Welcome to BCII lesson 10

Chimie Biologique II

Biological Chemistry II

BIO-213

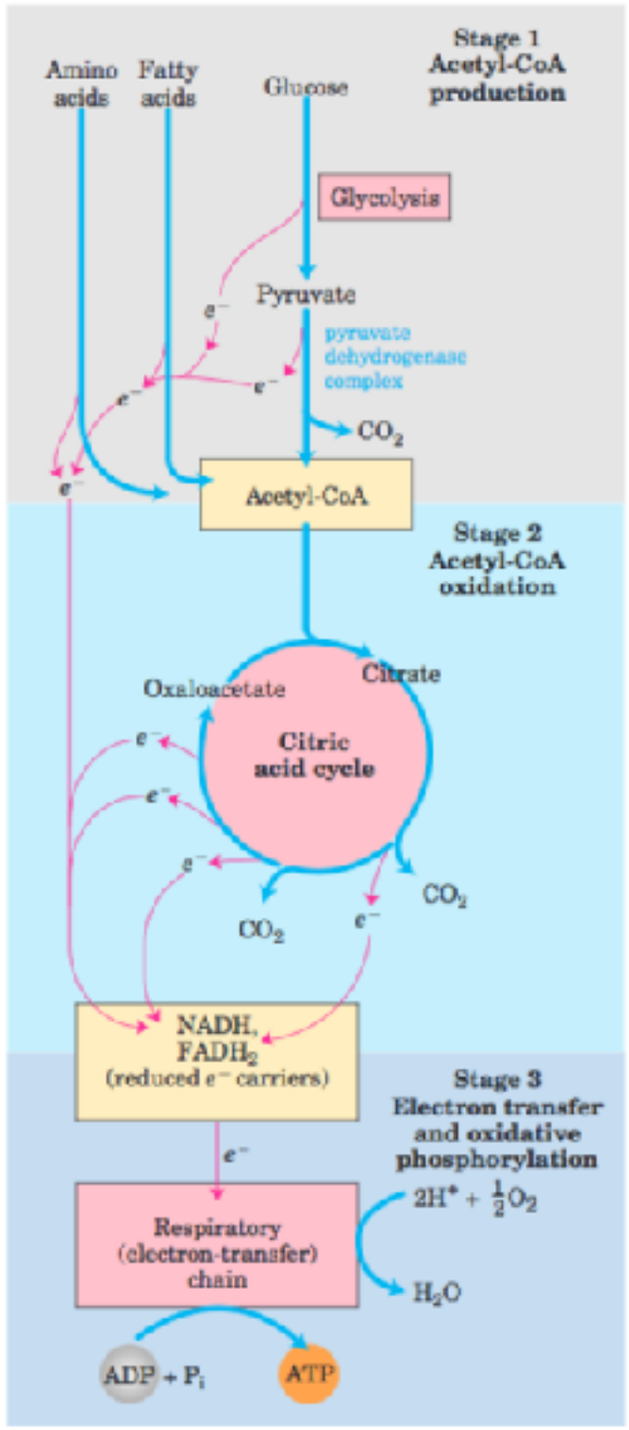
Teacher

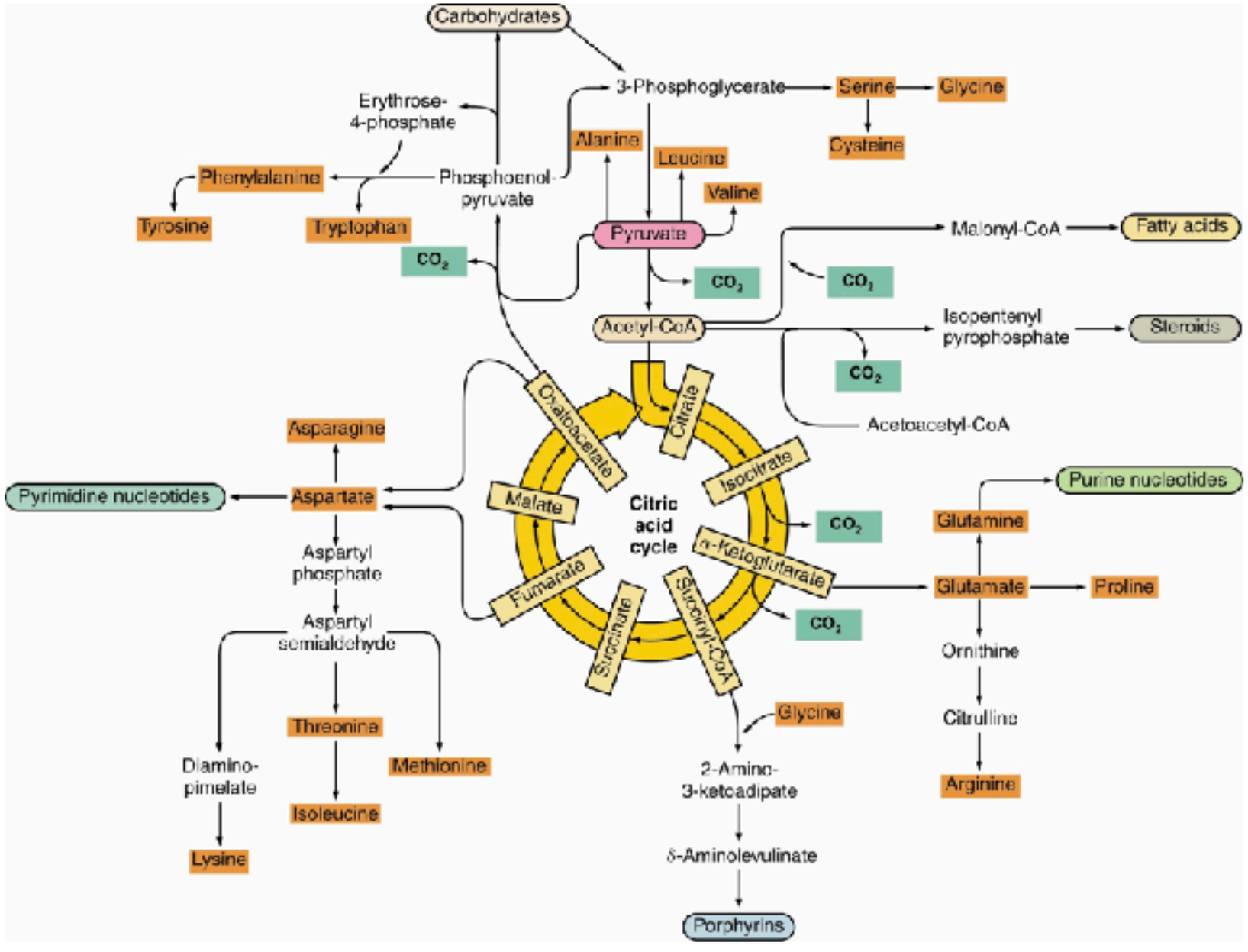
Giovanni D’Angelo, IBI

Lecture 10

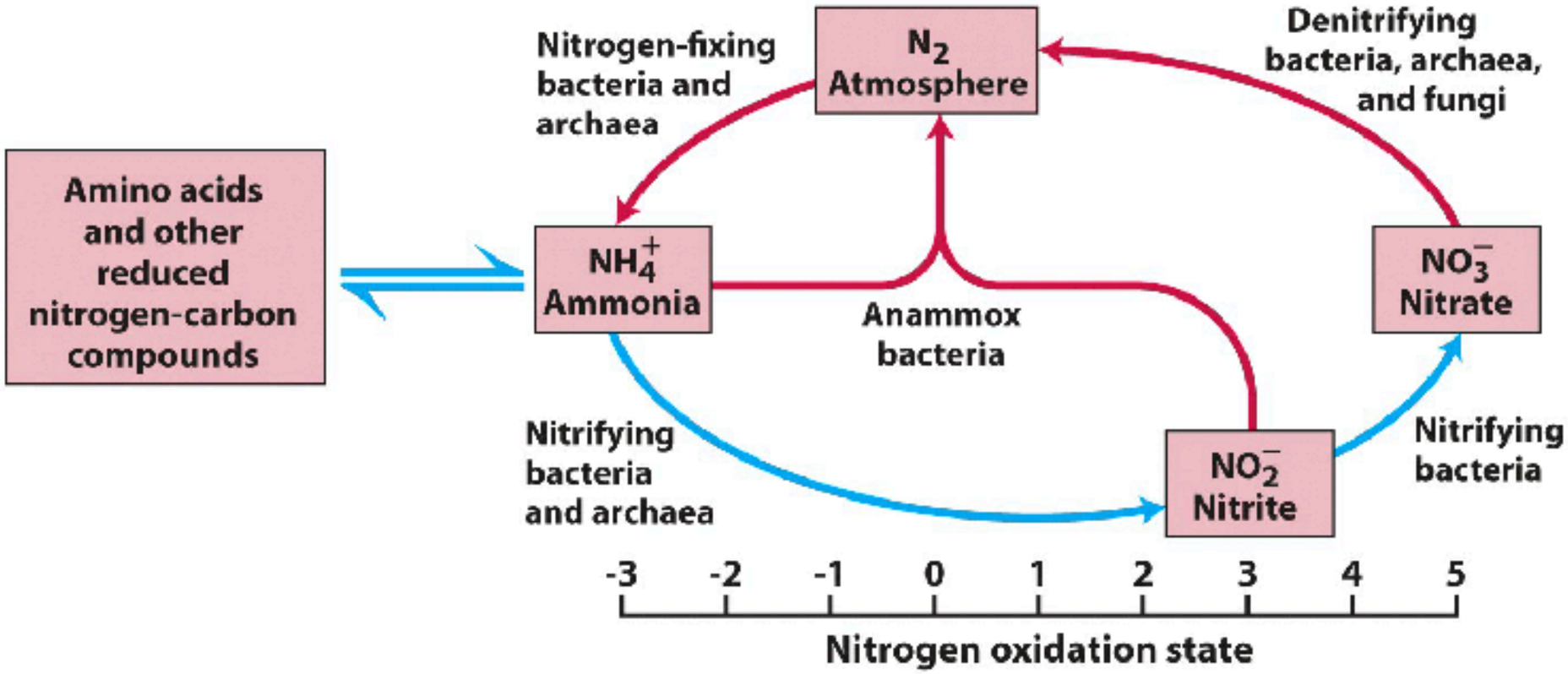
The biosynthesis of amino acids and nucleic acids

General overview





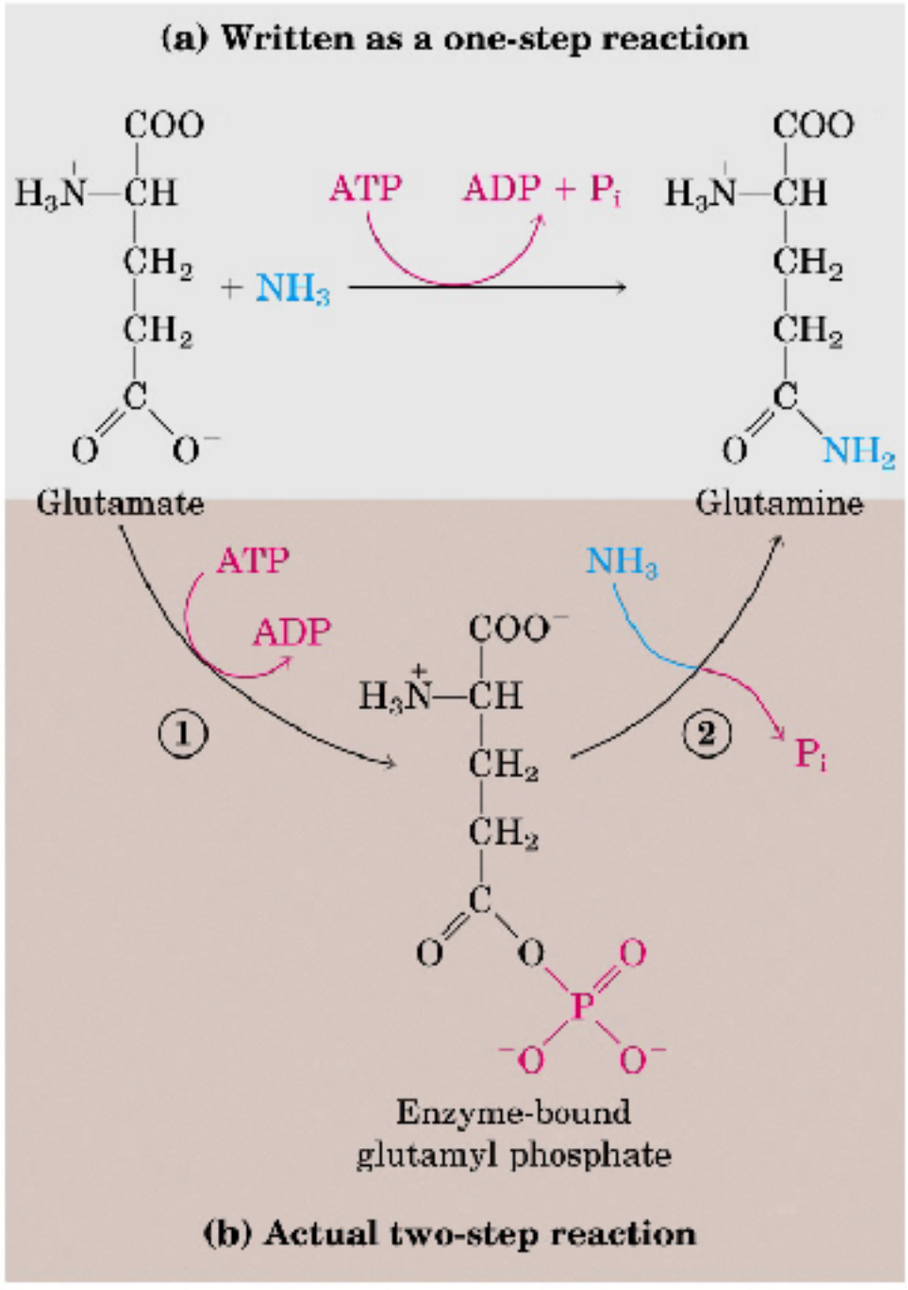
Amino Acids Biosynthesis



Both **amino acids** and **nucleotides** require nitrogen for their synthesis.

The nitrogen required for the *de novo* synthesis of these compounds is obtained through the nitrogen cycle whereby bacteria and archaea fix atmospheric N2 to produce ammonia. Ammonia then enters cell metabolism by being incorporated into **glutamate** and **glutamine**

Synthesis of Glutamate and Glutamine



The production of **glutamate** and **glutamine** happens in two steps:

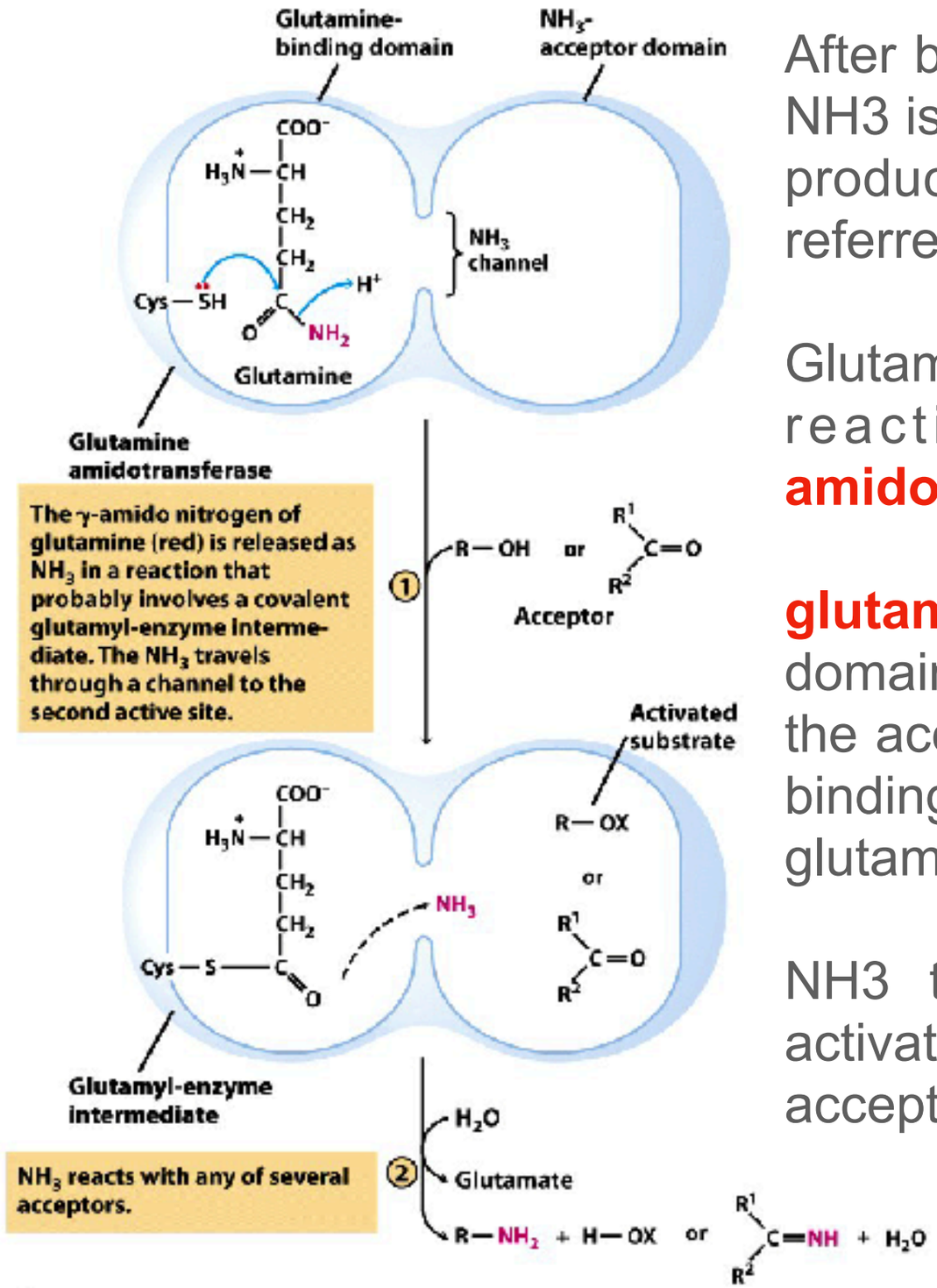
In the first step glutamate and NH4+ react to form **glutamine** in a reaction catalysed by the enzyme **glutamine synthetase** with consumption of one ATP molecule (you have seen this reaction already in previous lectures)

In the second step (in bacteria) 2 **glutamate** molecules are formed from a-ketoglutarate and glutamine by the enzyme **glutamate synthase** that transaminases a-ketoglutarate consuming a NADPH molecule.

