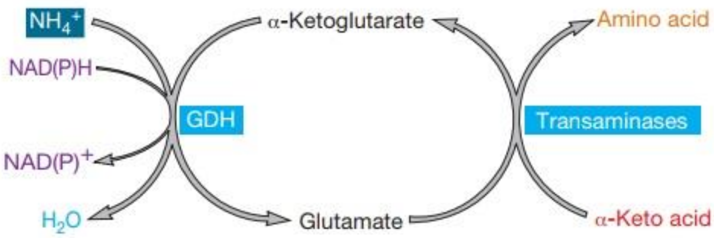
Ammonia assimilation

Biochemistry of Ammonia Assimilation:

Reduced nitrogen in the form of NH_4^+ is assimilated into amino acids and then into other nitrogen-containing biomolecules. Two amino acids, **glutamate** and **glutamine**, provide the critical entry point. Glutamate is the source of amino groups for most other amino acids, through transamination reactions. The amide nitrogen of glutamine is a source of amino groups in a wide range of biosynthetic processes. In most types of cells, and in extracellular fluids in higher organisms, one or both of these amino acids are present at higher concentrations-sometimes an order of magnitude or more higher-than other amino acids. An *Escherichia coli* cell requires so much glutamate that this amino acid is one of the primary solutes in the cytosol. Its concentration is regulated not only in response to the cell's nitrogen requirements but also to maintain an osmotic balance between the cytosol and the external medium. The biosynthetic pathways to glutamate and glutamine are simple, and all or some of the steps occur in most organisms. Ammonia is initially incorporated into carbon skeletons by one of two mechanisms: **reductive amination** or by the **glutamine synthetase-glutamate synthase (GS-GOGAT) system** (GS-GOGAT = glutamine synthetase - glutamine:2-oxoglutarate aminotransferase). Once incorporated, the nitrogen can be transferred to other carbon skeletons through transamination reactions by enzymes called transaminases. The **reductive amination pathway** involves the formation of glutamate from α-ketoglutarate in a single step process, catalyzed in many bacteria and fungi, by the enzyme **glutamate dehydrogenase** when the ammonia concentration is high.

$$\alpha\text{-ketoglutarate} + NH_4^+ + \text{NADPH (NADH)} + H^+ \rightleftharpoons \text{glutamate} + \text{NADP}^+(\text{NAD}^+) + H_2O$$



GDH = glutamate dehydrogenase

Once glutamate has been synthesized, the newly formed α- amino group can be transferred to other carbon skeletons by transamination reactions to form different amino acids. In eukaryotic cells, glutamate dehydrogenase is located in the mitochondrial matrix. The reaction equilibrium favors reactants, and the Km for NH_4^+(~1 mM) is so high that the reaction probably makes only a modest contribution to ammonia assimilation into amino acids and other metabolites. Concentrations of NH_4^+ requires to be high enough for the glutamate dehydrogenase reaction to make a significant contribution to glutamate levels and generally occur only when NH_3 is added to the soil or when organisms are grown in a laboratory in the presence of high NH_3 concentrations.

The most important pathway for the assimilation of NH_4^+ into glutamate is the **glutamine synthetase-glutamate synthase (GS-GOGAT) system** which requires two reactions. In the **first reaction**,

glutamine synthetase catalyzes the reaction of glutamate and NH_4+ to yield glutamine. This reaction takes place in two steps, with enzyme-bound γ-glutamyl phosphate as an intermediate.

$$\text{(1)}\quad \text{Glutamate} + \text{ATP} \longrightarrow \gamma\text{-glutamyl phosphate} + \text{ADP}$$

$$\text{(2)}\quad \gamma\text{-Glutamyl phosphate} + NH_4^+ \longrightarrow \text{glutamine} + P_i + H^+$$

$$\textit{Sum:}\quad \text{Glutamate} + NH_4^+ + \text{ATP} \longrightarrow \text{glutamine} + \text{ADP} + \text{Pi} + H^+$$

Glutamine synthetase is found in all organisms. In addition to its importance for NH_4+ assimilation in bacteria, it has a central role in amino acid metabolism in mammals, converting toxic free NH_4+ to glutamine for transport in the blood. In bacteria and plants, glutamate is produced from glutamine in the **second reaction** catalyzed by **glutamate synthase**. α-Ketoglutarate, an intermediate of the citric acid cycle, undergoes reductive amination with glutamine as nitrogen donor:

