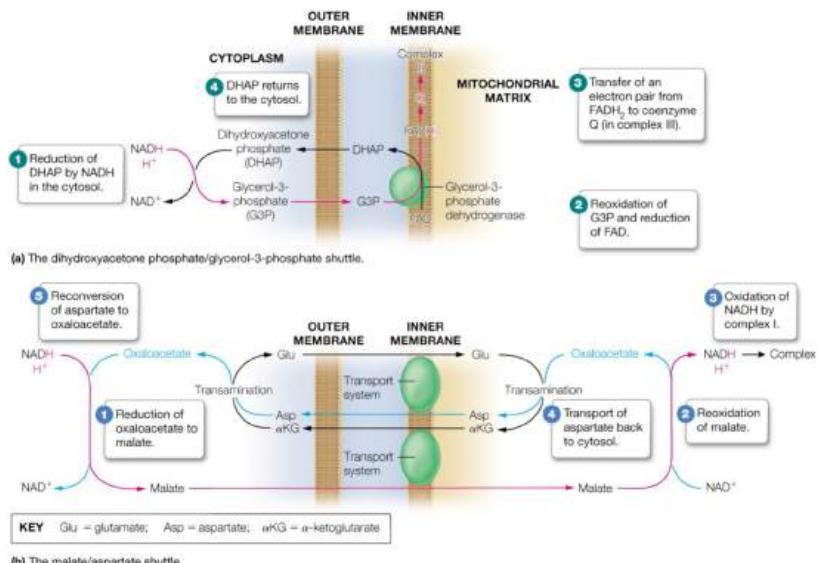


Figure 2.45: Shutting of NADH.

Malate/Aspartate Shuttle

First, oxaloacetate will be reduced into malate by NADH. This malate can pass through both mitochondrial layer to be inside the matrix. Here, the malate will be reoxidized into oxaloacetate but also making NADH for complex I. Now, the oxaloacetate along with the transported in glutamate will be transaminated into aspartate and αKG respectively.

An **antiporter** protein will couple the out-flux of aspartate with that of influx of glutamate. Meanwhile, another transporter will do the same for the influx of malate into the matrix and out-flux αKG to the cytosol. Once

⁸This is also why FADH₂ can only make 1.5ATP.

outside, the α KG and aspartate will be revert back into glutamate and oxaloacetate respectively.

2.7.5 ATP Synthesis

Observation 2.38 Let's look at an ATP synthase⁹. It has 2 portions that are: F_1 and F_O . The F_1 portion consists of a literal turbine that can rotate to produce ATP. The F_O portion, on the other hand, will have subunits to accept H^+ that uses its pmf to drive the rotation.

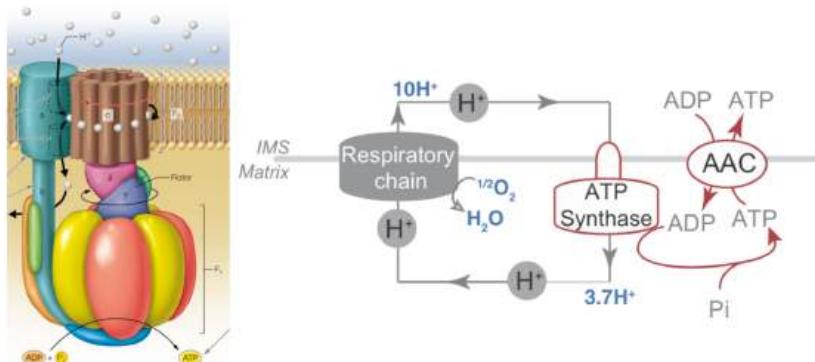


Figure 2.46: ATP synthase and P/O ratio

Definition 2.24. The **P/O ratio** is the stoichiometry of oxidative phosphorylation with P means ATP and O means oxygen i.e. P/O ratio means the amount of ATP made for every oxygen atom consumed.

Concept 2.2 The P/O ratio differs depending on the where the electrons comes from along the ETC.

Example 2.7.1. The P/O ratio of complex I is $10/3.7 \approx 2.5$ and this number is the same as 1 NADH to make 2.5ATP (evident since complex I is from e^- extraction of NADH). Similarly for complex II with $P/O = 6/3.7 \approx 2.5$ (Same for $FADH_2$ making 1.5ATP)

⁹It was called ATP synthase even though it's making ATP is because when it was first identified, it could hydrolyze ATP

Where do you get 10? It comes from the transport of H^+ by complex I-IV which is 10. If we're going from complex II, we will exclude the first 4 \Rightarrow 6.

Where do you get 3.7? Well...first let's look at the ATP synthase. We've said before that every 360° , 3ATP is made. Now, we need to know the amount of H^+ used to make 3ATP. Well, we realize that the amount of H^+ correlates to the amount of subunits in the F_O portion. For mammals this is around 8 \Rightarrow $8H^+$. Thus, $8/3 \approx 2.7$.

We also have to account for the fact that we make ATP from ADP \Rightarrow We need P_i . The thing with P_i is that it's highly negatively charged \Rightarrow To not influence the electrochemical gradient, you need to couple it with a positively charged particle: H^+ . So accounting we need $1H^+$, we'll get $2.7 + 1 = 3.7$.

2.8 Oxidative Phosphorylation II

In today's lecture, we will briefly go through the historical discovery of oxidative phosphorylative pathways and more on the mitochondria, diseases and control.

2.8.1 Historical Perspective

The 2 main scientists that play an important role in this discovery are: **Peter Mitchell and Edward Slater**. These 2 scientists independently developed their own hypothesis on how oxidative phosphorylation took place.

- Edward Slater: He believed and came up with the **chemical coupling hypothesis**. Essentially, ATP is made from a high energy intermediate of the respiratory chain directly.
- Peter Mitchell: He believed and came up with the **chemi-osmotic theory**. Basically, he theorized that the energy released by NADH oxidation will be stored in the membrane potential that will be later used to drive ATP synthesis.

Obviously from the few previous lectures, the chemi-osmotic theory is right. **How do we know that Peter Mitchell was right?** Well...because of the following:

1. The respiratory chain can still function without phosphate.
2. The number of ATP made through NADH was not an integer.
3. You need an intact IMM to make oxphos \Rightarrow membrane is important to the gradient.
4. Key electron transport proteins are embedded in the IMM.
5. There exists uncouplers like DNP to inhibit ATP synthesis and increase oxygen consumption.
6. You can generate an artificial H^+ gradient that drive ATP synthesis without electron transport chain.

So even though Edward Later was wrong, it still provided a framework to help with understanding oxidative phosphorylation.

2.8.2 Mitochondrial Poisoning

Looking at complex I, we want to know how much energy is driven or made.

Methods 2.1 What they did was giving **antimycin A** that blocks complex III. This is to isolate complex I and IV. Then, they added **ferricyanide**, a channel that allow electrons to pass through, in complex I. Essentially, electron can enter complex I and get out from ferricyanide.

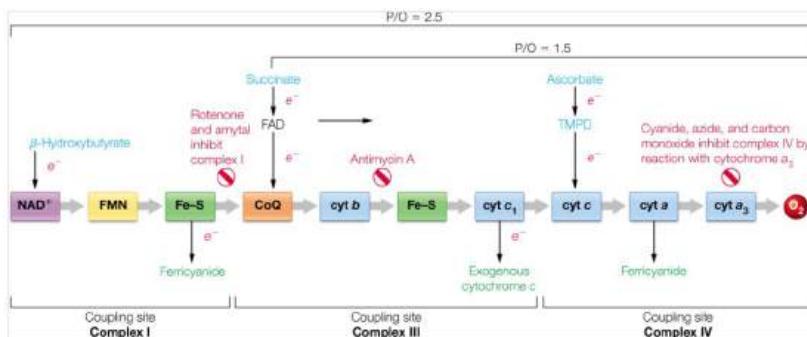


Figure 2.47: Measuring P/O ratio of each complexes.

Observation 2.39 What we'd measure is that with complex I alone it still has a P/O ratio of 1. Now, going to complex IV, with the same model as

above, we can first provide electrons to cytochrome c via ascorbate and build a ferricyanide channel at cytochrome *a*. We'd measure that strangely, complex IV has a P/O ratio of 1 also.

Remark 2.28. As of today, there's still debate whether complex IV is transporting 2 or 4H^+ . Nevertheless, the idea here is that you don't have to go from complex I to IV to make ATP.

Methods 2.2 Now, we can block complex IV with cyanide while adding succinate to provide electrons to complex III with an exogenous cyt C.

Observation 2.40 The P/O ratio of complex III was measured to be 0.5

Definition 2.25. **Dinitriphenol (DNP)** is a very toxic chemicals used as an uncoupler

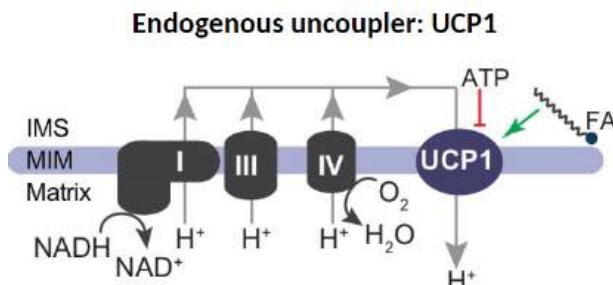


Figure 2.48: Endogenous uncoupler of the body.

Observation 2.41 Accordingly, DNP has a very small therapeutic window for weight loss. Another approach is through using endogenous uncoupler like **UCP1** that make the body to spend more energy which can lead to weight loss. The purpose of this uncoupler is for cell to generate heat.

2.8.3 Mitochondria and Diseases

The mt was believed to be a bacteria engulfed in a symbiotic relation with a ancient eukaryotic cells (proto-eukaryotes).

Observation 2.42 Mt has its own DNA of around 16k bp and 13 of them is just for proteins of the ETC. Wait...but mitochondria has 2000+ proteins,

where are those coming from? Well...it's imported in from the nuclear genome.

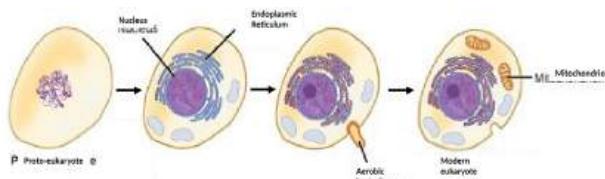


Figure 2.49: Mitochondria origin.

You can have diseases that relates to mt DNA mutations and there are lots of them, but mostly metabolic diseases that's related to muscle fatigue, mental illnesses, exercise intolerance, etc. Another thing that is fascinating is Mitochondrial proteins homeostasis as it has to coordinate with 2 different genomes to build its own proteins in the ETC.

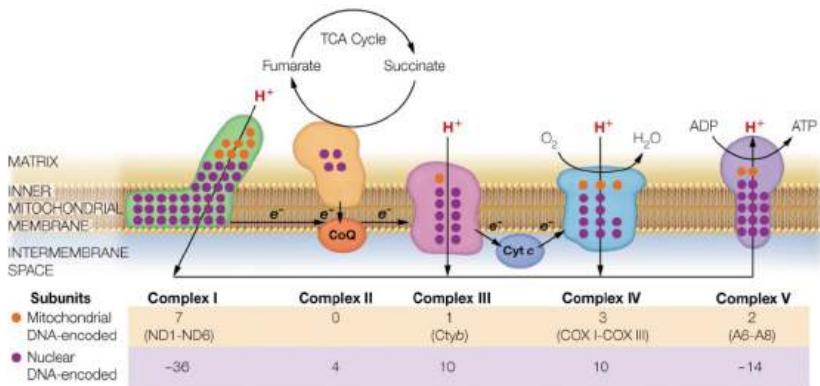


Figure 2.50: Mitochondrial protein homeostasis.

Remark 2.29. When looking at the assembly factors, we can get lots of different diseases from it having defective nuclear genes as well.

2.8.4 Respiratory Control

Definition 2.26. **Respiratory control** is simply the control of the respiratory chain.

Explanations. Suppose that you have lots of O_2 , a limiter to the flow of e^- through the ETC is ATP. This is because if H^+ flows and make ATP, electrons can then flow through the ETC. So, when $[ATP] \uparrow$, electrons stop flowing, v.v. and that is respiratory control. \square

Methods 2.3 Using an oxygen electrode you can measure the consumption of oxygen of the buffer in the machine. We first add glutamate to mitochondria and measure the O_2 consumption. Then, add $0.25\mu M$ of ADP and P_i .

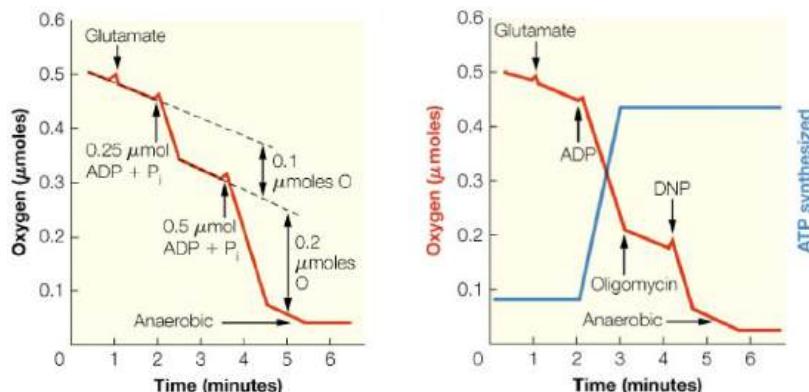


Figure 2.51: Respiratory control experiment.

Observation 2.43 When you added the glutamate, you can see that it does have a little consumption of O_2 , this is because you have a transamination pathway for glutamate to enter. When adding the $0.25\mu M$ of ADP and P_i , the consumption of O_2 will increase because the mitochondria is consuming O_2 to make ATP. However, it will slow down and this is because ATP is building up so there's no need to use more O_2 . Lastly, if added more and high enough level of ADP, it will begin anaerobic synthesis of ATP.

Observation 2.44 We can then overlay the ATP synthesis graph with the oxygen consumption graph (See Figure 2.51). As we added the ADP, we get a rise in ATP production while oxygen consumption decreases. Here, we also add DNP at the end, of which we can see that ATP synthesis plateau (i.e. stop) because it's an uncoupler which remove that membrane potential used to make ATP.

Lecture 14: October 2nd, 2024.

2.9 Oxidative Phosphorylation III

In this lecture, we'll have a much closer investigation on the ETC and oxidative phosphorylation.

2.9.1 ATP/ADP Carrier

We've discussed previously about the transport of inorganic phosphate along with H⁺ of which we've said that this is electronically neutral...**but isn't inorganic phosphate has a charge of -3?** Well...because *in vivo*, the P_i is transported in the form of H₂PO₄ which is only charged -1 \Rightarrow if transported with H⁺ will be neutral.

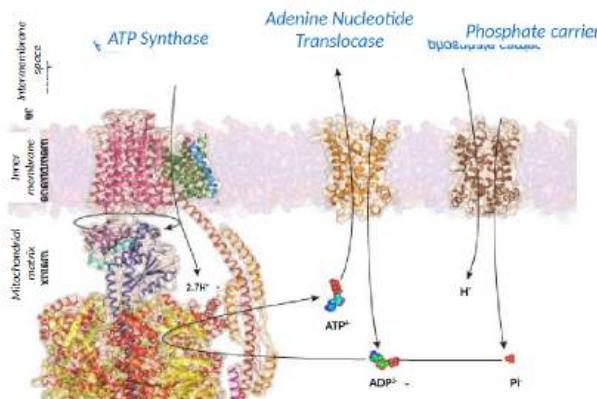


Figure 2.52: ANT and other transporters.

Observation 2.45 Another important transporter that's present is the **adenine nucleotide translocase (ANT)**. This ANT can transport ATP to the intermembrane while bringing in ADP. This can happen because the intermembrane space is positively charged which pull the ATP out. Notice too that ATP has charges of -4 while ADP has charge of -3 \Rightarrow with the transport of ATP and ADP, the membrane potential will be hyperpolarized.

Hold on...coming back to H₂PO₄, you're bringing in more H⁺ so **how is that going to impact everything?** Well...not that much this is because we're not just looking at the H⁺ gradient, we're looking at the electrochemical gradient. For you information, **the electrical gradient will play a higher**

importance than the chemical gradient. Thus, the addition of $2H^+$ would be negligible to the gradient.

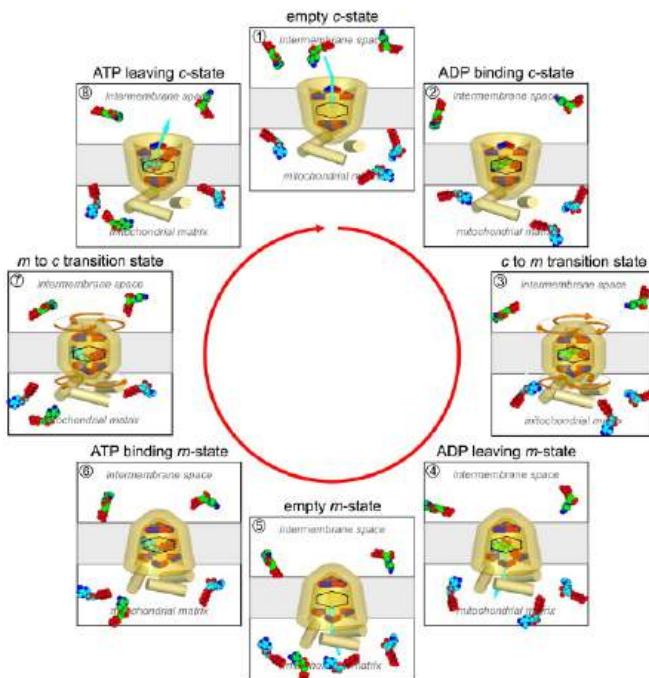


Figure 2.53: Mechanism of Action of ANT.

Mechanism of Action (ANT): we begin with the ANT in its empty **c-state** (open to the cytosolic side)

1. Here, ADP can come to bind to it and changes to conformation to the **m-state** (open to the matrix side).
2. In the matrix, the ADP unbind and allow an ATP to come and bind.
3. This binding trigger conformational change back to the c-state. Now, the ATP will unbind which return to the empty c-state ANT for ADP to bind.

2.9.2 ΔG for Each Complexes

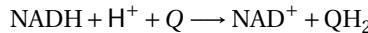
We'll now have a little look on how to calculate the $\Delta G^{\circ'}$ for each complexes. We need to introduce to you the Nernst equation as follow

$$\Delta G^{\circ'} = -nF\Delta E^{\circ'} \quad (2.4)$$

where n is the number of e^- , F is Faraday's constant given as 96.4kJ/V/mol and $\Delta E^{\circ'}$ is the different between reduction potential of the e^- acceptor and donor.

Complex I-IV

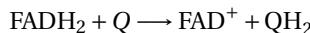
For complex I, we have the following reaction:



We have that $E^{\circ'}$ of NADH and CoQ is -0.315 and 0.045 which yield

$$\begin{aligned}\Delta G^{\circ'} &= -nF\Delta E^{\circ'} \\ &= -(2)(96.4)(0.045 - (-0.315)) \\ &= -(2)(96.4)(0.36) \approx -69.4\text{kJ/mol}\end{aligned}$$

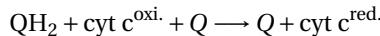
For complex II, we have the following reaction:



We have that $E^{\circ'}$ of FAD and CoQ is -0.040 and 0.045 which yield

$$\begin{aligned}\Delta G^{\circ'} &= -nF\Delta E^{\circ'} \\ &= -(2)(96.4)(0.045 - (-0.040)) \\ &= -(2)(96.4)(0.085) \approx -16.4\text{kJ/mol}\end{aligned}$$

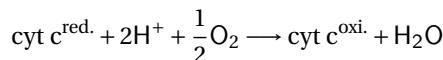
For complex III, we have the following reaction:



We have that $E^{\circ'}$ of Cyt C and CoQ is 0.235 and 0.045 which yield

$$\begin{aligned}\Delta G^{\circ'} &= -nF\Delta E^{\circ'} \\ &= -(2)(96.4)(0.045 - 0.235) \\ &= -(2)(96.4)(0.19) \approx -36.6\text{kJ/mol}\end{aligned}$$

For complex IV, we have the following reaction:



We have that E°' of Cyt C and O_2 is 0.235 and 0.815 which yield

$$\begin{aligned}\Delta G^\circ' &= -nF\Delta E^\circ' \\ &= -(2)(96.4)(0.045 - (0.235)) \\ &= -(2)(96.4)(0.58) \approx -112\text{ kJ/mol}\end{aligned}$$

So now, to determine the overall $\Delta G^\circ'$ of oxidative phosphorylation would be given as

$$\Delta G_1^\circ' + \Delta G_{\text{II}}^\circ' + \Delta G_{\text{III}}^\circ' + \Delta G_{\text{IV}}^\circ'$$

Which would give us around -220 kJ/mol .

2.9.3 F_1 Component

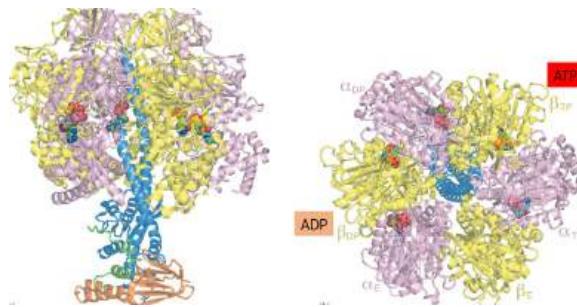


Figure 2.54: Structure of F_1 component

Why does a 360° turn of the ATP synthase make 3ATP? Well...it's because its F_1 's pseudo three-fold symmetry. To be specific, the portion is made from 6 different subunits 3 of which are α while the rest are β . There are only 3 spaces between these subunits where ATP can bind to \Rightarrow 3 ATP is synthesized every cycle.

In this chapter, we will look at all of everything about lipids.

3.1 Lipids

In today's lecture, we're mostly focusing on talking about the absorption, structure and function of lipids.

3.1.1 Structures and Functions

Look at the figure below, could you identify which are lipids?

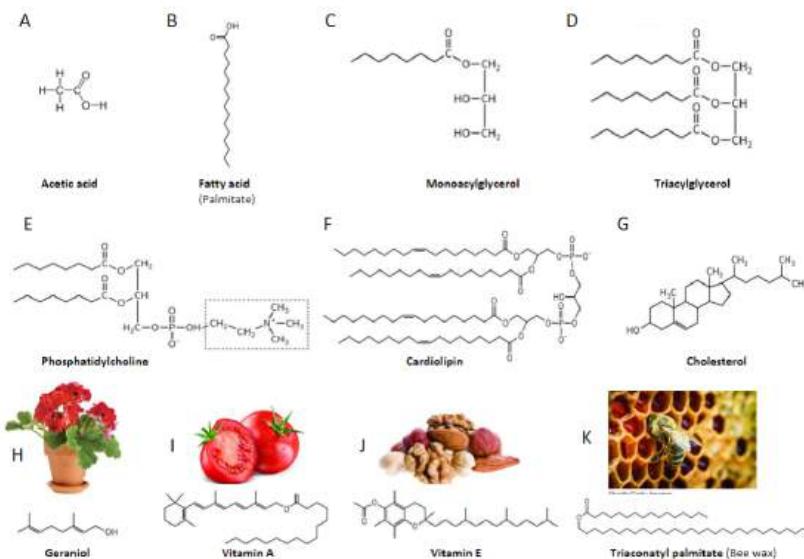


Figure 3.1: Lipids structural variations. **A:** acetic acid, **B:** fatty acid, **C:** monoacylglycerol, **D:** triacylglycerol, **E:** phosphatidylcholine, **F:** Cardiolipin, **G:** Geraniol, **H:** Vitamin A, **I:** Vitamin E and **K:** Triaconyl palmitate (bee wax).

Well...all of them are lipids. Some of the main features that these molecules

seem to shares are: hydrocarbon chain, have oxygen but most important, insoluble as a whole. We will now get to a more formal definition of them.

Definition 3.1. **Lipids** are a heterogeneous group of biological compounds, which includes fats, oils, waxes and steroids that are relatively insoluble in water

Observation 3.1 As the definition mentioned, their main properties are insoluble in water, however, they are soluble in nonpolar solvents e.g. ether and chloroform. Structurally, most have a polar head that are hydrophilic and a non-polar tail that is hydrophobic.

Function-wise, they're used in many different ways, such as

- Energy storage and high energy by nature
- Structural components of biomembranes
- Serve as thermal insulators, signalling molecules and are the precursors for hormones.

Definition 3.2. When lipids are present in water, they will cluster together in sphere-like structures, called **micelles**, with hydrophilic heads pointing out while the hydrophobic tails hiding within.

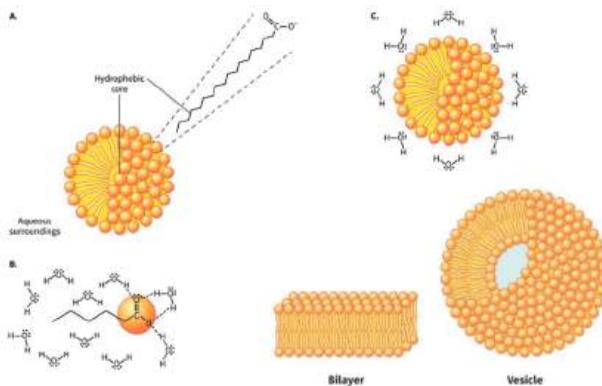


Figure 3.2: Micelles and vesicles.

Similarly, if it was phospholipids, it will form **vesicles** where the hydrophobic tails hides in a bilayer while the hydrophilic heads points outwards/inward (to the inner space).

Observation 3.2 These lipids are located practically everywhere in a cell. For once, they're the building block of the cell membrane and ER. They're also made synthetically in the cytosol, stored in lipid droplets and even used in β -oxidation by mitochondria.

Observation 3.3 Now that we've said all these good things about lipids, having an irregular amount of lipids in blood i.e. **dyslipidemia**, can be quite dangerous.

First, when consumed high-lipid food, the blood fraction will have quite opaque, milk-like plasma. This is not a big deal since the body will clear of this within hours. However, having too much lipid can lead to **xanthomas**, a condition where lipids are contained on skin lesions; or even **corneal leukoma (opacity)**, a condition where the cornea become opaque.

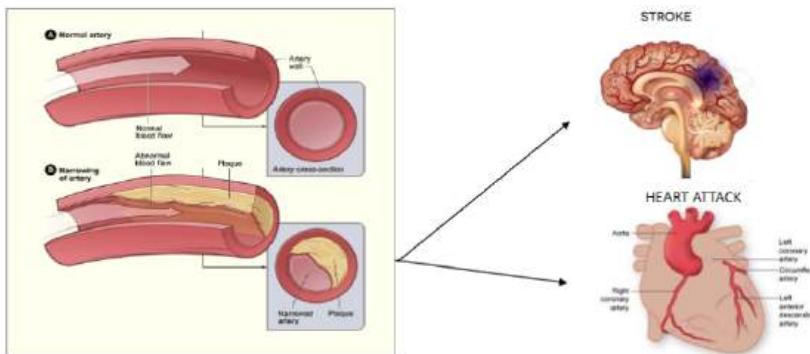


Figure 3.3: Atherosclerosis complications.

Lastly, the much more dangerous condition by high lipid level is building up of lipids within the vessel, called **atherosclerosis**, of coronary artery or in the brain which will lead to a **heart attack** or a **stroke**, respectively.

3.1.2 Absorption of Dietary Lipids

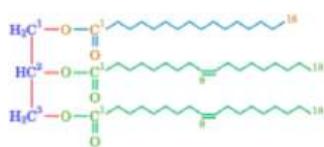
Where do lipids come from? Well...they're mostly coming from our diet. For the dietary lipids, we'll divide them into 2 types: "purified" and cellular lipids.

1. "Purified" lipids: These lipids comes from butter and oil. They're both composed of **triacylglycerol** and are neutral i.e. no polar head

nor nonpolar tail.

If they're similar why is butter solid while oil liquid? Well...it has to do with the saturation of the acyl/fatty acid chain (discuss more later on).

'Purified' Lipids



1-Palmitoyl-2,3-dioleoyl-glycerol
• Triacylglycerol

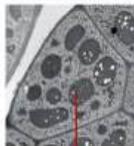
- Triacylglycerol

Neutral lipids

Cellular Lipids



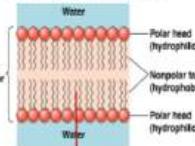
Lipid droplets



- Triacylglycerol
- Cholesteryl Ester

Neutral lipids

Membrane lipids



- Phospholipids

- Sphingolipids

- Glycolipids

- Cholesterol

Amphipatric

Figure 3.4: Dietary lipids.

2. **Cellular Lipids:** These are lipids comes from other forms of food like meats and vegetables. This is because they're and all cell have membranes which contain lipids.

In meats, you'd find lipids droplets which can consist of triacylglycerol and **cholesteryl esters** and are all neutral. In veggies, they're mostly membrane lipids which are phospholipids, sphingolipids, glycolipids and cholesterol which are all **amphipathic** i.e. has a hydrophobic tail and hydrophilic head.

Lipoprotein Transport

When the body absorbs lipids, it will be transported in **lipoproteins**.

Observation 3.4 Now, let's look briefly through the path of lipids through the body. From the small intestine, as the body finished absorbing lipids, it

will be transported by lipoproteins, more specifically called **chylomicron**. This chylomicron can travel through the blood stream to the muscle and adipose tissue where the cell's **lipoprotein lipase (LPL)** is located. Upon interaction, the lipids from within will be hydrolyzed and the chylomicron will become **chylomicron remnant**. This remnant can travel to the liver to the liver and bind to the remnant receptors to be reuptaken.

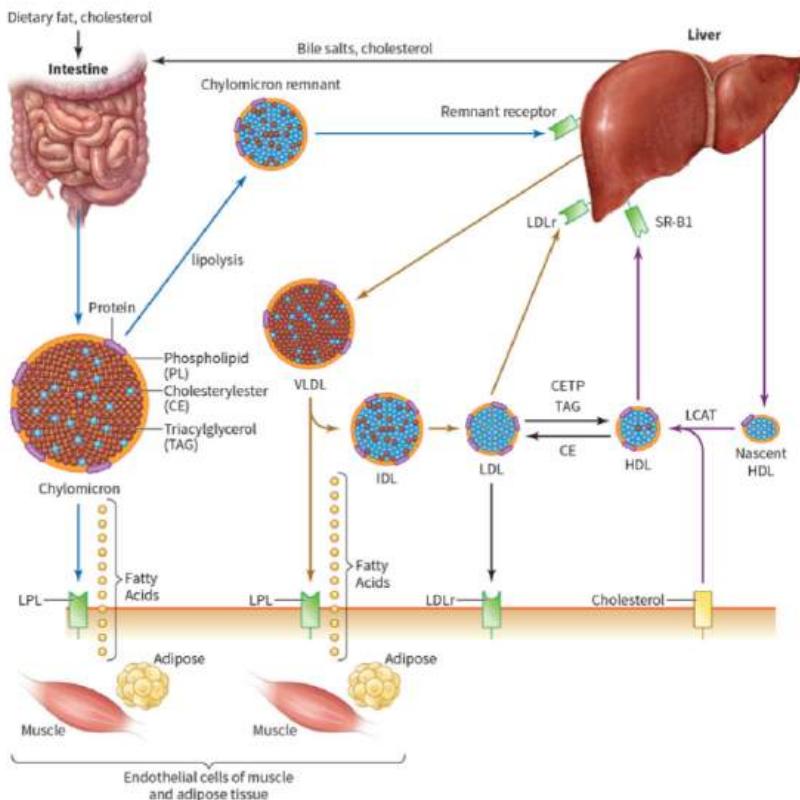


Figure 3.5: Lipids transportation via lipoproteins.

Observation 3.5 As you'd see later on that the liver can synthesize its own lipids and transported out in the form of **very low density lipoprotein (VLDL)**. This VLDL can then go through the process of mentioned above and then become **intermediate-density lipoprotein (IDL)**. IDL can interact with the liver and become **low density lipoprotein (LDL)**. LDL's main function is to

carry cholesterol ester to various tissues.

On the other hand, cholesterol can return back to the liver through a lipoprotein called **high density lipoprotein (HDL)** for the synthesis of **bile salt**.

Absorption of Lipids

Now, we previously saw that lipids are not soluble in water which means we need a way to break it down in order to absorb.

Observation 3.6 As lipids got into the small intestine, it will be broken down by a substance called **bile acid** that is also amphipatic and are stored in the gallbladder. The bile acids will come and solubilize the lipids to micelles or will coat the neutral lipid from the water.

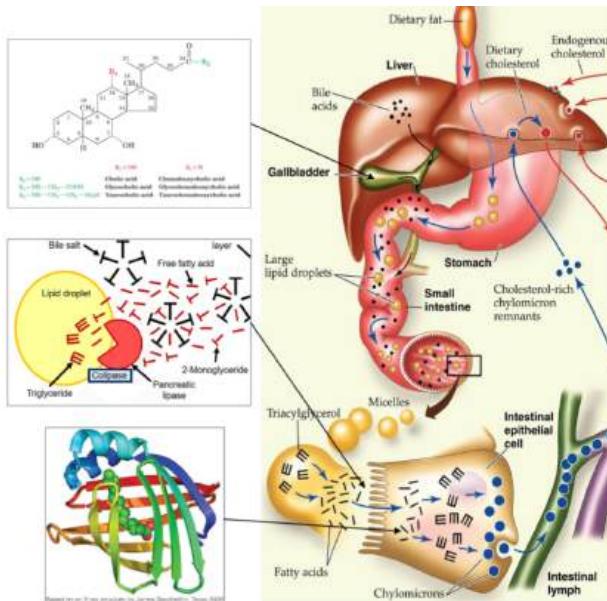


Figure 3.6: Dietary lipid observation.

As it travels down the intestine, the pancreas will release **pancreatic lipase** which can hydrolyzes these lipids (most of which are triglycerides) thus breaking it down into glycerol and acyl chains (fatty acids). These

components can be uptaken by the **brush border** of intestinal cells. Notably, the fatty acids can be brought into the intestinal cell via the **intestinal fatty acid binding protein (i-FABP)** [since they're hydrophobic].

Inside the intestinal cell, these constituents will be resynthesized into triglycerides and packaged into chylomicron to be sent into circulation.

Observation 3.7

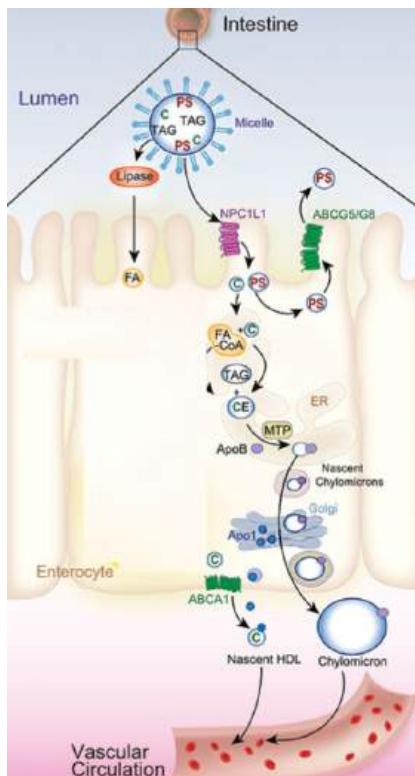


Figure 3.7: Absorption of sterols

erides and phospholipids.

Notion 3.1 Saturation of fatty acid allude to whether it has double bonds or not

Sterols, including phytosterols¹, are absorbed differently than majority of lipids. They're not broken down and rebuilt but are transported right away into the intestinal cells (enterocytes) via the **NPC 1L1**. Once inside the ER, the sterols (like cholesterol) and other lipids will be reassemble into chylomicron which are then secreted. Roughly, few hours after a meal, all the chylomicron would have been processed.

3.1.3 Fatty Acids

Definition 3.3. **Fatty acids** are lipids that has a carboxylic acid head group and a long hydrocarbon tail.

Observation 3.8

Since it's a type of lipid, it can be found in a lot of place in the cell e.g. mitochondria and cytosol. It's also a building block for triglycerides and phospholipids.

¹plants' sterols

Example 3.1.1. When a fatty acid is said to be *saturated*, it only consists of single bond along the hydrocarbon chain. Meanwhile, if it's *unsaturated*, it has at least 1 double bond along its hydrocarbon chain.

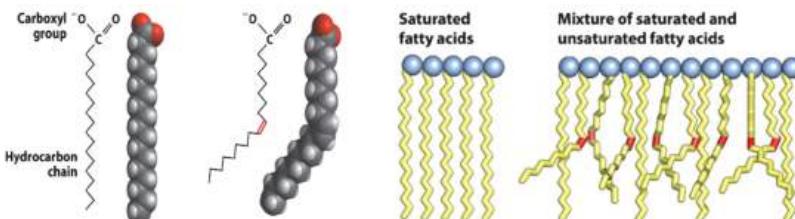


Figure 3.8: Saturated vs unsaturated fatty acids.

Observation 3.9 Saturated fatty acids has straight and organized hydrocarbon chain \Rightarrow it can be packaged more efficiently \Rightarrow it will be in its solid form at room temperature.

On the other hand, unsaturated fatty acids has double bonds which create kinks along the chain \Rightarrow inefficient packing \Rightarrow it will be in its liquid form at room temperature.

Saturated Fatty Acids

Saturated fatty acids only have single bonds along its hydrocarbon chain. Its chemical formula is often written as



Observation 3.10 We observed that as the number of carbon in the hydrocarbon chain increases, the stability of the chain also increases which lead to higher melting point.

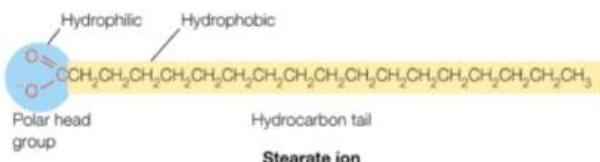
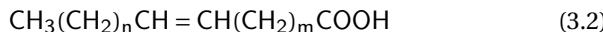


Figure 3.9: Saturated fatty acids.

Unsaturated Fatty Acids

Unsaturated fatty acids have at least 1 double bond along its hydrocarbon chain. Its chemical formula is often written as



When a fatty acid has 1 double bond only, we call it **mono-unsaturated**; while if it has multiple double bonds, it's called **poly-unsaturated**.

Observation 3.11 Similar to the saturated fatty acid, if the amount of carbon in the hydrocarbon increases, the unsaturated fatty acid's increases. Contrarily, if the amount of unsaturation (double bond), its stability decreases which decreases the melting point.

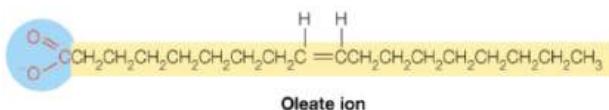


Figure 3.10: Unsaturated fatty acids.

Observation 3.12 Unsaturated fatty acids exist in 2 kind of isomers: **Cis-** and **trans-**. *Cis*-unsaturated fatty acids will have that kink (bent) at the unsaturation since the radicals are on the same side of the double bond. On the other hand, *trans*-unsaturated fatty acids will be a straight chain since the radicals are on opposite sides of the double bond.

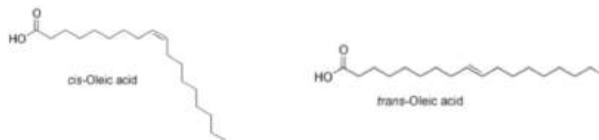


Figure 3.11: Cis vs trans oleic acid.

trans-unsaturated fatty acids are packed more regularly \Rightarrow higher melting point than *cis*-unsaturated fatty acids.

Methods 3.1 By performing partial hydrogenation of vegetable oils, we can convert some of the *cis* double bond into the *trans* double bonds thus generating *trans* fat.

Remark 3.1. *Trans fat increases the risk of cardiovascular disease*

So to resume the general idea of what we've discussed,

- When there's more carbon in the hydrocarbon chain \Rightarrow higher melting point for fatty acids.
- When there's an increase in unsaturation in the hydrocarbon chain \Rightarrow lower melting point
- When there's a *trans*-isomer \Rightarrow higher melting point than the *cis*-isomer.

Symbol ^a	Common Name	Systematic Name	Structure	mp (°C)
Saturated fatty acids				
12:0	Lauric acid	Dodecanoic acid	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$	44.2
14:0	Myristic acid	Tetradecanoic acid	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$	53.9
16:0	Palmitic acid	Hexadecanoic acid	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	63.1
18:0	Stearic acid	Octadecanoic acid	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	69.6
20:0	Arachidic acid	Eicosanoic acid	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$	77
22:0	Behenic acid	Docosanoic acid	$\text{CH}_3(\text{CH}_2)_{20}\text{COOH}$	81.5
24:0	Lignoceric acid	Tetracosanoic acid	$\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$	88
Unsaturated fatty acids (all double bonds are <i>cis</i>)				
16:1n-7	Palmitoleic acid	9-Hexadecenoic acid	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	-0.5
18:1n-9	Oleic acid	9-Octadecenoic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_9\text{COOH}$	12
18:2n-6	Linoleic acid	9,12-Octadecadienoic acid	$\text{CH}_3(\text{CH}_2)_{14}\text{CH}=\text{CHCH}_2_9(\text{CH}_2)_5\text{COOH}$	-5
18:3n-3	α -Linolenic acid	9,12,15-Octadecatrienoic acid	$\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_9(\text{CH}_2)_3\text{COOH}$	-11
18:3n-6	γ -Linolenic acid	6,9,12-Octadecatrienoic acid	$\text{CH}_3(\text{CH}_2)_6(\text{CH}=\text{CHCH}_2)_9(\text{CH}_2)_3\text{COOH}$	-11
20:4n-6	Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	$\text{CH}_3(\text{CH}_2)_{14}(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_3\text{COOH}$	-49.5
20:5n-3	EPA	5,8,11,14,17-Eicosapentaenoic acid	$\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_5(\text{CH}_2)_2\text{COOH}$	-54
22:6n-3	DHA	4,7,10,13,16,19-Docosahexenoic acid	$\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_6(\text{CH}_2)_3\text{COOH}$	-44
24:1n-9	Nervonic acid	15-Tetracosenoic acid	$\text{CH}_3(\text{CH}_2)_{23}\text{CH}=\text{CH}(\text{CH}_2)_{15}\text{COOH}$	39

^aNumber of carbon atoms: Number of double bonds. For unsaturated fatty acids, the quantity " *n-x* " indicates the position of the last double bond in the fatty acid, where *n* is its number of C atoms, and *x* is the position of the last double-bonded C atom counting from the methyl-terminal (*ω*) end.

Source: LipidBank (<http://www.lipidbank.jp>).

Figure 3.12: Table of saturated and unsaturated fatty acids.

We'll now be talking about the nomenclature of unsaturated fatty acids i.e. how to name it.

Concept 3.1 There are 2 ways to indicate the unsaturation

1. From the COOH: Start counting from the carbon of the COOH. When reaches to the first double bond, we will write Δ followed by the superscript of said number.

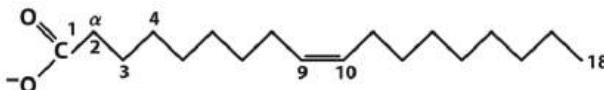
2. From the last carbon: Start counting from the last carbon of the chain. When reaches to the first double bond, we will write ω follow by the superscript of said number.

We can also write with the nomenclature the ratio of number of carbon along the chain to the number of double bond.

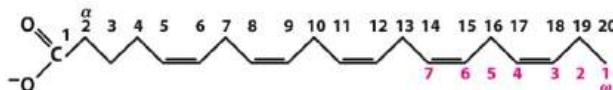
Example 3.1.2. Consider the following unsaturated fatty acid



This means the fatty acid has a double bond at the 9th carbon along the chain (if start counting from the COOH) and there's only 1 double bond. It will have the following structure



Consider the following unsaturated Eicosapentanoic acid:



We can have the following nomenclature: $20:5(\Delta^{5,8,11,14,17})$ Eicosapentanoic acid [if the count were from COOH]. Similarly, it's also named $20:(\omega^{3,6,9,12,15})$ Eicosapentanoic acid [if the count were from the last carbon].

Branched Fatty Acids

As the name implied, **branched fatty acids** are fatty acids with branchings along the hydrocarbon chain.

Observation 3.13 Branched fatty acids are quite uncommon in human and only present in bacteria and some ruminants. For us, we have 2 kinds: **pristanic** and **phytanic acid**. They typically undergo α -oxidation in peroxisomes instead of β -oxidation in mitochondria.

Example 3.1.3. Geranyl pyrophosphate (GPP) is a result from the α -oxidation of isoprenoids.

Definition 3.4. **Free fatty acids** refers to fatty acids that are free flowing and unbounded.

Observation 3.14 Typically, we do not have any free fatty acids in the blood stream. This is because we have lots of proteins called **albumin**, that has a hydrophobic site of which can bind to the fatty acid. Rather, you'd find them in the form of triglycerides or phospholipids.

3.1.4 Triglycerides and Phospholipids

Definition 3.5. **Triacylglycerol** or **Triglycerides** (TG) are lipids of which 3 acyl chains (fatty acids' hydrocarbon chain) are attached together with a glycerol backbone.

Observation 3.15 TAGs are full hydrophobic and is an excellent source of energy and transportation of fatty acids.

Definition 3.6. **Phospholipids** are lipids where 2 fatty acids (typically 1 saturated and 1 unsaturated) are attached together with a glycerol backbone.

Observation 3.16 In this form, it has a polar head group and a hydrophobic tail which allow them to form the bilayer of the cell membrane. Interestingly, because of the balance in saturated and unsaturated, **phospholipids will be semi-liquid at room temperature.**

Cholesterol also have a similar structure where there's a polar head group and a polar tail which allow them to form micelles under aqueous environment.

3.2 Triglycerides

In today's lecture, we will look at metabolism of triglycerides (TG) but before, we'll briefly go through the transportation of different kinds of lipids.

Fatty acids (FA) can combine together to make TG but also phospholipids. FA, by itself, has a hydrophobic tail and hydrophilic group \Rightarrow in aqueous environment, these FA will come together to form micelle of which its hydrophobic tail points inward while its hydrophobic head face

the environment.

For TG, they're completely hydrophobic \Rightarrow they need a completely separate environment. In this case, they're either stored in the cell through lipid droplets or transported through lipoproteins in the plasma. These lipoproteins (will discuss further in next lecture) and lipid droplets consist of a single layer of phospholipids and proteins around the surface to have some structural integrity.

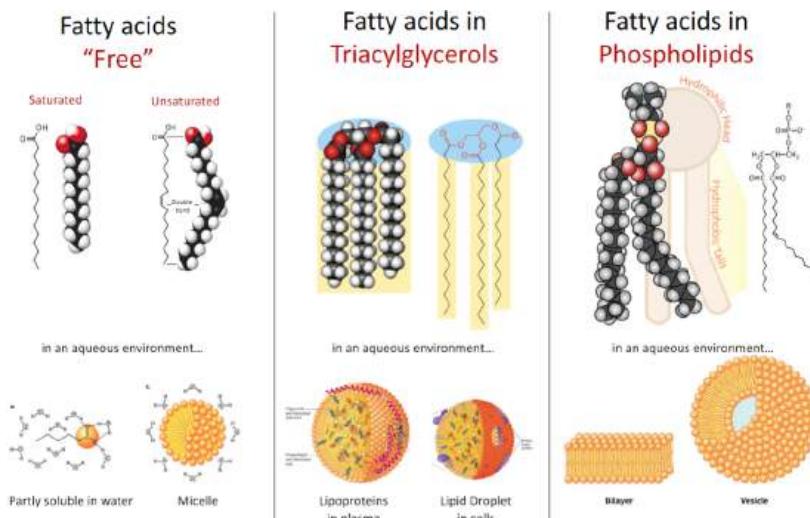


Figure 3.13: Lipids transportsations overview.

