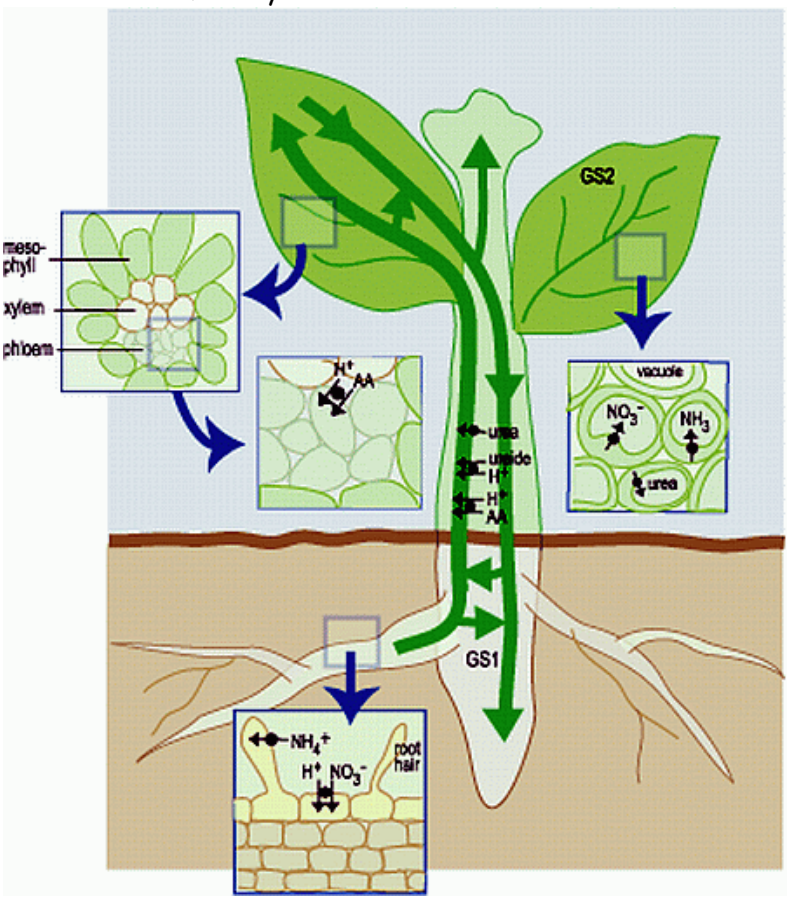
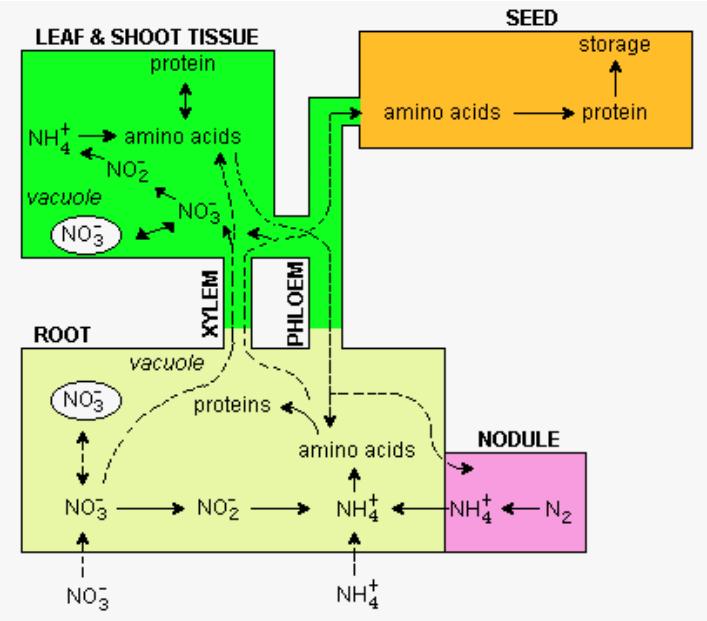
Nitrogen Metabolism

Nitrogen is a very important constituent of cellular components. Alkaloids, amides, amino acids, proteins, DNA, RNA, enzymes, vitamins, hormones and many other cellular compounds contain nitrogen as one of the elements. It is not exaggerating to say that Nitrogen is the key element for it is the most important constituent of proteins and nucleic acids. Thus N2 plays a significant role in the formation of the above said compounds which in turn control cellular activities. Without nitrogen, no living organism can survive. Paradoxically all the living organisms are virtually submerged in a sea of atmospheric nitrogen (i.e. 78%), but unfortunately not all organisms are endowed with the potentiality to utilize this abundantly available molecular N2. Only some organisms like certain bacteria, blue green algae and few fungi, have the potentiality to utilize molecular N2 directly. However, most of the plants are capable of utilizing other forms of nitrogen with ease and facility.

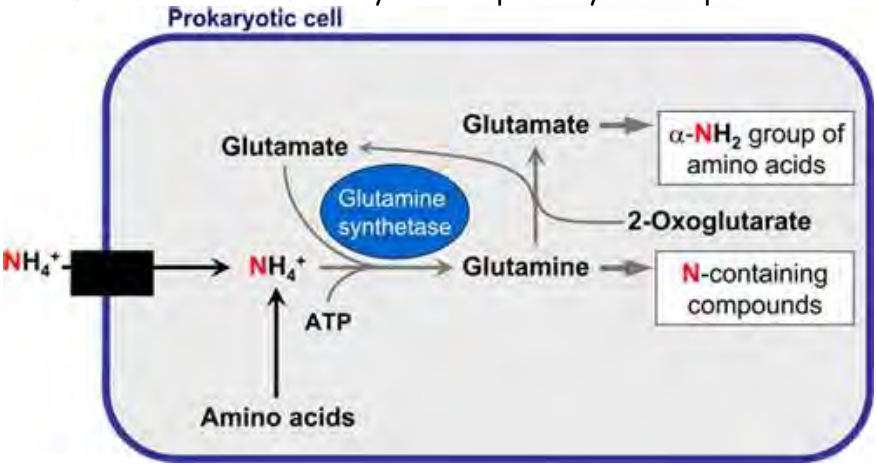




SOURCES OF NITROGEN

Ammonical and organic form of Nitrogen:

Ammonical form of N2 is available in soil in the form of urea or NH4 in free state. Urea, if present, is first split into NH4 and CO2, and NH4 is then utilized directly by metabolic pathways by higher plants. But recent studies indicate that urea can be directly used up by metabolic pathways in certain plants. It should be remembered here, that free ammonia is the only utilizable form of N2 that can be directly incorporated into amino acids. Whatever may be the source of nitrogen, first it has to be converted to NH3 and fixed into amino acid. It can be converted or transferred to other form by various pathways that operate in living systems.



The decay of dead plants and animals also releases different kinds of nitrogen compounds of which amino acids, nucleotides and other such simpler compounds constitute organic form of N2. The same are absorbed by the root system and utilized directly. Thus the decaying organic matter acts as the rich source of organic nitrogen that can be utilized by not only higher plants but also by micro-organisms.

NITRATE / NITRITE FORM

Invariably the N2 that is available in the soil is in the form of nitrates. And nitrites are also found but in small quantities. These forms are available as ions and the same are easily absorbed by the roots or cellular surfaces from its surrounding soil solution. The absorption of NO3 or NO2 ions is not by just diffusion process, but it is facilitated by specific carriers.

Once the nitrate or nitrite ions enter into cellular milieu they have to be converted to NH4, before the same can be incorporated into cellular components. Under normal conditions, nitrite is never accumulated in the soil in sufficient quantities and it is toxic to plants and to other microbes.

THE MECHANISM OF CONVERSION OF NO3 AND NO2 TO NH4

Plant structures like roots as well as leaves can utilize nitrates and the same can be converted to NH4. But more of nitrate reductive activity is found in leaves than in roots. However, the mechanism of nitrate and nitrite reduction is performed by different enzymes while NO3 is reduced by nitrate reductase enzymes and the NO2 is reduced by nitrite reductases.

NITRATE REDUCTION

Nitrate reduction to NH4 is not a single step process, but it is a series of reactions in which the first step is performed by nitrate reductase. This enzyme has been isolated and purified from various sources like Aspergillus, bacteria, chlorella, blue green algae, alfa alfa and other higher plants. The mol. Wt. of it is about 3.5 x 10^5 daltons. The enzyme is associated with 2 cofactors i.e. FAD and two molybdenum ions. The enzyme also requires reducing power supplied by NADH+H or NADPH+H. The former is available in non chlorophyllous tissues and the latter is found in chloroplast containing leaves.

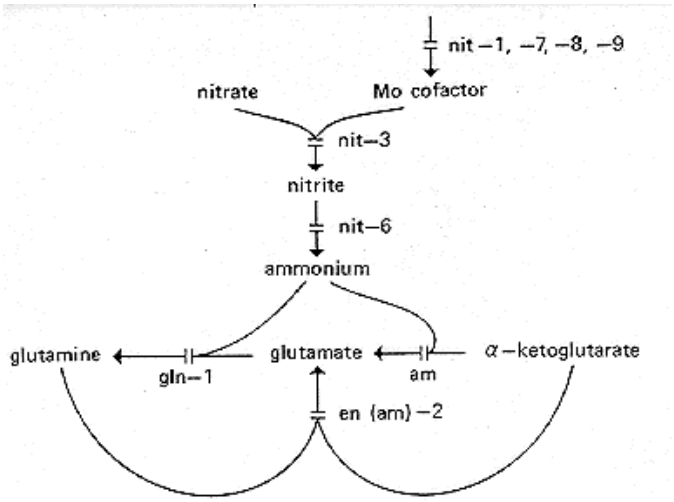
NITRATE REDUCTASE IS AN INDUCIBLE ENZYME

In the absence of NO3 the amount of this enzyme present in the tissues is very low. With the addition of NO3 as the substrate, the amount of this enzyme increases many fold. However, the induction requires light without which the enzyme induction is not possible to the fullest extent. The nitrate induced enzyme synthesis can be inhibited by the inhibitors of transcription and translation like actinomycin D and cycloheximide respectively, which indicates that NO3 acts as an inducer of nitrate reductase gene expression. How light modulates the gene expression is not yet clear.

Furthermore, phytohormones, particularly cytokinin also induces nitrate reductase synthesis denovo even in the absence of light and NO3. Cytokinin induced NO3 reductase activity can be inhibited with actinomycin or CHI. The mechanism of denovo synthesis of nitrate reductase, though not clear, it is fully accepted that the nitrate reductase is an inducible enzyme.

NITRATE REDUCTION

The reduction of nitrate to ammonia is a multistep reaction in which nitrates are reduced to nitrites, which are then converted to hyponitrites then to hydroxylamines and finally to ammonia.

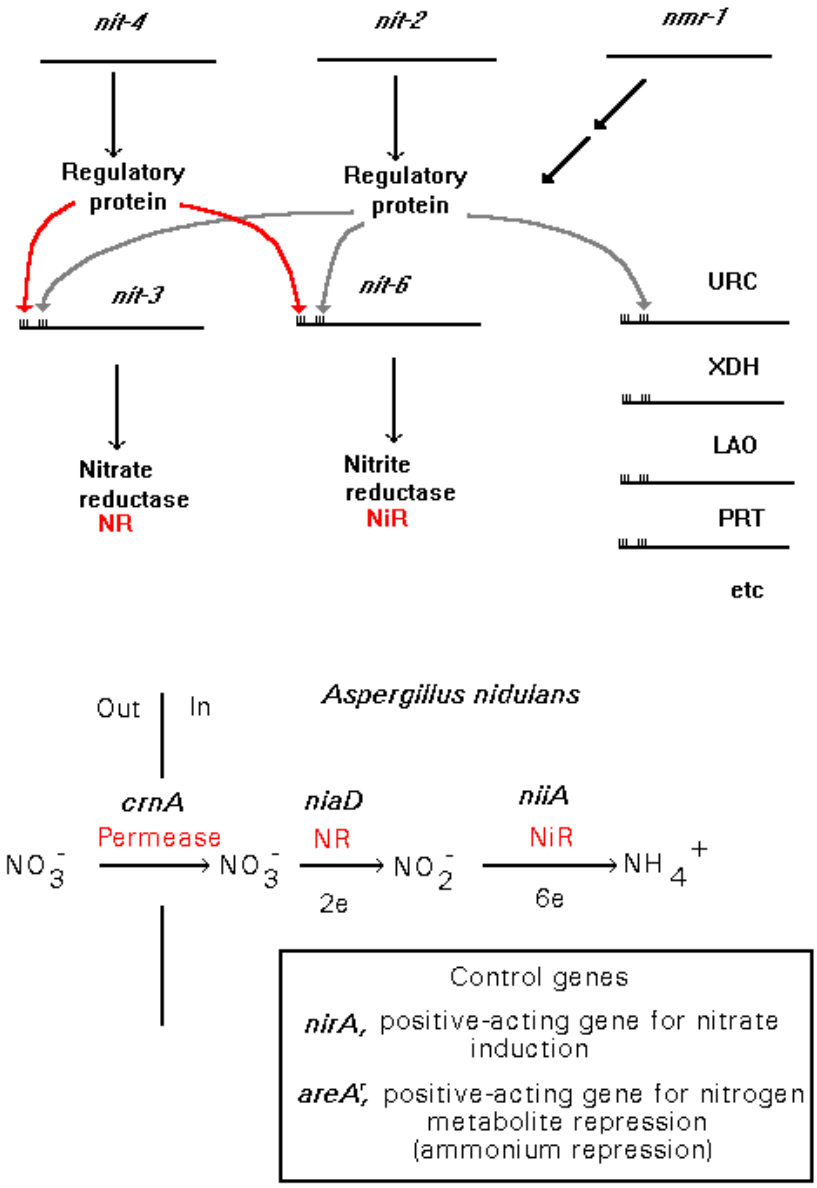


Depending upon the tissues involved nitrate reductase accepts NADH2 (roots) or HADPH+H (leaves), where hydrogen is transferred to the coenzyme FAD to form FADH2. In the next step, protons (H+) and electrons are transferred to NO3 simultaneously. However, electrons are transferred to NO3 through molybdenum ions.

For the maximal activity of nitrate reductase, it requires an optimal concentration of MO2+, Fe3+ and Ca2+ ions. Though calcium has no catalytic activity in this enzymatic reaction, unlike iron and molybdenum which are involved in electron transport and it facilitates the transport of nitrite across the chloroplast membranes. Thus the nitrite synthesized in this reductive step in the cytoplasm is transported into chloroplasts. But in roots, lower fungi and bacteria, the entire process takes place in the cytoplasm.

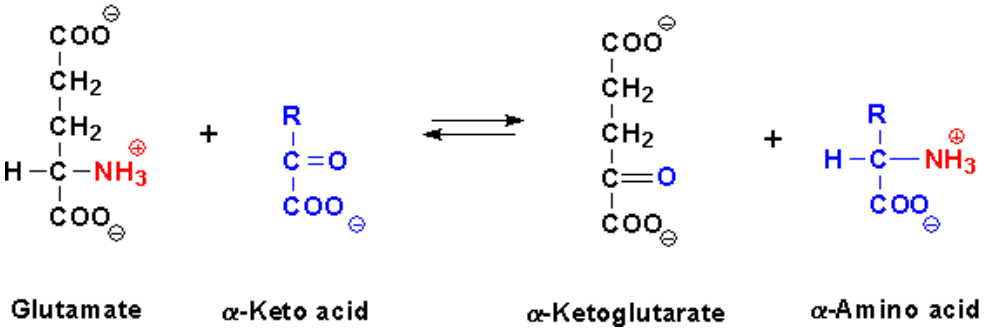
NITRITE REDUCTION

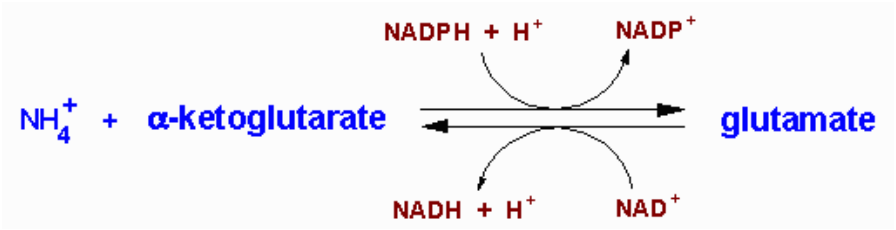
In most of the higher plants so far studied, the nitrites synthesized in cytoplasm or transported into plastids, where the nitrites are reduced to hyponitrite by an enzyme called nitrite reductase. The enzyme has a mol. Wt. of 60-70KD and it has a special heme component called siroheme detected in soret band. Actually there are two forms of nitrite reductases, of which one form uses NADPH+H as the proton/electron donor in photosynthetic tissues, but root tissues and others including bacteria and fungi use NADH+H as the hydrogen donors. The enzyme nitrite reductase possesses flavin and iron groups. Added to this, they are inducible enzymes. Strangely, these enzymes are induced by nitrates than nitrites. However, nitrite reductase brings about the reduction of nitrite to NH4 in a multistep reaction, where the intermediary products remain attached to the surface of enzyme; only the final product is release from the surface. In this process, a total of six electrons and six protons are transferred to nitrite to produce ammonia.

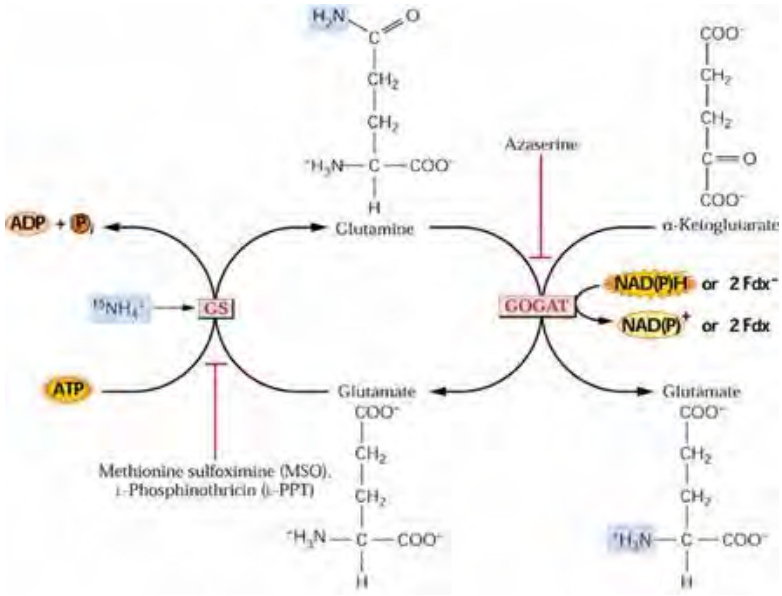


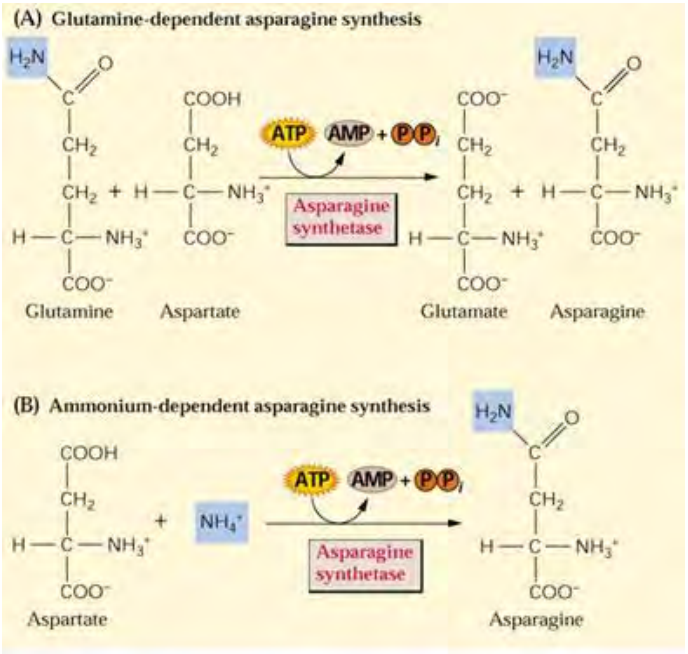
From: JR Kinghorn (1989) Genetic, biochemical, and structural organization of the *Aspergillus nidulans crnA-niiA-niaD* gene cluster. In JL Wray, JR Kinghorn eds "Molecular and Genetic Aspects of Nitrate Assimilation," Oxford Science Publications, Oxford, pp 69-87.

