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# 1 Amino Acids in Biosynthesis

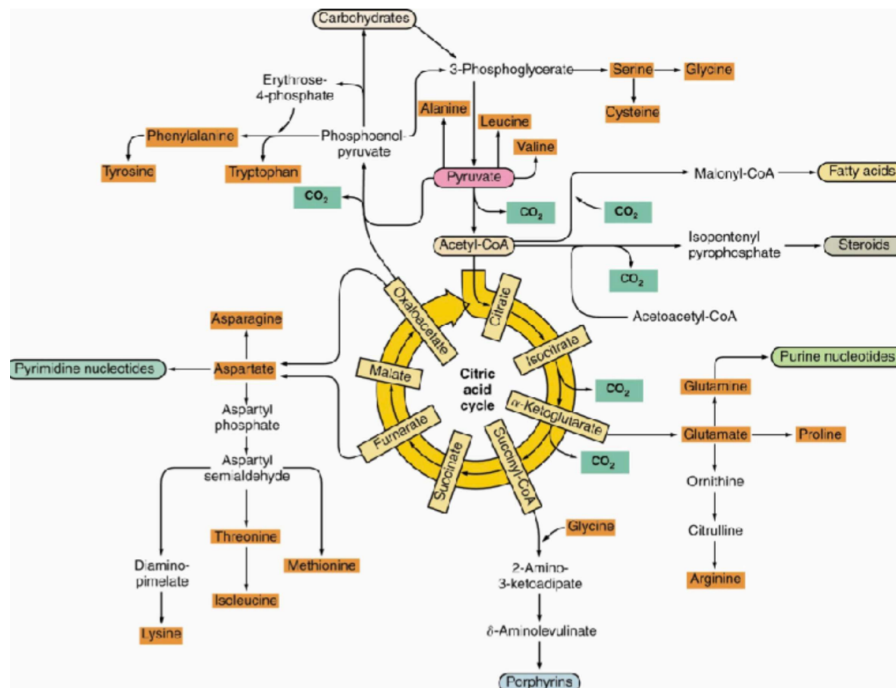


Figure 1: This image shows an overview of many of the products stemming from the citric Acid Cycle. This ranges from amino acids, to nucleic acids, porphyrins, and more.

## 1.1 The actual synthesis of Amino Acids

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

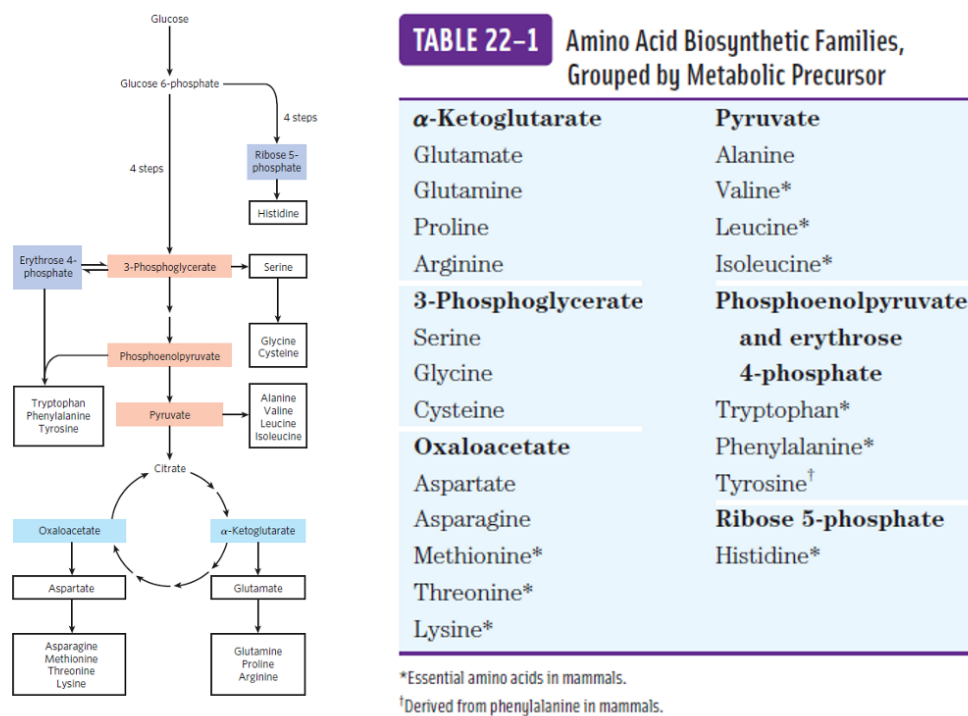


Figure 2: the image on the left shows in which part of glycolysis we derive the amino-acids from. The table on the right shows the precursor for each amino acid.

### 1.1.1 Prelude: Incorporating Nitrogen - Glutamate and Glutamine

Nitrogen is required in amino acids. However, we humans can't get it from the atmosphere. That means we rely bacteria and archaea to fix  $N_2$  from the atmosphere to produce ammonia. That ammonia then enters the cell metabolism and is incorporated into **Glutamate** and **Glutamine**.

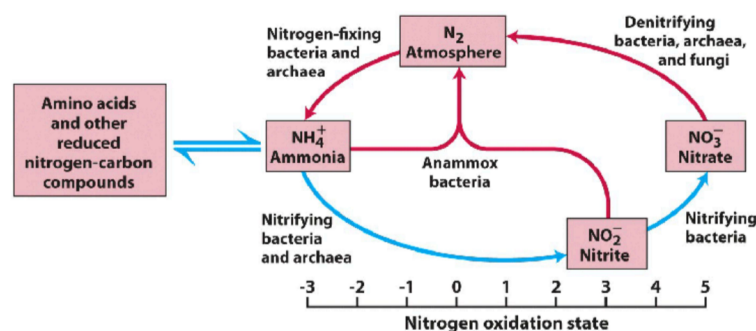
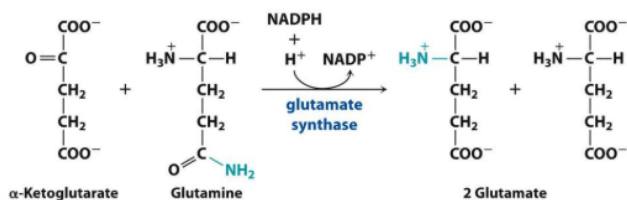


Figure 3: Shows the nitrogen cycle, which happens in bacteria and archaea. Humans can absorb ammonia.

