Nitrogen fixation

Dr. Howaida Nounou

Nitrogen Metabolism

- The biosynthetic pathways leading to amino acids and nucleotides share a requirement for nitrogen.
- Because soluble, biologically useful nitrogen compounds are generally scarce in natural environments, most organisms maintain strict economy in their use of ammonia, amino acids, and nucleotides.
- Free amino acids, purines, and pyrimidines formed during metabolic turnover of proteins and nucleic acids are often salvaged and reused.

Forms of Nitrogen

- Urea → $CO(NH_2)_2$
- Ammonia → NH_3 (gaseous)
- Ammonium → NH_4
- Nitrate → NO_3
- Nitrite → NO_2
- Atmospheric Dinitrogen → N_2
- Organic N

Nitrogen Cycle Maintains a Pool of Biologically Available Nitrogen

- The most important source of nitrogen is air.
- However, relatively few species can convert atmospheric nitrogen into forms useful to living organisms.
- In the biosphere, the metabolic processes of different species function to salvage and reuse biologically available nitrogen in a **nitrogen cycle.**

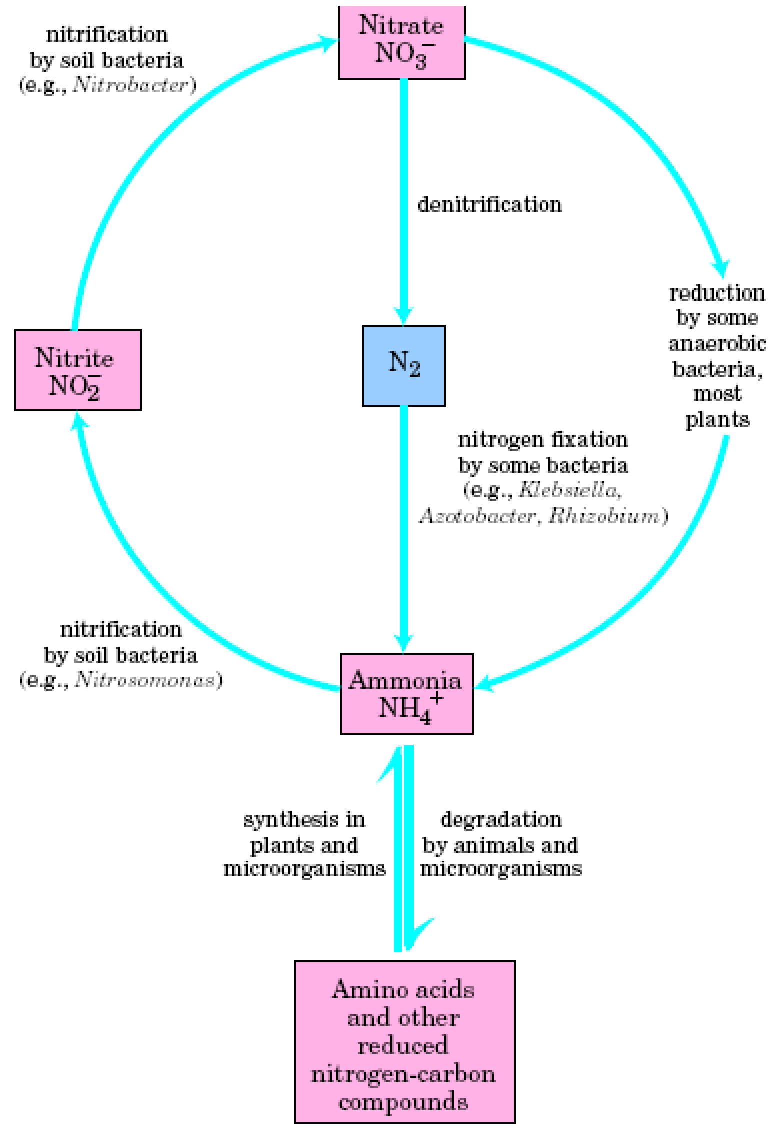
Biological nitrogen fixation

- Biological nitrogen fixation (**BNF**) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. The formula for BNF is:
- $N_2 + 6 H^+ + 6 e^- \rightarrow 2 NH3$

- The first step in the cycle is **fixation (reduction) of atmospheric** nitrogen by **nitrogen-fixing bacteria** to yield ammonia (NH3 or NH4).

