Understanding the Physiology of Heterocyst and Nitrogen Fixation in Cyanobacteria or Blue-Green Algae.

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Abstract: Ever since the man started thinking about his origin and evolution, he has sought answer on scientific facts and findings. In nature there are certain tiny microscopic blue-green algae or Cyanobacteria that have immense importance from many aspects ranging primarily from Nitrogen Fixation to the most coveted query – the origin of animal life on this planet. This paper is a peer review into the various physiological aspects of the nitrogen fixation and simultaneous Oxygen evolving mechanisms of blue-green algae. This paper makes the understanding of cyanobacterial nitrogen fixation in the Heterocyst easy, and also links the evolution of free Oxygen (g) from the splitting of water by its vegetative cells and ultimately, the role of Cyanobacteria in altering the primitive earth's reducing atmosphere into present day oxygenating and thus making animal life possible on earth. [Nature and Science. 2008;6(1):28-33]. ISSN: 1545-0740.

Key Words: Cyanobacteria; Blue-Green Algae; Heterocyst; Nitrogen Fixation.

Introduction:

The blue-green algae or Cyanobacteria are the most primitive form of algae under plant kingdom. These are basically a type of autotrophic bacteria, which are prokaryotic in their cellular structure. These are called as blue-green algae because they contain the photosynthetic pigments- ***c phycocyanin*** **(dominant pigment*)***, ***c phycoerythryin***, and ***chlorophyll a***, which are responsible for their characteristic **blue-green colour**. These are known by different names such as, ***Blue-Green Algae / Cyanobacteria*** **(Stainer and Cohen-Bazire, 1977),** ***Schizobacteria / Myxobacteria, Myxophyceae*** and ***Cyanophyceae***. These blue-green algae are the first plant forms, which got the power of chlorophyll in their thylakoids and started the life supporting process of photosynthesis in the planet earth. The common examples of blue-green algae are ***Nostoc, Anabaena, Rivularia, Gleotrichia, Gleocaspa, and Camptylonema*** etc.

The primitive environment of the earth was reducing i.e. Hydrogen rich (H_2 plus) or Oxygen deficient (O_2 minus). This primitive atmosphere was totally dominated by Hydrogen gas, and Oxygen gas was not present in free state. That time life was present in the form of simple primitive ***archaebacteria***, such as iron bacteria, sulphur bacteria, chemosynthetic bacteria and methane producing ***methanogens*** etc. They used to generate energy not from the sunlight, but from the different chemicals only, and there was no photosynthesis at all in the primitive earth due to the absence of any photosynthetic pigment. They were these blue-green algae or Cyanobacteria, which initiated the process of photosynthesis and started giving off free-Oxygen gas as a by-product of photosynthesis, by the splitting of water molecules with the help of **Photosystem II (PS II)**. And thus we can say that the blue-green algae were the pioneer in transforming the early earth's reducing atmosphere to present day oxygenating atmosphere, which ultimately made it possible for animals to breathe. Although, the evolution was also going on simultaneously, and the whole plant and animal kingdom was in a state of establishing itself.

Later on in the evolutionary process, the blue-green algae migrated inside a eukaryotic cell and got the designation of Chloroplast. This is how chloroplasts were formed, and that is why the chloroplasts are prokaryotic **(Endosymbiont hypothesis, Margulis, 1971;Bogorald, 1975;Mahler and Raff, 1975;Saccone and Quagliarello 1975;Bucher *et al.*, 1977; DeRobertis and DeRobertis 1984)**. Now, the resultant cell was the first unicellular, eukaryotic alga of the class Chlorophyceae **(F.E.Fritsch, 1944)**. Similar migratory Endosymbiont hypothesis is also given for the origin of mitochondria, where the free-living aerobic, ATP generating bacteria migrated inside a eukaryotic cell (cell with nucleus and other cell organelles) and transformed in the present day mitochondria. That is why both, chloroplast and mitochondria are prokaryotic in nature and have their own independent genetic material and specific protein synthesis.

Many of these blue-green algae have the power of nitrogen fixation, and for this nature has provided them a very special enlarged cell, which is called as **Heterocyst *(Gr. Hetero=different; Cyst=swollen and encapsulated cell), (Fay et al.* 1968), *(Stewart, 1967)*.** This Heterocyst is very much enlarged than its other body cells (vegetative cells).

