NITROGEN METABOLISM

- Atmosphere has **78%** nitrogen by volume.
- Plants can not utilise this dinitrogen because they do not have the enzymes that can break the bonds between the two nitrogen atoms of dinitrogen.
- Blue – Green algae and some bacteria able to utilise this dinitrogen.
- Higher plants absorb nitrogen from the soil in the form of **Nitrates ions, Ammonium ions** and **urea.**
- The absorbed form of nitrogen has to be converted to **Ammonium** ions. This is the first step in nitrogen metabolism.
- Nitrate is converted to Nitrite by Nitrate reductase by utililsing electrons and protons of the cell or organism.
- $NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + 2\ H_2O$
- Nitrite is converted to Ammonium ions by Nitrite reductase.
- $NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2\ H_2O$
- Urea is converted to Ammonium ions by **urease** enzyme.
- In nitrogen fixing organisms atmospheric nitrogen is converted to ammonia by nitrogenase.

b. SYMBIOTIC SYSTEMS :

Microorganisms	Symbiotic structures	Host
1. Bacteria		
A. **Rhizobium** sp.	Root nodules	Legume plants
B. **Rhizobium** sp.	Root nodules	Non-legume (*Parasponia*)
2. Actinomycetes		
Frankia sp.	Root nodules	Non-legumes such as *Alnus, Casuarina* and *Myrica*
3. Cyanobacteria		Ascomycetous and basidiomycetous fungi
Anabaena, Nostoc	Lichens	Bryophyte: *Anthoceros*
Nostoc		Pteridophyte: *Azolla*
Anabaena azollae		Gymnosperms: *Cycas*
Anabaena cycadae	Leaves	Angiosperms: *Gunnera*
Nostoc	Corolloid roots	
	Stem glands	

Symbiotic Nitrogen fixation in Legumes

- Mutual beneficial association of two organisms is known as symbiosis.
- The microbe in symbiotic association is called as Micro-symbiont and the plant as host.
- Root nodules of legumes are the most common type of symbiotic associations in plants. In this *Rhizobium* is micro-symbiont.
- *Rhizobium* gets carbon skeleton from the plant and the host plant obtains nitrogen from the bacterium.
- In the second step, ammonium ions are used for the synthesis of amino acids.
- In the third and final step amino acids are involved in the synthesis of Proteins.

Biological Nitrogen Fixation

- It is conversion of molecular nitrogen into Ammonia by microorganisms.
- It is catalysed by the enzyme **dinitrogenase.**
- It is also known as **diazotrophy** or **dinitrogen** fixation.
- Organsms that fix nitrogen are called as **Diazotrophs** or **Nitrogen fixers.**

Nitrogen fixing organisms

a. Free living organsms

- It is fixed by both free living and symbiotic organisms.
- *Azotobacter* is free living aerobic nitrogen fixing bacterium
- *Clostridium* is free living anaerobic nitrogen fixing bacterium.
- *Rhodospirillum* is free living anaerobic photosynthetic nitrogen fixing bacterium.
- *Anabaena* and *Nostoc* are free living nitrogen fixing Blue Green Algae.

Formation of Root nodules in Legumes

1. Roots of leguminous plants **secrete sugars, amino acids** and **flavanoids** into the soil. The Rhizobia are attracted by this. The compatible strains of bacteria move towards the root.

2. Rhizobia multiply in number around the root hairs and cause its **curling** by secreting a **curling factor.** This curling acition is called as **Shepherds cook**. The host recognises the Rhizobium by its specific proteins called as **lectins**.
3. Rhizobia secrete cell wall degrading enzymes such as **cellulases** and **pectinases** and hydrolyse the cell wall of root hair. Growth of root hair is ceased and the bacteria come in contact with the plasma membrane of root hair. Plasma membrane invaginates and the rhizobia enter into the invagination to form **infection thread.**
4. Infection reaches the innermost cortical cells. Phytohormones secreted by cortical cells induce divisions in cortical cells which leads to the formation of young **nodule**.
5. Rhizobia multiply in the host cell and form swollen structures known as **Bacteroids.** Bacteroids differ from Rhizobia of soil in structure and function. Rhizobia of the soil are **rod shaped** and its nitrogenase is functionless.
6. Bacteroids of cortical cell are surrounded by the host cell membrane known as **peribacteroidal membrane**.
7. The Nodule enlarges and matures. Vascular connection is established to the nodule and exchange of materials between the host and plant is possible.