- Some N2 fixing bacteria live **freely** in the soil. While others live in **symbiotic** relation with plants (leguminous plants, some tropical grass)

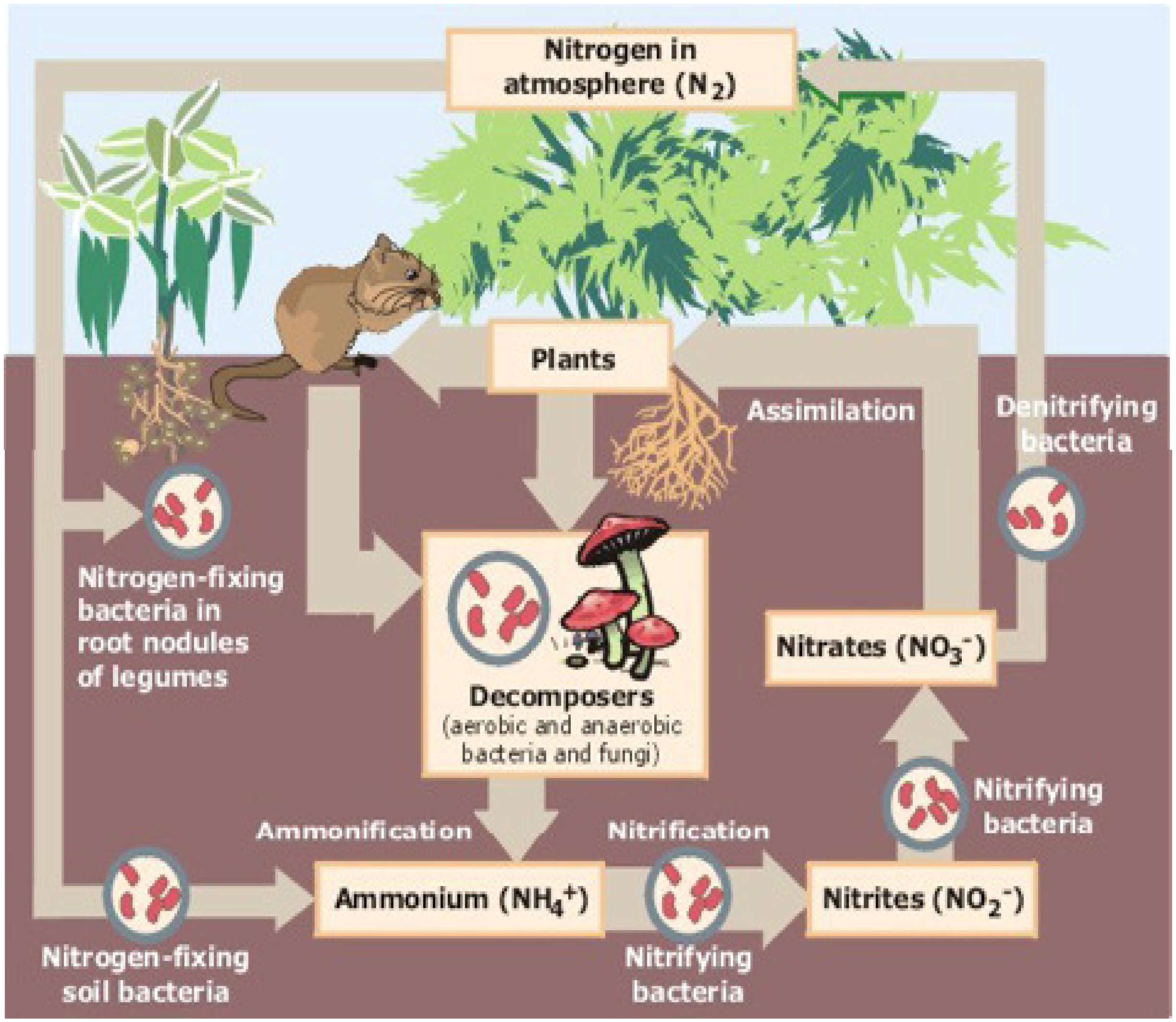
- **No plant is known that can fix N2**



- The first step in the cycle is **fixation (reduction) of atmospheric nitrogen by nitrogen-fixing bacteria to yield ammonia (NH_3 or NH_4^+).**
- **Soil bacteria** that derive their energy by **oxidizing ammonia to nitrite (NO_2) and ultimately nitrate (NO_3)** are so abundant and active that nearly all ammonia reaching the soil is oxidized to nitrate. This process is known as **nitrification.**

- **Plants and many bacteria** can take up and readily **reduce nitrate and nitrite** through the action of **nitrate** and **nitrite reductases**.
- The **ammonia** so formed is incorporated into **amino acids by plants**.
- **Animals then use plants** as a source of amino acids, both nonessential and essential, to build their proteins.
- When organisms die, **microbial degradation of their proteins returns ammonia to the soil**, where nitrifying bacteria again convert it to nitrite and nitrate.

