ALGOLOGY.

1.) Differentiation of heterocysts in Anabaena is triggered by nitrogen deprivation (ammonia, nitrate, nitrite).

2.) 2 step Process :- 1st Step - Reversible.
2nd Step - Irreversible.
(Adams, 2000 ; El-Shehawy & Kleiner, 2003)

3.)

Reversible step 1 (10 hours)

- Depletion of Combined Nitrogen in Medium
- Drop in Ammonia Level Results in Increase in 2-Oxoglutarate (α-Ketoglutarate)
- Protein NtcA is Activated
- Gene hetR produces protein hetR, Inducing Formation of Proheterocyst

Addition of Combined Nitrogen Results in Degradation of hetR & Reverses Cell to vegetative cells.

Protein hetR is the 'Master Switch'

Irreversible Step 2

Proheterocyst — (1) Loses Granulation But still has Thin Cell wall

(2) Nitrogenase Enzyme is formed (3)

(4) Thick Cell wall is Produced Limiting entrance of O_2 into Cell

PatS produces PatS

Oligopeptide

PatS diffuses from Proheterocyst into adjacent cells, (Preventing their dev. into Heterocyst)

Heterocyst

NH_3 → GS → Glutamate

Nitrogen is Fixed into Glutamine, which travels into adjacent Veg. Cells

Sucrose from vegetative cells travel into Heterocyst

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[Events leading to the formation of Heterocyst.]

4.) Absence of combined nitrogen leads to an increase in intracellular 2-oxoglutarate (α-Ketoglutarate). (because cyanobacteria lacks 2-oxoglutarate dehydrogenase that facilitates breakdown of 2-oxoglutarate).

5.) Increase in 2-oxoglutarate activates NtcA & results in a drop in the amount of Ca-binding protein CcbP & Rise in Ca^{2+} in the cells (Zhao et al. 2005)

6.) Heterocysts have 10 times more Ca^{2+} than vegetative cells.

7.) Rise in Ca^{2+} up-regulates het R genes that form het R, a serine-type protease. Het R induces the change of vegetative cell into a heterocyst.

8.) Lack of combined nitrogen for 10 hours results in continued production of het R protein & the cell becoming a proheterocyst.

9.) Het R protein also induces the production of an Oligopeptide called Pat S.

10.) Oligopeptide (PatS) diffuses into adjacent cells thus it prohibits the formation of het R protein in these cells & thus prevents conversion of veg. cells into proheterocyst.

11.) If combined nitrogen is added in the medium then proheterocyst reverts back to veg cell. Because N_2 presence switches off 'het R' gene & thus production of het R protein also stops.

Irreversible :

12.) The 2nd stage is irreversible even by the addition of combined nitrogen.

13.) In this the Proheterocyst transforms into a mature heterocyst.

14.) A thick wall is formed containing glycolipids & polysaccharides to reduce diffusion of O_2.

15.) N_2-fixing enzyme, Nitrogenase is activated when 11 kilobases are removed between repeat sequences flanking nitrogenase (nif) genes.

16.) Heterocyst differentiation is a terminal event & is a basic form of programmed cell death or (Apoptosis)