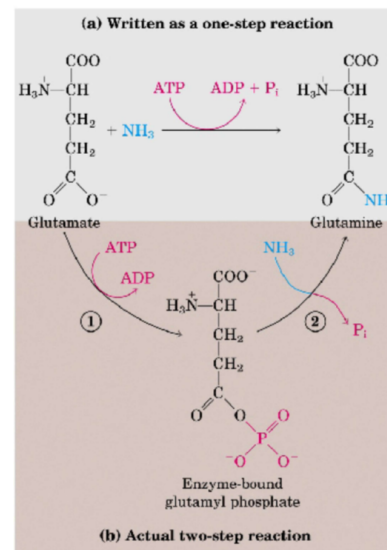
The production of glutamate in bacteria follows the production of glutamine. Here's what that looks like:

- Glutamine production (both in humans and bacteria):  $\text{Glutamate} + \text{NH}_4^+ \rightarrow \text{glutamine}$ , through the enzyme **Glutamine synthase** using a 1 ATP.

- ii) Glutamate production (in bacteria):  $\alpha$ -ketoglutarate + glutamine  $\rightarrow$  2 glutamate, through the enzyme **Glutamate synthase** using both an NADPH and an ATP. This reaction is a **transamination** and how bacteria produce more glutamate.



(a) Synthesis of Glutamate in bacteria using the enzyme glutamate synthase



(b) Synthesis of Glutamine in bacteria or humans using the enzyme glutamine synthase

Figure 4: Shows the synthesis of both glutamine and glutamate

Glutamate and glutamine are then used to transfer  $\text{NH}_3$  to a variety of different product, producing aminated molecules; these are called **transamination** reactions.

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

- This enzyme is constituted by two domains, one that binds glutamine, the other binds the acceptor substrate.
- A Cys residue in the Glutamine-binding domain breaks the acidic bond and forms a glutamyl-enzyme intermediate
- $\text{NH}_3$  travels to the  $\text{NH}_3$ -acceptor domain
- There an activated substrate (usually activated by ATP) is aminated and released.

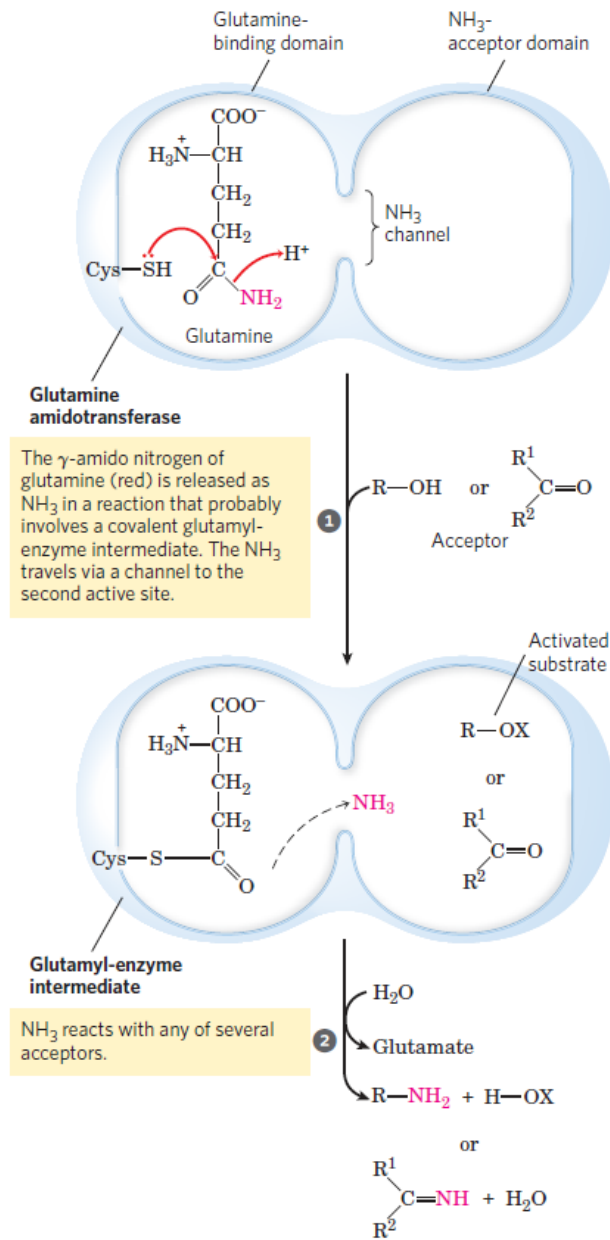


Figure 5: The enzyme glutamine amidotransferase's mechanism.

### 1.1.2 Proline and Arginine

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

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

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

**Arginine** is synthesized in a similar pathway:

- i) Glutamate is first acylated.
- ii) [*Proline – equiv.*] Acetylglutamate is phosphorylated.
- iii) [*Proline – equiv.*] Acetylglutamate-P is then reduced.
- iv) The acylation impedes the cyclisation. Instead through further transamination and de-acylation Ornithine is produced.
- v) Ornithine is then converted to Arginine in the urea cycle (seen in lecture on Lipid biosynthesis).