The overall reaction in bacteria reads:

a-ketoglutarate + NADPH + ATP +NH4+ => glutamate + NADP+ + ADP + Pi

Transamination



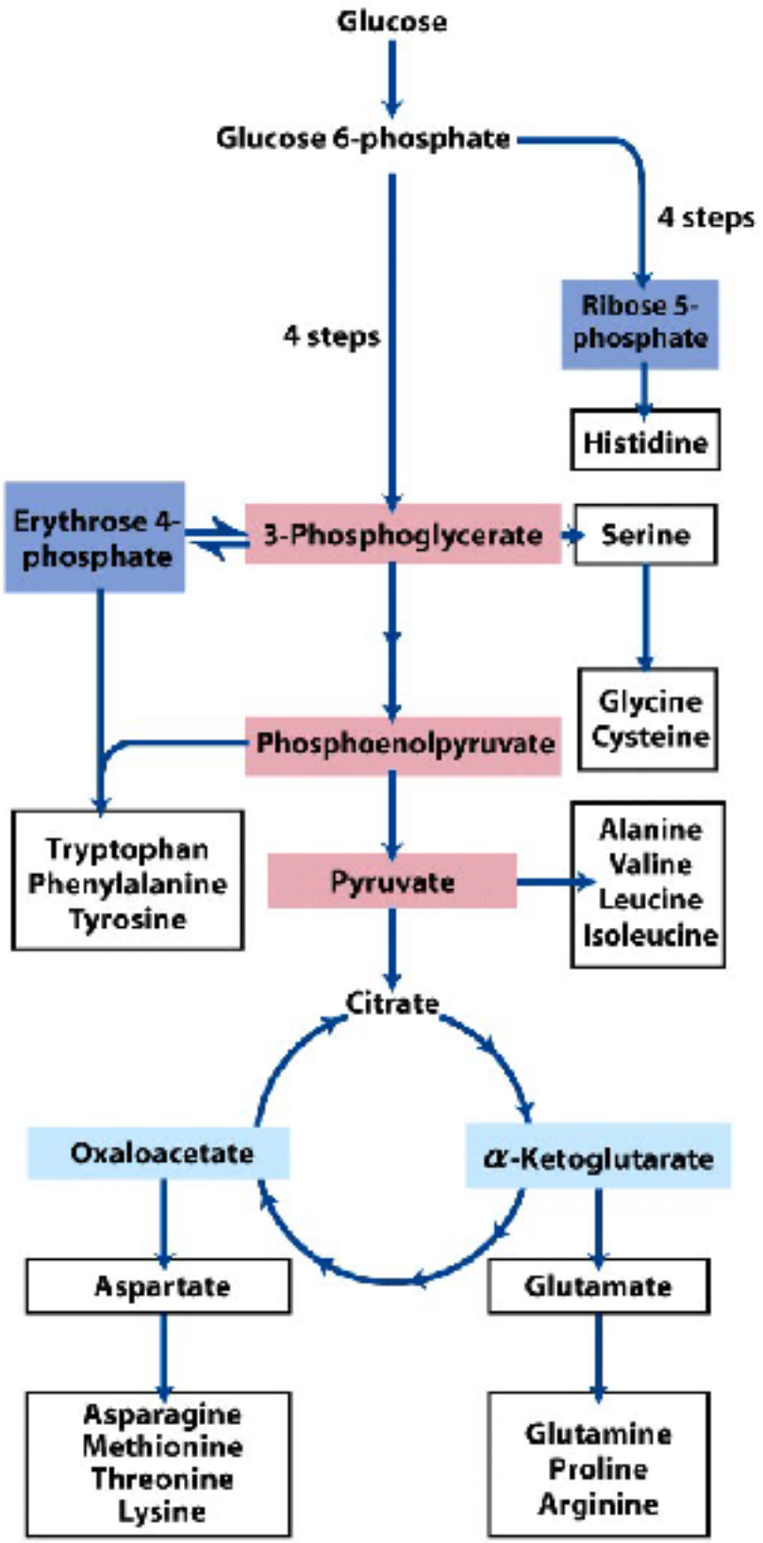
After being incorporated into **glutamate** and **glutamine** NH3 is transferred to a variety of different compounds to produce 'aminated' products. These reactions are referred to as **transamination reactions**

Glutamine serves as a NH3 donor in many such reactions that are catalysed by **glutamine amidotransferases**

glutamine amidotransferases are constituted by two domains one that binds glutamine the other that binds the acceptor substrate. A Cys residue in the Glutamine-binding domain breaks the acidic bond and forms a glutamyl-enzyme intermediate.

NH3 travels to the NH3-acceptor domain here an activated (usually by ATP to form an acylphosphate acceptor) substrate is aminated and released.

Amino Acids Biosynthesis



Amino Acids are produced starting from intermediates of **glycolysis**, of the **TCA** cycle or of the **pentose phosphate pathway**.

Nitrogen is transferred to these compounds from glutamine or glutamate

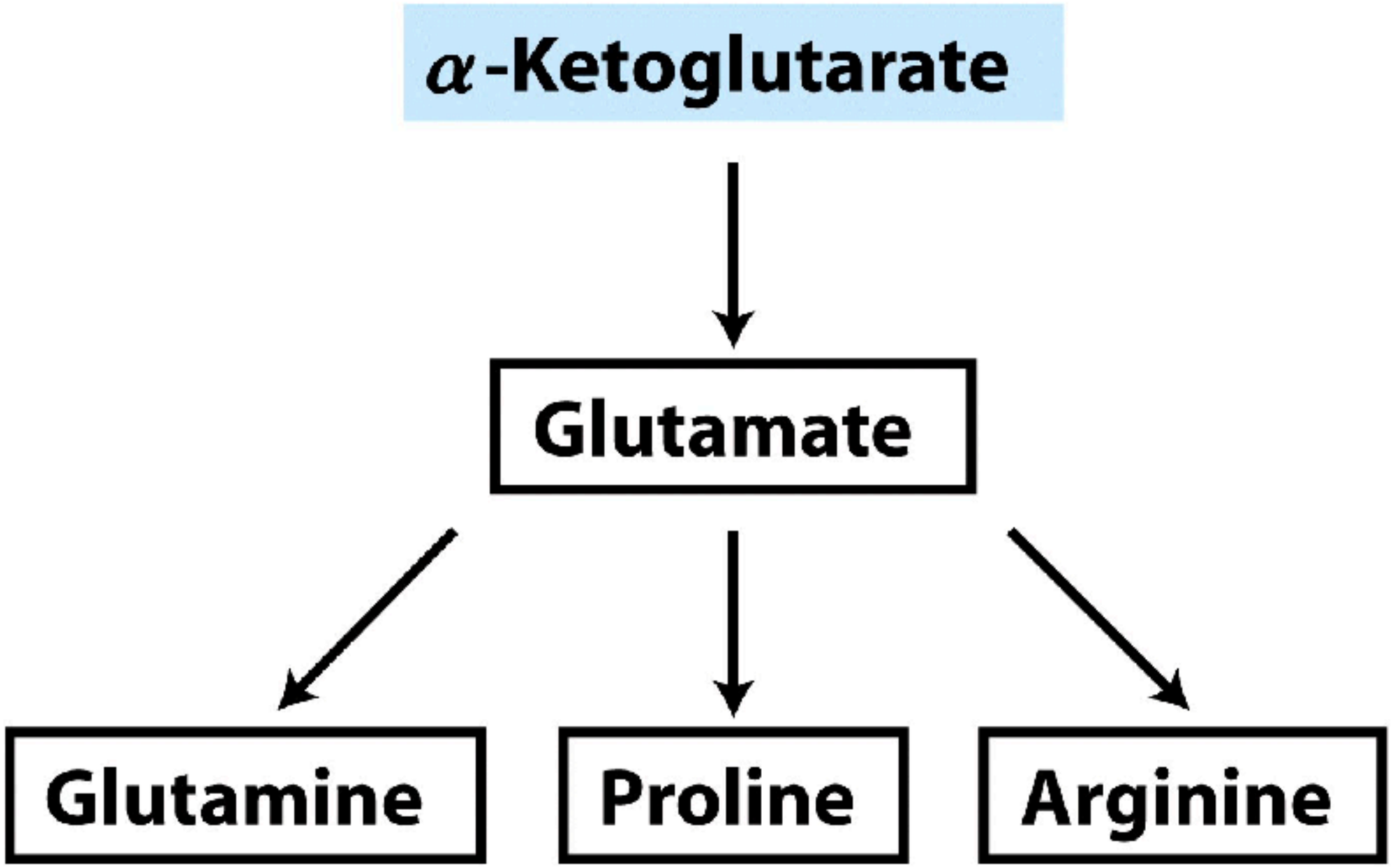
TABLE 22–1 Amino Acid Biosynthetic Families, Grouped by Metabolic Precursor

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

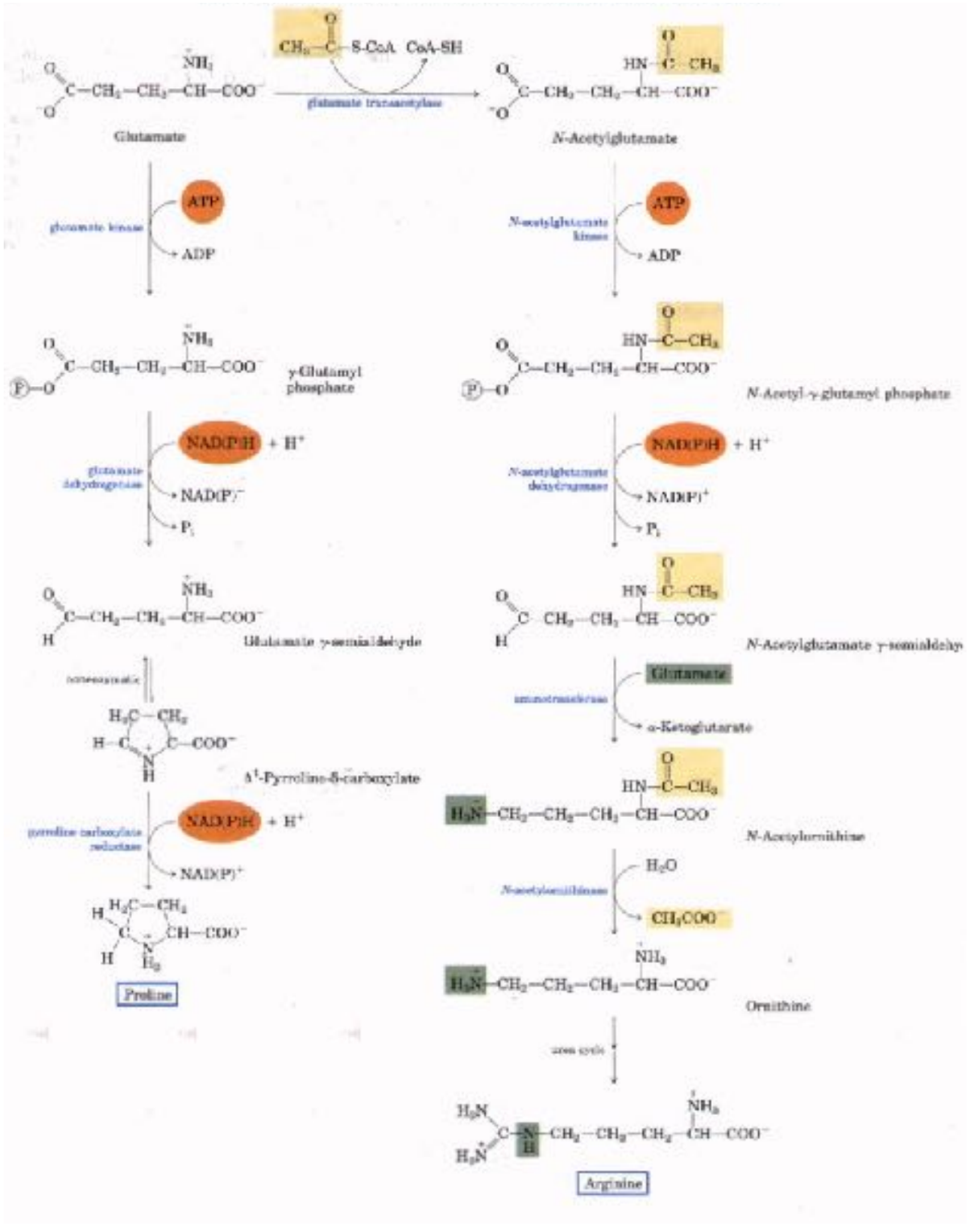
*Essential amino acids.

†Derived from phenylalanine in mammals.

Amino Acids Biosynthesis



Proline and Arginine

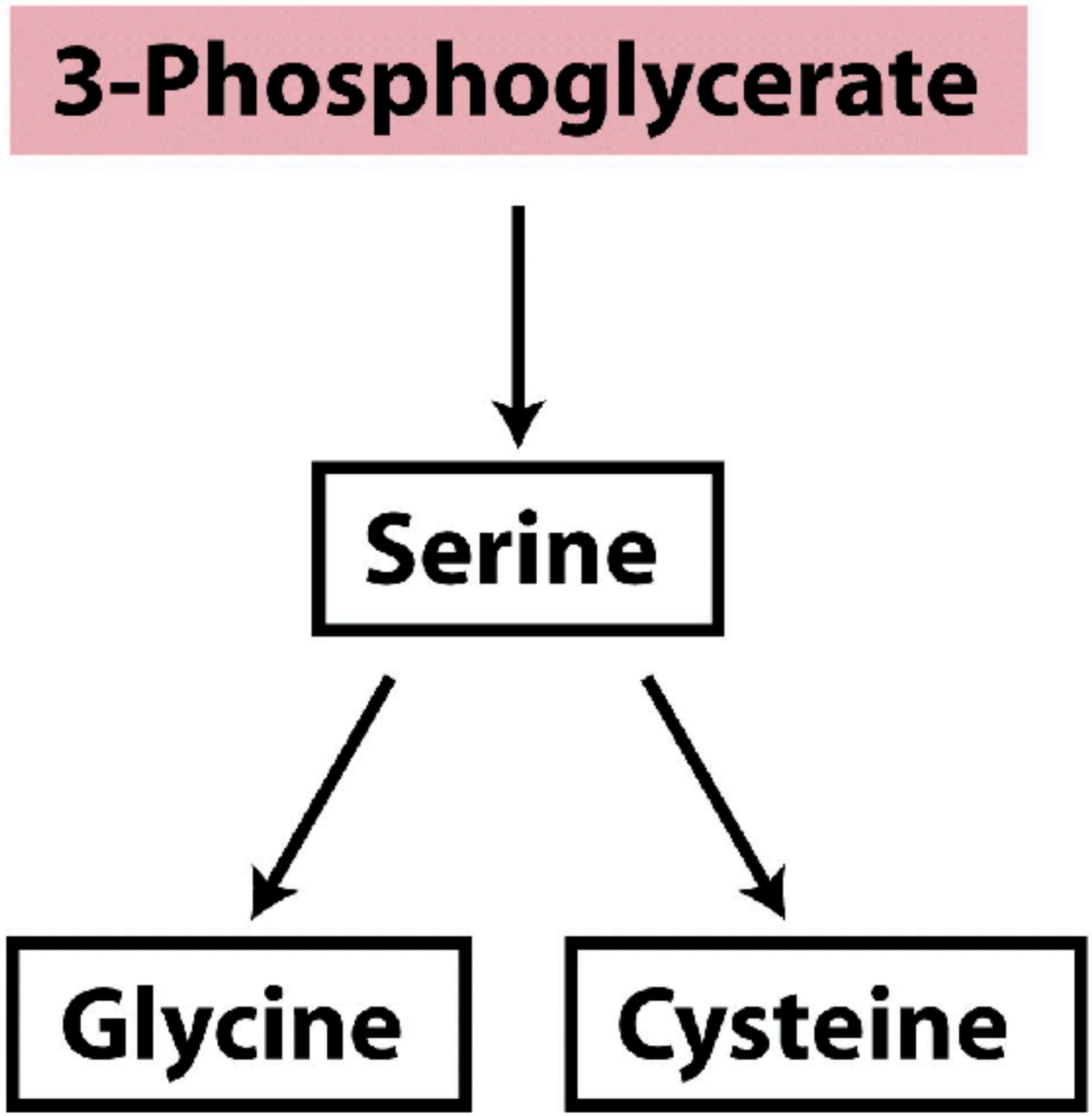


Proline is a cyclic derivative of glutamate. It is synthesised in four steps:

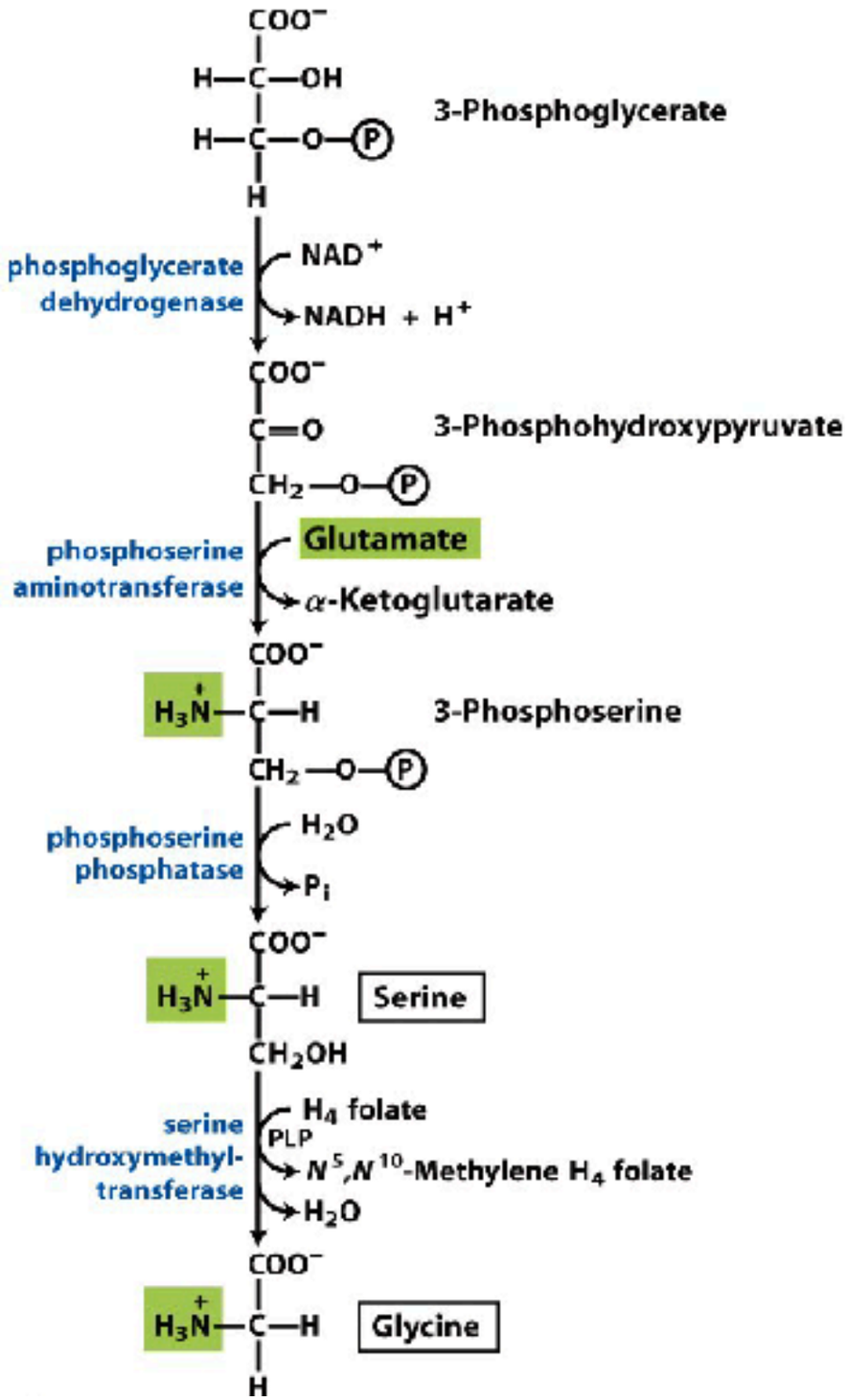
1) glutamate is phosphorylated
2) glutamyl-P is dephosphorylated and reduced
3) glutamate semialdehyde undergoes spontaneous cyclisation
4) pyrroline-5-carboxylate is reduced to **Proline**