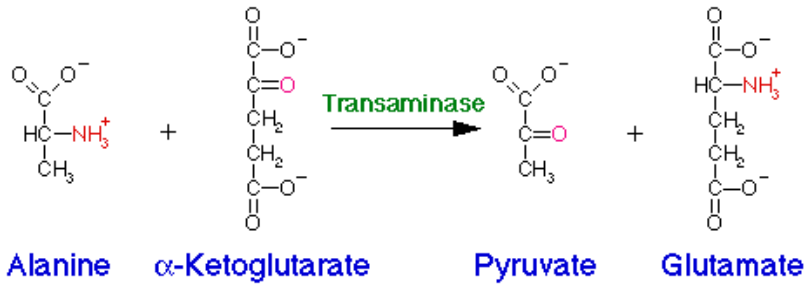
Nonetheless in some cases one of the intermediate products like hydroxylamine has been found to be converted to NH4 by the activity of hydroxylamine reductase. Such reactions have been observed in mesophyll tissues of higher plants, Neurospora, aspergillus and some bacteria. Whether or not, the enzyme nitrite reductase by itself is capable of converting hydroxylamine to NH4 is not clear. Still the overall pathway from NO3 or NO2 to NH4 is catalyzed by a group of enzymes or multienzyme complexes, but the synthesis of NH4 is very essential for amino acid synthesis.

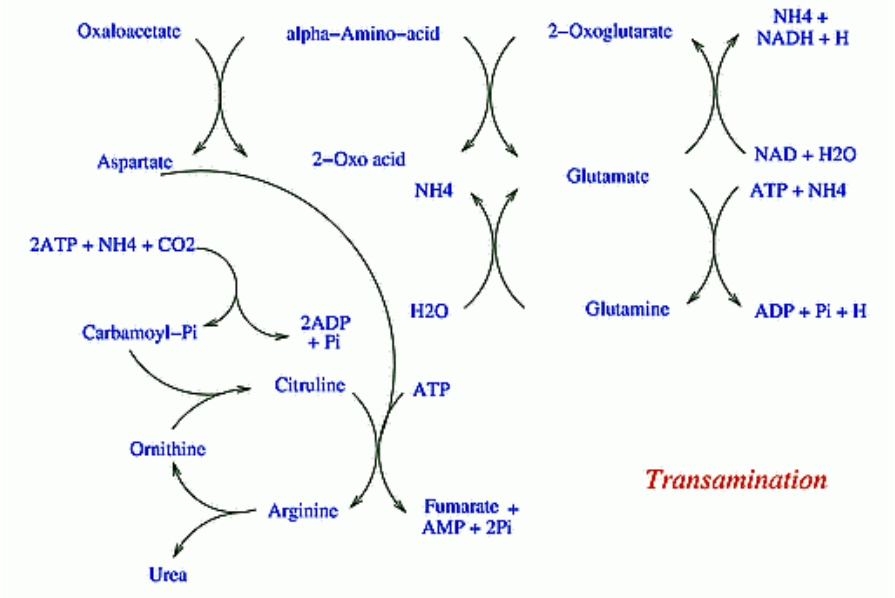

Glutamate
α-Keto acid
α-Ketoglutarate
α-Amino acid

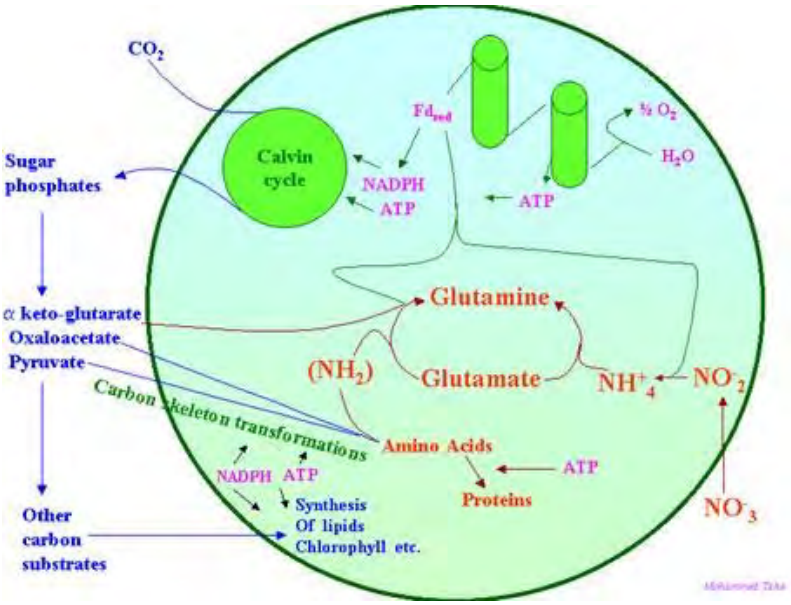

NADPH + H+
NADP+
NH4+ + α-ketoglutarate
glutamate
NADH + H+
NAD+

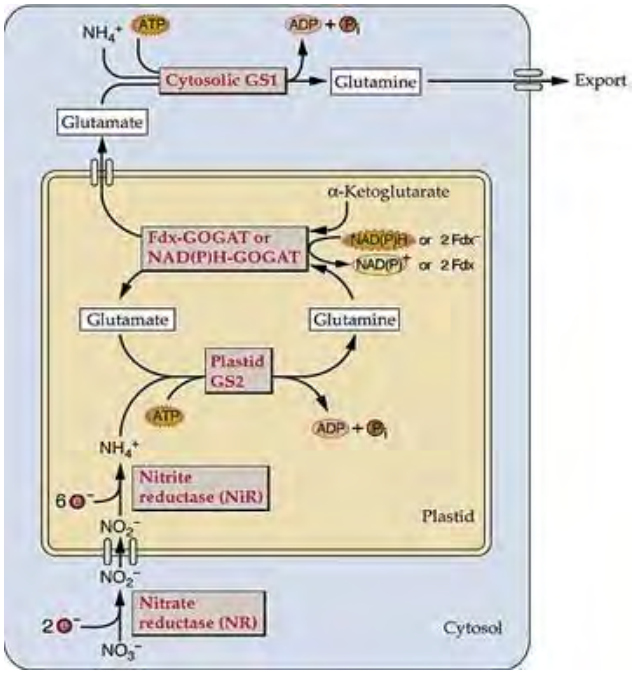

Azaserine
ADP + Pi
Glutamine
α-Ketoglutarate
15NH4+
GS
GOGAT
NAD(P)H or 2 Fdx-
NAD(P)+ or 2 Fdx
ATP
Glutamate
Glutamate
Methionine sulfoximine (MSO),
L-Phosphinothricin (L-PPT)

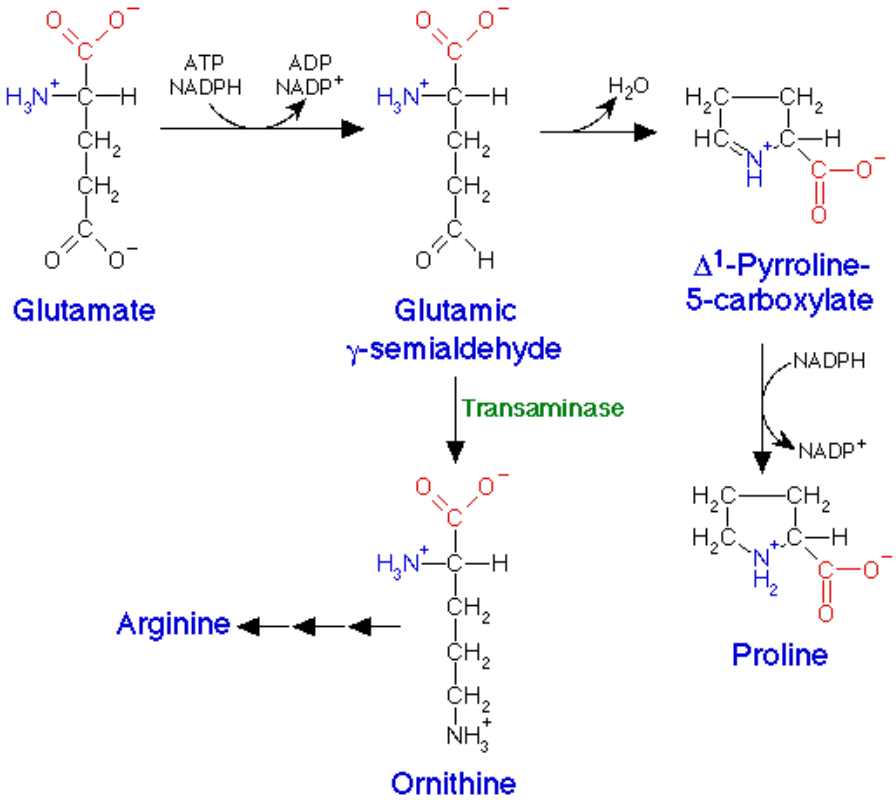

(A) Glutamine-dependent asparagine synthesis
ATP
AMP + PPi
Asparagine synthetase
Glutamine
Aspartate
Glutamate
Asparagine
(B) Ammonium-dependent asparagine synthesis
ATP
AMP + PPi
Asparagine synthetase
Aspartate
Asparagine

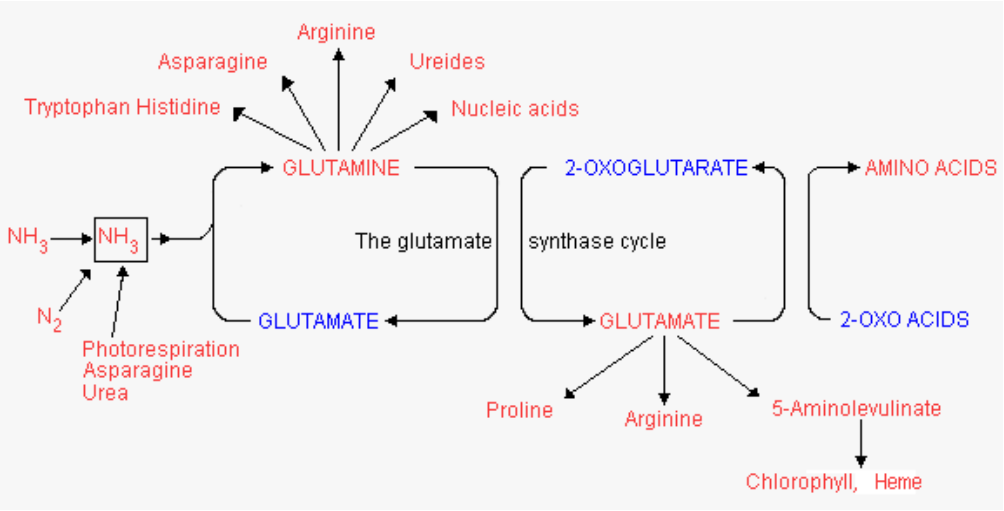

Transaminase
Alanine
α-Ketoglutarate
Pyruvate
Glutamate

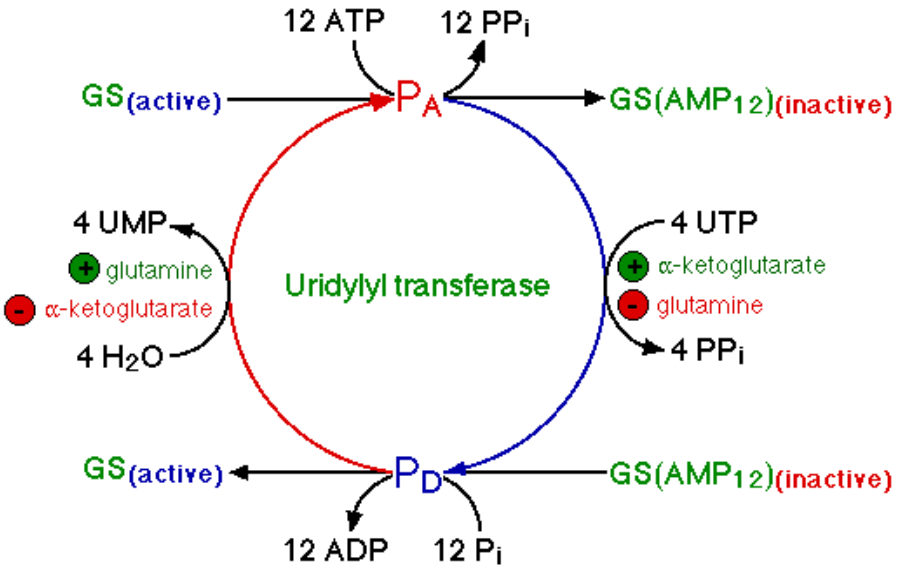

Oxaloacetate
alpha–Amino–acid
2–Oxoglutarate
NH4 + NADH + H
Aspartate
2–Oxo acid
NAD + H2O
Glutamate
NH4
ATP + NH4
2ATP + NH4 + CO2
H2O
Glutamine
ADP + Pi + H
Carbamoyl–Pi
2ADP + Pi
Citruline
ATP
Ornithine
Transamination
Arginine
Fumarate + AMP + 2Pi
Urea


CO_2
Calvin cycle
Fd_{red}
NADPH
ATP
½ O_2
H_2O
ATP
Sugar phosphates
Glutamine
α keto-glutarate
Oxaloacetate
Pyruvate
(NH_2)
Glutamate
NH^+_4
NO^-_2
Carbon skeleton transformations
Amino Acids
ATP
NADPH ATP
Proteins
Synthesis Of lipids Chlorophyll etc.
Other carbon substrates
NO^-_3

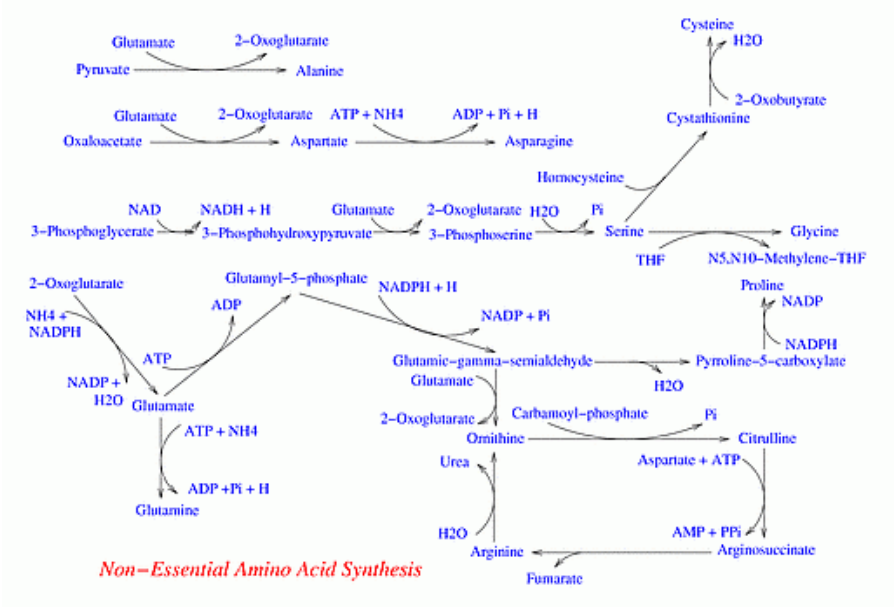

NH_4^+
ATP
ADP + P_i
Cytosolic GS1
Glutamine
Export
Glutamate
α-Ketoglutarate
Fdx-GOGAT or NAD(P)H-GOGAT
NAD(P)H or 2 Fdx$^-$
NAD(P)$^+$ or 2 Fdx
Glutamate
Glutamine
Plastid GS2
ATP
ADP + P_i
NH_4^+
6 e^-
Nitrite reductase (NiR)
NO_2^-
Plastid
NO_2^-
2 e^-
Nitrate reductase (NR)
NO_3^-
Cytosol

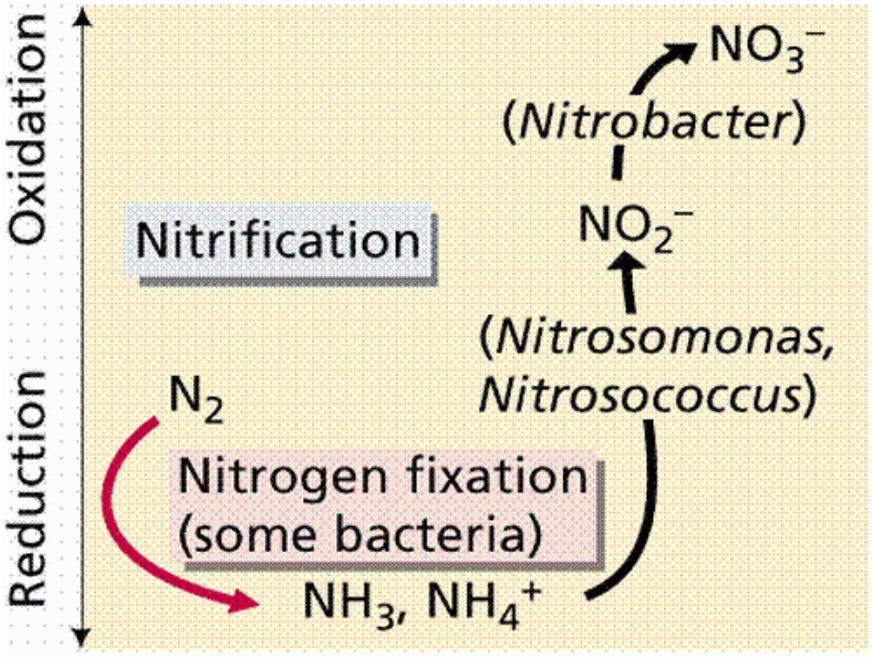

ATP
NADPH
ADP
NADP+
H2O
Glutamate
Glutamic
γ-semialdehyde
Δ1-Pyrroline-
5-carboxylate
NADPH
NADP+
Transaminase
Arginine
Proline
Ornithine


Arginine
Asparagine
Ureides
Tryptophan Histidine
Nucleic acids
GLUTAMINE
2-OXOGLUTARATE
AMINO ACIDS
NH3
NH3
The glutamate
synthase cycle
N2
GLUTAMATE
GLUTAMATE
2-OXO ACIDS
Photorespiration
Asparagine
Urea
Proline
Arginine
5-Aminolevulinate
Chlorophyll, Heme



Glutamine synhtase





MOLECULAR NITROGEN

Abundantly available molecular N2 is more or less inert. With the exception of some bacteria, fungi and blue green algae none of the higher plants are capable of utilizing molecular N2 directly. However, nature has devised mechanisms to fix this type of N2 into utilizable form of N2 i.e. NH4by non biological and biological methods.