Nitrogen cycle



- Enzymes responsible for nitrogenase action are very susceptible to destruction by oxygen. (In fact, many bacteria cease production of the enzyme in the presence of oxygen).
- Many nitrogen-fixing organisms exist only in anaerobic conditions

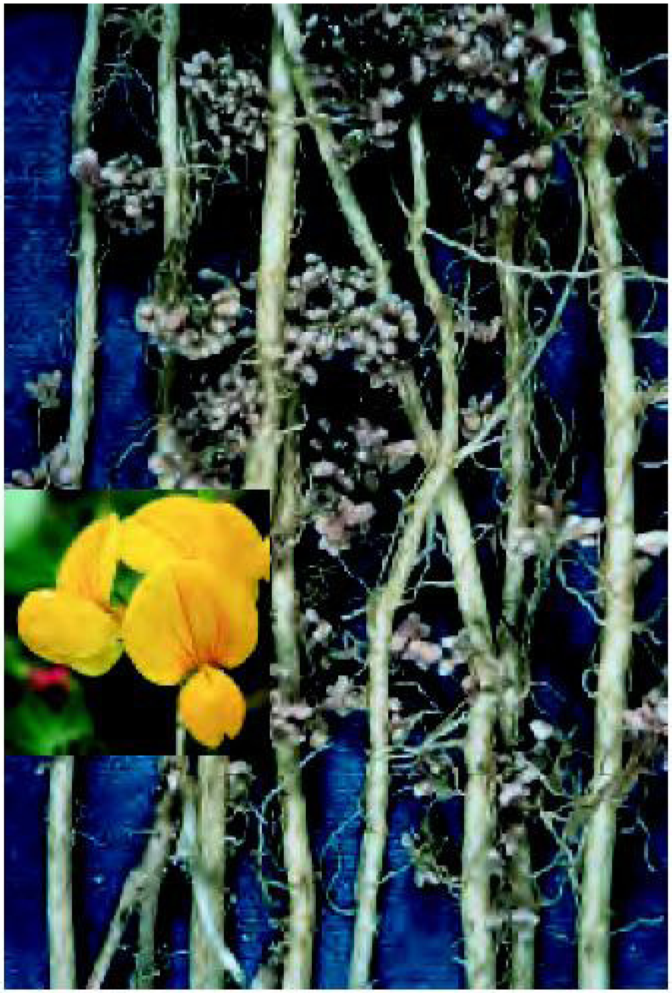
- A balance is maintained between **fixed nitrogen and atmospheric nitrogen by bacteria** that convert nitrate to N2under anaerobic conditions, a process called **denitrification** .
- These soil bacteria use NO3 rather than O2as the ultimate electron acceptor in a series of reactions that (like oxidative phosphorylation) generates a transmembrane proton gradient, which is used **to synthesize ATP.**

Nitrogen Is Fixed by Enzymes of the Nitrogenase Complex

- Only certain prokaryotes can fix atmospheric nitrogen.
- These include the **cyanobacteria** of soils and fresh and salt waters, other kinds of free-living soil bacteria such **as *Azotobacter*** *species, and the nitrogen-fixing bacteria that live as* ***symbionts in the root nodules of leguminous plants.***
- The first important product of nitrogen fixation is **ammonia**, which can be used by all organisms either directly or after its conversion to other soluble compounds such as **nitrites, nitrates, or amino acids**.
- The reduction of nitrogen to ammonia is an **exergonic** reaction:

$$N_2 + 3H_2 \longrightarrow 2NH_3 \qquad \Delta G'^{\circ} = -33.5 \text{ kJ/mol}$$

- **Nitrogen-fixing nodules. Root nodules of a legume.**
- Plants that contribute to nitrogen fixation include the legume family . They contain symbiotic bacteria called *Rhizobia* within nodules in their root systems, producing nitrogen compounds that help the plant to grow.
- When the plant dies, the fixed nitrogen is released, making it available to other plants and this helps to fertilize the soil.