Lastly, for phospholipids, they have a hydrophobic tail and hydrophilic head. They can bilayer vesicles where the hydrophobic head points toward each other to create a hydrophobic environment while the head points away to the aqueous environment.

3.2.1 Triacylglycerol Breakdown (Lipolysis)

We'll reiterate what we've previously seen about triacylglycerol from last lecture.

Definition 3.7. **Triacylglycerol** or **Triglycerides** (TG) are lipids of which 3 acyl chains (fatty acids' hydrocarbon chain) are attached together with a glycerol backbone.

Observation 3.17 TG are 100% hydrophobic and we can find them in many different form like purified lipids such as butter or oils; cellular lipids like lipid droplets or membrane lipids.

Because they're neutral lipids, they will be transported in chylomicron that will be produced right after a meal. This is mainly produced by the intestine however, liver also produce a similar transporter called VLDL that carry synthesized TG i.e. the dietary TG are exogenous while the synthesized TG are endogenous.

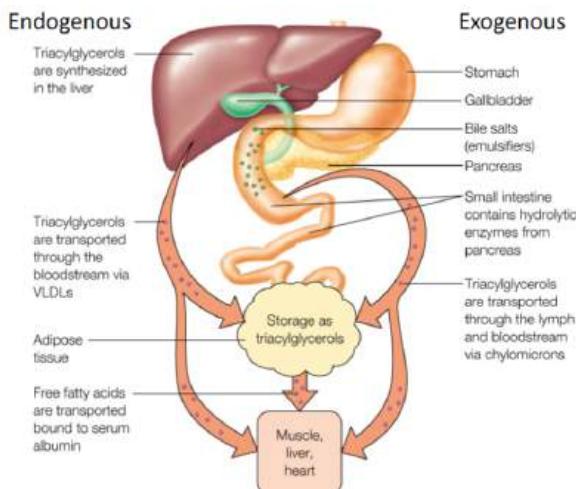


Figure 3.14: Endogenous and exogenous pathway of TG.

Mechanism of Action (Exo- and Endogenous Pathway): We first begin with the exogenous pathway of TG.

1a. TG is ingested as food and will be first solubilized by bile salts and then hydrolyzed by pancreatic lipase.

2a. After being hydrolyzed into FA, they're absorbed by enterocytes and then reassemble into TG inside chylomicron to be transported in the blood stream.

We'll now look at the endogenous pathway.

1b. TGs are synthesized in the liver when the body are in need.

2b. They're now packaged into VLDL to be transported in the blood stream.

For both pathway, they'll share the following:

3. Chylomicrons and VLDLs can be transported to adipose tissue for storage.
4. Or, they can be transported directly to muscle, heart and liver itself for energy usage.

Pancreatic Lipase

Now, we look at first step of degradation of TG that happens in the GI tract.

Observation 3.18 In the GI tract right after the meal (and even during), lipolysis will be occurring where pancreatic lipase will be released to break-down lipids into free FA.

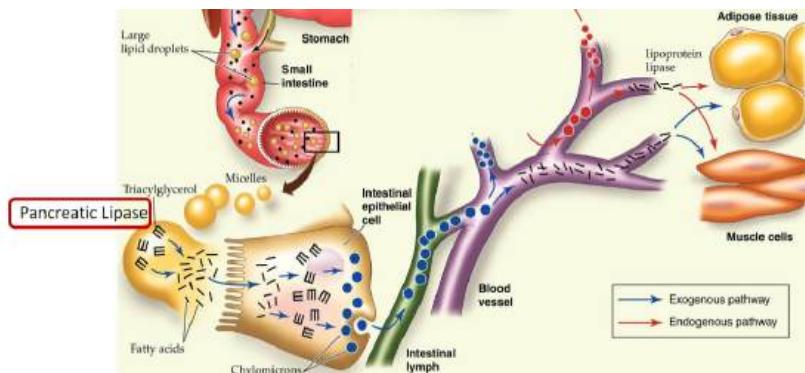


Figure 3.15: Pancreatic lipase.

Wait...the free FAs are only slightly more hydrophilic than TGm so **then how can the they move through water and cross the membrane bilayer?** Well...Most are moved in via transporters however some can cross the membrane but at a lower pace. The free FAs have a charged hydrophilic head group (in aqueous environment) which can be diffused or transported through.

Or, another way is through pinocytosis where FA are taken inside the cell in vesicles. Once inside, the vesicle acidify which will neutralize the charged head group and allow the FA to diffuse across the vesicle. Once in the cytoplasm, it becomes charged again.

Lipoprotein lipase

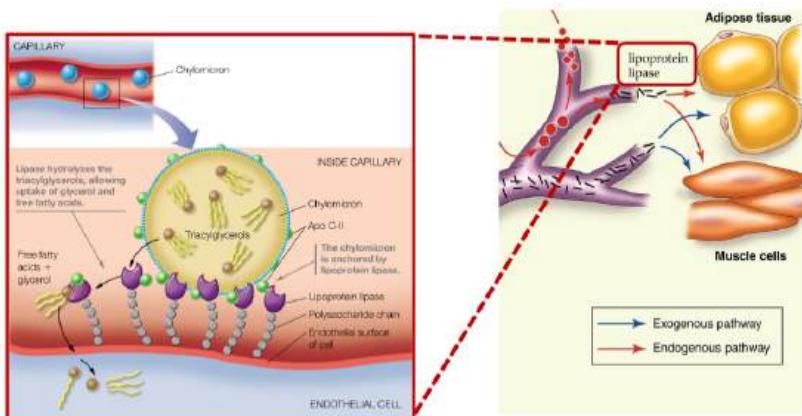


Figure 3.16: Lipoprotein lipase.

Observation 3.19 **Lipoprotein lipases** are located at the surface of muscle and adipose cells. Chylomicron that carry TG will be carried across the blood vessels into adipose tissues and muscle. Here, the lipoprotein lipase will catalyse a reaction that hydrolyze free FA and then it can cross and then reform TG in the cell.

Lipid Droplets

Definition 3.8. **Lipid droplets** are vesicles in muscle and adipose cells for storing TG and other lipids.

Notion 3.2 Lipid droplets will vary depending on the tissue types

Example 3.2.1. Lipid droplets in liver cells are very small and is used to stored esterified FAs and esterified cholesterol.

On the other hand, lipid droplets in adipocyte are larger (nearly to the size of the cell) and are used to store TG.

Observation 3.20 If you start doing exercise for a long while, you'd need lipid as a source of energy. First, the TG will be broken down to **diacylglycerol (DAG)** and then **monoacylglycerol (MAG)** which finally ends up with

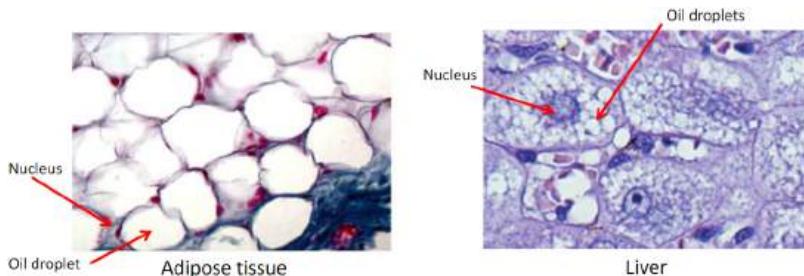


Figure 3.17: Lipid droplets of adipose tissue vs liver.

glycerol. Every step of TG catalysis, an FA is released and transported in the blood plasma via albumin.

Each of these steps involve an enzymes in said order: **adipose triglyceride lipase (ATGL)**, **hormone sensitive lipase (HSL)**, and **monoacylglycerol lipase (MGL)**.

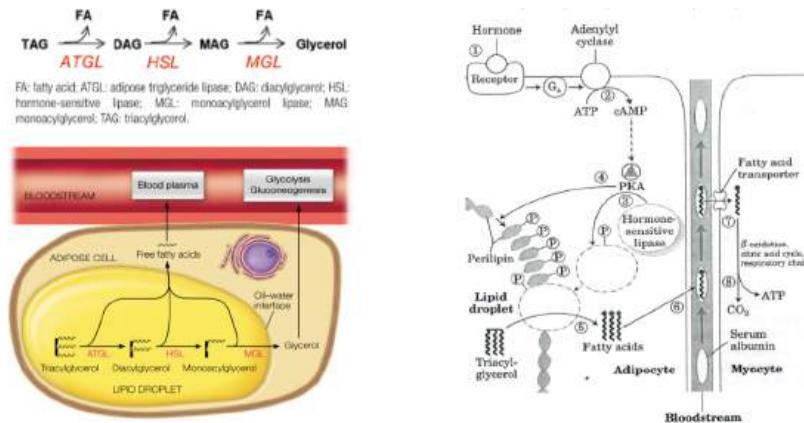


Figure 3.18: Lipolysis and regulation.

Observation 3.21 The catalysis process we've discussed above is tightly controlled.

During exercise and fasting, adrenaline will be released and bind to its receptor which lead to the activation of **adenylyl cyclase** to produce cAMP

from ATP \Rightarrow activate PKA. PKA will activate HDL². PKA will also phosphorylate **perilipin**, proteins that coat the lipid vesicle, which lead to a change of conformation and allow HSL to come into the vesicle. This consequently produce FA that can be used by other tissues as a source of energy.

3.2.2 Triacylglycerol Synthesis (Lipogenesis)

We will now look at how TG are synthesized in the body.

Enterocytes

We've previously talked about TG re-synthesis in the enterocytes which involves TG forming micelles in the intestine which will be hydrolyzed by pancreatic lipase to make free FA to be absorbed. The absorbed free FA will reassemble into TG and packaged in chylomicrons. We will focus on the mechanism of formation of these vesicles.

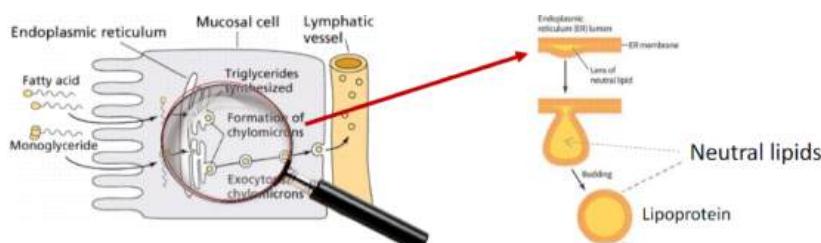


Figure 3.19: Formation of lipoprotein.

The only place that you can first store neutral lipids are in hydrophobic environment. **Where do you get these lipoproteins?** well...you get from the ER membrane bilayer. First, the membrane bulges out from the inter-membrane, then will bud off into a monolayer vesicle with a hydrophobic interior where TG can reside.

Now, **how do you reform TG from free FA?** Well...you use an enzyme called **Acyl-CoA synthase**.

²Because this enzyme is activated by hormone as we've shown, we call it hormone-sensitive lipase instead of diacylglycerol lipase

Mechanism of Action (TG Re-synthesis): First, free FAs will be transported into the cell.

1. Acyl-CoA synthase will catalyze a reaction between the FA and ATP to form acyladenylate intermediate. This reaction is driven forward by hydrolysis of PP_i into 2P_i via PP_i ase.
2. The intermediate will be reacted again with CoA by acyl-CoA synthase to form AMP and an acyl-CoA.
3. **2-monoacylglycerol (2-MAG)** converted to DAG via **2-MAG acyl-transferase** using acyl-CoA in the process.
4. DAG can be converted back to TG using **DAG acyltransferase (DGAT)** using acyl-CoA again.

Thus, you've made TG that can be packaged into chylomicrons.

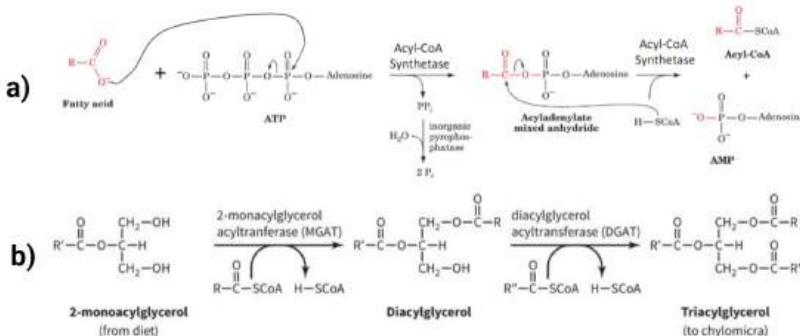


Figure 3.20: a) synthesis of Acyl-CoA via Acyl-CoA synthase. b) re-synthesize TG via the 2-MAG pathway.

De Novo Synthesis

Now, what if the cell need to synthesize TG that are not from lipids i.e. De Novo synthesis of TG? Well...first we need to make **glycerol-3-phosphate**.

Mechanism of Action (TG De Novo Synthesis): To make G3P, there are 2 ways: 1, performing gluconeogenesis to make DHAP which can then be turn into glycerol-3-phosphate. 2, make glycerol-3-phosphate from fructose-1-phosphate (in the liver).

- From here, glycerol-3-phosphate can be turned into **phosphatidic acid** by the addition of acyl-CoA. Phosphatidic acid can either be used to make phospholipid or TG.
- Phosphatidic acid can be dephosphorylate into DAG by **lipin** (a key regulatory enzyme).
- DAG can be converted back to TG by DGAT using acyl-CoA.

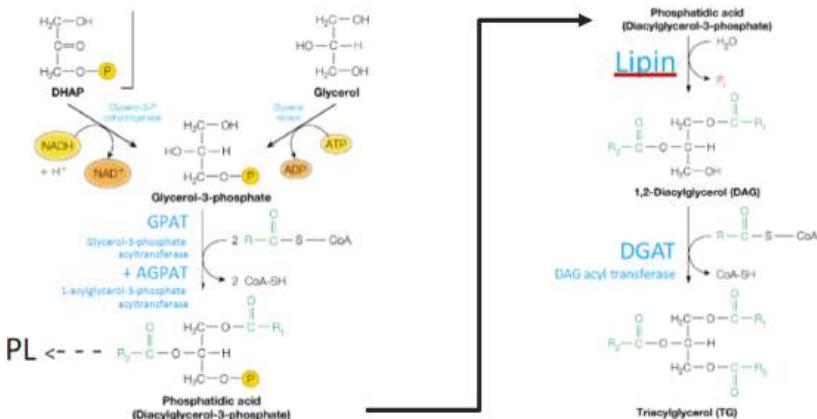


Figure 3.21: De Novo Synthesis of TG.

Regulation

Observation 3.22

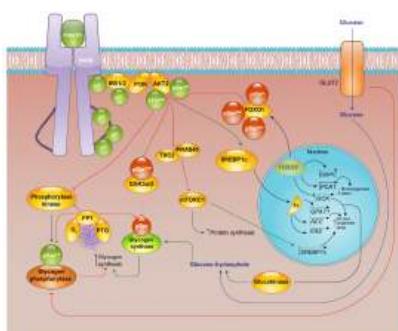


Figure 3.22: lipogenesis via insulin.

After a meal, enterocytes will transfer the glucose extracted to blood which will lead to [glucose] ↑ ⇒ insulin release. Insulin will stimulate the uptake of glucose through GLUT4 (adipose tissue and muscle), glycogen synthesis (in liver and muscle) and even lipogenesis (from FA to TG) through transcriptional control in adipose tissues.

3.3 Phospholipids

Definition 3.9. **Membrane lipids** are a class of amphipatic lipids that are used to build the membrane. This class include 3 important categories: phospholipids, glycolipids and sterols

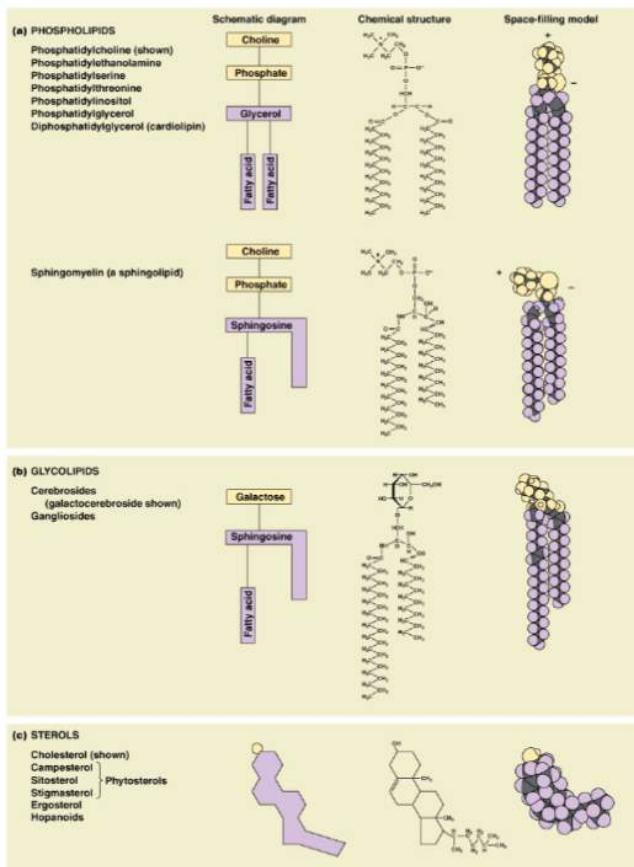


Figure 3.23: Membrane lipids categories.

Observation 3.23 Phospholipids can be further divided according to their structure. Normally, the typical phospholipid will have head group + phosphate attaching to a glycerol with 2 FAs. This is called **glycerolphospholipids**.

There is a specific structure of phospholipid consists of head group + phosphate attaching to a *sphingosine* with 1 FA instead. This is called **Sphingomyelin (sphingolipid)**.

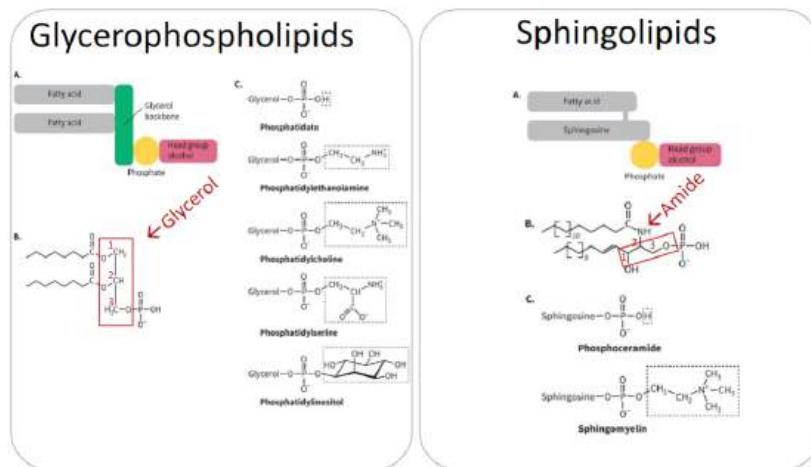


Figure 3.24: Glycerophospholipids and sphingolipids.

Notice that for glycerophospholipids, they can differ from one another by the head group e.g. phosphatidylserine and phosphatidylethanolamine are both phospholipids but their head groups are different (serine vs ethanamine).

Observation 3.24 Glycolipids have similar structure to that of sphingolipid but instead of head group + phosphate, it's galactose. Lastly, sterols are similar to the rest with a hydrophilic head group and hydrophobic tail.

Remark 3.2. You can also change the FA of these lipids to yield different properties.

Roles of Membrane Lipids

Concept 3.2 (Membrane Asymmetry). The membrane lipids are not randomly distributed but will have specific arrangement on both leaflets thus it's asymmetric.³

³By "leaflets", we meant each of the layers of the bilayer membrane

Notion 3.3 Lipids' transverse diffusion from 1 leaflets to the other is very slow compared to lateral diffusion from 1 position of the same leaflet to the next.

Observation 3.25 To maintain the membrane asymmetry, the cell uses protein transporter to bring whichever lipids from 1 leaflet to the other.

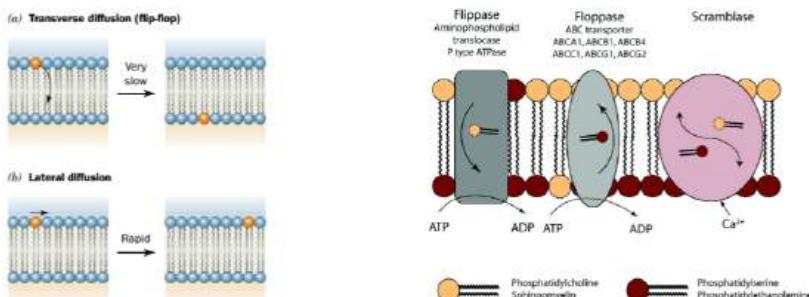


Figure 3.25: Membrane asymmetry.

Example 3.3.1. **Flippase** is a transporter that brings lipids to the cytosolic side while **flopase** will bring lipids to the extracellular side. We also have **scramblase** that does the job of flip- and flopase simultaneously.

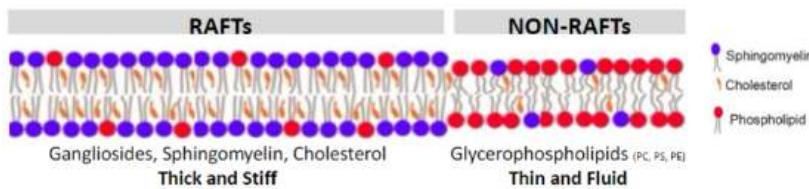


Figure 3.26: Raft vs non-raft microdomain.

Observation 3.26 Membrane lipids can assemble together to form **micro-domains** which are classified into 2 types: **raft** and **non-raft**.

In a raft microdomain, the membrane is thicker, stiffer and composed of sphingolipids, cholesterol and gangliosides.⁴

⁴Gangliosides are molecules consisting of both lipids and carbohydrates.

Contrarily, non-raft microdomain is thinner, more fluid and consists of glycerophospholipids (to be specific: PC, PS and PE; all of which we will discuss later). Not only that these glycerophospholipids' FAs are unsaturated and are shorter (thus thinner).

Observation 3.27 Some of these membrane lipids are also essential for signalling of molecules once broken down. e.g. phosphatidylinositol can be broken down into inositol triphosphate which is a second messenger.

3.3.1 Glycerophospholipids

We will now look at the synthesis of these phospholipids, starting with glycerophospholipids.

Example 3.3.2. First, consider the 4 following circumstances:

1. After a fatty meal
2. When cells are dividing
3. When fasting for > 12h
4. To maintain membrane rafts balance
5. In case of stress, to provide signaling molecules

When would phospholipids (PL) be synthesized? Well...when cells are dividing, maintain raft balance and to provide signalling molecules when under stress.

Observation 3.28 We can find PL every where in the cell that contain a membrane and they're mainly synthesized in ER.

Remark 3.3. *For the following syntheses, we'll be assuming that FAs and the head groups are available and we're just assembling them together.*

Now, we're going to discuss on the mechanism of synthesis of different glycerol-PL.

Synthesis of PI, PG and Cardiolipin

We're going to look at the synthesis of **phosphatidylinositol (PI)**, **phosphatidylglycerol (PG)** and **cardiolipin**.

Mechanism of Action (PI, PG and Cardiolipin Synthesis): For the first 2 steps, it's to make glycerol-3-P then add acyl-CoA making phosphatidic acid.^a

1. Phosphatidic acid will be combined by CTP forming **CDP-DAG** (a high energy intermediate) by **CDP-DAG synthase**.

Another thing to see from this is hydrolysis of PP_i immediately hence pushing reaction forward.

2. You can now either add inositol or glycerol-3-P which yield different results.

3a. Adding inositol to CDP-DAG using **PI synthase** will produce PI.

3b(i). Adding glycerol-3-P to CDP-DAG using **PG-3-P synthase** will form PG-P, which can then be dephosphorylated by the same enzyme to make PG.

3b(ii). The newly made PG can be deglycerolized by **cardiolipin synthase** to make cardiolipin.

^aDiscussed in previous lecture

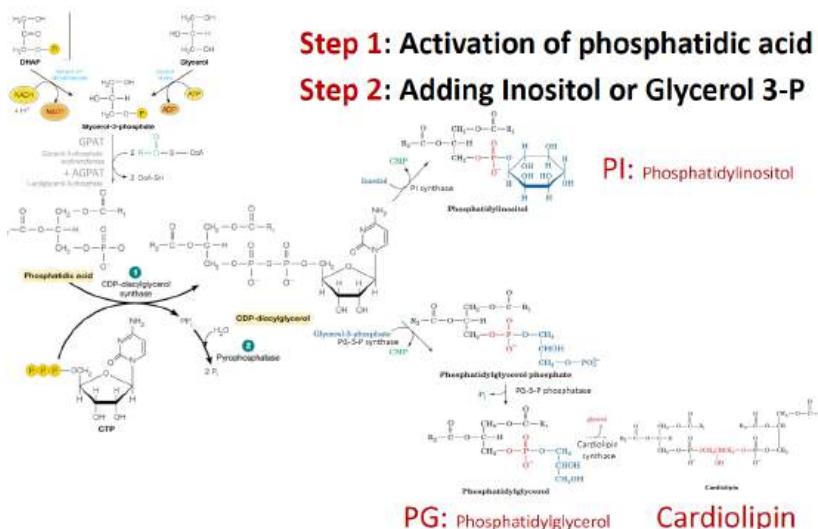


Figure 3.27: PI, PG and cardiolipin synthesis.

Synthesis of PE and PC

We're now going to look at the synthesis of **phosphatidylethanolamine (PE)** and **phosphatidylcholine/lecithin (PC)**. There are 2 pathways we'll be looking at: Kennedy pathway and one-carbon pathway.

1. The Kennedy pathway present in all cell types.

Mechanism of Action (The Kennedy Pathway): We first start with the head group instead of the phosphatidic acid.

1. The head group (ethanolamine or choline) is phosphorylated with ATP using their respective kinase: **ethanolamine or choline kinase** to form **phosphoethanolamine or phosphocholine**.

2. The phosphorylated headgroup is now activated by adding CTP and driven by PP_i to form **CDP-ethanolamine or CDP-choline** (high energy intermediate).

3. The activated head group will be added on with 1,2-DAG and releases CMP forming PE or PC respectively.

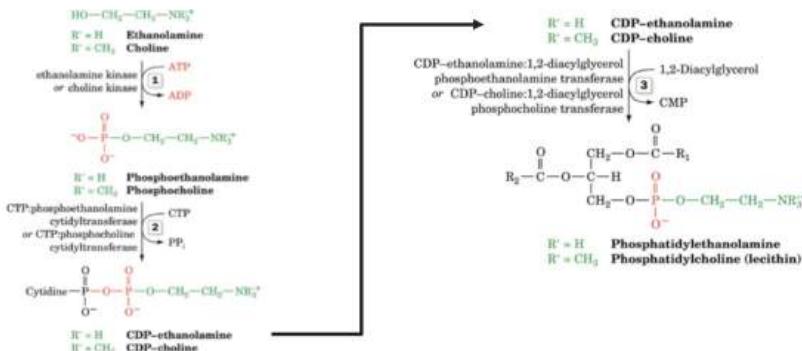


Figure 3.28: The Kennedy pathway.

2. The one-carbon pathway. Unlike the Kennedy pathway where either of the PE or PC can form. It will transform PE to PC. This is through the utilization of an enzyme called **PE methyltransferase (PEMT)**. Interestingly, this pathway covers around 30% of total PC production in the liver.

Mechanism of Action (one-carbon pathway): First, there must already be PE present.

1. PE is methylated using PEMT forming an intermediate.

2-3. Methylation repeated 2 more times using PEMT which result in PC formation

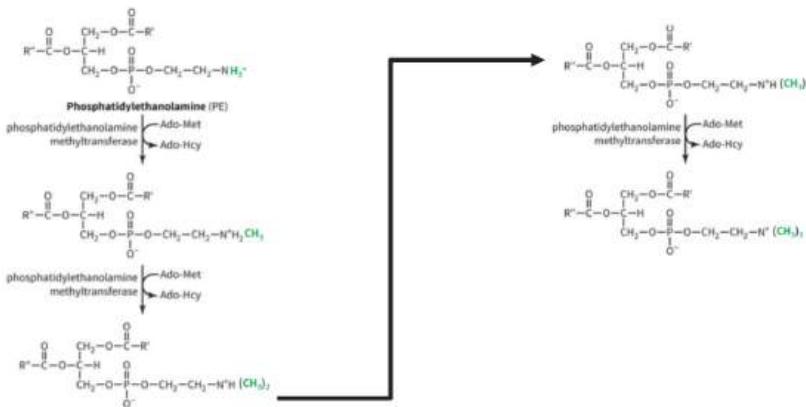


Figure 3.29: The one-carbon pathway

Where does all of these methyl group come from? Well...they originate from **S-adenosyl methionine** (another high energy intermediate) which is a combination of methionine and ATP

Problem-Based Learning

Question: Why does 30% of our PC is produced through PEMT?

Answer: Because of bile production. This is because bile is a mixture of bile salts, cholesterol, PC (highest concentration), and others. It's important that **functional/good bile can only be produced at the right ratio of these components**. If there's too much of cholesterol or PC or bile salts \Rightarrow crystallization \Rightarrow **gallstone**.

Question: What is the high energy intermediate of one-carbon pathway from PE to PC?

Answer: S-adenosyl methionine.

Question: What phenotype does a person with PEMT deficiency will have?

Answer: Enlarged liver since you cannot effectively produce bile \Rightarrow accumulate lipids in liver \Rightarrow liver enlargement.

PE to PS and PS to PE

We will now look at the conversion of PE to **phosphatidylserine (PS)** and vice versa.

Remark 3.4. *The conversion back and forth is NOT 1 pathway but 2 separate ones with 2 different enzymes.*

Mechanism of Action (PE to PS and PS to PE): We will first begin from PE (1a) then back from PS (1b).

- PE can have its ethanolamine group removed by **PE serine transferase** and add on a serine group \Rightarrow PS is formed.
- PS can have its serine group decarboxylated by **PE decarboxylase** \Rightarrow PE is formed.

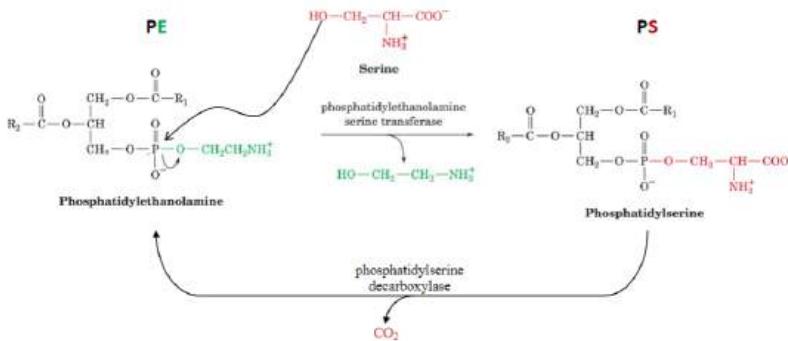


Figure 3.30: Conversion from PE to PS and PS back to PE.

3.3.2 Sphingolipids

Now, we will look at the synthesis of sphingolipids.

Mechanism of Action (Sphingolipids Synthesis): The FA in focused is **palmitic acid** which has been combined with CoA forming

palmitoyl-CoA.

1. Palmitoyl-CoA is combined with serine backbone to form an intermediate that through many steps will form **ceramide**.

2a. Ceramide can be added with a phosphocholine head group to make sphingolipid.

2b. Or, ceramide can be added with UDP-glucose (high energy intermediate) to form **cerebroside** (a glycosphingolipid).

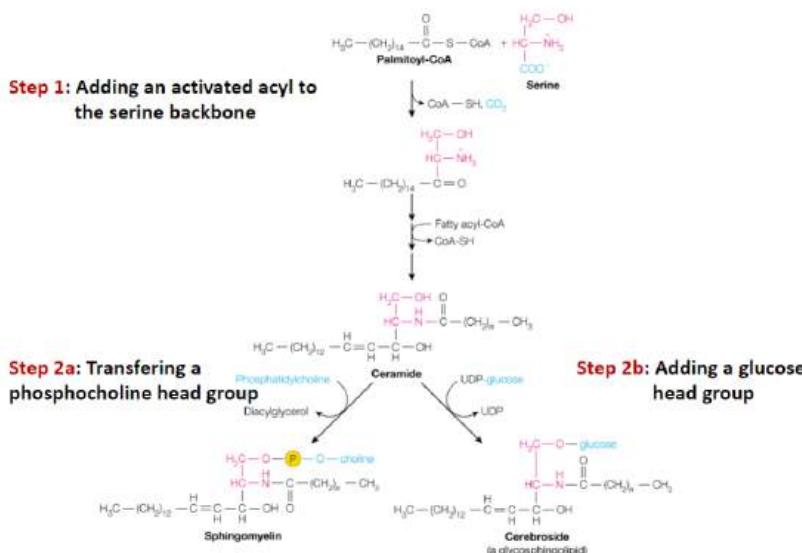


Figure 3.31: Sphingolipid synthesis.

3.3.3 Phospholipases

Definition 3.10. **Phospholipases (PLase)** are enzymes localized mainly in the plasma membrane to cleave PL.

Observation 3.29 The reason they cleave PL could be due to variety of reasons like generate signalling molecules, convert 1 phospholipids to another and even modulate the shape of the plasma membrane.

The body wants a specific ratio of each PL thus by having PLase, it can help controlling this ratio \Rightarrow PLase deficiencies will often be associated with certain diseases e.g. Deficiency in Sphingolipidase can cause **Nieman-Pick Type A/B disease**.

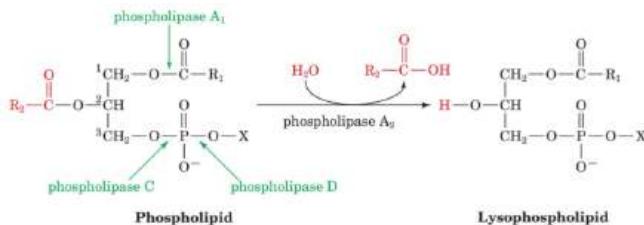


Figure 3.32: Different PLase and their cleavage positions.

Observation 3.30 We've previously discussed the function of PLase for signalling in the previous chapter. What's more interesting is the **Lands' cycle** which is the deacylation/acylation of PL. In this instance, regular phospholipids can be cleaved by PLase A_2 to release 1 FA and **lysophospholipids** which can be converted back to PL by **lysoPL acyltransferases**. Notice that **lysophospholipids are missing 1 FA \Rightarrow induce curvature on the membrane**. This is essential when the cell needs to move, perform pinocytosis or phagocytosis, and etc.

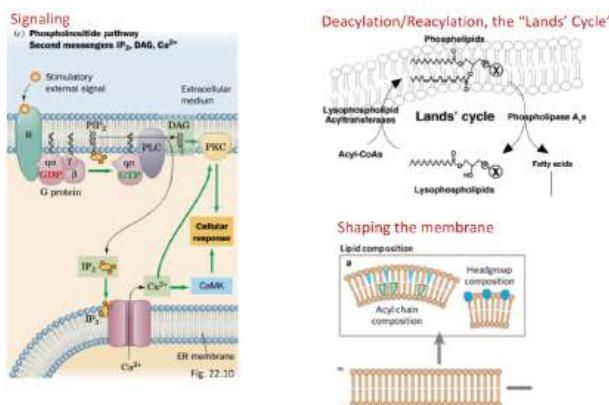


Figure 3.33: Function of PLase.

3.4 Fatty Acid Breakdown

In this lecture, we will focus on the breakdown of FAs, in particular, it's the β -oxidation that will happen in the mitochondrion.

3.4.1 Origins

Example 3.4.1. Which of the following is(are) correct right after a meal?
Select ALL that apply.

1. Liver will breakdown fatty acids to generate ATP
2. Adipose tissue will store fatty acids from the diet in lipid droplets.
3. Muscle will breakdown fatty acids to regenerate glucose.
4. Liver will uptake fatty acids from chylomicron remnants

Answer: 2 and 4.

Which of the following metabolic circumstances require(s) breaking down fatty acids to generate energy? Select ALL that apply.

1. During prolonged exercise.
2. After a fat-rich meal.
3. After a glucose-rich meal.
4. After a fructose-containing meal.
5. Exhausted glycogen stores in muscle.

Answer: 1 and 5.

Let's ask ourselves first, **where does FA come from?** Well...there are 3 main sources of FA: 1, you can get through dietary lipids or synthesized from the liver (transported in chylomicron or VLDL respectively.) 2, you can get through lipolysis of TG in lipid droplets. 3, the least favoured, you can get FA through phospholipase A₁ and A₂ reactions.

3.4.2 Fatty Acid Transport into Mitochondria

Now, we can see that FA will have a hard time getting through the membrane of mitochondria, especially for those with more than 16 carbon chain. To combat this, we will use a series of reactions and transports

Mechanism of Action (Transport of FA into Mitochondria): We start in the cytoplasm near the mitochondria where FA is present.

1. FA is phosphorylated by acyl-CoA synthase using ATP to make **acyladenylylate**. PP_i is used to drive the reaction forward. Phosphorylation also helps to keep FA inside the cell and make a gradient to pull more in.

Acyladenylylate will be dephosphorylated and added with a CoA simultaneously forming **acyl thioester (acyl-CoA)** and releasing AMP.

2. This newly made acyl-CoA will be coupled to **carnitine** using **carnitine palmitoyl transferase I (CTP I)** forming **acylcarnitine**. **This is the rate-limiting step.**

3. The acylcarnitine can cross the outer membrane via the **acylcarnitine translocase**, of which at the same time it, a carnitine is transported out to the cytoplasm.

In the intermembrane, acylcarinitine crosses the inned membrane with the same transporter (+ releasing 1 carnitine).

4. One inside the matrix, acyl carnitine will have its carnitine removed (returned back to the cytoplasm) and added on with CoA forming Acyl CoA (can be used in β -oxidation) using **CTP II**.

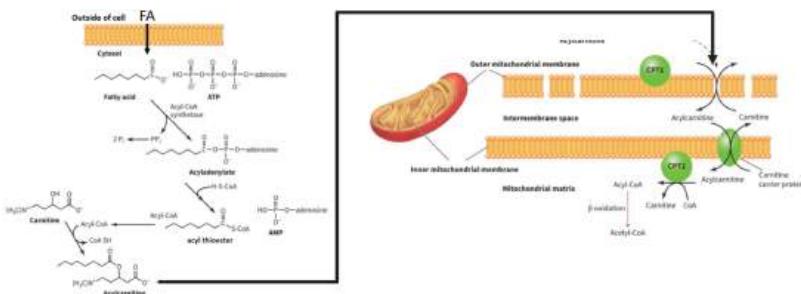


Figure 3.34: FA transport into mitochondria.

It must be noted that when you're synthesizing FA, you do not want to break it down at the same time. Turns out the body have a mechanism for

this which is the inhibition of CTP I (rate limiting step) by high [malonyl-CoA] in the cytosol because of FA synthesis.

3.4.3 β -Oxidation

So now that we've effectively transported FA into the matrix, we can begin to break it down using β -oxidation. Remember: FA exists in many form and we'll discuss how to break each form down.

Saturated FA β -Oxidation

We shall begin with β -oxidation of saturated FA. The basic strategy is cutting the α carbon from the β carbon; then, we oxidized the β . This will yield 1 acetyl-CoA and oxidized β carbon chain, of which can be cleave more until the end.⁵ Let's pick palmitoyl-CoA as an example:

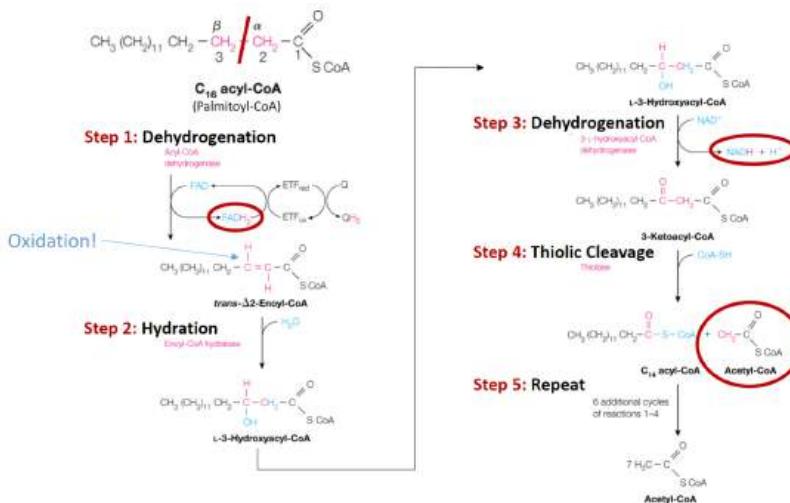


Figure 3.35: Degradation of palmitoyl-CoA.

Mechanism of Action (Palmitoyl-CoA degradation): We've transported palmitic acid into the matrix as palmitoyl-CoA.

⁵Since the oxidized β carbon will now be considered as the new α carbon and the next down the chain is the new β carbon.

1. palmitoyl-CoA will be dehydrogenated into ***trans*- Δ^2 -enol-CoA** using **acyl-CoA dehydrogenase**. At the same time, FAD will be reduced to FADH₂ which can reduce ETF thus transport e^- to CoQ of the ETC.

2-3. The intermediate will be hydrated by **enol-CoA hydratase** then dehydrogenated again by **3-L-hydroxyacyl-CoA dehydrogenase** yielding **3-ketoacyl-CoA** and 1 NADH.

4-5. **Thiolase** will cleave 3-ketoacyl-CoA into a 14-carbon acyl-CoA chain and 1 acetyl-CoA. The cycle repeat until there's no acyl-CoA chain left, in this case, 6 more times.

In the end, after the repetition, we will yield 8 acetyl-CoA, 7 FADH₂ and 7 NADH.

Using calculation from last chapter, we would realized that 8 acetyl-CoA, 7 FADH₂ and 7 NADH will give us 108 ATP but remember that we need to expend 2 ATP to transport the palmitic acid in \Rightarrow 106 ATP is made through its degradation.⁶

Mono-Unsaturated FA β -Oxidation

For mono-unsaturated FA, its mechanism is the exact same as the saturated, the only different is that in step 1, it's isomerization instead of dehydrogenation \Rightarrow no FADH₂ produced for mono-unsaturated FA.

Poly-Usaturated FA β -Oxidation

For poly-unsaturated FA, we need to consider 2 positions: unsaturation point is at **an odd numbered carbon or an even numbered carbon (counting from the first α carbon)**.

Observation 3.31 As we keep cleaving the chain of a poly-unsaturated FA, we will encounter an odd unsaturation point. In this case, the will perform β -oxidation the same like mono-unsaturated FA (no FADH₂ is made).

For the even unsaturation point, we need to **convert it into an odd saturation point and this will produce 1FADH₂ but cost 1 NADPH**. This is not a problem since $1\text{NADPH} = 0\text{ATP}$!

⁶1 acetyl-CoA can go through the 1 CAC making 10ATP, 1 NADH is equivalent to 2.5 ATP and 1 FADH₂ is equivalent to 1.5 ATP.

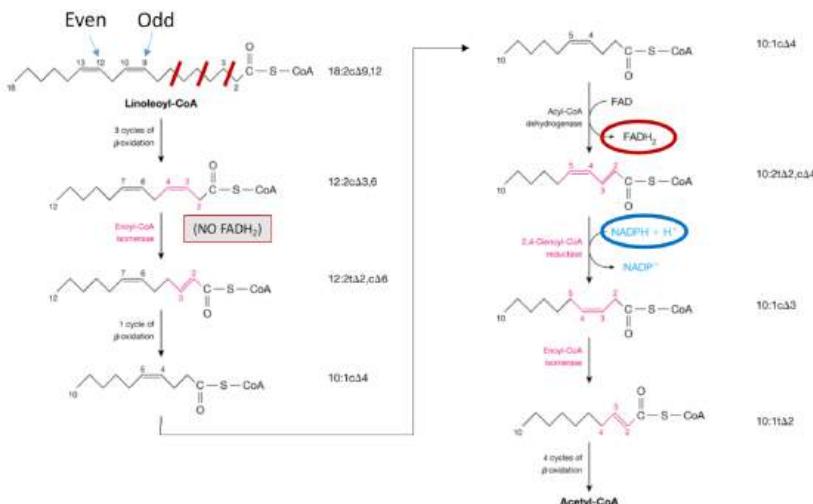


Figure 3.36: Poly-unsaturated FA β -oxidation.

Odd-Chain FA β -Oxidation

Mechanism of Action (Odd-Chain FA β -Oxidation): β -oxidation will proceed normally until the last 3-carbon chain.

1. The last 3-carbon chain will be released from β -oxidation as **propionyl-CoA**.
2. Propionyl-CoA can go through a series of reaction to convert it into **succinyl-CoA**.
3. Succinyl-CoA can enter CAC (midway) along with the previously made acetyl-CoA (beginning of CAC).

Example 3.4.2. Margaric acid is a FA with 17-carbon chain. So, it will undergo β -oxidation for the first 14 carbons forming 7 acetyl-CoA. The last 3 will be converted into 1 succinyl-CoA and enter CAC midway through.

Branched Chain FA β -oxidation

For branched chain FA (e.g. phytanic or pristanic acid), it's quite simple for β -oxidation:

- Any section with normal chain will be converted into acetyl-CoA.

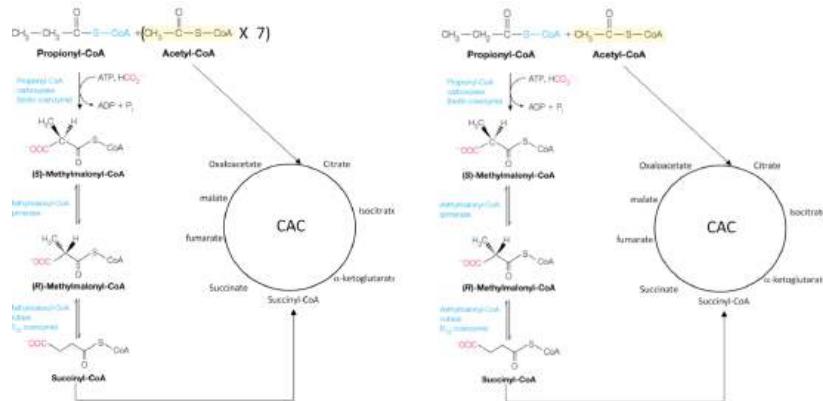


Figure 3.37: Odd-chain FA β -oxidation: margaric acid (left) and valeric acid (right) with 17 and 5 carbon-chain respectively.

- Any section with a branched point will be converted into propionyl-CoA.

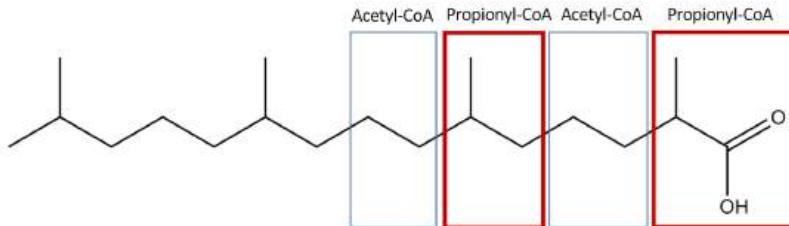


Figure 3.38: Degradation of branched FA.

3.4.4 Ketone Bodies (Ketogenesis)

Observation 3.32 When the body is undergoing starvation or fasting, there will be a low (or even no) carbohydrate intake \Rightarrow oxaloacetate will eventually be depleted and effectively stop CAC. To combat this, the body will degrade FA to make acetyl-CoA for CAC.