NITROGEN FIXATION

Non biological Method:

Electrical discharges in atmosphere due to lightening leads to the formation of various oxides and reductants of N2. In the presence of water vapors they dissolve and produce nitrous and nitric acids. These inturn, come down to earth along with rain water. Later they get converted to nitrates. Annually many billion tons of atmospheric N2 is fixed by this non biological process.

BIOLOGICAL METHOD – ASYMBIOTIC PROCESS

Among the living plant world, some free living bacteria, fungi and blue green algae are capable of fixing molecular nitrogen into utilizable form of N2 i.e. NH4. Ex. Azatobactor veinlandi, Clostridium pasteurianum, Rhodospirullum rubrum, Chromatium, Nostoc, Anabaena, Rivularia, etc. When the above said organisms are allowed to multiply in the soil, under favourable conditions they easily fix 15-40 kg. of N2 per acre per year. In recent years, the above said organisms are made available to farmers as bio-fertilizers.

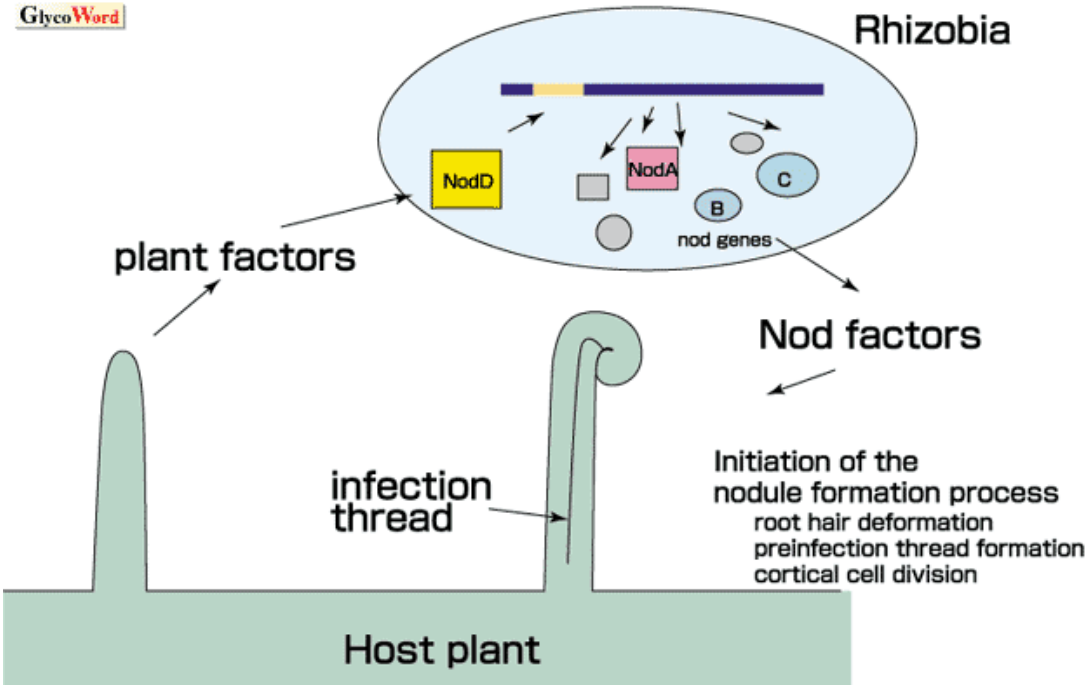
When the cultures of them are spread in the fields and allowed to grow, they enrich the soil with a lot of nitrogen as a natural fertilizer. The mechanism by which molecular N2 is converted to NH4 is described elsewhere. One important aspect of it is to maintain moisture in the soil. This living fertilizer renewable and enriches the soil all the time.

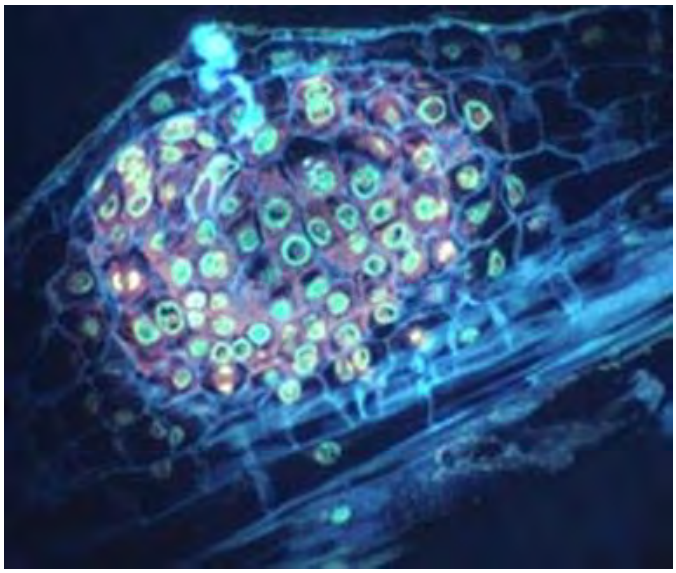
SYMBIOTIC PROCESS

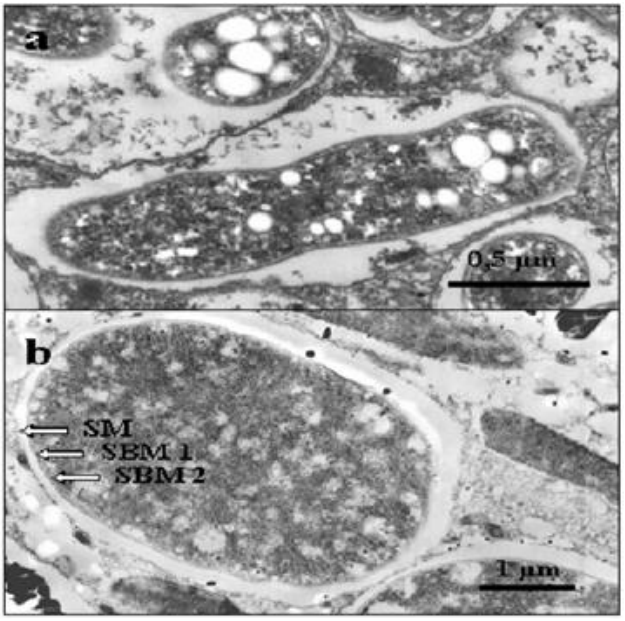
None of the known crop plants, or any other angiosperms are capable of utilizing molecular N2 directly, but some have developed a method by which they obtain nitrogen through symbiotic association with bacteria. It is widely known that many species of bacteria and also some blue green algal colonies live in association with higher plants, either in the roots, leaves, lichens, liverworts and coralloid roots. But the roots of leguminous plants possess characteristic root nodules in which nitrogen fixing bacteria called Rhizobium are present. These bacteria, on infecting host roots induce the development of characteristic pink colored root nodules. In their symbiotic association, bacteria obtain carbohydrates and other minerals from host cells and host cells in return obtain nitrogen fixed by bacteria. So by growing leguminous plants in the fields the soil will be enriched with nitrogen fertilizers up to the tune of 40-80 kg./acre./year.

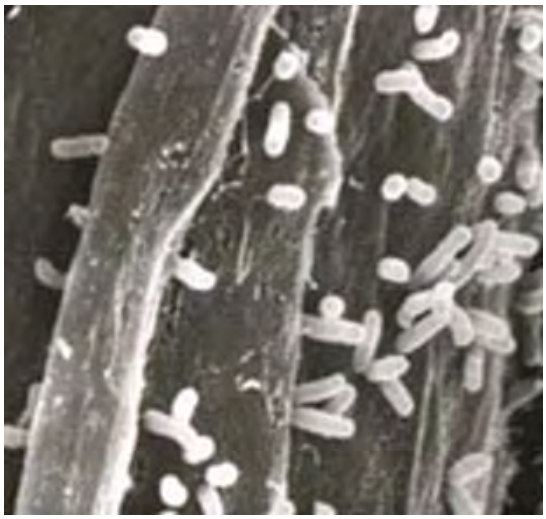
SPECIFICITY OF BACTERIA AND HOST ASSOCIATION

The symbiotic association between bacteria and the host is highly specific. For example, Rhizobium phaseolin infects phaseolus species only but not others. Similarly, Rhizobium trifoli infects Trifolium repens but not others. The host bacterial specificity is due to the presence of glycoproteins as receptors in host root cell surface which recognizes some proteins found on the bacteria cell wall. These recognize each and other as in the case of enzyme recognizing its specific substrate.





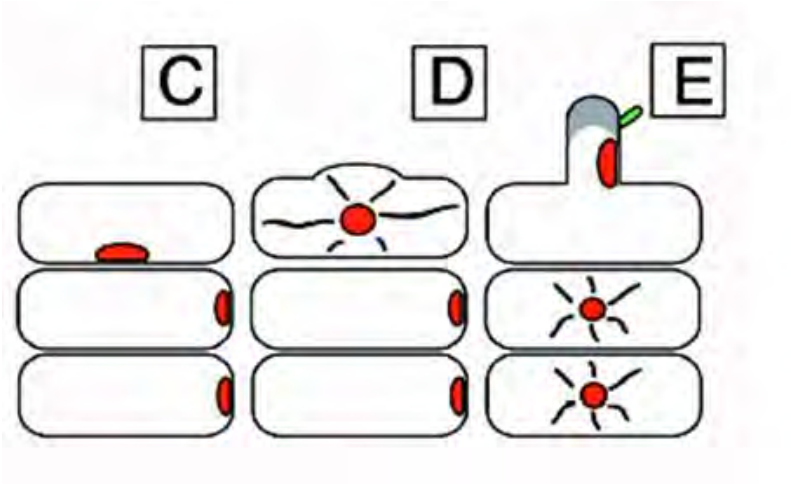

a
0,5 µm
b
SM
SBM 1
SBM 2
1 µm

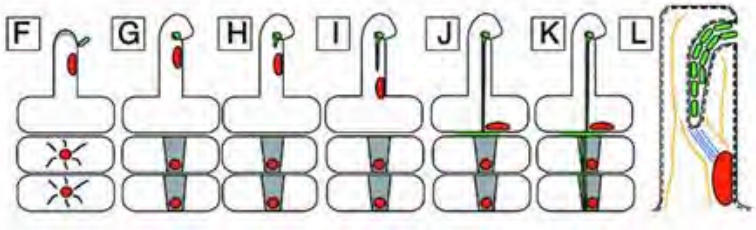


DEVELOPMENT OF ROOT NODULES

Infection:

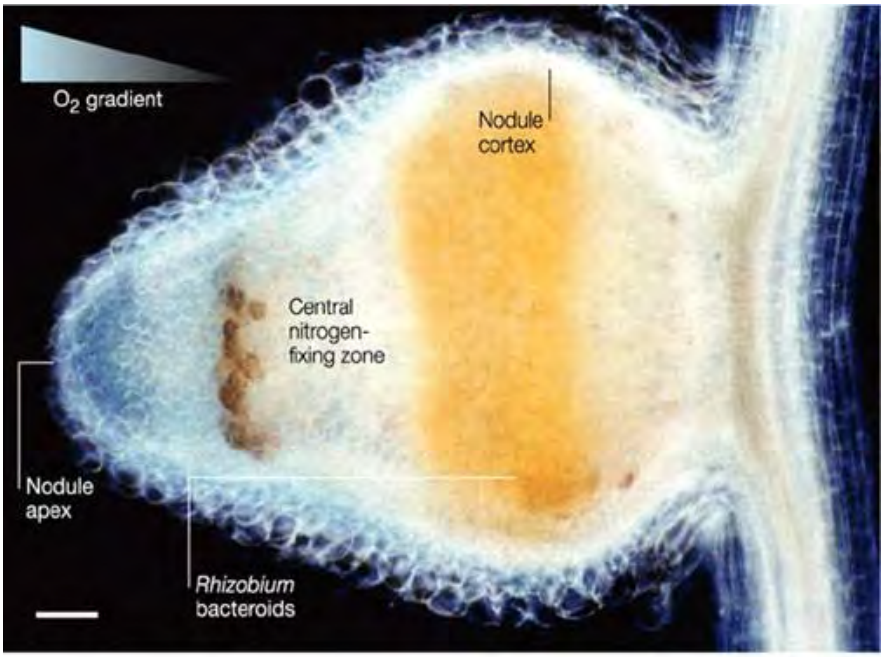
Once a particular specific rhizobial strain binds to the host root hair cell the bacteria induces the formation of infection thread.





Bacterial cells can also enter into the hair cells at the injured surfaces. The infection thread develops from the inner primary cell wall, which grows inwards in the form of invagination enclosing bacterial cells. The infection thread further grows inwards and invades the cortex and finally it finds its way into pericyclic region where the end of the infection thread bursts open releasing bacterial cells. As the infection grows inwards bacterial cells multiply by cell division and the process of multiplication continues even after they are released into the host cells. Bacterial cells

assume various shapes and also they aggregate into groups. Such bacterial clusters surrounded by a thin membrane are called bacteroids.

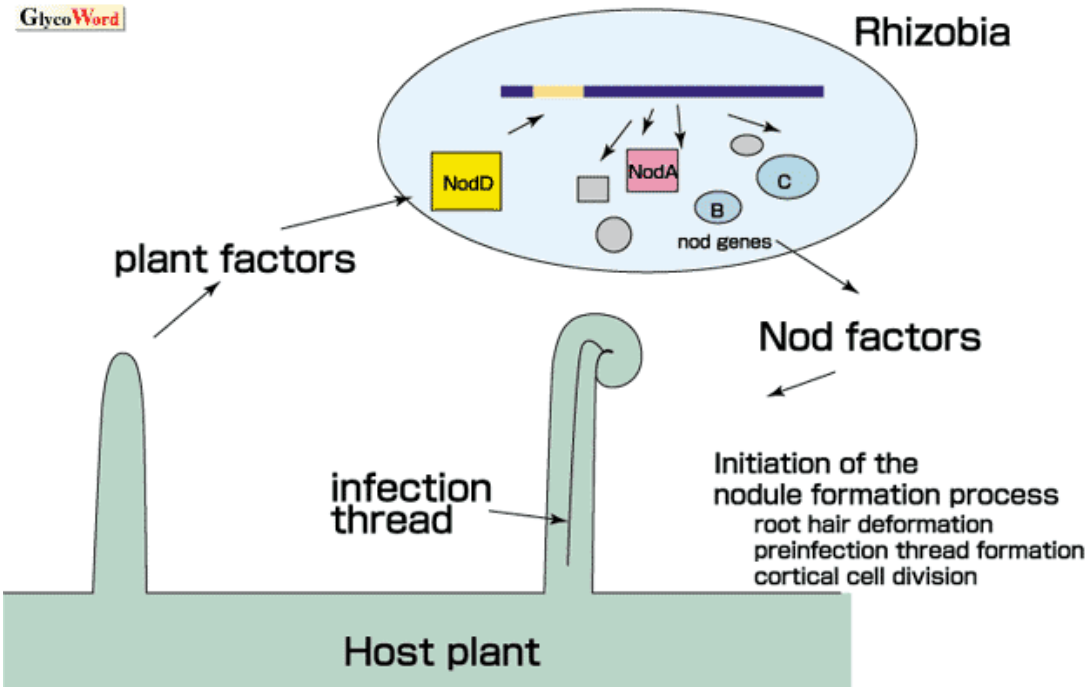


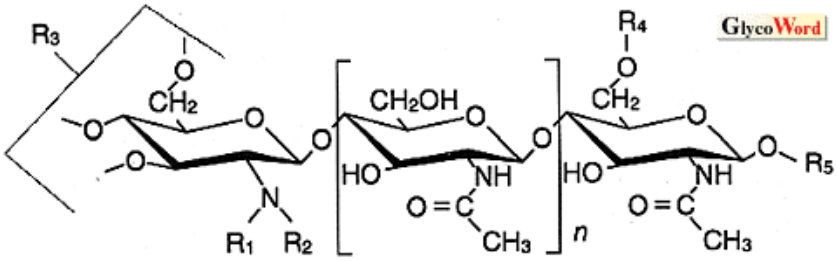
NODULE FORMATION

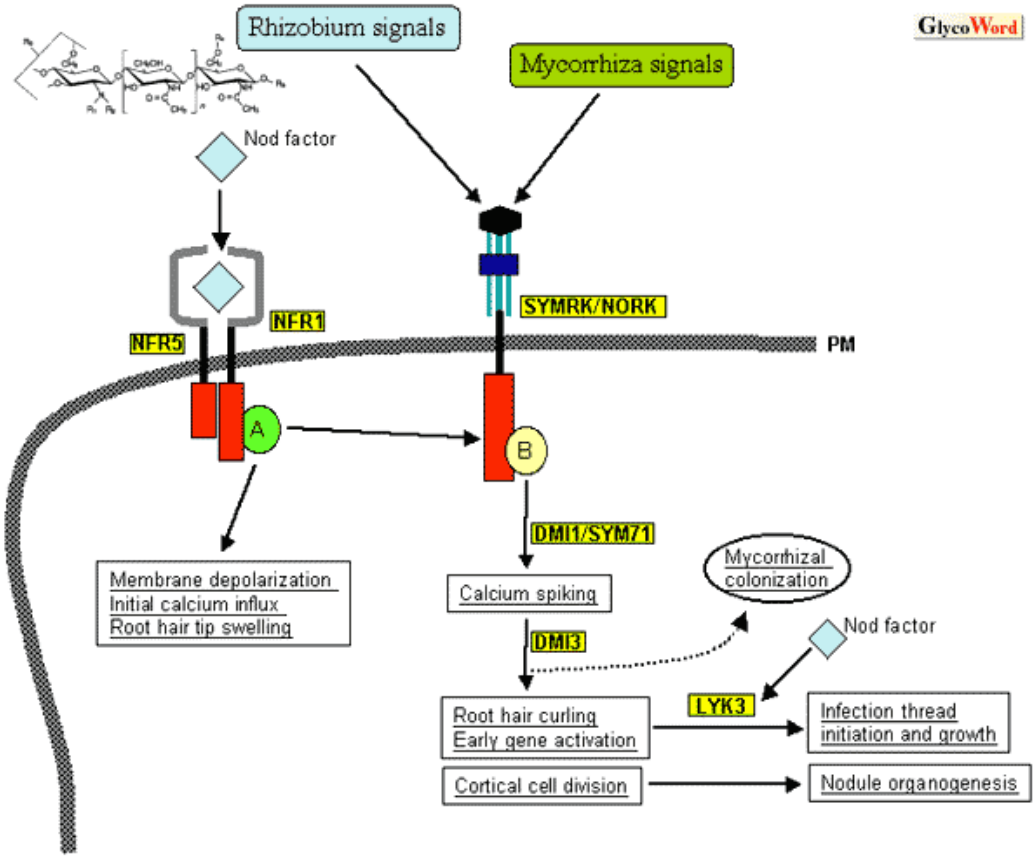
With the entry of rhizobial cells into pericyclic cells, if the host cell is a tetraploid cell, the cell undergoes transformation into actively dividing cells otherwise they do not respond to bacterial infection. However, the infected tetraploid cells then divide and redivide to produce a mass of cells which assume nodular form. The growth and the development of a nodule requires the secretion of indole acetic acid (IAA) by the bacterial cells.