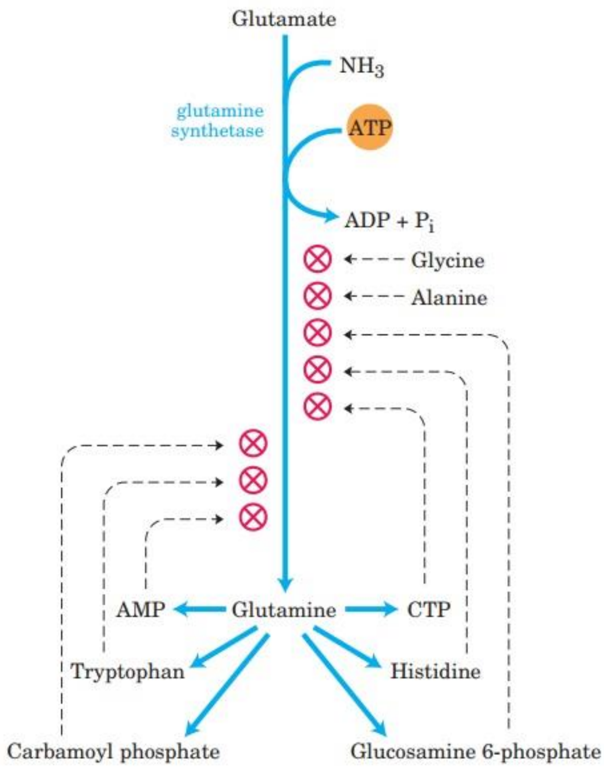
$$\alpha\text{-Ketoglutarate} + \text{Glutamine} + \text{NADPH} + H^+ \longrightarrow 2\ \text{Glutamate} + \text{NADP}^+$$

The net reaction of glutamine synthetase and glutamate synthase is:

$$\alpha\text{-Ketoglutarate} + NH_4 + \text{NADPH} + \text{ATP} \longrightarrow \text{L-Glutamate} + \text{NADP}^+ + \text{ADP} + \text{Pi}$$

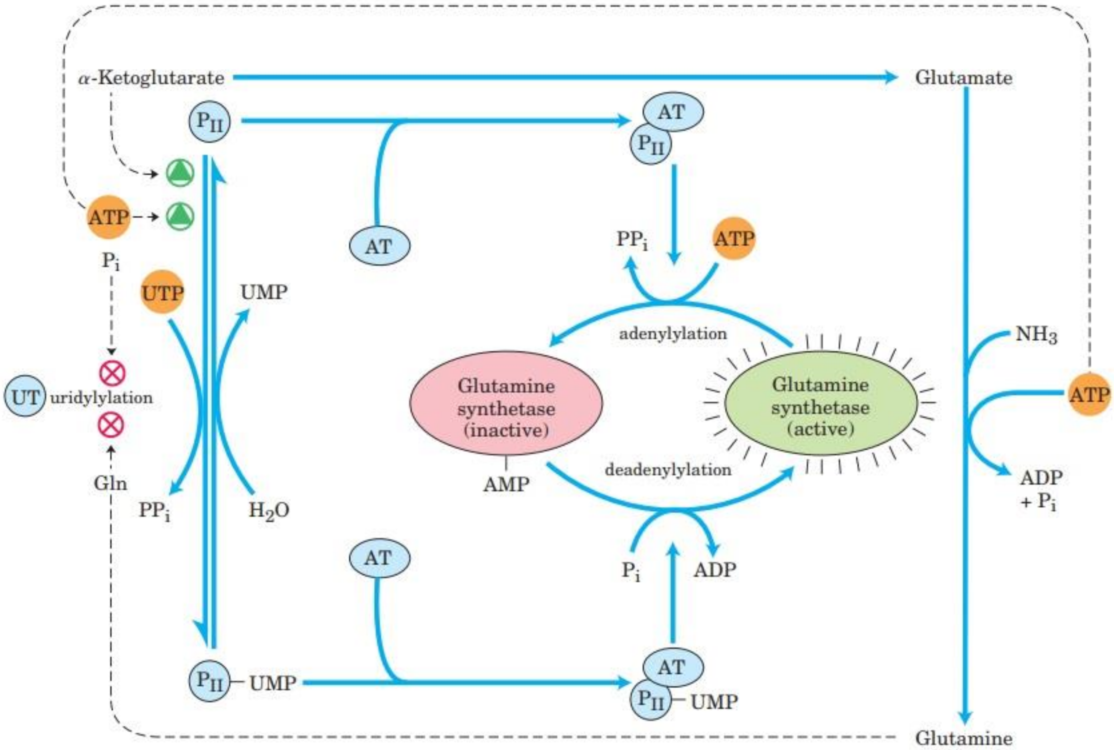
Glutamate synthase is not present in animals, which, instead, maintain high levels of glutamate by processes such as the transamination of -ketoglutarate during amino acid catabolism.