The problem here is that this is primarily in the liver and acetyl-CoA can barely cross the cell membrane...so **how can you transport them to other**

tissues? Well...you use ketone bodies.

Basically, acetyl-CoA will go through a series of reaction until it reaches the 3 followings molecules: **acetoacetate**, **acetone** and **β -hydroxybutyrate**; these molecules are called **ketone bodies** and entire process of making them is called **ketogenesis**. These ketone bodies can travel through the membrane to different organs like the brain and heart where it can be converted back to acetyl-CoA, called **ketolysis**.

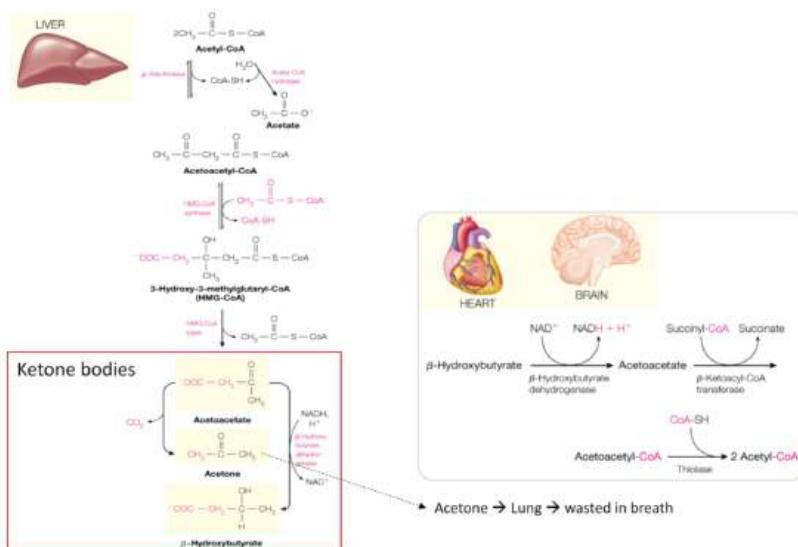


Figure 3.39: Ketogenesis.

When does ketogenesis begin? When our blood glucose is dropping below 5.5mM and the glucose storage is depleted. Ketogenesis would be required to transport acetyl-CoA where the body is mostly needed of energy like heart and brain.

3.5 Fatty Acid Synthesis

In the previous lecture, we talked about how to breakdown FA. In this lecture, we will look at their synthesis. Before that, we have some little activities for revision.

Example 3.5.1. What is the output of β -oxidation?

1. Acetyl-CoA
2. FADH₂
3. NADH
4. NADPH
5. CPT1

Answer: 1,2,3 only.

When does ketogenesis occur?

1. To initiate cell division
2. Glycogen stores are exhausted
3. After a fat-rich meal
4. After a glucose-rich meal
5. When blood [Glucose] is low

Answer: 2, 5

Which of the following metabolic circumstances require(s) to synthesize fatty acids?

1. To initiate cell division
2. After a fat-rich meal
3. After a glucose-rich meal
4. After a fructose-containing meal
5. Glycogen stores are exhausted
6. While running a marathon

Answer: 1, 3 and 4.

3.5.1 Shuttling of Acetyl-CoA

The FA synthesis is in the cytosol thus we have to bring out acetyl-CoA inside the mitochondria.

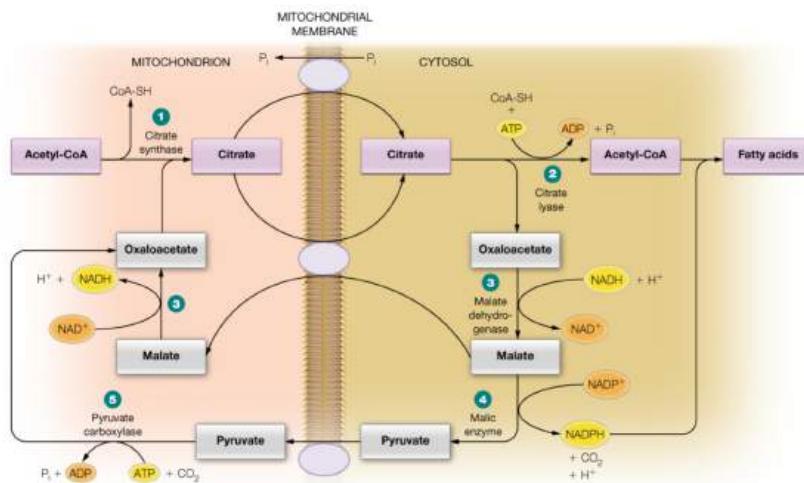


Figure 3.40: Transportation of acetyl-CoA into the cytoplasm.

Mechanism of Action (Shuttling of Acetyl-CoA): Acetyl-CoA is combined with oxaloacetate using **citrate synthase** which converted into citrate.

1. Citrate crosses the mitochondria membrane (through diffusion) into the cytoplasm.
2. Citrate is then reverted to acetyl-CoA and oxaloacetate using **citrate lyase** and ATP.
3. Acetyl-CoA can be used to FA synthesis while oxaloacetate is recycled back to the mitochondria matrix.

3.5.2 Fatty Acid (Palmitate) Synthesis

We will divide the process of synthesizing FA into 3 separate steps.

Remark 3.5. *The body commonly synthesize a 16-carbon chain FA called palmitate.*

Synthesis of Malonyl-CoA

The step to synthesize of malonyl-CoA is mediated by an important enzyme called **acetyl-CoA carboxylase (ACC)** and this is the rate-limiting step and is irreversible. Interestingly, this enzymes have 2 sites:

1. *Biotin carboxylase site*

2. *Transcarboxylase site*

Both of which will participate in making malonyl-CoA⁷

Mechanism of Action (Synthesis of Malonyl-CoA): We begin with the biotin carboxylase site.

1. The biotin molecule is carboxylated via the **biotin carboxylase** site using bicarbonate and ATP.
2. The carboxylated biotin is then transferred to the **transcarboxylase** site where it will be decarboxylated.
3. The released CO₂ is used to carboxylate acetyl-CoA making malonyl-CoA.

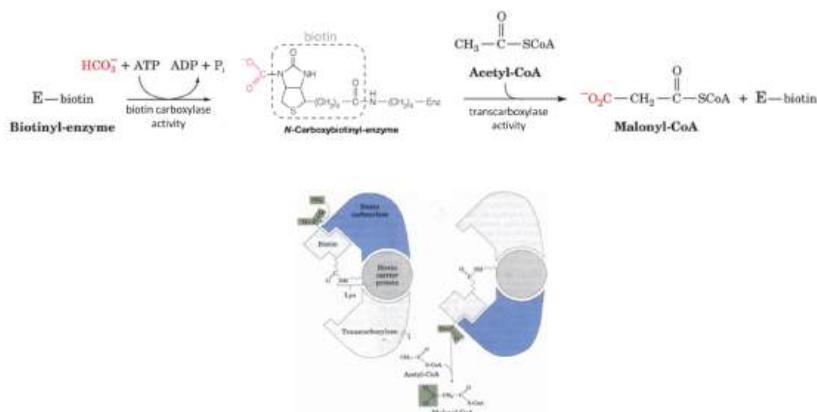


Figure 3.41: ACC reaction.

Remark 3.6. Malonyl CoA is a regulator, more specifically inhibitor of CPT1.

Coupling of ACP to MAT

The next reaction we'll be doing is adding ACP to acetyl-CoA and malonyl-CoA.

⁷This is similar to pyruvate carboxylase reaction using biotin.

Observation 3.33 Basically, to synthesize FA, we need to generate a high energy intermediate and prevent it from diffusing to other compartment. To create the high energy intermediate, they will be coupled to a substrate called **acyl carrier protein (ACP)**.

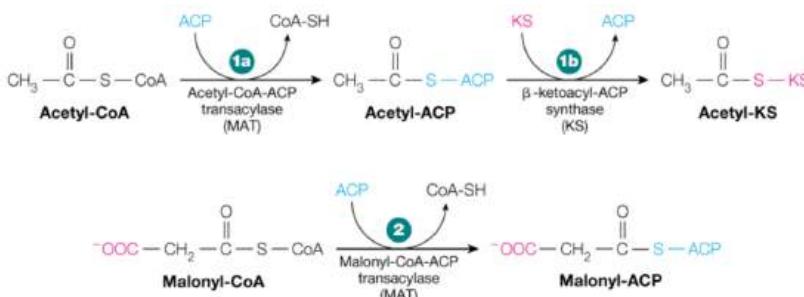


Figure 3.42: Coupling of ACP to acetyl- and malonyl-CoA.

The overall process consists of acetyl-CoA converted into acetyl-ACP using **Malonyl/Acetyl-CoA transferase (MAT)**, of which will then be further converted to acetyl-KS via β -ketoacyl-ACP synthase (KS). Similarly, malonyl-CoA is converted to malonyl-ACP using MAT.

Elongation

Now, we've primed the molecule, it's time to elongate it. The enzyme that mediates this reaction is called **fatty acid synthase**.

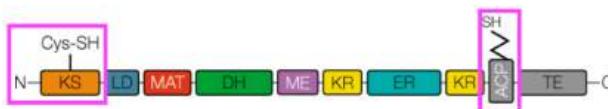


Figure 3.43: Domains of FA synthase.

Observation 3.34 The reaction is simple. We begin with acetyl-KS undergoing condensation reaction with malonyl-ACP. To be specific, malonyl-ACP will decarboxylate (3 carbon become 2 carbon) and simultaneously, acetyl-KS will denote its 2 carbons forming β -ketoacyl-ACP.

β -ketoacyl-ACP goes through many other steps to finally form **butyryl-KS** (4-carbon chain). This step will cycle back again with addition of more

acetyl-CoA for 6 more times in order to form palmityl-ACP that can undergo hydrolysis to make palmitate.

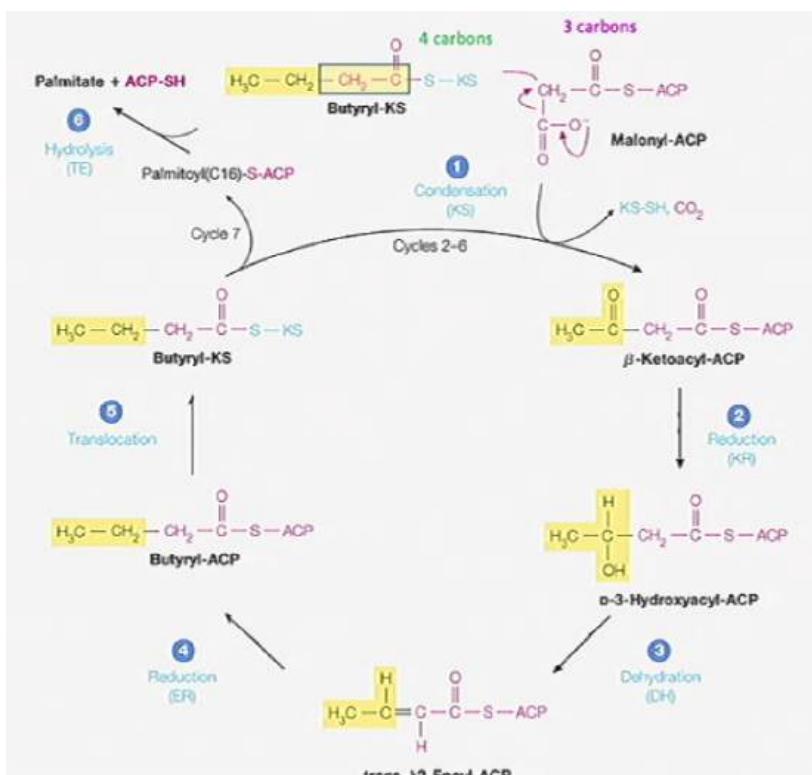


Figure 3.44: Elongation to make palmitate.

Observation 3.35 For your information, FA synthase is a really big molecule that have many catalytic sites that are all located on 1 monomer (see Figure 3.43). There are 2 domains to make thioesteric body where the one to make ACP is the main one.

Furthermore, the enzymes is made from 2 homodimer organized upside down from each other with lots of interaction between the domain. Because the catalytic domains/sites are fairly close, the reaction is really fast.

Remark 3.7. Though it's not mentioned here, 1 cycle of adding 2 extra carbons will need to invest 2 NADPH (from PPP).

3.5.3 Synthesis Regulation

We've mentioned that the rate-limiting step is ACC thus it'll be the one to be regulated. There are 2 ways it can be regulated: allosteric and hormonal.

With allosteric regulation, we begin with ACC in its low activity which can then become very active in the presence of citrate. On the other hand, it will be inhibited by FA.

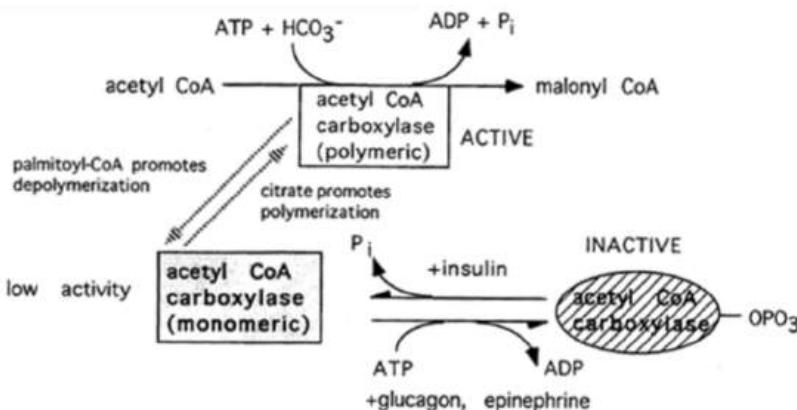


Figure 3.45: Regulation of ACC.

For hormonal regulation, glucagon can lead to its inhibition by phosphorylating it through a PKA. This is because you're having no glucose in circulation thus we need should not make more FA. On the other hand, insulin will lead to its activation by stimulate glucose uptake and pyruvate dehydrogenase thus making more acetyl-CoA.

3.5.4 Elongation and Desaturation of Fatty Acids

Palmitate is not the only one FA that we produce so...**how do we elongate or desaturate it?** Well...we do them as follows

- **Elongation:** For this, we will add acetyl-CoA onto the palmitate through the reversal of FA oxidation.

- **Desaturation:** We have desaturase that will induce FA unsaturation point at Δ^9 , Δ^6 , Δ^5 and Δ^4 . In mammals, we do not have desaturase that goes beyond the Δ^9 point \Rightarrow linoleic and lenolenic acids can not be made and must be ingested from the diet.

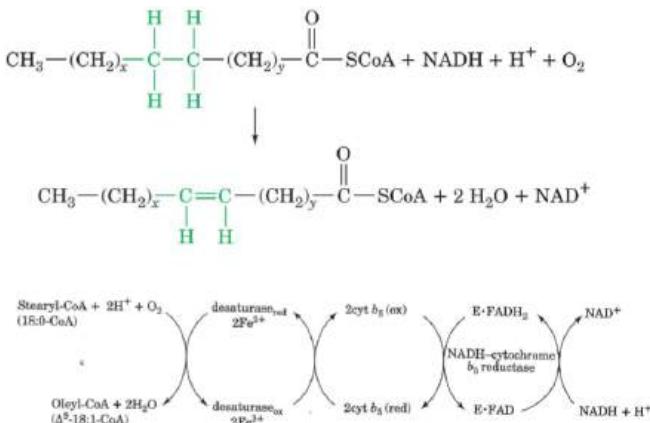


Figure 3.46: General reaction of desaturation .

Remark 3.8. *We need to make the FA then do elongation before desaturation in said order.*

3.5.5 Fatty Acids as Precursors

FA not only can be stored, we also use them as precursors of different molecules that is important for the body.

Example 3.5.2. Linolenic acid can be converted to arachidonic acid which is the precursor for prostaglandin and other molecules that induce an inflammatory response.

Interestingly, many **non-steroidal anti-inflammatory drugs (NSAIDs)** function by blocking the synthesis of certain FA \Rightarrow blocking the precursors of inflammatory chemicals \Rightarrow inflammation reduce.

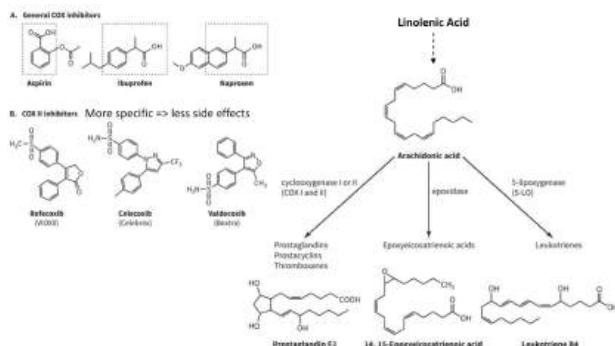


Figure 3.47: FA as precursors and NSAIDs

Summary

To summarize the breakdown and synthesis of FA, consider the following figure:

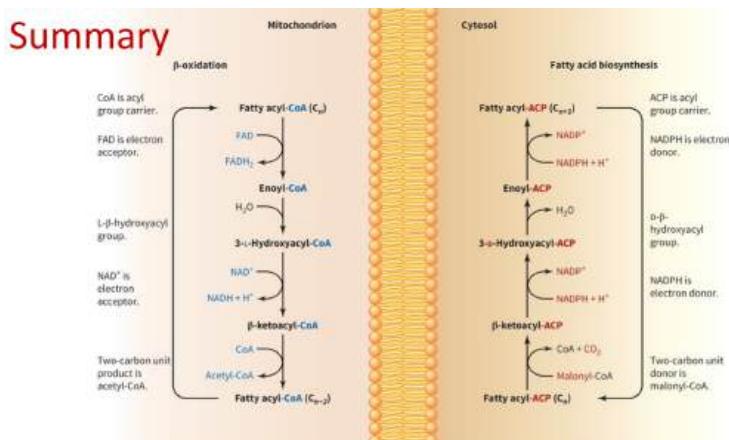


Figure 3.48: Summary of FA synthesis and degradation.

To breakdown FA, we first need to activated it and shuttles it in the mitochondria. It's regulated by substrate availability, compartmentalization, CPTI and can be inhibited by malonyl-CoA. For FA synthesis, we will shuttle it out and activated by ACP. It's regulated by ACC, allosterically citrate (+) and FA (-) while hormonally insulin (+) and glucagon (-).

3.6 Lipoproteins

In this lecture, we will focus on lipoproteins. Before that, there's an interesting research of lipoprotein lipase.

Observation 3.36 With the emergence of cardiovascular diseases that are linked with high lipids level in the body, researchers have tried to decrease its absorption by inhibiting lipid lipase. If you inhibit lipid lipase, you'd not be breaking FA down which decrease absorption by the brush border thus decreasing circulating chylomicron. Now...here's a problem, if you do not absorb lipids properly, it would lead to **steatorrhea** which is an excessive amount of fat in feces leading to diarrhea.

3.6.1 Structure of Lipoproteins

Definition 3.11. **Lipoproteins** are transporters/carriers of lipids that has a neutral/hydrophobic interior covered by 1 monolayer of phospholipids with at least 1 apolipoprotein inserted.

Definition 3.12. **Apolipoproteins** are amphipatic proteins inserted along the cover of lipoproteins, which they can help with their structural integrity but also serve as ligands for docking and recognition .

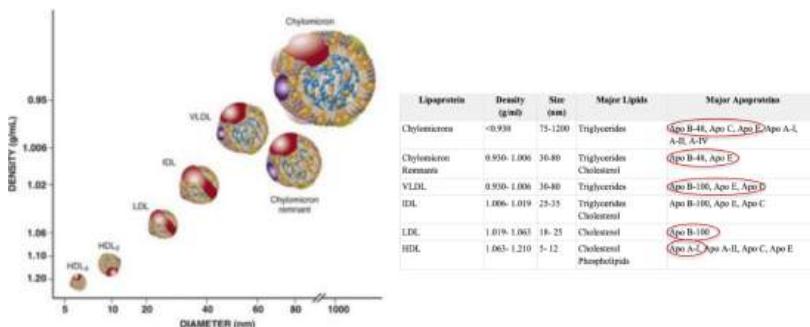


Figure 3.49: Variants of lipoproteins.

Observation 3.37 These lipoproteins are named in accordance to their density (with the exception of chylomicron which has the lowest density) e.g. lipoprotein that has high density is called *high-density lipoprotein (HDL)*.

3.6.2 Lipoproteins Metabolism

Since there are many variants of lipoproteins, we will look at the metabolism of each of them.

Chylomicron Metabolism

This is mostly from the exogenous pathway which would be formed right after the meal.

Observation 3.38 After you've had a meal, lipids will be broken down, absorbed and packed into chylomicron in intestinal cells. These **chylomicrons** have 3 different apolipoproteins include: ApoC (II and III), ApoE and ApoB-48. Once in circulation, these apolipoproteins can interact with **lipoprotein lipase (LPL)**, located along the capillaries of muscle and adipose tissues, which can digest the TG⁸ in the chylomicrons.

Once LPL has finished its reaction, we get **chylomicron remnants** that can travel to the liver and dock onto either receptors for ApoE or ApoB-48. The liver will uptake this leftover and thus we've cleared all of the dietary lipids from circulation.

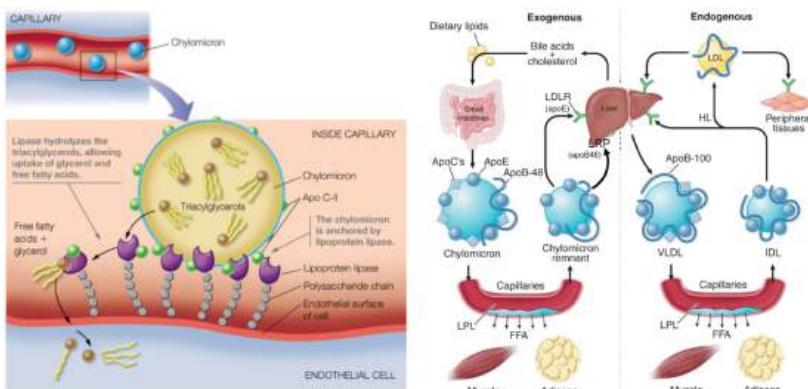


Figure 3.50: LPL and Lipoprotein metabolism.

Remark 3.9. The 48 of ApoB represents the percentage of the full length i.e ApoB-48 means 48% of ApoB.

⁸Abbreviation of triglycerides.

VLDL and LDL Metabolism

This is the endogenous pathway involving synthesizing and delivering lipids by the liver (See Figure 3.51, Endogenous pathway).

Observation 3.39 Very-low density lipoproteins (VLDL) have all of the same ApoC, ApoE but now with ApoB-100. Its job is to carry all of leftover TG of the liver and sent it off into circulation. Because it has the similar ligands as before, it can then activate LPL and TG can be taken up by muscle and adipose tissue. The remnant of this interaction is called **intermediate-density lipoproteins (IDL)**. IDL can either go back to the liver or be made into another type of lipoproteins.

Observation 3.40 Low-density lipoproteins (LDL) has all of the similar structure as the lipoproteins we've discussed but now with only ApoB-100. It's basically the remnants of IDL and their main job is to deliver cholesterol to peripheral tissues (especially tissue that synthesize hormones).

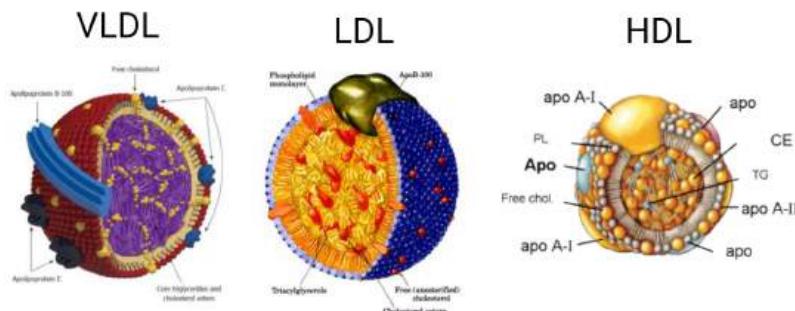


Figure 3.51: VLDL, LDL and HDL structure.

To be specific, they're taken up by cells through clathrin-mediated endocytosis. First, LDL receptors will recognize the LDL and initiate the endocytosis by creating a clathrin-coated pit surrounding the LDL. This later forms the clathrin-coated vesicle where the receptors will be recycled back to the surface. The vesicle will be acidified and breaks down LDL to extract the cholesterol that can be brought into the ER (Here, cholesterol will be regulated, next lecture).

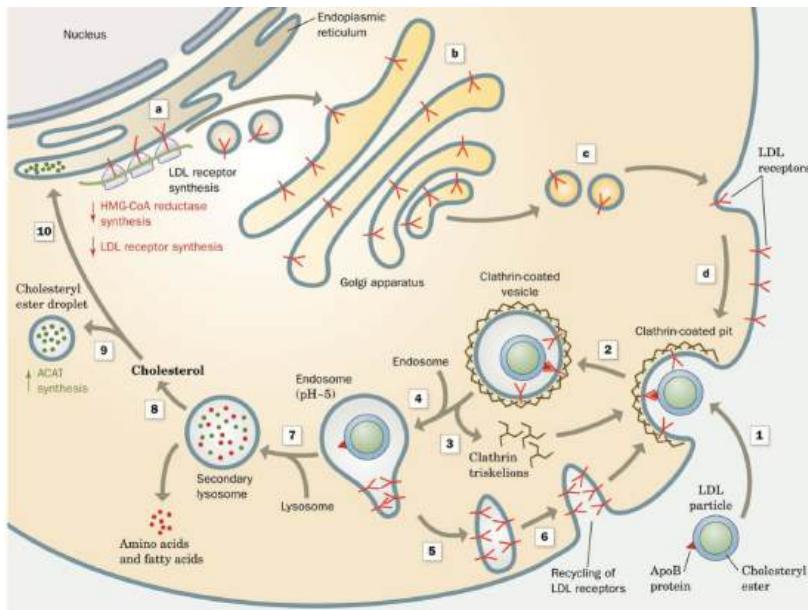


Figure 3.52: Clathrin-mediated endocytosis of LDL.

HDL Metabolism

Observation 3.41 **High-density lipoproteins (HDL)** has the same structure but now they have only ApoA I and II and ApoE. Their main job is to return the lipids back into the liver.

Mechanism of Action (HDL Metabolism): We first begin with forming the nascent HDL.

1. A floppase called **ABCA1** will use ATP to efflux lipids onto the lipid-free ApoA-I thus forming **nascent (pre) HDL** [a puck-like structure].
2. **Lecithin-cholesterol acyltransferase (LCAT)** will esterify the cholesterol by adding FA onto it thus making it completely neutral
⇒ The cholesterol ester (CE) will want to move into the nascent HDL interior which makes the nascent HDL larger, called **HDL₃**.
3. A protein called **CE transfer protein (CETP)** [a tube-like protein] will mediate the transport of TG into HDL₃ from VLDL (also trans-

port CE into VLDL).

The end product is HDL₂ which can return to the liver and interact a receptor called **SRB1** and allow the uptake of TG. The lipid-free ApoA-1 can then return back to the to produce HDL again.

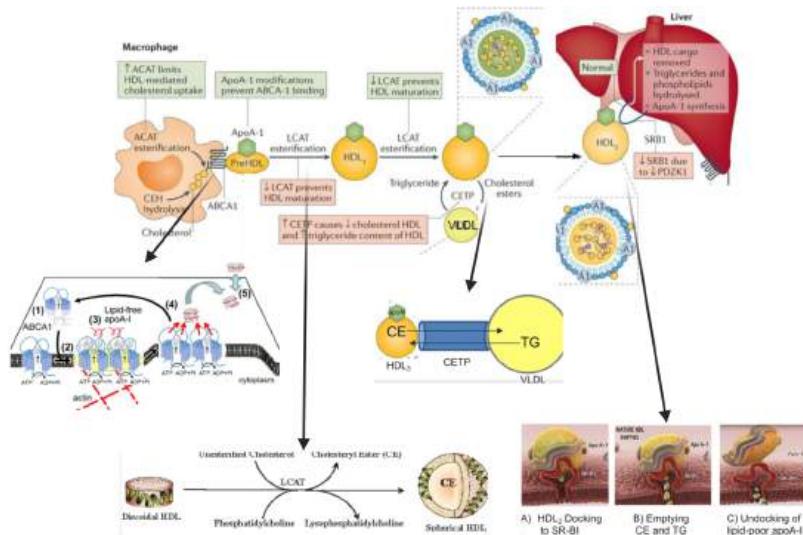


Figure 3.53: HDL metabolism.

Biosynthesis of Bile Salts

Now that the liver has more lipids, what is it going to do? Well...either make VLDL, which we've discussed, or make bile salts. First, cholesterol is transformed into 7α -hydroxycholesterol which, through many steps, will make glycocholate and taurocholate (both are bile acids)

Summary

The following figure constitute of what we've just talked about on lipoproteins metabolism.

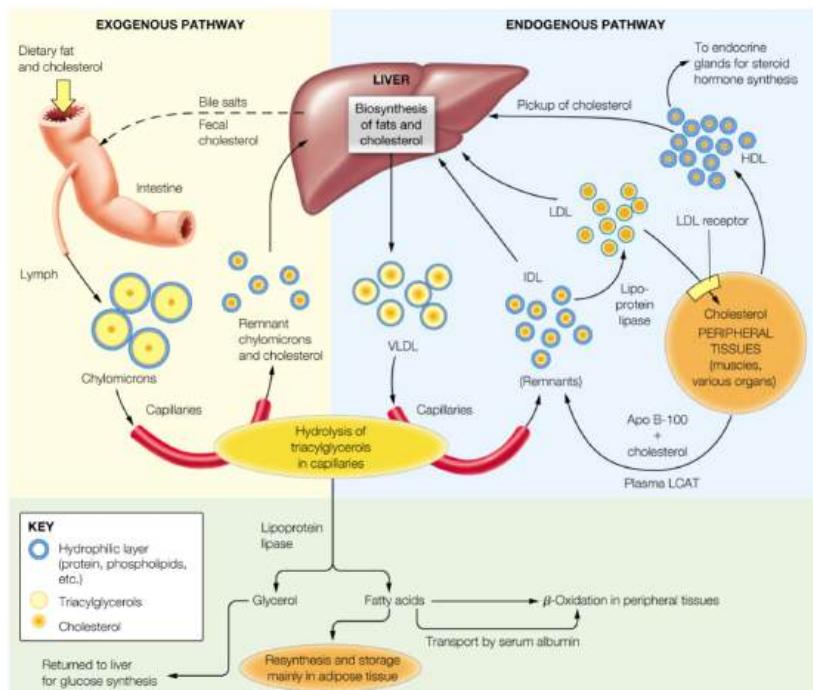


Figure 3.54: Summary of lipoprotein metabolism.

3.6.3 Diseases

We've discussed at the beginning of this module/chapter that there are serious consequences when there's irregular lipids level in blood (dyslipidemia).

So now... **What is good or bad cholesterol?** Well... the bad cholesterol is the LDL since it carries cholesterol to the tissue and when there's too much of LDL it starts to form plaque. The good cholesterol is HDL since it takes cholesterol away. Basically, there's no immediate correlation to what we eat to what considered to be bad or good cholesterol.

Observation 3.42 When there's an over accumulation of LDL in the arteries, macrophages will come and engulf them. They engulf so much so undergo apoptosis which will trigger the immune response to come and clear the debris from the cell death event.

More dangerously, when there's an over accumulation of lipids in the arteries, they will form plaque that would eventually block the arteries. This is called **atherosclerosis** and if it happens in the heart \Rightarrow heart attack; or in the brain \Rightarrow stroke.

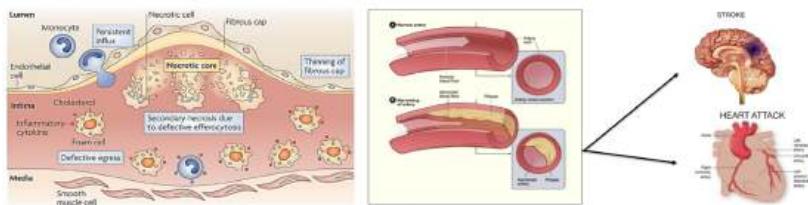


Figure 3.55: Over accumulation of LDL and lipids.

To summarize our above ideal. When there's a deficiency in LDL-receptors (LDL-R), we cannot uptake the LDL and its content \Rightarrow accumulation of LDL \Rightarrow **cardiovascular diseases (CVD)** risk increases. Likewise, when there's a deficient in ABCA1, we cannot make more HDL \Rightarrow no reuptake of excess lipids \Rightarrow increases CVD risk!

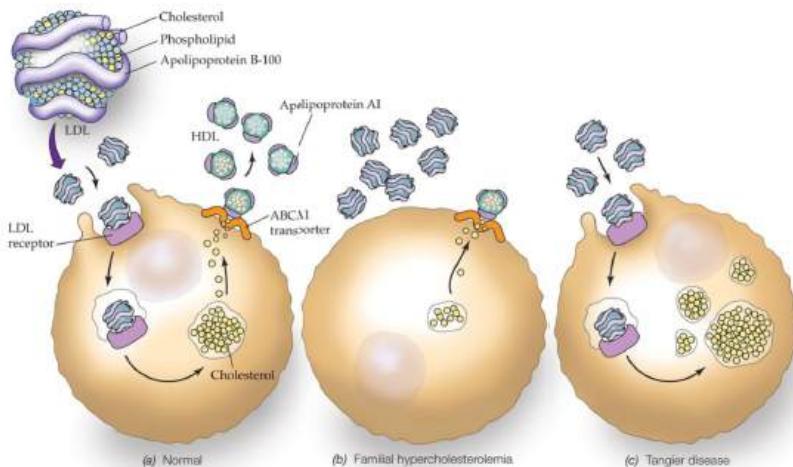


Figure 3.56: LDL-R and ABCA1 deficiency.

End of Lecture —

3.7 Cholesterol

Before talking about cholesterol, try the following questions: Hypercholesterolemia can be caused by...

- A) LPL deficiency C) ABCA1 mutations
- B) ApoC-III mutations D) ApoA-I deficiency

Answer: A and B

Question: Abnormally high [LDL] in plasma can be caused by loss of function of...

- A) LDL-R D) Lipoprotein lipase
- B) ApoB-100 E) Pancreatic lipase
- C) ABCA1

Answer: A, B, and D.

Question: Low [HDL] may result from mutations in...

- A) LDL-R D) ABCA1
- B) Lipoprotein lipase E) ApoA-I
- C) LCAT

Answer: C, D and E

Remark 3.10. We can make cholesterol from acetyl-CoA but never the converse.

3.7.1 Cholesterol Synthesis

Cholesterol is made from isoprene subunits starting from acetyl-CoA with some investment of ATP and NADPH.

Methods 3.2 The original way to discover that cholesterol was built from acetyl-CoA was by using radiolabelling. First, scientists radiolabel the carbons of the acetyl-CoA and then later, they track those same carbon to be in those of cholesterol.

Mevalonate Pathway

Before cholesterol synthesis, we will begin another pathway called the **mevalonate pathway** that will convert a molecule called mevalonate into substrates that would be used to make cholesterol.

Mechanism of Action (Cholesterol Synthesis): Before entering mevalonate pathway, we will first make mevalonate.

1. Acetyl-CoA combines with acetoacetyl-CoA making **HMG-CoA** which can then be turned into acetoacetate (This is the 1 of pathway of ketogenesis!).
2. The **rate-limiting step of cholesterol synthesis is that of HMG-reductase (key regulatory enzyme)** that reduce HMG-CoA into **mevalonate** using NADPH.

Now, we will begin the mevalonate pathway then synthesize cholesterol.

3. Mevalonate will further be modified into **farnesyl pyrophosphate** (These modification steps invest 3ATP)^a.
4. Farnesyl pyrophosphate can be turned into **squalene** which then turned into **lanosterol** (3 more steps) then finally 1 **cholesterol** (19 more steps).

^aThis molecule can also help with protein anchoring in the membrane.

What's the most striking about this pathway is that it requires **36ATP** and **16NADPH** just to make 1 cholesterol molecule \Rightarrow it's precious and shouldn't be broken down.

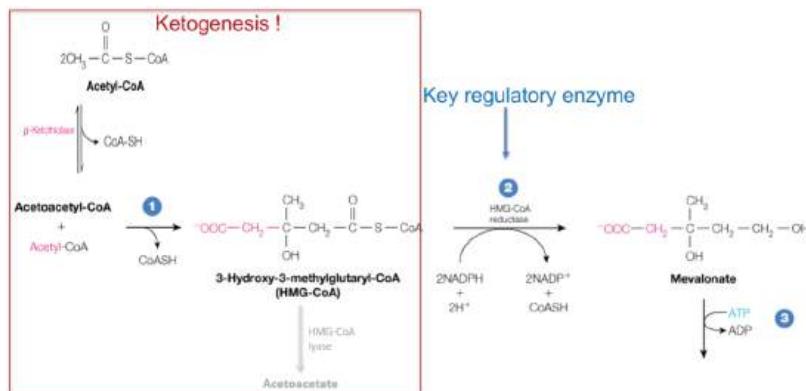


Figure 3.57: Synthesis of mevalonate molecule.

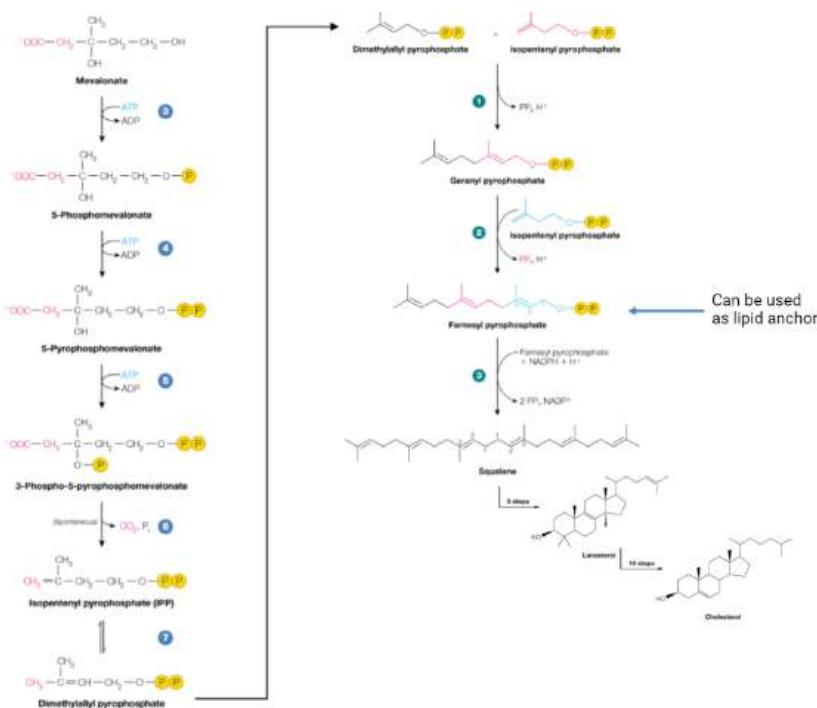


Figure 3.58: Mevalonate pathway and synthesis of cholesterol.

3.7.2 Regulation

Since cholesterol requires lots of energy to synthesize, it needs to be regulated. There are 2 levels of regulation of cholesterol: **by the body and by the cells.**

Observation 3.43 In the body, we ingest around 300mg of cholesterol per day. The cholesterol pool in our body is around 100mg and we synthesize around 700mg per day. Normally, we excrete cholesterol as bile acids and biliary cholesterol of 1000mg \Rightarrow We require a good balance of dietary input of cholesterol and endogenous synthesis.

At the cellular level, the synthesis of cholesterol depends on the energy state of the cell \Rightarrow **it requires lots of energy to start synthesis.** When you're

in a low energy state \Rightarrow low ATP and high AMP⁹ \Rightarrow **AMP kinase (AMPK)** is activated which will phosphorylate HMG-CoA reductase which makes it less active than before.

On the other hand, when there's high ATP \Rightarrow protein phosphatase is active \Rightarrow dephosphorylate the inactive HMG-CoA thereby making it active.

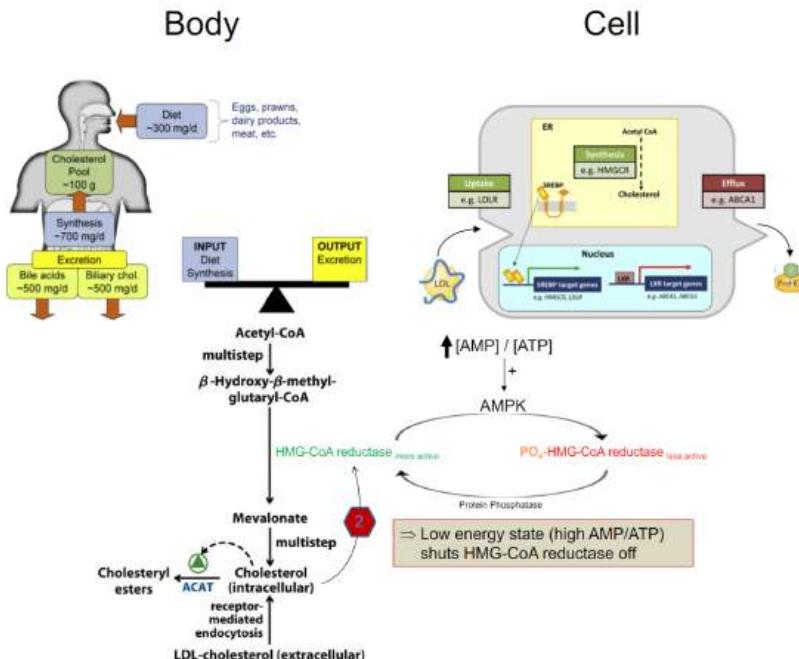


Figure 3.59: Body vs cellular regulation of cholesterol synthesis.

Observation 3.44 Another way is through transcription control of the amount of HMG-CoA reductase. This depends on the concentration of cholesterol in the ER. In general, when the body is low in cholesterol, protein called **sterol-response element binding element (SREBP)** in the ER will travel to the nucleus and activate genes that are involved in the production of cholesterol (e.g. HMG-CoA reductase gene).

⁹Same as saying $[AMP]/[ATP]$ is large.

Mechanism of Action (Transcriptional Control of Cholesterol Synthesis): We begin with low level of cholesterol

1. A protein called **scap**, that is bound to SREBP at the ER, has a cholesterol sensing domain does not detect it (low level).
2. Once it detect a low level of cholesterol, scap will transport the enter complex of SREBP into the golgi apparatus.
3. An enzyme called **site-1 protease (S1P)** will cleave the SREBP at position 1 releasing a part of SREBP.
4. Another enzyme called **site-2 protease (S2P)** will cleave the released form of SREBP at site 2 and thus release its head domain called **basic helix-loop-helix (bHLH)**.
5. bHLH can travel to the nucleus and bind to SRE response elements and promoter that lead to activation of genes involved in cholesterol synthesis.

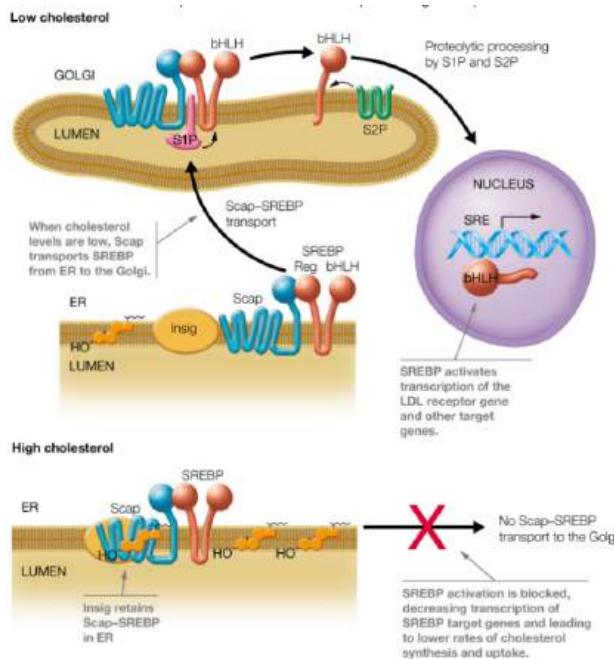


Figure 3.60: Transcriptional control of cholesterol synthesis.

In the end, you'd be making lots of HMG-CoA reductase thus increases

the level of cholesterol synthesis.

Remark 3.11. Another gene that is regulated by cholesterol level is that of *LDL-R* \Rightarrow low cholesterol leads to increase *LDL-R* \Rightarrow increase *LDL absorption*.

Observation 3.45 Now, that was for low cholesterol level. For high level, we can deduce the opposite. The detecting domain of scap will be bound to cholesterol \Rightarrow it will not transport anyway \Rightarrow no transcription.

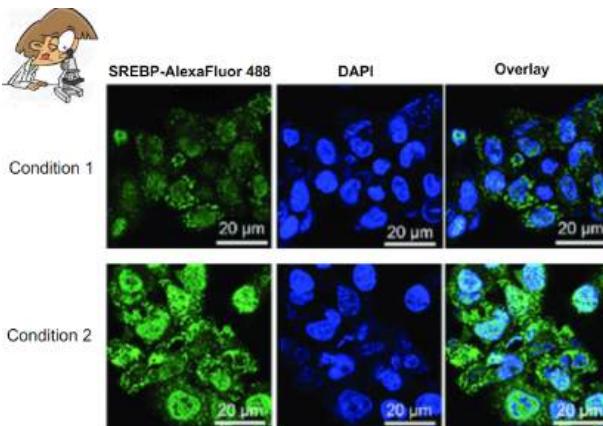
Pharmacological Perspectives

How can cholesterol synthesis be regulated pharmacologically?

- A) HMG-CoA synthase inhibitor
- B) HMG-CoA synthase activator
- C) HMG-CoA reductase inhibitors
- D) HMG-CoA reductase activators

Answer: C, HMG-CoA reductase inhibitors and the molecules in question are called **statins**. Not only do they inhibit HMG-CoA reductase activities, they also increase the synthesis of *LDL-R*.

Question: DAPI is a fluorescent tag used to light up the nucleus while SREBP-AlexaFluor 488 (in the nucleus) is to lightup the SREBP. Look at the following fluorescent microscopy images and determine which condition (low or high cholesterol) are the cell in.



Answer: Condition 1 is where the cell has high amount of cholesterol and condition 2 is where the cell has low cholesterol.

Explanations. In condition 1, we can see that very little amount of SREBP is located in the nucleus \Rightarrow it did not get transported there \Rightarrow cholesterol is bound to scap \Rightarrow high level of cholesterol. For condition 2, it follows a similar but opposing argument. \square

3.7.3 Sterol Derivatives

Cholesterol is never broken down! However it can be stored in the form of cholesterol-ester or used as a backbone for the synthesis other molecules.

Cholesteryl Ester

First off, we can store it in many part of the body.

Example 3.7.1. In the intestine, it's used to packaged into chylomicrons, in the liver¹⁰, it's in the VLDL and in adipose tissue, it's lipid vesicle.

The main enzyme that does this esterification process is **acyl-CoA-cholesterol acyltransferase (ACAT)** in order to make it lipid neutral. It can also be esterified in nascent HDL using LCAT as discussed above.