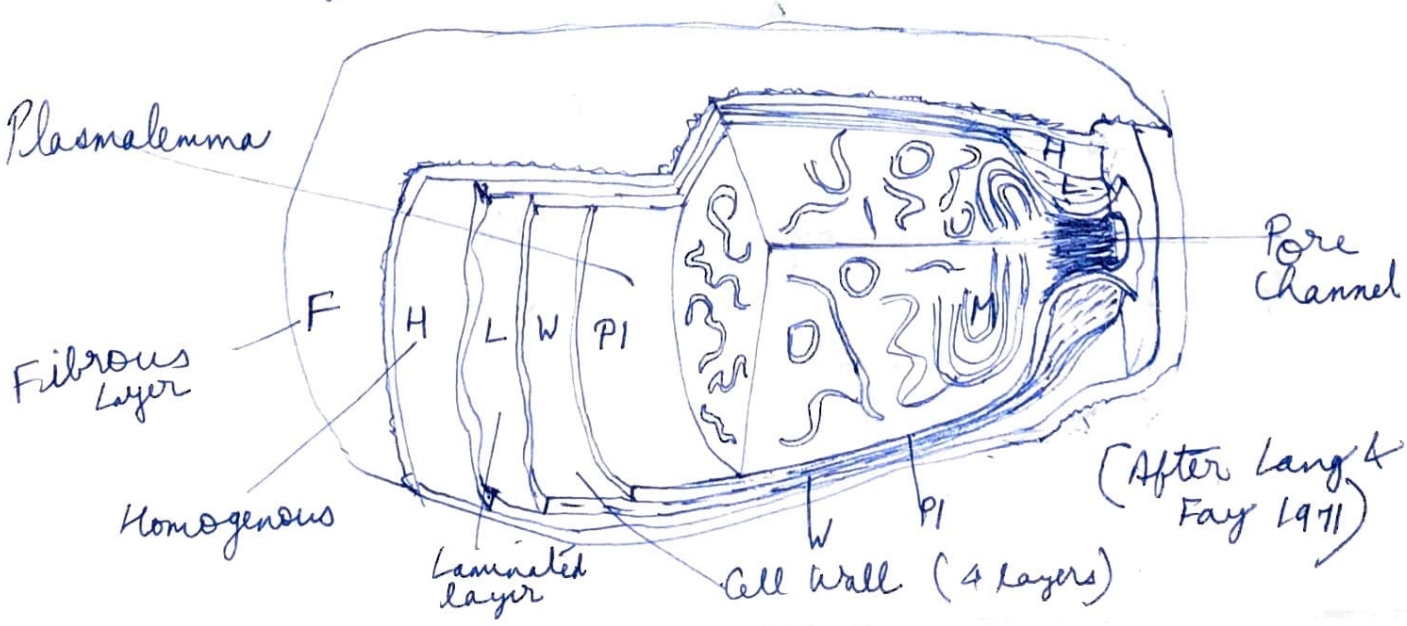
17.) Heterocysts are unable to divide, have a limited period of physiological activity & have a limited life.

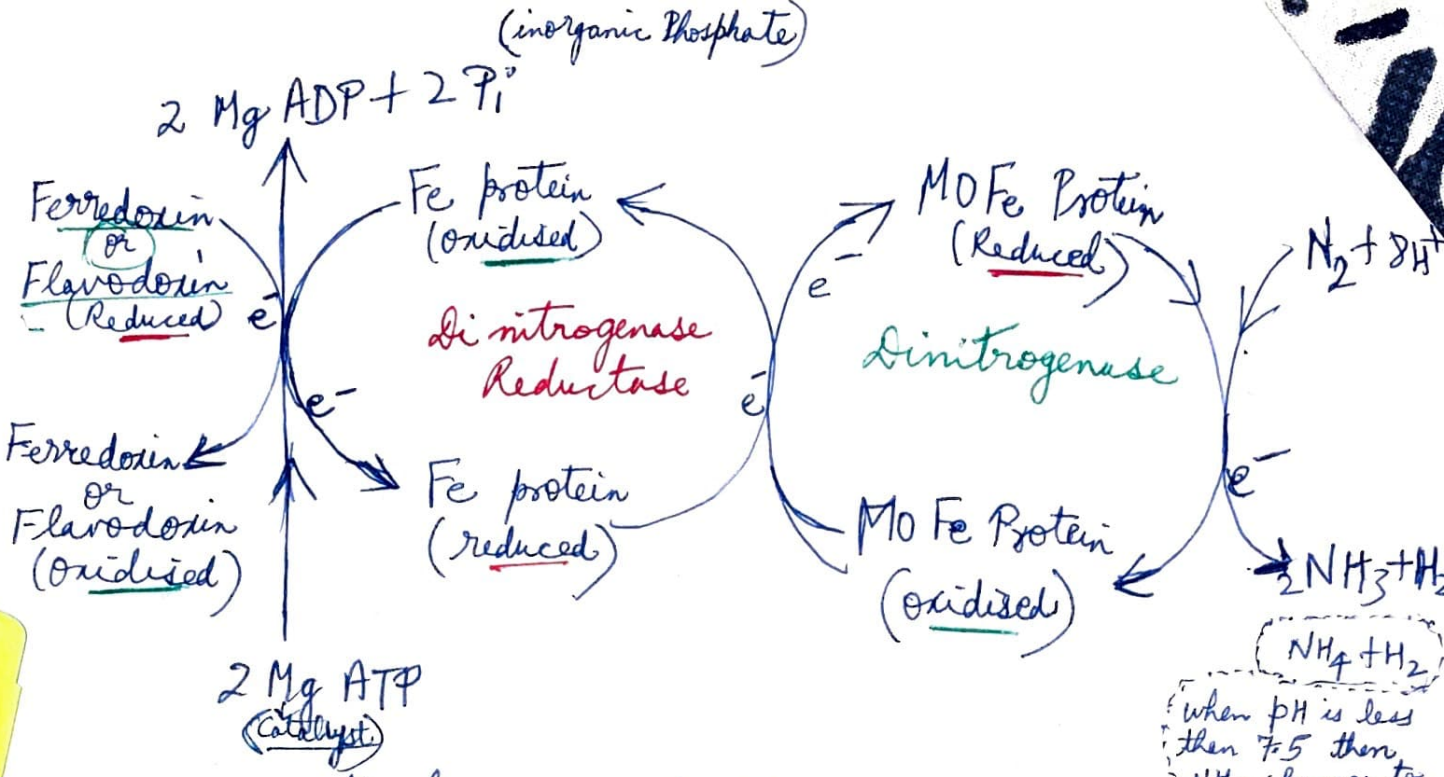
18.) Senescent heterocysts undergo vacuolation & break off from the filament, causing fragmentation of the filament.