Interestingly this Heterocyst is very unique in itself as it is specially designed by nature and has many important features to facilitate the nitrogen fixation. There are different factors, which control the Heterocyst formation, for example: The production of Heterocyst increases in the conditions of low light intensity and increase in the amount of phosphate in the medium **(Fay et al., 1968).** It has also been reported that the Heterocyst formation depends upon the availability of carbon intermediaries and ATP. The former are supplied by photosynthesis and the later by oxidative metabolism **(Tyagi, 1973). Singh and Trehan (1973)** found that the Heterocyst differentiation is inhibited in the presence of combined sources of nitrogen (nitrate and ammonium nitrogen), but is induced in the presence of nitrogen gas. However they further declared that the differentiation of Heterocyst, hormogones and spores in blue-green algae is genetically controlled. It was also found that in ***Camptylonema lahorensis***, Heterocyst differentiation is genetically controlled but its phenotypic expression is dependent on growth conditions in the medium.

According to **Tyagi (1973)**, apart from microbial nitrogen fixation, which is its prime function, the Heterocyst is also credited with many other biological functions of Cyanobacteria or blue-green algae. **Geitler (1921)** supposed that originally Heterocyst were developed for reproduction. This claim has also been backed by the occurrence of endospore formation in the germinating Heterocyst of *Anabaena cycadeae* **(Spratt, 1911)**. But of all these above discussed functions, the first and foremost work assigned to Heterocyst is the **cyanobacterial nitrogen fixation**.

Characteristic features of Heterocyst:

1. The Heterocyst is the site for cyanobacterial nitrogen fixation which is an enlarged cell, and may be present terminally or intercalary in the filamentous cyanophycean algae.
2. In the process of cyanobacterial nitrogen fixation, hydrogen gas (H_2) is also evolved as a by product and 40% of it is recycled by the ***hup*** gene (hydrogen uptake gene), **(Margheri *et al* 1990), (Howarth and Codd, 1985)** whereas remaining 60% hydrogen gas can be used by biotechnologists as a source of future clean fuel. **(Dutta *et al.* 2005)**
3. The Heterocyst is made up of three (3) different cell wall layers- the outer fibrous and middle homogenous layers are made up of ***non-cellulose polysaccharide***. Whereas, the inner laminated layer is made up of ***glycolipids***. **), (Lang, 1968)**
4. On one hand, these special cell wall layers permit the atmospheric N_2 (g) to diffuse inside, whereas on the other hand they stop the atmospheric O_2 (g) to come inside.
5. This is a **damage-control mechanism** for the enzyme ***nitrogenase***, as the *nitrogenase* is sensitive to O_2 and cold, and cannot function in the presence of O_2 (g).
6. Moreover the **Photosystem II (PS II), is also absent** from the Heterocyst, because PS (II) does the Photolysis of water and generates free O_2 gas.

   That is why **only PS (I) is present here**, which generates assimilatory powers ATP, which helps in nitrogen fixation. Had there been PS (II) also in the Heterocyst, then it would have done the Photolysis of water in the Heterocyst and free oxygen gas (O_2) would have stopped the functioning of ***nitrogenase*** enzyme, thus ultimately checking the whole cyanobacterial nitrogen fixation.
7. Absence of PS (II) helps in maintaining the O_2 (-), or H_2 (+) internal environment of Heterocyst.
8. If by chance, some oxygen gas (O_2) also enters the Heterocyst from polar plugs, then the enzyme ***Oxidase*** present inside Heterocyst executes reaction between this entering oxygen gas and hydrogen molecules, and ultimately makes the water, thus helping in maintaining the internal environment of Heterocyst as reducing and not oxygenating.

And this is how the marvel-cell Heterocyst of Cyanobacteria performs its unique biochemical mechanism of nitrogen fixation and is valuable in the present life status of the mother planet earth. First in making the Precambrian earths reducing environment, to present day oxygenating, and also functioning as bio-fertilizers. So, the whole biota of the earth is indebt to the blue-green algae for helping in establishing both, the plant and animal kingdom.

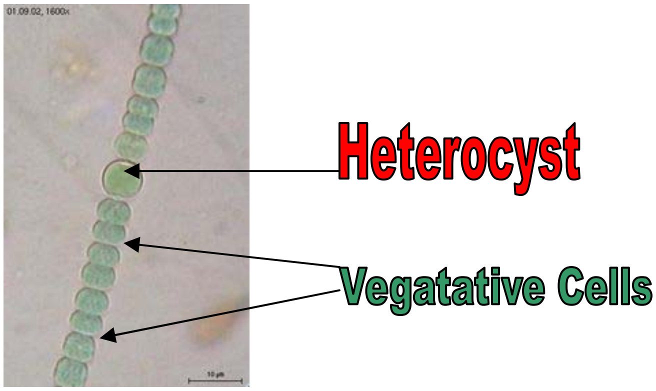

Fig 1: Cyanobacteria: *Anabaena sperica*

Cyanobacteria belong to the family Cyanophyceae (Algae), and as prokaryotes they represent the most primitive group of living organisms. They are extremely simple organisms that can exist as single cells, as slender filaments like the ones seen here, or as simple colonies. Cyanobacteria are capable of enduring a wide variety of environmental conditions ranging from freshwater and marine habitats to snowfields and glaciers. They are capable of surviving and flourishing even at extremely high temperatures.