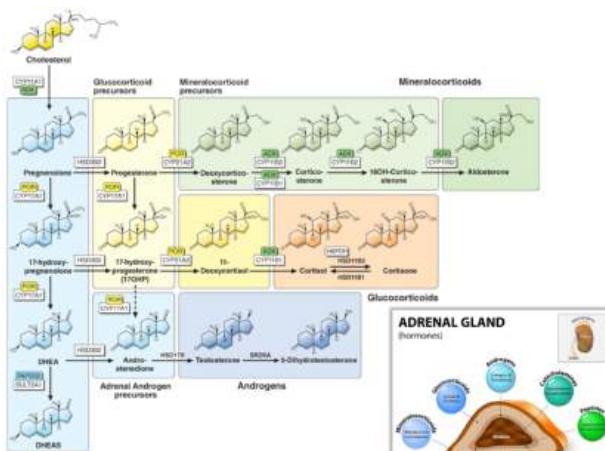


Figure 3.61: Cholesterol used to make hormones.

¹⁰It's mostly in the liver's adipocytes that cholesterol is stored

Bile Acids

We can use cholesterol to make bile salts where the rate-limiting step is that of **cholesterol 7 α -hydroxylase** (Cyp7 α 1).

Steroid Hormones

We can use cholesterol as the backbone to synthesize different hormones in the body.

Vitamin D

Lastly, cholesterol is also a starting material to make vitamin D. In the skin, when exposed to UV light, 7-dehydrocholesterol can be converted to cholecalciferol then through many steps into vitamin D₃.

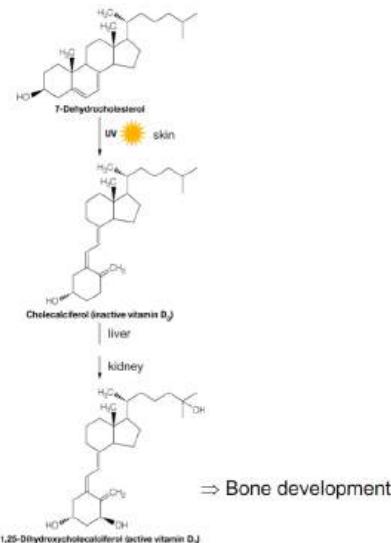


Figure 3.62: Synthesis of vitamin D₃ from cholesterol.

Remark 3.12. All in all, cholesterol is now degraded but is transformed.

3.8 Integration of Pathways

In this lecture, we will put all of the pathway we've learned together.

The usage of energy depends on the organ. The following table provides the energy usage and storage by each organ and

Tissue	Fuel Reserves	Preferred Fuel	Fuel Sources Exported
Brain	None	Glucose (ketone bodies during starvation)	None
Skeletal muscle (resting)	Glycogen, protein	Fatty acids	None
Skeletal muscle (during exertion)	None	Glucose	Lactate
Heart muscle	None	Fatty acids	None
Adipose tissue	Triacylglycerols	Fatty acids	Fatty acids, glycerol
Liver	Glycogen, triacylglycerols	Glucose, fatty acids, amino acids	Fatty acids, glucose, ketone bodies

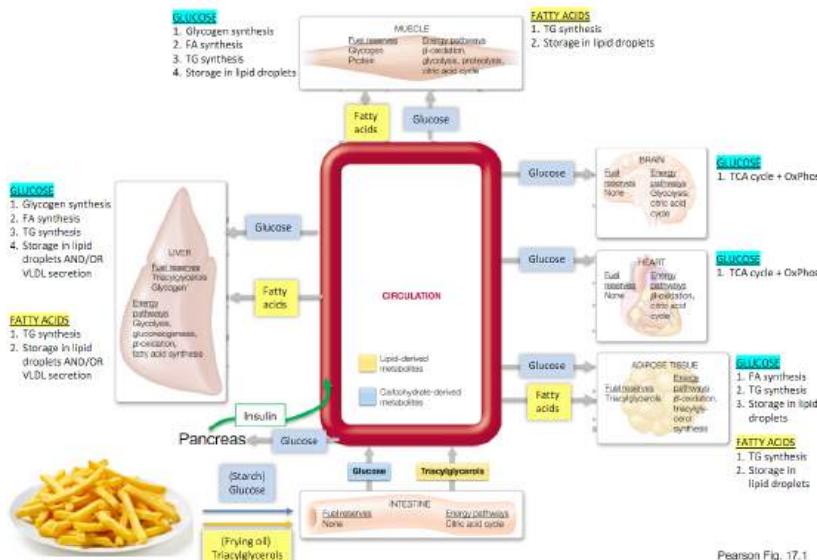
3.8.1 After A Meal and At Rest

Observation 3.46 After a meal and we're at rest, the glucose entering our body will be absorbed by intestinal cells and sent into circulation. This elevate blood [glucose] above 5.5mM which induces the release of insulin. Insulin will then stimulate glucose reuptake via transporter (GLUT, can be insulin-dependent or -independent).

In the liver, glucose is converted into glycogen for storage. Once glycogen storage is filled up, it will synthesize FA \rightarrow TG and stored in lipid droplets. All of the excess TG will be packaged into VLDL and sent off to be stored else where. In the muscle, it also follows the same pattern, the only different is that it will not have VLDL to sent excess synthesized TG.

In the adipose tissue, glucose is converted directly into FA \rightarrow TG which are then stored in lipid droplets. Organs like the brain and heart will use the glucose as a source of energy to drive CAC and oxidative phosphorylation directly.

Observation 3.47 The above are for glucose, for fats, it will be absorbed by broken down into FA and the reassemble into TG and sent into circulation as chylomicron. In the liver, the FA will be converted into TG and stored in lipid droplets (excess are sent away in VLDL). In the muscle, FA is converted into TG and stored in lipid droplets; this is similar in adipose tissues. There won't be any storage of TG in heart nor brain.



Pearson Fig. 17.1

Figure 3.63: Integration of pathways: after a meal and at rest.

3.8.2 At Exercise or Stress

Now, let's look what happens when the body is at stress or exercise. We'll begin with the perspective of glucose.

Observation 3.48 In the liver, glycogen will be broken down and gluconeogenesis begin releasing glucose. The released glucose can be used by muscle for anaerobic glycolysis, CAC and oxidative phosphorylation (it can breakdown glycogen for energy too). These 2 processes can be induced by adrenaline during stress.

The bi-product from the muscle is lactate that can be reverted back into glucose in the liver. In the heart and brain, glucose made from the gluconeogenesis will be used up as well. In adipose tissue, there are no such glucose.

Observation 3.49 Now, for the perspective of FA. In the liver, FA is produced by lipolysis and exported to blood. In the muscle, it can also produce FA through lipolysis and then use the FA for β -oxidation for CAC and oxidative phosphorylation. In the adipose tissue, FA is produced by lipolysis and transported out. There are no FA made in the brain nor heart.

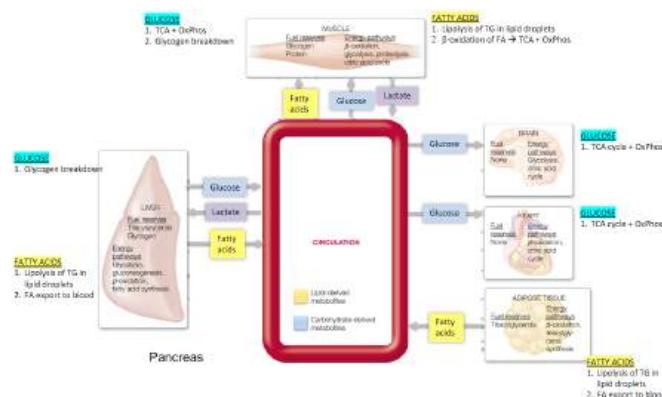


Figure 3.64: Integration of pathways: at exercise or stress.

3.8.3 Prolonged Exercise or Starvation

Now, you're exercising for a really long time or even starving yourself. The body has finally depleted its source of glucose.

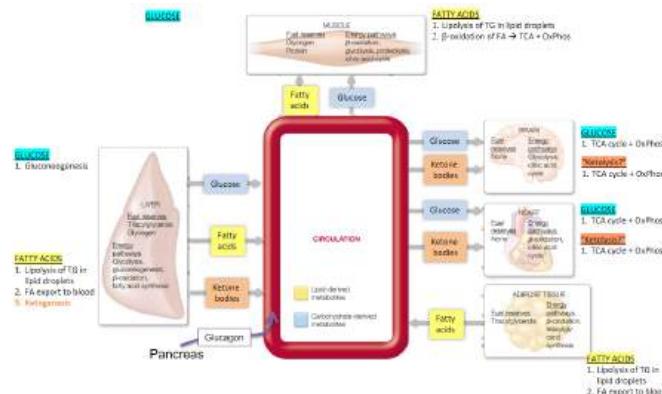


Figure 3.65: Integration of pathways: prolonged exercise or starvation.

Observation 3.50 During prolonged exercise or starvation, blood [glucose] drops below 5.5mM, the pancreas will release glucagon to stimulate the liver to enter gluconeogenesis to feed glucose for organs like brain and heart. It can also make FA to sent off to other organs through lipolysis.

Wait...but we've mentioned that FA can't be done much in the heart and brain, so **how does this help?** Well...along with FA synthesis, the liver produce ketone bodies for the heart and brain for ketolysis and generating energy.

The following figures are other ways to summarize the idea that we've just talked about.

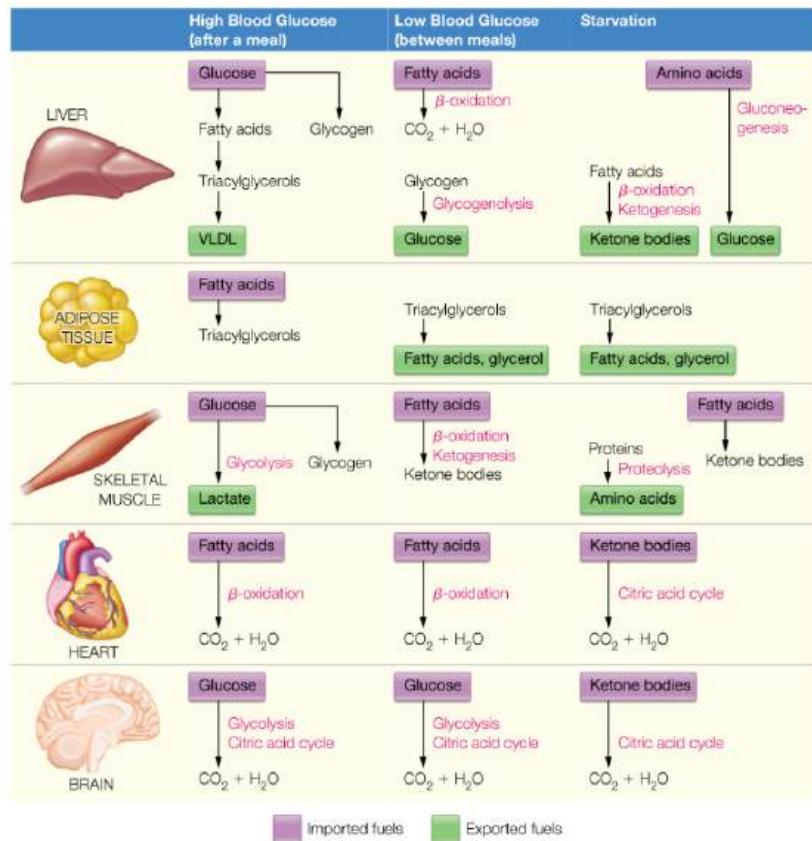


Figure 3.66: Metabolism during fed and unfed state.

Another way to look at the metabolism is at the cellular level:

High Energy Conditions	Pathways or enzymes upregulated	Pathways or enzymes downregulated	Low Energy Conditions	Pathways or enzymes upregulated	Pathways or enzymes downregulated
High $[ATP]/[ADP] + [AMP]$	Gluconeogenesis	Glycolysis PDC	Low $[ATP]/[ADP] + [AMP]$	Glycolysis PDC TCA cycle β -oxidation AMPK	Gluconeogenesis
High $[NADH]/[NAD^+]$	Minor effects	PDC TCA cycle β -oxidation	Low $[NADH]/[NAD^+]$	PDC TCA cycle β -oxidation Sirtuins	Minor effects
High $[GTP]$	Gluconeogenesis	Glycolysis	Low $[GTP]$	Glycolysis	Gluconeogenesis
High $[ACETYL-CoA]$	Gluconeogenesis (via PEPCK and PCK) Glycolysis (via PK)	Fatty acid β -oxidation	Low $[ACETYL-CoA]$	PDC β -oxidation	Minor effects
High $[MALONYL-CoA]$	Fatty acid synthesis (via carnitine shuttle)	Fatty acid oxidation (via carnitine shuttle)	Low $[MALONYL-CoA]$	Fatty acid oxidation (via acyltransferase)	Fatty acid synthesis (via acetyl-CoA carboxylase)
High CITRATE	Fatty acid synthesis (via acetyl-CoA carboxylase)	Glycolysis (via PFK)	Low CITRATE	Minor effects	Fatty acid synthesis (via acetyl-CoA carboxylase)

Figure 3.67: Biochemistry of cellular metabolic state.

3.8.4 Hormonal Control

We'll be talking more about hormonal control in module 5 but we'll briefly go through some of it.

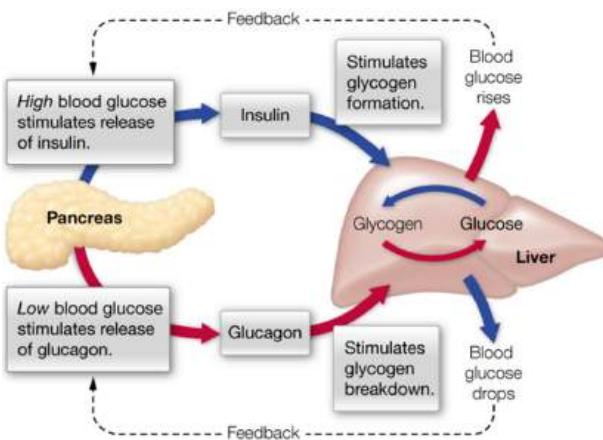


Figure 3.68: Opposing nature of glucagon and insulin.

Observation 3.51 As we've just discussed above, you have 2 insulin and glucagon that will be released after a meal and during starvation respectively.

Insulin, when released at high blood [glucose], will induce the uptake of glucose and conversion of it to glycogen. Glucagon, when released at low blood [glucose], will induce the breakdown of glycogen to make glucose. To be specific, increase in glucagon secretion will increase [cAMP] \Rightarrow increase phosphorylation of certain enzymes that lead to glycogenolysis, gluconeogenesis to increase blood [glucose].

Hormone	Biochemical Actions	Enzyme Target	Physiological Actions
Insulin	<ul style="list-style-type: none"> ↑ Glucose uptake (muscle, adipose tissue) ↑ Glycogenesis (liver, muscle) ↑ Acetyl-CoA production (liver, muscle) ↑ Glycogen synthesis (liver, muscle) ↑ Triacylglycerol synthesis (liver) ↓ Gluconeogenesis (liver) ↓ Lipolysis ↓ Protein degradation ↑ Protein, DNA, RNA synthesis 	<ul style="list-style-type: none"> GLUT4 PFK-1 (via PFK-2/FBpase-2) Pyruvate dehydrogenase complex Glycogen synthase Acetyl-CoA carboxylase FBPase-1 (via PFK-2/FBpase-2) 	<ul style="list-style-type: none"> Signals fed state: ↓ Blood glucose level ↑ Fuel storage ↑ Cell growth and differentiation
Glucagon	<ul style="list-style-type: none"> ↑ cAMP level (liver, adipose tissue) ↑ Glycogenolysis (liver) ↓ Glycogen synthesis (liver) ↑ Triacylglycerol hydrolysis and mobilization (adipose tissue) ↑ Gluconeogenesis (liver) ↓ Glycolysis (liver) ↑ Ketogenesis (liver) 	<ul style="list-style-type: none"> Glycogen phosphorylase Glycogen synthase Hormone-sensitive lipase, adipose triglyceride lipase FBPase-1 (via PFK-2/FBpase-2), pyruvate kinase, PEPCK PFK-1 (via PFK-2/FBpase-2) Acetyl-CoA carboxylase 	<ul style="list-style-type: none"> Signals fasting state: ↑ Glucose release from liver ↑ Blood glucose level ↑ Ketone bodies as alternative fuel for brain
Epinephrine	<ul style="list-style-type: none"> ↑ cAMP level (muscle) ↑ Triacylglycerol mobilization (adipose tissue) ↑ Glycogenolysis (liver, muscle) ↓ Glycogen synthesis (liver, muscle) ↑ Glycolysis (muscle) 	<ul style="list-style-type: none"> Hormone-sensitive lipase, adipose triglyceride lipase Glycogen phosphorylase Glycogen synthase Glycogen phosphorylase, providing increased glucose 	<ul style="list-style-type: none"> Signals stress: ↑ Glucose release from liver ↑ Blood glucose level

Figure 3.69: Major hormones controlling fuel metabolism in mammals.

Metabolic abnormalities

Definition 3.13. Type I diabetes is an autoimmune disease resulting in the destruction of β -cells in the pancreas leading to the inability to produce insulin.

Remark 3.13. This is commonly developed by children (which is why it was called juvenile diabetes)

Definition 3.14. Type II diabetes is condition where the pancreatic cells has become desensitized to high blood [glucose] that it stop producing as much insulin as before. Or, the cells are less responsive the produced insulin.

In both cases, an injection of exogenous insulin is required with the difference is that type I diabetes require insulin injection because there's no insulin, whereas type II diabetes requires insulin injection to add on to the already made insulin in the body.

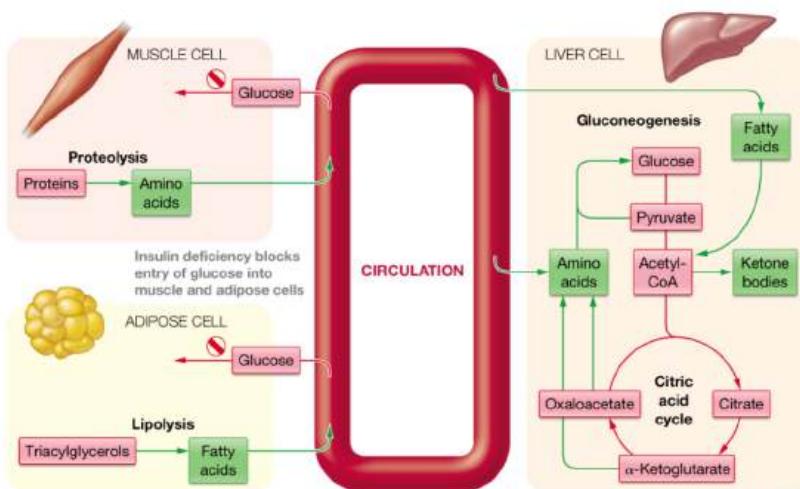


Figure 3.70: Metabolic abnormalities in diabetes.

Amino Acids and Nucleotides

In this chapter, we will look at amino acid and nucleotides.

Definition 4.1. A ***de novo pathway*** of nucleotides is a synthesis of nucleotides from starting materials of amino acids, ribose, etc. It's highly conserved.

Definition 4.2. The ***salvage pathway*** of nucleotides is the recovery of bases for making new nucleotides. It's more divergent amongst organisms.

Remark 4.1. *1 way to make antibiotics is to shut down the *de novo pathway* since bacteria mostly use it.*

There will be 2 *de novo* pathways and 1 is for purines the other for pyrimidine. In today's lecture, we begin with the *de novo* pathway of purine synthesis.

4.1 ***De Novo Pathway of Purine***

The material involved in the *de novo* pathway of purine includes: PRPP, glutamine, aspartate, CO₂, glycine and formate; which would be to make IMP.¹ Now, before talking about the pathway itself, we need to introduce terminology and nomenclature to simplify the process.

4.1.1 **Nomenclature and Terminology**

Observation 4.1 (Nomenclature). Nucleotides have a "**base**" which is the aromatic part. Different nucleotides will have different bases and they're divided into 2 types: **purines** and **pyrimidines**. There are 2 different riboses (deoxyribose and ribose) that the base can be put on which will form **nucleosides**. If we add a phosphate group to nucleosides, it becomes **nucleotides**.

¹For pyrimidine, it will be a shorter list with CO₂, glutamine, aspartate and PRPP to make UMP

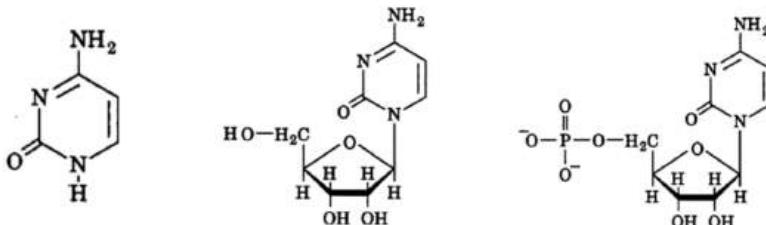


Figure 4.1: Base (left), nucleoside (middle) and nucleotide (right).

Purine has 2 fused ring of 6- and 5-membered ring. The nitrogen will always be on the odd number (beside 5) and the **position 9** is where it links to the ribose. **Pyrimidine**, on the other hand, has a single 6-membered ring. Similarly, its nitrogen will be odd number position but now the **ribose** is at position 1.

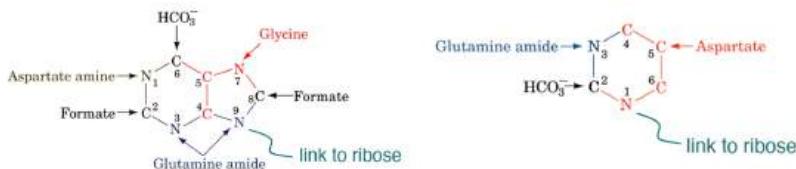


Figure 4.2: Purine (left) vs pyrimidine (right). The illustration also shows all the starting materials to make up the base.

There are 2 kind of ribose: **ribose** and **deoxyribose**. In both cases, they are 5-membered sugar ring (**furanose**) and is considered a D sugar. The link between the ribose and the base is called the **β -glycosidic bond** at 1' carbon while the phosphate group is linked at 5' carbon. The linkage at 1' carbon can either above (called β -position) or below (called α -position) since this carbon is an *anomeric* carbon.

In general, the only difference between these 2 riboses are 2'OH i.e. with ribose, you have the 2'OH while with deoxyribose, you only have a 2'H.

Remark 4.2. *It's important that when numbering the carbon position, we will include the ' to differentiate it from the base counting. e.g. 1 vs 1'*

The table below provides 5 different bases, 2 of which are purine (ade-

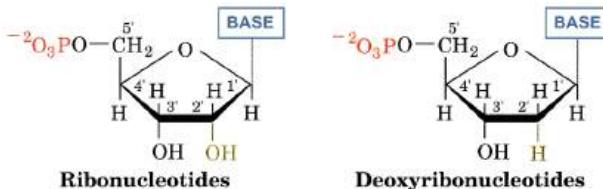


Figure 4.3: Ribose vs deoxyribose.

nine and guanine) while the rest are pyrimidines (cytosine, uracil and thymine). All 3 adenines, guanine cytosine can be found in both DNA and RNA; only uracil is found in RNA and similarly for thymine is only DNA.

CAUTION: It will be tempted to assume the naming configuration from bases to nucleoside is to add a suffix "-sine" e.g. adenine (base) vs adenosine (nucleoside). **But this is not always right** e.g. uracil (base) vs uridine (nucleoside); or even cytosine (base) vs cytidine (nucleoside). ² Also, thymine IS NOT the same as thiamine!

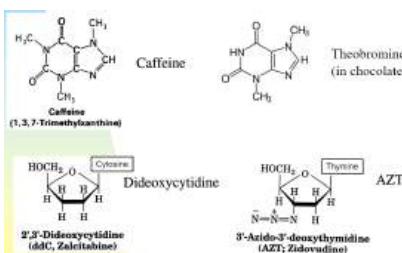
Metabolites

Observation 4.2 Before making these nucleotides, we need to make its metabolites. This includes: xanthine, hypoxanthine, orotic and uric acids.

The mentioned nucleotides are not just for making RNA and DNA. They are also important as drugs with different therapeutic usages.

Example 4.1.1.

Caffeine is an important chemicals to help you stay awake. Theobromine is found in chocolate. ddC is used mostly in Sanger's sequencing and AZT is used as antivirals.



1 thing that we will be using a lot in the following lecture is "the cube". This is just the mapping pyrimidine.

Figure 4.6: Therapeutic usages of nucleotides beside making DNA and RNA.

²i.e. the best ways to remember them is through memorization...

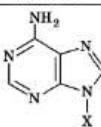
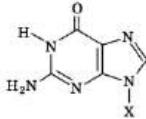
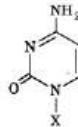
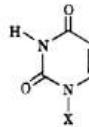
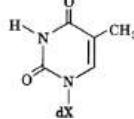
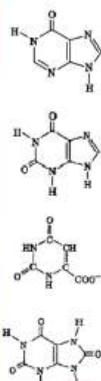
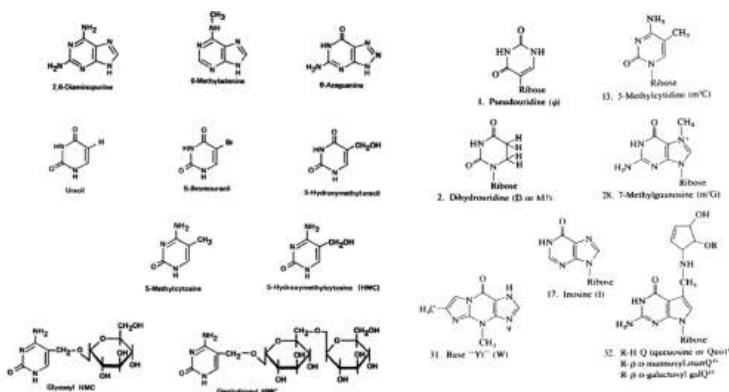
Base Formula	Base ^a X = H	Nucleoside X = ribose ^a	Nucleotide ^b X = ribose phosphate ^a
	Adenine Ade A	Adenosine Ado A	Adenylic acid Adenylate Adenosine monophosphate AMP
	Guanine Gua G	Guanosine Guo G	Guanylic acid Guanylate Guanosine monophosphate GMP
	Cytosine Cyt C	Cytidine Cyd C	Cytidylic acid Cytidylate Cytidine monophosphate CMP
	Uracil Ura U	Uridine Urd U	Uridylic acid Uridylate Urdine monophosphate UMP
	Thymine Thy T	Deoxythymidine dTd dT	Deoxythymidylic acid Deoxythymidine monophosphate dTMP Thymidylate Deoxythymidylate

Figure 4.4: Table of bases, their formed nucleosides and nucleotides name.

Interestingly, our ribosomes are made of RNA but up to 25% in tRNAs are heavily modified (rRNA are also modified but not as much, mostly methylated bases).

Base	Nucleoside	Nucleotide
Hypoxanthine	Inosine	Inosinate (IMP)
Xanthine	Xanthosine	Xanthylate (XMP)
Orotate (orotic acid)	Orotidine	Orotidylate (OMP)
Uric acid	—	—


Figure 4.5: Metabolites as building block for the biosynthesis of bases.**Figure 4.8:** Modification of Bases in RNA and DNA

Furthermore, only 1 – 2% of animal and 5 – 8% of plant DNA bases are 5-Me-cytosine, 1 – 2% of *E. Coli* are 6-Me-adenine.

4.1.2 Formation of 5-phosphoribosylamine

Concept 4.1 Almost always, biochemical pathway are regulated at the start.

How are we going to synthesize purine? Well...let's briefly go through

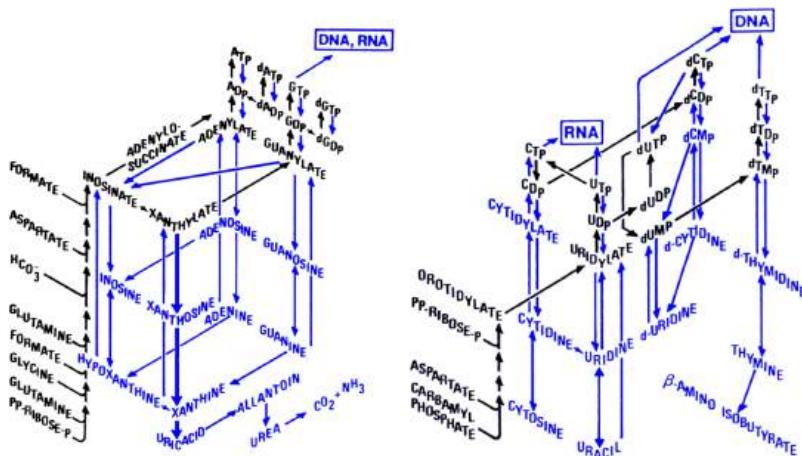


Figure 4.7: Purine and pyrimidine cube. Black arrows represent the *de novo* pathway while blue represents the degradation and salvage pathway.

all of the sources and then we'll see how they're put together sequentially. First , we will get the 1, and 2,9 nitrogen from aspartate amine and glutamine amide respectively. The other 7-nitrogen will be from glycine (along with 5,4-carbon). 6-carbon will be taken from HCO_3^- and 2,8-carbon from formate.

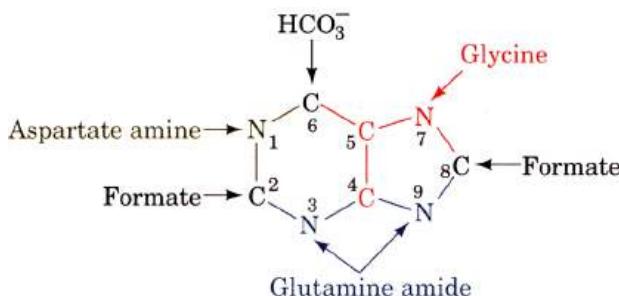


Figure 4.9: Origin of atoms on purines.

Mechanism of Action (Synthesis of 5-phosphoribosylamine): We will first begin synthesizing PRPP^a

1. α -D-Ribose-5-phosphate (i.e. 5-phosphoribose) will be converted into **Phosphoribosyl pyrophosphate (PRPP)** by **ribose phosphate pyrophosphokinase** using ATP.
2. PRPP can then be converted into **5-phosphoribosylamine** by amidophosphoribosyl transferase using glutamine and water.

Both of these steps are subjected to regulation.

^aIn some textbook, the synthesis of PRPP is step 1 other argue that it's already there.

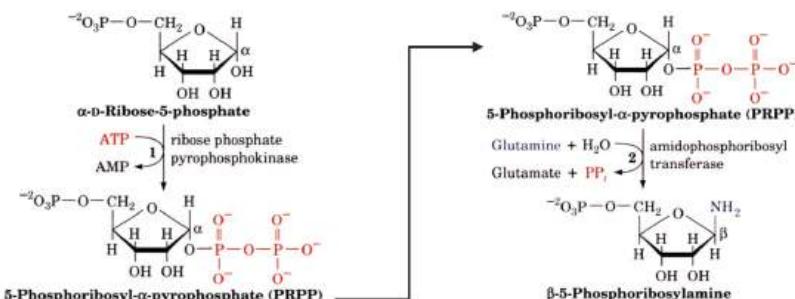


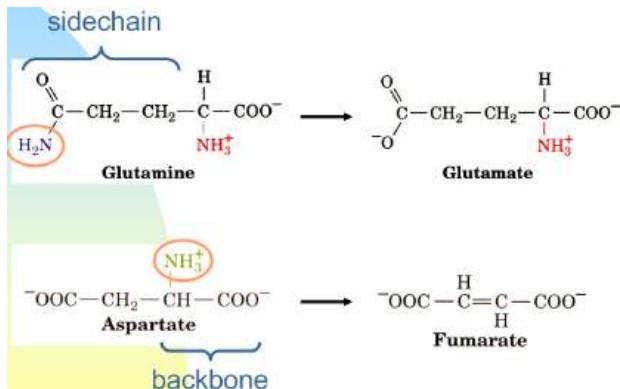
Figure 4.10: Synthesis of 5-phosphoribosylamine.

Explanations. The reason we have 2 regulation steps is that 1 for regulation while the other one is to commit to the *de novo* synthesis. □

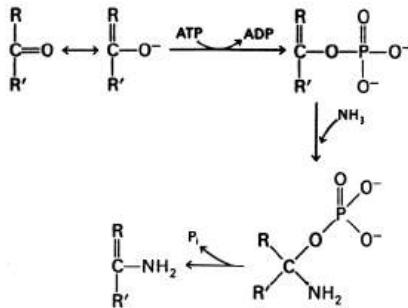
Observation 4.3 In PRPP, the pyrophosphate is at the α -position of the ribose. PRPP will be converted into 5-phosphoribosylamine using the nitrogen of glutamine and the amine group will be at the β position (pyrophosphate will be detached from the α -position). Note too that PRPP is a key intermediate in nucleic acid and amino acid biosynthesis.

Why are we wasting pyrophosphate? Well...The reason we have pyrophosphate here is used to drive the reaction forward and never synthesize backward. Thus, this reaction would be irreversible.

Remark 4.3. Nitrogen can only comes from 2 places: **glutamine and asparatic acid.**

**Figure 4.11:** Main nitrogen donors.

Whenever there's an attachment of nitrogen, we first phosphorylate the position with ATP or GTP and then add in the nitrogen. This will form a phosphoester intermediate that change that will release the inorganic phosphate and thus resulting in the position attaching to the nitrogen.

**Figure 4.12:** Process of attaching nitrogen

4.1.3 Synthesis of IMP

From last lecture, we stopped at the synthesis of 5-phosphoribosylamine. In today's lecture, we will finish off the entire *de novo* synthesis of purine. We begin with synthesizing IMP, an intermediate base for purines.

Mechanism of Action (Synthesis of IMP): We're starting with the already made 5-phosphoribosylamine.

3. 5-phosphoribosylamine will be converted into **glycinamide ribotide (GAR)** by *GAR synthase* using glycine and ATP.

4. More carbon is added onto GAR turning into **formylglycinamide ribotide (FGAR)** by *GAR transformylase* using N^{10} -Formyl-THF.

5. A nitrogen is donated onto FGAR making **formylglycinamidine ribotide (FGAM)** by *FGAM synthase* using glutamine, ATP and water.

6. Using ATP, *AIR synthase* will begin a cyclization reaction. Specifically, the enamine 9N will perform a nucleophilic attack on the adjacent C = O group and this form and imidazole ring i.e. FGAM is converted into **5-aminoimidazole ribotide (AIR)**.

7. a bicarbonate group is added onto AIR by *AIR carboxylase* forming **carboxyaminoimidazole ribotide (CAIR)**.

8. CAIR is converted into **SACAIR** by *SACAIR synthase* using aspartate and ATP.

9. SACAIR will have the fumarate removed by *adenylsuccinate lyase* leaving behind only the N, the molecule formed is **AICAR**.^a

10. An additional carbon will be added onto AICAR via *AICAR transformylase* using N^{10} -Formyl-THF, forming **FAICAR**.

11. Lastly, the other 6-membered ring will be formed using *IMP cyclohydrolase* which turn FAICAR into IMP. *Notice:* The base is hypoxanthine.

^aBasically, the addition of N using aspartate involves 2 step: adding the entire aspartate then remove the fumerate leaving the N.

Remark 4.4. AICAR, or acadesine, or ZMP are banned substances in competitive sports (it's a performance-enhancing drug) as it can stimulate AMP-dependent protein kinase (AMPK).

4.1.4 Synthesis of Purine Bases

This synthesized IMP will be the intermediate to form purine bases. Since there are 2 types of purine, IMP is also the branch point into forming these 2. Because it's a



Figure 4.14: We're currently here on the purine pathway.

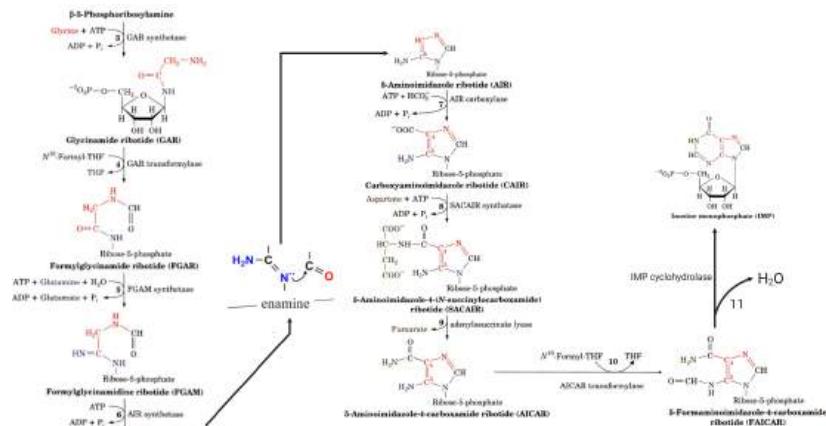


Figure 4.13: Synthesis of IMP.

branch point for them 2, it's also a point of regulation.

Observation 4.4 To make the AMP from IMP, we simply add in an amine group. This is done via addition of aspartate and GTP, then removal of fumerate (using the same enzymes used above).

To make GMP, we first dehydrogenate IMP into **XMP** (similar structure with an additional = O) using *IMP dehydrogenase*. XMP will have an amine group attached using glutamine and ATP by *GMP synthase* to finally form GMP.

Notice that the energy source for formation of AMP and GMP is opposite in nature i.e. GTP is used to form AMP and ATP is used to form GMP. **Why is that?** Well...because we need a balance of them 2. When there's too much of one, the synthesis of the other will use up the excess.

Observation 4.5 After synthesis the monophosphate, we need to finally reach the final form of triphosphate.

Formation of ADP and GDP will be base-dependent i.e. there's 1 specific enzyme for either synthesizing ADP or GDP. These enzymes are: **adenylate kinase** and **uridylate kinase** for synthesis of ADP and GDP respectively.

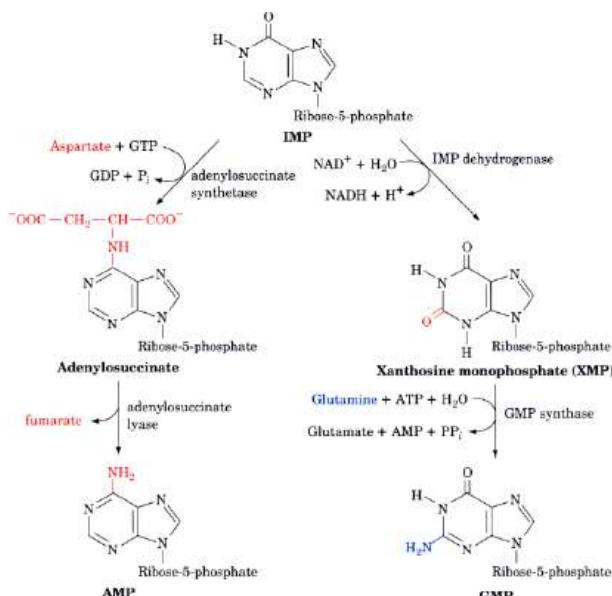
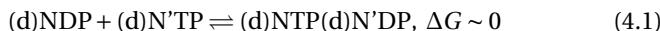


Figure 4.15: Synthesis of AMP and GMP from IMP.

tively; and they will give the following reactions:



Lastly, the addition of 1 final phosphate will be done using 1 universal enzyme called **nucleoside diphosphate kinase** (for synthesis of ATP and GTP).



These enzymes operate close to equilibrium, transferring phosphate groups between diphosphates. For example, they can move a phosphate from ATP to another molecule without any net gain or loss of energy, maintaining the balance of free energy. The cell's energy charge — more ATP than ADP or AMP — drives this process. As a result, the ratio $\frac{[\text{ADP}]}{[\text{ATP}]}$, as well as between $\frac{[\text{ADP}]}{[\text{AMP}]}$, are equal.

$$\text{adenylate kinase: } \frac{[ADP][ADP]}{[AMP][ATP]} = 1 \implies \frac{[ADP]}{[ATP]} = \frac{[ADP]}{[AMP]}$$

But why is that? Well...Because enzymes are catalysts, these reactions are reversible, simply redistributing phosphates rather than breaking them down. This keeps the ratio ADP:AMP similar to the ratio of ATP:ADP, and similar patterns are seen across different nucleotide types, like uracil and adenine nucleotides.

Observation 4.6 We can also see this when measuring the different level of nucleotides and PRPP in *E. Coli*. When you look at the ratio between RNA and DNA, we can see a ratio of 7 to 1 (RNA to DNA) and this magic number reoccur for nucleotides as well e.g. there's ~ 7 times more GTP than GDP and similarly for GDP and GMP. We can generalize this over the nucleotides as



If you multiply this together for ATP vs AMP, you get roughly 50 times more ATP than AMP. In all cases, just remember that 7 is an important number in this case.

Intracellular amounts of nucleotides & PRPP in *E. coli*

Compound ^a	μmol/g (dry wt)	Intracellular concn (μM)	Compound ^b	μmol/g (dry wt)	Intracellular concn (μM)
RNA			UTP	2.08	894
AMP	165		UDP	0.22	93
GMP	203		UMP	0.33	142
CMP	126		dTTP	0.18	77
UMP	136				
DNA			ADPGlc	0.01	5
dAMP	24.7		GDPMAN	0.04	18
dGMP	25.4		UDPGlc(Gal)	1.33	570
dCMP	25.4		UDPGlcNAc	0.38	164
dTMP	24.4		UDPAcMurP ₃	0.55	234
			dTDPGlc	0.58	248
ATP	7.00	3,000			
ADP	0.58	250	NAD + NADH	1.88	806
AMP	0.24	105	NADP + NADPH	0.47	200
dATP	0.41	175	FAD	0.12	51
			FMN	0.21	88
GTP	2.15	923			
GDP	0.30	128	AcCoA	0.54	231
GMP	0.05	20	SucCoA	0.03	15
dGTP	0.28	122	MalCoA	0.03	15
IMP	0.38	162			
CTP	1.20	515	cAMP	0.01	6
CDP	0.19	81	ppGpp	0.07	31
dCTP	0.15	65	pppGpp	0.04	18
			PRPP	1.24 ^c	472

Remark 4.5. You also get 7 times more NTP vs dNTP.

4.1.5 Regulation

We've mentioned already which is the regulation of purine is done at step 1 and 2. The regulation is done by **feedback product inhibition** i.e. The synthesized nucleotides will be the inhibitor of the first 2 steps. At PRPP, you have a feed-forward reaction via pyrophosphate that commit the substrate into becoming nucleotides. At the level of IMP into AMP and GMP, you also have another feedback inhibition by nucleotides.

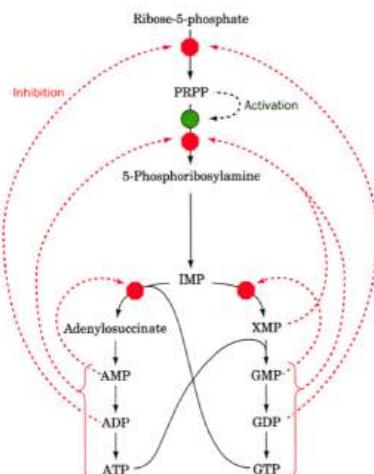


Figure 4.16: Regulation of purine de novo synthesis.

4.1.6 Folates

Definition 4.3. **Tetrahydrofolate (THF)**³ is the most versatile enzyme co-factor known and is used in the purine *de novo* pathway twice.

The reason that it's considered the most "versatile" is because it can carry carbon in many ways: at N¹⁰ and N⁵. Of the carbon that it can carry, there can exist in 3 different oxidation states. And out of the 3 oxidation states, there are also 5 different chemical forms

Observation 4.7 THF and folic acid is a target for some antibiotics e.g. **Sulfonamide**. This is because bacteria do not get its folate from the diet but

³They got their name from folic acid that can be obtained through our diet (in leaves).

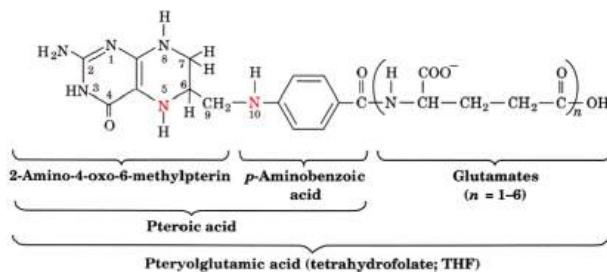
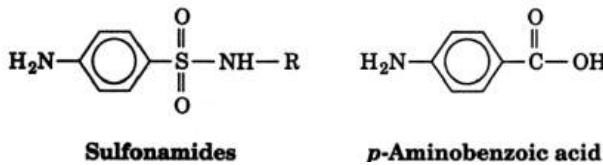


Figure 4.17: THF structure.

synthesize *de novo* from **p-aminobenzoic acid**. Thus, by inhibiting its production, the bacteria won't be able to grow.



Let's look at the 3 oxidation level and the 5 chemical forms by THF. Looking at the table provided below, you have the most reduced (has the most hydrogen) form of carbon called **methanol** and the chemical forms associated to said carbon is the *methyl group*. The next level will be less reduced \Rightarrow more oxidized which is **formaldehyde** with the *methylene group*. Then, the highest oxidation level is **formate** with, *formyl*, *formimino* and *methenyl group*.

TABLE 24-1. OXIDATION LEVELS OF C₁ GROUPS CARRIED BY THF

Oxidation Level	Group Carried	THF Derivative(s)
Methanol	Methyl ($-\text{CH}_3$)	N^5 -Methyl-THF
Formaldehyde	Methylene ($-\text{CH}_2-$)	N^5, N^{10} -Methylene-THF
Formate	Formyl ($-\text{CH}=\text{O}$)	N^5 -Formyl-THF, N^{10} -formyl-THF
	Formimino ($-\text{CH}=\text{NH}$)	N^5 -Formimino-THF
	Methenyl ($-\text{CH}=$)	N^5, N^{10} -Methenyl-THF

Remark 4.6. It's been studied that folate supplement can reduce the risk of neural tube defect (a rare but detrimental disease) by 70%.



4.2 Ribonucleotide Reductase

In this lecture, we talk about ribonucleotide reductase and the *de novo* pathway of pyrimidine.

Before getting directly into talking about Ribonucleotide reductase, let's ask **why is DNA the genetic material?** Well...we're not so sure but there are some possible explanations: By removing the 2'OH from RNA, the DNA phosphodiester backbone is more stable than that of RNA.

Methods 4.1 To purify DNA in lab, you might have a step where the DNA solution pH is raised by adding NaOH. The reason we do this is because the base will hydrolyze the RNA \Rightarrow RNA will be cleaved into pieces while DNA are resistant.

Another hypothetical reason for DNA being better suit for genetic material is because of deamination. **Deamination** is a common biochemical breakdown of nucleic acids e.g. for RNA you get deamination from cytosine to uracil. If DNA uses uracil and deamination happens, it would not detect this error hence leading to more error down stream. To combat this DNA uses thymine which has an extra methyl group \Rightarrow if deamidation happens, detection is possible.

Note: Synthesis of deoxyribose is done at the level of nucleotide diphosphate (NDP). From NDP, we will remove the OH in place of an H making dNDP. DNA is more reduced.

Definition 4.4. **Ribonucleotide reductase (RR)** is the main enzyme that mediate the synthesis of deoxyribonucleotides that are essential for DNA synthesis.

The main driver of this reduction is the reduction of NADPH which will transfer through different co-factors, enzymes and eventually the RR.

Remark 4.7. *Do not confuse this with NADH. A way to remember is look at the concentration of its reduced and oxidized form:*

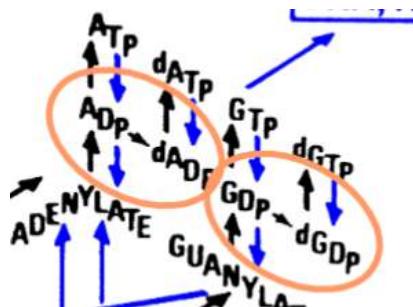


Figure 4.18: Purine cube: synthesis of dNTP .

$$\frac{\text{NADPH}}{\text{NADP}^+} = 100 \quad \frac{\text{NAD}^+}{\text{NADH}} = 1000$$

We can see that there's more NADPH (reduced form of NADP^+) in the cell which means it'll be thus used for reduction. On the other hand, there's more NAD^+ (oxidized form of NADH) in the cells which it'll be thus used for oxidation.