19.) Heterocysts are dependent on a supply of substrates from adjacent vegetative cells through cytoplasmic connections (microplasmodesmata)

20.) These cytoplasmic connections convey nitrogen fixed in the form of Glutamine by heterocyst to veg. cells.

21.) Veg. cells transfer photosynthate to heterocysts since ~~Veg~~ are incapable of C-fixation.

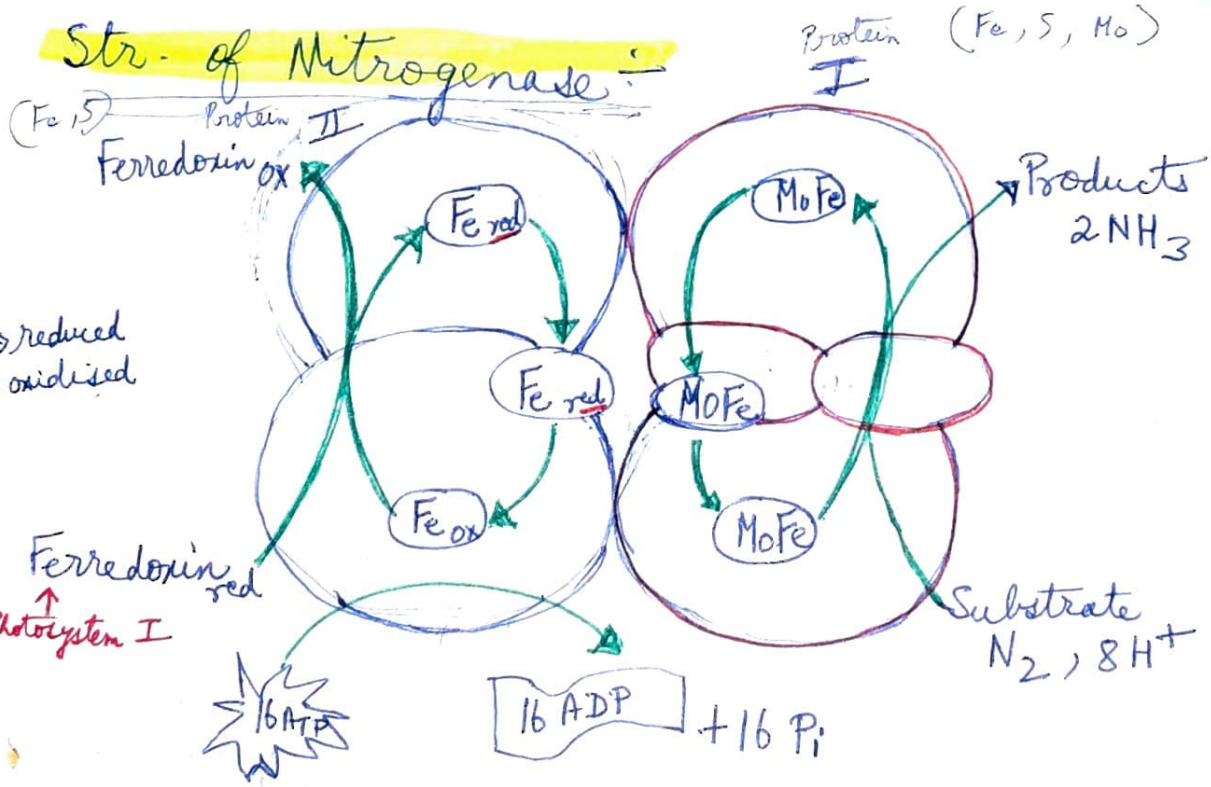




Mechanism of Biological nitrogen Fixation.

e⁻ transfer in Nitrogenase.