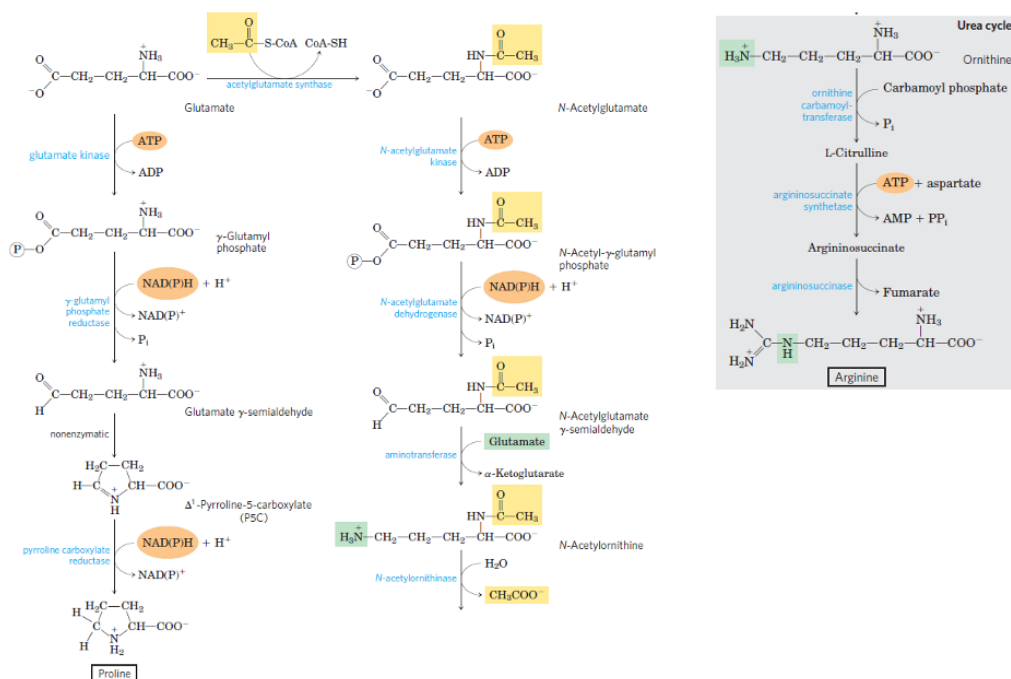


Figure 6: Shows the pathway for the production of both Proline and Arginine, stemming from glutamate.

### 1.1.3 Serine, Glycine, and Cysteine

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

First the synthesis of Serine:

- i) Oxidation of 3-phosphoglycerate
- ii) Transamination of 3-phosphohydroxypyruvate
- iii) Dephosphorylation of 3-phosphoserine

To form **Glycine** from serine, the enzyme **serine hydroxymethyltransferase** removes a one-carbon unit (a hydroxymethyl group,  $-\text{CH}_2\text{OH}$ ) from serine. For this it uses tetrahydrofolate, as well as PLP (activated vitamin B6) as a **cofactor**.

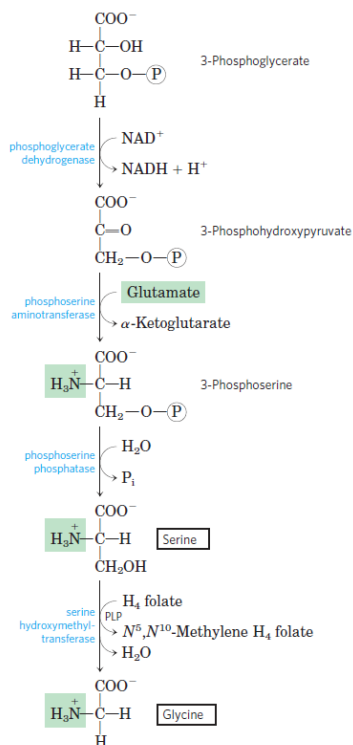


Figure 7: The synthesis of Glycine and Serine. Glycine is a derivate of Serine.

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

- i) Through a series of reactions Methionine becomes homocysteine.
- ii) Homocysteine is condensed to bond with Serine to form cystathionine.
- iii) Cystathionine is hydrolysed with a loss of  $\text{NH}_4^+$  to form cysteine and **alpha-ketobutyrate**.

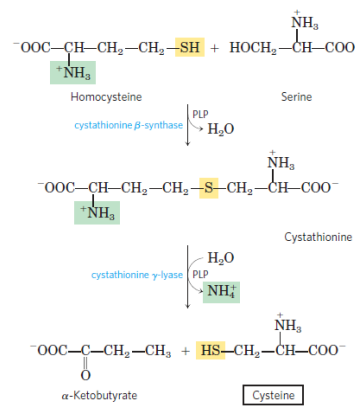


Figure 8: The synthesis of Cysteine from Serine and Methionine.

#### 1.1.4 Aspartate, Asparagine, and Alanine

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

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

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

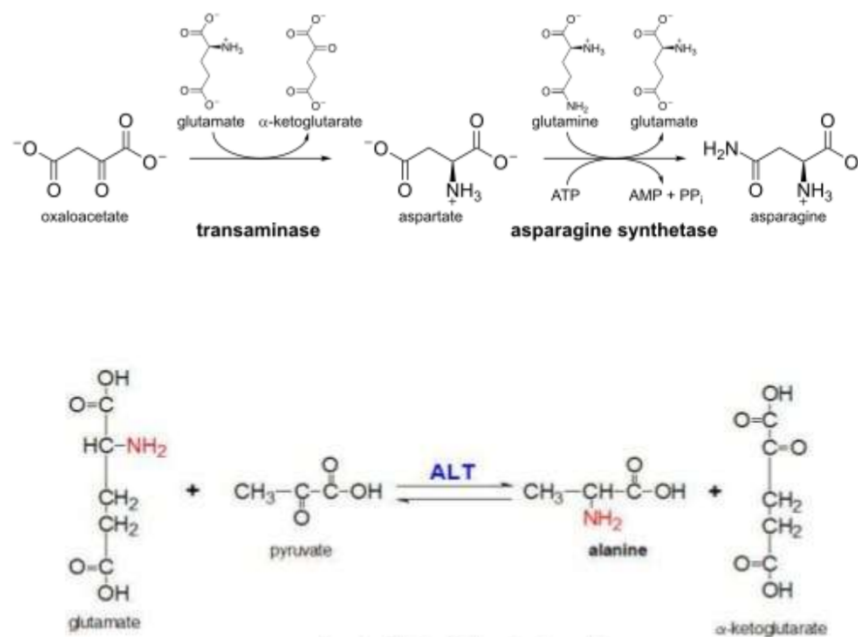
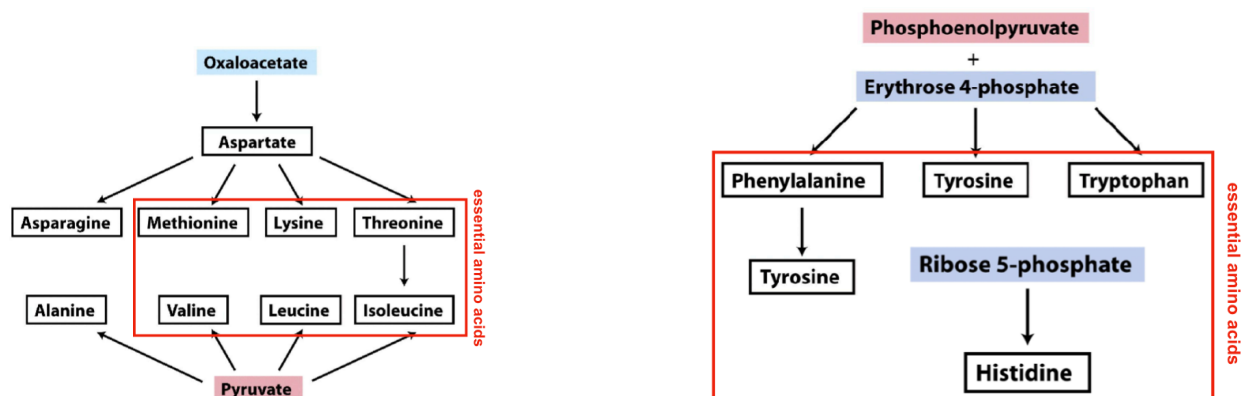


Figure 9: The synthesis of Aspartate (to become Aspartic Acid), Asparagine, and Alanine.