Observation 4.8 There are lots of different RR but we will focus on the human's one. The human RR is a heterotetramer with 1 homodimer regulatory subunit and 1 homodimer catalytic subunit.

On the regulatory subunits, there are 2 regulatory sites which are regulated at 2 different levels: 1 site is regulated by the ration of ATP to dATP, the other is regulated by the type of dNTPs (e.g. dATP, dGTP, etc.) The regulation is done through allosteric binding.

On the catalytic subunits, there are unusual stable enzyme tyrosine radical which are often used as therapeutic target. The drug that inhibits this is called **hydroxyurea** and they're used to shut down virus, bacteria and even cancer cell production and growth.

There are 2 types of feedback regulation to maintain proper and equilibrated levels of dNTPs for DNA synthesis

4.2.1 Base Excision Repair

Let's have a brief look at what happens during the deamination process where cytosine is converted into uracil in DNA.

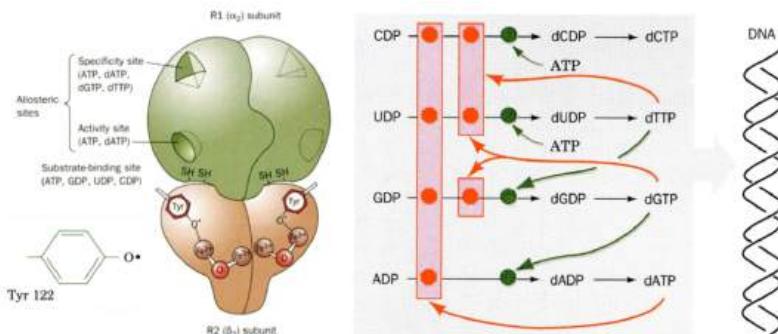


Figure 4.19: RR structure and regulation.

Observation 4.9 The pathway where uracil is removed is called **base excision repair**. An enzyme called **uracil-DNA glycosylase** will be present and remove the uracil base and you'll end up with an **apurinic site** (DNA without the base). This apurinic DNA will be cleaved further using **apurinic /apyrimidinic (AP) endonuclease** and then resynthesize later.

Remark 4.8. When there's too much repair, there's an increase risk of having mutations.

4.3 *De Novo* Pathway of Pyrimidine

Now, we will look at the *de novo* pathway of pyrimidine. Basically, we're trying to make UMP but we do not want the uracil to end up in DNA but only RNA. To do so, we need to prevent the formation of dUTP and the cell does this by **UTPase**.

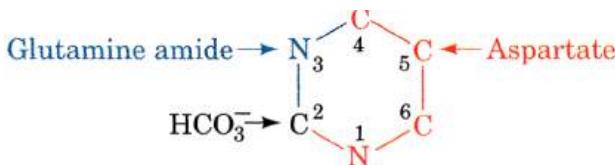


Figure 4.20: Origin of atoms for pyrimidine.

Let's look at the origin of atoms for pyrimidine. Compared to purine, it's much simpler, it will have its nitrogen from glutamine amide, 1 car-

bon from bicarbonate and the rest is from aspartate. Different from purine where PRPP is assembled with the base in the same pathway together, however, for pyrimidine, its ring is assembled separately then phosphoribose by PRPP is added in the end.

Note: Bases are hydrophobic so it'd diffuse out. To prevent this diffusion, we need to charge it. For pyrimidine, it doesn't matter since there's a charge on all of the intermediate.

4.3.1 Synthesis of UMP

Similar to the synthesis of pyrimidine, the synthesis pathways can be regulated in the first 2 steps.

- In animals, the enzyme to make carbamoyl phosphate is part of the urea cycle on mitochondria but is also part of pyrimidine synthesis in cytosol. Thus, it's the one to be regulated
- In bacteria, they only have to regulate the second step of making carbamoyl aspartate.

Observation 4.10 Interestingly, the enzyme regulated in the second step called **aspartate transcarbamoylase (ATCase)** was the first enzyme that people studied to really understand what allosteric regulation is. This enzyme can be activated by ATP and inhibited by CTP.

Basically, in the presence of CTP, more aspartate is required as compared to the presence of ATP.

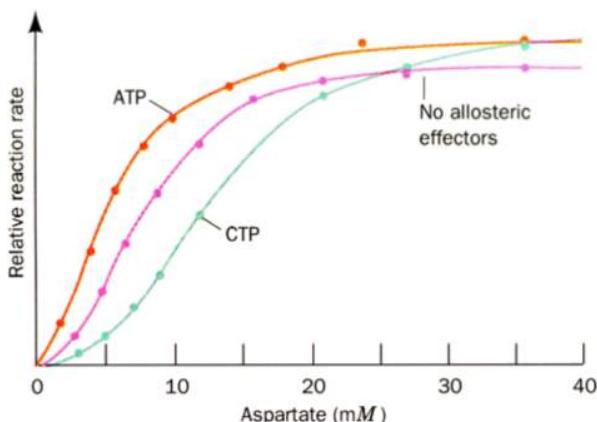


Figure 4.21: ATCase activity with ATP and CTP.

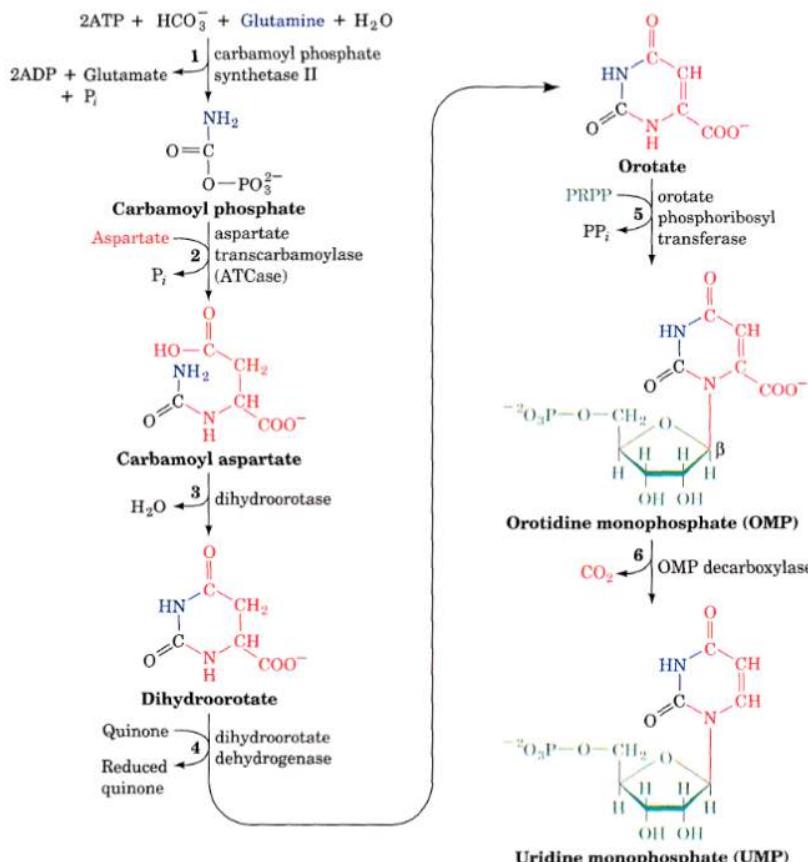


Figure 4.22: Synthesis of UMP.

Mechanism of Action (Synthesis of Pyrimidine): It must be noted that these substrates are channelled through a very large enzyme complex i.e. these enzymes sit together in a megacomplex. Specifically, the first 3 enzymes sit in proximity to each other and similar to the 5 and 6 one.

1. Glutamine along with bicarbonate, ATP and water is converted into **carbamoyl phosphate** by *carbamoyl phosphate synthase II*.
 2. Carbamoyl phosphate is converted into **carbamoyl aspartate** using *ATCase*.
 3. Carbamoyl asparate is then dehydrated into **dihydroorotate** by *dihydroorotase*.
 4. At the mitochondrial membrane, with the reduction of quinone, dihydroorotate is converted into **orotate** using *dihydroorotate dehydrogenase*.
 5. Orotate will be attached with PRPP using *orotate phosphoribosyl transferase* making *orotidine monophosphate (OMP)*.
 6. OMP is finally decarboxylated into **uridine monophosphate (UMP)** using *OMP decarboxylase*.

The reason they live in proximity is to increase output and decreases the cellular concentration of intermediate. Interestingly, bacteria does not have this channeling.

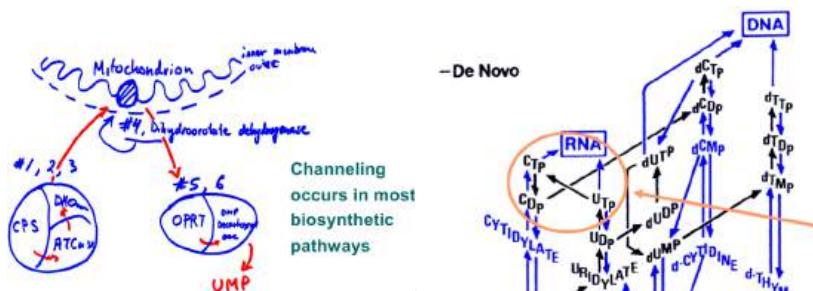


Figure 4.23: Channeling of UMP synthetic pathway (left). We're here in the pyrimidine cube (right).

Remark 4.9. The loss of the fifth and sixth enzyme can lead to build up of orotate that would be eliminated in the urine. This condition is called **orotic aciduria**

4.3.2 Synthesis of CTP and dNDPs

Observation 4.11 CTP is synthesized at the triphosphate level. First, we need to phosphorylate UMP to UDP via UMP kinase and then finally converted to UTP via nucleotide diphosphate kinase. With the UTP made, we can then use glutamine, ATP and water to make CTP using **CTP synthetase**.

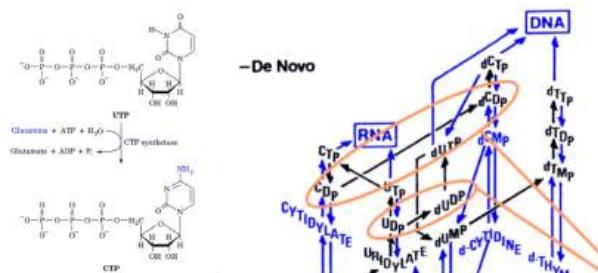
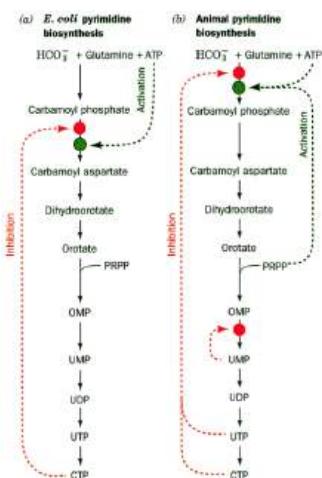


Figure 4.24: CTP and dNDP synthesis.

To make dNDPs, RR will catalyze the reaction and this enzyme is universal i.e. it will convert CDP to cCDP and UDP to dUDP.

4.3.3 Regulation of Pyrimidine Synthesis



Like we've said before, there are 2 ways to regulate the synthesis pathway of pyrimidine: via ATCase in bacteria and carbamoyl phosphate synthase II in animals. In either case, you have activation by ATP and inhibition by products (CTP).

Observation 4.12 From last lecture, we need to ask ourselves, why would we make dUTP in the first place? Well...it's because 1, RR is not base-specific will can convert any NDP to dNDPs; 2, UMP kinase is not specific for either ribose or deoxyribose form of UMP; and 3, dUTP can happen because of deamination of dCTP. To undo this, we have dUTPase and in case that it's on the DNA strand, it would be base excision repair.

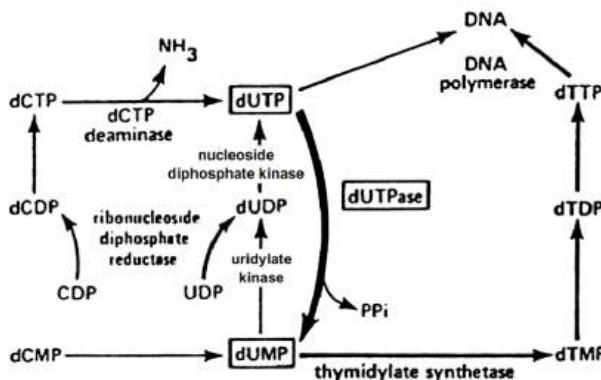


Figure 4.26: dUTP back to dUMP.

4.3.4 Thymidylate Synthase

In today's lecture, we will look at an enzyme that convert dUMP into dTMP which is the **thymidylate synthase**⁴.

Observation 4.13 The synthesis from dUMP to dTMP is an important pathway as it will not affect RNA synthesis but DNA synthesis. Thus, it's an important target for drugs against cancer and bacteria.

The way thymidylate convert dUMP to dTMP is to attach a methyl group and this is obtained from methylene THF. This is where the weird part coming from is that it could've taken methyl from the most reduced form which is methanol THF but it doesn't. Because it doesn't, THF have to be oxidized into dihydrofolate.

The dihydrofolate (DHF) can then be reduced back into THF by **dihydrofolate reductase**. Since this is a reduction, we need an H⁺ source which is from NADPH

⁴Some older sources refer to it as "synthetase"

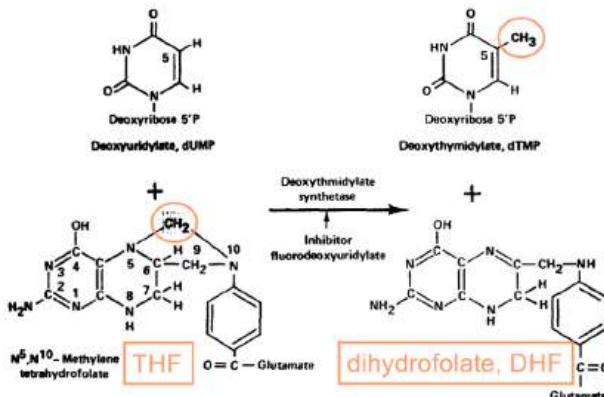


Figure 4.27: conversion of dUMP to dTMP.

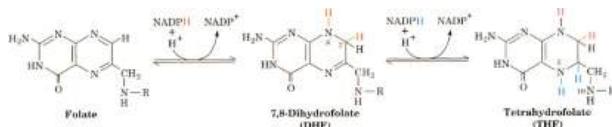


Figure 4.28: DHF reversion to THF.

Observation 4.14

Thymidylate synthase's is in a cycle. First, dTMP is made from dUMP using methylene THF, which would be oxidized to DHF. DHF will be reduced back into THF using DHF reductase. A methyl group from serine will be attached onto the THF restoring the methylene THF.

There has been drugs developed to block these critical step that lead to DNA synthesis. We have **fluoro-dUMP (FdUMP)** can be used to inhibit thymidylate synthase. It acts like other dUMP substrate but upon binding, the fluoride will

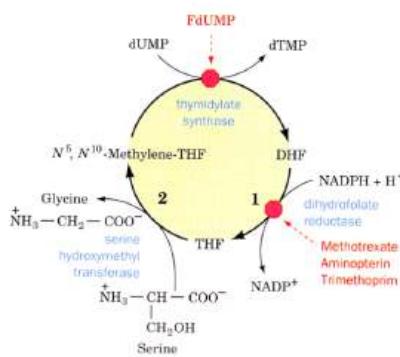


Figure 4.29: Thymidylate synthase cycle

create a covalent bond to the enzyme and shut it down. This is called a *suicide inhibitor*.

Definition 4.5. Suicide inhibitor is an inhibitor inhibit a biological process by creating a covalent bond to the enzyme mediating that process.

There are other drugs like **methotrexate** and **trimethoprim⁵** which are inhibitors of DHF reductase and they look oddly similar to DHF. This is because they're substrate mimics

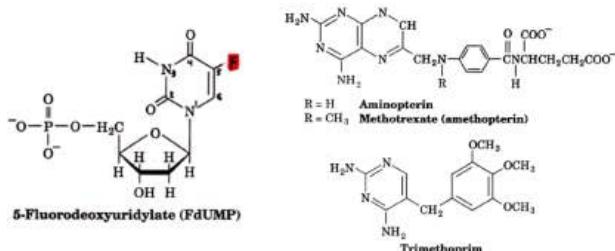


Figure 4.30: Drugs that inhibit the thymidylate synthase.

Definition 4.6. Substrate mimics are substrates that look like the original substrate, and can bind more tightly but does no modification.

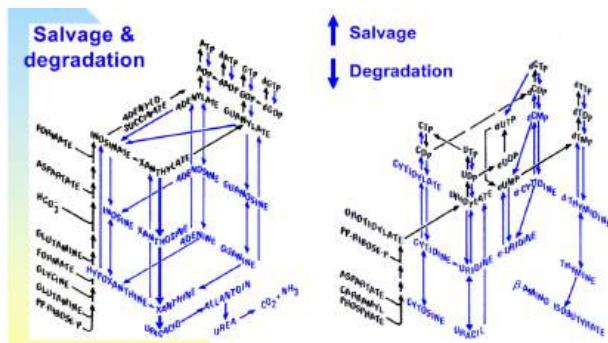


Figure 4.31: Salvage pathway

⁵Methotrexate can be used as anti-cancer while trimethoprim can be used as anti-bacterial.

4.4 Salvage Pathways

Now, we look at the salvage pathway where we will recover bases to make new nucleotides. This pathway is less conserved and vary from different organisms. When looking at the cubes, the salvage pathways are blue arrows that go up. Contrarily, going down would be degradation. We will not go into much detail but the table below includes different enzymes that can shuffle around the bases which lead to the formation of nucleosides/nucleotides

Pathway	Reaction	Enzymes
(i) Conversion of base to ribonucleotide	PRPP + base X \rightarrow rNMP + PP _i	phosphoribosyl transferase **
(ii) Interconversion of bases and nucleosides	ribose 1-P + base N \rightleftharpoons nucleoside N + P _i deoxyribose 1-P + base N \rightleftharpoons deoxynucleoside N + P _i	nucleoside phosphorylase
(iii) Conversion of nucleoside to nucleotide	nucleoside N + ATP \rightarrow NMP + ADP	nucleoside kinase
(iv) Base exchange into deoxynucleosides	base X + deoxynucleoside Y \rightleftharpoons base Y + deoxynucleoside X	nucleoside transglycosylases
(v) Interconversion by base alterations	adenine \rightarrow hypoxanthine cytosine \rightarrow uracil	deaminases
(vi) Reutilization of nucleotides	rNMP \rightarrow rNDP; dNMP \rightarrow dNDP rNDP \rightleftharpoons rNTP; dNDP \rightleftharpoons dNTP	nucleoside monophosphate kinase * nucleoside diphosphate kinase *

Figure 4.32: Salvage pathways enzymes. (*) already seen as part of *de novo* pathway, (**) one example already seen in pyrimidine *de novo* pathway.

Observation 4.15 Lesch-Nyhan disease is a genetic disease characterized by loss of **hypoxanthine/guanine phosphoribosyl transferase (HPRT)** activity. Some of the symptoms include gout and mental retardation. There are also be loss of other enzymes such as those for adenine and uracil as well.

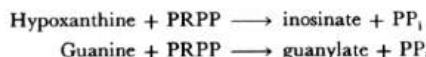
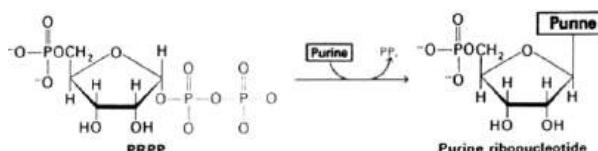


Figure 4.33: Activity of HGPRT.

The following is an illustration of enzymes involved in making nucleotides/nucleosides.

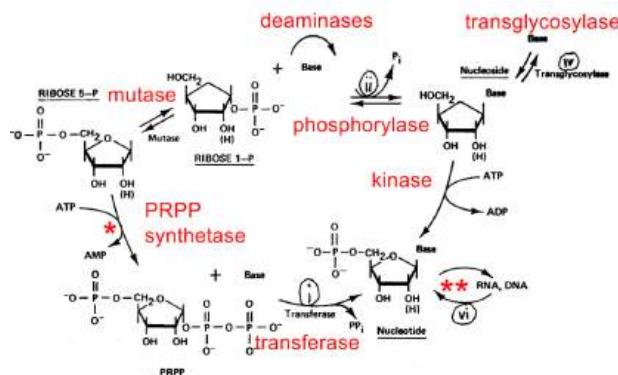


Figure 4.34: Enzymes illustration.

4.4.1 Purine degradation

We will now look at the degradation pathways of purine and pyrimidine.

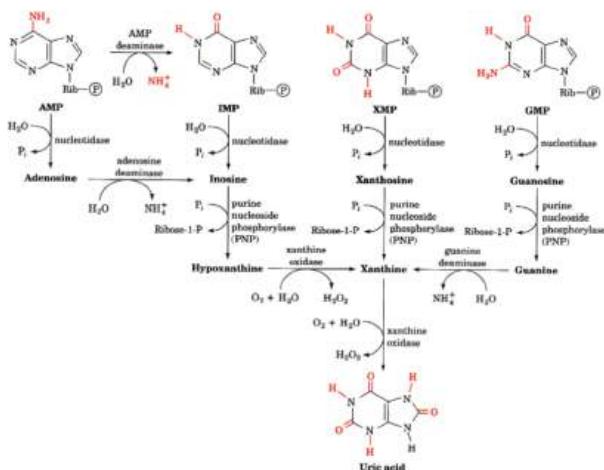


Figure 4.35: Purine degradation pathway.

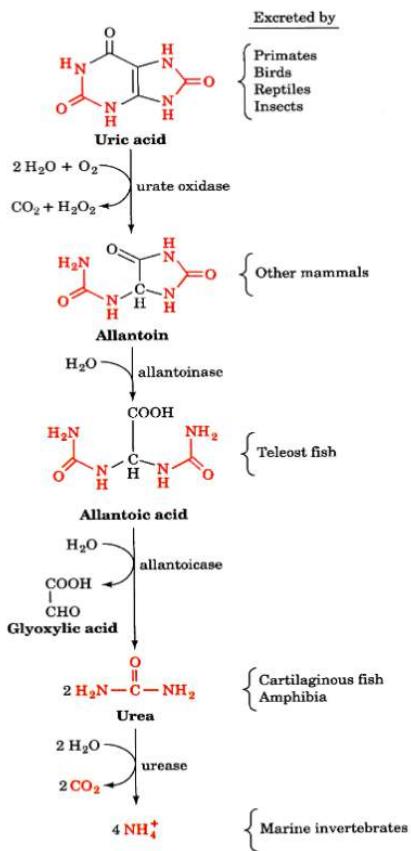
Observation 4.16

Figure 4.36: Excretion of nitrogen by different animals.

We can deaminate AMP to make IMP then become inosine, or go to adenosine to deaminate into inosine. Inosine can then be converted into hypoxanthine which will then make xanthine. For XMP, it can be converted into xanthosine which will then converted into xanthine. For GMP, it can be converted into guanosine then guanine, and finally xanthine. Basically, xanthine is the universal degraded substrate in the end, and it will further be degraded into **uric acid** by *xanthine oxidase* (see Figure 4.35).

Severe combined immunodeficiencies (SCID) is a very rare genetic disease characterized by the loss (of function) of adenosine deaminase, but also the loss (of function) of purine nucleoside phosphorylase. Interestingly, excess of uric acid in blood could lead to its deposition in joint which manifest in the body as a disease called **gout**. Nevertheless, it's not as severe as other disease and can be treated by inhibiting xanthine oxidase.

It was commonly believed that gout was formed by excess meat consumption. However, it's now more linked with inadequate kidney function thus unable to eliminate uric acid effectively. Another metabolic consequences that **lead to the development of gout is high PRPP level**.

Observation 4.17 Uric acid is the final product from purine degradation. Depending on the type of animal, they will secrete this by-product differ-

ently (see Figure 4.36).

- In the least developed animal like fish where the environment is rich in nitrogen, they will eliminate it as ammonia.
- In humans, our main way to get rid of excess nitrogen is urea, though we do produce some uric acid (during the breakdown of nucleic acid, specifically, when there's purine).
- For birds, urea is not the ideal way as it requires too much water. **They make purines which can then be concentrated as uric acid and released without or even with little water.** They do this by lowering uric acid pH which will precipitate it out⁶

Interestingly, uric acid was originally, naturally obtained from **guano**, which is a nicer way of saying accumulated excrement and remains of birds. They're used to make fertilizer and especially gunpowder. As of the current moment, they're used to make facial cream e.g. *Uguisu no Fun*.

4.4.2 Pyrimidine Degradation

For pyrimidine degradation, CMP is converted into cytidine which is then made into uridine. UMP and converted directly to uridine. Uridine can then be converted into uracil then dihydrouracil.

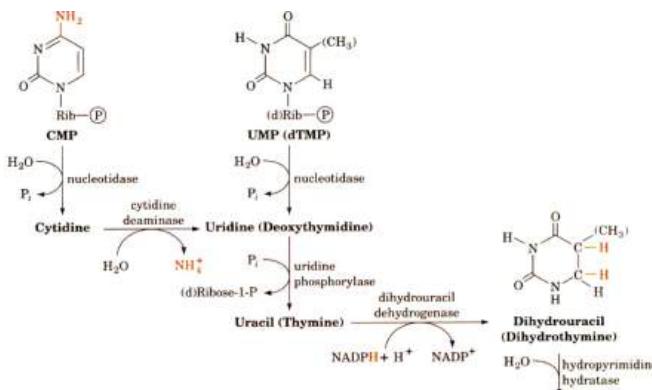


Figure 4.37: Pyrimidine degradation.

⁶uric acid is less soluble at low pH.

In the second part of this chapter, we will look at how to synthesize and degrade amino acids.

4.5 Nitrogen

Definition 4.7. **Amino acids (aa)** are compounds that contain both carboxylic and amino group. They can combine together to form proteins.

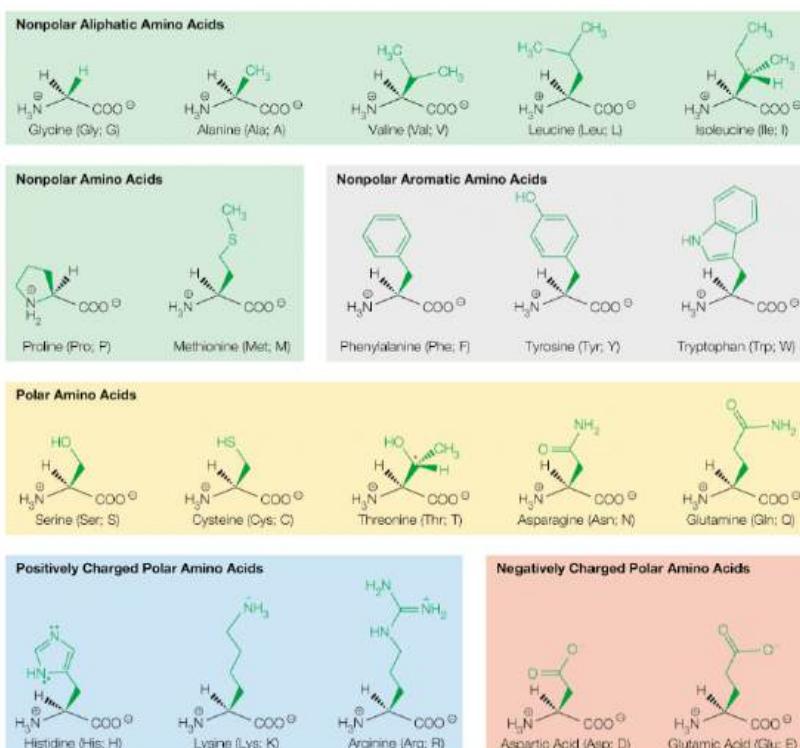


Figure 4.38: 20 amino acids.

Observation 4.18 There are 20 different amino acids. These amino acids contains nitrogen along with other **nitrogen-containing compounds** such as metabolites, purines, heme and hormones. There are various disease linked to the problems of metabolism of amino acids, see the table below.

Disorder	Pathway	Effect or Symptoms
Urea cycle disorders	Urea cycle	Six disorders related to deficiencies of the enzymes of the urea cycle. Patients experience hyperammonemia and nerve damage. Often severe to fatal.
Maple-syrup urine disease	Branched-chain amino acid metabolism (α -keto acid dehydrogenase)	Deficiency of branched-chain α -keto acid dehydrogenase leads to elevated levels of branched-chain amino acids and branched-chain α -keto acids, causing neurological damage and death. The disease is named for the sweet maple odor produced by the catabolite soliton found in the urine of affected subjects.
Phenylketonuria (PKU)	Tyrosine metabolism (phenylalanine hydroxylase)	A deficiency in phenylalanine hydroxylase leads to elevated levels of phenylpyruvate, which is found in the urine of affected subjects. If left untreated, the disease can lead to mental retardation and neurological damage, but the disease is currently diagnosed in infants and successfully treated with a low phenylalanine diet.
Albinism	Tyrosine metabolism (tyrosinase)	Tyrosinase deficiency results in the inability to convert tyrosine into the pigment melanin. Subjects lack pigmentation in skin, hair, and eyes.
Hyperprolinemia	Proline catabolism	A deficiency in one of the enzymes that catabolize protein leads to proline levels 3 to 10 times normal, resulting in neurological problems.

Remark 4.10. Anaplerotic and cataplerotic reactions do not limit to just the CAC but for other pathways too.

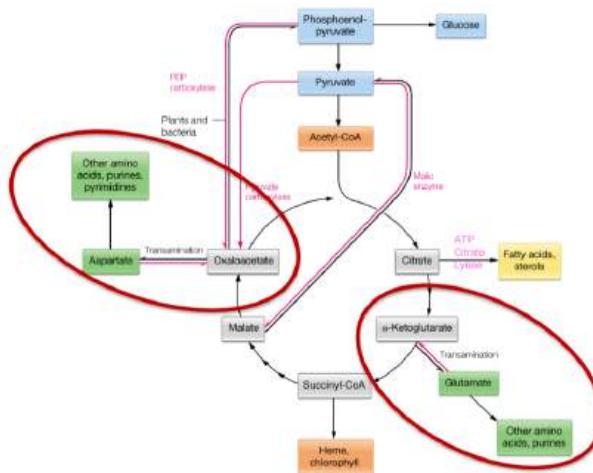


Figure 4.39: Anaplerotic and cataplerotic reaction reminder.

Example 4.5.1. With the reference of CAC, synthesizing glutamate is a cataplerotic reaction but from the stand point of glutamate, it's an anaplerotic reaction. This is because by definition, cataplerotic reaction is the depletion of substrates of a pathway in reference while anaplerotic is the replen-

ishing.

The point here is that, CAC could be considered as the starting point to synthesize some amino acids as they're composed of carbon, oxygen and hydrogen but no nitrogen. So now we need to ask, **where do we get our nitrogen?** Well...mostly from diet.

4.5.1 Nitrogen, from Air to Food

Nitrogen in the air is very stable and it's very hard to break it and attach it to something else. Plants also have a hard time to get these nitrogen from air. Nevertheless, there's a symbiotic relation between bacteria and yeast, called **diazotroph**, that associate with the root. These microorganisms have the necessary enzymes called **nitrogenase** that can fixate nitrogen from the air, in the following reaction.



Definition 4.8. **Fixation** is the reaction from N_2 to NH_3 .

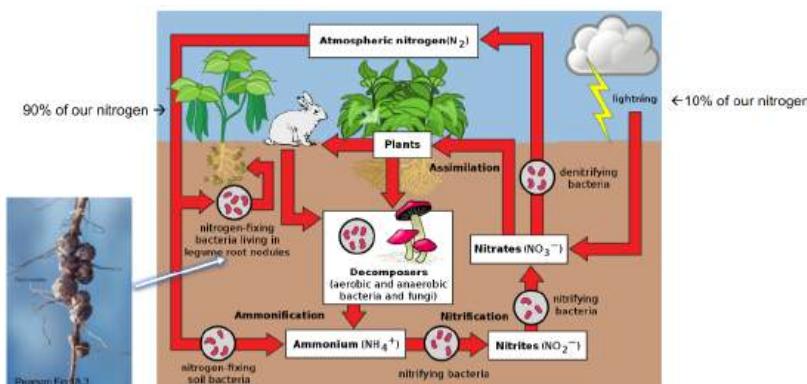
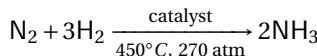


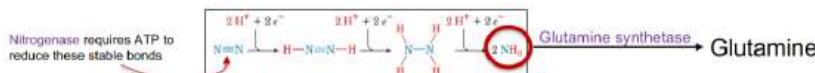
Figure 4.40: The nitrogen cycle.

Remark 4.11. N_2 is a stable molecule so to fixate it into NH_3 , it would require lots of energy and heat.

Example 4.5.2. **Haber-Bosch** reaction is a common industrial nitrogen fixation process to make fertilizer. Normally, it takes around 450°C at high pressure for the reaction to take place, as in the following reaction.



The newly made NH₃ by microorganisms will be transported into plants. In plants, they have **glutamine synthase** that would use up this NH₃ to make glutamine, as in the following reaction:



Definition 4.9. **Assimilation** is a reaction where the cell takes the fixated NH₃ by bacteria and incorporate it into amino acid.

Observation 4.19 While there's only 1 fixation reaction, there are many assimilation reactions: **glutamine, glutamate, asparagine and carbonyl phosphate synthesis**.

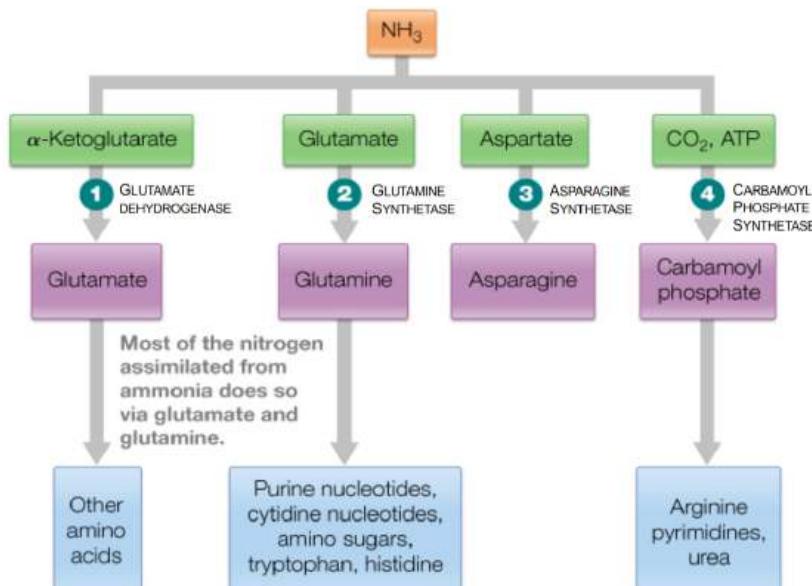


Figure 4.41: Assimilation reactions.

4.5.2 Dietary Amino Acids

When we eat food, they may contain different content of aa. But in general, they all contain proteins and amino acids (see table below).

g/100g Food	Beef	Skim Milk	White Rice	Corn	Tofu	Egg
Total protein	25.9	3.4	2.7	2.5	6.6	12.6
Glutamine	1.2	0.3	0.3	0.4	0.6	0.6
Glutamate	2.7	0.4	0.2	0.05	0.7	1.0
Leucine	2.2	0.4	0.2	0.4	0.5	0.9

Remark 4.12. *It's a misconception that banana does not have protein, they do have some level but very few.*

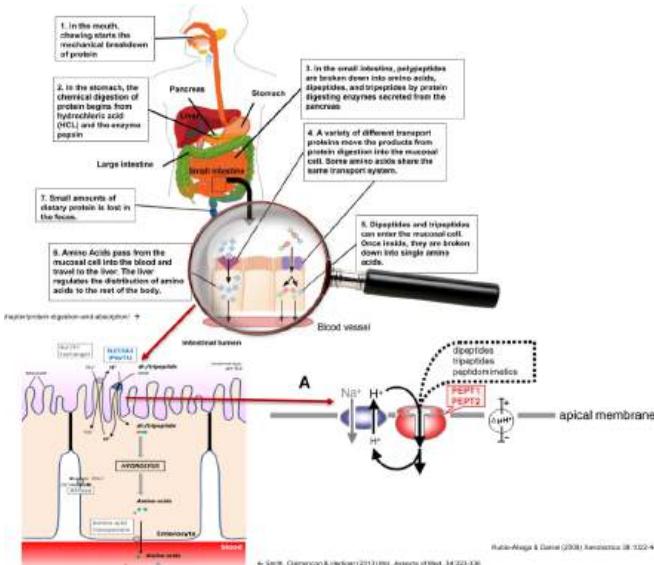


Figure 4.42: Absorption of amino acid .

Observation 4.20 Proteins are broken down in the mouth through chewing, then they're broken down in the stomach by gastric acid. In the intestine, they're further degraded into peptides and absorbed. The common

enzymes that will process proteins are: **pepsin**, that will breakdown complex proteins into polypeptides. In the intestine, **trypsin** and **chymotrypsin** (made by the pancreas) will further breakdown the polypeptides to smaller residues, including di- and tripeptides. These residues are then broken down into aa by **aminopeptidase carboxypeptidase A**.

The di-, tripeptides and aa will be absorbed into enterocytes using transporters **PEPT1** and **2**. This is a symport and will be transported with the movement of H^+ . These peptides are further broken down into aa which can be transported in the circulatory system without the need of chylomicron as they're water-soluble (see Figure 4.42).

Remark 4.13. *There are specific transporter for each of the 20 AAs.*

Observation 4.21 Though we've said that that proteins needs to be broken down into smaller peptides to be absorbed; There's an exception to this. In newborns, they can transcytose the milk protein from the intestine directly into enterocytes, digested there in the lysosome, and then to blood.

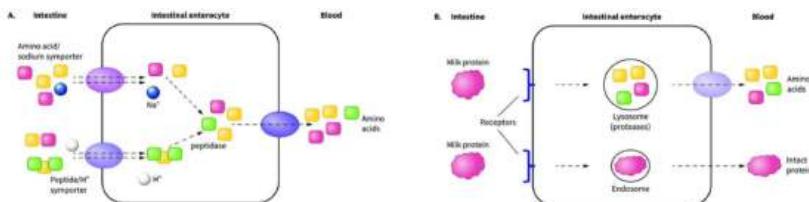


Figure 4.43: Absorption of digested proteins (A) vs in neonatals (B).

Essential aa are aa that we cannot synthesize while the **non-essential are** those we can synthesize endogenously. **Conditionally essential aa** are those that require the presences of essential aa to be a substrate to make said aa i.e. we can make that aa but we do not have the starting material which is another essential aa.

Nonessential	Conditionally essential*	Essential
Alanine Asparagine Aspartate Glutamate Serine	Arginine Cysteine Glutamine Glycine Proline Tyrosine	Histidine Isoleucine Leucine Lysine Methionine Phenylalanine Threonine Tryptophan Valine

Figure 4.44: Types of aa

4.5.3 Metabolism of Amino Acids

Remark 4.14. The 20 aa we have are in its L-conformation⁷

Observation 4.22 Typically, when dietary aa absorbed, they'll go into the aa pool of the body. In normal condition, these aa will be used as body proteins and build muscles. However, when the body is in need to energy, these body proteins will be degrade back into the aa pool to be used to generate energy e.g. FA synthesis and gluconeogenesis.

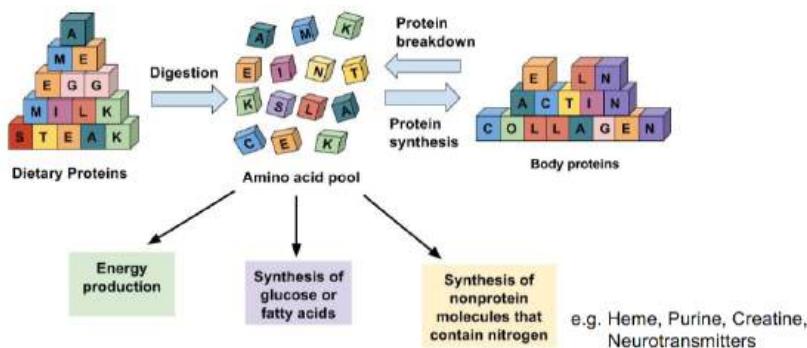


Figure 4.45: Amino acid pool.

The aa pool is **homogenous** i.e. doesn't matter the source, the aa would be the same. It's dynamic meaning that it's constantly breakdown and synthesized. It's constant in size as the synthesis and breakdown is in equilibrium. This leads directly to the regulation of this aa pool:

- **Healthy adult:** The intake equal outflows
- **Growth, pregnancy, weightlifting, etc.:** The intake is larger outflows i.e. ingesting more aa than degradation/elimination.
- **Illness, surgery trauma, cancer cachexia:** The intake is less than outflows i.e. there's more degradation and elimination of aa than ingesting.

Concept 4.2 Unlike carbs and lipids, nitrogen-containing molecules cannot be stored. Thus, it needs to be replenished through diet.

⁷For each aa, they have either the L- or D-conformation.

The following illustrations are an overview of synthesis and degradation pathways of aa (We'll be looking into this further in next lectures).

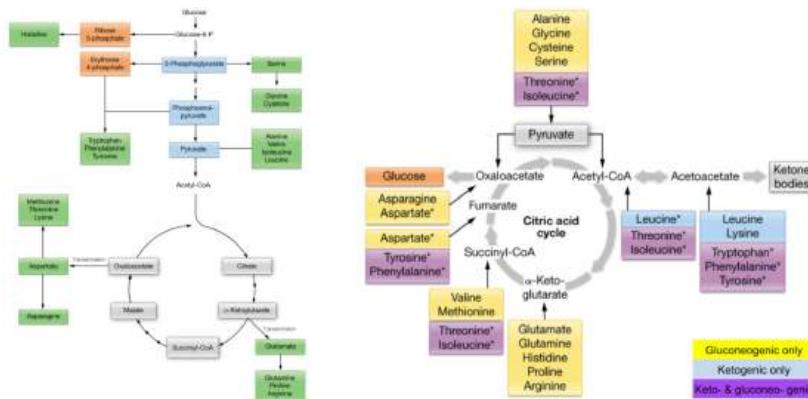
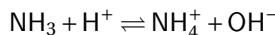


Figure 4.46: Synthesis (left) and degradation (right) pathway of amino acid.

Notice that when we degrade aa and its carbon backbone is recycled into glucose and CAC intermediates, the aa needs to be deaminated. Then, **where does the amino group of the aa go?** Well...it needs to be released out of the body because amino group on its own is **ammonia/ammonium which is highly toxic**. Normally, $[NH_3] < 50\mu M$ and if it was to be higher, patient would experience disturbance of consciousness, coma, convulsions and even death.

Remark 4.15. We'll probably using the term ammonia (NH_3) and ammonium (NH_4^+) interchangeably which is fine as both forms are in equilibrium in blood.



Summary of Amino Acids Metabolism

aa from our diet enter the blood, adding to the circulating pool of aa. In this pool, **alanine and glutamine are the most abundant, along with notable amounts of glycine**. These aa are distributed to various tissues based on need. For example, muscles use aa to build proteins after weightlifting. However, excess aa are catabolized by the liver through the urea cycle, generating urea, which is then excreted in urine.

If aa are broken down during exercise, the NH_3 byproduct needs safe transport back to the liver for excretion. Here, glutamine and alanine play a crucial role in transporting NH_3 safely.

Two enzymes are central to this process: **glutamine synthetase** and **glutaminase**. In peripheral tissues, **glutamine synthetase** combines toxic NH_3 with glutamate to form glutamine, enabling safe transportation of NH_3 to the liver. Once in the liver, **glutaminase** breaks down glutamine, releasing NH_3 , which then enters the urea cycle and is excreted.

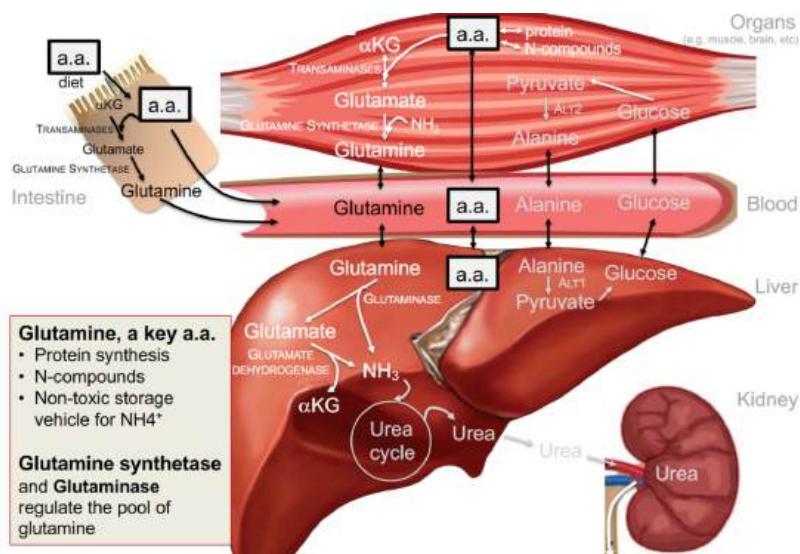


Figure 4.47: Summary of amino acid metabolism.

In summary, glutamine synthetase in peripheral tissues binds NH_3 to glutamate, forming glutamine for safe transport, while glutaminase in the liver releases NH_3 , which is then excreted.

4.6 De- and Transamination Reactions

Before talking about the reactions of today, let's first answer some questions:

A loss of function mutation in which of the following enzyme may cause delayed absorption of dietary amino acids in the GI-tract?

- | | |
|----------------------|-----------------------|
| A. Endopeptidase H | D. Carboxypeptidase A |
| B. Chymotrypsin | E. Pepsin |
| C. Pancreatic lipase | |

Answer: B, D and E.

Which is a non-toxic and abundant carrier of nitrogen in circulation?

- | | |
|---------------------------------|-----------------------|
| A. NH ₃ | D. Alanine |
| B. NH ₄ ⁺ | E. Glutamate synthase |
| C. Glutamine | |

Answer: C and D.

4.6.1 Amination: Glutamine Synthetase

Observation 4.23 Just to recap from last lecture, amination happens when you make glutamine from glutamate with NH₃. The NH₃, which is toxic, comes from the breakdown of aa during exercise to generate energy (this is after the glucose and lipid is becoming depleted).

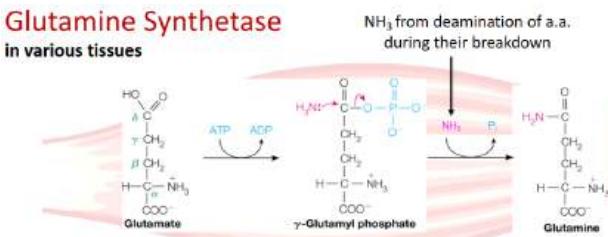


Figure 4.48: Glutamine synthetase action.

The enzyme mediating this path is called **glutamine synthetase**. To be specific of its mechanism, glutamate will be first phosphorylated using

ATP into a high-energy intermediate and then NH_3 will be incorporated forming glutamine.