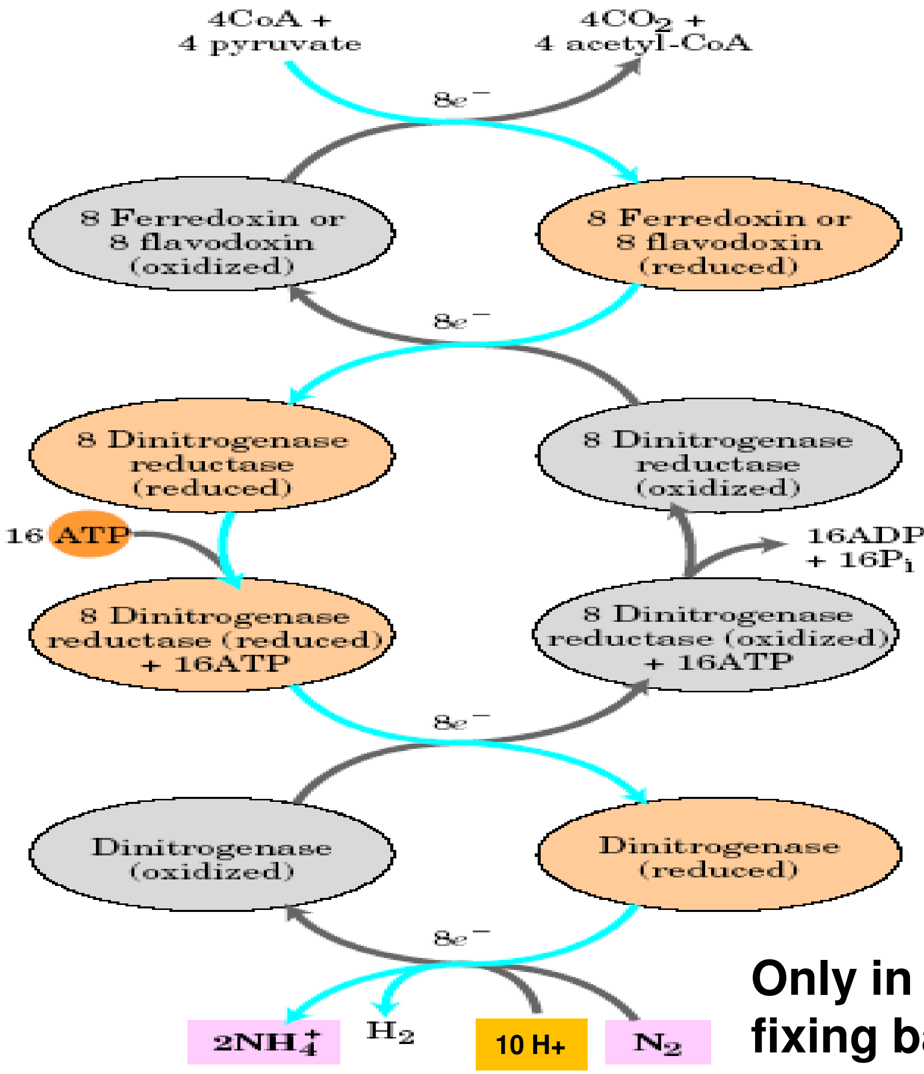
- Biological nitrogen fixation is carried out by a highly conserved complex of proteins called the **nitrogenase complex, It is made of 2 key components: dinitrogenase reductase (DNR) and dinitrogenase (DN)**

- **Dinitrogenase reductase** (*MW 60,000) is a dimer of two identical subunits. It contains 4Fe-4S center, and can be oxidized and reduced by one electron.*

- It also has two binding sites for ATP/ADP (one site on each subunit).

- **Dinitrogenase** (*MW 240,000), a tetramer , it contains both iron and molybdenum (Mo).*

- Nitrogen fixation is carried out by highly reduced form of dinitrogenase; and it requires 8 electrons (e-); 6 for reduction of nitrogen and 2 to produce H2.
- Dinitrogenase (DN) is reduced by the transfer of e- from the dinitrogenase reductase (DNR).
- The required 8 e- are transferred to dinitrogenase one at a time with the reduced reductase binding and the reduced reductase dissociating from dinitrogenase at a cycle.