Arginine is synthesised through a similar pathway whereby glutamate is first acylated and then undergoes the same reactions as in the Proline synthetic pathway. At the stage of semialdehyde, acylation impedes spontaneous cyclisation, and through a further transamination, and de-acylation Ornithine is produced. Ornithine is then converted to Arginine in the urea cycle (see previous lecture)

Amino Acids Biosynthesis



Serine, Glycine and Cysteine

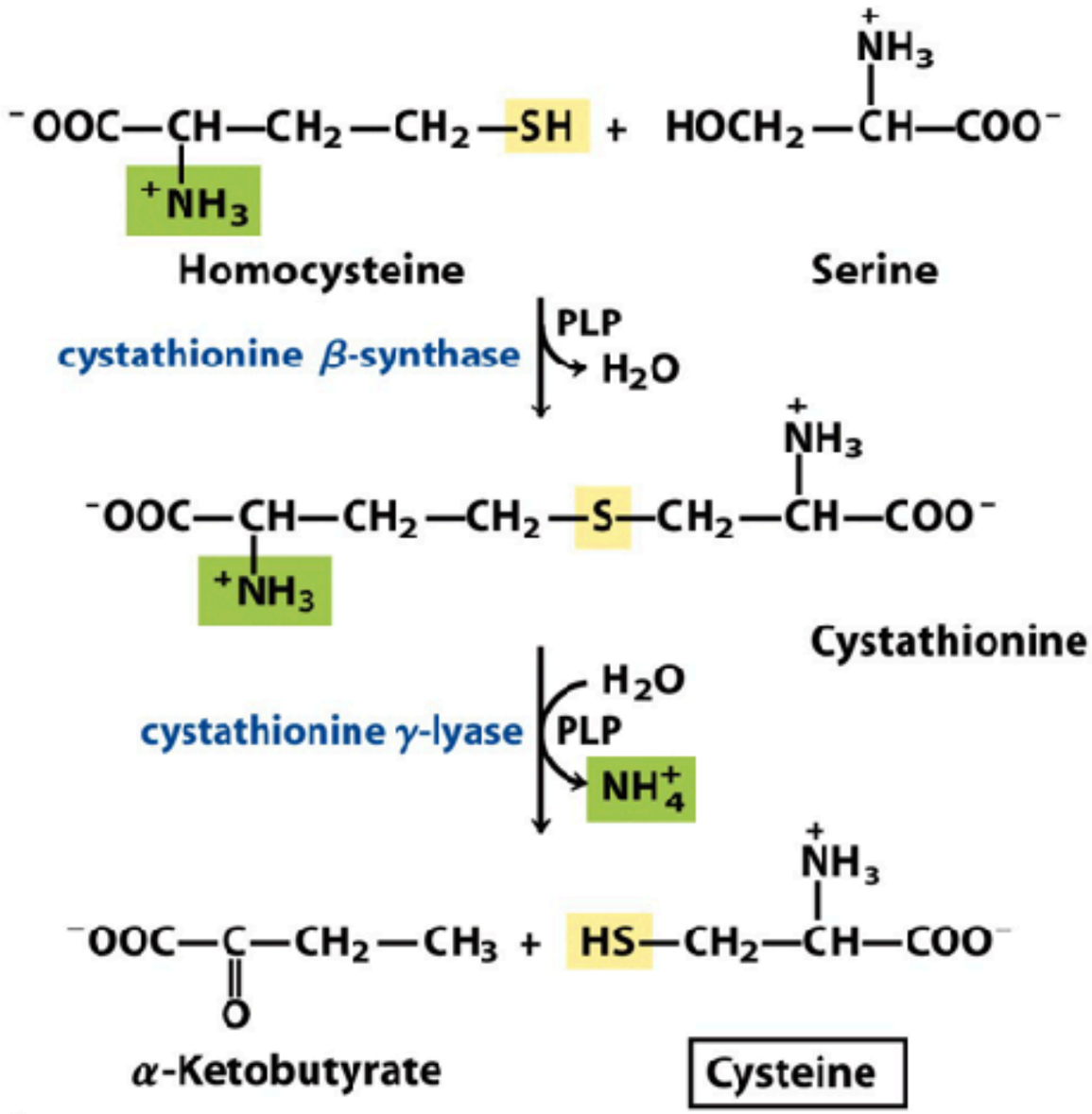


Serine is formed from 3-phosphoglycerate (an intermediate of glycolysis; see lecture 1) in 3 steps:

1) oxidation of 3-phosphoglycerate
2) transamination of 3-phosphohydroxypyruvate
3) dephosphorylation of 3-phosphoserine

Once formed, serine can be converted to **glycine** by the enzyme **serine hydroxymethylt-transferase**. this enzyme removes a carbon atom from glycine. It uses tetrahydrofolate and requires PLP as a cofactor

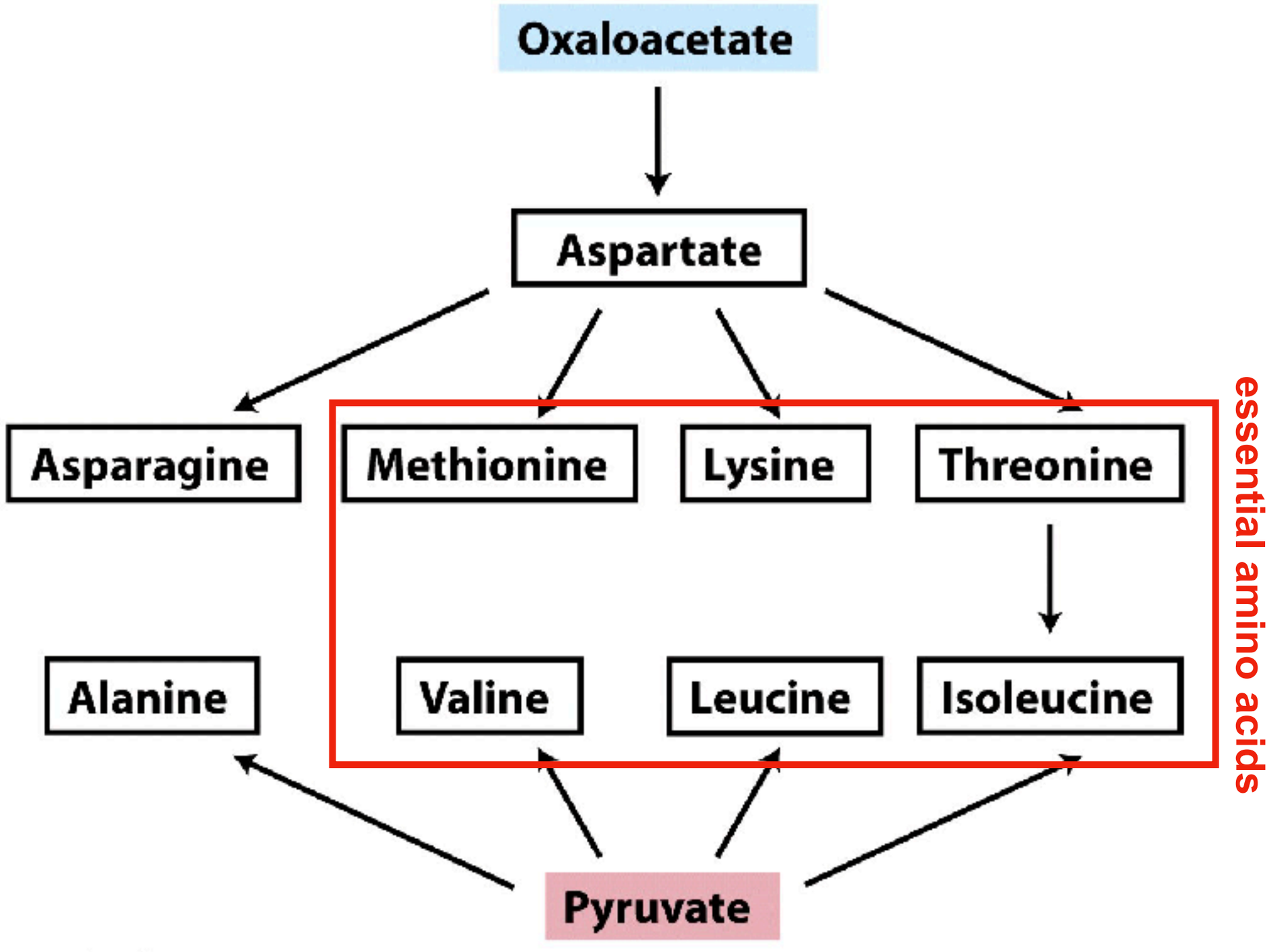
Serine, Glycine and Cysteine



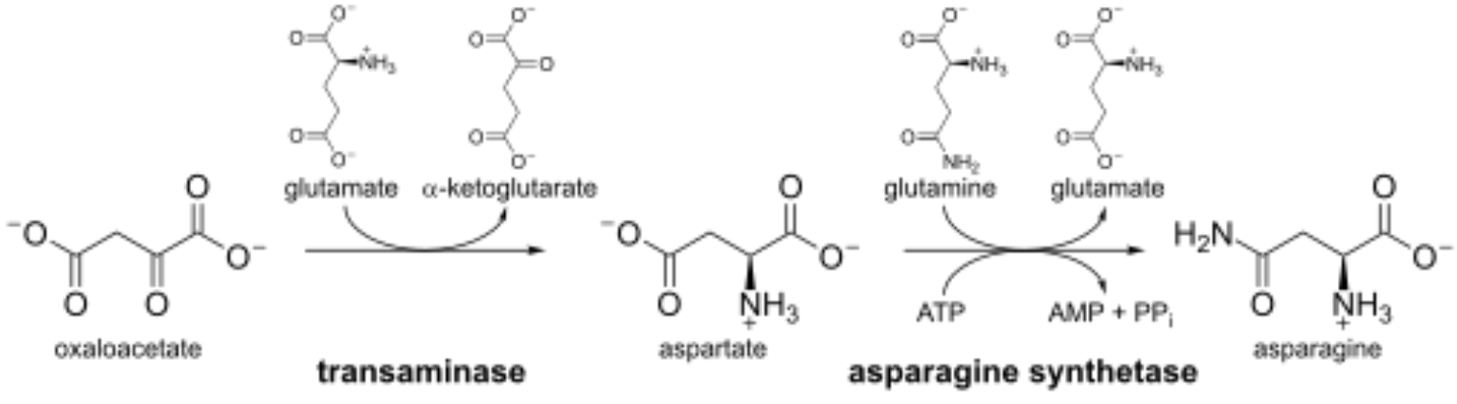
In mammals **Cysteine** is formed from serine and methionine (that provides the sulfur atom).

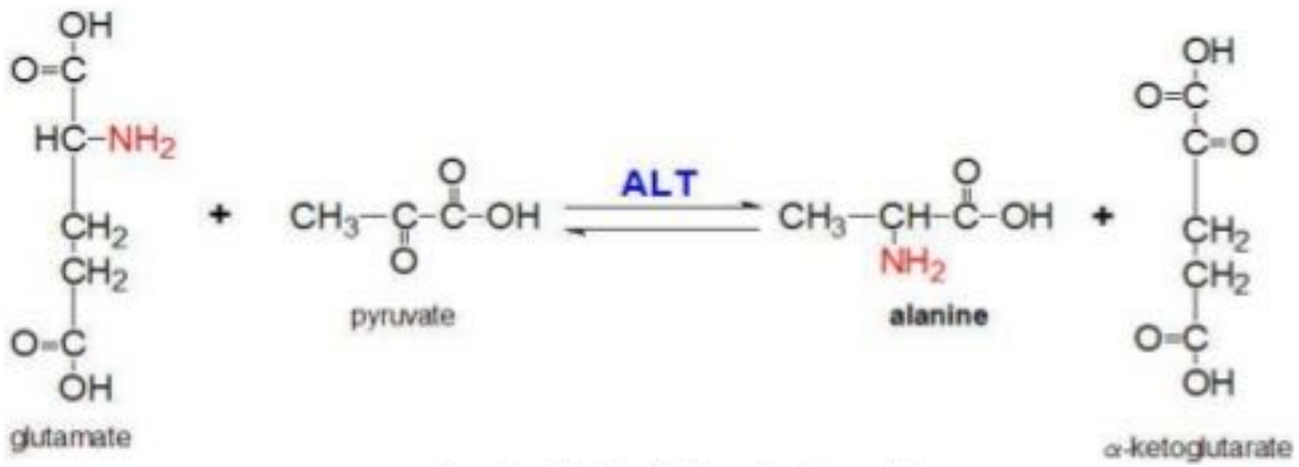
1) Through a series of reactions Methionine is converted into homocysteine
2) Homocysteine is condensed to serine to form cystathionine
3) cystathionine is hydrolysed with loss of an NH4+ to form cysteine and a-ketobutyrate

Amino Acids Biosynthesis



Aspartate, Asparagine and Alanine



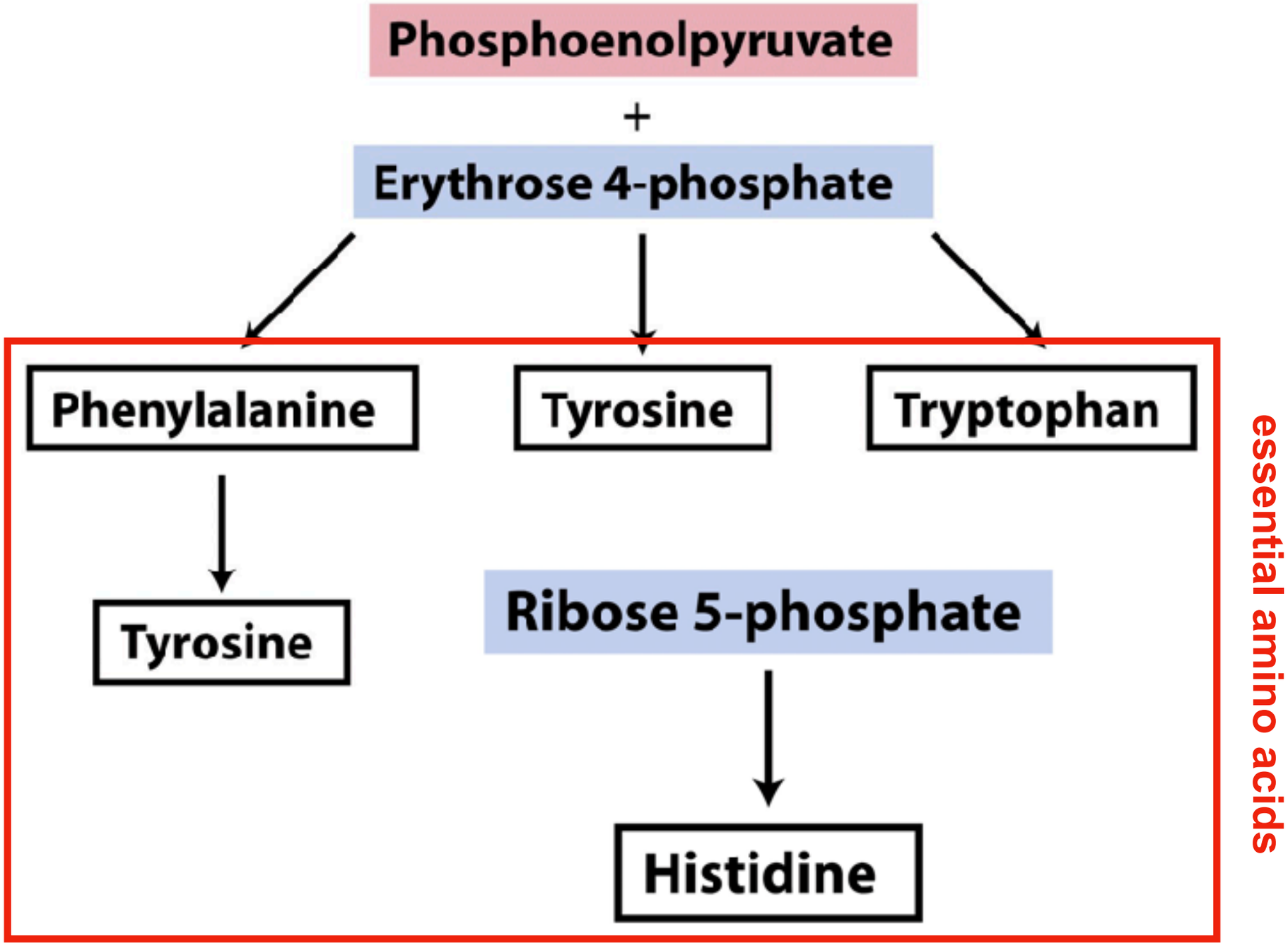


Aspartate is produced by transamination of oxaloacetate in a reaction catalysed by **aspartate transaminase** (**AST**).

Asparagine is produced by amidation of aspartate by glutamine in a reaction catalysed by **asparagine synthase** where an ATP is hydrolysed to AMP and PPi

Alanine is produced by transamination of pyruvate in a reaction catalysed by **alanine transaminase** (**ALT**)

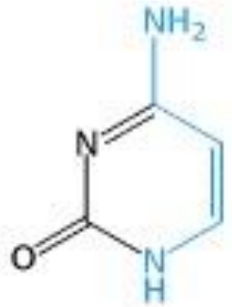
Amino Acids Biosynthesis



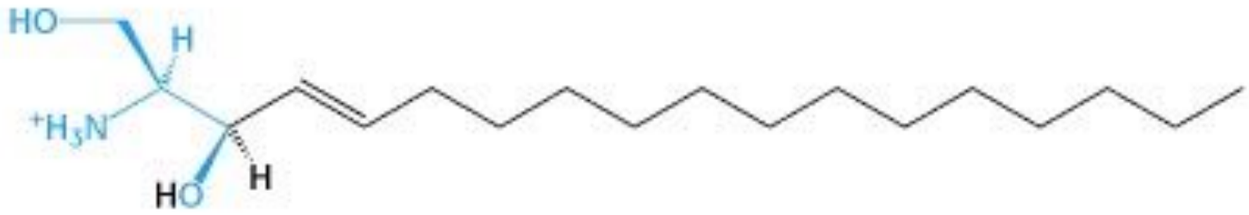
Amino Acids derived Biomolecules

In addition to being the building blocks of proteins and peptides, amino acids serve as **precursors of many kind of small molecules** that have important and diverse biological roles.