Remark 4.16. *Synthetase uses ATP while synthase does not use ATP.*

4.6.2 Deamination Reaction

Deamination reaction involves in 2 steps: 1, removal of the NH_4^+ ; 2, resynthesize $\alpha\text{-KG}$. The first step is carried out by **glutaminase**, which is mostly expressed in the liver but some can be in kidney.

Observation 4.24 In the liver, glutaminase will hydrate glutamine making it release the NH_4^+ which will leave and feed the urea cycle. The consumption of NH_4^+ by the urea cycle drives the reaction forward.

The regenerated glutamate can then be reverted to $\alpha\text{-KG}$ using **glutamate dehydrogenase (GDH)**, which are often expressed in the mitochondrial matrix. Specifically, it would remove the NH_3 group using the reduction of either NAD^+ or NADP^+ to NADH or NADPH , respectively. Interestingly, **this reaction is driven forward by the consumption of NH_4^+ by the urea cycle and $\alpha\text{-KG}$ by CAC.**

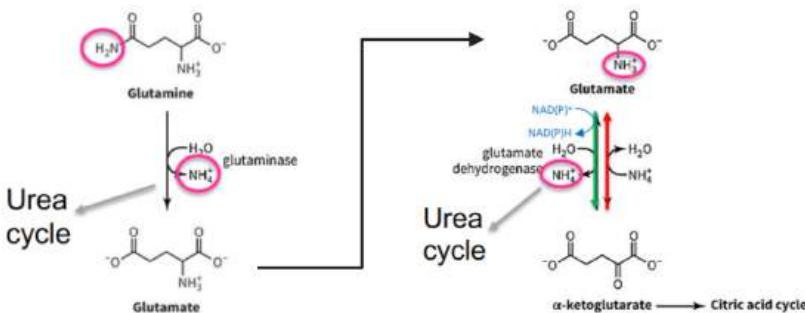


Figure 4.49: Glutaminase and GDH in deamination.

This is especially important since it's an endergonic reaction with $\Delta G = 30 \text{ kJ/mol}$ i.e. it's not spontaneous⁸.

⁸The reason that it would be driven forward is because there's a constant supply of aa needed to degraded which lead to feeding of NH_4^+ and $\alpha\text{-KG}$.

Regulation

GDH is regulated by the energy state of the cell:

- Low energy state: ADP (and NAD(P)⁺) acts as an allosteric activator.
- High energy state: GTP (and NAD(P)H) acts as an allosteric inhibitor.

Explanations. When the body needs energy, ADP will activate GDH which lead to more conversion of glutamate back to α -KG. This α -KG can be fed into CAC that can generate ATP later on in oxphos. Similarly but opposite would happen for GTP. □

Observation 4.25 In cancer cells, they treat alpha-KG, glutamine and glutamate as sources of energy instead of treating them like waste products as in normal cells.

4.6.3 Transamination Reaction

The main principle of transamination reaction is taking an amino group of one aa and attach it to another aa i.e. we do not metabolize the NH₃.

Example 4.6.1. Adding amino group to α -KG will make glutamate while adding amino group to oxaloacetate will make aspartate.

Definition 4.10. **Keto acids (ka)** are molecules with similar structure to aa however instead of having the amino group, they have *ketone* group (C = O).

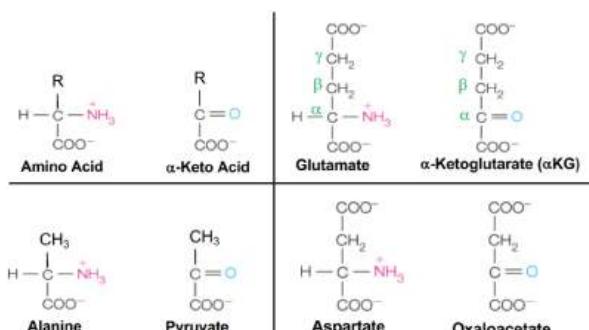


Figure 4.50: Transamination pair examples.

Definition 4.11. When a transamination can occur and transform a ka into an aa, we call said ka and aa, a **transamination pair**.

Example 4.6.2. α -KG is a ka while glutamate is an aa. Transamination reaction can happen and transform α -KG into glutamate, thus they're a transamination pair. Similarly, oxaloacetate and aspartate is a transamination pair; and, alanine and pyruvate is a transamination pair.

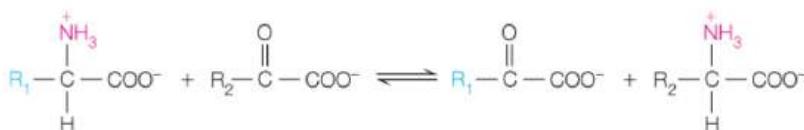


Figure 4.51: Transamination reaction.

Observation 4.26 Interestingly, you can combine 2 transamination pair in a transamination reaction. The basic idea behind this is that the amino group of 1 aa will be used to transaminate to another ka. The original aa will become the new ka, while the transaminated ka will be come the new aa.

Example 4.6.3.

aa can donate its amino group to α -KG. This transamination resulting in the original aa becoming the new ka while α -KG becomes glutamate.

Definition 4.12. Transaminases (aminotransferases) are enzymes that transfer an amino group from 1 aa to another ka i.e. It forms the transamination reaction. They ultimately generate a new set of aa and ka.⁹ All transaminases have **pyridoxal phosphate (PLP)** as their coenzyme.

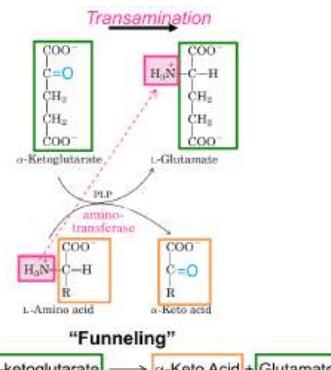


Figure 4.52: Reaction mediated by transaminase.

⁹This is because post-transamination, the original aa will lose its amino group and now become the new ka, similarly for the original ka becoming the new aa. (Observation 4.26)

See Example 4.6.3 for the brief outline of how transaminases will perform a transamination reaction.

Definition 4.13. The process of transferring a substrate from 1 molecule to the next is called **funneling**.

Pyridoxal Phosphate

PLP is derived from Vitamin *B*₆, to be specific, it has same structure but with addition of a phosphate group, and aldehyde group instead of alcohol. It's involved in a wide range of reactions but most relevant to this lecture is transamination. Their key components include:

1. **C4' aldehyde group:** Used to form covalent bond with the amino-group of the enzyme.
2. **Phosphate group:** Used for binding to PLP enzyme.
3. **3' OH group:** Used to improve catalytic efficiency
4. **Pyridine ring:** Used to stabilize carboanion intermediate.

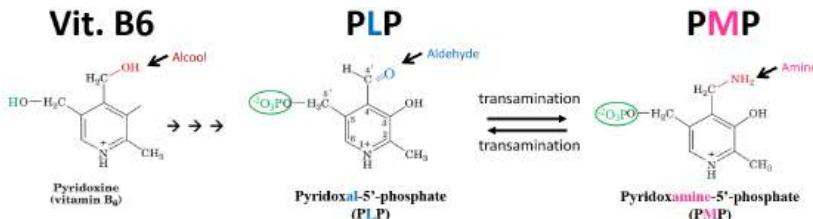


Figure 4.53: PLP and Vitamin B6.

Definition 4.14. **Ping-pong mechanism** is an enzymatic reaction in which no ternary complex is formed i.e. a double replacement reaction.

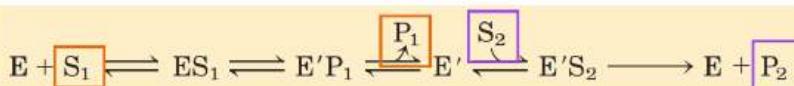


Figure 4.54: Ping pong mechanism.

Mechanism of Action ("Ping Pong" Mechanism of Transamination): Normally, PLP forms an internal **Schiff base** with the transaminase.

1. An aa, call it aa#1, forms an external Schiff base using PLP, which subsequently disrupt the Schiff base of the enzyme to PLP.
2. Hydrolysis reaction happens and the aa#1 will now leave as ka#1. The PLP is now transformed into **Pyridoxamine phosphate (PMP)** because the amino group from aa#1 is transferred onto PLP.
3. A different ka, call it ka#2, will come and form Schiff base using PMP.
4. The PMP reverts back to PLP, reassociates with its enzyme, and release the ka#2 attaching to the amino group, thus it's now aa#2.

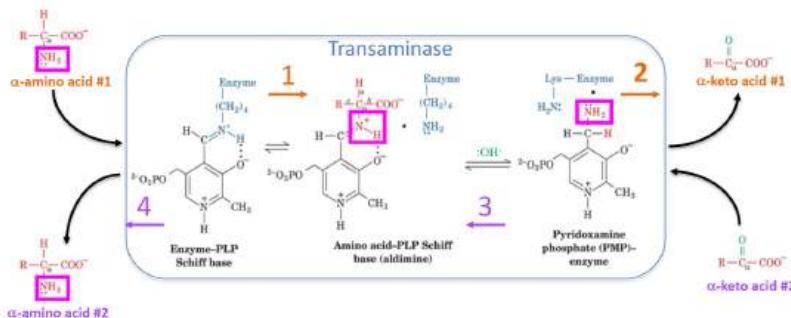


Figure 4.55: Transamination reaction mechanism.

Remark 4.17. *The most frequently used transamination pair is α-KG as the ka#2 (and glutamate as the aa#2).*

Observation 4.27 Type II Tyrosinemia is an inherited autosomal recessive defect in tyrosine catabolism (non-sense mutation). It's caused by a deficiency in tyrosine aminotransferase i.e. you cannot metabolize tyrosine well. Some of the symptoms that will manifest with this disease include: photophobia, sensitive skin, mental retardation, and behavioural problems.

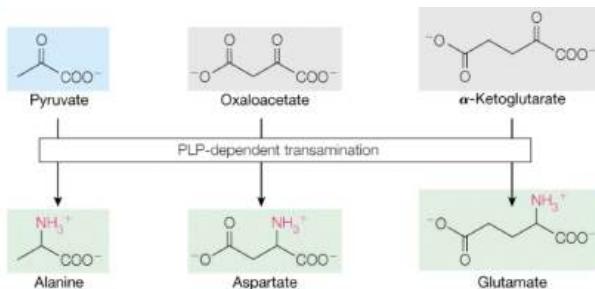


Figure 4.56: Common transamination reaction that are PLP-dependent.

4.6.4 Glucose-Alanine Cycle

Definition 4.15. **Cahill cycle** is a series of reactions that lead to the transferring of amino groups in muscle to liver using alanine.

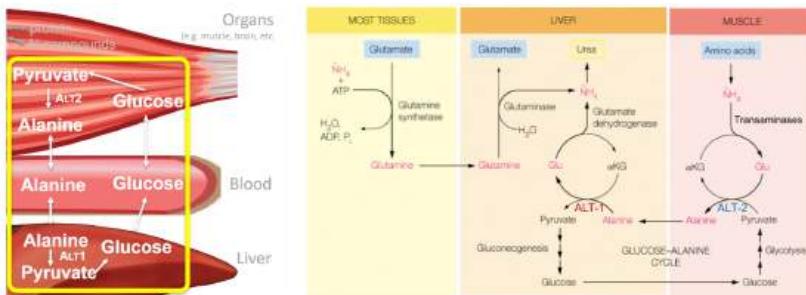


Figure 4.57: Cahill Cycle.

Observation 4.28 It's quite similar to Cori cycle, in term of recycling nutrients and substrates from the muscle to the liver, v.v. Notice that the usage of alanine here is essential as its transamination pair is pyruvate.

The general mechanism of Cahill cycle is as follow: In muscle, during exercise, glucose will be broken down through glycolysis and ultimately producing pyruvate. Pyruvate can be transaminated into alanine using **alanine transaminase 2 (ALT-2)** with amino group from glutamate¹⁰. Alanine

¹⁰The glutamate comes from glutamine detoxifying NH_3 , previously discussed

will leave the muscle and go to the liver. In the liver, alanine is transaminated back to pyruvate using **alanine transaminase 1 (ALT-1)** with α -KG as the ka. The pyruvate in the liver will be used as a precursor for gluconeogenesis. When glucose is made, it will be used again by muscle and the cycle continues.

Clinical Importance

Observation 4.29 For both ALT-1 and 2, they are mostly found in their respective places i.e. they're almost never found free flow in circulation.

Thus, **an elevated ALT-1 is a good indicator for liver damage**, this is because there shouldn't be ALT-1 in the blood. On the other hand, **leakage of ALT-2 is an indicator of muscle damage**.

4.7 Urea Cycle

In today's lecture, we will look at the urea cycle, but before that, let's answer some questions.

Which of the following is (are) involved in net deamination reactions?

- A. Glutamine synthetase
- B. Glutaminase
- C. Aspartate
- D. Alanine transaminase
- E. Glutamate dehydrogenase

Answer:

What prosthetic group(s) serve(s) as co-factor(s) for transaminases?

- A. PLP
- B. PMP
- C. PNP
- D. POP
- E. PPP
- F. Vit B_6
- G. Vit B_{12}

Answer: A and B

Which of the following prefer(s) α -KG as a substrate?

- A. Glutaminase
- B. Glutamate dehydrogenase
- C. ALT2
- D. Aspartate transaminase
- E. Tyrosine aminotransferase
- F. Proline transaminase

Answer: A and E

4.7.1 Overview

Historical Context: The urea cycle was discovered by Sir Hans Adolf Krebs and Kurt Hanseleit in 1932. They were simply measuring the urea production from liver slices.

Observation 4.30 The urea cycle happens partially in the mitochondria and partially in the cytosol i.e. It's compartmentalized. In the mitochondria, we will produce NH_4^+ (from glutamine or glutaminase¹¹ or deamination of glutamate) which will be incorporate with HCO_3^+ (which is the CO_2 from the CAC). This incorporation will generate a molecule called *carbamoyl phosphate* which will be transported out to the cytosol and pass through many intermediates until urea.

Remark 4.18. The rate limiting step is the enzyme that synthesizes carbamoyl phosphate.

4.7.2 Carbamoyl Phosphate Synthetase 1

Observation 4.31 In the mitochondria, **carbamoyl phosphate synthetase 1 (CPS1)** an enzyme that turns HCO_3^+ attaching with NH_4^+ using ATP to form **carbamoyl phosphate**, which is a high energy intermediate.

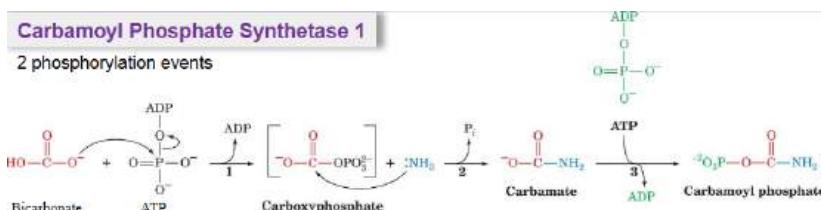


Figure 4.58: CPS1 reaction.

The reaction by **CPS1** is the rate-limiting step; in particular, this reaction is irreversible. It's regulated by an allosteric activator called **N-acetyl-glutamate (NAG)**. In turn, NAG is produced by an enzyme called **NAG synthase (NAGS)** using glutamate and acetyl-CoA. NAGS is stimulated by the

¹¹NOT from transamination!

level of arginine \Rightarrow NAG production is proportional to the [aa.] \Rightarrow the abundance of aa. will activate CPS1.

In some previous lecture, we've mentioned slightly about CPS2 but it's slightly different. For once, CPS2 is localized in the cytoplasm and is used for pyrimidine synthesis. Additionally, it doesn't need NAG to be activated and even use NH_3 from glutamine.

Carbamoyl Phosphate Synthetase 2

CPS-2

- Pyrimidine synthesis
- Cytoplasmic
- No need for NAG
- Uses Glutamine as NH_3 donor

Carbamoyl Phosphate Synthetase 1

CPS-1

- UREA synthesis
- Mitochondrial
- Absolutely needs NAG
- Uses NH_4^+

Essentially, compartmentalization ensures that both end products are made

Hyperammonia

Observation 4.32 Hyperammonia is a condition where there's elevated ammonia concentration in blood. This condition has many causes but in the context we're talking about, here are some:

1. **CPS-1 deficiency:** A missense mutation of CPS-1 gene. It can also lead to liver damage and even death at a very early age.
2. **NAGS deficiency:** A non-sense mutation of CPS-1 gene. Similar resemblance to CPS-1 deficiency.
3. **GDH gain-of-function:** A mutation that dissable GDH from shutting down thus producing more NH_4^+ than normal.

4.7.3 The Urea Cycle Steps

Mechanism of Action (Urea Cycle): Now that carbamoyl phosphate is made, we will use that to begin the urea cycle.

1. An intermediate called **ornithine** will be transported in the mitochondria via a transporter. Here, **ornithine transcarbamoylase** will

transfer the "carbamoyl" group from carbamoyl phosphate onto ornithine forming **citrulline**, which can be transported out to the cytosol^a.

^amutations in these transporters can lead to hyperammonemia

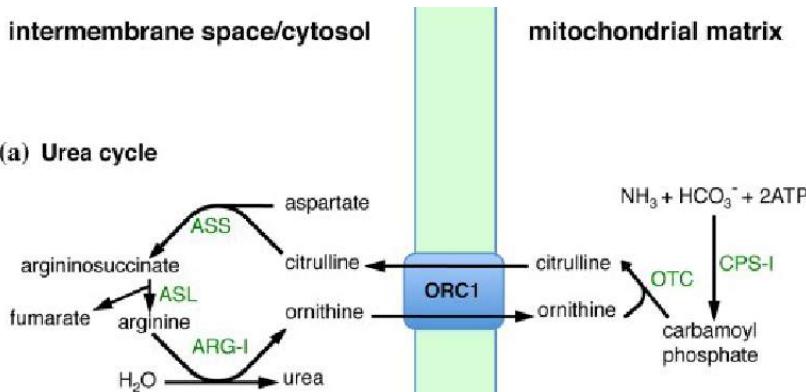


Figure 4.59: Citrulline and ornithine transporter. Specifically, it's an antiporter i.e. citrulline is transported out while ornithine is transported in to the mitochondria.

Continuation from the urea cycle.

2a. Citrulline will be phosphorylated by **arginosuccinate synthase (ASS)** which will form **citrullyl-AMP**. The release PP_i can then be used to drive the reaction forward.

2b. ASS can then remove the AMP by forming a nucleophilic attack by the α -amino group of aspartate. This attachment of aspartate (which also carry another NH_3 group) will form **arginosuccinate**.^a

3. Arginosuccinate will be converted to arginine by **arginosuccinate** with the release of fumarate.

4. Arginine is then hydrolyzed back into ornithine and at the same time releasing urea by **arginase**.

The ornithine is then go back in the cycle while the urea is transported to the kidney through blood. Here, it's excreted as urine.

^aMutations of ASS can cause hyperammonemia e.g. Type I hypercitrulinemia.

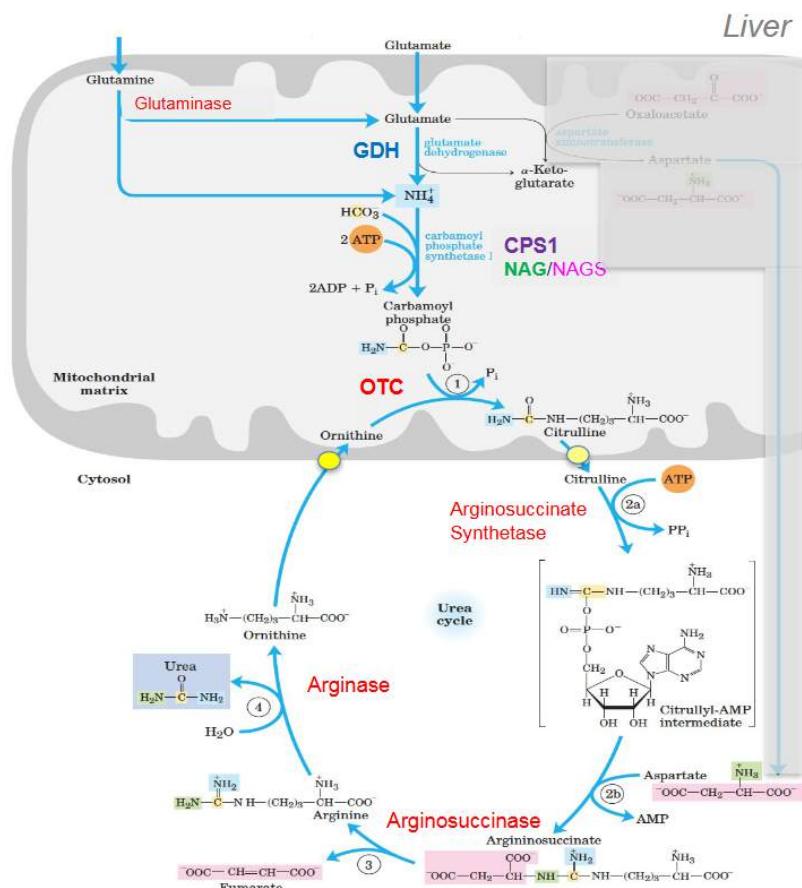


Figure 4.60: The urea cycle.

Now, you might be wondering...**where does the aspartate coming from (used in step 3)?** Well...It comes from the transamination reaction between glutamate to α -KG and oxaloacetate to aspartate, which happens in the mitochondria. So, in the theoretical sense, both of the amino group that's used to finally formed the urea originate from glut-

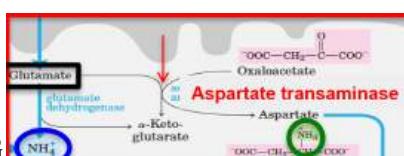


Figure 4.61: Synthesis of aspartate.

mate.

Remark 4.19. The enzyme that mediates the transamination reaction is **aspartate transaminase**, which is found in the liver's mitochondria \Rightarrow if it's found in the blood, it's a strong indication of liver damage.

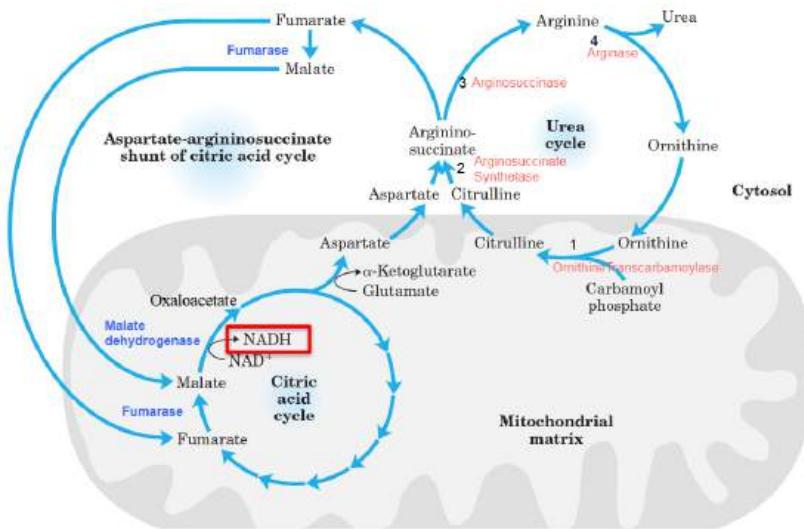


Figure 4.62: Links between urea and citric acid cycle.

Observation 4.33 You can then ask, well then **where's the oxaloacetate coming from?** Well...it's from the end product of CAC. Similarly, the generated fumerate will also feed into the CAC. So we can see that **the urea cycle depends on the contribution of α -KG to make aspartate; and nothing is wasted!** In fact, if we remember the NADH shuttle in past lecture, it contributes to this cycle.

Net Energy of Urea Cycle.

Like other cycle, let's see the net energy within this cycle i.e. the amount of energy used and released in making 1 urea molecule

Observation 4.34 To begin with, at the beginning, you have CPS2 consume 2ATP. Then, later on in the cycle, you need to invest another equivalent of 2 ATP (PP_i). So in total, you'd be investing 4 ATP in total.

Now, for the energy released. First, GDH can make $\alpha - KG$ which can be used in the CAC and potentially make 10ATP. Next, the generated fumarate can be used in the CAC and convert into oxaloacetate, and this can make 1NADH or 2.5ATP. In total, 12.5ATP made

So, the net energy is $12.5 - 4 = 8.5$ ATP. Basically, even though we invested some energy, the cycle will release some energy more than invested.

4.8 Amino Acids Synthesis and Degradation

Before beginning with today's lecture, let's answer some questions:

Is all the CO_2 generated during metabolism is excreted from the body by exhalation?

Answer: False, it can also be excreted by urine.

How does the body avoid NH_3 toxicity? Select all that apply.

- A. Glutamine synthetase incorporates NH_3 to glutamine
- B. Muscle pyruvate is converted to alanine
- C. Carbamoyl-Phosphate Synthetase assimilates NH_4^+
- D. Two NH_3 moieties are incorporated in urea, which is excreted by the kidney
- E. None of the above

Answer: A, C, D and E

How is urea production regulated? Select all that apply.

- A. High [arginine] stimulates urea production
- B. CPS-1 is downregulated in presence of NAG
- C. CPS-2 downregulates CPS-1-mediated production of urea
- D. CPS-1 is activated by substrate availability
- E. None of the above

Answer: A and D

What are the metabolic fates of the products of urea cycle? Select all that apply.

- A. Deamination of aspartate generates α -KG which serves in the CAC
 B. Arginosuccinate generates Succinate which serves in the CAC
 C. Urea is exported to the kidney
 D. The recycling of fumarate to the CAC permits the production of NADH
 E. None of the above

Answer: C and D.

4.8.1 Amino Acid Catabolism

Observation 4.35

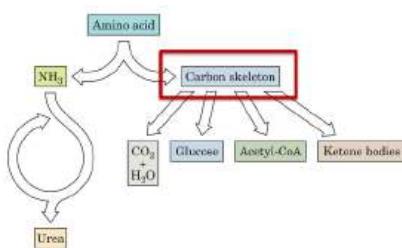


Figure 4.63: Amino acid catabolism.

Since there are 20 different aa., there would be 20 of these degradation pathways, we'll understand them instead (the general scheme). First, when aa. is degraded, 2 things will be released: NH_3 or NH_4^+ , and carbon skeleton, which will be eliminated in the urea cycle and used for other processes, respectively.

Although they can be broken down into these 2 compounds, the carbon skeleton part can actually vary greatly between aa. Nevertheless, some of these 20 aa. (~ 18) carbon skeleton can be recycled back to the 7 amphibolic intermediates of the CAC: pyruvate, acetyl-CoA, α -KG, succinyl-CoA, fumarate and oxaloacetate.

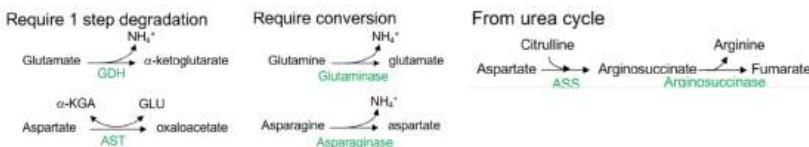
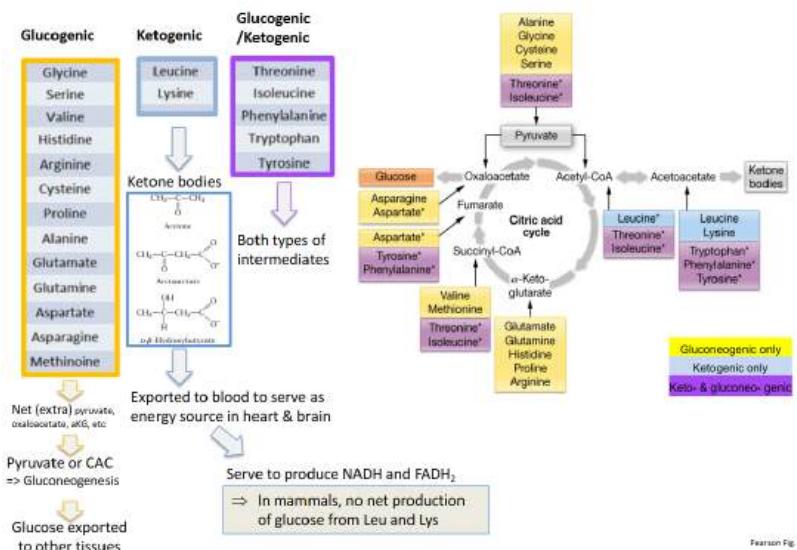


Figure 4.64: Amino acids degradation examples.

Example 4.8.1. We've seen some of these aa that only require 1 step degradation such as glutamate to α -KG and asparagine into oxaloacetate. Some

would require conversion to another aa. such as glutamine to glutamate and asparagine to aspartate. Even 1 that can be converted from the urea cycle such as aspartate to arginosuccinate and then fumarate.

Observation 4.36 Additionally, when the aa. is broken down to its carbon skeleton, this structure can be either: glucogenic, ketogenic or even both.



Pearson Fig. 18.12

Figure 4.65: Ketogenicity and glucogenicity of amino acids.

Glucogenic amino acids are aa. that can be broken down to become directly an intermediate of the CAC i.e. the degradation of glucogenic aa. is anaplerotic for the CAC. They all will undergo gluconeogenesis and the glucose synthesized can be exported to other tissues.

Ketogenic amino acids are aa. that can be converted into ketone bodies, which can be used as energy sources for the heart and brain (produce NADH and FADH₂) i.e. they're not used to make glucose.

For amino acids that are both glucogenic and ketogenic, they can do either as mentioned above.

4.8.2 Branched-Chain Amino Acids

Observation 4.37 Now, we have 3 amino acids that are branched: valine,

isoleucine and leucine, of which we will look at its degradation, which **does not happen in the liver**. First, all 3 of these aa. will undergo a transamination reaction into its α -Keto acid (α -ka) form using **branched-chain aminotransferase**. The α -ka can then be converted to acyl-CoA derivatives using **branched-chain α -ka dehydrogenase (BCKD) complex**. When there's disruption of the BCKD complex's activity, **maple syrup urine disease** will manifest.

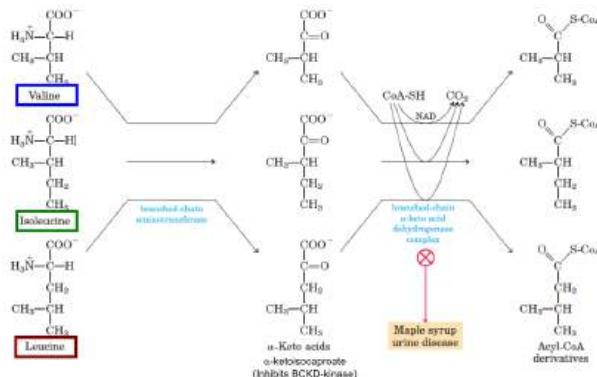


Figure 4.66: Degradation of branched-chain amino acids.

Now, it must be noted that the BCKD complex is quite similar to that of the PDC, especially the E2 and E3 complex which can be considered the exact same. BCKD complex can be inhibited by the activation of **BCKD kinase** i.e. when the BCKD complex is phosphorylated, it's inactivated (similar to how PDC is inhibited when its kinase phosphorylates it).

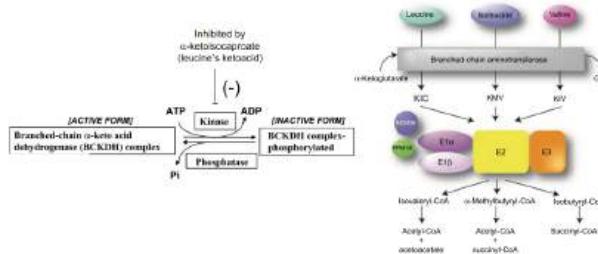


Figure 4.67: BCKD complex.

The regulator of the BCKD kinase is leucine's α -ka, to be specific it's

the inhibitor of BCKD kinase. Thus, when there's an abundance of leucine's α -ka, BCKD is shut off \Rightarrow BCKD complex is activated and begin to process the ketoacids.

4.8.3 Synthesis of Non-Essential Amino Acids

We will now look at the synthesis of the non-essential aa. We won't have too look for long as we've looked at these reactions many times before

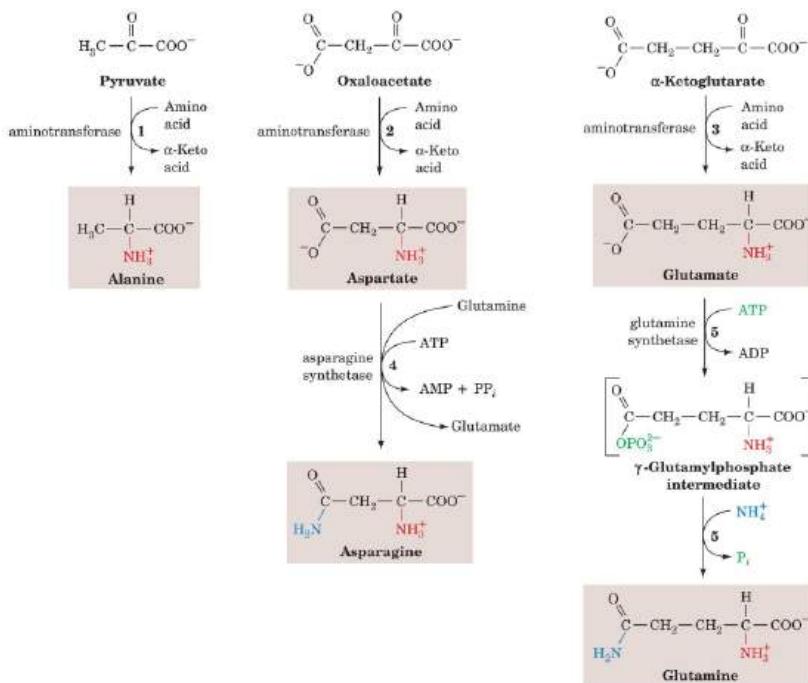


Figure 4.68: Non-essential amino acids synthesis.

Example 4.8.2. Pyruvate can be converted to alanine, Oxaloacetate can be converted to aspartate, and α -KG can be converted to glutamate. All of these reactions are transamination. The aspartate can be converted to asparagine and similarly glutamate can be converted to glutamine.

4.8.4 Metabolism of Methionine

Observation 4.38 **Methionine** is a non-essential aa.. We will now look at its synthesis. Methionine is very important as it's the start codon for protein synthesis, it's the precursor for cysteine. It's involved in the one-carbon metabolism, and is involved in the methylation of DNA.

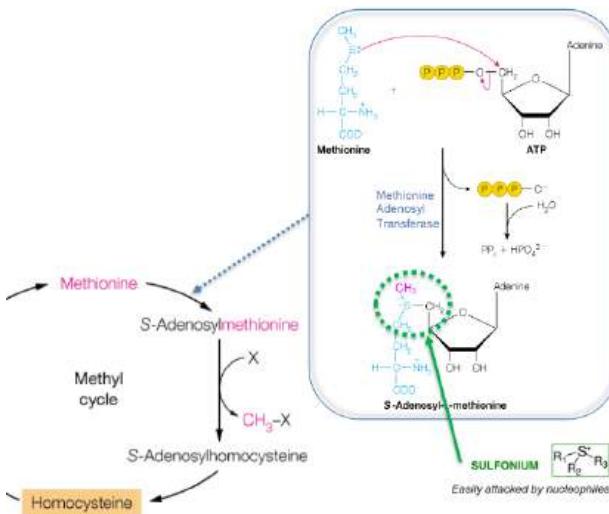


Figure 4.69: Methionine to S-adenosylmethionine.

Now, methionine can be degraded, or more like converted, into homocysteine which can be turned into cysteine. Conversely, homocysteine can be reverted back to methionine. If we focus on the pathway from methionine to homocysteine, we see that it first converted into **S-adenosylmethionine** by **methionine adenosyl transferase**, which will attach an AMP onto the sulfur position. Notice that this attachment is a sulfonium, which can be easily attacked by nucleophiles; and evidently, **it's used in the PE to PC one-carbon pathway as a methyl donor.**

Homocysteine

Observation 4.39 Now, the S-adenosylmethionine can be turned into **S-adenosylhomocysteine** after donating its methyl group which will then be turned into **homocysteine**. Homocysteine can undergo many reactions

with different intermediates which finally arrive to succinyl-CoA of the CAC. At the same time, when undergoing these reactions, cysteine is made and released.

Remark 4.20. *Methionine and homocysteine are homogenic.*

Observation 4.40

Hyperhomocysteinemia is a condition where there's an elevated homocysteinemia in plasma and this is due to the imbalance between the rate of its production and degradation. To be specific, this imbalance can be caused by:

- Vitamin B_{12} and folic acid deficiency.
- Mutation of enzyme converting homocysteine to cystathione.

Hyperhomocysteinemia is often associated with the following diseases:

1. **Cardiovascular disease:** Homocysteine can impair the formation of connective tissues \Rightarrow defect of blood vessel.
2. **Cognitive impairment/ dementia**
3. **Developmental defects:** Such as **neural tube defects** and **anencephaly**. Both are often severe and can cause be fatal to infant. This is also the reason why it's ideal for pregnant mothers to take vitamin B_{12} and folic acid supplement.

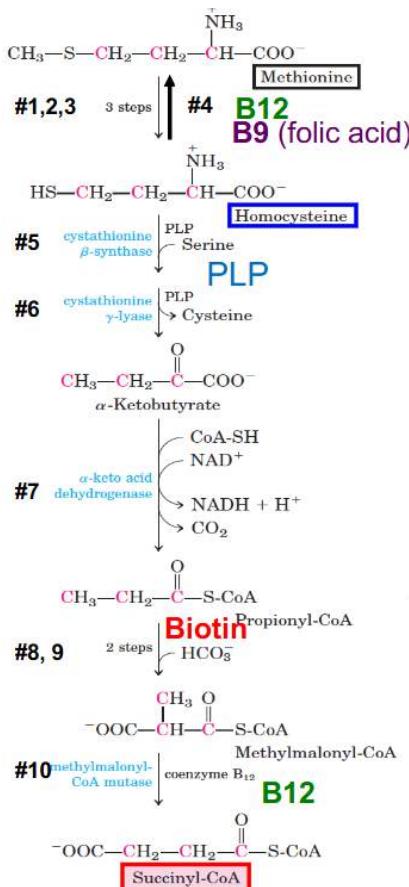


Figure 4.70: Methionine to succinyl CoA.

This figure highlights the role of **Vitamin B₁₂** and **Folic acid** in the metabolism of Methionine to Succinyl-CoA, which is a key component of the Citric Acid Cycle (CAC).

Cysteine

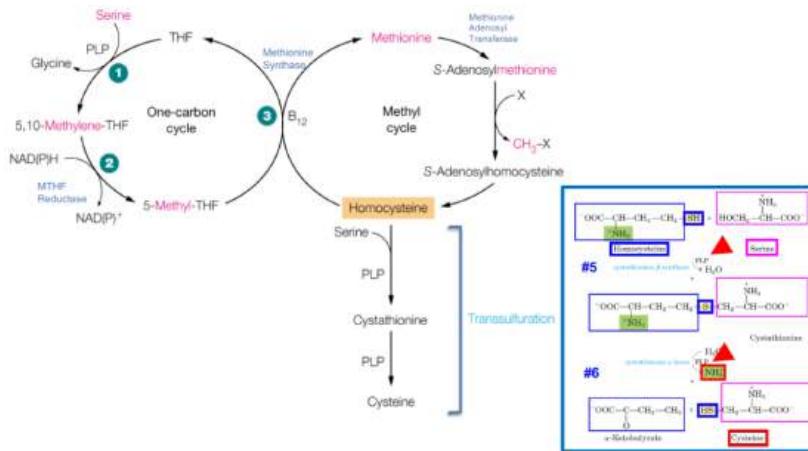


Figure 4.71: Homocysteine to cysteine.

The reaction forming cysteine is called **transsulfuration** and it's mediated by the coenzyme that we've previously known called PLP using serine. To put it simply, serine and homocysteine will undergo a first reaction where the homocysteine is attached to the serine at the sulfur, this intermediate is called **cystathionine**. Next, the cystathionine will be broken down into α -ketobutyrate and cysteine, which is a conditional essential aa.

Observation 4.41 MTHF reductase (MTHFR) is the enzyme that is the rate-limiting step for making intermediates to create 5,10-methylene-THF, which is important for the metabolism of methionine and homocysteine.

Vitamine B6, 8, 9, and 12

The metabolism of methionine is interesting as it requires many co-factors (we'll be also referencing the reaction numbers from figure 4.70):

1. **Vitamin B6** is required for PLP activity i.e. it required in reactions 5 and 6.
2. **Vitamin B8 (Biotin)** is required for reaction 8.
3. **Vitamin B9 (Folic acid)** is required for the MTHFR reaction and also in reaction 4.

4. **Vitamin B12** is required in 2 enzymatic reactions: reaction 4 and 10. In fact, this is the only 2 reactions that vitamin B12 is used in mammals.

Observation 4.42

Vitamin B12 deficiency can lead to hyperhomocysteinemia and other health problems. Nevertheless, it's very rare because the amount of vitamin B12 needed by the body is quite low. Vitamin B12 is produced by bacteria and sufficient amounts are often provided from a normal diet. The adjacent is symptoms of vitamin B12 deficiency (mild and severe).

• Symptoms of mild Vitamin B12 deficiency:	
Anemia, Nausea, Constipation, Gas	
• Symptoms of severe Vitamin B12 deficiency:	
Numbness/tingling hands and feet	eye problems
Insomnia	Headaches
Memory loss	Hallucinations
Dizziness	Inflamed tongue
Lack of Balance	Breathing difficulties
Depression	Palpitations
Digestive problems	Neurological damage
Liver enlargement	Tinnitus or ringing in the ears

The following illustration is the summary of amino acid catabolism

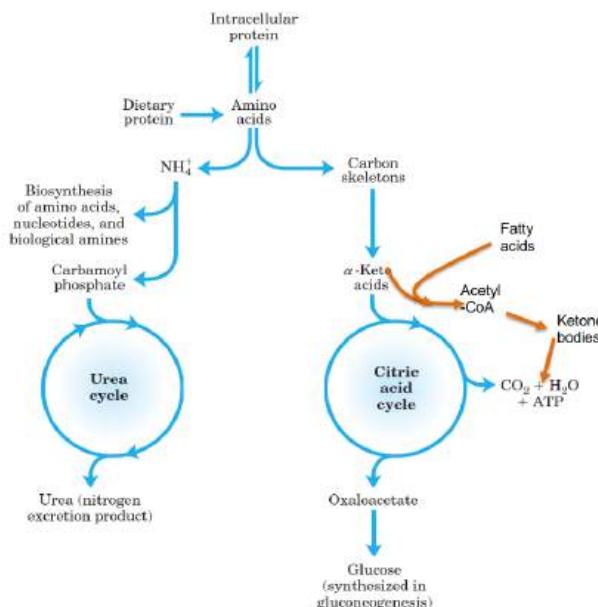


Figure 4.72: Amino acid catabolism summary.

Lecture 31: November 18th, 2024.

4.9 Integrated Metabolism II

In today's lecture, we will be looking at the integrated metabolism of all the pathways we've just looked at. In the figure below, you have the integrated metabolic pathway, of which we will focus on the amino acids. We see that aa. can be broken down into pyruvate which can be used to regenerate glucose, into acetyl-CoA for CAC or even to feed the urea cycle.

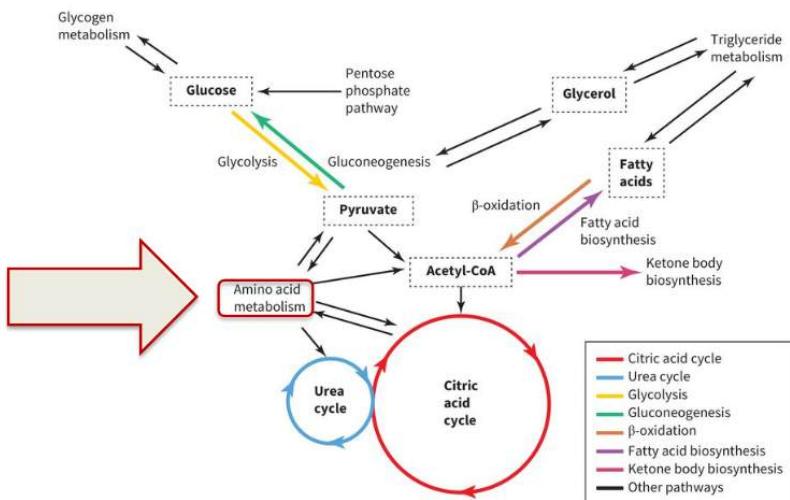


Figure 4.73: Integrated metabolism illustration.

Observation 4.43 Suppose that you just had a meal. In the liver, the aa pool will increase and so to get rid of the excess, it will begin catabolize it. The amino group will be get rid of in the urea cycle while the carbon backbone group is used to generate energy (feeding CAC, gluconeogenesis).

If you're exercising, in the muscle some of the protein will be broken by sheer stresses. The released aa will be carry to the liver and detoxified. Similarly, in other tissue, it can also provide some aa that contribute to the aa pool.

The metabolism of aa. takes place first in the mitochondria (first step of urea cycle) and then later of in the cytosol.

Remark 4.21. *The carbon backbone of aa metabolism is used to make oxaloacetate from the CAC which is used in gluconeogenesis.*

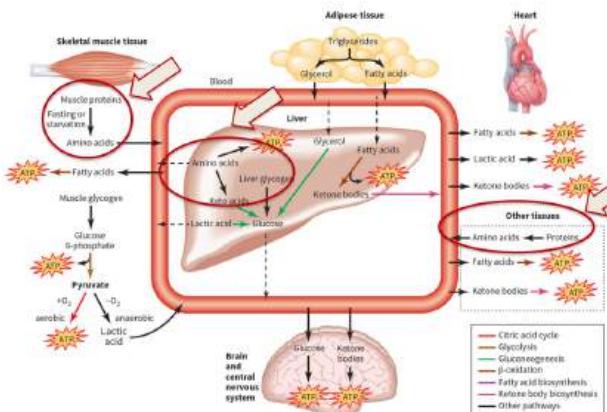


Figure 4.74: Integrated metabolism of amino acids .

4.9.1 Metabolism by Fed and Unfed State

We'll now take a look at the body's metabolism by fed and unfed state.

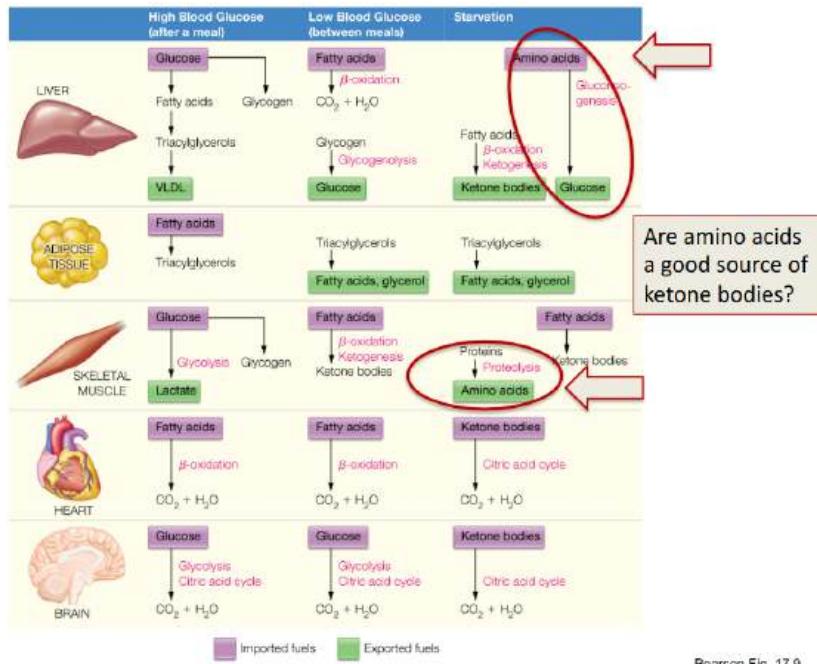
Observation 4.44 We will begin with fed state and blood glucose is high. In the liver, glucose will be broken down and stored as glycogen; the excess is converted to FA and TG, which is delivered by VLDL. In adipose tissues, FA is turned into TG and stored in lipid droplets of the cell. In the muscle, glucose is converted to glycogen and the rest will be used for glycolysis. Notice in the figure that the heart uses FA as a preferred fuel, **why is that?** Well...compared to the muscle, which when exercise will begin to use up the ATP, aerobic and anaerobic glycolysis and then finally aerobic lipolysis (using FA).