Table: Enzymological comparison between Heterocyst and Vegetative cell in ***Anabaena variabilis***, grown aerobically **(after C.P. Wolk, 1973)**

Activity mainly or entirely in Heterocyst	**Activity mainly or entirely in Vegetative Cell**
Enzymes 1. *Nitrogenase* (encoded by ***nif*** gene) 2. *Glutamine Synthetase* 3. *Glucose-6-Phosphate dehydrogenase* (in Oxidative Pentose Phosphate Pathway) 4. *Uptake hydrogenase* (encoded by ***hup*** gene) 5. *Bi-directional* or *Reversible hydrogenase* (encoded by ***hox*** gene) **6.** *Oxidase* in high concentration.	***Enzymes*** **1.** *Glutamate Synthase* (in GOGAT- Glutamine Oxalo gluterate Amino Transferase Pathway). **2.** *RUBP carboxylase* **3.** *Oxidase* in low concentration.
Main Metabolic Pathways 1.Nitrogen fixation Pathway 2. Oxidative Pentose Phosphate Pathway. 3.Only Photosystem I (PS I) present and Photosystem II (PS II) is absent from Heterocyst.	**Main Metabolic Pathways** **1.** Calvin Cycle (Carbon Fixation) or Reductive Pentose Phosphate Pathway **2.** Photosystem II (PS II) present. Photolysis of water and evolution of O2 gas occurs.