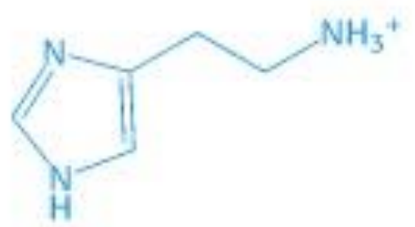
Adenine



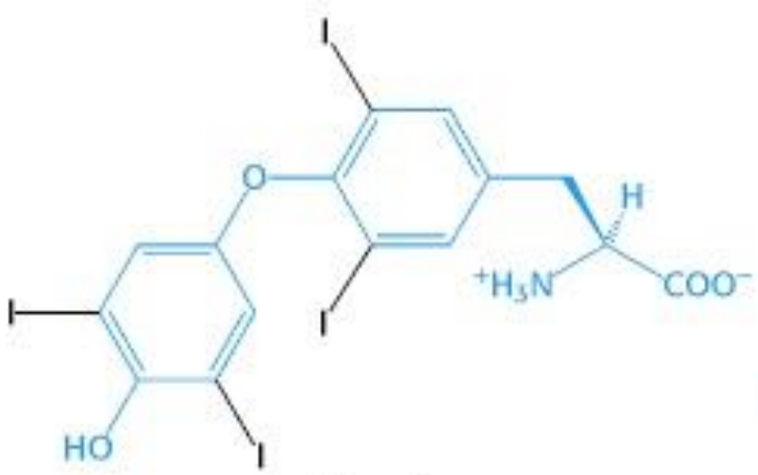
Cytosine



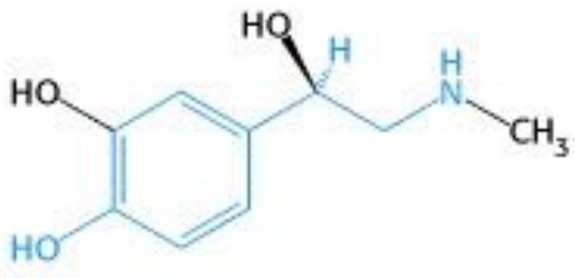
Sphingosine



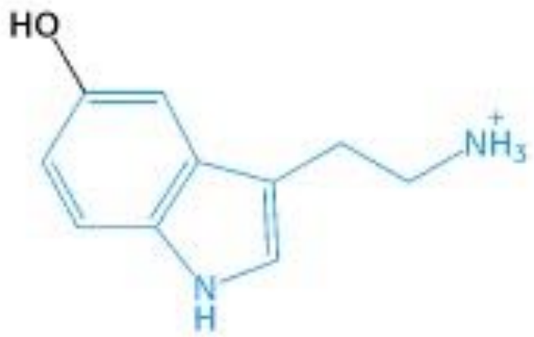
Histamine



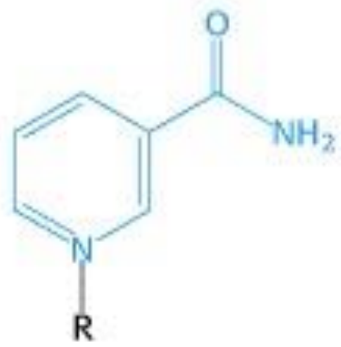
Thyroxine (Tetraiodothyronine)



Epinephrine

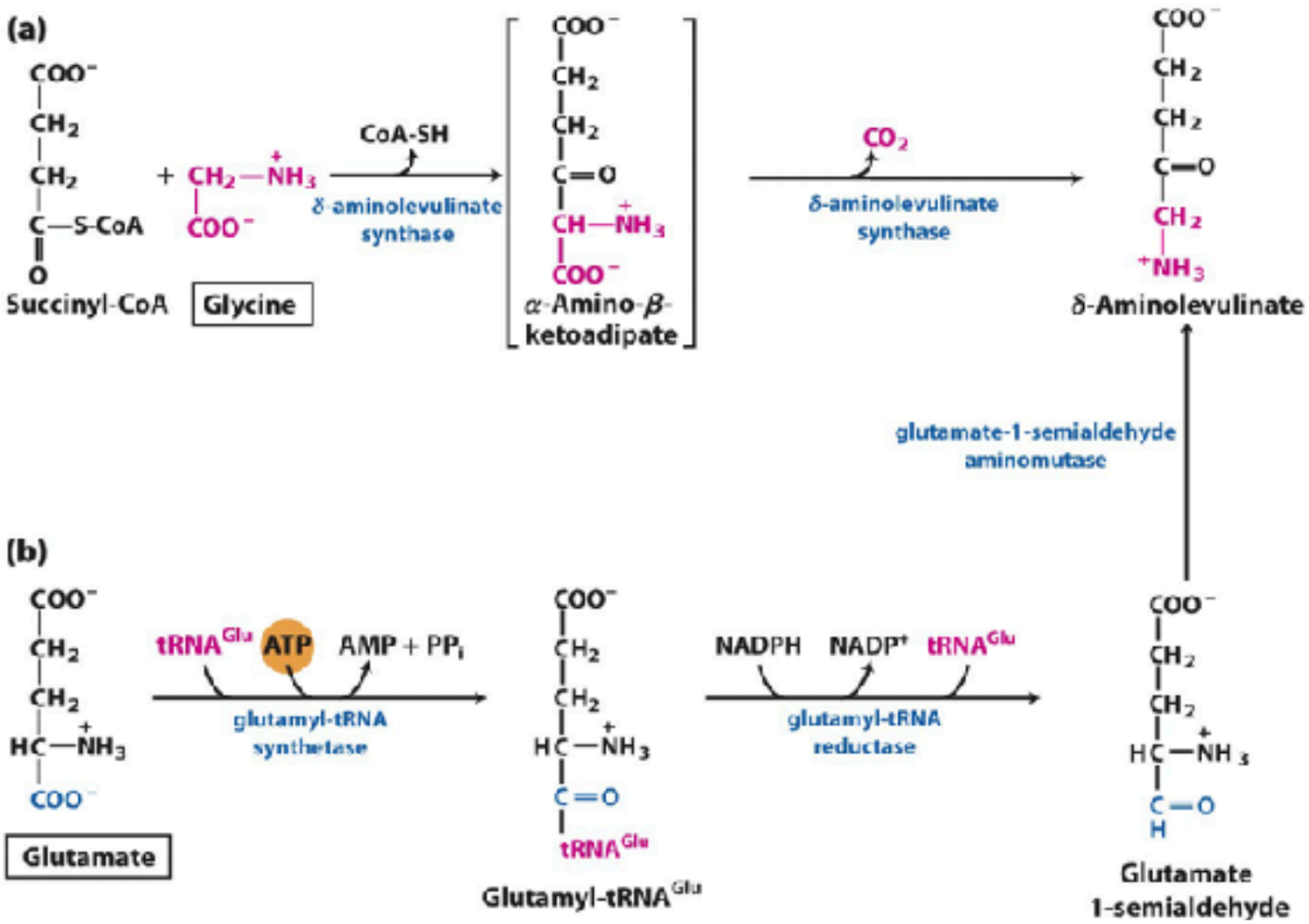


Serotonin



Nicotinamide unit of NAD^+

Porphyrins



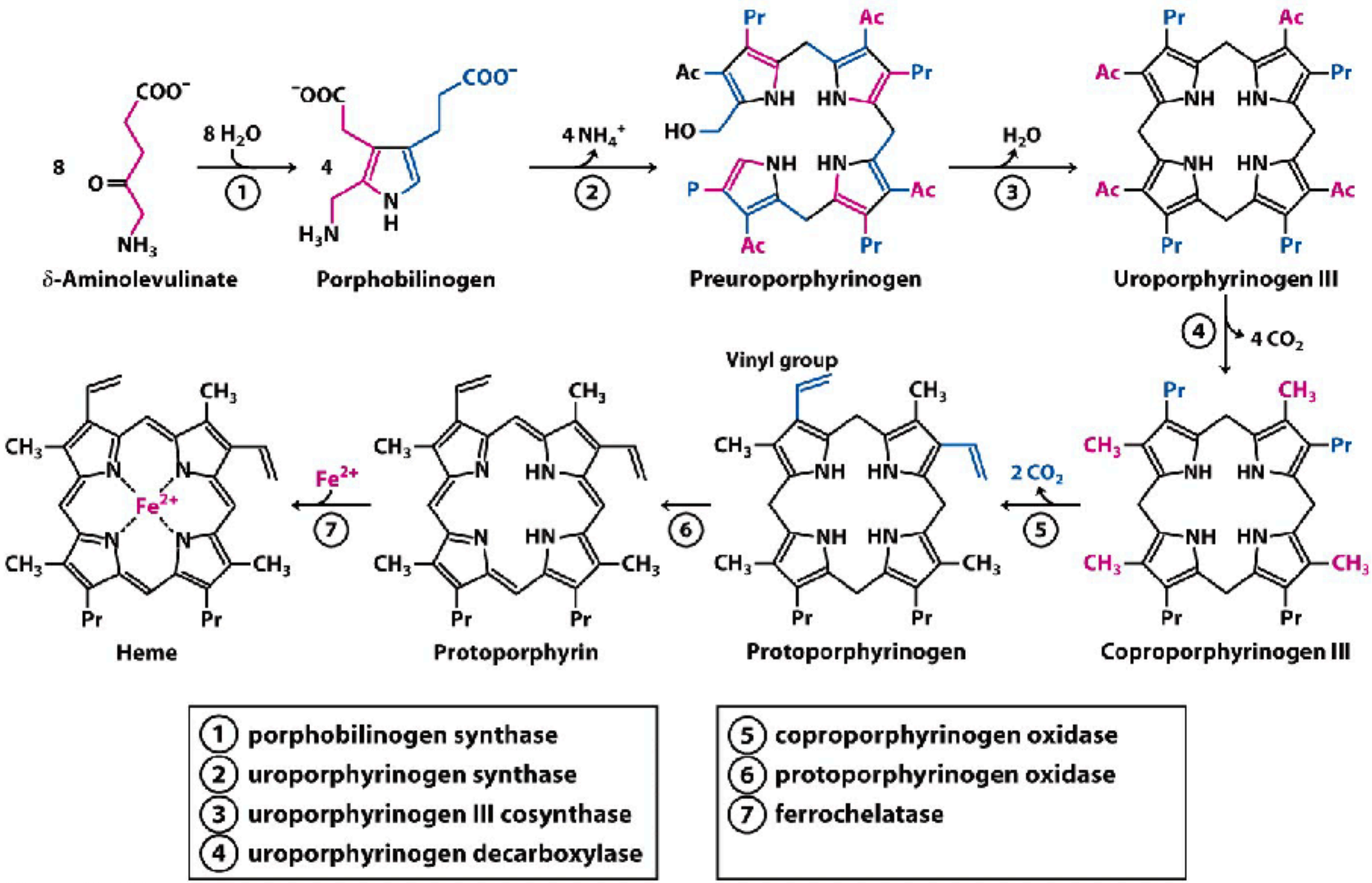
Porphyrins *are a group of heterocyclic compounds, that absorb strongly in the visible region of the electromagnetic spectrum, i.e. they are coloured. Metal complexes derived from porphyrins occur naturally. One of the best-known families of porphyrin complexes is heme, the pigment in red blood cells, a cofactor of the protein hemoglobin.*

Glycine is the main precursor for the synthesis of porphyrins.

In the first reaction glycine reacts with succinyl-CoA to form a-amino-ketoadipate that then is decarboxylated to **d-aminolevulinate**.

Glutamate is an alternative source of **d-aminolevulinate** that can ne produced by reduction of Glutaminyl-tRNA and isomerization of glutamate semialdehyde

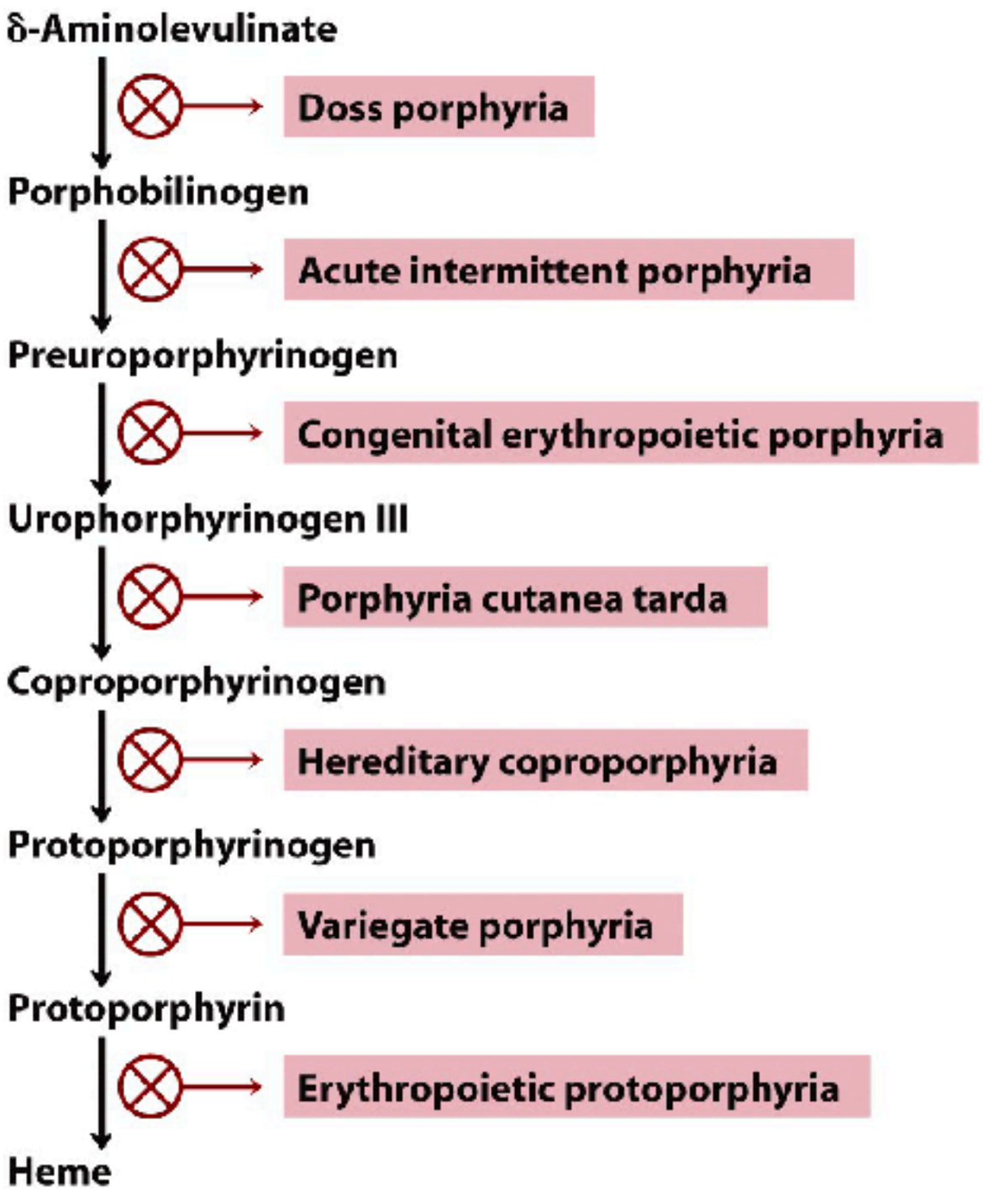
Porphyrins



1. porphobilinogen synthase
2. uroporphyrinogen synthase
3. uroporphyrinogen III cosynthase
4. uroporphyrinogen decarboxylase

5. coproporphyrinogen oxidase
6. protoporphyrinogen oxidase
7. ferrochelatase

Porphyrins

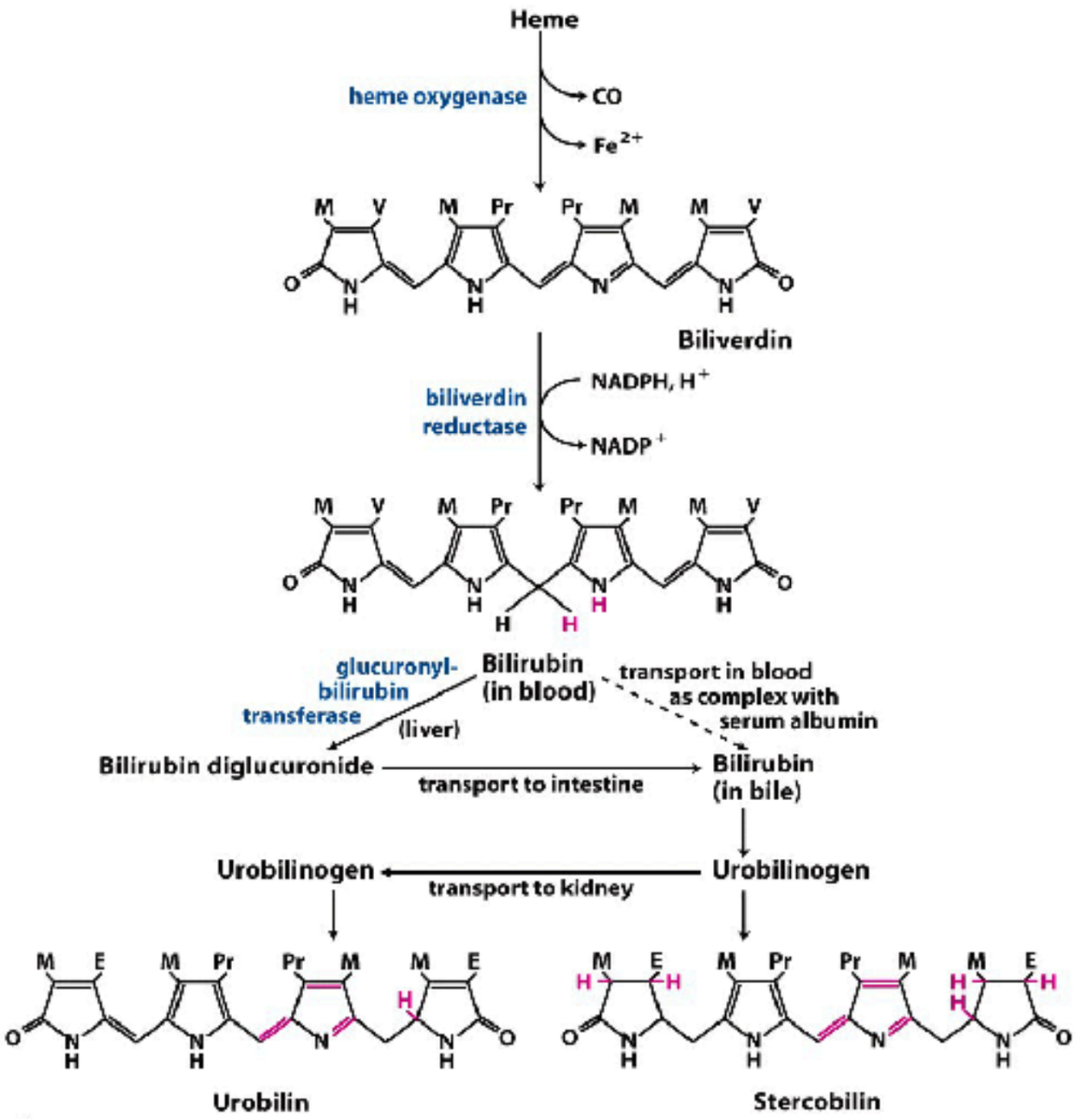


Porphyria is a group of diseases in which intermediates of the synthesis of porphyrins build up, negatively affecting the skin or nervous system.