Only in N-fixing bacteria

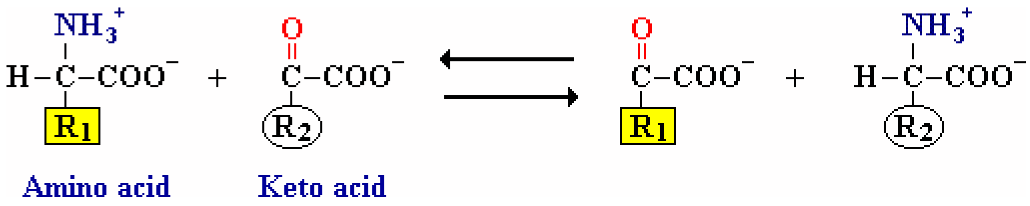
- The process is coupled to the hydrolysis of 16 equivalents of ATP by the reductase and is accompanied by the co-formation of one molecule of H_2.

- The role of ATP is not thermodynamic in the reaction carried out by DNR, both ATP binding and ATP **hydrolysis bring about important protein conformational change** required to bring about the reaction.

- N2-fixation is an **anaerobic reaction** , DNR is inactivated by oxygen.
- The energy required for N2 fixation was probably the **evolutionary driving force for the association of plants with bacteria**. (such as legumes plants).
- The bacteria in root nodules have access to a large reservoir of energy in the form of the abundant carbohydrate available by the plant.
- Because of this energy ,the **symbiotic bacteria may fix hundreds of times more nitrogen** than free bacteria.

- NH_3/NH_4^+ is used in the transamination reactions

α-amino acid+α-keto acid ⟹ new α- amino acid+ new α-keto acid



- NH_4^+ salts can be used by plants to synthesis all the **20 amino acids**, which are used for the synthesis **of protein**.
- N_2 is inorganic form of nitrogen. It is converted to organic compounds (urea, proteins, N compounds) then other organic compounds are converted to inorganic form.

- In some agricultural countries , micro-organisms are used to generate nitrogen.

(micro-organisms are found normally in the soil)

The overall N_2- fixation reaction (in bacteria)

$$N_2 + 10\ H^+ + 8\ e^- + 16\ ATP \longrightarrow 2\ NH_4^+ + 16\ ADP + 16\ Pi + H_2$$

- Plants can absrob NH_4^+ salts , NO_3^-, NO_2^- from the soil through the roots and use it for the formation of N- containing compounds [protein, nitrogen bases (DNA,RNA), heme, cytochromes]
- When animals, humans eat plants, they get a source of N_2.
- Humans also get N_2 from animals.

- In free-living diazotrophs, the nitrogenase-generated **ammonium** is assimilated into **glutamate** through the glutamine synthetase/glutamate synthase pathway

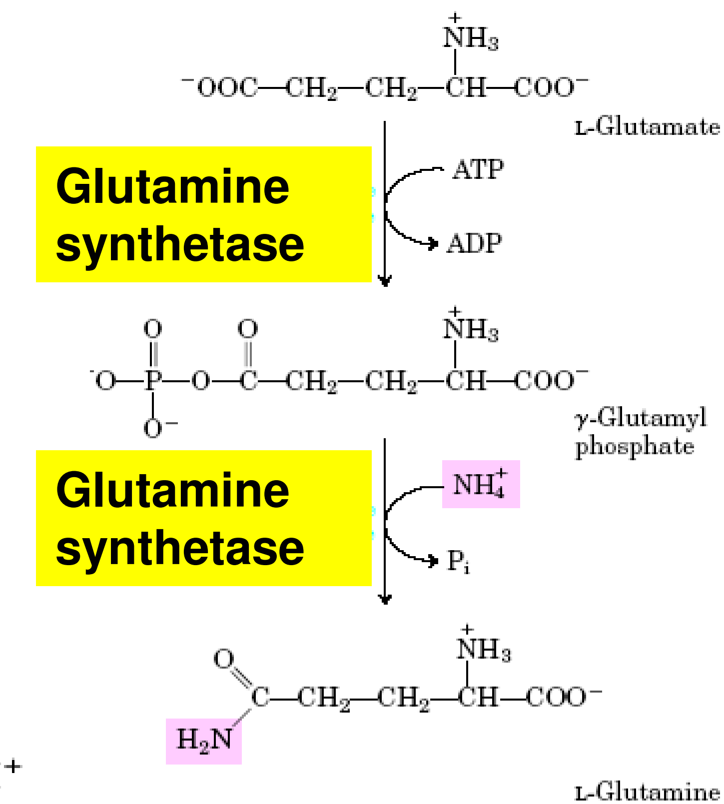
- **Ammonia Is Incorporated into Biomolecules through Glutamate and Glutamine**
- Reduced nitrogen in the form of NH_4^+ is assimilated into amino acids and then into other nitrogen-containing biomolecules.
- Two amino acids, **glutamate and glutamine, provide the critical entry point.**
- these same two amino acids play central roles in the catabolism of ammonia and amino groups in amino acid oxidation.
- **Glutamate is the source of amino groups** for most other amino acids, through **transamination** reactions.