Regulation of Ammonia Assimilation:



Ammonia assimilation is regulated by regulating the activity of glutamine synthetase in virtually all organisms—not surprising, given its central metabolic role as an entry point for reduced nitrogen. In bacteria such as *E. coli*, the regulation is unusually complex. The enzyme has 12 identical subunits of Mr 50,000 each and is regulated both allosterically and by covalent modification. Alanine, glycine, and at least six end products of glutamine metabolism (AMP, CTP, Histidine, Tryptophan, Carbamoyl phosphate, and Glucosamine 6-phosphate) are allosteric inhibitors of the enzyme. Each inhibitor alone produces only partial inhibition, but the effects of multiple inhibitors are more than additive, and all eight together virtually shut down the enzyme. This control mechanism provides a constant adjustment of glutamine levels to match immediate metabolic requirements.

Inhibition by covalent modification is carried out by adenylylation of (addition of AMP) to Tyr397, located near the enzyme's active site. This covalent modification increases sensitivity to the allosteric inhibitors, and activity decreases as more subunits are adenylylated. Both adenylylation and deadenylylation are promoted by **adenylyltransferase**, part of a complex enzymatic cascade that responds to levels of glutamine, -ketoglutarate, ATP, and Pi. The activity of adenylyltransferase is modulated by binding to a regulatory protein called **PII**, and the activity of PII, in turn, is regulated by covalent modification (uridylylation), again at a Tyr residue. The adenylyltransferase complex with uridylylated PII (PII-UMP) stimulates deadenylylation, whereas the same complex with deuridylylated PII stimulates adenylylation of glutamine synthetase. Both uridylylation and deuridylylation of PII are brought about by a single enzyme, uridylyltransferase. Uridylylation is inhibited by binding of glutamine and Pi to uridylyltransferase and is stimulated by binding of -ketoglutarate and ATP to PII.



The regulation does not stop there. The uridylylated PII also mediates the activation of transcription of the gene encoding glutamine synthetase, thus increasing the cellular concentration of the enzyme; the deuridylylated PII brings about a decrease in transcription of the same gene. This mechanism involves an interaction of PII with additional proteins involved in gene regulation. The net result of this elaborate system of controls is a decrease in glutamine synthetase activity when glutamine levels are high, and an increase in activity when glutamine levels are low and α-ketoglutarate and ATP (substrates for the synthetase reaction) are available. The multiple layers of regulation permit a sensitive response in which glutamine synthesis is tailored to cellular needs.

Anammox

Aerobic ammonia-oxidizing prokaryotes such as *Nitrosomonas* oxidize ammonia into nitrite and ultimately to nitrate by nitrite oxidizers (by *Nitrobacter*) by the process known as nitrification. But NH_3 can also be oxidized under anaerobic conditions. This process of oxidizing ammonia in anoxic condition is called *anammox* (for *an*oxic *amm*onia *ox*idation) and is catalyzed by an unusual group of obligately anaerobic *Bacteria*. Ammonia is oxidized in the anammox reaction using NO_2^- as the electron acceptor to yield N2:

$$NH_4^+ + NO_2^- \longrightarrow N_2 + 2H_2O$$

A major anammox organism, *Brocadia anammoxidans*, is a species of the *Planctomycetes* phylum of *Bacteria*. *Planctomycetes* are unusual *Bacteria* in that they lack peptidoglycan and their cytoplasm contains membrane-enclosed compartments of various types. In cells of *B. anammoxidans* this compartment is the *anammoxosome*, and it is within this structure that the anammox reaction occurs. In addition to *Brocadia*, several other genera of anammox bacteria are known, including *Kuenenia*, *Anammoxoglobus*, *Jettenia*, and *Scalindua*, all of which are related to *Brocadia* and also contain anammoxosomes. Like aerobic ammonia oxidizers, anammox bacteria are also autotrophs, but they do not fix CO_2 using the pathways employed by aerobic ammonia oxidizers. Instead, anammox bacteria fix CO_2 by way of the acetyl-CoA pathway, an autotrophic pathway widespread among some obligately anaerobic autotrophic *Bacteria* and *Archaea*.