INTERACTION BETWEEN BACTERIA AND HOST CELLS

With entry of bacterial cells into host cells, bacterial cellular components stimulate host genome, where globin genes and other related genes get expressed. As a result globin proteins and other factors are synthesized in significant quantities. The globin protein produced in leguminous root nodules is called leghemoglobin, whose amino acid sequence and structure is similar to that of animal globin proteins.



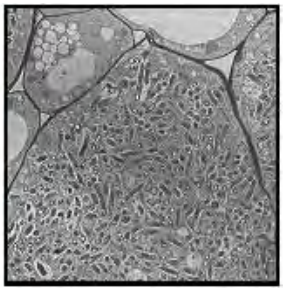


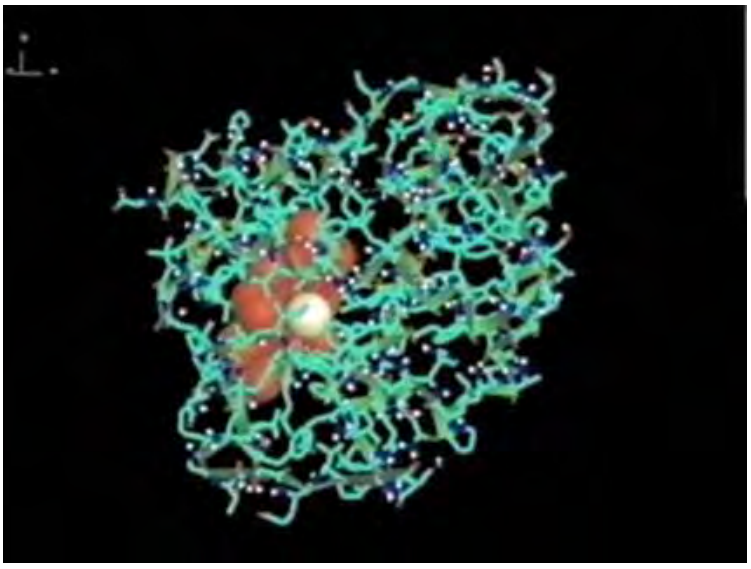


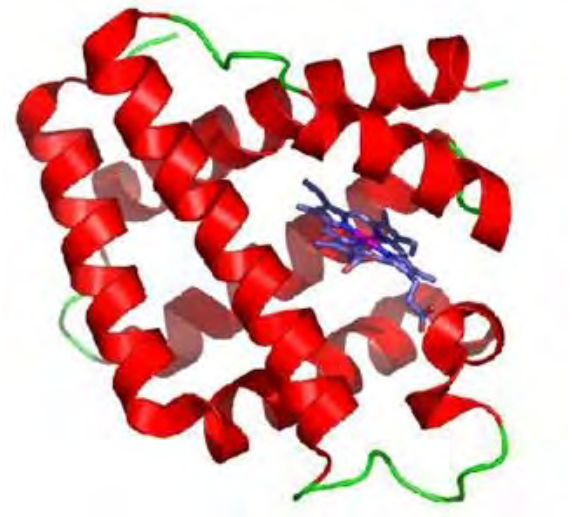
On the contrary, host cellular factors inturn activate the expression of nitrogen fixing genes found

in rhizobial cells. The nif genes remain unexpressed if the rhizobial cells are free from host cells. Though the bacterial cells are associated with the host cells, the genes remain unexpressed if the nitrogen sources like nitrate and ammonia are present in the medium. Only in the absence of them, the N2 fixing genes are expressed. Hence the nitrogenase and other related enzymes are considered as inducible enzymes. Thus the interaction between the host cellular components and bacterial cellular components is very important in the expression of each other's genomes for N2 fixation.









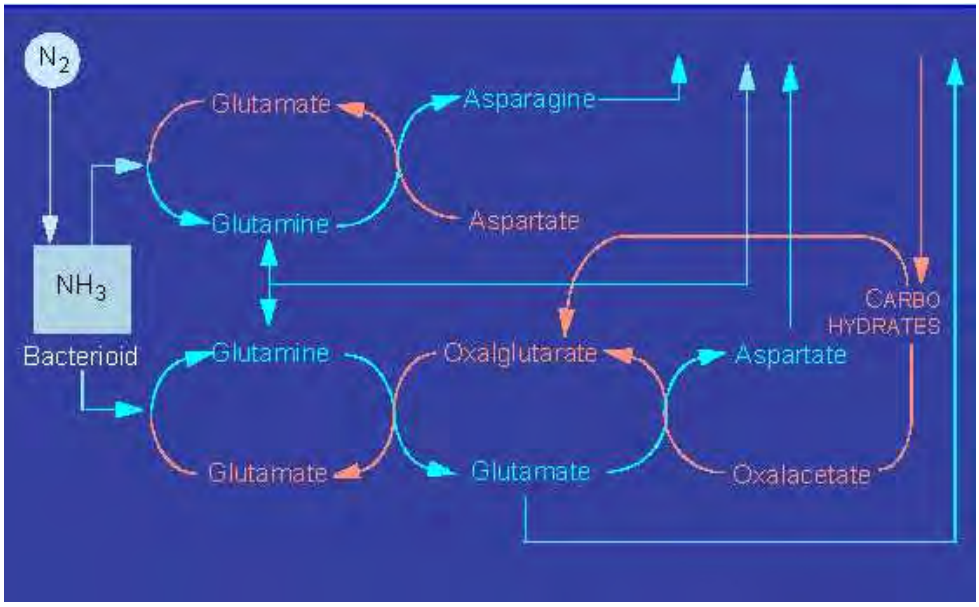
NIF GENES AND NITROGENASE

Nitrogen fixing genes are a family of 17 genes. They are located in the bacterial chromosomes but scattered over a length. Among them 10-11 genes are mainly responsible for the synthesis of functional enzyme complex which is made up of larger subunits, smaller subunits and cofactors. Rest of the genes code for the other factors, some of which induce leghemoglobin gene expression in the host cells and the rest are involved in nitrogen fixing activity.

Table 1. The *nif-gene* Products and Their Role (Known or Proposed) in Nitrogen Fixation

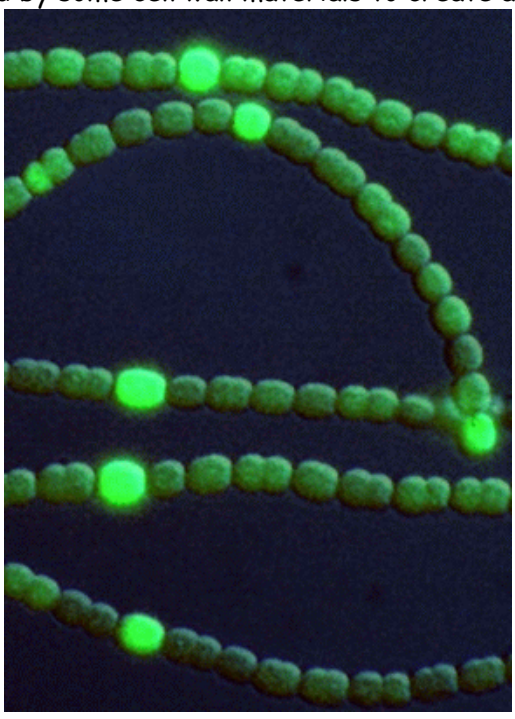
nif-GENE	IDENTITY/ROLE
nifH	Dinitrogenase reductase. Obligate electron donor to dinitrogenase during nitrogenase turnover. Also is required for FeMo-co biosynthesis and apodinitrogenase maturation
nifD	α subunit of dinitrogenase. Forms an $a_2ß_2$ tetramer with ß subunit. FeMo-co, the site of substrate reduction, is present buried within the α subunit of dinitrogenase
nifK	ß subunit of dinitrogenase. P-clusters are present at the ß subunit-interface
nifT	Unknown
nifY	In *K. pneumoniae*, aids in the insertion of FeMo-co into apodinitrogenase
nifE	Forms $a_2ß_2$ tetramer with NifN. Required for FeMo-co synthesis. Proposed to function as a scaffold on which FeMo-co is synthesized
nifN	Required for FeMo-co synthesis
nifX	Involved in FeMo-co synthesis. Specific role is not known
nifU	Involved in mobilization of Fe for Fe-S cluster synthesis and repair
nifS	Involved in mobilization of S for Fe-S cluster synthesis and repair
nifV	Homocitrate synthase, involved in FeMo-co synthesis
nifW	Involved in stability of dinitrogenase. Proposed to protect dinitrogenase from 0_2 inactivation
nifZ	Unknown
nifM	Required for the maturation of NifH
nifF	Flavodoxin. Physiologic electron donor to NifH
nifL	Negative regulatory element
nifA	Positive regulatory element
nifB	Required for FeMo-co synthesis. Metabolic product, NifB-co is the specific Fe and S donor to FeMo-co
fdxN	Ferredoxin. In *R. capsulatus*, serves as electron donor to nitrogenase
nifQ	Involved in FeMo-co synthesis. Proposed to function in early MoO_4^{2-} processing
nifJ	Pyruvate:flavodoxin (ferredoxin) oxidoreductase. Involved in electron transport to nitrogenase

The mol. Wt. of nitrogenase enzyme is 250-320 kd. It consists of complex II with 4 large subunits and two small subunits. The small subunit is in association of iron and Mo2 ions as activators. The enzyme is highly sensitive to oxygen and it will be irreversibly destroyed in the presence of oxygen. For its stability and activity, the enzyme has to be maintained in an anaerobic environment within the cell itself. In root nodules, however, leghemoglobin proteins have a dual role to play. While leghemoglobin mop up all the oxygen present in the environs of nitrogenase enzyme enclosed in a membranous vesicle called microsomes, it also provides oxygen for other cellular structures for oxidative mechanisms. In the case of obligate anaerobic bacteria which live in free state, how cells maintain anaerobic condition intracellular is not known and the same is true with the root nodules.

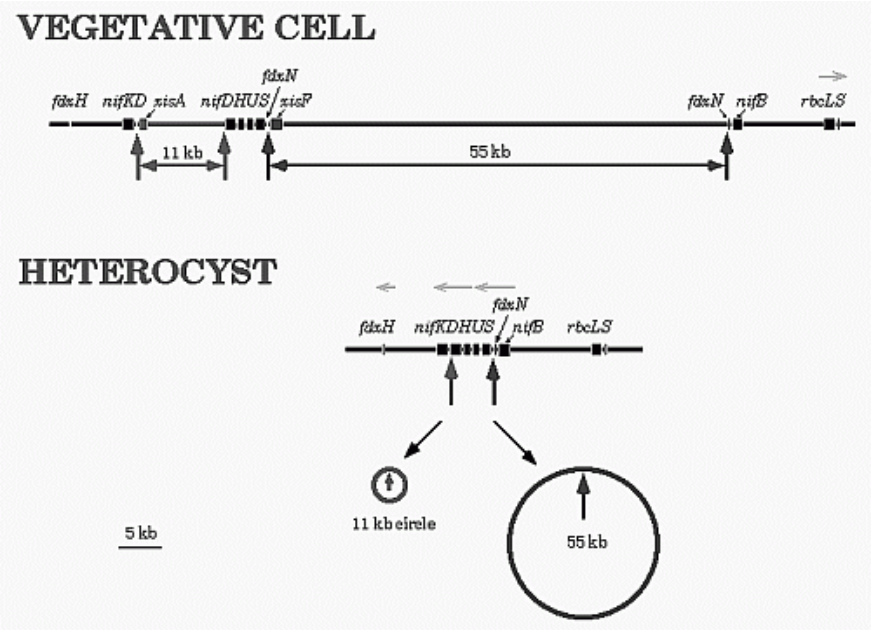


NITROGENASE IS ALSO AN INDUCIBLE ENZYME

As mentioned earlier, nitrogenase enzyme is also an inducible enzyme. In the presence of nitrogen sources like NO3, nitrites and ammonia from cellular environment, this enzyme is expressed through gene activation. In blue green algae, like Nostoc and anabaena, in the absence of above said nitrogen sources, the vegetative cells get transformed into large hyaline cells, whose polar ends will be plugged by some cell wall materials to create an anaerobic environment within the cells.

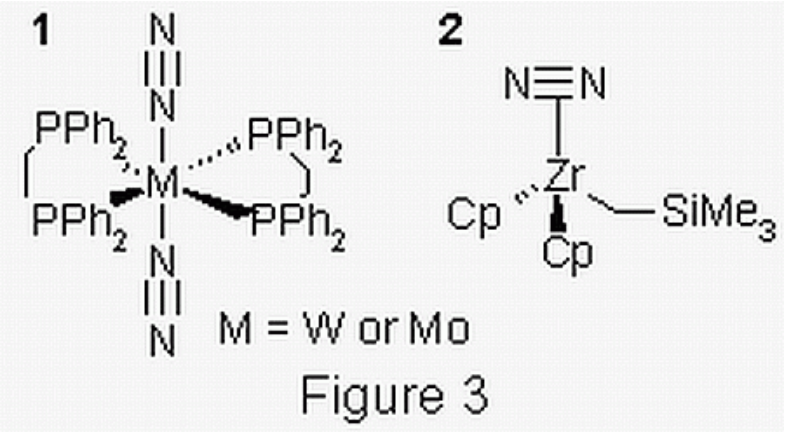


At the same time, hitherto unexpressed nitrogenase and other related genes get expressed for fixing the molecular N2.



MECHANISM OF NITROGEN REDUCTION TO NH3

In order to explain the mechanism of reduction of inert molecular nitrogen to utilizable form i.e. NH4 various theories have been proposed in the past. However, recent studies support the view that the molecular N2 is reduced on the surface of the enzyme nitrogenase in a multistep process. The intermediate products remain bound to the enzyme surface and only the final product ie NH4 is released from the enzyme.



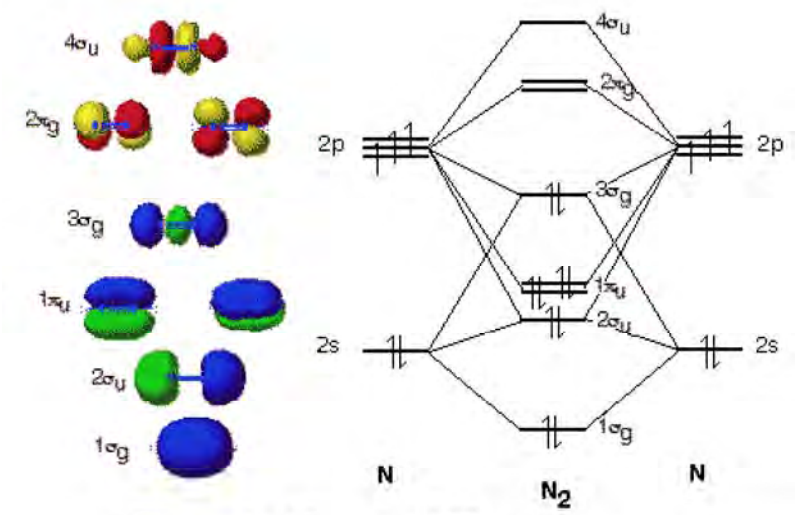
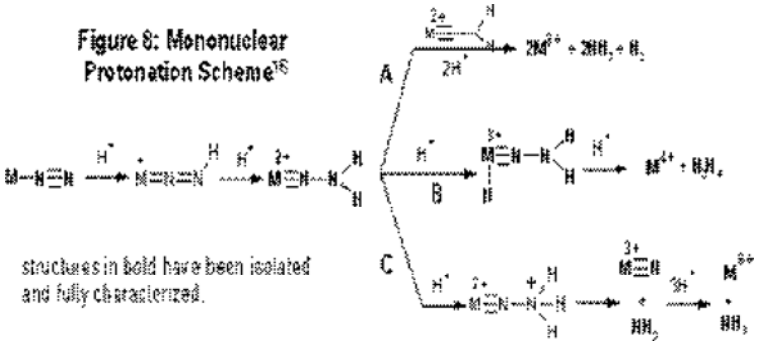
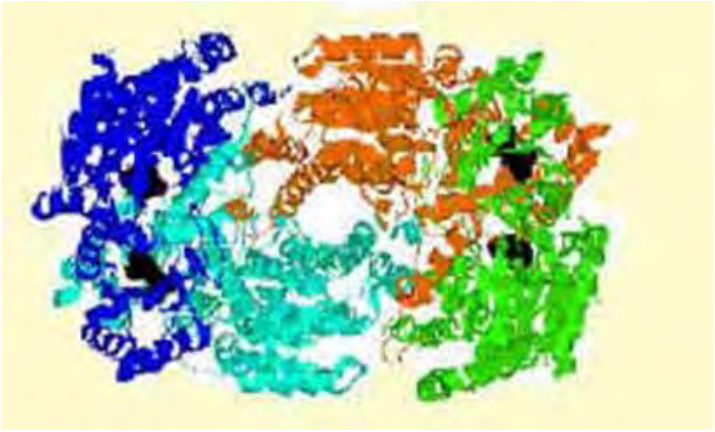
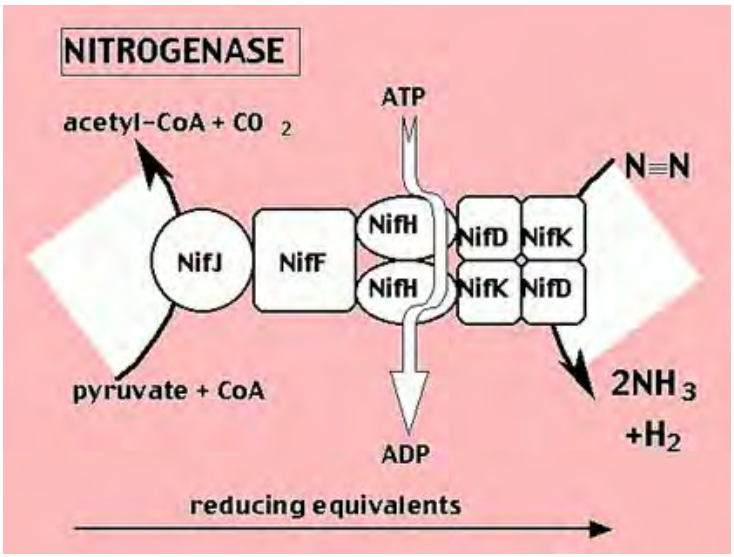


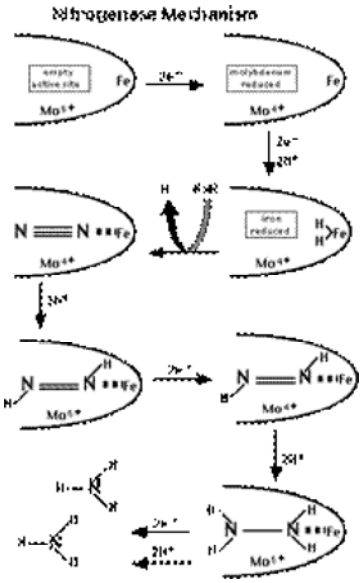
Figure 1:- Nitrogen MO scheme



The reductive power i.e. NADH2 (or NADPH+H) and ATP energy required for this process is supplied by the products of glycolytic pathway or HMP pathway. To begin with, the large subunit part of the nitrogenase enzyme is activated by ATP.