Mechanism of Biological Nitrogen Fixation

- It is catalysed by the enzyme **dinitrogenase** present only in Prokaryotic cells.
- Dinitrogenase is sensitive to **oxygen** and its synthesis is regulated by a set of genes known as **Nif genes.**
- In symbiotic systems the enzyme is protected by **leg-haemoglobin** and in non-symbiotic system by **metabolic compartmentation**. Leg-haemoglobin helps in controlled supply of oxygen to Rhizobia. In blue green algae it is protected in **heterocysts.**
- It has an **Fe-protein** part and **Fe-Mo-protein** part.
- The source of electrons, type of electron carrier to transfer electron to dinitrogen and energy source differ in different organisms. But the basic mechanism is same in all organism.
- The over all mechanism of biological nitrogen fixation can be represented as
- $N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$
- In many nitrogen fixing organisms the reducing power ($8e^-$, $8H^+$) is usually supplied by pyruvic acid (end product of glycolysis).
- The pyruvic acid undergoes “Phosphoroclastic Cleavage” in the presence of pyruvic dehydrogenase forming acetyl phosphate and CO_2.
- The electrons released in this reaction are accepted by ferredoxin.
- **Ferridoxin** receives electrons from respiratory substrates in bacteroids of root nodules. It transfers electrons to Fe- protein part of Nitrogenase and later to Fe-Mo- Protein part and ultimately to N_2 during which protons are also accepted to form Ammonia or Ammonium ions. The energy present in ATP is utilised in the presence of Mg dependent **ATP ase.**

Genetic Code

- DNA is genetic material and it directs the synthesis of various types of proteins at different times in an organism.
- Proteins are formed by **20** different types of Amino acids.
- The number and sequence of aminoacids is different in different types of proteins.
- The nature of proteins is determined by the number and sequence of amino acids which in return determined by the number and sequence of nucleotides in DNA.
- DNA has only 4 types of Nitrogen bases or Nucleotides.
- A set of nucleotides that determine an amino acid is called as **Codon.**
- The relationship between the sequence of amino acids and nitrogen bases or nucleotides of DNA or RNA is called as **Genetic code.**
- If the genetic code is singlet code, the **4** nucleotides are not sufficient to determine the 20 amino acids.
- A doublet code forms **16** codons. It is also not adequate to specify the 20 amino acids.
- A tiplet code forms **64** codons. These 64 codons are sufficient to indicate 20 amino acids inovolved in protein synthesis.

Genetic code – properties

1. **It is triplet code:** A triplet code has 3 nitrogen bases or nucleotides that specify an amino acid. e.g. UUU codes for **Phenyl Alanine** and GGG codes for **Glycine**.
2. **It is degenerate:** It is an amino acid having more than one codons. e.g. **Serine** has codons UCA, UCG, UCC and UCU; **Proline** having codons CCU, CCC, CCA and CCG; **Lysine** having codons AAA and AAG.
3. **It is non overlapping:** A nitrogen base of one codon is not involved in the formation of next codon.
4. **It is commaless:** No nitrogen base is wasted in between to adjacent codons. It is continuous and does not require any punctuations.
5. **It is non - ambiguous:** A particular codon indicates only a particular amino acid but not 2 or more than 2 amino acids.
6. **It is Universal:** It is applicable to all organisms and does not change from organism to organism.

7. **Starting codons:** It has polypeptide chain initiating codons. The starting codon is **AUG** in all eukaryotes and many prokaryotes. It indicates amino acid **Methionine** in Eukaryotes and **formyl methionine** in prokaryotes. In some prokaryotes, it is **GUG** and indicates **formyl methionine**. As later codon it indicates **Valine**.
8. **Stop codons:** These are also known as Chain terminating codons. They are present at the end of coding region of mRNA. They terminate the synthesis of polypeptide chain. These are UAA (**Ochre**), UAG (**Amber**) and UGA (**Opal or Umber**) codons.

PROTEIN BIOSYNTHESIS

- Proteins are macromolecules made of **20** different types of Amino acids.
- **Peptide bonds** are formed between the amino acids. These are formed between the **carboxyl group** of first amino acid and **amino group** of next amino acid.
- The general chemical formula of amino acid is **$RCHNH_2COOH$.**
- **Zamecnic** discovered the involvement of ribosomes in protein synthesis.
- Usually polysomes are involved in protein synthesis. The number of ribosomes in a polysome dependent on the length of mRNA. These ribosomes are arranged in linear or rosette manner.
- Binding or dissociation of ribosomal sub units is dependent on **Mg^+ ion** concentration.
- The maechanism of protein synthesis in prokaryotes (E. coli) is described here.
- It has two stages known as **Transcription** and **Translation.**

Transcription

- It is synthesis of mRNA on one of the strands of DNA.
- It is catalysed by the enzyme **RNA polymerase** or **Transcriptase.**
- It takes place in the nucleoid or Nucleus.
- The two strands of DNA are separated at the site of the gene to be transcribed.
- One of the strands of DNA acts as template on which the mRNA is synthesised. This strand is called as **Antisense strand** or Code complement. The other strand is called as sense strand or code copy.
- Ribosome pulls away the synthesised mRNA from DNA template and translation is started at one end while transcription is in progress on another strand.