Remark 4.22. *It's not that each energy pathway (ATP, creatine phosphate, aerobic glycolysis, etc.) is getting turned on 1 by 1 but their relative contribution can differ in time.*

For the heart, it's constantly beating which is unlike the muscle that has a beginning to the exercise \Rightarrow **the heart is always in aerobic lipolysis.** Nevertheless, the heart can always go back to use glycolysis and especially when it ran out of oxygen (emergency source of energy).

Example 4.9.1. When a patient suffers from a myocardial infarction, the heart will run low on oxygen and thus it will begin to use glycolysis as begin

to produce lactate. In fact, elevated lactate is an indicator for myocardial infarction in clinical setting.



Pearson Fig. 17.9

Figure 4.75: Metabolism differs in fed and unfed state.

Observation 4.45 Now we will look at when the body is in between meals with low blood glucose. In the liver, FA will undergo β -oxidation and glycogen will undergo glycogenolysis into glucose. In adipose tissues, TG is reverted back to FA and glycerol. In skeletal muscle, FA is broken down into ketone bodies. In the heart, FA continue to be used as fuel and in the brain glucose will be used as fuel.

Observation 4.46 Now, suppose the body is undergoing starvation. In the liver, aa will be degraded and used for gluconeogenesis; and FA is broken down into ketone bodies. Everything would be the same for other tissues except in the muscle, you have proteins broken down into aa to be used to make glucose; and in the brain, we'll be using ketone bodies.

Are amino acids a good source of ketone bodies? Well...yes but they're not the primary source (FA mostly).

Observation 4.47 Interestingly, if you were to plot the different metabolites concentration during starvation, you'd see the build up and drop of certain metabolites. However, when looking at amino acids, they're relatively stable overtime.

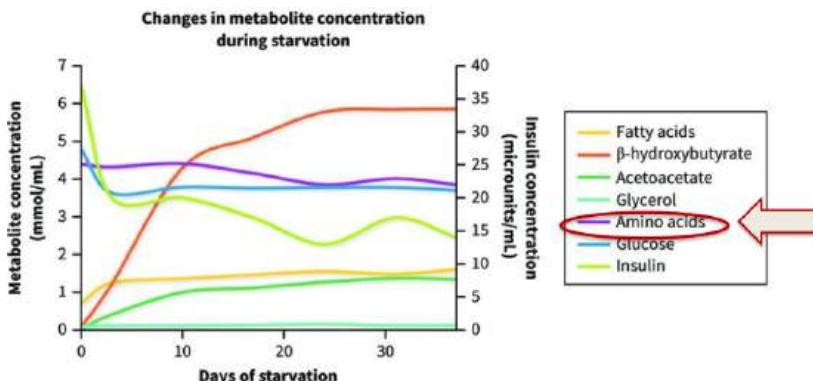


Figure 4.76: Amino acid stability during starvation.

4.9.2 Deficiencies

In this part, we will look at some clinical cases and deficiencies relating to the metabolism of the body. Before that, let's look at the regulation of the urea cycle.

Observation 4.48 Enzymes involved in the regulation of amino acid metabolism, particularly those managing the urea cycle, play a critical role in maintaining nitrogen balance. In the short term, aa. can significantly impact metabolism. For instance, mutations in enzymes like GDH can have profound effects. A gain-of-function mutation in GDH results in excessive production of NH_4^+ , leading to hyperammonemia, a potentially life-threatening condition.

Another key enzyme is CPS-1, which catalyzes the rate-limiting step of the urea cycle. A mutation in CPS-1 can impair the conversion of NH_4^+ into carbamoyl phosphate, causing a buildup of NH_4^+ and resulting in hyperam-

monemia. This is especially critical in cases of complete CPS-1 deficiency.

Example 4.9.2. Consider a baby born with complete CPS-1 deficiency. Such a condition leads to severe hyperammonemia shortly after birth. Without the ability to detoxify ammonia, the baby quickly experiences liver failure, brain damage, and eventually death, typically within hours.

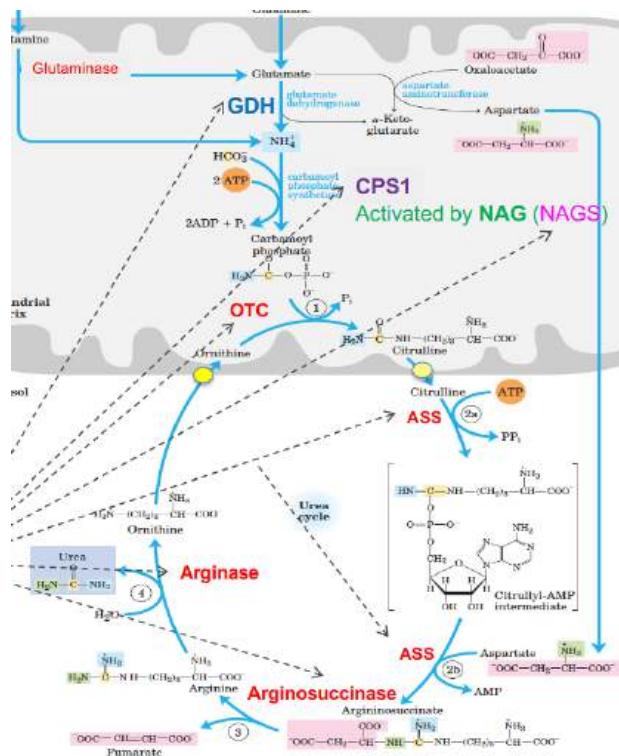


Figure 4.77: Urea cycle reminder. Mutations in all of the enzymes that the dashed arrow pointed at (and transporter ORNT1) will lead to hyperammonia, with mutation in earlier step having worst prognosis.

But then, **how does the baby survive in the womb?** Well...it's thanks to the mother. While in utero, the mother's functional CPS-1 processes the baby's ammonia, effectively shielding the baby from the toxic effects. However, after birth, the baby loses this support and is unable to handle

ammonia independently, leading to fatal consequences.¹²

Recall: CPS-1 activity is regulated by NAG, which in turn, depends on the enzyme NAGS. Deficiencies in NAGS can impair CPS-1 function, further exacerbating issues in NH_3^+ processing. Regulation of the urea cycle also involves transcriptional control e.g. during prolonged starvation, the body upregulates the transcription of genes involved in the urea cycle to enhance the breakdown of amino acids and manage nitrogen excretion effectively.

Remark 4.23. While complete CPS-1 deficiency is lethal (as both parental alleles are defective), other mutations may have less severe clinical effects. In some cases, individuals can survive but may face metabolic challenges, especially under stress or during metabolic crises.

Case Study 1

A 48-year-old woman who presented with lethargy, weakness, and altered mental status following prolonged nausea and vomiting despite an esophageal dilatation procedure 3 weeks prior. Further investigation with assistance from the genetics consult team revealed a partial enzyme deficiency associated with urea cycle disorder.

Lab	On Admission	After treatment	Reference Range
Glucose	96	159	65-99 mg/dL
Ammonia	406	29	11-51 $\mu\text{mol/L}$
Urine ketones	2+	Negative	Negative
AST	13	19	11-33 unit/L
ALT	11	15	6-37 unit/L
Barbiturates screen	Negative	N/A	Negative
Benzodiazepine screen	Negative	N/A	Negative
Tricyclic antidepressant screen	Negative	N/A	Negative
Ethanol	<0.01	N/A	<0.01 g/dL
Methanol	<10	N/A	<10 mg/dL

Questions:

- What are the possible genetic disorders in this patient?
- What treatment is available and how does it work?
- Can you explain the clinical values before/after the treatent in the table above?

¹²The normal range of ammonia is $30 - 70 \mu\text{M}$ while hyperammonemic range is $\geq 240 \mu\text{M}$.

Answer:

- Some possible genetic disorder that this patient experience includes: partial dysfunction of CPS-1, gain-of-function mutation of GDH.
- Treatment can include reduced protein intake and stimulate other pathway for NH_4^+ secretion using phenylacetate + benzoate ("ammonul").
- All of the patient lab value return to normal range, especially with the 2 previous elevated value of ammonia and urine ketone. With that we also have an elevated level in glucose. (Personal thought: could've been that the patient was put on a high glucose diet and protein was removed completely to avoid further complication).

Observation 4.49 (Treatment of Hyperammonemia). With that, we want to look further into the treatment of hyperammonemia. The most obvious thing to do is reduce protein intake \Rightarrow reduce aa. intake \Rightarrow lower NH_3 enter the body to be cleared. Next is to stimulate other pathways to clear NH_4^+ and this can be done by 2 compounds:

1. **Phenylbutyrate:** Used to regenerate the consumed glutamine \Rightarrow NH_4^+ will be taken up by the glutamine synthase reaction.
2. **Benzoate:** Used to regenerate the consumed glycine \Rightarrow NH_4^+ will be taken up by the glycine synthase reaction.

The by-product of these reactions are **hippurate** and **phenylacetylglutamine** by benzoate and phenylbutyrate, respectively. Both of them carry NH_4^+ and are both non-toxic to be released in urine.

Typically, during treatment, clinicians will use a combination of phenylacetate and benzoate called **ammonul**, which can stabilize patient however they're still at risk of a **sudden onset hyperammonemic coma**.

Case Study 2

[...] Here, we report the case of a young woman with severe, recurrent thrombo-embolic events associated with severe hyperhomocysteinemia ($111 \mu\text{mol/L}$).
[...] Family study shows segregation of elevated homocysteine in heterozygous relatives for the mutation in the cystathionine β -synthase gene.

Questions:

- What are the potential genetic defects that can cause hyperhomocysteinemia?
- What are the clinical consequences of those mutations?
- What are the non-genetic causes of hyperhomocysteinemia?
- How to prevent the irreversible effects of hyperhomocysteinemia?

Answer:

- The potential genetics defects could be: problems with MTHFR, loss-of-function in cysteine β -synthase, gain-of-function in methionine adenosyl transferase, and loss-of-function in methionine synthase.
- Elevated level of homocysteine
- For the non-genetic causes, it could've been deficiencies in the different vitamin involves in the homocysteine metabolic pathway.

Observation 4.50 (Treatment of Hyperhomocysteinemia). There are many ways to treat homocysteine imbalance. For this instance, we'll focus on the loss-of-function mutation in cysteine β -synthase. In this particular case, it can actually help by giving patients high dose of vitamin B6. (500 – 1000mg/day)¹³. However, **this is only possible if patient having this disease is "vitamin B6-responsive".**

When the patient having this disease is B6-resistant, we will use folic acid instead. This is to stimulate the re-methylation of homocysteine and methionine formation.

¹³The recommended normal intake is only 1.3mg/day

5.1 Transcriptional Control of Metabolism I

Definition 5.1. **Energy homeostasis** is the balanced of fuel intake, storage and expenditure.

Observation 5.1 When there's a disturbance to the energy homeostasis, it can lead to consequences like anorexia or obesity which are the causes of many metabolic syndrome leading to diabetes, heart and kidney failure, etc. Hence, energy homeostasis should not be "messed" with and this is done through metabolic control by the body.

Remark 5.1. *In more serious consequences of the disturbance, you can have development of fatty liver that ultimately lead to cancer.*

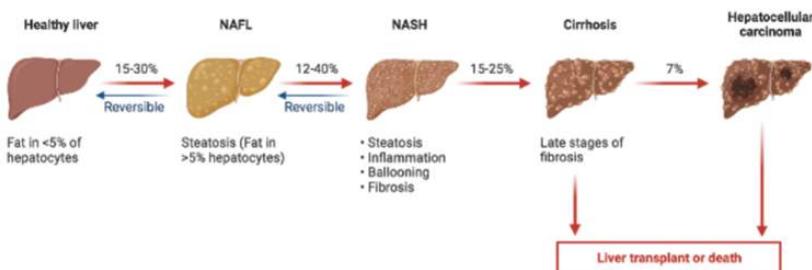


Figure 5.1: From healthy liver to liver cancer.

When it comes to the control of metabolism, we can divide them into 2 categories: **short- and long-term**.

- **Short-term regulation:** Mostly through allosteric control and **post-translational modifications (PTMs)** of enzymes that respond to metabolic changes.
- **Long-term regulation:** Mostly through transcriptional regulation of metabolic genes.

Nevertheless, most metabolic regulations benefit the highest through correct coordination of these regulatory mechanism. So now we need to ask ourselves **how does transcriptional control of metabolism work?** Well...it's through signals that can be transduced to the genes that are targeted to be regulated. We can divide this into 3 stages:

1. Pre-regulation of genes Here, signals from outside of the nucleus will activate some signaling pathway in the cell that ultimately route to the nucleus.
2. Regulation of genes: Once the signal is correctly transduced to the nucleus, necessary factors, proteins, etc. will come and regulate the expression of the genes in question.
3. Post-regulation of genes: Once the genes are regulated, signals from this regulation will be sent back out and target the correlated enzymes or other proteins.

For the first step, we've basically been talking about it for the past 4 chapters, which are just signalling molecules that can lead to changes. We will begin with step 2.

5.1.1 Metabolic Transcription Factors

Definition 5.2. **Metabolic transcription factors (mTFs)** are proteins that receive signals and bind to the specific DNA that express a certain gene to be regulated.

There are many mTFs but we will look at some of the key players for metabolic transcriptional control.

6 Different mTFs and Their Activation

Definition 5.3. **cAMP response element bind (CREB)** along with its members CREM and ATFI, *leucin-zipper*¹ transcription factors.

Observation 5.2 They're activated by PTM through phosphorylation via PKA when [cAMP]↑ after the action by glucagon. Once activated through phosphorylation, they will work with a co-activator called **CBP**. In fact, they're considered to be the "first responders" to activate gluconeogenesis during fasting.

¹a protein motif consists of has a leucine residue at every 7th position forming an α -helical conformation.

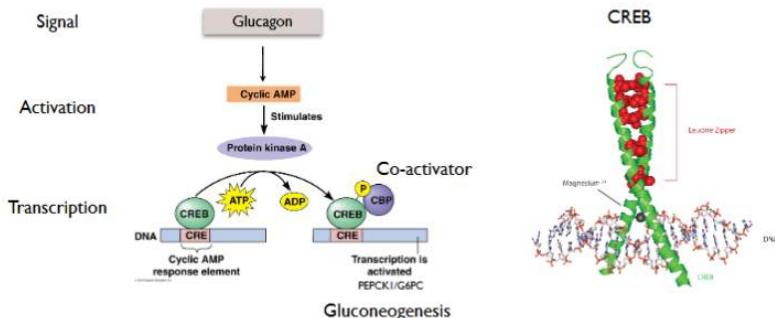


Figure 5.2: CREB structure and activation.

Definition 5.4. **Carbohydrate response element binding protein (ChREBP)** is a *bHLH-leucine zipper* transcription factor.

Observation 5.3 When ChREBP is phosphorylated by PKA, it will be inactivated. On the other hand, it can be activated through dephosphorylation (PTM also) mediated by an phosphatase called **PP2A**. It can regulate both glucose and lipid metabolism.

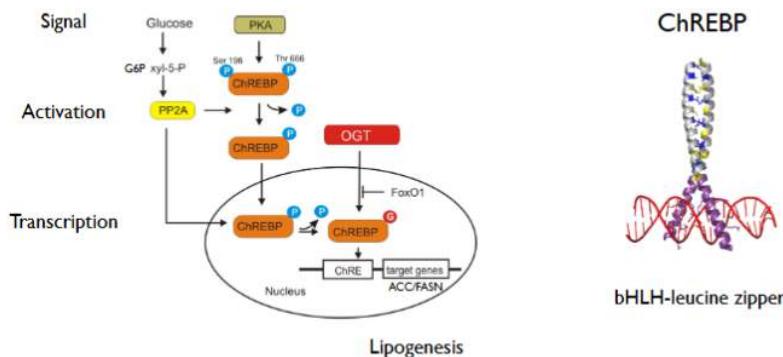


Figure 5.3: ChREBP structure and activation .

Example 5.1.1. Glucose enters the body and metabolized to G6P or even Xu-5-P (intermediates of PPP). These intermediates can activate PP2A which can dephosphorylate ChREBP and thereby activate it. Activation of ChREBP this way can lead to lipogenesis using glucose.

Definition 5.5. Sterol response element binding protein 1c (SREBP-1c) is a *bHLH-leucine zipper* transcription factor.

Observation 5.4 SREBP-1c can be activated by proteolytic cleavage that is stimulated by sterols and unsaturated FAs. They can target genes that are involved in lipid metabolism. Normally, SREBP-1c is found to be bound in a complex with another protein called SCAP (refers back to lecture on cholesterol); all of which sit at the ER membrane.

Example 5.1.2. In the presence of sterol, SREBP-1c and SCAP are bounded together with another protein called **INSIG** and stay at the ER. In the presence of low sterol, SREBP-1c/SCAP complex breaks away from INSIG and is transported elsewhere² of which the SREBP-1c will undergo proteolytic cleavage by S1P and then S2P. This ends up with 2 subunits of SREBP-1c: transmembrane and cytoplasmic subunits. The cytoplasmic portion of SREBP-1c is now free flowing and can enter the nucleus to activate the appropriate gene for lipogenesis.

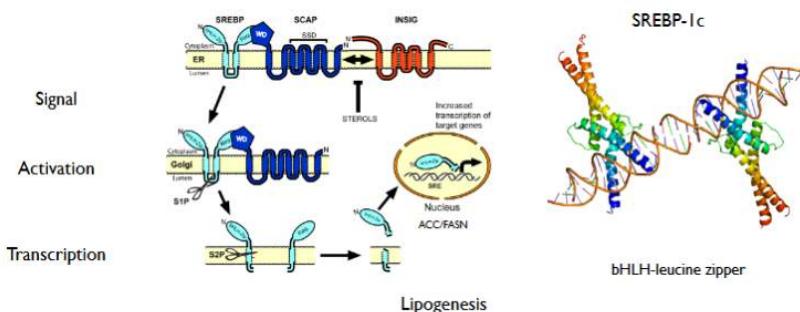


Figure 5.4: SREBP-1c structure and activation.

Definition 5.6. Forkhead box (FOXO) proteins, consisting of FoxO1, O3, O4, O6 and A2, are transcriptional factors involved in hepatic glucose production.

Observation 5.5 Fox proteins are regulated by PTMs, including phosphorylation and acetylation. Specifically, it's inhibited through phosphorylation by **AKT** in response to insulin signalling. When there's low glucose, FOXO

²Though not mentioned in class, this "elsewhere" is the golgi apparatus

can freely enter the nucleus and activate genes relating to gluconeogenesis.

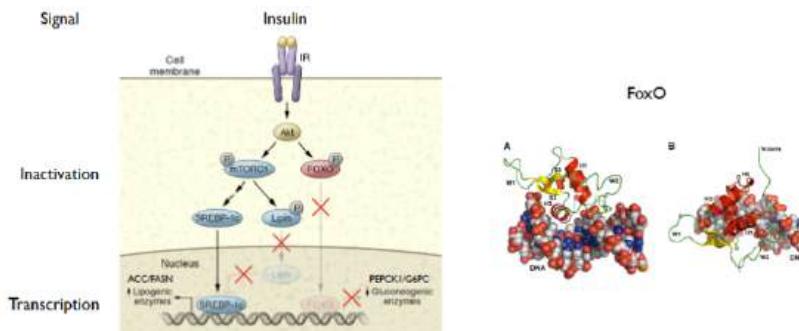


Figure 5.5: FOXO structure and regulation.

Definition 5.7. **CCAAT-enhancer binding proteins (CEBP)** are also *leucin-zipper* transcription factor. There are 6 different but related proteins of this category.

Observation 5.6 Their activity is constitutive and they play a major role in the response to fasting by their signal-independent high expression in the liver. In particular, during fasting, CEBP will not be modified but its production will increase.

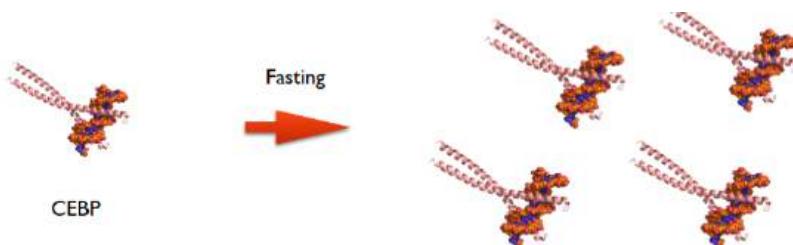


Figure 5.6: CEBP structure and regulation.

Definition 5.8. **Nuclear receptors (NRs)** is a family of 48 ligand-response

*zinc finger*³ transcription factors, of which many work as "metabolic sensor".

Observation 5.7 Majority of NRs are activated directly by their ligands i.e. ligand come and bind to the NR which can activate it. They can also be regulated through phosphorylation and other modification which can activate or suppress them. Like other factors, they will need a co-regulator to work.

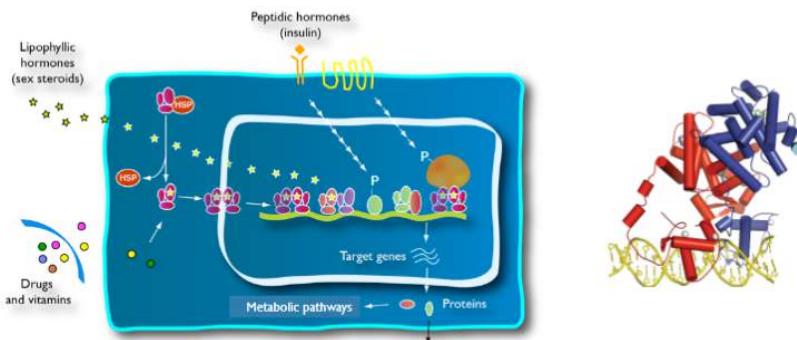


Figure 5.7: NRs regulation .

Remark 5.2. *Transcription factors are only there to find the right gene and recruit the transcription machinery.*

5.1.2 Metabolic Coregulators

Definition 5.9. **Metabolic coregulators** are proteins that interact with transcription factors to either repress or activate a gene related to metabolism. They don't have a DNA-binding domain and don't recognize anything on the chromatin directly.

Observation 5.8 Coregulators either act as scaffolds to recruit other coregulators or the transcription machinery, and/or have enzymatic activity to modulate chromatin accessibility and/or the activity of transcription factors and other coregulators. They can be classified into 2 types: **coactivators and cosuppressor** which will help activate and represess the gene,

³Zinc finger domain are small protein motifs containing zinc and have finger-like protrusions

respectively.

For NRs, we have coactivators like **nuclear coactivator (NCOA)** which has 3 member in this group (NCOA1-3). They are large proteins, about 2000kDa, and have many domains to interact with other proteins. Contrarily, we have corepressors like **nuclear corepressor (NCOR)** with 2 members (NCOR1-2).

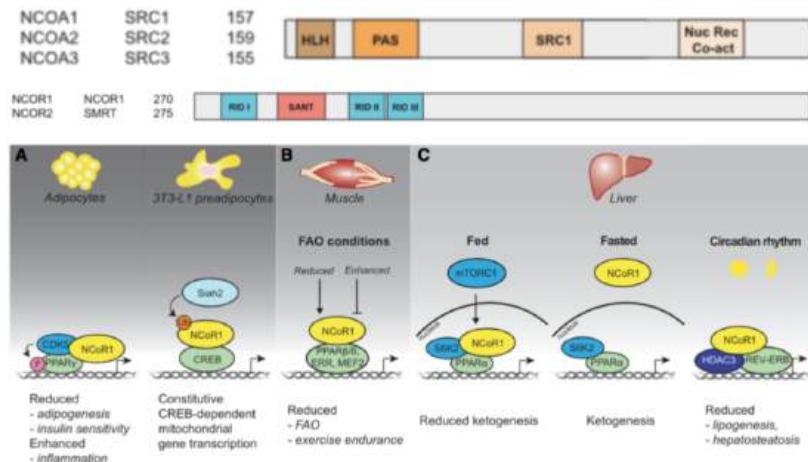


Figure 5.8: NCOA and NCOR.

Remark 5.3. While they were first discovered to work with NRs, they can also work with other mTFs, such as CREB.

Example 5.1.3. In adipocytes, NCOR can regulate pathways related to adipogenesis and etc., depending on which NRs it interacts with, such as PPAR. Removing NCOR from cells can lead to changes in metabolic gene transcription, reduced adipogenesis, or other tissue-specific effects.

In muscle, for instance, removing NCOR reduces FA oxidation and exercise endurance. This highlights how different coregulators influence distinct biological outputs in different tissues.

Peroxisome proliferator-activated receptor gamma coactivator

Definition 5.10. **PPARG coactivator-1 (PGC-1)** is another important coregulator of metabolism and comes in 2 forms: PGC-1 α and PGC-1 β .

Observation 5.9 These are "master" coactivators interacting with multiple transcription factors, especially NRs like PPARs and ERRs. They regulate mitochondrial biogenesis, energy metabolism, and heat production in brown fat.

Example 5.1.4. When the body receives Signals like cold exposure or glucagon, it can activate PGC-1 α via cAMP, PKA, etc. This activation will lead to mitochondrial DNA replication, changes in the TCA cycle, thermogenesis, and etc.

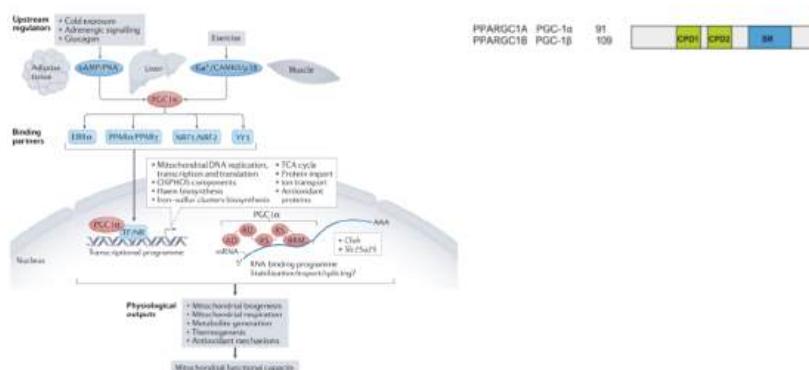


Figure 5.9: PGC-1 α actions.

Observation 5.10 Regulation of coactivators like PGC-1 α often involves PTMs. You have enzymes like KAT2A and B⁴, can acetylate PGC-1 α and decrease its activity. It's used to weaken/blunt gluconeogenesis made by PGC-1 α . Conversely, a sirtuin⁵ called SIRT1, which is a NAD $^+$ -dependent deacetylase can reactivate PGC-1 α .

5.1.3 Nuclear Receptors

We will now focus more on nuclear receptors (NRs). They're the master regulator of metabolism. NRs are particularly interesting because they bind small molecules, making them therapeutic targets for drugs. This family

⁴They're lysine acetyl transferase but more official know with the names: GCN5 and PCAF, which are histone acetyltransferase.

⁵A family of proteins/enzymes involved in metabolic regulation.

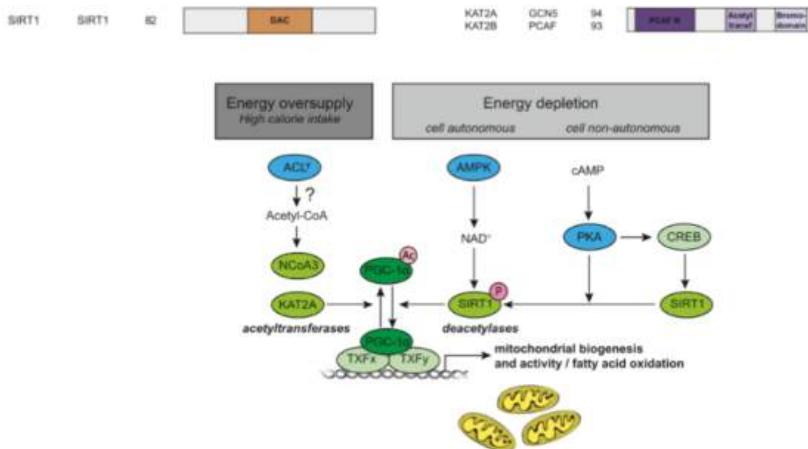


Figure 5.10: Regulation of PGC-1 α .

of 48 receptors responds to ligands like FAs, bile acids, cholesterol, and vitamins. Some receptors remain “orphan,” meaning their ligands are unknown, suggesting more discoveries are yet to be made.

Classic Hormone Ligands for NRs

Observation 5.11 **Classic ligands** for nuclear receptors include thyroid hormones, which regulate energy homeostasis; cortisol, which influences glucose levels; and sex hormones like estrogen and androgens, which play roles in muscle building and reproductive metabolism.

Adopted and Foster Homes NRs

Definition 5.11. **Adopted NRs** are NRs who were previously thought to have no known endogenous ligand that can activate it but was later discovered.

Observation 5.12 We have discovered new ligands for certain NRs (turning them from orphan to adopted NRs), and one of these is 7 α -hydroxycholesterol. When you remove the receptor called **LXR (Liver X Receptor)** from a mouse, you observe that within a few days, the mouse develops **fatty liver disease**. This aligns with what we’ve seen earlier: by removing just one of these two key receptors, you trigger fatty liver disease.

In the next lecture, we'll discuss **CDCA (chenodeoxycholic acid)**, which is a bile acid. Until recently, bile acids were not known to function in this way. It was discovered that they work through a nuclear receptor called **FXR (Farnesoid X Receptor)**. Additionally, many phospholipids also act through similar NRs. These receptors interpret signals originating from the cell membrane. Another one is PPARs which recognize long-chain FAs and they do not bind exclusively to one type of fatty acid.

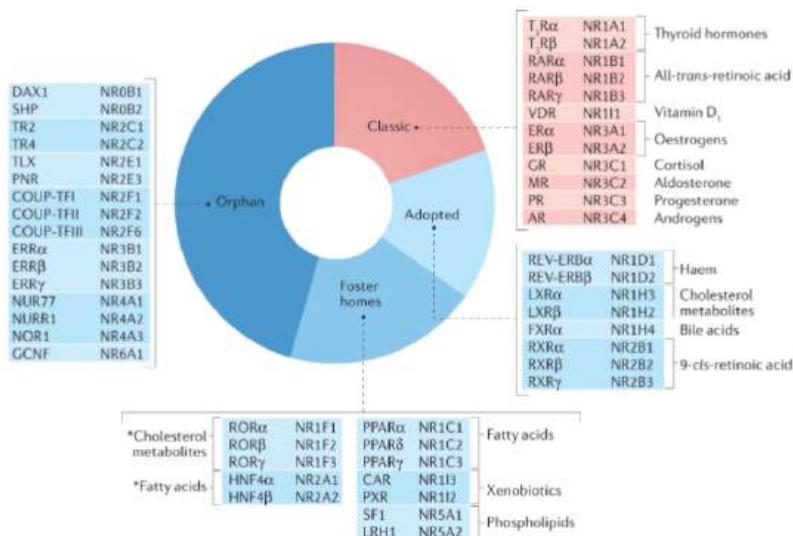


Figure 5.11: Superfamily of NRs classified by the type of ligand they bind to.

Orphan NRs

Definition 5.12. **Orphan NRs** are NRs that does have (or at least we haven't discovered 1) an endogenous ligand that bind to it.

Observation 5.13 These NRs are regulated by the level of expression, PTMs and protein stability. Though we've have no clue if there's a ligand that binds to them or not, we've developed drugs that work on these NRs suggesting there must indeed be an endogenous ligand for them.

5.1.4 Regulating of Gene Expression by NRs

We will now briefly look at how do nuclear receptors work.

Observation 5.14 Looking at an NR, it has a **DNA-binding domain**, to recognize a very specific sequence on DNA. Unlike other transcription factors, they also have this **ligand binding domain**, which recognizes small molecules like thyroid hormone, estrogen, androgen, etc. They have 2 transactivation domain called **AF-1** and **2**, which is used to recognize the coregulators, such as PGC1, NCOR, and SRC1. And so all of these domains can also target by PTMs. For instance, if you phosphorylate the DNA-binding domain, you'll inhibit its DNA binding activity because a phosphate group will inhibit it.

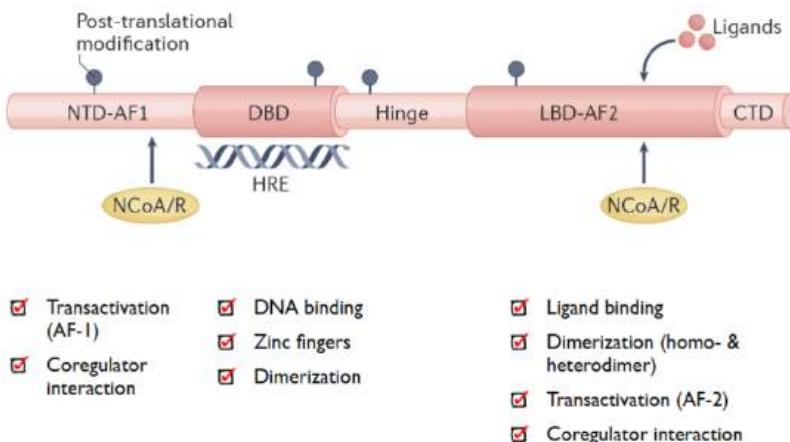


Figure 5.12: Domains of NRs.

Observation 5.15 So now we need to know **how would they bind to the DNA?** Well...they'd have to interact with a specific sequence of DNA, like all transcription factors do. The problem with the NRs, all of them bind basically the same sequence in DNA. So **how do the 48 receptors know which genes to activate or repress?** Well...first it's not truly because of the sequence, **but the way these sequences are arranged.** They can bind as dimers which can be homodimer, heterodimer, or even monomer. So, it's not truly the sequence itself; part of it is, but most of it is the way that the elements are arranged, and that will give you the specificity of recognition of the gene in the genome.

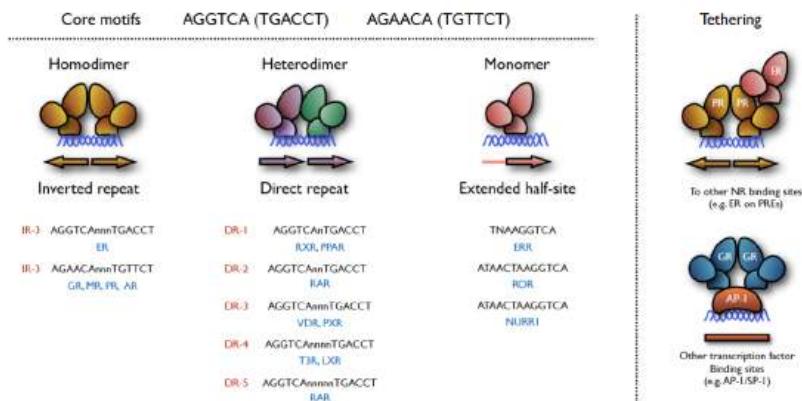


Figure 5.13: NRs and DNA interactions.

ChIP Sequencing

How do you know which gene it binds to in the genome? Well...We use a technique called **ChIP sequencing**.

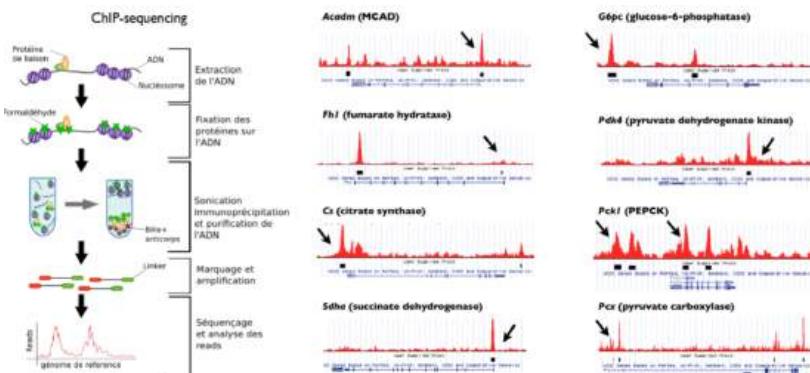
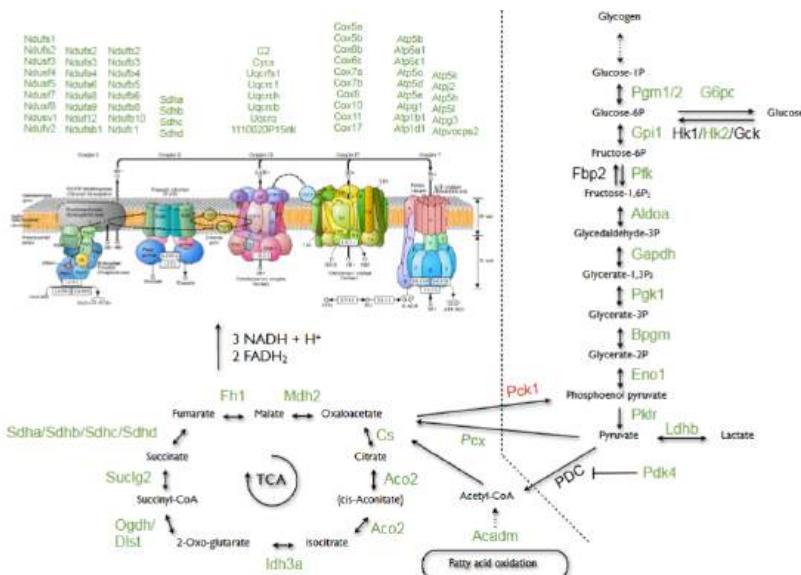


Figure 5.14: ChIP sequencing of *ERRα*.

Methods 5.1 We cross-link the receptor to the DNA. We cut the DNA into small pieces. We use an antibody against the receptor. We precipitate the DNA, remove the protein, and sequence this DNA. Finally, we match those sequences with the genome from the database.

Explanations. If you see you have a lot of the sequence, this means you precipitated a lot of that sequence and sequenced a lot of it. So, a lot of small fragments and you can see here and thus you've determined the sequence that the receptor bind to. □

Example 5.1.5. If we were to do ChIP sequencing with an NR called **ERR α** , we'd see all the ones in green, we all bound by this receptor (see Figure below). So you can see all the promoters are bound to this receptor. So that means this guy regulates glycolysis, the TCA cycle, and phosphorylation.



the energy metabolism program that will lead to more ATP, and that responds to all kinds of signals from the outside or from the inside.

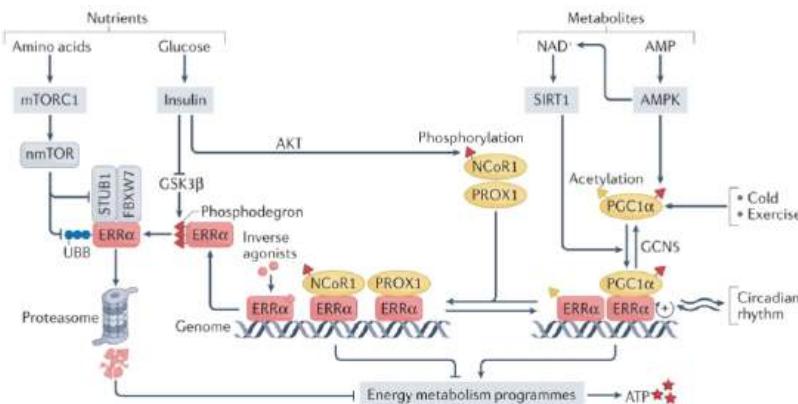


Figure 5.16: Integration of multiple metabolic signals and control.

End of Lecture

5.2 Transcriptional Control of Metabolism II

To start off today's lecture, we will look at a brief example of Ozempic (Not on the exam).

Observation 5.16 Ozempic is a drug that's been all over the news. It's a medication based on a hormone called **glucagon-like peptide (GLP-1)**. What it does is help reduce your appetite, so if you take it, you don't feel like eating, which leads to weight loss. This works through the brain, but we still don't fully understand how it happens or the exact pathways involved. Normally, this hormone is released by your intestines and stomach as a way to tell you that you've eaten enough.

Originally, Ozempic was used for diabetes because it also helps with insulin signaling, meaning it helps your body respond better to insulin and lowers blood sugar levels. But over time, doctors noticed an interesting side effect: people lost weight. Many patients reported not only losing weight but also experiencing lower heart attacks, strokes, and less kidney dysfunction—conditions often linked to obesity.

One of the most interesting effects of Ozempic is that it reduces cravings for unhealthy food, alcohol, tobacco, and even sex. People on Ozempic have said they go to the supermarket and end up buying almost nothing because they just aren't hungry. It's the opposite of when you go to the store on an empty stomach and fill your cart with things you don't need.

This reduction in cravings is so powerful that some believe it could help tackle some of the biggest health problems, like obesity, heart disease, and even cancer related to being overweight. By reducing cravings for bad foods and unhealthy habits, it could also help with issues like smoking and alcohol consumption. And as a bonus, if people eat less and have fewer cravings, it could even help reduce global warming by lowering food production and deforestation, as less meat would be needed. So, in a way, a single drug like Ozempic could help improve public health, reduce diseases, and even have a positive impact on the planet.

5.2.1 Genetic Evidence of Transcription Factors in Metabolic Control

Let's look at how important transcription factors (TFs), specifically metabolic TFs, can be in the development of diseases.

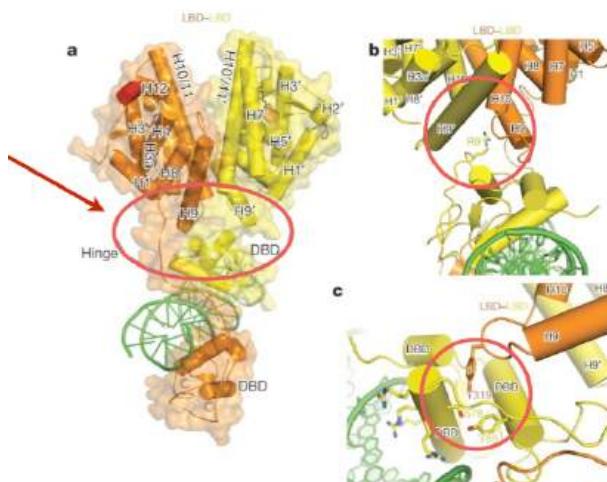


Figure 5.17: NFH4 structure.

Example 5.2.1. MODY (Maturity Onset Diabetes of the Young) is a genetic form of diabetes that appears in teens. People with MODY don't have diabetes at birth, but they develop it as they grow older, typically starting in their teenage years.

This condition is caused by a mutation in a nuclear receptor called **HNF4**. This receptor responds to lipids, and when researchers studied its structure, they found that it works by binding to lipids. HNF4, like other NRs, has a ligand-binding domain (LBD), a DNA-binding domain (DBD), and end regions that connect these domains. Recent studies show they do, and this interaction is crucial for the receptor's function. In fact, this is called the central zone is where both activating and repressive signals are transmitted.

It can be activated and inhibited through PTMs:

- **Activation:** When an arginine residue in the receptor are methylated (by **PRMT1**), the receptor binds very tightly, allowing it to interact effectively with DNA. This tight binding enables the receptor to properly regulate gene expression.
- **Inhibition:** When the S78 residue of the HNF4 is phosphorylated by PKC, it will create a "clash" with another aa residue (Y319) and thereby disconnect the DBD and LBD.

Remark 5.4. A complete loss of HNF4 would be fatal because it's necessary for liver development and function.

Observation 5.17 In patients with MODY, the mutations don't typically affect the DBD. Instead, many mutations occur in the central region and the LBD. These mutations impair the receptor's ability to respond to fatty acids properly. As a result, people with MODY can still live, but their liver doesn't function properly in the long term. Here are some of the mutations:

1. **Mutation of central zone:** cause MODYI and **hyperinsulinaemic hypoglycemia**.
2. **Mutation of R127W/D126Y:** Prevent the 2 DBD bind effective to the DNA.
3. **Mutations of I314F and R324H:** Reduce DNA binding affinity.

All of these mutations will lead to the development of MODY.

Concept 5.1 Germ-line mutations in TFs responsible for inherited metabolic diseases show the biological significance of transcription in the control of hepatic metabolism.

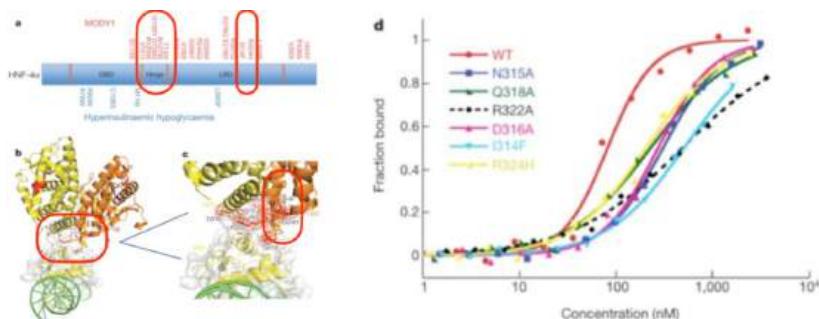


Figure 5.18: MODY, Mutations and its response to FA compared to WT.

Observation 5.18 Interestingly, these mutations reduce the receptor's ability to respond to FA by about tenfold (as compared to wild-type). This reduction is why people with MODY can survive. If we can develop new ligands or modify the signaling pathways to compensate for the mutations, it might be possible to restore the receptor's function, potentially treating the disease.

5.2.2 Bile Acid and Nuclear Receptors

Observation 5.19 **Bile acids (BAs)** are steroid molecules produced by the liver and secreted into the intestine, where they form micelles to absorb dietary lipids and fat-soluble vitamins like vitamin A and D. Once absorbed, these components are transported back into the liver via the portal vein for further metabolism.

BAs are derived from cholesterol, but unlike most steroids, they cannot diffuse freely due to the hydrophilic groups attached to them. As a result, specific transporters are required to move them across cell membranes. Approximately 95% of bile acids are recirculated, with only 5% being excreted daily. This recirculation (enterohepatic circulation) is crucial for ab-

sorbing dietary lipids and vitamins. A deficiency in bile acid transporters can lead to vitamin deficiencies.

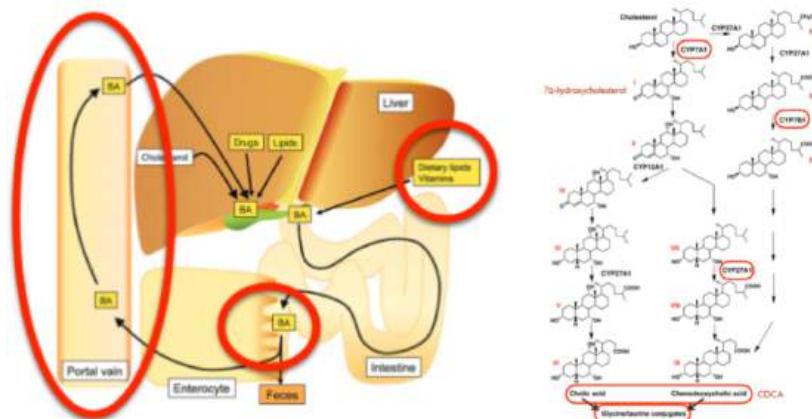


Figure 5.19: BA circulation and synthesis of BAs.