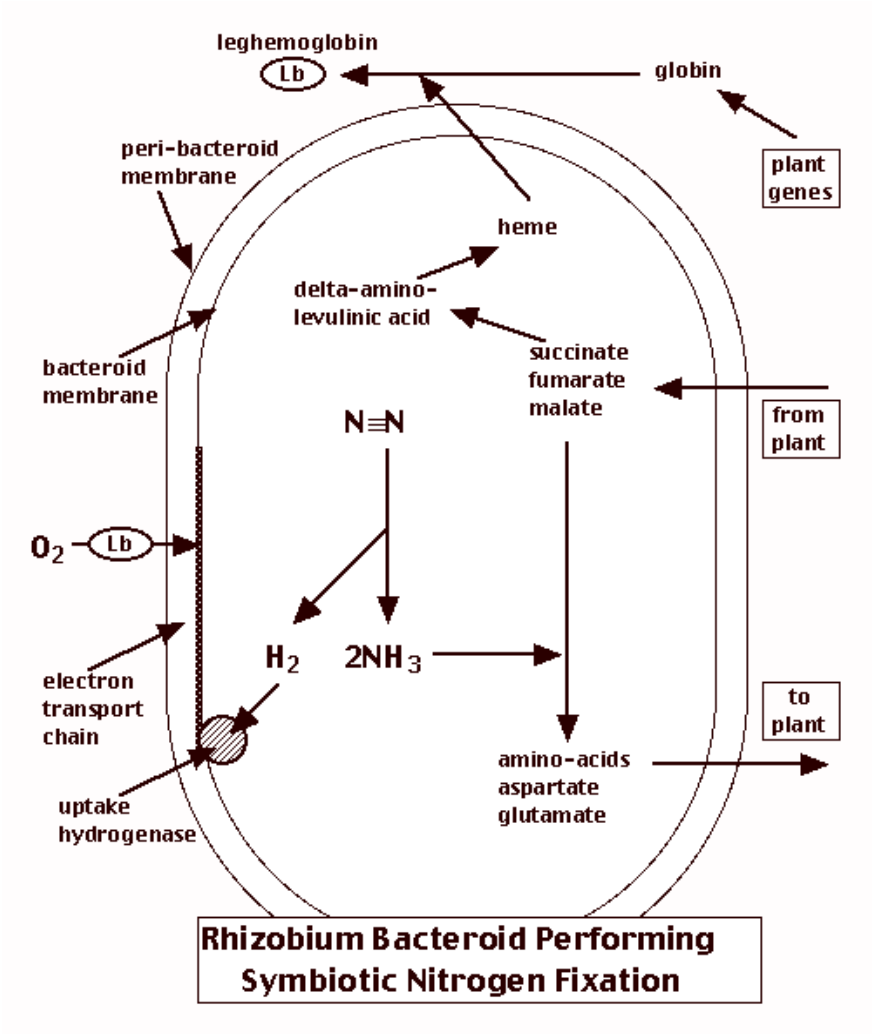






The activated enzyme now accepts molecular N2 and the same binds to the enzymatic surface at specific sites. The energized enzyme now loosens the triple bonds between N atoms, probably through conformational change in the protein structure. Thus the inert triple bonded N2 is rendered active N=N ready for reductive step. At this juncture, the large complex part of nitrogenase enzyme containing Fe2+ and Mo2+ is reduced by NADH+H. In this oxidation reduction step, the electrons are conveyed to the activated N=N, through Fe/Mo2+. A the same time the activated N=N gets reduced to HN-NH called diimide by another reduction reaction.

With another round of activation of enzyme by ATP, and reduction of HN-NH, is further reduced to Hydrazine i.e. H2N=NH2. In the final round of activation and reduction, the hydrazine gets reduced to 2NH3 which are immediately freed from the surface of the enzyme. So the reduction of one mole of N2 to two moles of NH3 requires 10-12 moles of ATP, and 3 moles of NADH+H+.

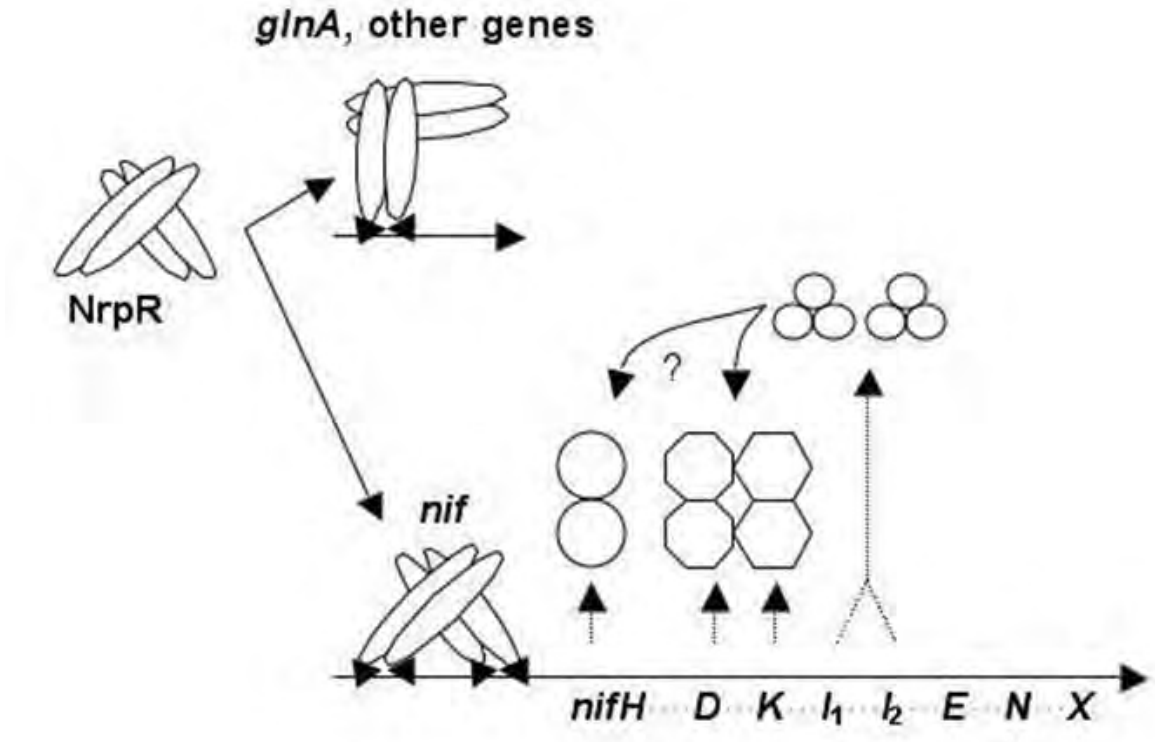


The NH3 thus produced within the bacterial cell is assimilated into glutamate, which is then released into host cells. This way, leguminous plants fix molecular nitrogen to utilizable form of nitrogen.

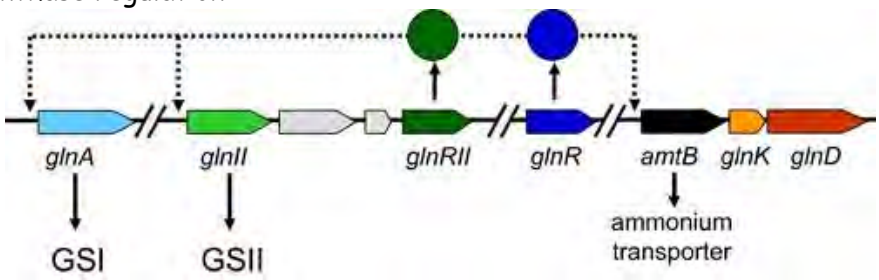
GENETIC ENGINEERING OF NIF GENES

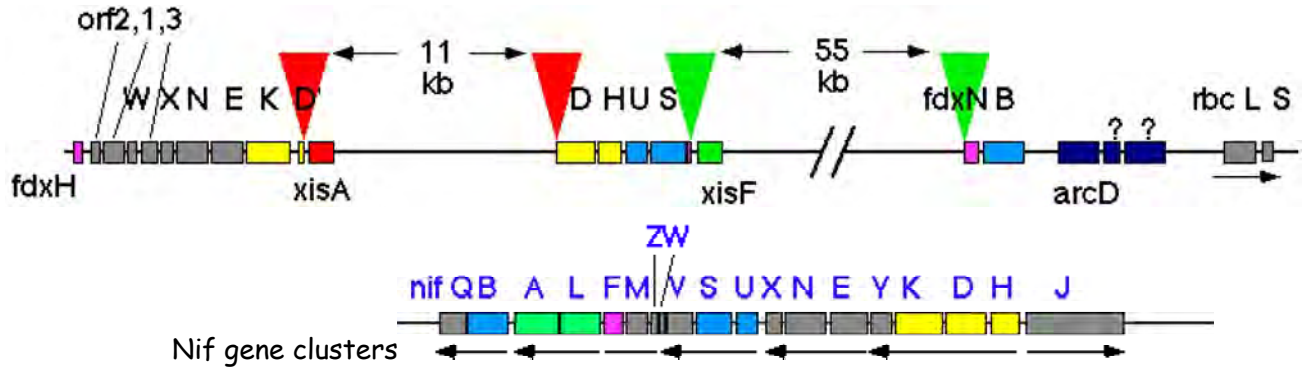
Nitrogenase genes have been identified and isolated by recombinant DNA techniques from various N2 fixing bacterial cells. Transfer of such genes into higher plant cells is a formidable task. Inspite of it, biologists have succeeded in incorporating such genes into protoplasts of higher plants by incubating protoplasts in a medium containing exogenously supplied nif genes through plasmids, but what is disconcerting in these experiments is that the incorporated genes do not express in the plant cells. Probably the expression of incorporated nif genes required many regulatory gene products and other factors that maintain intracellular anaerobic atmosphere. People are making attempts to make non leguminous plants like paddy, sorghum, wheat, corn, etc.,

compatible for rhizobial infection to their root system. In some labs, plant genetic engineers are making attempts to hybridize heterocyst cells of Nostoc with higher plant cells. The success of these experiments, if it happens, brings about another super green revolution in the field of agriculture. We have to wait and see for that D-day to be dawn.