The biosynthetic pathways to glutamate and glutamine are simple, and all or some of the steps occur in most organisms.

•The most important pathway for the assimilation of NH4+ into glutamate requires **two reactions.**

•First, **glutamine synthetase catalyzes the reaction of glutamate and NH4+ to yield glutamine.**

•This reaction takes place in two steps, with **enzyme-bound γ-glutamyl phosphate** as an intermediate



(1) Glutamate + ATP $\longrightarrow$ γ-glutamyl phosphate + ADP

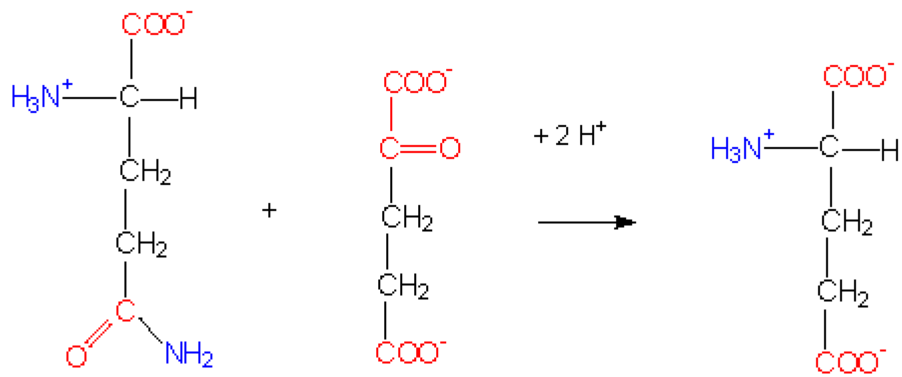
(2) γ-Glutamyl phosphate + $NH_4^+ \longrightarrow$ glutamine + P_i + H^+

Sum: Glutamate + NH_4^+ + ATP $\longrightarrow$
glutamine + ADP + 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 a reaction catalyzed by **glutamate synthase.**

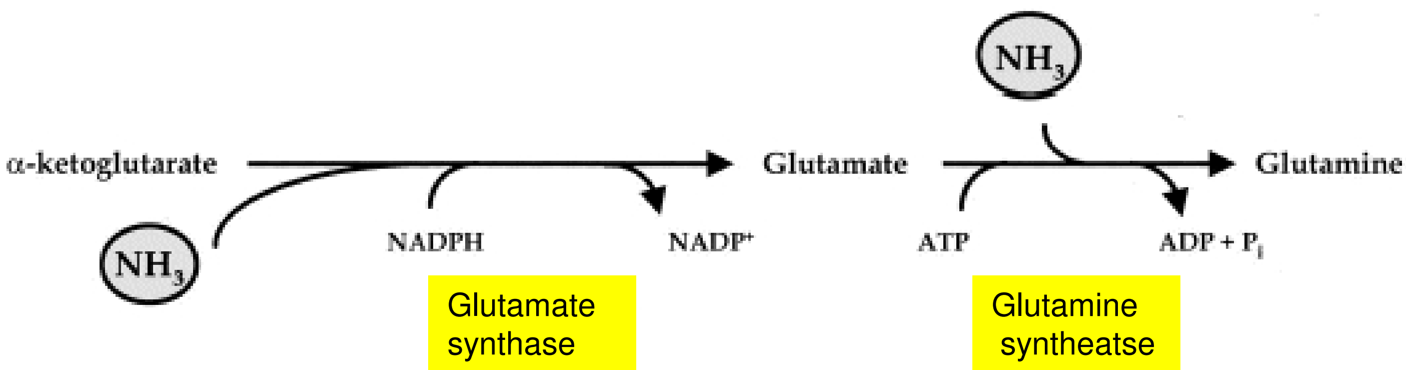
- **α-Ketoglutarate**, an intermediate of the citric acid cycle, undergoes reductive amination with glutamine as nitrogen donor:

$$\alpha\text{-Ketoglutarate} + \text{glutamine} + \text{NADPH} + \text{H}^+ \longrightarrow 2\ \text{glutamate} + \text{NADP}^+$$