Str. of Nitrogenase :-



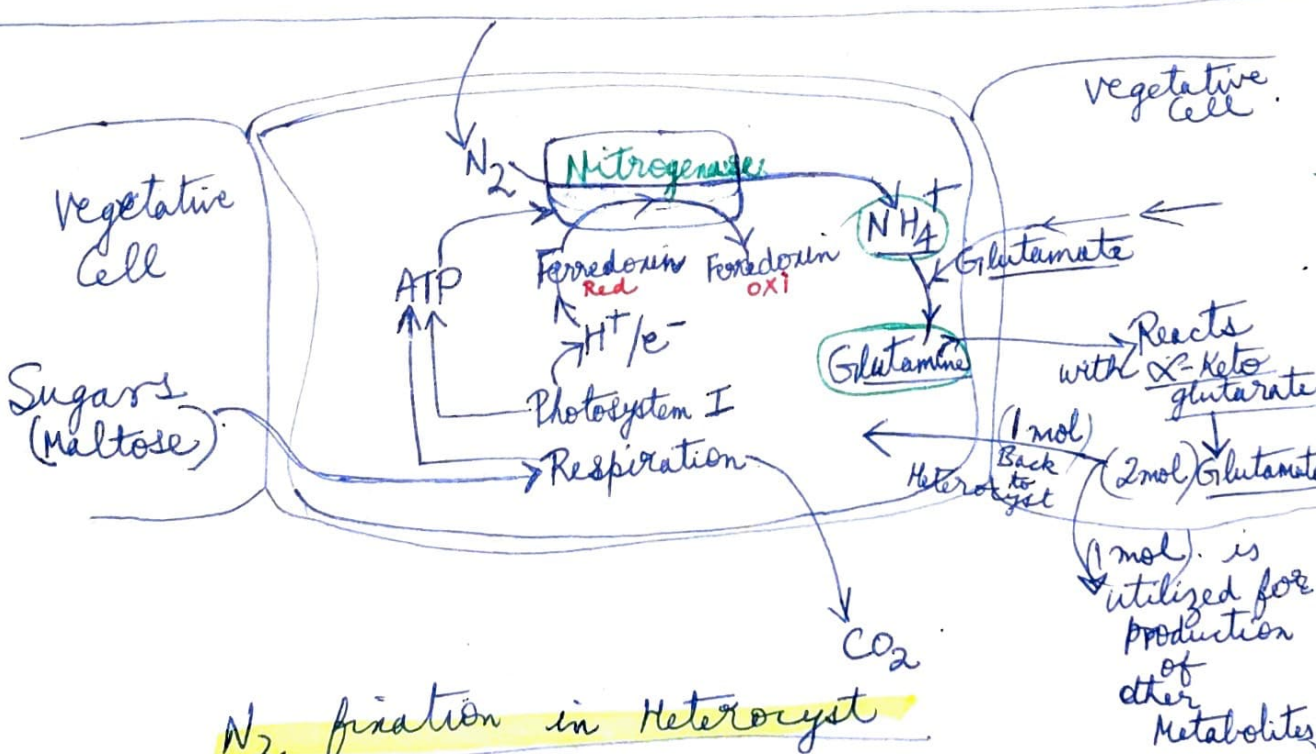
I → Dinitrogenase — (Molybdoferredoxin) (Mo-Fe Protein) is an alpha 2 | beta 2 tetramer. α & β subunits are similar yet distinct.

→ α & β subunits are encoded by genes nifK & nifD.

→ Each tetramer contains 2Mo & several Fe S (iron-Sulphur) groups.