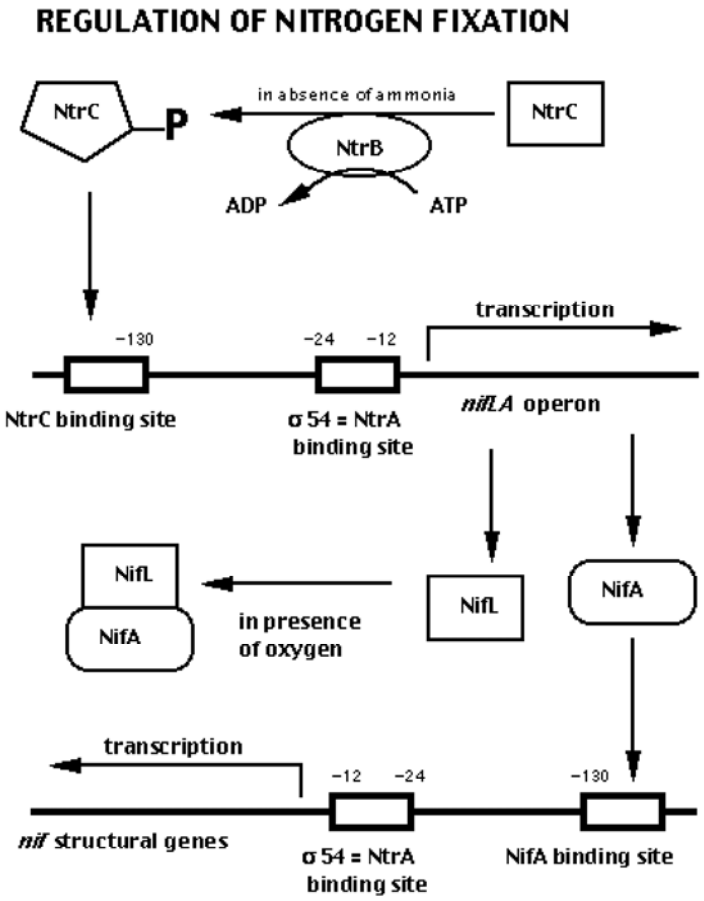


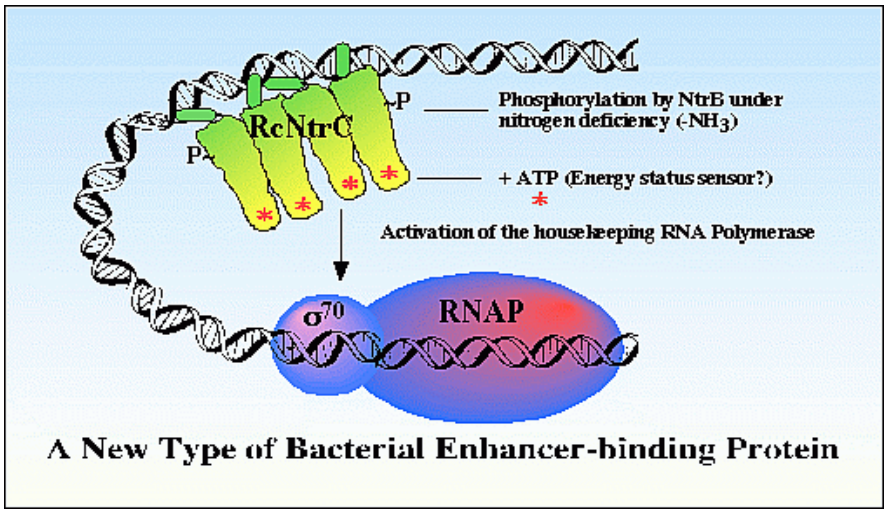
Gln synthase regulation





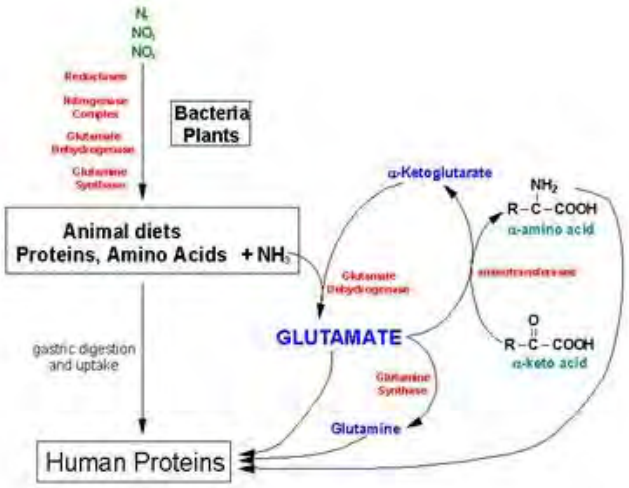
Klebsiella nif gene clusters

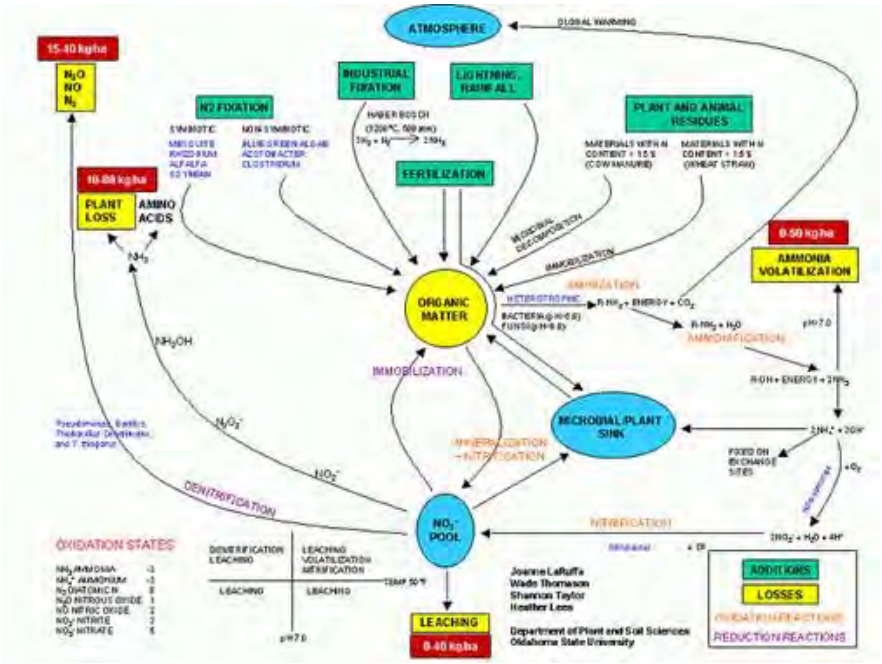


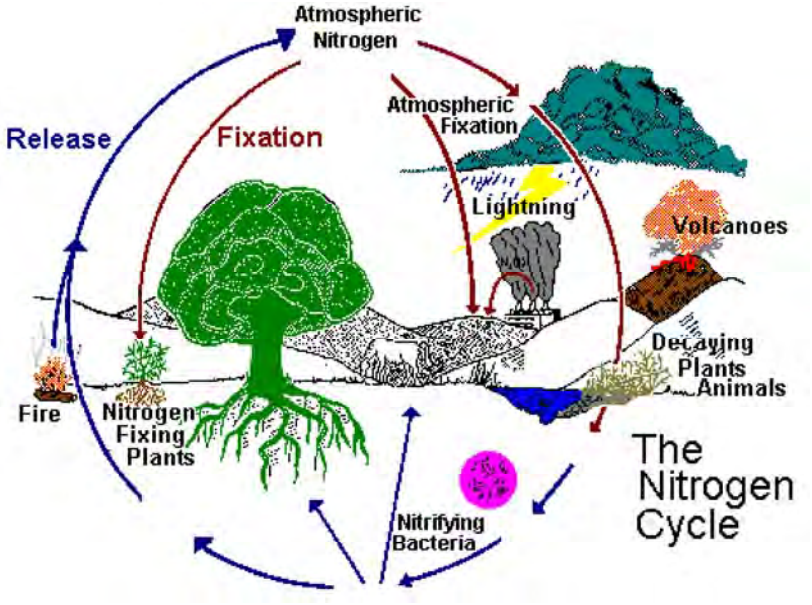


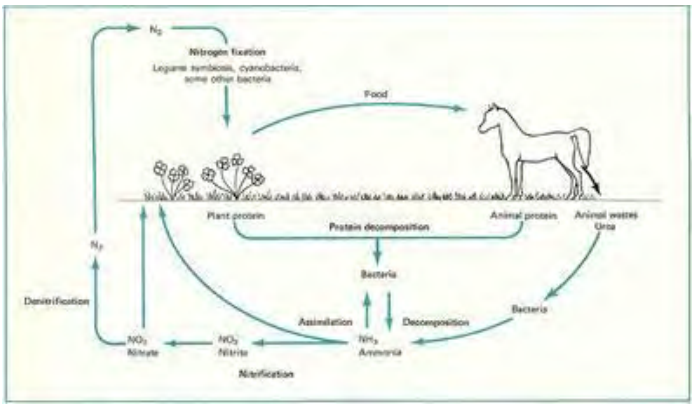
NITROGEN CYCLE

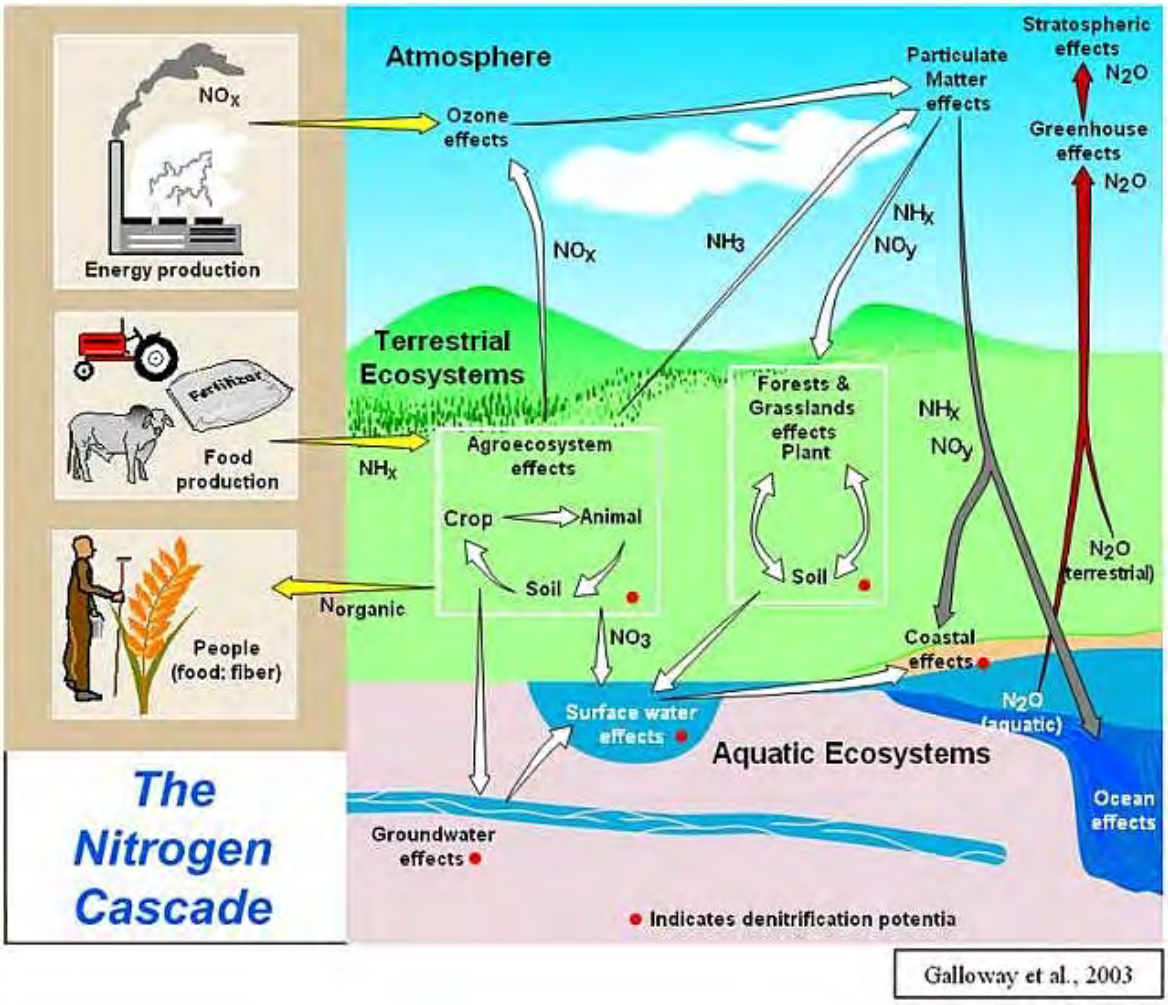
As shown in the self explanatory figures, plants, animals and soil micro organisms bring about the interconversions of inert N2 to utilizable form of N2 and back to inert N2 by various ways and means.

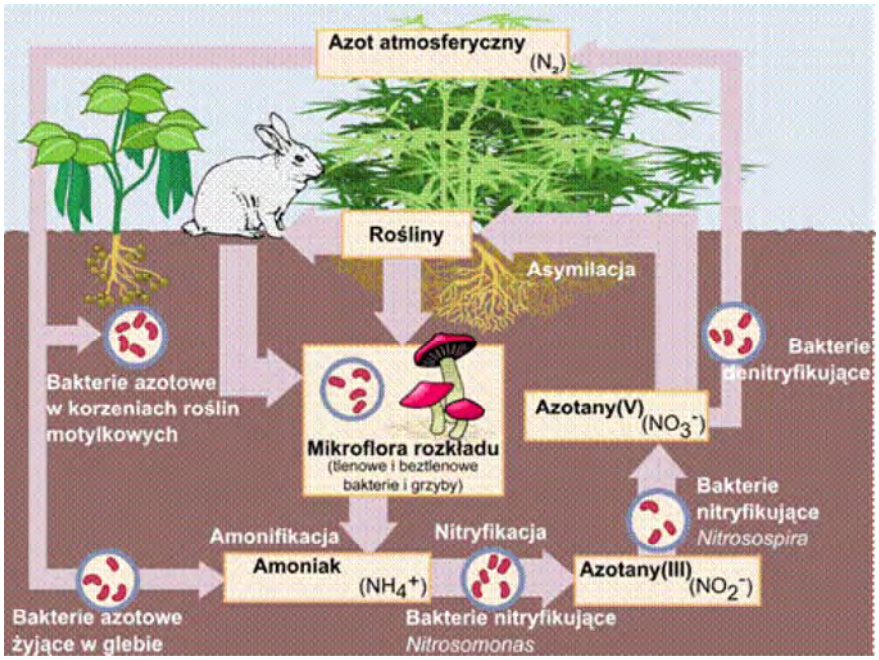

Bacteria
Plants
Animal diets
Proteins, Amino Acids +NH3
gastric digestion
and uptake
α-Ketoglutarate
α-amino acid
α-keto acid
GLUTAMATE
Glutamine
Human Proteins


ATMOSPHERE
INDUSTRIAL FIXATION
LIGHTNING
PLANT AND ANIMAL RESIDUES
N2 FIXATION
FERTILIZATION
PLANT LOSS
AMINO ACIDS
AMMONIA VOLATILIZATION
ORGANIC MATTER
IMMOBILIZATION
MICROBIAL/PLANT SINK
DENITRIFICATION
NO3- POOL
NITRIFICATION
OXIDATION STATES
LEACHING
ADDITIONS
LOSSES
REDUCTION REACTIONS


Atmospheric Nitrogen
Release
Fixation
Atmospheric Fixation
Lightning
Volcanoes
Decaying Plants Animals
Fire
Nitrogen Fixing Plants
Nitrifying Bacteria
The Nitrogen Cycle


Nitrogen fixation
Food
Plant protein
Protein decomposition
Animal protein
Animal wastes
Urea
Bacteria
Denitrification
Assimilation
Decomposition
Bacteria
Nitrate
Nitrite
Ammonia
Nitrification


Atmosphere
NOx
Energy production
Ozone effects
Particulate Matter effects
Stratospheric effects
N2O
Greenhouse effects
N2O
NOx
NH3
NHx
NOy
Terrestrial Ecosystems
Fertilizer
Food production
NHx
Agroecosystem effects
Crop
Animal
Soil
Forests & Grasslands effects Plant
Soil
NHx
NOy
N2O (terrestrial)
Norganic
People (food: fiber)
NO3
Coastal effects
Surface water effects
N2O (aquatic)
Aquatic Ecosystems
Ocean effects
Groundwater effects
The Nitrogen Cascade
Indicates denitrification potentia
Galloway et al., 2003



AMINOACID METABOLISM

Organic acids containing an amino group at one end and a carboxyl group at the other and are called amino acids. They are one of the most important of cellular components, because they are used in the synthesis of proteins, nitrogenous bases (for nucleic acids), alkaloids, phenolic compounds, porphyrin compounds, flavinoids, pigments, etc. Thus amino acids play a central role in cellular structures and cellular metabolism. More than 150 amino acids have been identified from various plant sources, but only 20 of them are involved in the formation of proteins, others have different functions.

PROPERTIES

Amino acids when extracted appear as amorphous powder. They are sparingly soluble in water. With the exception of glycine all others show an asymmetric carbon atom to which one amino group, one carboxyl group, one R-group and one hydrogen are linked. So they exhibit the properties of chirality and isomerism. They also show optical property like D and L forms. Almost every amino acid found in plant or animal proteins have been identified as L amino acids. It is really paradoxical to observe that living organisms have chosen L forms of amino acids for cellular metabolism but with regard to carbohydrates they have selected D forms as carbohydrate units. It is difficult to explain why and how life forms have selected L-form of amino acids and D forms of carbohydrates. Interestingly the D forms of amino acids are used in the production of some important cyclic or linear proteins, some of which are antibiotics.

Amino acids, because of their ionizable property show different electrical charges in the same molecule. Under different pH conditions, they can exist either as basic ions, acidic ions or neutral ions. The neutral ions are also called amphoteric or Zwitter ions. In fact, the pH at which an amino acid exists as a Zwitter ion is referred to as isoelectric point. Different amino acids show

different isoelectric points and they have to be determined by titration against known concentration of a base or an acid. Amphoteric amino acids are also called ampholytes and they are of greater use in chemical industry and medical research.

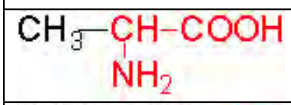
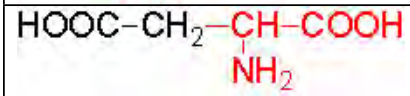
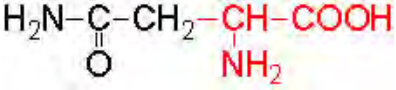
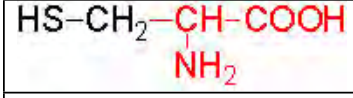
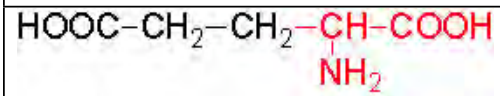
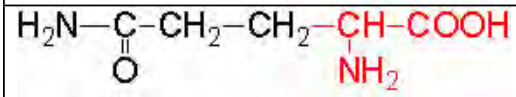
Further more, the R groups present vary from amino acid to amino acid, because they may contain additional basic amino groups, carboxyl groups, sulfhydril groups, hydroxyl groups, aromatic groups or CH3 groups. Depending upon the nature of R groups different amino acids have been identified.

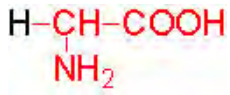
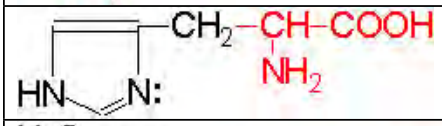
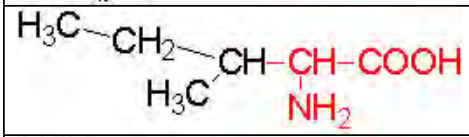
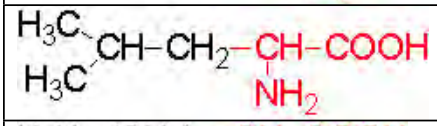
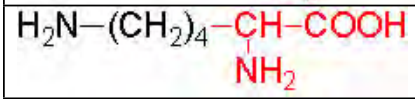
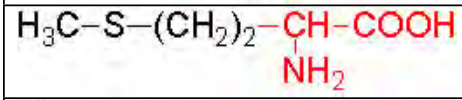
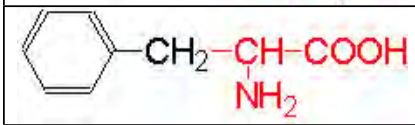
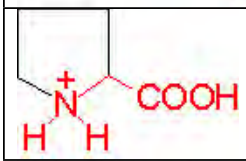
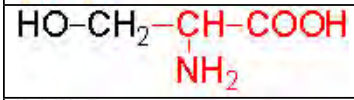
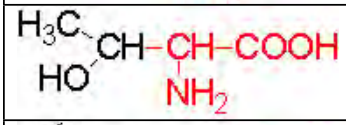
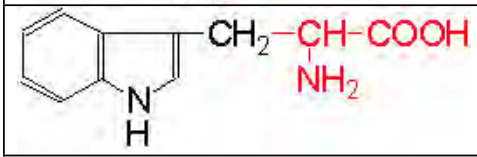
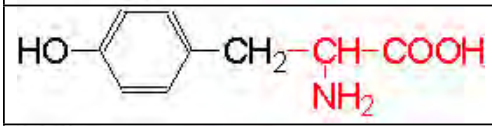
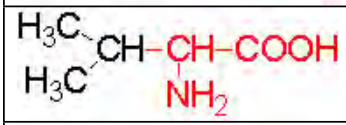
DETECTION OF AMINOACIDS

Almost all amino acids react with Ninhydrin, a coloring reagent, and produce purple coloration. Proline ad hydroxyproline produce yellow color action with Ninhydrin reagent. Using Ninhydrin reaction methods, it is possible to identify individual amino acids but also they can be estimated quantitatively. Mixtures of amino acids can be separated by paper chromatography or by automatic amino acid analyzers and they can be identified as well as estimated. Another interesting method that is employed for identification of specific amino acids is by the use of 1' fluoro 2.4 dinitro benzene which under mild alkaline conditions react with amino acids to produce 2,4 dinitrophenyl derivatives. In finger printing of a polypeptide chain this method is very useful.

CLASSIFICATI8ON OF AMINOACIDS

Almost every amino acid found in cellular proteins has been identified as L form. The total number of such amino acids found in all biological system is just twenty. But one more is added, i.e. Selenocysteine.
The said twenty one different amino acids can be easily identified because of different R groups. Based on this L form of amino acids has been classified into the following types and each one of them shows characteristic features; aliphatic, aromatic, heterocyclic, hydroxyl, acidic, basic and sulphur containing.
The said twenty different amino acids can be easily identified because of different R groups. Based on this L form of amino acids has been classified into the following types and each one of them show characteristics features.

Amino Acid structure	Name	Symbol
	Alanine	A
	Aspartic	D
	Aspargine	N
	Cysteine	C
	Glutamic	E
	Glutamine	Q

	Glycine	G
	Histidine	H
	Isoleucine	I
	Leucine	L
	Lysine	K
	Methionine	M
	Phenylalanine	F
	Proline	P
	Serine	S
	Threonine	T
	Tryptophan	W
	Tyrosine	Y
	Valine	V
	Selenocyteine	Sc

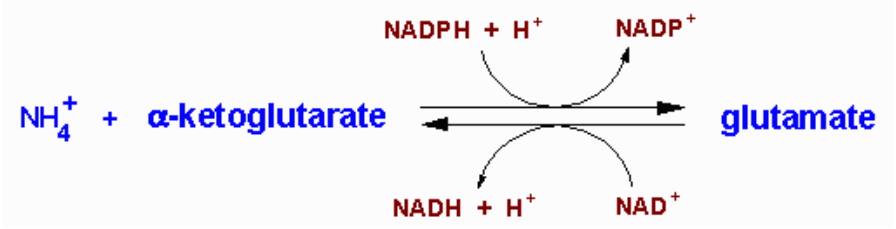
Biosynthesis of amino acids

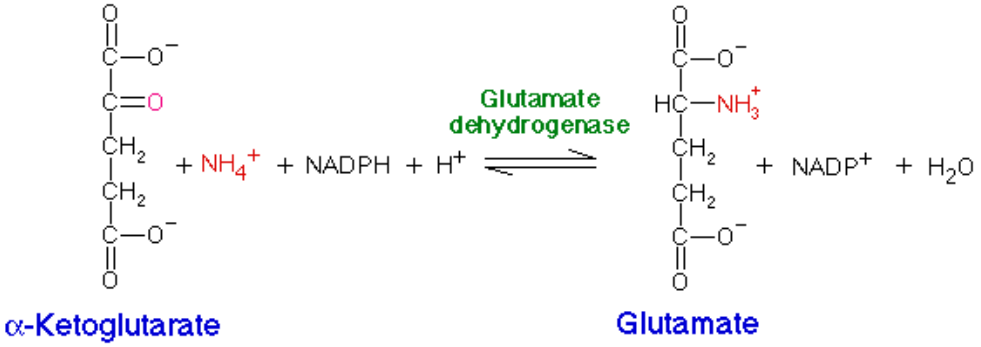
Plants are capable of synthesizing amino acids in every living cell but most of the primary amino acids are synthesized in roots and leaves. The ammonia produced by the reductive steps of NO2, NO3, or N2 are toxic if they are accumulated in the cells. Hence, the ammonia is immediately used up in the synthesis of amino acids. If there is any excess of NH3 it is stored in amides, from which the same can be recovered.