These are caused by genetic defects in genes encoding the enzymes of the pathway. The types that affect the nervous system are also known as acute porphyria, as symptoms are rapid in onset and last a short time.

Cutaneous porphyria includes forms of the disease that cause skin symptoms as a result of sensitivity to sunlight, but these forms don't usually affect your nervous system.

Porphyrins



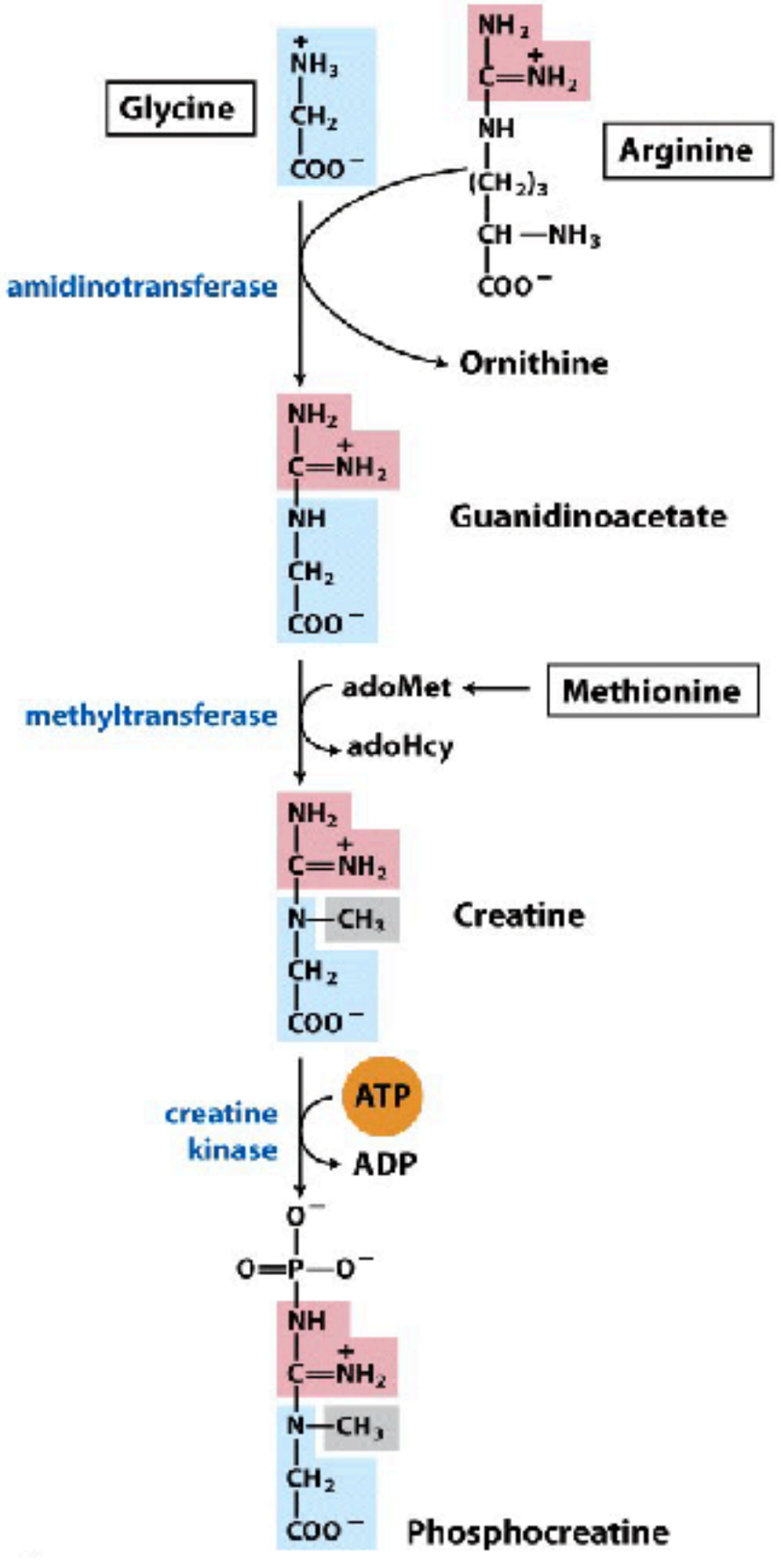
Heme destined for degradation and excretion comes from senescent erythrocytes while a minor component is derived from other cell types.

Senescent erythrocytes are degraded in the spleen. Here, Heme is first converted to bilirubin in a two-step enzymatic process which employs "Biliverdin" as an intermediate. These steps result in oxidation and opening of the Heme ring. Bilirubin is then excreted into the plasma.

Within hepatocytes, one or two molecules of glucuronic acid are attached to bilirubin, generating bilirubin mono- or di-glucuronide. These are excreted into bile *canaliculi* from where they are secreted into the duodenum as a component of bile.

Break

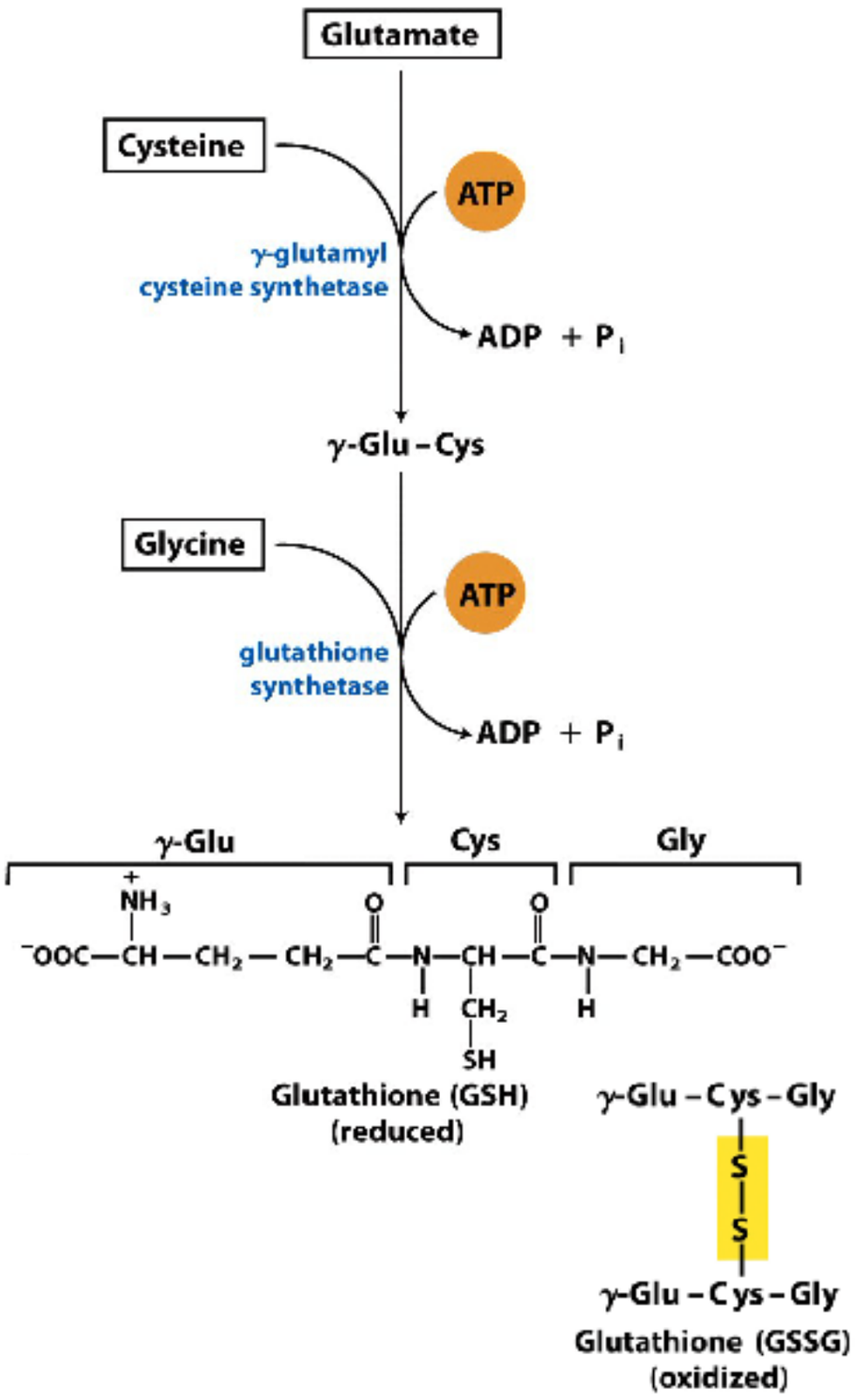
Phosphocreatine



Phosphocreatine, also known as **creatine phosphate** (**CP**) or **PCr** (**Pcr**), is a phosphorylated creatine molecule that serves as a rapidly mobilisable reserve of high-energy phosphates in skeletal muscle, myocard and the brain to recycle adenosine triphosphate, the energy currency of the cell.

Creatine the direct precursor of phosphocreatine is produced from glycine and arginine with participation of methionine as a donor of methyl group

Glutathione

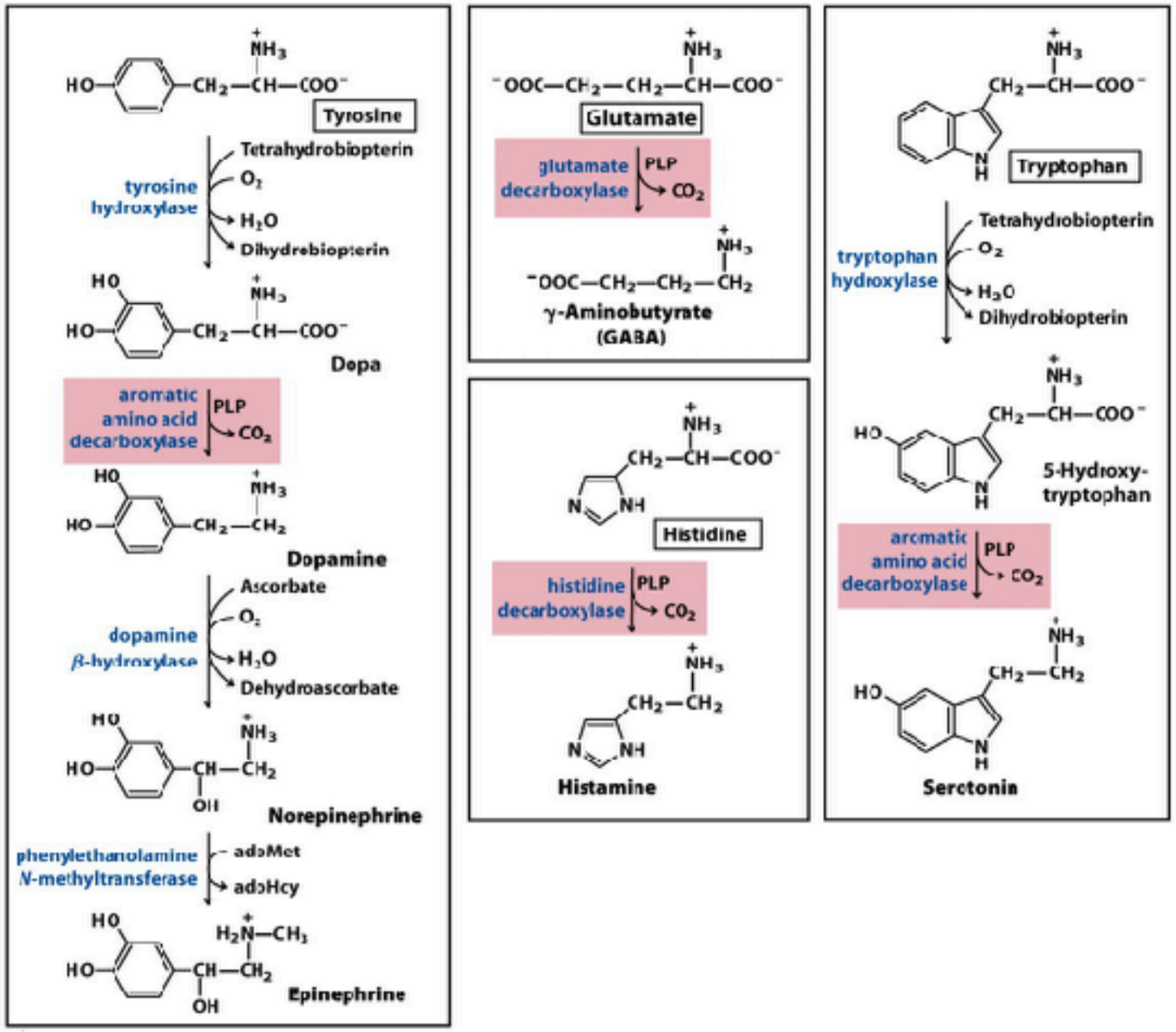


Glutathione (**GSH**) is an antioxidant capable of preventing damage to cellular components caused by reactive oxygen species. It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side chain and cysteine. The carboxyl group of the cysteine residue is attached by normal peptide linkage to glycine.

GSH biosynthesis involves two ATP-dependent steps:

- First, ***gamma*-glutamylcysteine** is synthesized from L-glutamate and cysteine. This conversion requires the enzyme **glutamate–cysteine ligase** (GCL).
- Second, glycine is added to the C-terminal of *gamma*-glutamylcysteine. This condensation is catalyzed by **glutathione synthetase**.

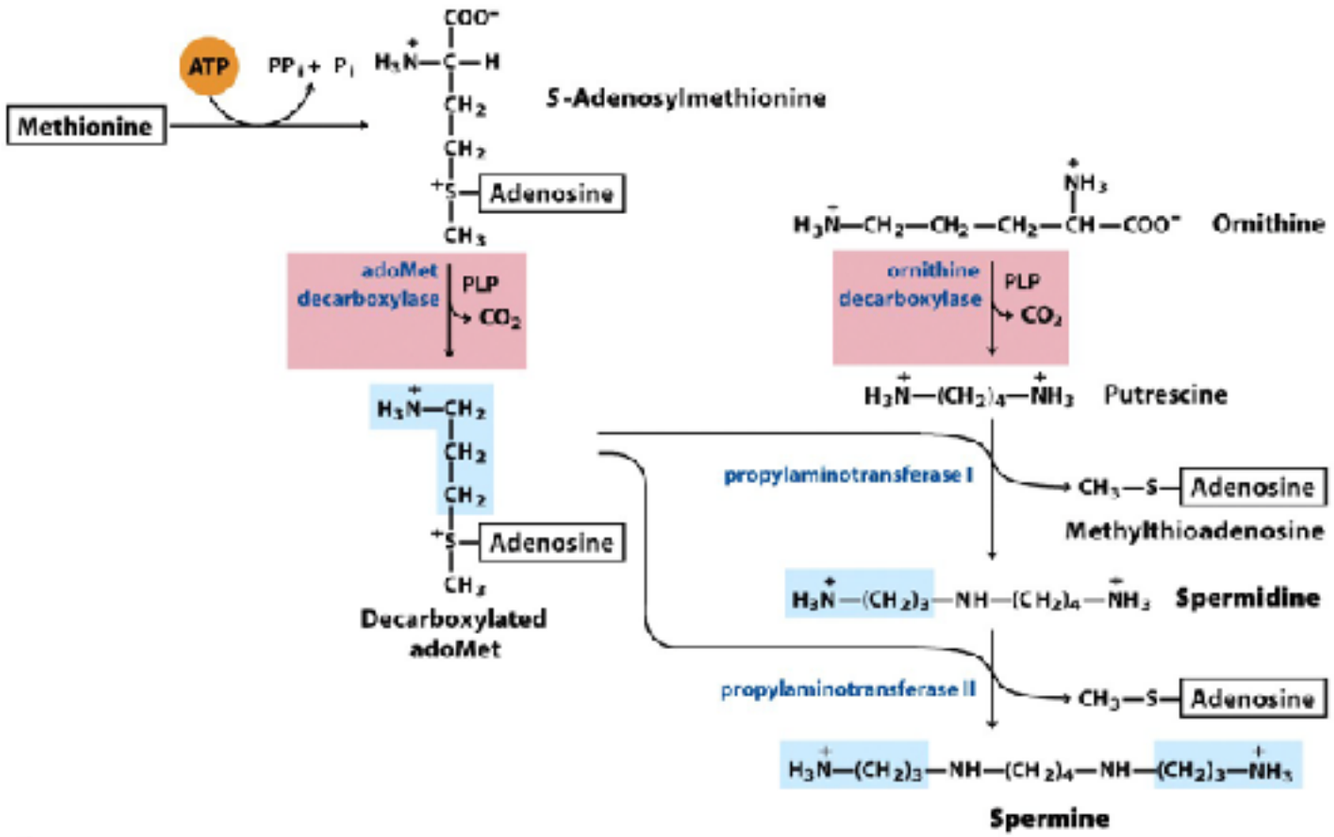
Biogenic Amines



Biogenic amines are organic bases with low molecular weight are produced in many different tissues (for example: adrenaline in adrenal medulla or histamine in mast cells and liver). Many of the **Biogenic amines** function as neurotransmitters (including acetylcholine, serotonin, histamine, epinephrine, norepinephrine, and dopamine) or as agonists for dedicated receptors.

Biogenic amines are produced by modifications (mostly decarboxylation and hydroxylations) of different amino acids.