*Remark 1.1.* In the blood ALT and AST concentrations are measured to assess **liver health**. When liver cells are damaged more of these enzymes will be in the blood. Recall the liver is "central bank" of nitrogen management.

### 1.1.5 Essential Amino Acids

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



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

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

Figure 10:



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## 1.2 Amino Acids derived Biomolecules

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

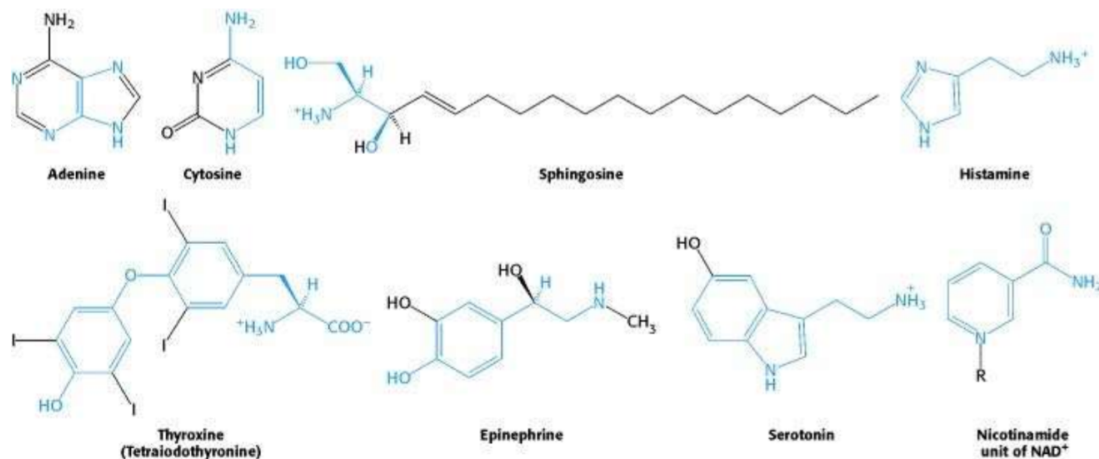


Figure 11: Some examples of amino acids being precursors for other biomolecules.

### 1.2.1 Porphyrins

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

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

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

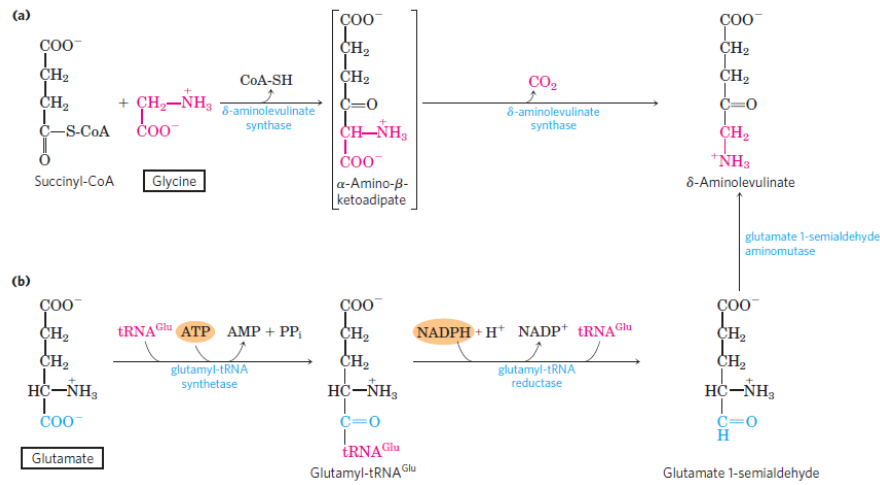


Figure 12: The biosynthesis of delta-aminolevulinic acid. mammals use glycine, while plants use glutamate.

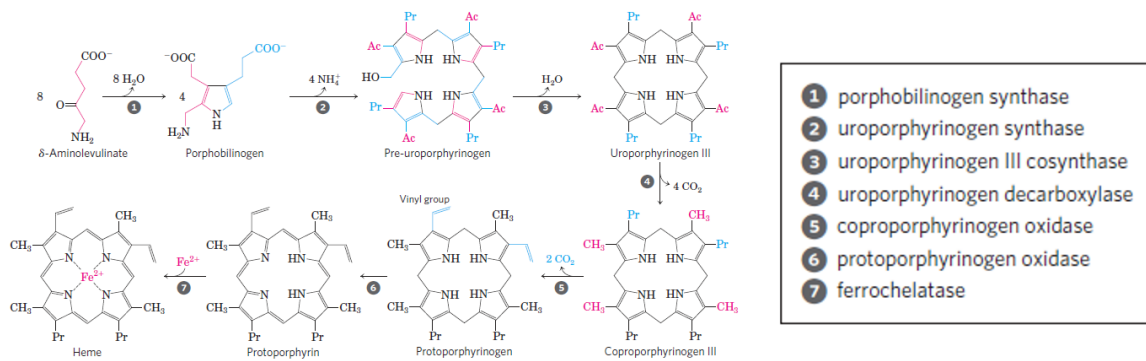


Figure 13: Biosynthesis of heme from delta-aminolevulinic acid.

**Remark 1.2 (Porphyria).** **Porphyria** is a group of diseases caused by the buildup of intermediates in the porphyrin biosynthesis pathway, which negatively affects the skin or nervous system. This accumulation results from genetic defects in the enzymes responsible for the pathway.