Observation 5.20 (Synthesis Pathway of BAs). The synthesis of BAs begins with cholesterol, progressing through various steps that involve cytochrome P450 enzymes, particularly **CYP7A1**, which is the rate-limiting enzyme in BA production and initiate the neutral pathway, which then metabolize cholesterol to 7α -hydroxycholesterol. This leads to the formation of bile acids like **cholic acid** and **chenodeoxycholic acid (CDCA)**, which can be conjugated to aa. glycine and taurine.

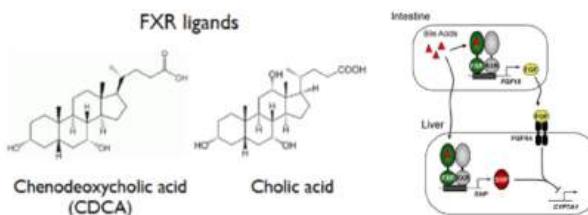


Figure 5.20: FXR ligand and its action.

CDCA and cholic acid can act as a ligand for FXR that regulate the BA pathway. Furthermore, this receptor acts as a sensor for BA levels in the body. When [BA] are high, FXR represses further synthesis. In the absence

of FXR, such as in KO mice, [BA] rise significantly, leading to increased cholesterol, triglycerides, and eventually fatty liver disease.

The feedback mechanism involves FXR activation in the intestine and liver. In the intestine, FXR binds to its partner receptor, RXR, to stimulate the production of **FGF15**. In the liver, where FXR induce the expression of SHP. SHP and together with the signal from FGF15, CYP7A1 is inhibited.

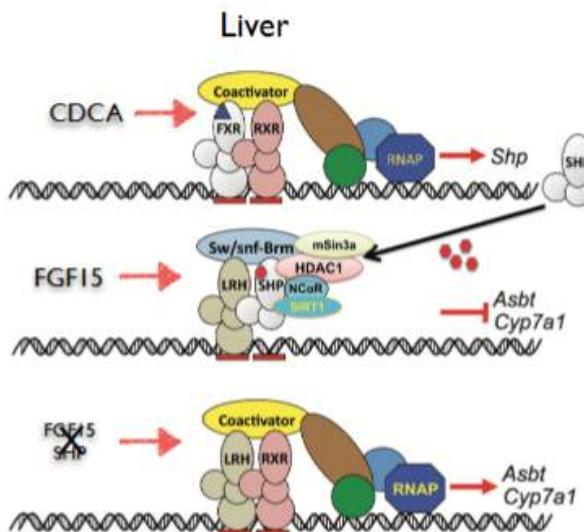


Figure 5.21: Control of CYP7A1 and ASBT.

Mechanism of Action (Control of CYP7A1): Let's begin with when the [BA] level is high.

- 1a. In the intestine, FXR forms a heterodimer with RXR (FXR/RXR) which induce the expression of SHP. SHP will bind to another orphan NR called **LRH**.
- 1b. In the liver, in response to BAs, FXR will also form the heterodimer FXR/RXR thereby inducing the expression of FGF15.
2. FGF15 will travel to the intestine and bind to its receptor thus sending a signal.
3. The signal will recruit other corepressor to form a complex with LRH/SHP. Together, they will inhibit CYP7A1 and thus inhibit BA.

When [BA] is low, FXR won't be active and SHP and FGF15 won't be expressed and thus CYP7A1 won't be inhibited.

Remark 5.5. *In fact, this is the exact same mechanism but for the BA transporter **ASBT** (encoded by Asbt).*

Observation 5.21 In the presence of CDCA, the transcription of Asbt gene will be inhibited by LHP/SHP complex and FGF15 signal. On the other hand, in the absence of CDCA, there's no inhibition and thus the transcription of ASBT can happen.

All of this is just to highlight the fact that you have 2 signals involved in regulating the cascade of TFs resulting in transcriptional control of BAs synthesis and transport.

5.2.3 Fructose Metabolism and Obesity Epidemic

Definition 5.13. **Fructose** is a naturally occurring lipogenic sugar that found in many fruit. **High-fructose corn syrup (HFCS)** is an artificial sweetener from corn starch with high concentration of fructose.

Observation 5.22 HFCS is found in everything, every junk food (especially junk food) because that creates some kind of addictive effect that you have, and that works probably on the brain. What worst is that it causes metabolic syndrome. **Metabolic syndrome** leads to obesity, diabetes, liver disease, arteriosclerosis, calcium metabolism issues, kidney disease, and, like I said, cancer.

Observation 5.23 HFCS is directly associated with the epidemic of obesity, especially in the US. Now, over 60% of people in the US are obese. That's directly linked to the consumption of HFCS.

Fructose is a very harmful micronutrient and contributes to thousands of deaths in America—and probably Canada—every year due to the diseases

Biochemistry of Fructose Metabolism

Observation 5.24 Fructose enters its metabolic pathway, and especially in the first step, as fructose-1-phosphate (F1P) then goes directly to lipogenesis. This is one of the reasons studies show that as soon as you add fructose

to the diet, there's a huge increase in the flux of metabolites going directly to lipids.

Fructose influences the capacity for making fatty acids. When F1P is given chronically, expression and activities of enzymes involved in lipid synthesis, like pyruvate dehydrogenase (PDH) and FA synthase (FAS), will be up-regulated massively.

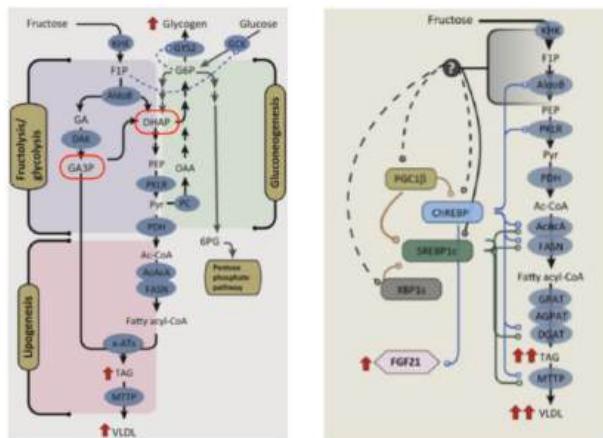


Figure 5.22: Fructose as metabolites .

Not only that, TFs like SREBP, ChREBP, and PGC-1 β are directly stimulated by fructose however we do not know the mechanism in between.

Concept 5.2 What you eat can directly influence transcription factor cascades. This, in turn, regulates critical metabolic pathways and affects your overall health. So be careful what you eat.

5.2.4 Transcriptional Control of Hepatic Glucose Metabolism

Observation 5.25 We will now look at the response to fasting. During fasting, [glucose] decreases, glucagon will be secreted by your pancreas, and even glucocorticoids.

First, fasting will induce glycogenolysis, where glycogen is broken down into glucose. That's usually enough to sustain you for a while. Then if you fast for longer, gluconeogenesis will kick in to make new glucose. Lastly, if you fast for a couple of days, then ketogenesis will kick in. Gluconeogenesis relies on aa derived from the muscle \Rightarrow you'd lose muscle during very long fasting period. Then they can also use glycerol from fat once you begin to degrade your fat.

All 3 pathways are regulated at the transcriptional level by the concerted action of TFs. They all respond to specific signals and mechanisms.

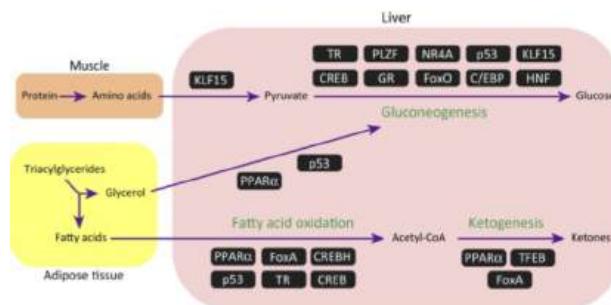


Figure 5.23: Glucose metabolism during fasting.

In the liver, gluconeogenesis relies on TFs such as: thyroid hormone receptor, CREB, FOXO, and HNF4 α . The same thing applies to β -oxidation. We have the PPARs, FOXO, CREB, and others, like thyroid hormone receptor. So all of these again are involved in making this happen. Nevertheless, it's CRB and glucose receptor that are the most important response that will receive the signal from glucagon and glucocorticoids.

Observation 5.26 When you're fed, your glucose levels are high, insulin goes high. Then, through factors like, SREBP (sterol sensor), LXR α (cholesterol sensor), and ChREBP (glucose sensor), you induce lipogenesis, FA synthesis, and BA metabolism. All of these make the response to insulin possible.

Meanwhile, During fasting, glucagon will be secreted by the pancreas and glucocorticoids will be secreted by the adrenal glands. Glucagon and glucocorticoids will activate CREB and glucose receptor respectively, and this act as the initiation of TF cascade. In the end, you'd get gluconeogenesis and etc.

Concept 5.3 A simple signal composed of two hormones, glucagon and

glucocorticoids, initiates a molecular cascade of events necessary for the regulation of a series of interconnected metabolic pathways: gluconeogenesis, β -oxidation, and ketogenesis.

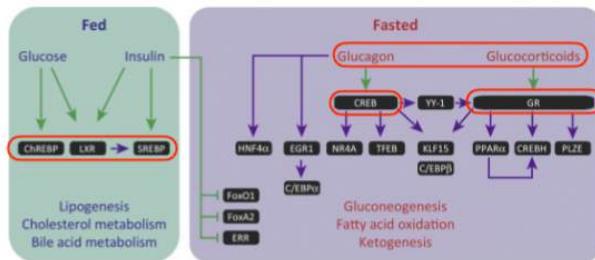


Figure 5.24: Fed vs unfed state and transcription

5.2.5 Transcriptional Control of Circadian Cycle-dependent Metabolism

In the first lecture, we've started by saying that transcription is important for long-term metabolic changes. One of those changes is what happens during the day—from morning to night—or even during the seasons.

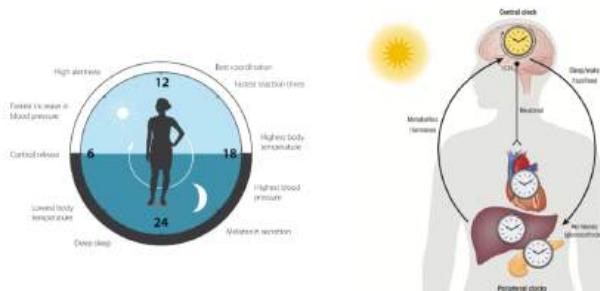


Figure 5.25: Central clock and circadian metabolism.

Example 5.2.2. Right now (approaching winter), we have less and less light. We respond to that through our brain, but it also naturally influences our metabolism, which changes a lot. In fact, when the light starts decreasing in July or August, bears begin eating more and more to prepare for hibernation. Humans are a bit less subject to this, but we're still affected. People

experience depression due to lack of light.

Interestingly, we've found that people's weight tends to peak in October and November, which aligns with a tendency to eat more unconsciously, much like bears. We might not be as extreme, but there's still this subtle pattern of preparing for winter through increased eating.

Observation 5.27 Before waking up—around 5 to 6 in the morning—our body experiences a surge of glucocorticoids. This prepares us for the day by providing energy in the form of glucose. At the same time, blood pressure rises, and we become alert. Throughout the day, these processes build up, and you reach peak alertness. As evening approaches, **melatonin** secretion begins, leading to deep sleep, and the cycle starts again. During this time, metabolism varies significantly and is centrally controlled by the central clock in the brain. This clock is sensitive to light and coordinates processes across various organs, including the heart, liver, and pancreas.

Remark 5.6. *Many metabolites and hormones can both disrupt and regulate this central clock, which in turn influences when certain hormones, like glucocorticoids, are secreted.*

Observation 5.28 Disruptions to this clock can have various causes, including aging, shift work, jet lag, obesity, and serious illnesses like cancer. Each of these disruptors affects how well the clock regulates functions like mood, behavior, and metabolism. For instance, shift workers—like nurses or flight attendants—are often out of sync with natural cycles, which can lead to issues like obesity, chronic stress, or even tumors.

Methods 5.2 (Resetting the Central Clock). One way to reset the central clock and avoid the metabolic consequences by disrupting it is through **time-restricted feeding**, which focuses on when you eat rather than what you eat. In particular, **eating twice a day—around 10 AM and 6 PM—and avoiding food after 6 PM** is suggested

Example 5.2.3. While this can be challenging, especially with family or social obligations, it's an effective method to synchronize the metabolic clock. Studies show benefits like reduced fat mass and improved glucose homeostasis when people adopt this pattern.

Example 5.2.4. There are also experiments with mice that highlight the importance of timing. Mice, which naturally eat at night, were fed during the day in a controlled study. This reversed their behavior, leading to

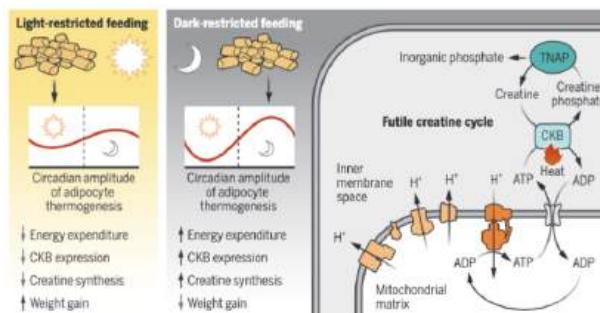


Figure 5.26: Time restricted feeding.

weight gain and poor health. When they were allowed to return to their natural eating cycle, they lost weight and improved their energy expenditure.

Observation 5.29 The central clock in the brain is known as the **suprachiasmatic nucleus (SCN)**, and it synchronizes peripheral clocks in other organs, like the liver, through **rhythmic signals** involving hormones, metabolites, feeding, and body temperature. The SCN itself is synchronized by the light-dark cycle, and these signals are interpreted by the "**immediate early genes**" which convey the information to the peripheral clocks. These output genes (OG) are the **core clock controlled (CCC)** gene of said organ which will then regulate the expression of "**local clock-controlled (LCC)**" OG.

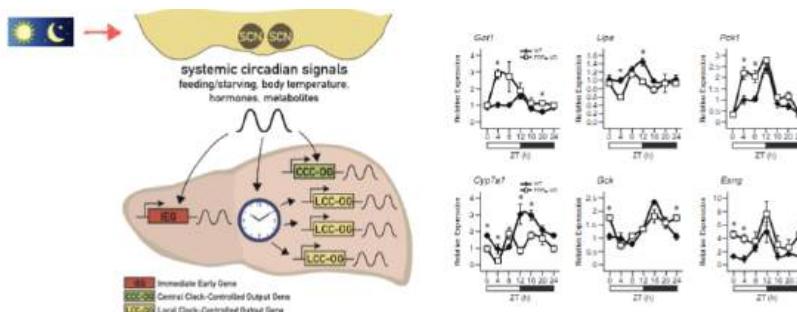


Figure 5.27: Immediate early gene relay information on to CCC-OG then LCC-OG.

Definition 5.14. The **core clock controlled (CCC)** gene is the clock that can self-regulate but also regulate the metabolic output of genes.

Observation 5.30 These genes operate in a feedback loop which consists of a activator and repressor loop. Activator loop consists of proteins like **Bmal1/Clock**. These 2 activators can then activate the repressor loop consists of **Cry/Per** and **Rev-erba α/β** (NRs). These repressors, in turn, inhibit the activator loop. This cycle takes about 8 hours, creating a rhythmic pattern of gene expression throughout the day. All of the CCC gene are TFs and the circadian clocks control physiology and metabolic homeostasis through transcription.

Adaptive Metabolic Clock

Observation 5.31 In addition to the central clock, we also have the **adaptive (metabolic) clock** which can be influenced by hormones and responses because these systems are under the control of various factors, primarily NRs, which can respond to BAs, oxysterols, glucocorticoids, fatty acids, and other ligands. Once activated, these receptors bind to response elements in front of target genes, either activating or repressing them depending on the ligands involved.

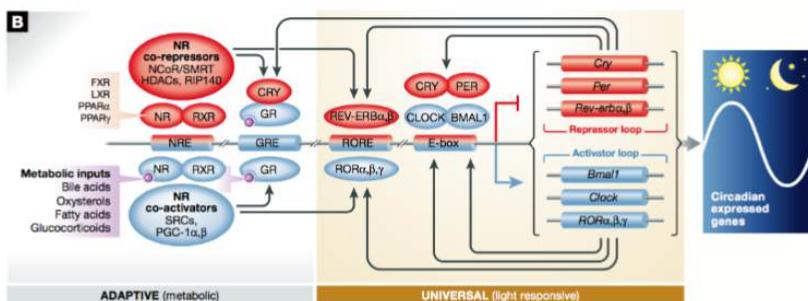


Figure 5.28: Adaptive (metabolic) clock.

Example 5.2.5. Eating a pizza at midnight introduces FA, which could induce or repress certain reactions. This leads to insulin spikes and disrupts your clock due to receiving inputs at an inappropriate time. This disruption underscores why restricted feeding is effective and why eating at irregular times, such as late at night, is detrimental.

Note: Several activators and co-repressors, including PGC-1 α and others, also influence these processes.

Concept 5.4 Nutrient-sensitive and hormone-sensitive transcription factors, such as nuclear receptors, regulate the adaptive clock and circadian metabolism.

Thus, what you eat and when you eat can affect your sugar metabolism and clock system, making timing and food type critical for maintaining proper metabolic functions.

Through evolution, our systems have been designed to respond to light and a relatively simple lifestyle. Modern disruptions, including diverse food types and irregular eating patterns, impact ATP levels, clock factors, and metabolic signaling pathways. These changes disrupt the intricate balance of our internal clock.

5.3 Hormones I

Concept 5.5 (Principle of Hormones Functions). Here are some of the typical functions of hormones:

- Specificity: each hormone will only bind to 1 receptor
- Amplification: Upon activation, there would be an amplification of signal.
- Duration of Effect: short-term or long-term regulation.
- Desensitization/Adaptation:

In today's lecture, we will look at 2 kind of receptors (a specific example of the type) and this include: **receptor enzyme** and **serpentine receptor**.

Remark 5.7. *Serpentine receptors is a shorter name of GPCRs, while receptor enzymes are just receptors that upon activation will activate (an) enzymatic activities (in today's lecture, it'll be receptor tyrosine kinase).*

We've describe a receptor tyrosine kinase, specifically insulin receptors, in previous chapters. Upon binding by insulin, it will recruit GLUT4 to the surface in adipose and muscle tissue (NOT liver!) in addition too activation of lipogenesis and glycogen synthesis (muscle). In the liver, activation

of insulin receptor, will activate glycogen synthesis and lipogenesis. On the contrary, we've also seen a type of GPCR that does the opposite effect and that are: β and α -adrenergic receptors, which will promote lipolysis, glycogenolysis and gluconeogenesis.

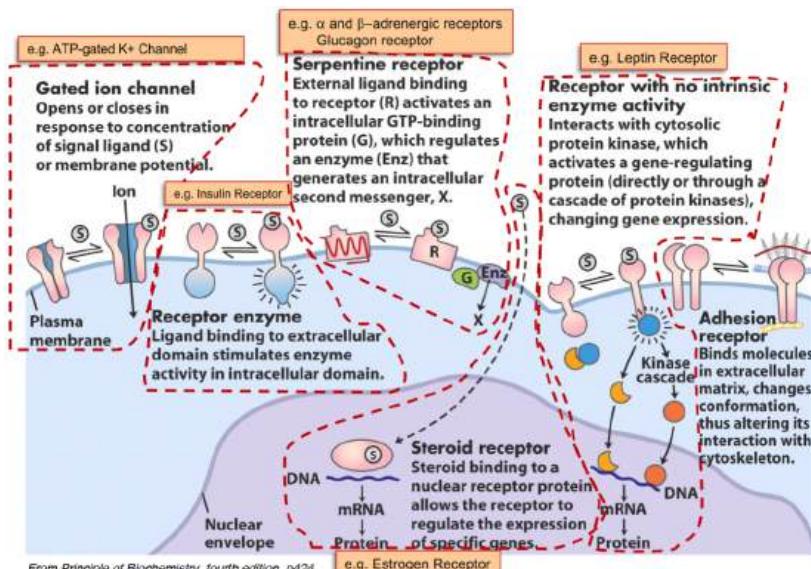


Figure 5.29: Classification of receptors.

5.3.1 Receptor Tyrosine Kinase: Insulin Receptor

Definition 5.15. Receptor tyrosine kinase (RTK) is a type of surface receptor where upon activation its own tyrosine residue will be phosphorylated and activate further pathway.

There's a range of different RTK but they all have the same structure: extracellular ligand-binding domain (LBD) and intracellular tyrosine kinase-domain (TKD). Normally, RTK will exist as its own monomer and upon activation, majority of them will dimerize. In our case, we will focus on the insulin receptor

Observation 5.32 We will briefly run though what insulin receptor does. First, the main signalling of insulin is tell the body to store energy. Upon binding, the receptor will go through a cascade of event that lead to the

activation protein kinases. These protein kinases will deactivate glycogen synthase kinase and thereby activate glycogen synthase and begin glycogenesis.

Observation 5.33 The insulin receptor works as a homodimer linked together by a disulfide bond. Each monomer consists of an α (where the LBD is) and β chain (where TKD is). Once bound to insulin, it will undergo conformational change that will be transmitted to the β chain where the catalytic subunit are. Here, the tyrosine kinase will perform **transphosphorylation** on all of the β chain's tyrosine.

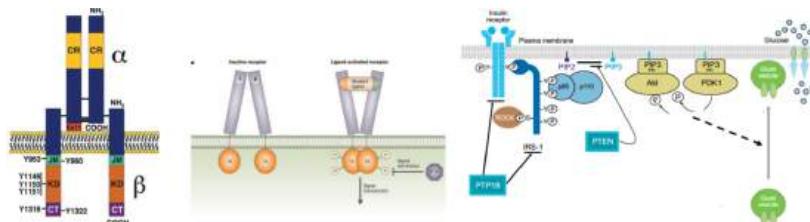


Figure 5.30: Insulin receptor structure and activation.

Next, **insulin receptor substrate (IRS)**, which has **SH2 domain** that recognize phosphorylated tyrosine, will be recruited to bind to it. The IRS will have all of its tyrosine residue phosphorylated. This phosphorylation of IRS will recruit other enzymes that phosphorylate **PIP2** into **PIP3**. PIP3 can then be recognized by **Akt** which will activate downstream event, one of which will recruit GLUT4 to the surface.

Methods 5.3

Take cells and tag its GLUT4 receptor with fluorescent label. Separate the cell into the control group, where we does nothing to it, and a +insulin group where will we put insulin into the environment.

Explanations. We can see that in the control group, the GLUT4 receptors localize in vesicle together (big glowing blob in the middle of the cell in the figure) inside the cell. In the +insulin group, the GLUT4 receptors move to the plasma membrane (smaller glowing spots along the cell boundary) in response to insulin stimulation.

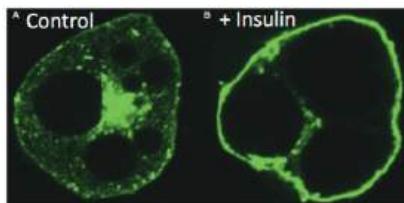


Figure 5.31: GLUT4 transporter localization experiment

+insulin group, the GLUT4 will be spread out at the membrane of the cell indicating that the transporter has been recruited to the surface. □

Observation 5.34 We've also saw that insulin can also turn the inactive form of ACC into a slightly less inactive form which will be fully activated when there's citrate promoting polymerization. The activated ACC will be used to convert acetyl-CoA to malonyl-CoA that will be used for FA synthesis.

Insulin Injection vs Inhalation

Observation 5.35 Typically, with diabetic patients, they have to take insulin before a meal or even when blood glucose is high. The insulin is delivered through needle however many people think **why not through inhalation?** Well...they did develop insulin inhalator that does indeed work however there are some complications:

1. Preparation before inhalation is complicated.
2. The inhaler is more bulky than the injection pen.
3. For smoker, the delivery of insulin can be inconsistent.
4. For smoker and former smoker, there's a higher incidence of **lung cancer**.

Remark 5.8. *It must be interpreted carefully that insulin does not cause cancer but because these patients already have damaged lungs (and respiratory DNA) and insulin somehow has an effect to exacerbate this.*

Observation 5.36

From the previous remark, we can further find out that insulin can also activate the recruitment of key regulatory pathways such as MEC and MAP kinases that lead to mitosis. i.e. When insulin bind to its receptor, not only metabolic signalling but also mitogenic signalling is activated.

Stopping Insulin Receptors

The activated insulin receptor will need to be deactivated to be reused at another but also that we do not need a constant activation of insulin receptor for no reason at all.

Observation 5.37

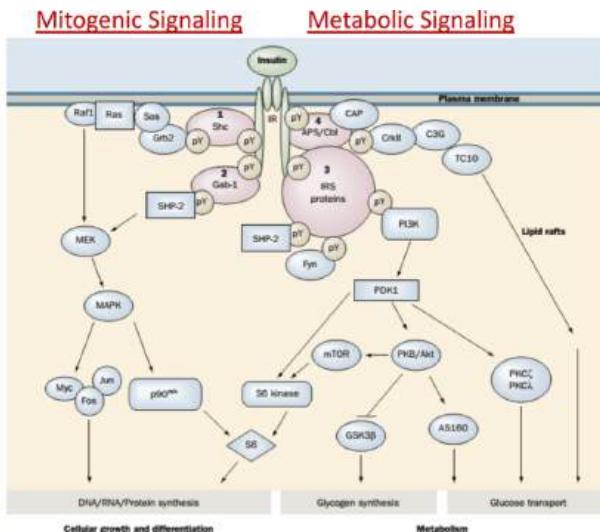


Figure 5.32: Insulin signalling.

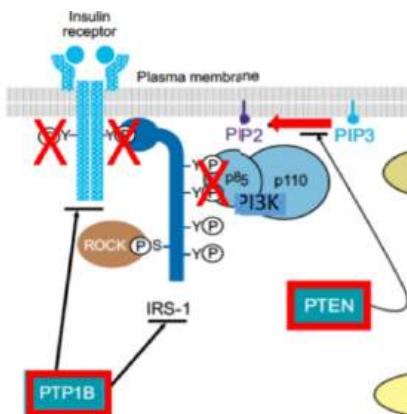


Figure 5.33: PTP1B and PTEN action.

An enzyme called **protein tyrosine phosphatase 1B (PTP1B)** is used to dephosphorylate the tyrosine phosphate along the β chain and the IRS. This dephosphorylation will shut down insulin receptor and thereby stoping the signalling cascade. There's also **phosphatase and tensin homolog (PTEN)** that revert PIP3 to PIP2. This also stop the signalling cascade. These 2 enzymes together will help resetting insulin receptors for the next binding event.

Definition 5.16. Insulin sensitivity refers to how responsive your cells are to insulin. If a person has low sensitivity, the person will not response as much as the normal ones to a certain dose of insulin. On the other hand, if a person has high insulin sensitivity, the person will response more strongly than normal at a given

dose.

Remark 5.9.

*When a condition where one doesn't respond as they should to insulin, is also called **insulin resistance**.*

What would happen if we inhibit PTP1B? Well...the body would increase sensitivity of insulin. This is because PTP1B is required to reset the insulin receptor, if we inhibit its action, insulin receptor will stay activated for longer.

Could PTEN inhibitor increase insulin sensitivity? Why? Well...it would increase sensitivity since you did not reset the system like before.

Observation 5.38 Now, that was a temporary way to shut down the insulin receptor or resetting it to the basal state (dephosphorylate it), but there's also a much more permanent way to shut down the system. This is done through degradation of the insulin receptor after being endocytosed into the cell.

5.3.2 GPCRs: Adrenergic Receptors

Glucagon will activate similar pathway to that of β -adrenergic receptor however they're not the same and one ligand will not bind to the other receptor.

Remark 5.10. *Glucagon will only activate glucagon receptor while epinephrine will only activate to adrenergic receptor.*

Definition 5.17. **Glucagon**⁶ is a 29-aa peptide hormone that's released by the pancreatic α -cell as signal to generate energy when blood glucose is

⁶It basically the main molecule to have counter effect of insulin. In fact, insulin/glucagon ratio helps in controlling hepatic glucose by controlling the rate of gluconeogenesis vs glycogenolysis.

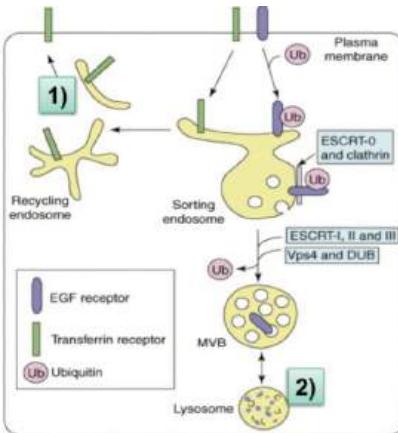


Figure 5.34: Insulin receptor temporary (1) and permanent shut down (2).

low. Meanwhile, **epinephrine (EPI)** is a hormone released by the adrenal cortex as a signal to mobilize energy.

G_{αs}-coupled GPCRs: Glucagon Receptor

Observation 5.39 For the glucagon receptor, upon binding by glucagon, it will be activated. The GDP bound G_{αs} subunit will be phosphorylated GTP bound G_{αs} which dissociate from the receptor. This GTP-G_{αs} will come and activate adenylyl cyclase to synthesize cAMP from ATP. This cAMP can activate various kinase that will ultimately lead to a cellular response (linked with glucagon signalling).

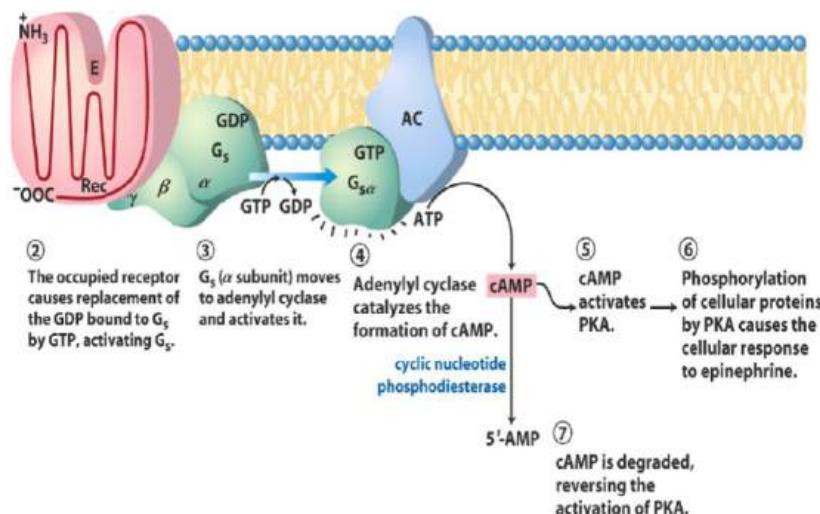


Figure 5.35: G_{αs} coupled receptor structure and activation.

Remark 5.11. cAMP can be broken into 5'-AMP using **cyclic nucleotide phosphodiesterase**.

G_{αs}-coupled GPCRs: β-Adrenergic Receptor

Observation 5.40 The β₃-adrenergic receptor will share the same kind of activation pathway as that of glucagon receptor however β₃-adrenergic receptor is activated by EPI instead. They're mostly found in **brown adipoc-**

ytes⁷ Upon activation of this receptor, hormone sensitive lipase that is necessary for lipolysis. Not only that, this receptor is important for the pathway that lead to **thermogenesis** (generate heat for warmth).

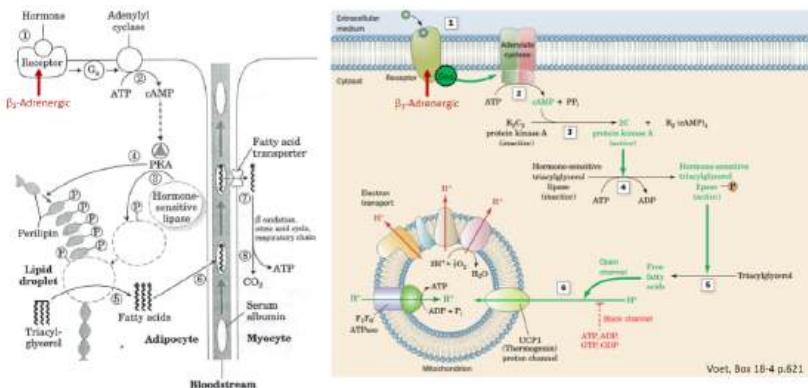


Figure 5.36: Pathways activated by β_3 -adrenergic receptor.

G_{αq}-coupled GPCRs: α-Adrenergic Receptor

We've described the action of α -adrenergic receptor before, we'll just briefly go through it here.

Observation 5.41 When the GPCR is activated, GDP-G_{αq} subunit will become GTP-G_{αq} and dissociate. The GTP-G_{αq} will come and activate phospholipase C (PLC), of which will turn membrane-bound PIP₂ into DAG (stay membrane-bound) and IP₃ (free-flowing). The IP₃ can act as a signal to open a Ca^{2+} channel releasing Ca^{2+} into the cytosol. The Ca^{2+} together with DAG will activate protein kinase C (PKC) which will phosphorylate different protein that lead to a cellular response.

Stopping

We will now look at the inhibition of these receptor.

Observation 5.42 First, we can turn off the β -adrenergic receptor through phosphorylation using **β -adrenergic receptor kinase (βARK)**⁸. The phos-

⁷It's a type of adipose tissue used to keep us warm during cold and also stores energy and help burn calories.

⁸Activated by PKA when the receptor is overstimulated

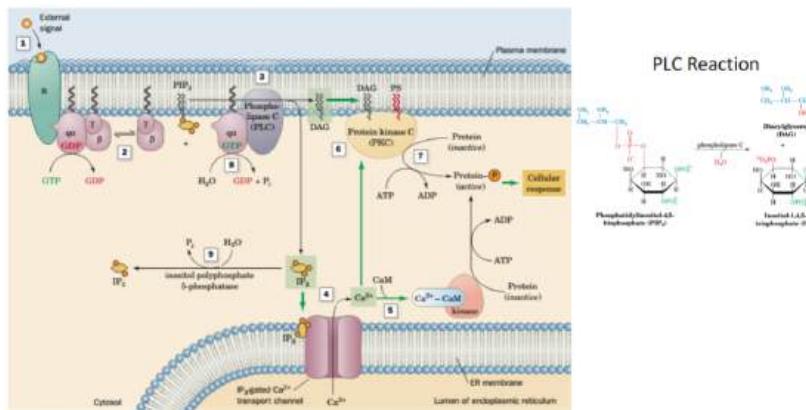


Figure 5.37: α -adrenergic receptor structure and activation .

phorylation will recruit **β arrestin** to further downregulate the system. In particular, upon binding to β arrestin, the receptor will be internalized into vesicles of these receptors. This process is *desensitization* as the receptor is no longer on the surface and no matter how much EPI you give, there will be no response.

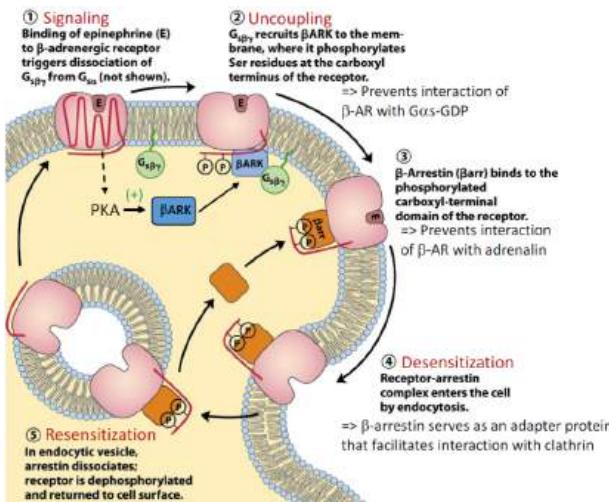


Figure 5.38: Inhibition of β -adrenergic receptor.

Definition 5.18. **Desensitization** is the mechanism by which a receptor can no longer respond to its ligand after persistent exposure.

Remark 5.12. *This is also why many drug fails as receptor can desensitize to the drug molecules which cause the patient to increase the dosage of said drug overtime.*

5.3.3 Summary

Hormone	Receptor	Biochemical Actions	Enzyme Target	Physiological Actions
Insulin	Insulin-Receptor (Tyrosine Kinase)	<ul style="list-style-type: none"> ↑ Glucose uptake (muscle, adipose tissue) ↑ Glycolysis (liver, muscle) ↑ Acetyl-CoA production (liver, muscle) ↑ Glycogen synthesis (liver, muscle) ↑ Triglyceride synthesis (liver) ↓ Glucogenesis (liver) ↓ Lipolysis ↓ Protein degradation ↑ Protein, DNA, RNA synthesis 	<ul style="list-style-type: none"> GLUT4 PFK-1 (via PFK-2/FBPAse-2) Pyruvate dehydrogenase complex Glycogen synthase Acetyl-CoA carboxylase FBPAse-1 (via PFK-2/FBPAse-2) 	<ul style="list-style-type: none"> Signals fed state: ↓ Blood glucose level ↑ Fuel storage ↑ Cell growth and differentiation
Glucagon	Glucagon-Receptor (GPCR)	<ul style="list-style-type: none"> ↑ cAMP level (liver, adipose tissue) ↑ Glycogenolysis (liver) ↓ Glycogen synthesis (liver) ↑ Triglyceride hydrolysis and mobilization (adipose tissue) ↑ Gluconeogenesis (liver) ↓ Glycolysis (liver) ↑ Ketogenesis (liver) 	<ul style="list-style-type: none"> Glycogen phosphorylase Glycogen synthase Hormone-sensitive lipase, adipose triglyceride lipase FBPAse-1 (via PFK-2/FBPAse-2), pyruvate kinase, PEPCk PFK-1 (via PFK-2/FBPAse-2) Acetyl-CoA carboxylase 	<ul style="list-style-type: none"> Signals fasting state: ↑ Glucose release from liver ↑ Blood glucose level ↑ Ketone bodies as alternative fuel for brain
Epinephrine	Adrenergic-Receptors (GPCR)	<ul style="list-style-type: none"> ↑ cAMP level (muscle) ↑ Triglyceride mobilization (adipose tissue) ↑ Glycogenolysis (liver, muscle) ↓ Glycogen synthesis (liver, muscle) ↑ Glycolysis (muscle) 	<ul style="list-style-type: none"> Hormone-sensitive lipase, adipose triglyceride lipase Glycogen phosphorylase Glycogen synthase Glycogen phosphorylase, providing increased glucose 	<ul style="list-style-type: none"> Signals stress: ↑ Glucose release from liver ↑ Blood glucose level

Figure 5.39: Summary of insulin, glucagon and EPI, along with its receptor.

5.4 Hormones II

5.4.1 Stopping of GPCR

Professor just went through exactly what we've said from the previous lecture without adding anything new... (Went through the pathway of α -adrenergic receptor [see Observation 5.41, p.270] and how to stop GPCR [see Observation 5.42, p.270-272])

5.4.2 Regulation of Food Intake

There are 2 hormones that can regulate food intake of which we will take a look in today's lecture: **ghrelin and leptin**.

Ghrelin

Definition 5.19. **Ghrelin** is a small peptide hormone secreted by empty stomach in order to stimulate appetite.

Observation 5.43 Ghrelin will first be activated through acylation using **ghrelin-O-acyltransferase (GOAT)** into its *octonoylated* form. Once activated, it can then travel through the blood stream into the hypothalamus where it can then bind to **GHS receptor (GHS-R)⁹** located on **NPY/AgRP neuron**. The GHS-R is a type of $G_{\alpha q}$ -coupled receptor (mechanism we've seen before) so it will activate PLC which then lead to the production of DAG and IP₃, all of which will lead to the activation of a hunger response.

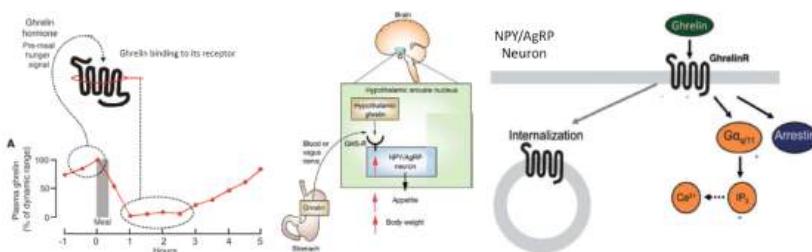


Figure 5.40: Ghrelin plasma concentration overtime and its receptor signalling.

As we've seen for GPCR, it can be overstimulated and undergo desensitization. Indeed, this is what we also see when we first experience hunger and if we continue to let the hunger persist for a few hours, it will become less "severe" compared to initially.

Observation 5.44 If we measure the plasma level of ghrelin, we can see that it will peak when we begin to eat and then drop quickly after meal. Then, it will reach a low value where ghrelin begins to rise again until it peaks again a few hours after the first meal.

Knowing about the hunger response induced by ghrelin, **can we find a way to use it to help with obese patients?** Well...theoretically, yes. We could

⁹Also known as ghrelin receptor.

try to make a drug to block the GHS-R or even a drug that binds to ghrelin itself to prevent it from binding. **Why don't we make it then?** Well...it's theoretically correct however, ghrelin doesn't just induce hunger response, it can have all sort of effect on different organs i.e. blocking it can have serious complications and side effects. This will lead to *pleiotropic effect*.

Definition 5.20. **Pleiotropic effect** is the action of a drug that can have more than 1 primary effect that may or may not be therapeutic. In the context of genes, pleiotropy means a gene that can affect 2 different (may be unrelated) phenotype.

Example 5.4.1. Ghrelin is pleiotropic as it can induce hunger (in the hypothalamus) but also increase cardiac output (in the heart).

Parabiosis Experiment

Before talking about leptin mechanism, we will look at the *parabiosis experiment*.

Definition 5.21. **Parabiosis** is the anatomical joining of 2 individuals, especially artificially in physiological research.

Definition 5.22. **ob/ob mice** denotes mice that cannot produce any leptin (leptin deficient). Meanwhile, **db/db mice** denotes mice that have normal leptin production but have no leptin receptor (leptin-receptor deficient).

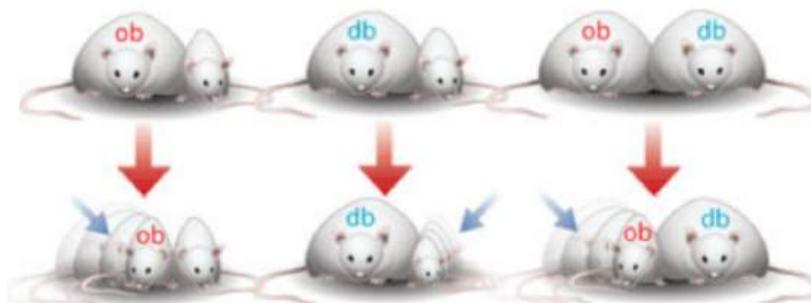


Figure 5.41: Parabiosis experiment.

Methods 5.4 We will create 3 scenarios with the ob/ob, db/db and +/+ (wild-type) mice. This is done by performing parabiosis on db/db with +/+

mice, db/db with ob/ob mice, and ob/ob with +/+ mice. After performing parabiosis, we will then track the size/weight of the parabiosed mice overtime.

Explanations. First, in the parabiosed db/db and +/+ mice, the +/+ mouse will see a massive weight loss and eventually death from starvation. This is because in addition to the leptin it self-produced, it will also be receiving the leptin from the db/db mouse, thereby stopping it from feeling hungry even more.

Next, in the parabiosed ob/ob and +/+ mice, the ob/ob mice will experience the weight loss. This is because it will now receive the leptin from the +/+ mice, and it has functioning leptin receptor to detect these leptin hence preventing hunger.

Finally, in the parabiosed db/db and ob/ob mice, the ob/ob mice will experience the weight loss. This has the similar cause to the previous as db/db produce leptin but cannot detect but ob/ob can. (see Figure 5.41) □

Remark 5.13. *Basically, the only mouse that would survive in all cases is the db/db mouse. However, they couldn't walk since they're severely obese.*

Leptin

Definition 5.23. **Leptin** is a 16kDa protein produced by adipose tissue and by stomach upon food intake to reduce hunger.

Observation 5.45

When hunger is subsided by eating, leptin is secreted toward the gastric lumen into the gastric juice. The leptin then enter circulation in the duodenum and travel up the brain. In the hypothalamus, leptin binds to **leptin receptor (OB-R)**¹⁰ of the NPY/AgRP neurons and this lead to the inhibition of hunger.

Remark 5.14. *The mutation where the body is unable to produce leptin (ob/ob) is very rare in human, compared to OB-R mutation (db/db), which has more incidence.*

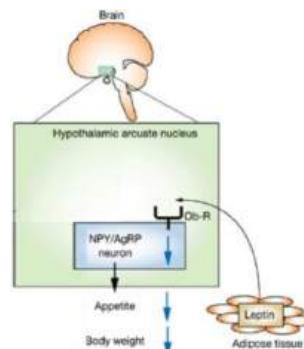


Figure 5.42: Leptin signalling.

¹⁰A receptor with no intrinsic enzyme activity.

Can you treat obese people with leptin? Well...depending on the case. Suppose that the person is obese due to the ob/ob mutation (loss of leptin production). Then, giving them leptin will actually help. However, suppose that the person is obese but has no deficiency. Then, giving them leptin will not help them lose weight. This is because OB-R can be desensitized.

Observation 5.46 The previously talked about ob/ob mice was discovered by Jackson Lab in 1949. These mice lack the functional leptin and was **hyperphagic** which is when the mice have extreme feeling of hunger leading to uncontrollable overeating, therefore they became obese. **Can you treat these obese mice by giving them leptin?** Well...yes.

Observation 5.47

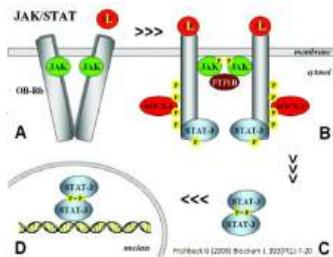


Figure 5.43: OB-R coupled to Jak/STAT pathway

Now, OB-R is indeed a non-enzymatic receptor however, it's coupled to enzymatic pathway that lead evidently to enzymatic effects. The pathway coupled to it is **Jak/STAT pathway** where Jak is **Janus kinase** while STAT is **signal transduction and activation of transcription** protein.

Mechanism of Action (Jak/STAT): We begin with leptin binds to OB-R and change the conformation of the receptor.

1. The change in conformation of OB-R will lead to its dimerization thereby activating the Jak.
2. Jak can autophosphorylate its tyrosine and also the OB-R.
3. This phosphorylation will recruit and activate STAT-3.
4. Upon activation of STAT-3, it will travel to the nucleus and increase the transcription of genes involved in appetite repression.

Remark 5.15. PTP1B, from previous lecture, can also reset Jak through de-phosphorylation.

Observation 5.48 Similar but slightly different from ob/ob mice, the db/db mice lack leptin receptor which also leading it to become hyperphagic and then obese. **Can you treat these obese mice with leptin?** Well...no since there's no leptin receptor for the given leptin to bind to.



The generation of metabolic energy in higher organisms with an emphasis on its regulation at the molecular, cellular and organ level. Chemical concepts and mechanisms of enzymatic catalysis are also emphasized. Included: selected topics in carbohydrate, lipid and nitrogen metabolism; complex lipids and biological membranes; hormonal signal transduction.