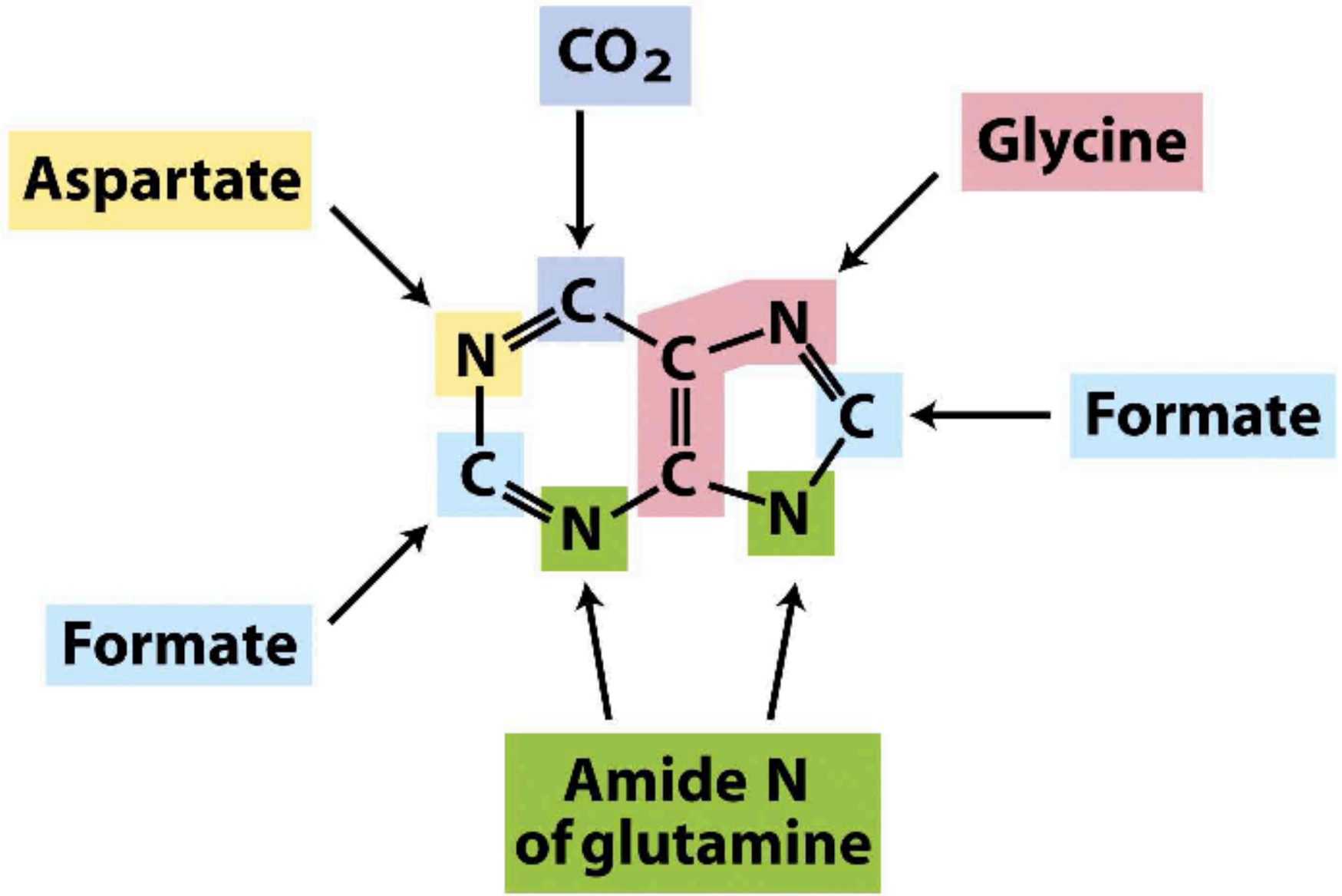
Polyamines



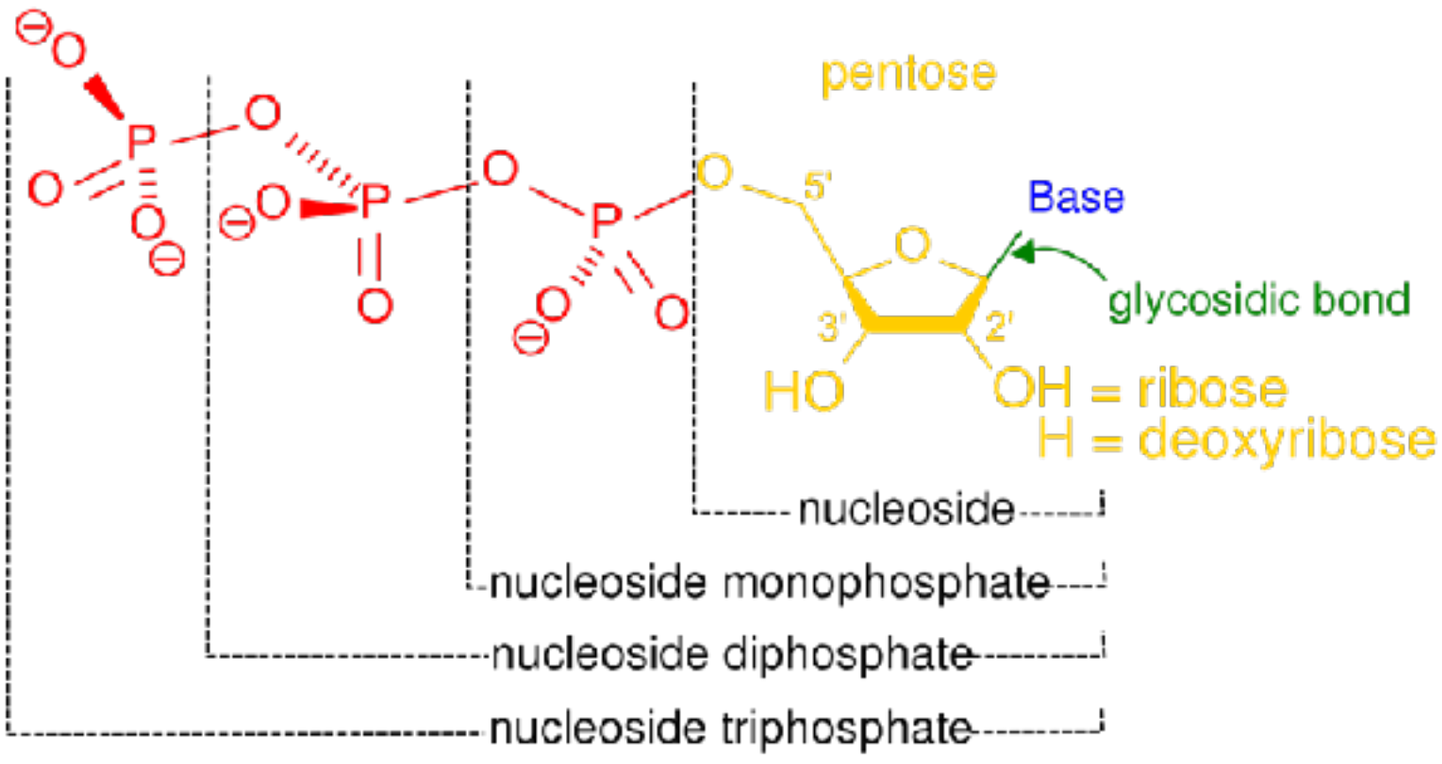
Although it is known that the biosynthesis of polyamines is highly regulated, the biological function of polyamines is only partly elucidated. In their cationic ammonium form, they bind to DNA, and, in structure, they represent compounds with cations that are found at *regularly spaced intervals*. They have also been found to act as promoters of programmed ribosomal frameshifting during translation.

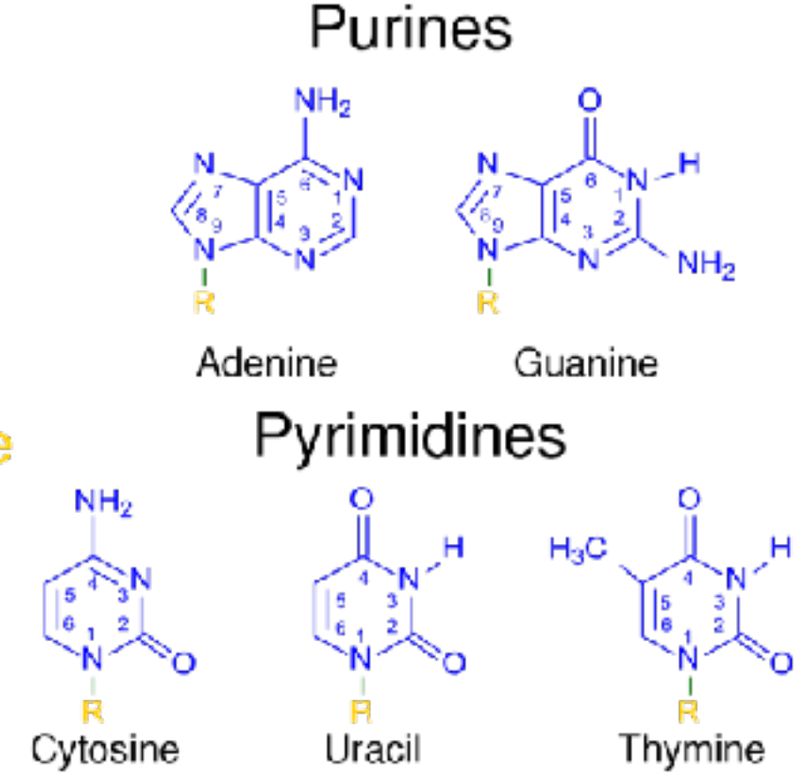
Spermidine and spermine are synthesised starting from ornithine. Ornithine itself is obtained from arginine in the urea cycle. Spermidine is synthesized from putrescine, using an aminopropyl group from decarboxylated *S*-adenosyl-L-methionine (SAM). The reaction is catalyzed by spermidine synthase. Spermine is synthesized from the reaction of spermidine with SAM in the presence of the enzyme spermine synthase.

Biosynthesis of Nucleotides



Biosynthesis of Nucleotides

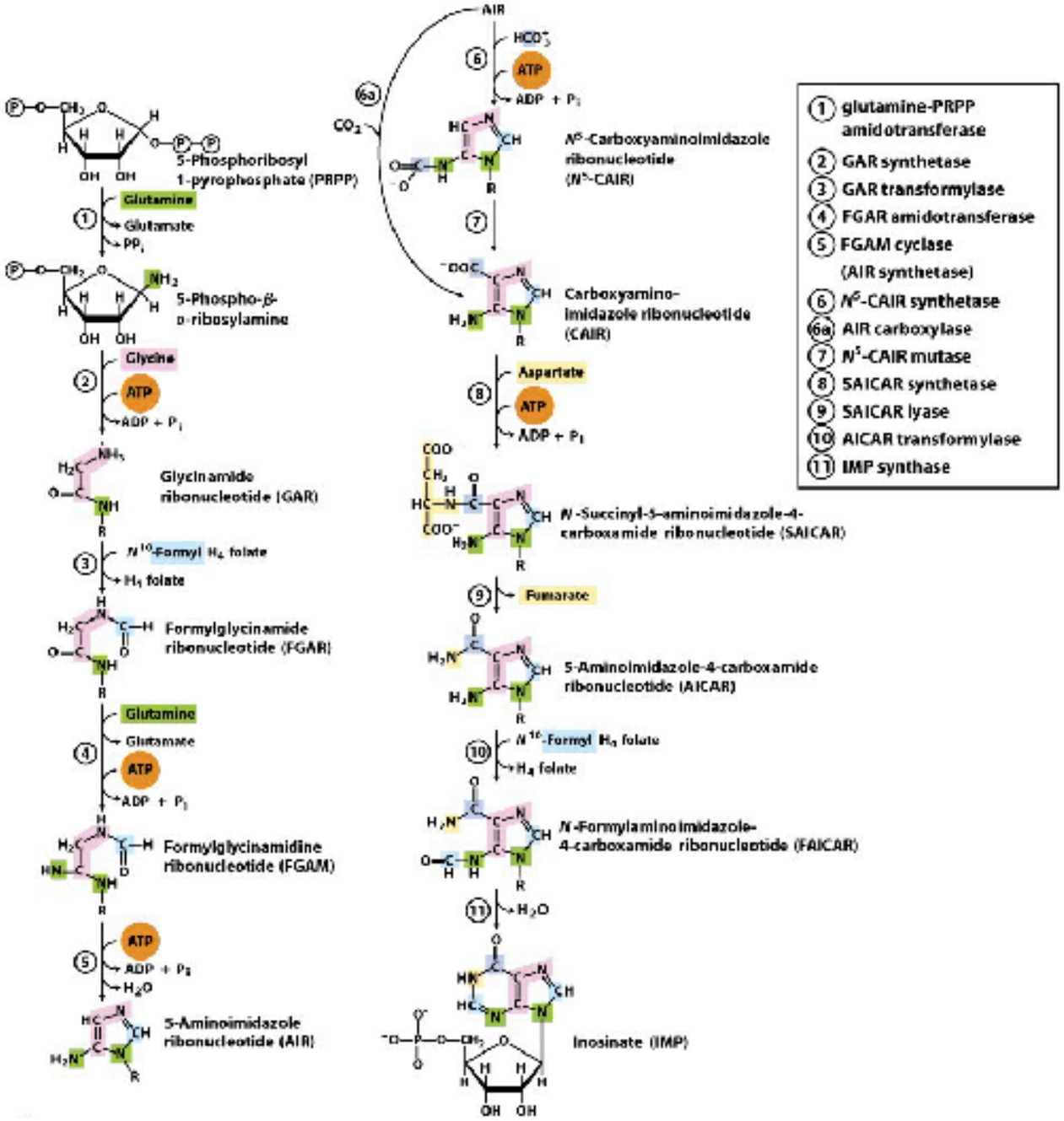




Nucleotides are molecules consisting of a nucleoside and a phosphate group. They are serve as units for forming the **DNA** and the **RNA**, they are precursors of energy molecules such as **ATP**, **GTP**, **CTP**, and **UTP** of second messengers such as **cAMP** and **cGMP** and of key enzyme cofactors such as **CoA**, **FAD**, **FMN**, **NAD+**, and **NADP+**.

Nucleotides contain either a purine or a pyrimidine base. **Pyrimidine** is an aromatic heterocyclic organic compound **Purine** is a heterocyclic aromatic organic compound that consists of a pyrimidine ring fused to an imidazole ring.

Biosynthesis of Purines



The pathway starts with the formation of 5-Phosphoribosyl pyrophosphate (PRPP) from 5-Phosphoribose, which is formed by the **pentose phosphate pathway**.

In the first reaction thePRPP's pyrophosphate group is displaced by an amide donated from either glutamine .

Next, a glycine is incorporated

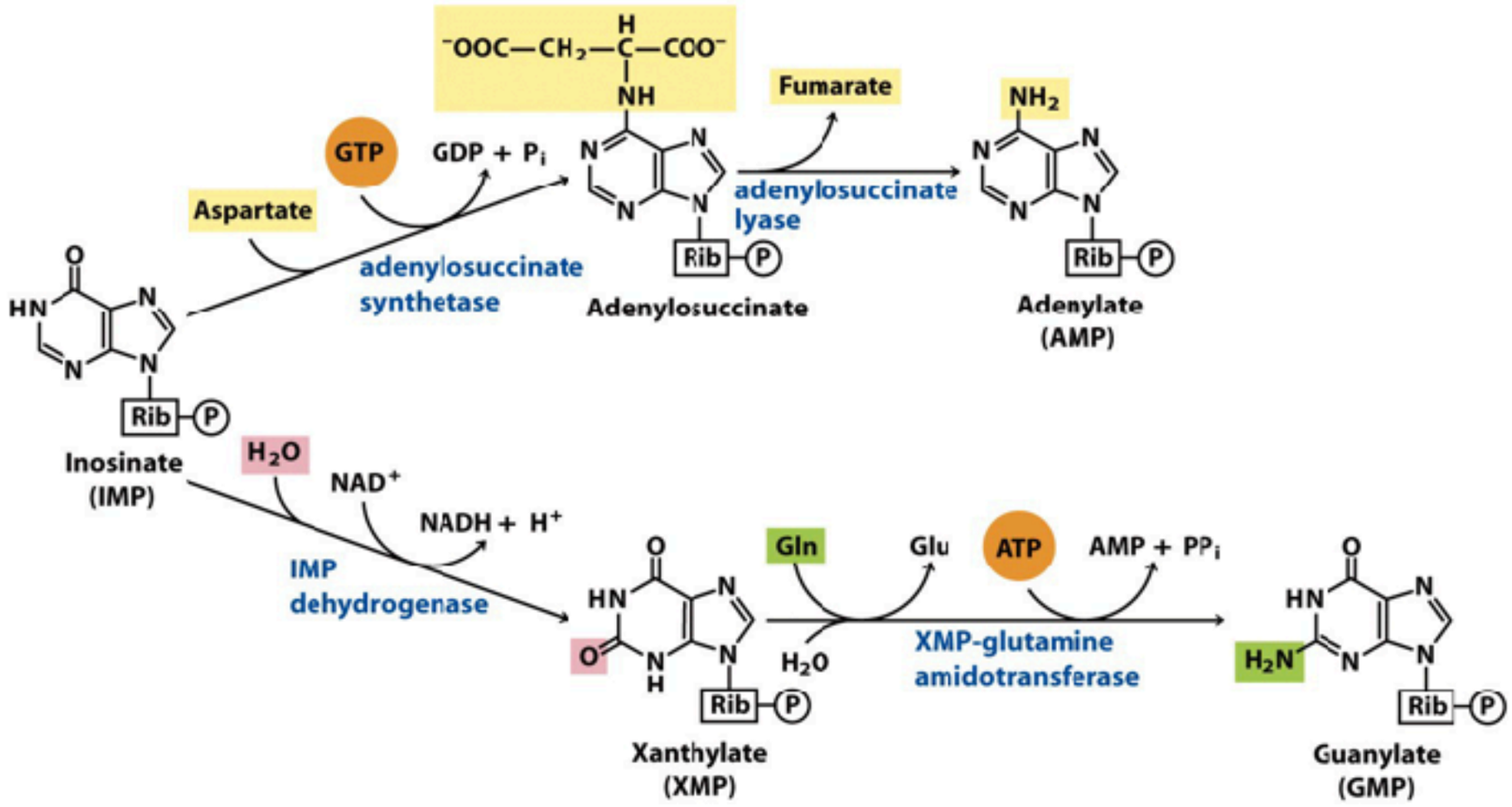
One-carbon unit from folic acid coenzyme N_{10}-formyl-THF is then added

A second NH_2 group is transferred from a glutamine to the first carbon of the glycine unit.

The ring is closed

Carboxylation of the second carbon of the glycine unit is concomitantly added. This new carbon is modified by the additional of a third NH_2.
Finally, a second one-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).

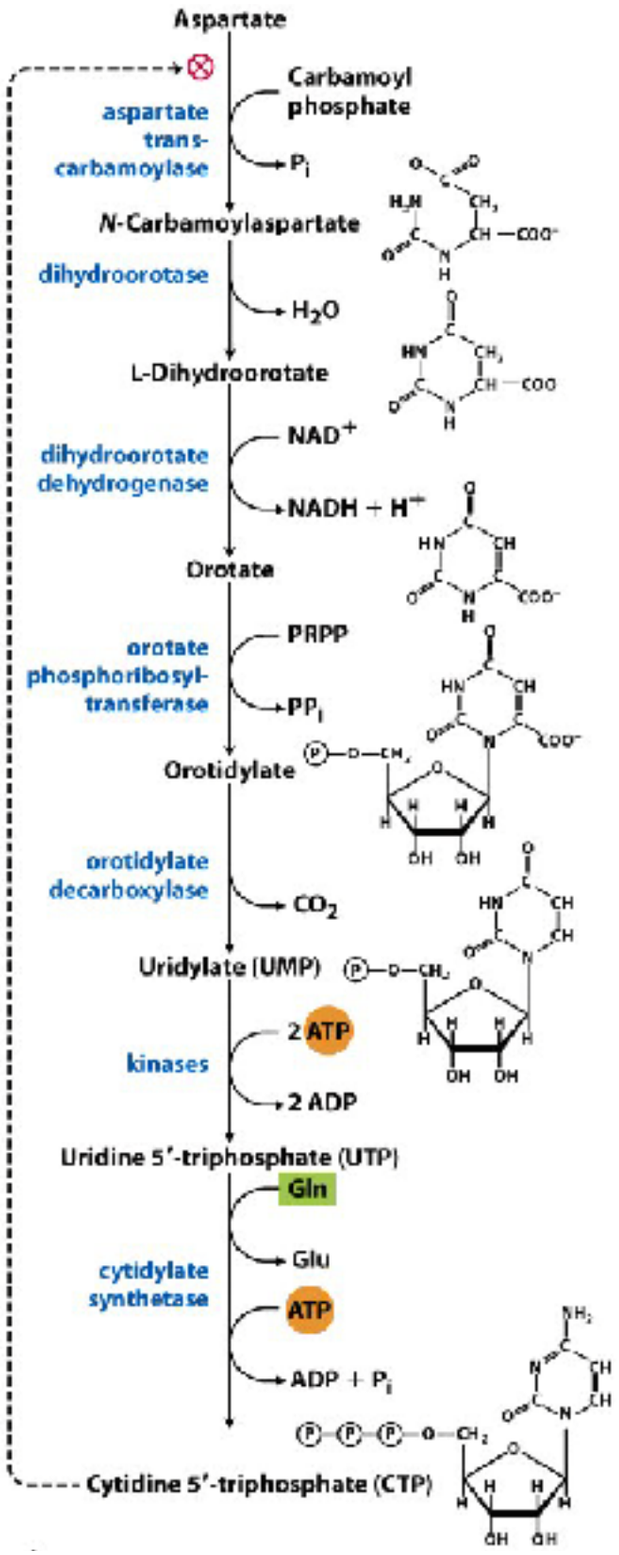
Biosynthesis of Purines



IMP is converted to **adenosine monophosphate** in two steps.
First, GTP hydrolysis fuels the addition of aspartate to IMP by adenylosuccinate synthase, substituting the carbonyl oxygen for a nitrogen and forming the intermediate adenylosuccinate.
Fumarate is then cleaved off forming adenosine monophosphate. This step is catalysed by **adenylosuccinate lyase**.