Porphyrias that affect the nervous system are known as acute porphyrias, characterized by a rapid onset of symptoms that typically last a short time. In contrast, cutaneous porphyrias primarily involve skin-related symptoms, such as photosensitivity, and usually do not affect the nervous system.

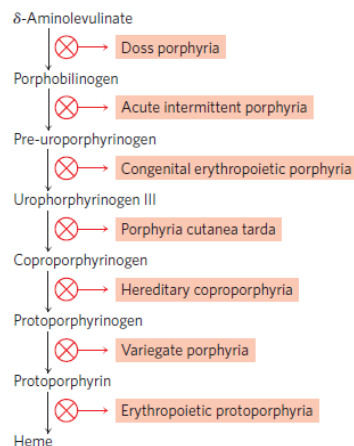


Figure 14: This image shows the types of diseases that stem from a certain enzyme defect. Accordingly the symptoms will vary, as the built up molecule will be different.

*Remark 1.3 (Degradation and excretion of heme).* **Heme** from senescent erythrocytes are the origin for degraded hemes. It can also be degraded coming from other cell types. Senescent erythrocytes are degraded in the spleen.

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

**Note** if there are too much calcium salts salt and bilirubin, **pigment stones** (type of gallstones) might get produced.

### 1.2.2 Phosphocreatine

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

**Creatine** (the gym-bro stuff) is the direct precursor of Phosphocreatine is produced from glycine and arginine with participation of Methionine as donor of a methyl group.

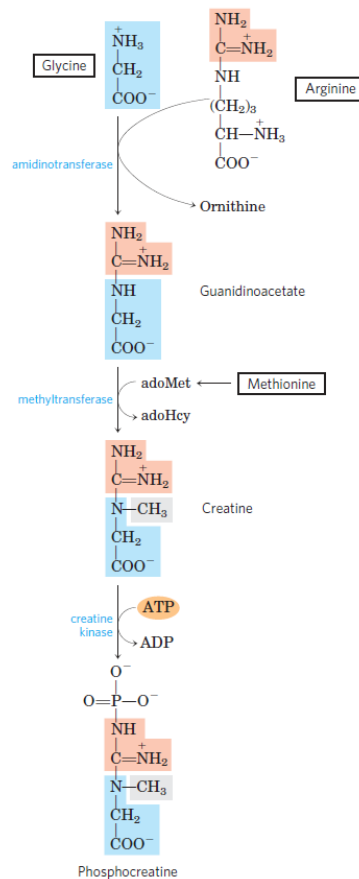


Figure 15: Biosynthesis of Creatine and phosphocreatine.

### 1.2.3 Glutathione

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

The GSH biosynthesis involves **two ATP-dependent steps**:

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

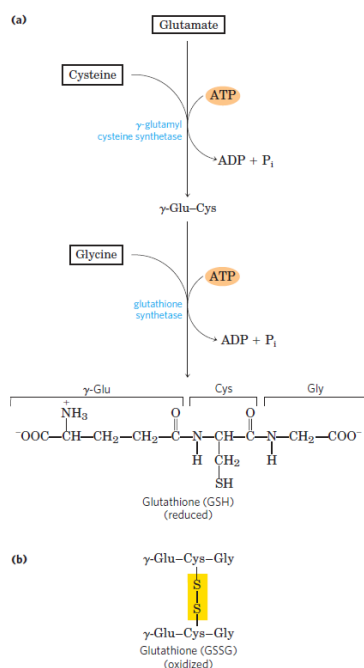


Figure 16: The top picture shows the biosynthesis of GSH. The bottom picture shows the oxidized form of glutathione.

#### 1.2.4 Biogenic Amines

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

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

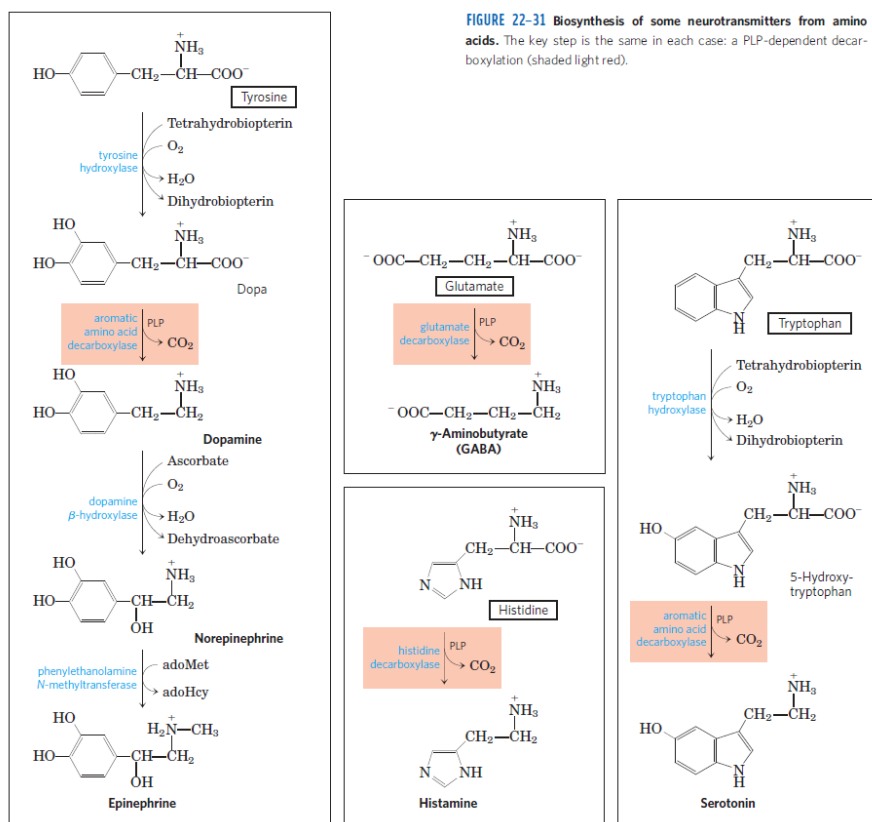


Figure 17: Biosynthesis of some neurotransmitters from amino acids.