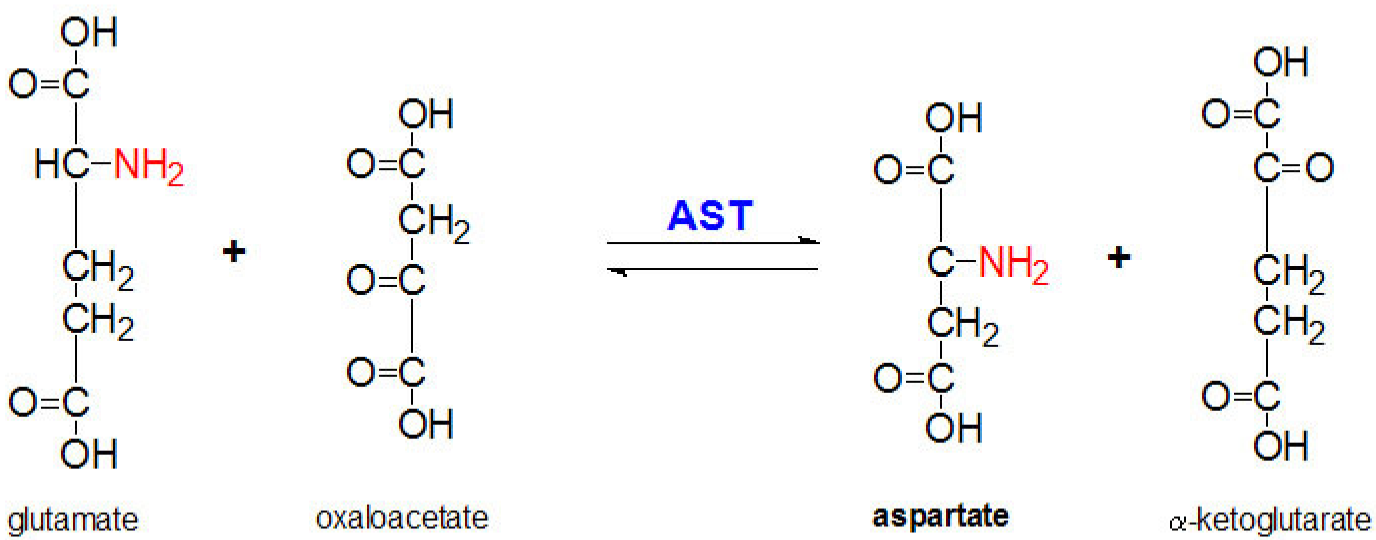


Glutamate synthase reaction (a transamination). The amino group of an amino acid (glutamine) is transferred to an *alpha*-ketoacid (*alpha*-ketoglutarate).

- The net reaction of glutamine synthetase and glutamate synthase is:



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

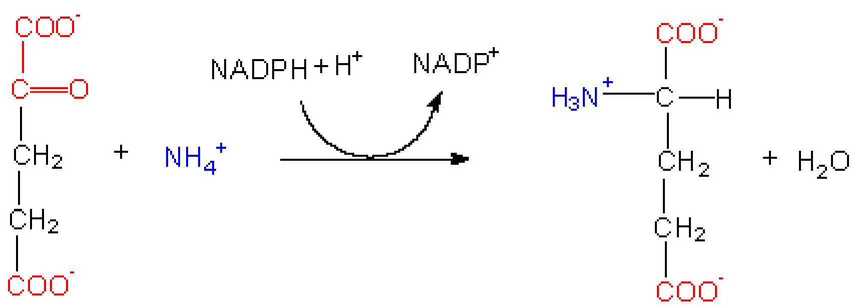


AST= aspartate transaminase

- Glutamate can also be formed in yet another pathway:
- the reaction of α-ketoglutarate and NH4+ to form glutamate in one step.
- This is catalyzed **by L-glutamate dehydrogenase**, an enzyme present in all organisms.
- Reducing power is furnished by NADPH:

$$\alpha\text{-Ketoglutarate} + NH_4^+ + NADPH \longrightarrow \text{L-glutamate} + NADP^+ + H_2O$$