IMP is converted to **guanosine monophosphate** by the oxidation of IMP forming xanthylate, followed by the insertion of an amino group at C_2. NAD^+ is the electron acceptor in the oxidation reaction. The amide group transfer from glutamine is fuelled by ATP hydrolysis.

Biosynthesis of Pyrimidines



The synthesis of the pyrimidines starts with the formation of **carbamoyl phosphate** from glutamine and CO_2.

Next, **aspartate carbamoyltransferase** catalyzes a condensation reaction between aspartate and carbamoyl phosphate to form **carbamoyl aspartic acid**.

This is cyclized into **4,5-dihydroorotic acid** by **dihydroorotase**.

The latter is converted to **orotate** by **dihydroorotate oxidase**.

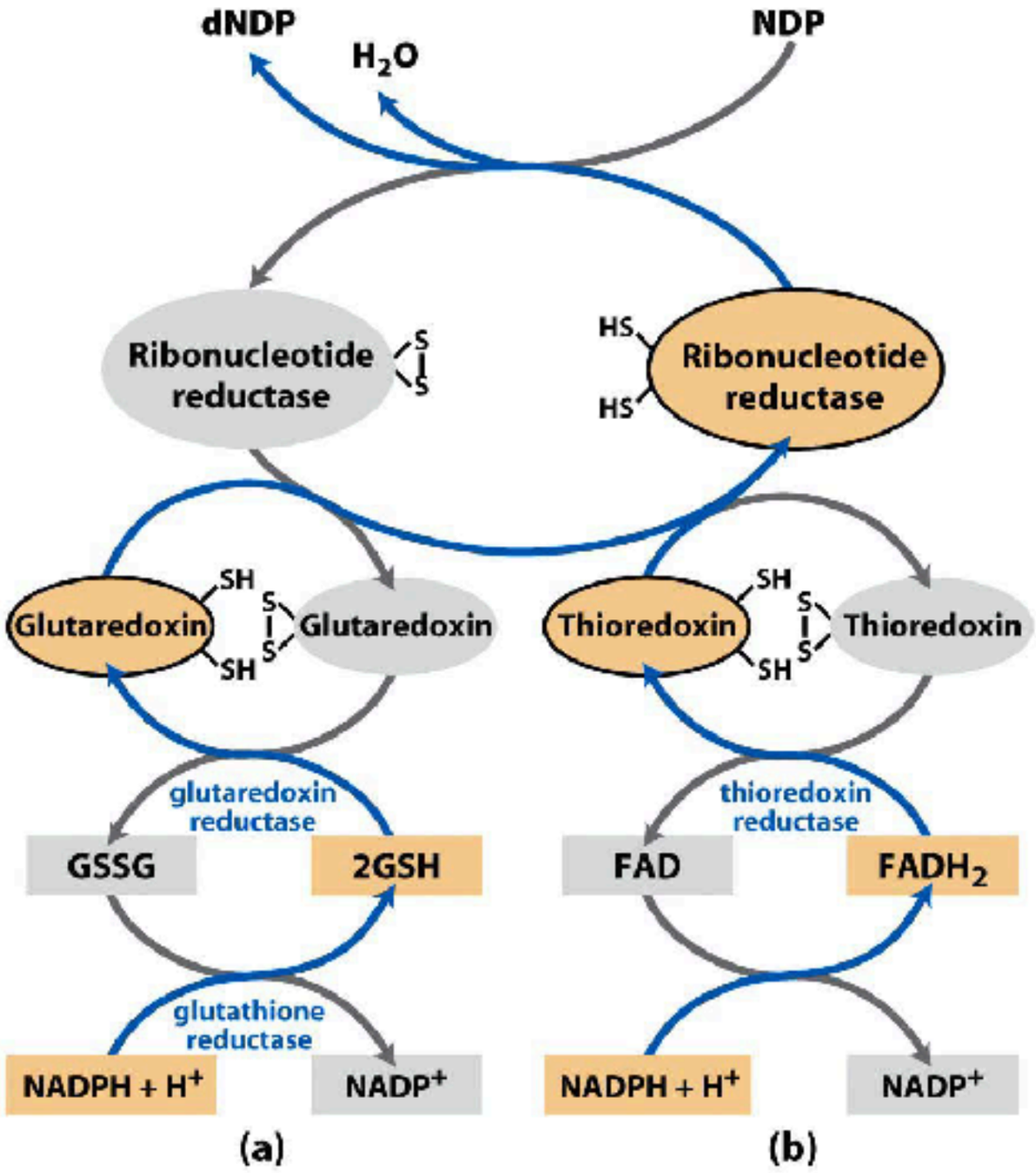
Orotate is covalently linked with a phosphorylated ribosyl unit.

Orotidylate is decarboxylated to form **uridine monophosphate** (**UMP**).

UMP is phosphorylated by two **kinases** to **uridine triphosphate** (**UTP**) via two sequential reactions with ATP.

CTP is subsequently formed by amination of UTP by the catalytic activity of **CTP synthetase**. Glutamine is the NH_3 donor and the reaction is fueled by ATP hydrolysis.

Reduction of Ribonucleotides to Deoxyribonucleotides



Ribonucleotide reductase (RNR) catalyzes the formation of deoxyribonucleotides from ribonucleotides by removing the 2'-hydroxyl group of the ribose ring of nucleoside diphosphates. Following a single reduction, RNR requires electrons donated from the dithiol groups of the proteins thioredoxin or glutaredoxin. Regeneration of thioredoxin occurs when nicotinamide adenine NADPH provides two hydrogen atoms to FAD. FADH2 is then used to reduce the disulfide groups of thioredoxin.

Similarly regeneration of glutaredoxin occurs when nicotinamide adenine NADPH is used to reduce glutathione. Reduced glutathione is the oxydised to reduce the disulfide groups of glutaredoxin

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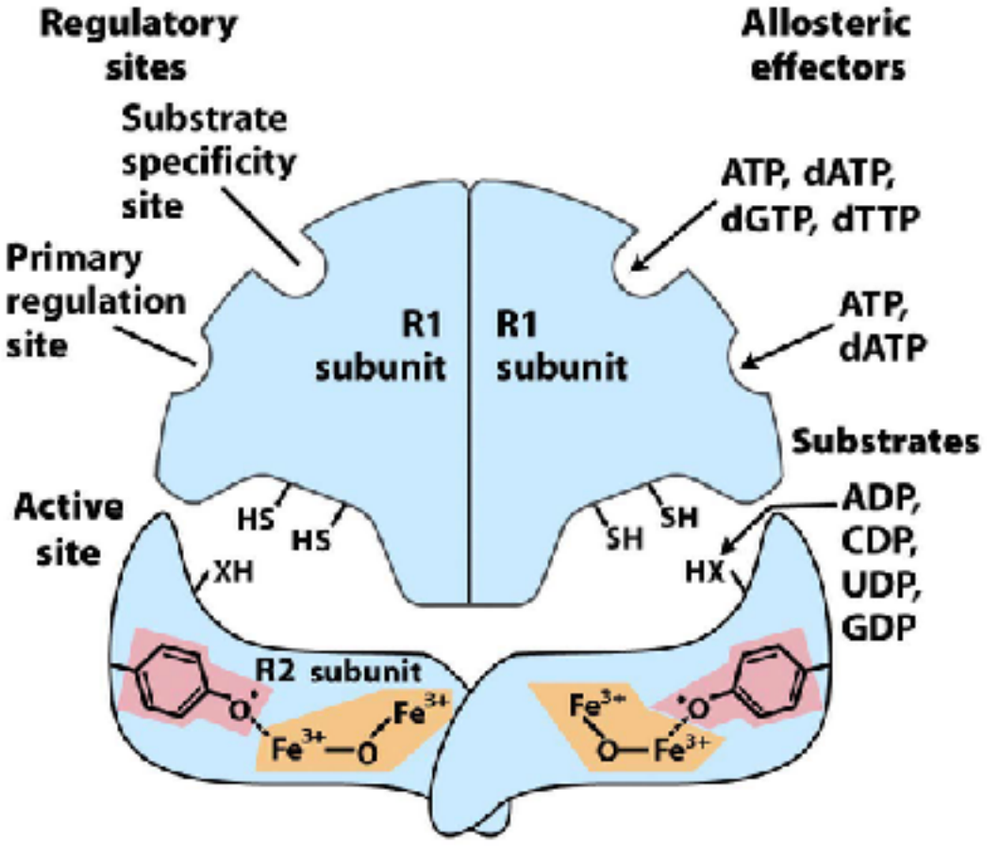
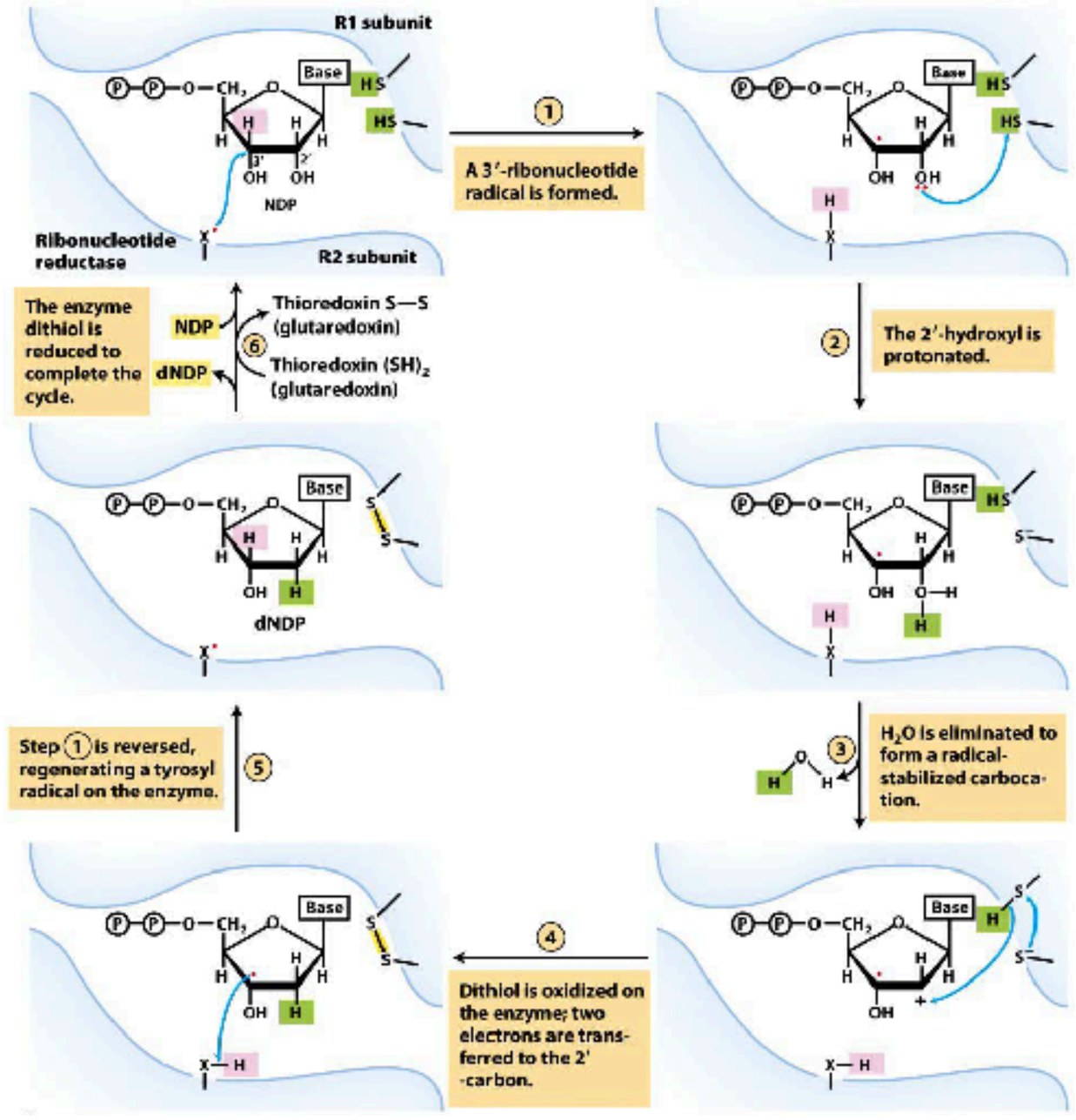
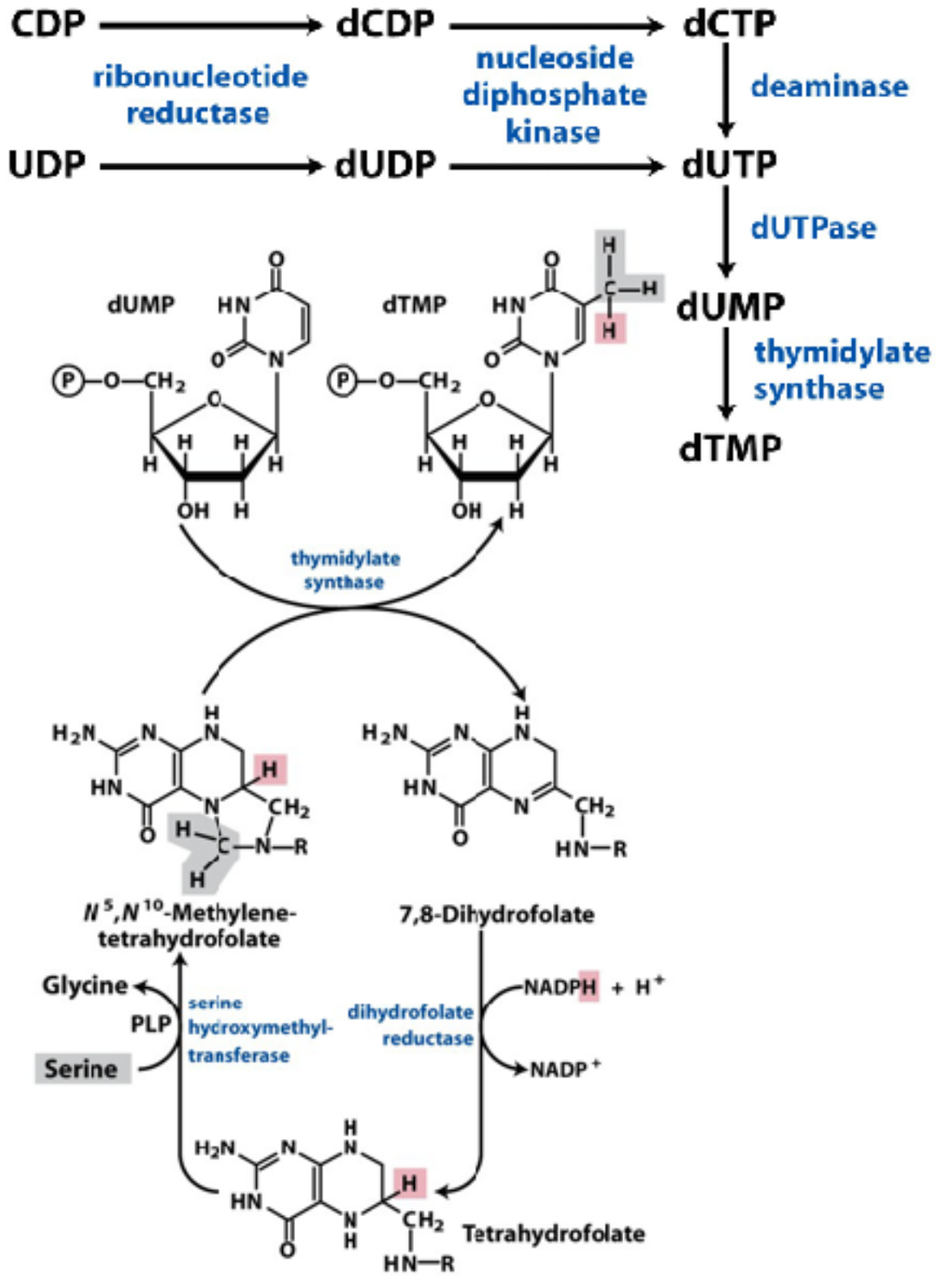