### 1.2.5 Polyamines

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

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

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

## 2 Nucleic Acids in Biosynthesis

### 2.1 Biosynthesis of Nucleic Acids

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

- **DNA** and **RNA**
- energy molecules such as **ATP**, **GTP**, **CTP**, and **uridine triphosphate (UTP)**

- 
- second messengers such as **cAMP** and **cGMP**
  - key enzyme cofactors such as **CoA**, **FAD**, **NAD<sup>+</sup>**, and **NADP<sup>+</sup>**

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

### 2.1.1 Biosynthesis of Purines

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

The pathway of Purine production:

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

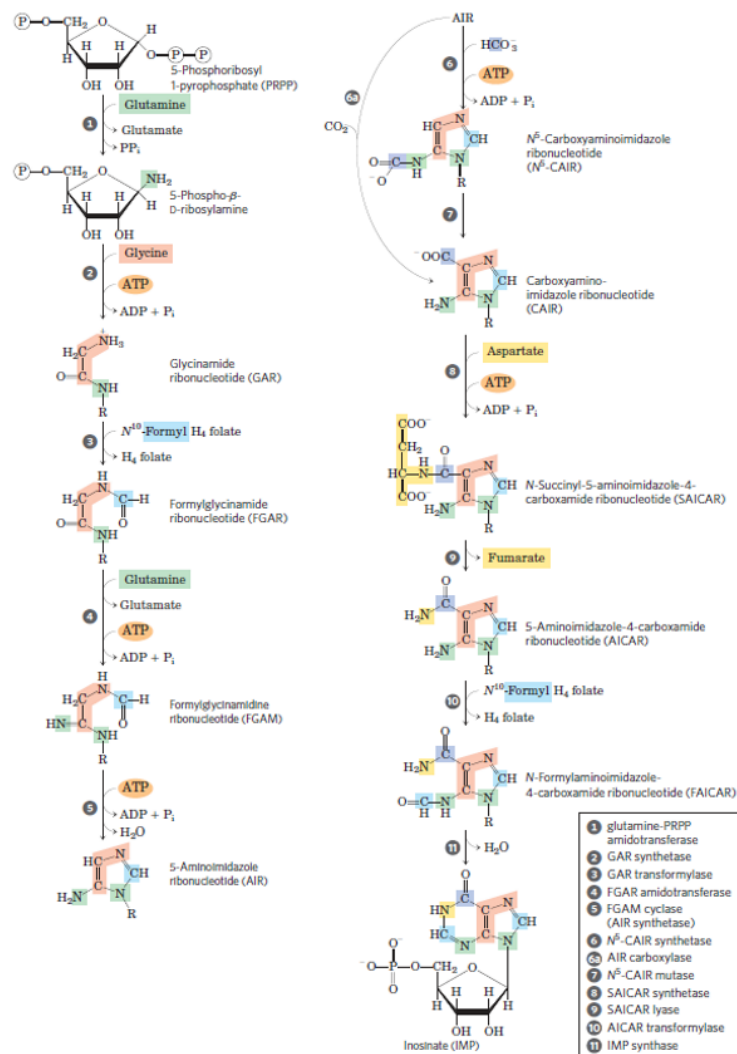


Figure 18: De novo synthesis of purine nucleotides: construction of the purine ring of IMP. On the bottom right one can see all the involved enzymes.

Creating the individual nucleic acids:

- IMP is converted to **adenosine monophosphate (AMP)** in two steps:
  - i) GTP hydrolysis fuels the addition of Aspartate to IMP, through the substitution of a carbonyl oxygen for a nitrogen forming the intermediate adenylosuccinate by **adenylosuccinate synthase**.
  - ii) Fumarate is then cleaved off forming AMP, catalyzed by **adenylosuccinate lyase**.
- IMP is converted to **guanosine monophosphate** by:
  - i) the oxidation of IMP forming xanthylate.  $\text{NAD}^+$  is the electron acceptor.
  - ii) An amino group is inserted at the  $\text{C}_2$ , which is fuelled by ATP hydrolysis.



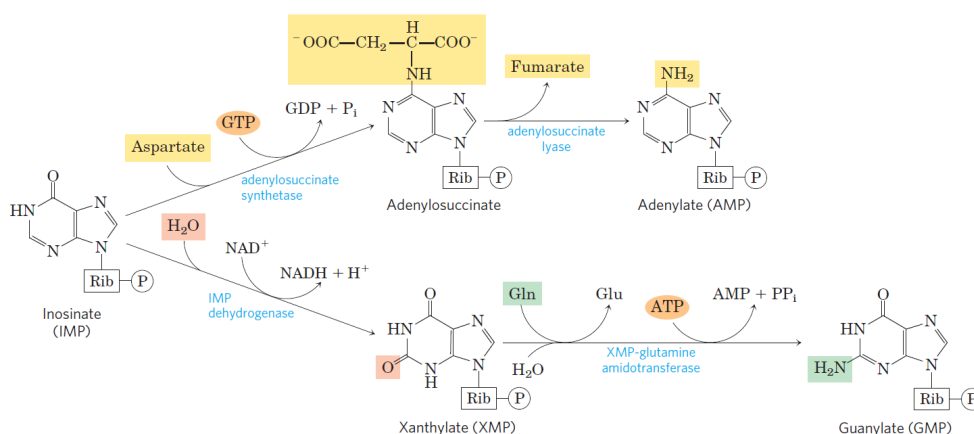


Figure 19: Biosynthesis of AMP and GMP from IMP.

### 2.1.2 Biosynthesis of Pyrimidines

**Pyrimidine** is an aromatic heterocyclic organic compound.

Here is the pathway:

- i) It starts with the formation of **carbamoyl phosphate** from glutamine and  $CO_2$ .
- ii) **Aspartate carbamoyltransferase** catalyzes a condensation reaction between aspartate and carbamoyl phosphate to form **carbamoyl aspartic acid**.
- iii) This is cyclized into **4,5-dihydroorotic acid** by **dihydroorotase**.
- iv) which is then converted to orotate by **dihydroorotate oxidase**.
- v) Orotate is covalently linked with a phosphorylated ribosyl unit.
- vi) Orotidylate is decarboxylated to form **uridine monophosphate (UMP)**.
- vii) UMP is phosphorylated by two **kinases** to form **uridine triphosphate (UTP)** via two sequential reactions with ATP.
- viii) **CTP** subsequently formed by the amination of UTP by the **CTP synthetase**, where glutamine is the  $NH_3$  donor and is fueled by ATP hydrolysis.