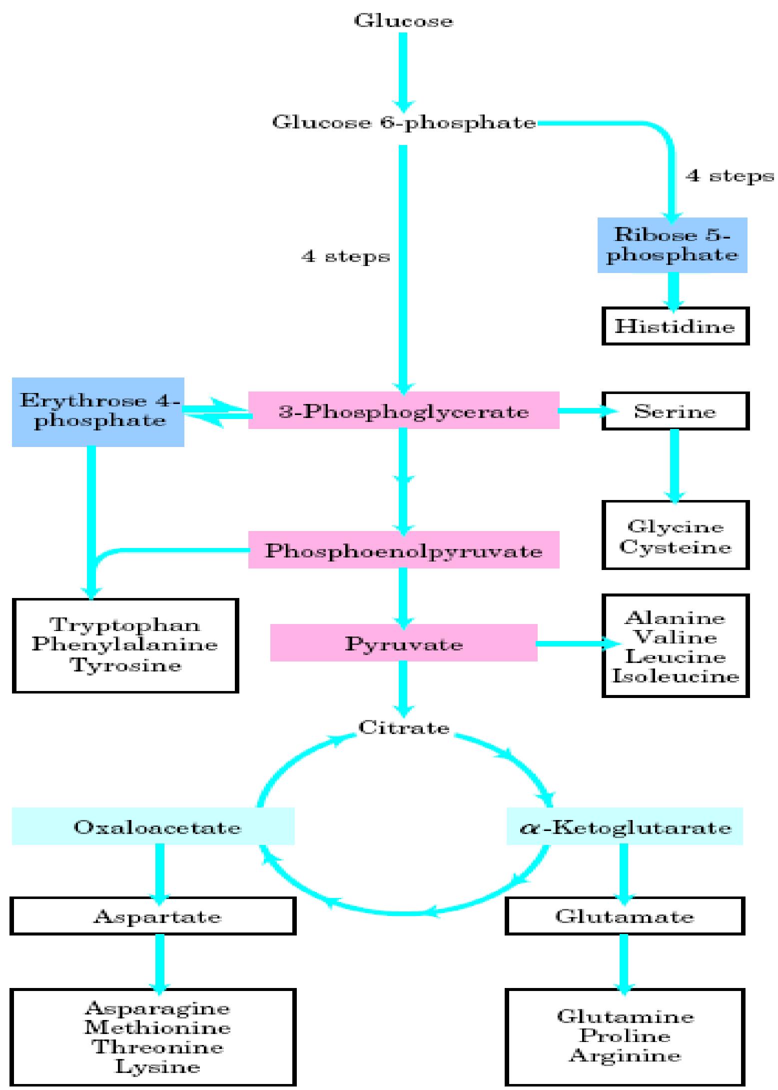
L-glutamate dehydrogenase reaction



Glutamate dehydrogenase reaction: The starting compounds are *alpha*-ketoglutarate and NH_4^+.

Biosynthesis of Amino Acids

- All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway .
- Nitrogen enters these pathways by way of ***glutamate and glutamine.***
- Some pathways are simple, others are not. Ten of the amino acids are just one or several steps removed from the common metabolite from which they are derived.
- The biosynthetic pathways for others, such as the aromatic amino acids, are more complex.


Glucose
Glucose 6-phosphate
4 steps
4 steps
Ribose 5-phosphate
Histidine
Erythrose 4-phosphate
3-Phosphoglycerate
Serine
Glycine
Cysteine
Phosphoenolpyruvate
Tryptophan
Phenylalanine
Tyrosine
Pyruvate
Alanine
Valine
Leucine
Isoleucine
Citrate
Oxaloacetate
α-Ketoglutarate
Aspartate
Glutamate
Asparagine
Methionine
Threonine
Lysine
Glutamine
Proline
Arginine

Organisms vary greatly in their ability to synthesize the 20 common amino acids.

•Whereas most bacteria and plants can synthesize all 20, mammals can synthesize only about half of them—generally those with simple pathways. These are the **nonessential amino acids, not needed in the diet**

•The remainder, the **essential amino acids, must be obtained from food.**

TABLE 18–1 Nonessential and Essential Amino Acids for Humans and the Albino Rat

Nonessential	*Conditionally essential**	*Essential*
Alanine	Arginine	Histidine
Asparagine	Cysteine	Isoleucine
Aspartate	Glutamine	Leucine
Glutamate	Glycine	Lysine
Serine	Proline	Methionine
	Tyrosine	Phenylalanine
		Threonine
		Tryptophan
		Valine