Translation

- It is synthesis of polypeptide chain on ribosome surface according to the information present on mRNA.
- Ribosomes**, mRNA, amino acids** and **specific tRNA** are involved in this process.
- It has the following 4 stages.

Transfer of Amino acids to the site of Protein synthesis

- An amino acid is activated and transferred to tRNA before its involvement in protein synthesis.
- It is catalysed by specific **amino acyl tRNA synthetases**. These enzymes have **two** binding sites. One for **amino acid** and another one for tRNA.
- **One ATP** is utilised for the activation of each amino acid.
- It has **two** steps. In the first step amino acid, enzyme and ATP react with each other and form **amino acyl Adenylate enzyme complex** and Pyrophosphate. It is represented as
- Amino acid + ATP + Amino acyl synthetase $\rightarrow$ Amino acyl adenylate enzyme complex + PPi
- In the second step the above complex reacts with specific tRNA and forms **Amino acyl tRNA**, AMP and free enzyme. Now the tRNA is referred as **charged tRNA** or **aminoacyl tRNA.**
- Aminoacyl adenylate enzyme complex + tRNA $\rightarrow$ Amino acy tRNA + AMP + Enzyme
- The first activated amino acid is **methionine** or **formyl methionine.**

Polypeptide chain initiation

- It requires methionyl or formyl methionyl tRNA, ribosome, a GTP, mRNA and initiation factors **IF_1, IF_2** and **IF_3**. it has the following steps.
- The smaller sub unit of ribosome and **IF_3** combine and form smaller **subunit-IF_3 complex**. It later combines with IF_1 and forms **smaller subunit-IF_3-IF_1 complex.**
- GTP and IF_2 form GTP-IF_2 complex. This complex combines with the above complex to form **smaller subunit-IF_3-IF_1-GTP-IF_2 complex.**
- Now mRNA is attached to the above complex and later the formyl methionyl tRNA. In between the codon of mRNA and anticodon of tRNA **hydrogen bonds** are formed.
- The larger sub-unit of ribosome is attached to the above complex and GTP is hydrolysed. This **separates the three initiation** factors.
- Formyl methionyl tRNA occupies the **Peptidyl site** of larger subunit. Aminoacyl site is unoccupied.

Chain elongation

- During this, the aminoacids are attached one after the other by peptide bond formation catalysed by the enzyme **peptidyl transferase**. This results in the elongation of polypeptide chain.
- It requires amino acy tRNAs, GTP, Elongation factors and Peptidyl transferase.

- In the presence of Elongation factor T, the second amino acyl tRNA charged with second amino acid occupies Amino acyl site of larger subunit by utilising a molecule of GTP. EF T is released.
- The enzyme peptidyl transferase forms a peptide bond between the carboxylic group of formyl methionine and amino group of second amino acid. A molecule of H_2O is released. This act breaks the bond between the first tRNA and first amino acid.
- The first tRNA at P site leaves the ribosome.
- The ribosome moves a codon distance towards the 3′ end of mRNA. It is called as **translocation.** The factor required for this is **EFG** or **translocase**. During this a molecule of GTP is utilised. It releases EFG.
- The above movement shifts the amino acyl tRNA (now known as Peptidyl tRNA) from Amino acyl site to Peptidyl site.
- In this way the successive amino acids are brought to the A-site and, peptide bods are formed between them and later the tRNA with polypeptide chain is shifted to P-site of ribosomes. It results in chain elongation. It takes place till the non-sense codon is exposed at the A-site.

Chain termination

- One of the non-sense codons is exposed at the A-site. Due to this no amino acid is brought to the A-site.
- The non sense codon is recognised by the **Releasing factor 1** or Releasing factor 2. these bind to the A-site.
- RF_1 or RF_2 are recognised by RF_3 and the factors are removed from A-site.
- The enzyme peptidyl transferase removes the polypeptide chain and last tRNA from P-site. In this way the synthesis of Polypeptide chain is terminated.