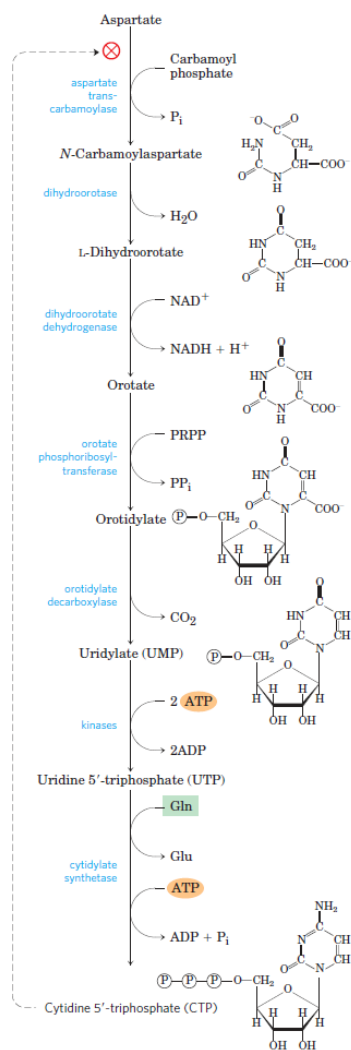


Figure 20: De novo synthesis of pyrimidine nucleotides. Biosynthesis of UTP and CTP from orotidylate.

### 2.1.3 Reduction from Ribonucleotides to Deoxyribonucleotides

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

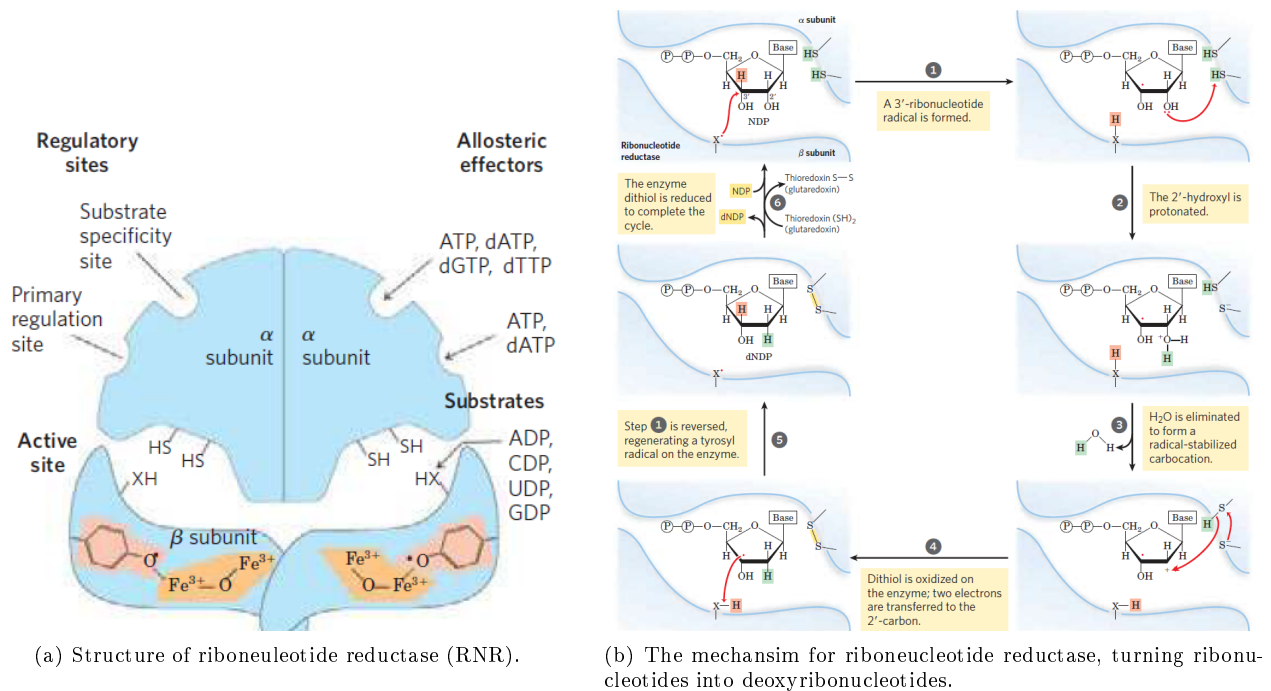


Figure 21: The structure and mechanism of RNR.

**Remark 2.1 (Regeneration of Ribonucleotide reductase (RNR)).** In order for RNR to catalyze the next reduction it has to be regenerated. This means the disulfide bond has to be broken into two sulfide groups. For this we have an electron chain which has two possible pathways:

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

Or:

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

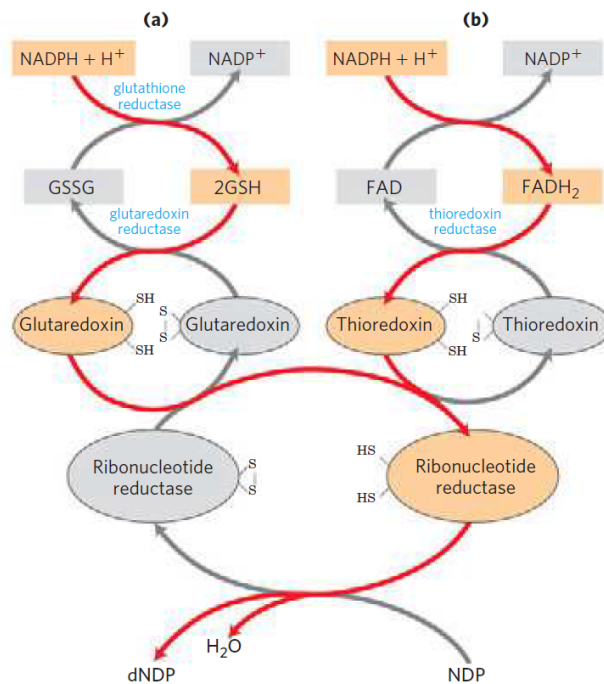


Figure 22: Shows the regeneration of RNR. Red arrows indicate electron movement, orange molecules are the reduced molecule, grey the oxidized. At the very bottom is the reduction of ribonucleotides to deoxyribonucleotides.