The **anammoxosome** is a unit membrane–enclosed structure and in this respect is technically an organelle in the eukaryotic sense of the term. Lipids that form the anammoxosome membrane are not the typical lipids of *Bacteria* but instead consist of fatty acids constructed of multiple cyclobutane (C_4) rings bonded to glycerol by both ester and ether bonds. These *ladderane lipids*, as they are called, aggregate in the membrane to form an unusually dense membrane structure that prevents diffusion of substances from the anammoxosome into the cytoplasm. The strong anammoxosome membrane is required to protect the cell from toxic intermediates produced during anammox reactions. These include, in particular, the compound *hydrazine* (N_2H_4), a very strong reductant. In the anammox reaction, NO_2^- is first reduced to nitric oxide (NO) by nitrite reductase, and then NO reacts with ammonium (NH_4^+) to yield N_2H_4 by activity of the enzyme hydrazine hydrolase. N_2H_4 is then oxidized to N_2 plus electrons by the enzyme hydrazine dehydrogenase. Some of the electrons generated at this step enter the anammoxosome electron transport chain, and electron transport reactions yield a proton motive force; other electrons feed back into the system to drive earlier anammox reactions. ATP is formed from the proton motive force by ATPases in the anammoxosome membrane.

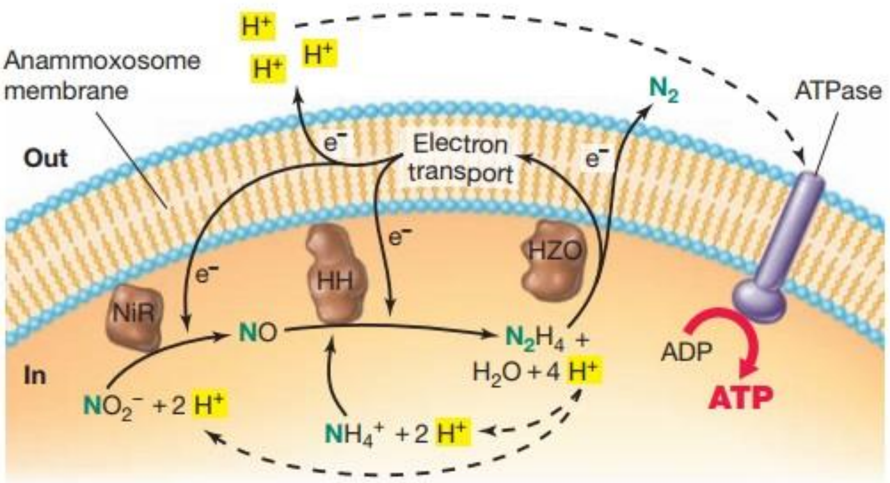


Figure: Reactions in the anammoxosome. NiR, nitrite reductase, HH, hydrazine hydrolase; HZO, hydrazine dehydrogenase

Ecology of Anammox

In nature the source of NO_2- for the anammox reaction is presumably aerobic ammonia-oxidizing *Bacteria* and *Archaea*. These organisms coexist with anammox bacteria in ammonia-rich habitats such as sewage and other wastewaters. The suspended particles that form in these habitats contain both oxic and anoxic zones in which ammonia oxidizers of different physiologies can coexist in close association. In mixed laboratory cultures, high levels of oxygen inhibit anammox and favor classic nitrification, and thus it is likely that in nature, the fraction of ammonia oxidation catalyzed by anammox bacteria is governed by the concentration of O_2 in the habitat.

From an environmental standpoint, anammox is a very beneficial process in the treatment of wastewaters. The anoxic removal of NH_3 and amines by the formation of N_2 helps reduce the input of fixed nitrogen from wastewater treatment facilities into rivers and streams, thereby maintaining higher water quality than would otherwise be possible. The activities of marine anammox organisms likely account for the significant fraction (>50%) of NH_3 known to disappear from marine sediments, a process which has previously been unexplained. At least some ammonia-rich freshwater lake sediments also support anammox, and thus it appears that anammox can occur in any anoxic environment in which NH_3 and NO_2- coexist.

Comammox organisms

Nitrification, the oxidation of ammonia via nitrite to nitrate, had always been considered as a two-step process (ammonia to nitrite and then nitrite to nitrate) catalyzed by chemolithoautotrophic microorganisms oxidizing either ammonia or nitrite. No nitrifier had been known to carry out both steps, although complete nitrification should be energetically advantageous, until 2015. Then a bacterium from the genus *Nitrospira* (*N. inopinata*) have been discovered by metagenomic analysis to contain genes for both ammonia oxidation and nitrite oxidation (e.g. *amoA* gene and *hao* cluster). Later, ***Nitrospira inopinata***, the first complete nitrifier was isolated in pure culture and characterized. As this organism- or other species of *Nitrospira* which- completely oxidizes ammonia to nitrate, it is known as **Comammox** (COMplete AMMonia OXidiser) organism.