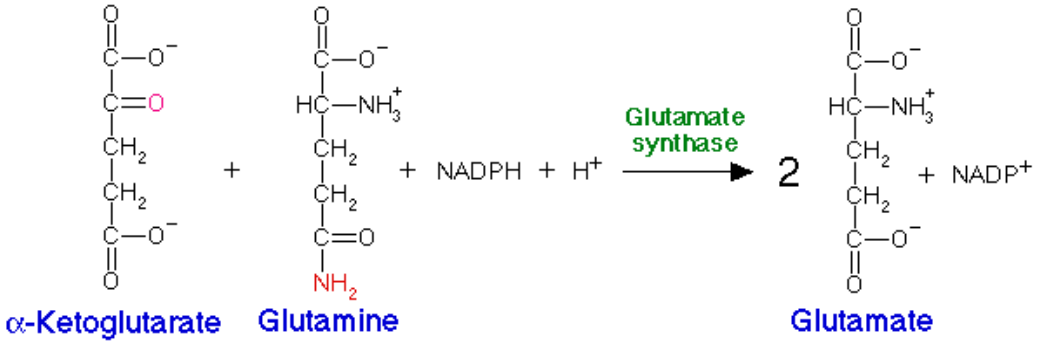
The most important pathway by which amino acids are synthesized is reductive amination leading to the synthesis of glutamate. The other pathways like transamination and carbonyl phosphate reactions are called secondary pathways.

REDUCTIVE AMINATION PATHWAY

The most important carbon compound that acts as an acceptor of amino group is ketogluterate which is an intermediate product of Kreb's cycle. Though alfa ketogluterate is mainly used in Kreb's cycle to generate energy, depending upon the need of the cells or tissues, it is also drawn into reductive amination pathway for the synthesis of glutamate. This pathway is catalyzed by an enzyme known as ketogluterate dehydrogenase which brings about amination as well as reduction. The mol. Wt. of the enzyme is 320,000 Daltons and it is an allosteric enzyme. That means it is a regulatory enzyme modulated by specific factors. The reducing power used in this reaction is NADH2 in non chlorophyllous tissue) or NADPH2 in chlorophyllous tissue.







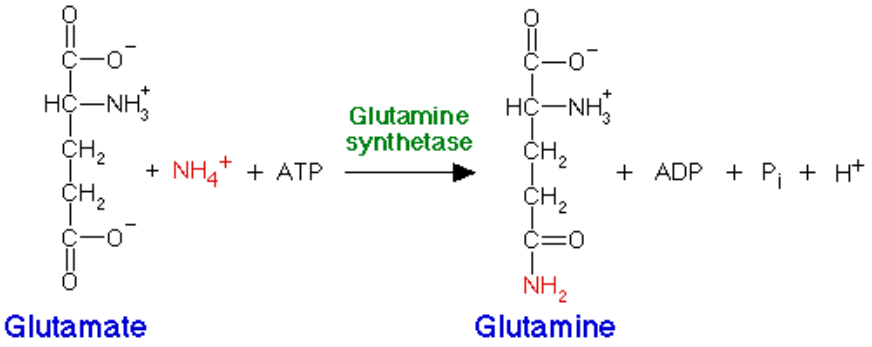
To begin with alfa ketogluterate, in the presence of NH3 spontaneously reacts and gets converted to alfa immunogluterate. Then alfa imminogluterate is reduced by the dehydrogenase to produce glutamate.

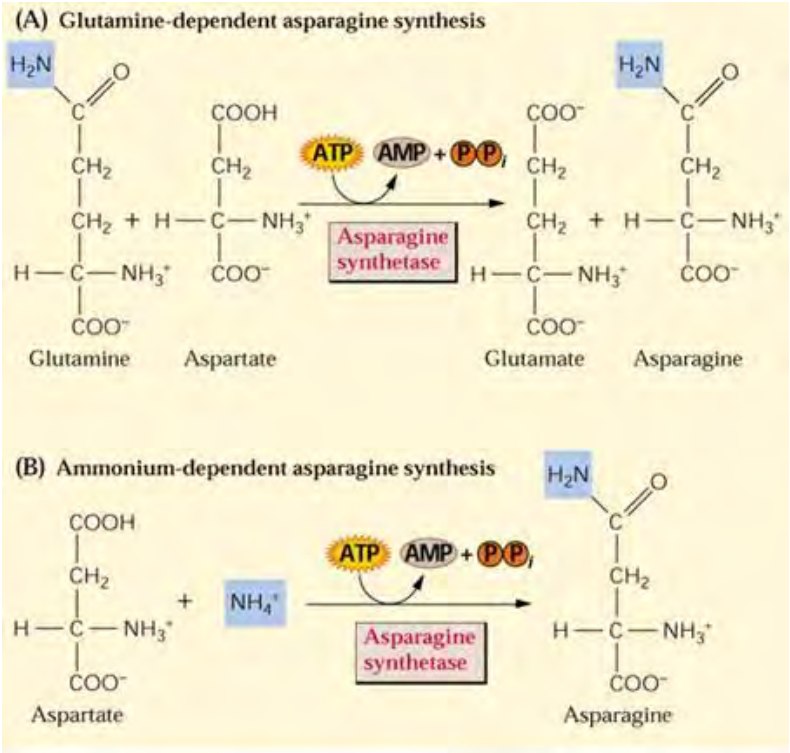
Similarly, the other keto acids like pyruvate and oxoloacetate are also used in reductive amination

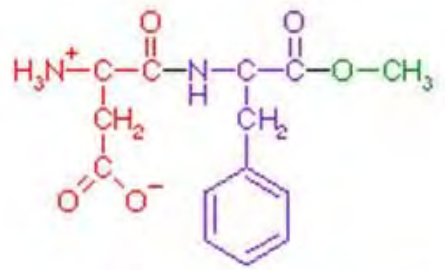
by specific amino-reductases resulting in the formation of respective amino acids. Pyruvate yields alanine and *OAA* yields aspartate. But when labeled NH3 is provided to plant tissues, most of the label (90%) ends up in glutamate; only a little quantity is found in alanine and aspartate. Thus glutamate synthesis acts as the major of pathway in amino acid synthesis. Nonetheless glutamate acts as the donor of amino group for the synthesis of other amino acid.

AMIDE SYNTHESIS:

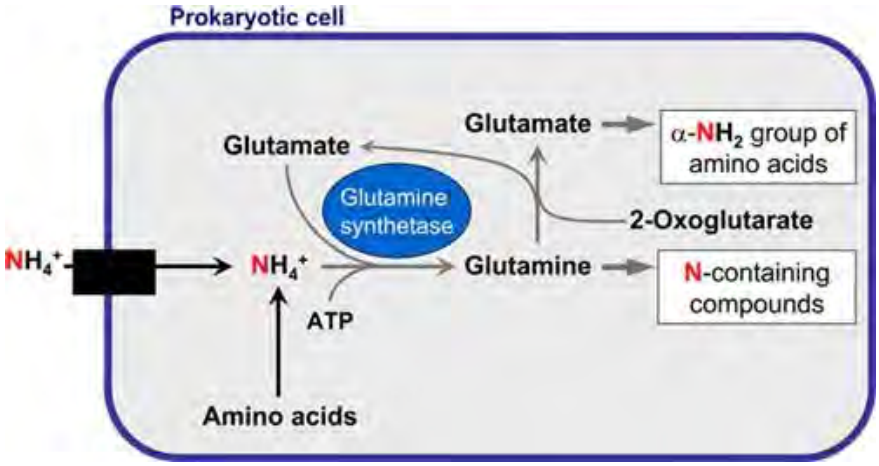
Plants have some unique mechanism by which excess NH3 is fixed as amides such as glutamine and asparagine. The amide synthesis is performed by specific amide synthetases. The glutamine synthetase is a complex allosteric enzyme regulated by specific factors. This enzyme requires ATP as the energy source for its activity in which extra NH3 group is added onto the additional carboxyl unit present in R group. When ammonia is present in sufficient quantities, most of the labeled ammonia ends up in glutamine and glutamicacid.







Aspartame

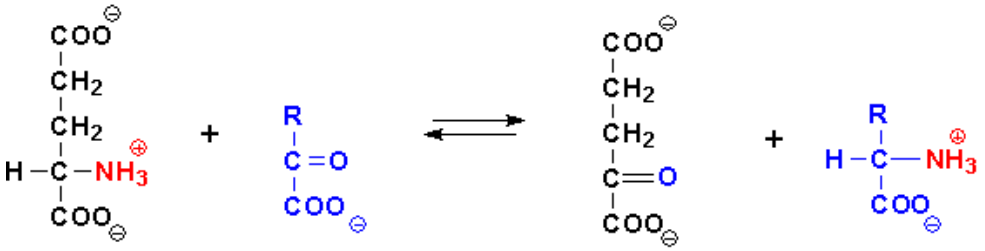


Similarly, Aspargine is also synthesized by amination reaction, which also requires the activation energy supplied by the hydrolysis of ATP. The enzyme involved in this reaction is Aspargine synthetase, which is also an allosteric enzyme.

The amides thus synthesized act as reserve components. Whenever there is a need for NH3 for the synthesis of amino acids either by reductive amination or by other processes, glutamine and Aspargine are subjected to deamination reactions by the activity glutamine or Aspargine deaminase enzymes and the NH3 released is used. It is important to note that synthesis and glutamine hydrolysis is not reversible reactions because the enzymes involved are different.

TRANSAMINATION PATHWAY

The transaminase enzymes are capable of transferring amino group from the donor amino acid to the acceptor ketonic organic acids. The coenzyme involved in this reaction is pyridoxal phosphate, which has a unique role in picking only amino group and then transferring to the ketonic group of the acceptor molecule.



In plants, of the activities of aspartate amino transferase and alanine amino transferase have been studied. Similarly, the synthesis of serine in peroxisomes found in C3 plants is also brought about by transaminase activities.

SYNTHESIS OF CARBOMOYL PHOSPHATE AND ARGININE

Plants have another unique pathway where they utilize respiratory CO2 and free NH3 to synthesize carbomoyl phosphate. The enzyme responsible for this process is carbomoyl phosphate synthetase. It is a mitochondrial enzyme and its activities has been studied in the leaves of phaseolus, pea and castor plants. The mechanism and properties of this enzyme is similar to that of animal mitochondrial carbomoyl phosphate synthetase. This enzyme requires ATP for its activity.

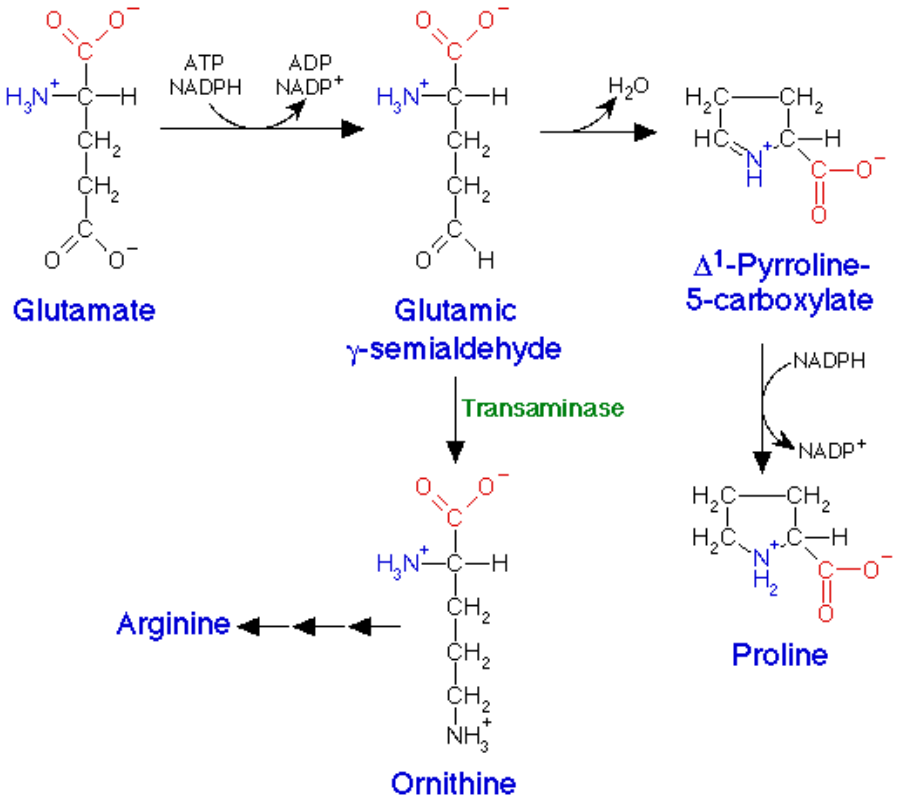
Carbamoyl phosphate is a very important compound; it is used in the synthesis of ornithine, citruline and Arginine. It is also used in the synthesis of nitrogenous bases.

Plants have been known to utilize urea as the source of NH3 for amino acid syntheses. But recent experiments, on chlorella pyrenoids and chlorella ellipsoides, have clearly demonstrated that urea is directly used in a condensation reaction with ornithine to produce arginine. However, the other properties of this enzyme have not been fully characterized.

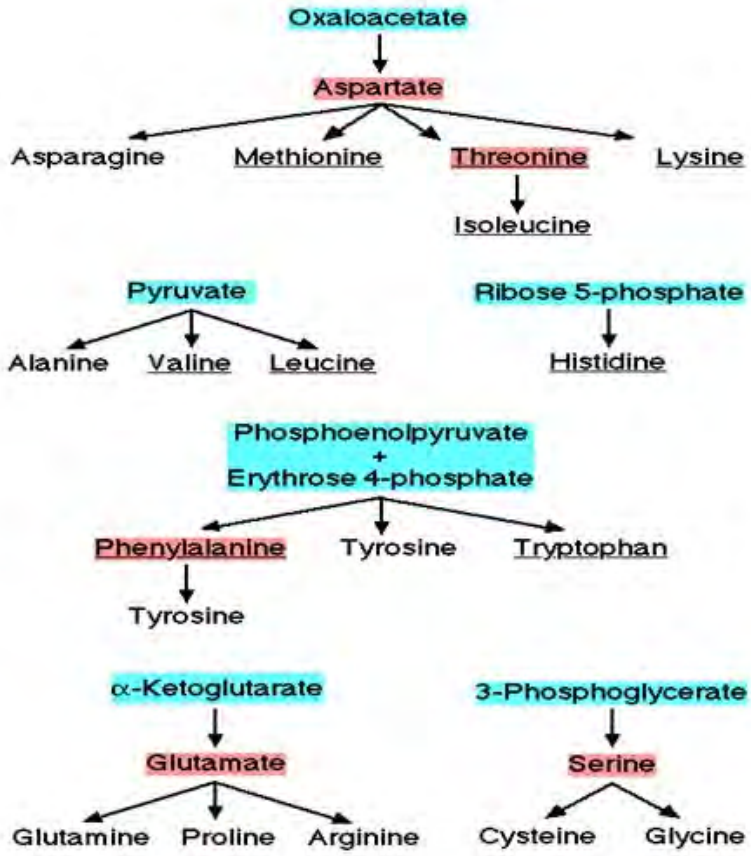
Urea + ornithine -> Arginine

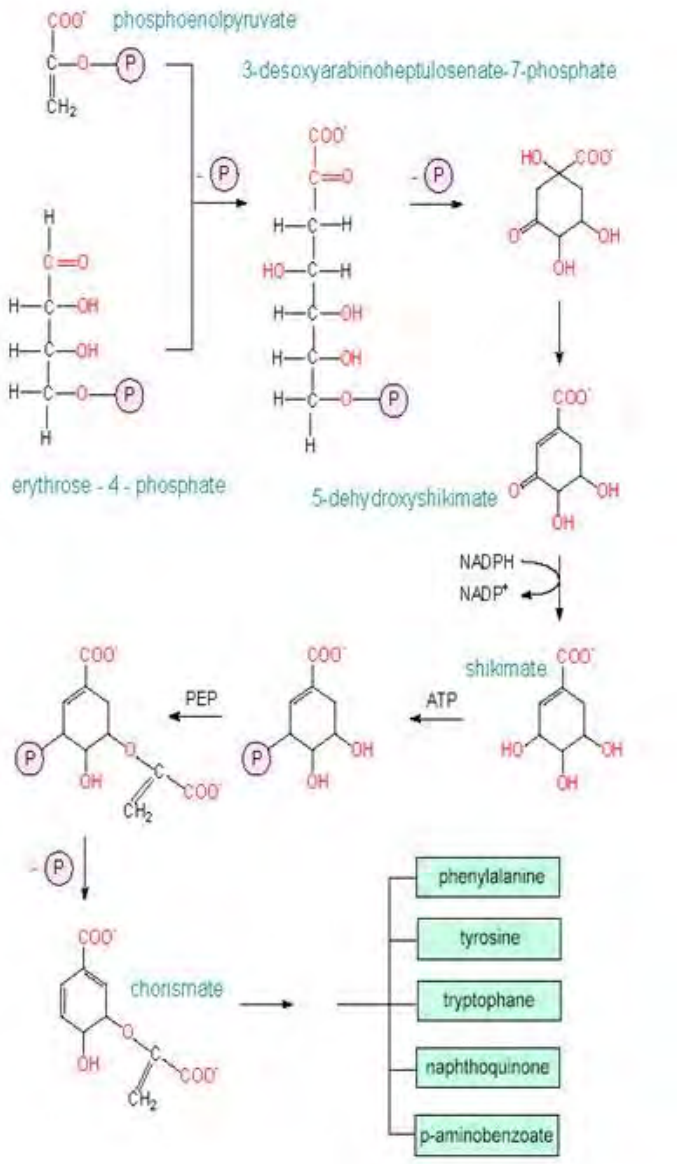
INTERCONVERSIONS:

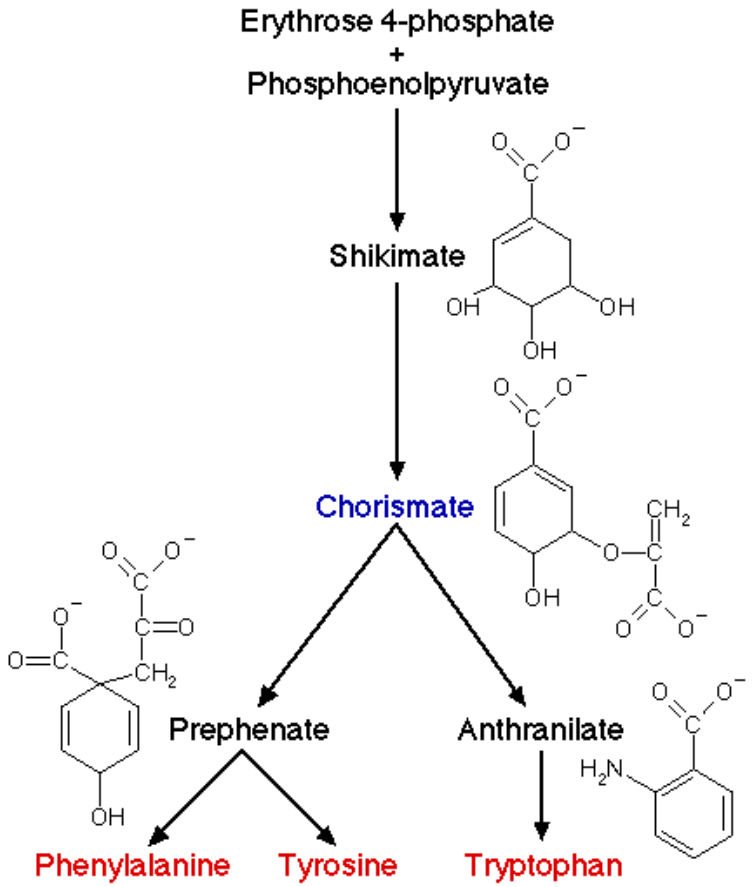
Plants possess various metabolic pathways in which starting from simple amino acids and other organic acids, they are capable of synthesizing all amino acids required for protein synthesis and other metabolic processes. Similarly, amino acids are also used in gluconeogenesis produce some intermediary compounds or to produce more energy. However these pathways are very well regulated.