#### 2.1.4 Biosynthesis of Thymidylate

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

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

*Remark 2.2 (Consequences of dTMP lacking).* The lack of dTMP can have some consequences, due to the DNA being messed up:

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

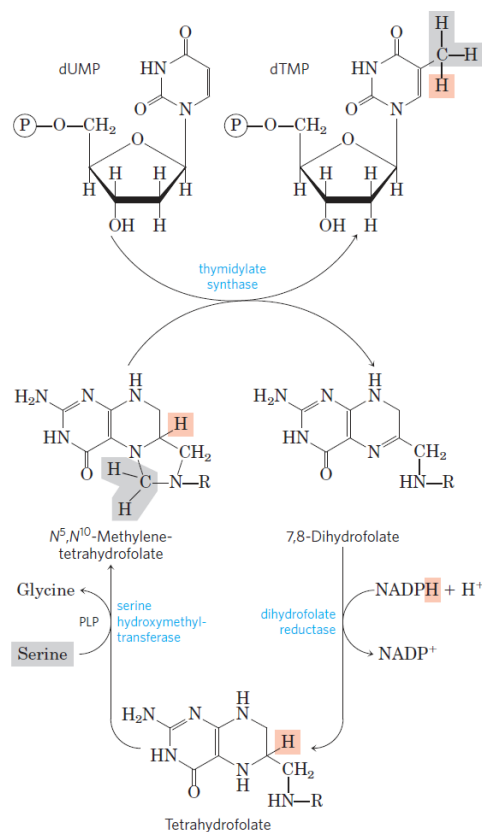


Figure 23: The conversion of dUMP to dTMP by TYMS and DHFR.

## 2.2 Disposal of Nucleic Acids

### 2.2.1 Purines Disposal

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

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

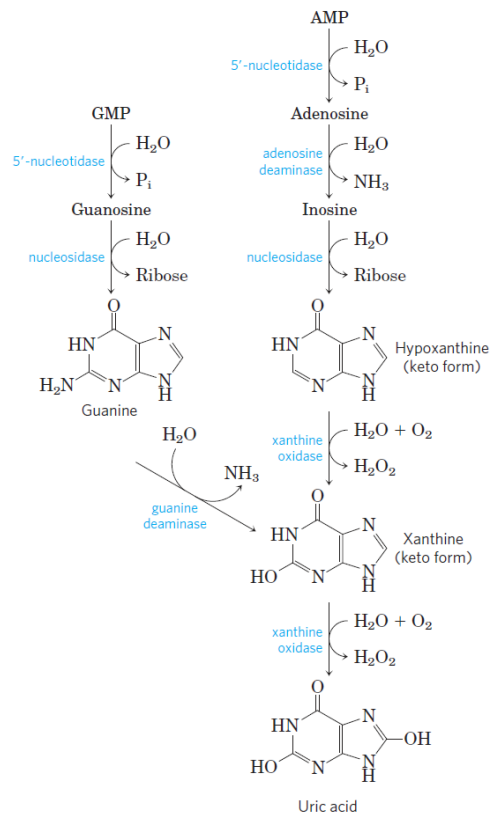


Figure 24: Catabolism of purine nucleotides.

**Remark 2.3 (Degradation Purine).** GMP follows a similar pathway, just that it has one deamination less in its degradative pathway.

### 2.2.2 Pyrimidines Disposal

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

Let's start with Cytosine:

- Cytosine gets broken down into Uracil.
- Uracil gets further broken down to N-carbamoyl- $\beta$ -alanine.
- That gets broken down to  $\beta$ -Alanine, by **beta-ureidopropionase**, with  $CO_2$  and ammonia as by-products.
- They are further broken down to  $\beta$ -alanine and from there to **Malonyl-CoA** to **Acetyl-CoA** where they can enter the **TCA cycle**.

Thymine's catabolism:

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

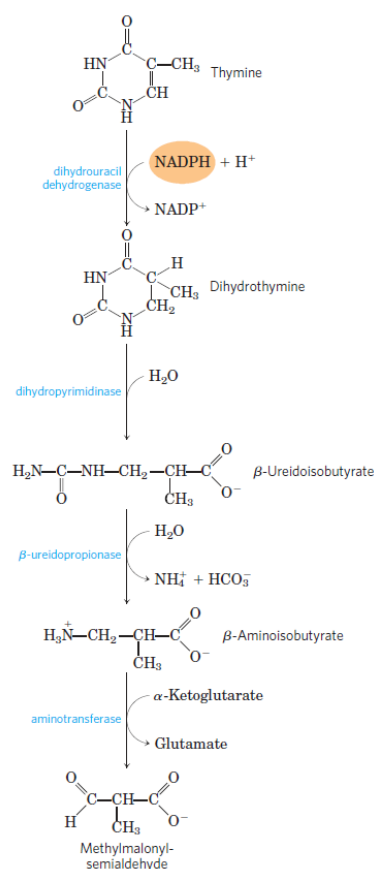


Figure 25: The catabolism of pyrimidines.

**Remark 2.4 (Consequences of Genetic Defects: Severe combined immune deficiency).** Genetic defects in the catabolism lead to diseases in humans. Defects in the enzyme adenosine deaminase leads to **severe combined immune deficiency (ADA-SCID)**. Why immunodeficiency? Because the defect results in an accumulation of deoxyadenosine, which in turn leads to a buildup of dATP in all cells. This in turn inhibits ribonucleotide reductase, preventing DNA cells. This means cells can't divide. T and B cells, with their high mitotic rate are very susceptible to this condition.

### 2.2.3 Salvage Pathways

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

For **Pyrimidines**:

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

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

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

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

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

i) **adenine phosphoribosyltransferases (APRT)**,

ii) **hypoxanthine-guanine phosphoribosyltransferases (HGPRT)**.

## 2.3 Chemotherapies Targeting Nucleotide Metabolism

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

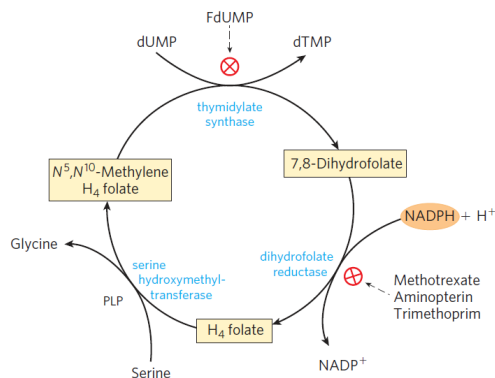


Figure 26: Thymidylate synthesis and folate metabolism as targets of chemotherapy.