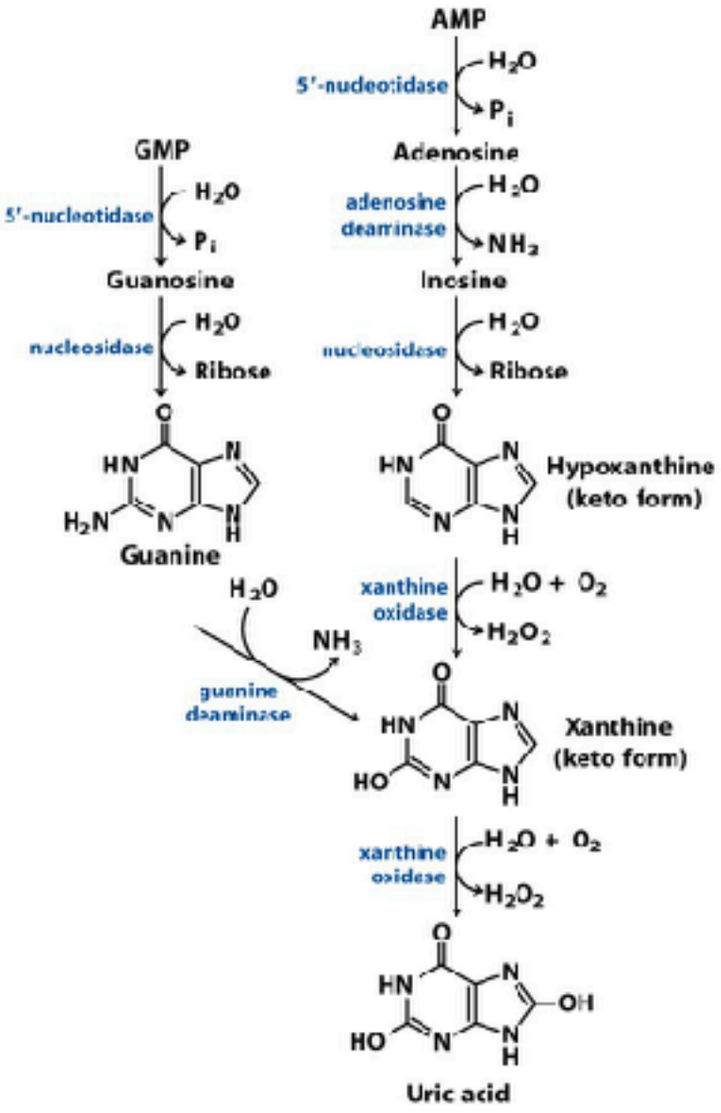


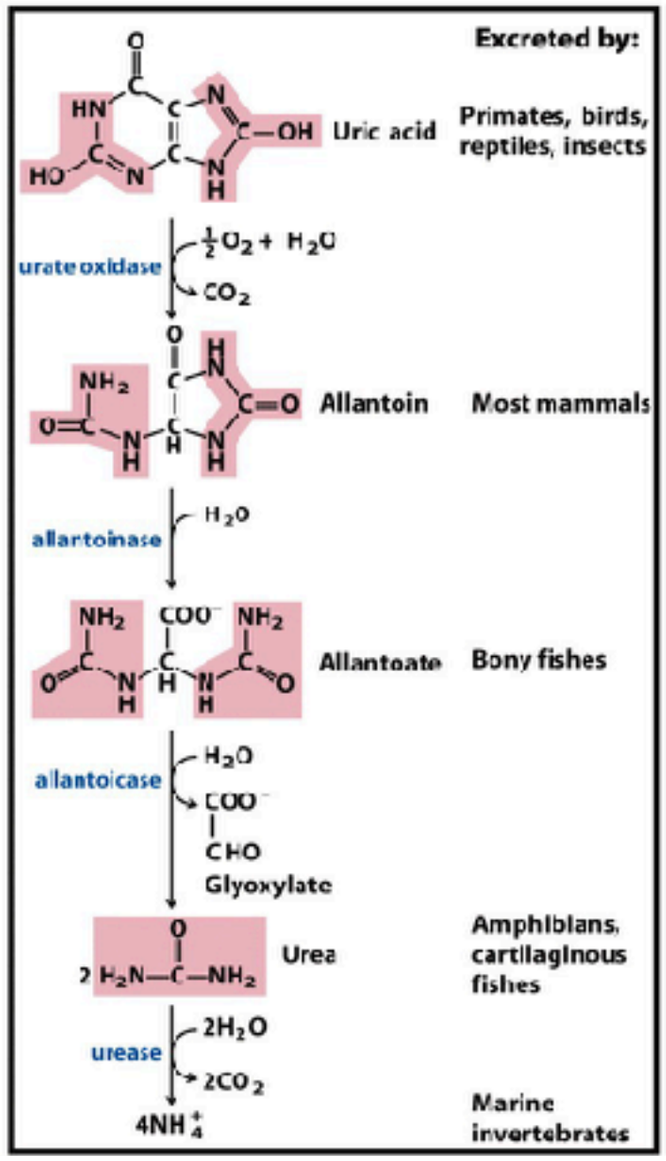
Biosynthesis of Thymidylate



Thymidylate dTMP is a component of DNA and is it synthesised *de novo* from deoxyuridylate and methylenetetrahydrofolate by thymidylate synthase (TYMS) by means of reductive methylation, yielding dihydrofolate as a secondary product. The *de novo* synthesis pathway forms a nuclear complex at the nuclear lamina at sites of DNA replication. DNA polymerases do not distinguish between deoxyuridylate and thymidylate, and when thymidylate synthesis is insufficient, uracil is misincorporated into DNA. This can lead to futile cycles of DNA repair and subsequent DNA single- and double-strand breaks. Impaired *de novo* thymidylate synthesis can result in neural tube defects, megaloblastic anaemia and immunodeficiency. Inhibitors of TYMS and DHFR impair cell replication and have been used in the treatment of cancer.

Purines disposal





Purines are degraded to **Uric acid.**

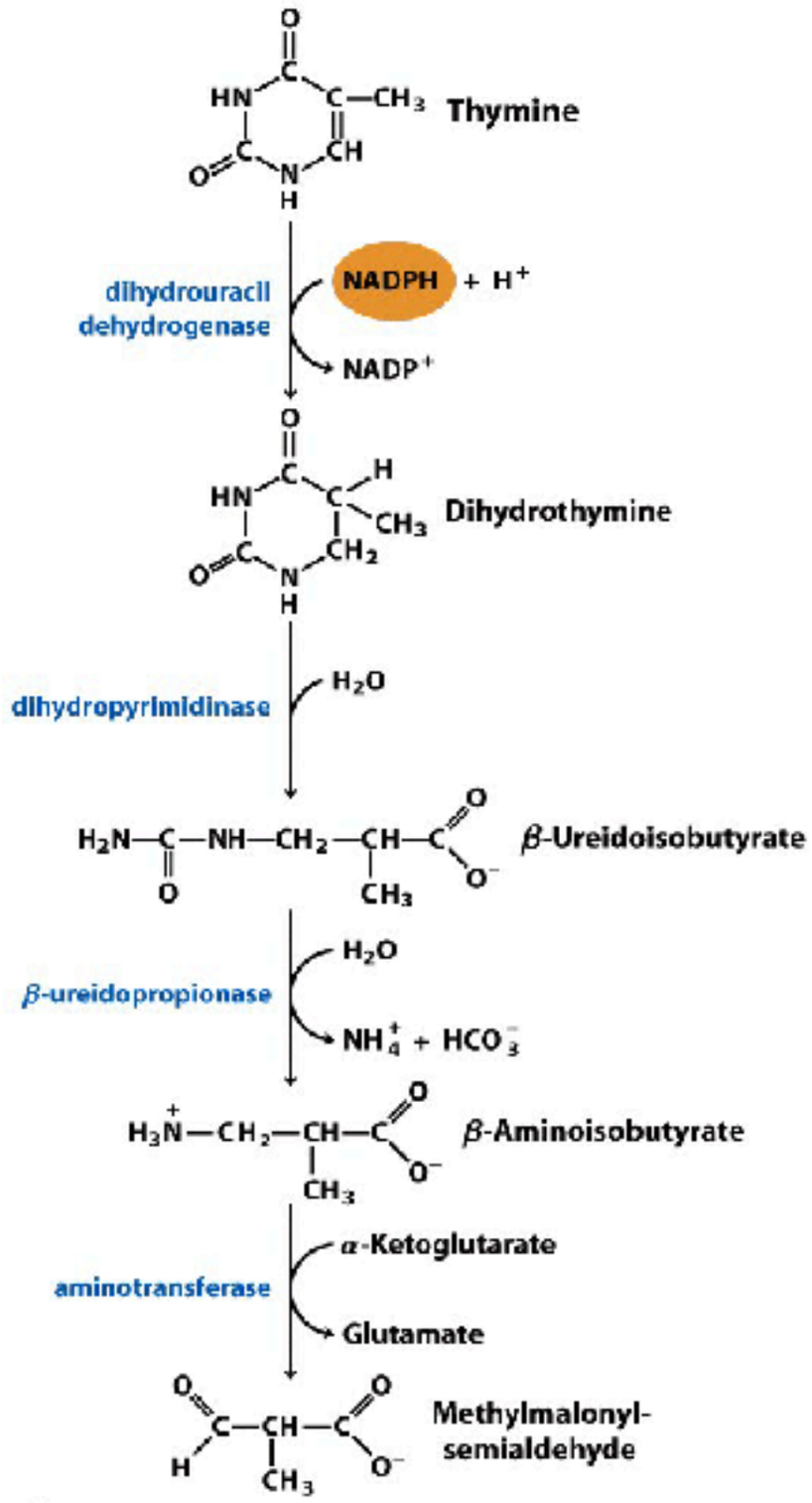
First the phosphate is hydrolysed by **5'-nucleotidase**.

Then **adenosine** is deaminated to form **inosine**.

Inosine loses the ribose to form **hypoxanthine** that through two oxidative reactions is converted into **uric acid**.

GMP follows a similar pathway having one deamination less in its degradative pathway.

Pyrimidines disposal



Pyrimidines are ultimately catabolized to **CO_2**, **H_2O**, and **urea**. Cytosine can be broken down to uracil, which can be further broken down to N-carbamoyl-β-alanine, and then to beta-alanine, CO_2, and ammonia by **beta-ureidopropionase**. Thymine is broken down into **β-aminoisobutyrate** which can be further broken down into **methylmalonyl semialdheyde** (an intermediate of valine catabolism) that is then converted into **succinyl-CoA** and thus enters the citric acid cycle.

Genetic defects in the catabolism of nucleotides lead to diseases in humans. For example defects in the enzyme adenosine deaminase lead to a severe combined immune deficiency (ADA-SCID).
This deficiency results in an accumulation of deoxyadenosine, which, in turn, leads to a buildup of dATP in all cells, which inhibits ribonucleotide reductase and prevents DNA synthesis, so cells are unable to divide. Since developing T cells and B cells are some of the most mitotically active cells, they are highly susceptible to this condition.

Salvage Pathways

Nucleotide salvage pathways are used to recover bases and nucleosides that are formed during degradation of RNA and DNA. The salvaged products can then be converted back into nucleotides.

Pyrimidines

Uridine phosphorylase or **pyrimidine-nucleoside phosphorylase** adds ribose 1-phosphate to the free base uracil, forming **uridine**. **Uridine kinase** (aka uridine–cytidine kinase) can then phosphorylate this nucleoside into **uridine monophosphate** (**UMP**)

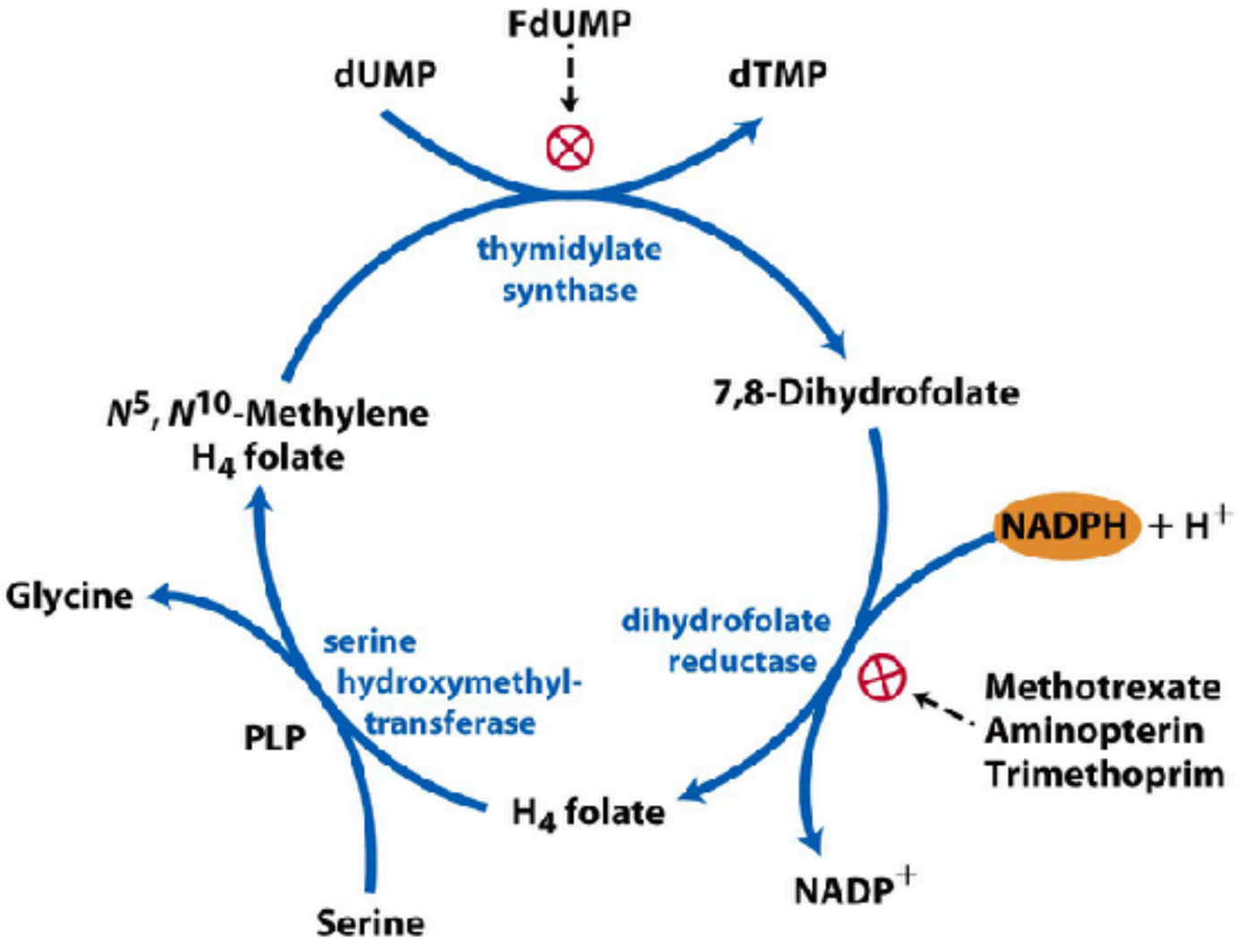
Thymidine phosphorylase or **pyrimidine-nucleoside phosphorylase** adds 2-deoxy-alpha-D-ribose 1-phosphate to thymine, forming **thymidine**. **Thymidine kinase** can then phosphorylate this compound into **thymidine monophosphate** (**TMP**).

The nucleosides cytidine and deoxycytidine can be salvaged along the uracil pathway by **cytidine deaminase**, which converts them to **uridine** and **deoxyuridine**, respectively. Alternatively, **uridine–cytidine kinase** can phosphorylate them into **cytidine monophosphate** (**CMP**) or **deoxycytidine monophosphate** (**dCMP**).

Purines

Phosphoribosyltransferases add Phosphoribosyl pyrophosphate, to bases, creating nucleoside monophosphates. There are two types of phosphoribosyltransferases: **adenine phosphoribosyltransferase** (APRT) and **hypoxanthine-guanine phosphoribosyltransferase** (HGPRT).

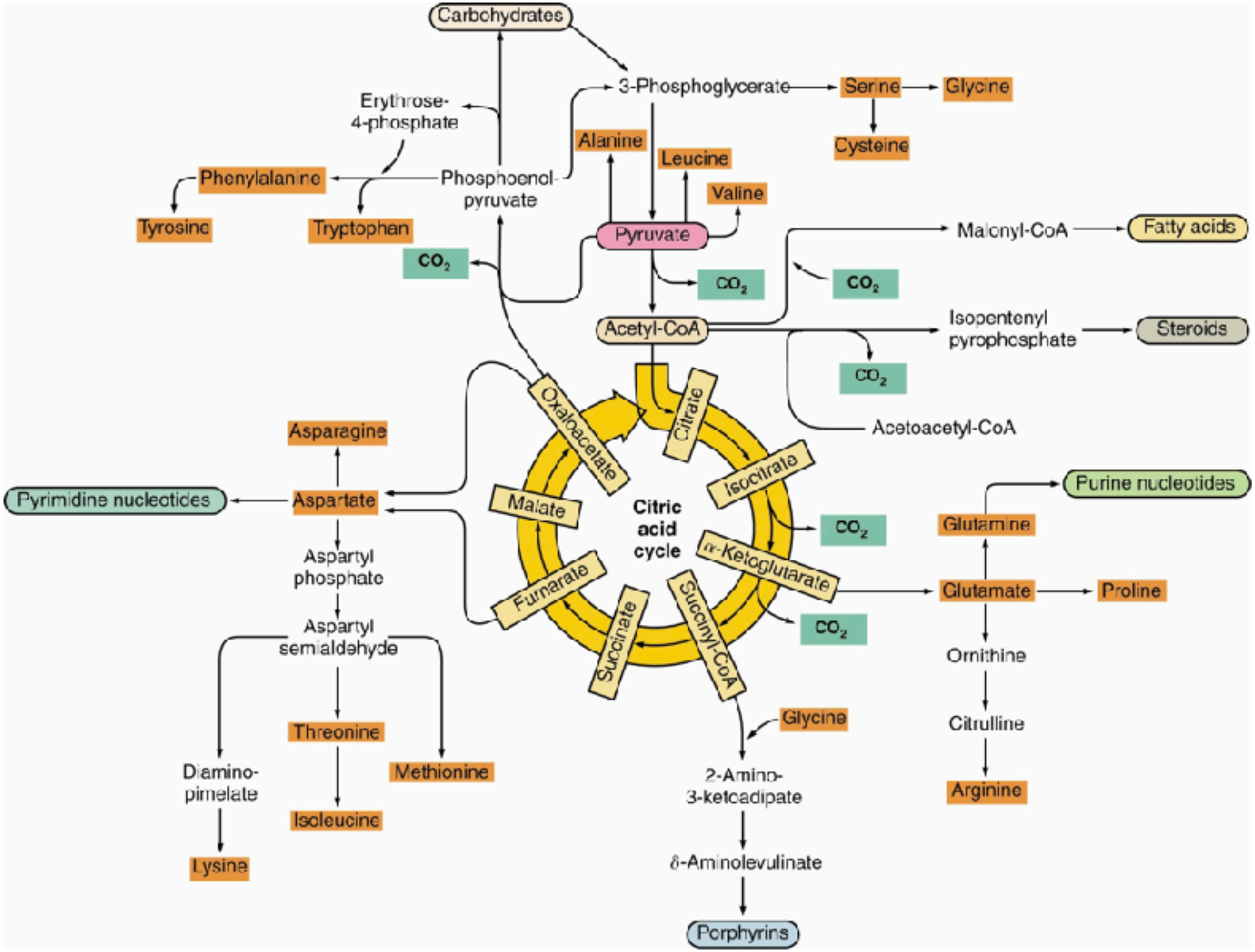
Some chemotherapics target nucleotide metabolism



Cancer cells usually grow at a faster rate than their normal counterparts. As a consequence of their need for nucleotides (for their DNA replication and RNA transcription), cancer cells are more susceptible to the inhibition of nucleotide synthesis.

For example some commonly used anti cancer drugs (i.e., methotrexate, aminopterin , trimethoprim and, fluodeoxyrouracile [FdUMP]) inhibit thymidylate synthesis.

General overview



Take home messages

- Amino acids are produced by transamination of intermediates of glycolysis, TCA cycle or pentose phosphate pathway.

- Porphyrines (Heme) phosphocreatine, neurotransmitters, polyamines, and glutathione are produced from amino acids

- Nucleotides are synthesised by de novo pathways that involve amino acids as precursors or by salvage pathways