→ Mo (2 atoms/mol)
Fe (32 atoms/mol)
S (30 atoms/mol)

II → Dinitrogenase Reductase (Fe-Protein) — (Azoferrodoxin) is a dimer.
→ Has identical subunits.
→ Subunits encoded by nifH genes.
→ Contain single Fe & S4 group per dimer.
i.e Fe (4 atoms/mol)
S (4 atoms/mol).
→ Has one Fe–S cluster which acts as ReDox site
→ Both subunits are linked together by 2 pairs of Cystine & a site for Nucleotide binding & cleaving



N_2 fixation in Heterocyst

→ In addition to Nitrogenase, the N_2 reducing system requires Mg ATP as a source of energy & a reductant (e^- donor) to catalyse reduction of substrates.
→ Whenever energy is needed, ATP undergoes enzymatic hydrolysis to form ADP + Pi (inorganic Phosphate ion)
→ Mg^{++} functions as Catalyst

Nitrogenase Mechanism

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1.) Both metalloproteins, nitrogenase (Mo-Fe proteins & Dinitrogenase Reductase (Fe-Protein) are essential for Nitrogenase activity.

2.) Fe-protein (Azoferrodoxin) interacts with ATP & Mg^{++}.

3.) Mo-Fe protein catalyses reduction of N_2 to NH_3, H^+ to H_2 & Acetelyne to Ethylene.

4.) The reduced ferredoxin or flavodoxin serves as a source of reductant for e⁻ transfer during N_2 fixation

→

5.) From reduced form of ferredoxin, e^- flows to Fe-protein which in turn reduces Mo-Fe protein (in Nitrogenase enzyme complex) with subsequent release of inorganic phosphate (P_i)

This enzyme complex gets its energy from Mg-ATP which is produced after cyanobacterial respiration.

6.) Finally Mo-Fe protein passes on the electron to reducible substrate i.e N_2.

7.) $N_2 + 10H^+ + 8e^- + 16ATP \xrightarrow{\text{Nitrogenase}} \underset{(2NH_3)}{2NH_4^+} + 16ADP + 16P_i + H_2\uparrow$

When pH is less than 7.5 then NH_3 changes to NH_4^+.

Self Regulatory Mechanism :

1.) H_2 is evolved during N_2-fixation which inhibits N_2 fixation reaction. Cyanobacteria possess enzyme Hydrogenase which recycles the H_2 produced. H_2 recitilization produces more ATP & improves efficiency of N_2 fixation.

2.) NH_4^+ salts help in vegetative growth but in excess amount inhibits Nitrogenase thus hampers N_2-fixation process. So, NH_3 generated is rapidly converted into organic nitrogen compound (Amino acids).

3.) IN heterocystous Cyanobacteria, Compartmentation of photosynthesis & N_2 fixation takes place. Thylakoids (Photosynthetic lamellae) are nearly absent in heterocyst.
Thus Nitrogenase is not inhibited by O_2 evolved during photosynthesis.

4.) However, the heterocysts lack

O_2 evolving photosystem II apparatus, ribulose biphosphate carboxylase & photosynthetic biliproteins.

5.) Wall of Heterocysts contain O_2 binding glycolipids which together with respiratory O_2 consumption maintain anaerobic condition or O_2 defficient conditions.

In Cyanobacteria :- (Assimilation of NH_4 salts).

1.) Glutamine is the main product of N_2-fixation.
2.) Glutamine is translocated from heterocyst to Vegetative cells.
3.) Transfer of Glutamate from veg. cell to heterocyst governs the formation of glutamine.
4.) (Maltose, Succinate, Fumarate) the end products of photosynthesis moves to heterocyst from veg. cells.
5.) They are metabolised to glucose-6-phosphate & oxidised by Oxidative pentose pathway.
6.) In this pathway the reduced NADPH combines with O_2 & creates an environment for reduction of ferredoxin. Ferredoxin in turn is also reduced by PS I.

7.) e^- are donated to nitrogenase by reduced ferredoxin.

8.) Nitrogenase reduces N_2 to NH_4^+ & releases $H_2 \uparrow$.

9.) The (HuP genes) & Hydrogenase present in heterocysts recycles H_2 evolved.

10.) A high amount of Glutamine Synthatase (GS) & low amount of GOGAT (Glutamine Oxo-Glutarate Amino Transferase) is present in heterocysts.

11.) Glutamine enters vegetative cells & reacts with α-Ketoglutarate to form 2 molecules of Glutamate.

12.) 1 molecule of Glutamate returns into heterocyst 2nd molecule takes part in production of other metabolites

13.) This Glutamate reacts with NH_4^+ & forms Glutamine.