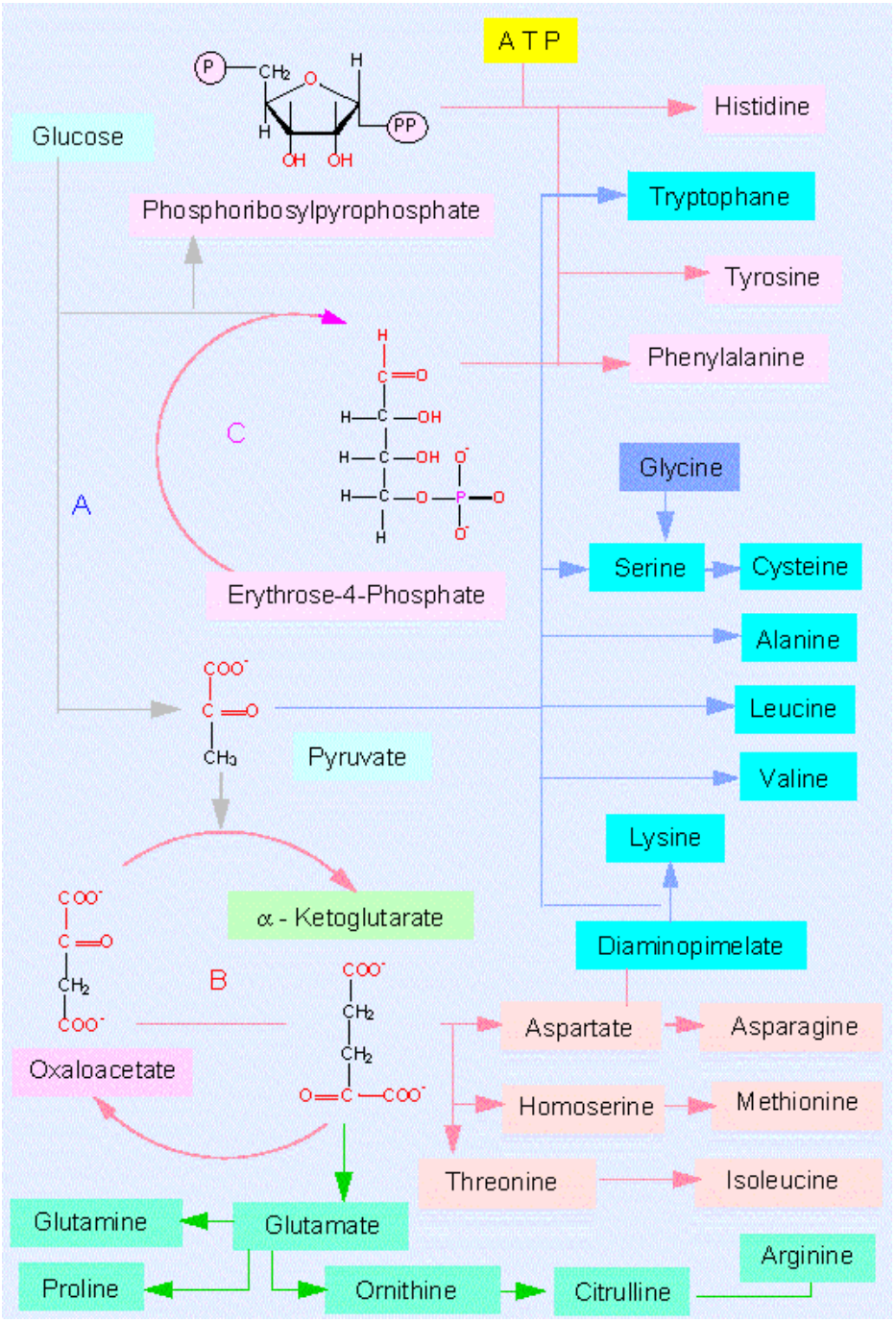


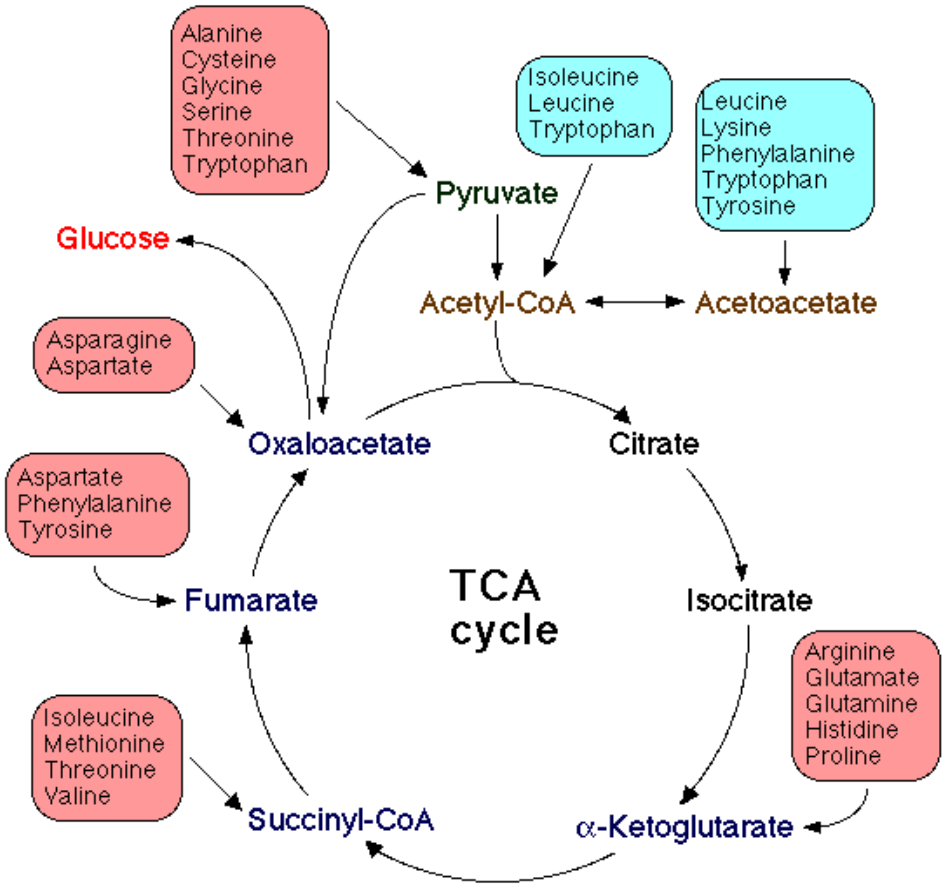
Most of the pathways that lead to the synthesis of various amino acids other than glutamic acid, aspartate, alanine, are multistep reactions. For example, glutamic acid is used to synthesize amino acids like proline, arginine, tryptophan or histidine in multistep reactions. Similarly, aspartate is metabolized to produce threonine or lysine; pyruvate is used in the synthesis of valine; phosphoenol pyruvate is converted to phenylalanine or tyrosine and anthralinic acid is metabolized to produce tryptophan, etc. Most of these pathways are regulated either at the enzymatic level or at the level of gene expression. For example, the synthesis of proline starting from glutamate exhibits feedback inhibition. Similarly, the pathways leading to the synthesis of threonine and lysine starting from aspartate are inhibited by their and product.

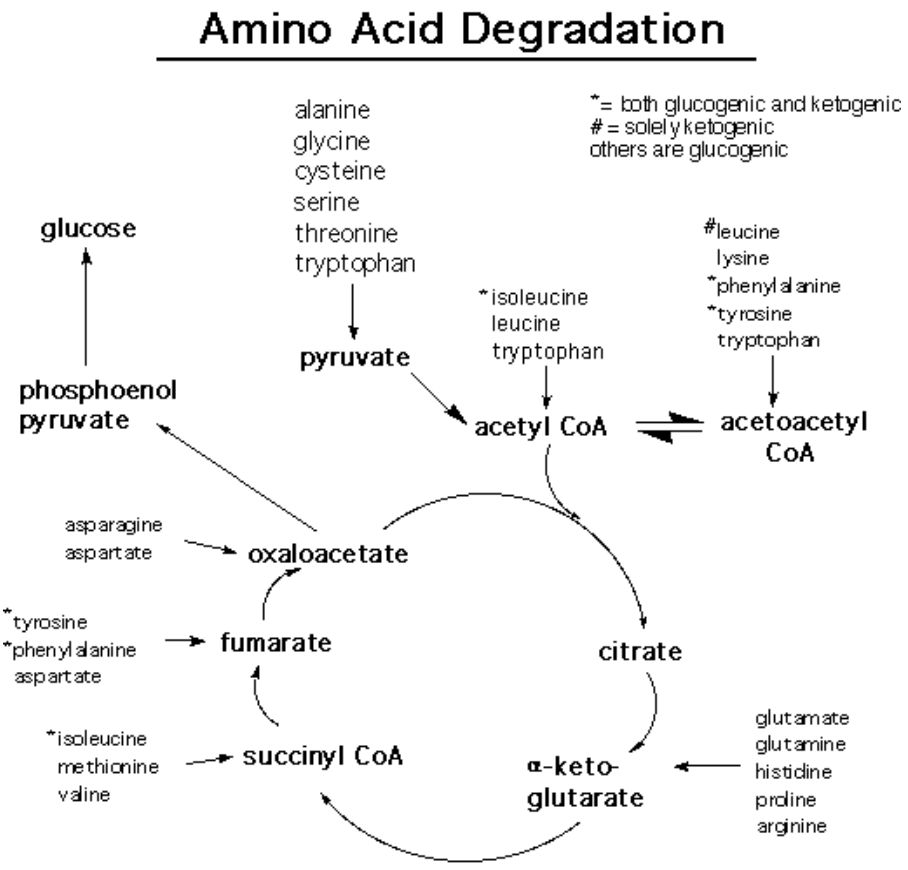

Oxaloacetate
Aspartate
Asparagine
Methionine
Threonine
Lysine
Isoleucine
Pyruvate
Ribose 5-phosphate
Alanine
Valine
Leucine
Histidine
Phosphoenolpyruvate
+
Erythrose 4-phosphate
Phenylalanine
Tyrosine
Tryptophan
Tyrosine
α-Ketoglutarate
3-Phosphoglycerate
Glutamate
Serine
Glutamine
Proline
Arginine
Cysteine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


phosphoenolpyruvate
3-desoxyarabinoheptulosenate-7-phosphate
erythrose - 4 - phosphate
5-dehydroxyshikimate
NADPH
NADP+
shikimate
ATP
PEP
chorismate
phenylalanine
tyrosine
tryptophane
naphthoquinone
p-aminobenzoate


Erythrose 4-phosphate
+
Phosphoenolpyruvate
Shikimate
Chorismate
Prephenate
Anthranilate
Phenylalanine
Tyrosine
Tryptophan


ATP
Glucose
Histidine
Phosphoribosylpyrophosphate
Tryptophane
Tyrosine
Phenylalanine
C
A
Glycine
Serine
Cysteine
Erythrose-4-Phosphate
Alanine
Leucine
Pyruvate
Valine
Lysine
α - Ketoglutarate
Diaminopimelate
B
Aspartate
Asparagine
Oxaloacetate
Homoserine
Methionine
Threonine
Isoleucine
Glutamine
Glutamate
Arginine
Proline
Ornithine
Citrulline


Alanine
Cysteine
Glycine
Serine
Threonine
Tryptophan
Isoleucine
Leucine
Tryptophan
Leucine
Lysine
Phenylalanine
Tryptophan
Tyrosine
Pyruvate
Glucose
Acetyl-CoA
Acetoacetate
Asparagine
Aspartate
Oxaloacetate
Citrate
Aspartate
Phenylalanine
Tyrosine
Fumarate
TCA
cycle
Isocitrate
Arginine
Glutamate
Glutamine
Histidine
Proline
Isoleucine
Methionine
Threonine
Valine
Succinyl-CoA
α-Ketoglutarate


Amino Acid Degradation
alanine
glycine
cysteine
serine
threonine
tryptophan
*= both glucogenic and ketogenic
= solely ketogenic
others are glucogenic
glucose
#leucine
lysine
*phenylalanine
*tyrosine
tryptophan
*isoleucine
leucine
tryptophan
pyruvate
phosphoenol
pyruvate
acetyl CoA
acetoacetyl
CoA
asparagine
aspartate
oxaloacetate
*tyrosine
*phenylalanine
aspartate
fumarate
citrate
*isoleucine
methionine
valine
succinyl CoA
α-keto-
glutarate
glutamate
glutamine
histidine
proline
arginine

Synthesis of histidine is not only regulated at the enzyme level but also it is controlled at the gene level. In the absence of histidine in the culture medium, entire sequence of genes responsible for all the enzymes that are required for histidine synthesis are expressed and the pathway operates leading to the production of histidine. Once histidine accumulates in sufficient quantities it binds to the first enzyme of the pathway and inhibits its activity. This is called as feedback inhibition. At the same time, histidine also binds to the inactive apo-repressor and makes it active. Then the active repressor binds to histidine operator gene, thereby the whole sequence of genes responsible for histidine synthesis are repressed (Refer regulation of gene expression is prokaryotes).

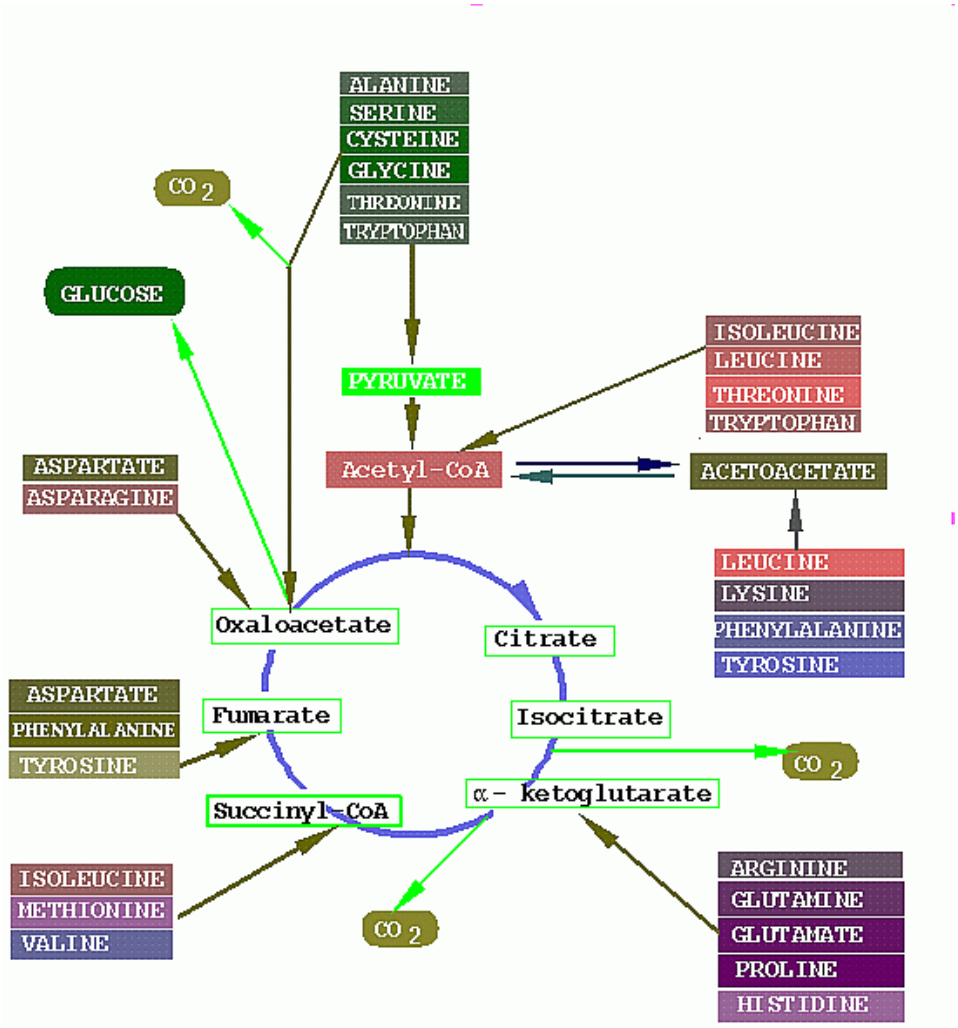
Such feed back inhibition of multistep pathways is also found in threonine to isoleucine and tryptophan pathways. With regard to tryptophan as an end product not only acts as an inhibitor at the enzyme level but also acts as the co-repressor in its gene expression.

TABLE 14–4 Glucogenic Amino Acids, Grouped by Site of Entry

Pyruvate	**Succinyl-CoA**
Alanine	Isoleucine*
Cysteine	Methionine
Glycine	Threonine
Serine	Valine
Threonine	**Fumarate**
Tryptophan*	Phenylalanine*
α-Ketoglutarate	Tyrosine*
Arginine	**Oxaloacetate**
Glutamate	Asparagine
Glutamine	Aspartate
Histidine	
Proline	

Note: All these amino acids are precursors of blood glucose or liver glycogen, because they can be converted to pyruvate or citric acid cycle intermediates. Of the 20 common amino acids, only leucine and lysine are unable to furnish carbon for net glucose synthesis. *These amino acids are also ketogenic (see Fig. 18-21).

On the other hand, depending upon the demand or the intracellular conditions, many amino acids found within the cells are interconverted or metabolized to various organic compounds and some of them are virtually drawn into citrate cycle or they may be used in glycogenic pathways. As shown in the figure one can find how different groups of amino acids are drawn into Kreb's cycle. Thus interconversions also help in producing many key intermediary products required for specific metabolic pathway or products. Such pathways are highly regulated.


ALANINE
SERINE
CYSTEINE
GLYCINE
THREONINE
TRYPTOPHAN
CO 2
GLUCOSE
PYRUVATE
ISOLEUCINE
LEUCINE
THREONINE
TRYPTOPHAN
ASPARTATE
ASPARAGINE
Acetyl-CoA
ACETOACETATE
LEUCINE
LYSINE
PHENYLALANINE
TYROSINE
Oxaloacetate
Citrate
ASPARTATE
PHENYLALANINE
TYROSINE
Fumarate
Isocitrate
CO 2
α- ketoglutarate
Succinyl-CoA
ISOLEUCINE
METHIONINE
VALINE
CO 2
ARGININE
GLUTAMINE
GLUTAMATE
PROLINE
HISTIDINE