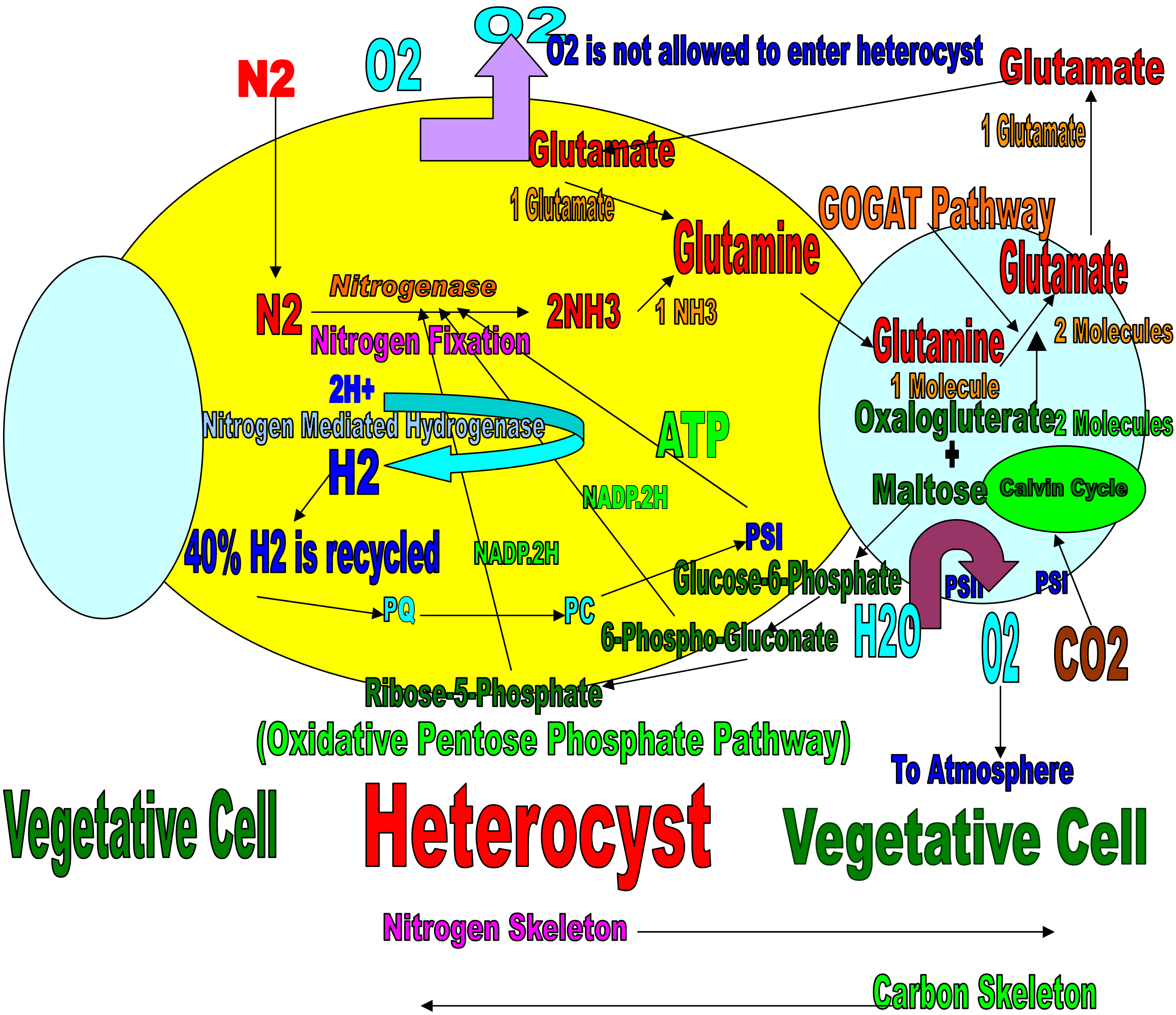

Figure 2: The central bulging cell is the Heterocyst, which is attached to two normal vegetative cells. The biochemical nitrogen fixation is going on in the Heterocyst, whereas in the vegetative cell the carbon fixation is in progress.

Different Biochemical Reactions of Heterocyst:

The different biochemical reactions going on, in the cyanobacterial Heterocyst and the adjoining vegetative (normal) cell are explained in the **Figure2**.

The Central bulging Yellow colored cell is the Heterocyst, which is the surrounded by two light blue colored vegetative cells. As mentioned earlier, the cell wall of Heterocyst is three layered, which gives the diffusion passage only to atmospheric Nitrogen (g) and stops the entry of Oxygen (g) inside the Heterocyst. So that it helps in maintaining the early earth's reducing environment inside the Heterocyst. As we know that the activity of ***Nitrogenase*** enzyme present inside the Heterocyst is stopped in the presence of Oxygen, so it's a defense mechanism evolved by the nature for the ***nitrogenase*** enzyme. Now the ***nitrogenase*** enzyme combines the atmospheric Nitrogen and Hydrogen molecules (present inside the Heterocyst) to form two molecules of Ammonia (NH_3). Now after this, one molecule of Ammonia is changed into Glutamine, which further migrates to the adjoining vegetative cell for other biochemical pathways.

In Vegetative Cell:

On the other hand, Carbon fixation cycle (Calvin Cycle) is going on simultaneously in the adjoining vegetative cell, which has got both PS (I) and PS (II) for this purpose. Atmospheric CO_2 is taken up by the ***RUBISCO (Ribulose Biphosphate Carboxylase)*** enzyme and changed into 3-PGA (3-Phosphoglyceraldehide), which further goes on in the Calvin Cycle, and in this way the Carbon is fixed in the adjoining vegetative cell of Blue-Green Algae. The PS (II) here splits the water (H_2O) molecules and generates Oxygen (O_2) (g), which is then released into the atmosphere. The Calvin Cycle also produces Maltose and Oxalo gluterate.

In Heterocyst:

Now after that the maltose and Oxalo gluterate are formed in the vegetative cell, two molecules of Oxalo gluterate react with one molecule of Glutamine and enter into the **GOGAT Pathway (Glutamine Oxalo Gluterate Amino Transferase).** Now as a result two molecules of Glutamate are formed and out of theses two, one molecule of Glutamate is cycled back to the Heterocyst and the cycle goes on.

On the other hand the Maltose is sent to the Heterocyst through different intermediate Carbon compounds as a Carbon Skeleton. These intermediate carbon compounds are: Glucose-6-Phosphate, which is changed into 6-Phospho-Gluconate, which is ultimately changed into Ribose-5-Phosphate. Now at each and every step of transformation of these different Carbon Compounds, they give rise to the sufficient amount of energy molecules i.e. **ATP (Adenosine Tri Phosphate)** to help in the fixation of atmospheric Nitrogen into solid Ammonia molecule (Nitrogen Fixation).

The Hydrogen ions (H^+) formed in the energy transfer process are taken up by the enzyme ***Nitrogen Mediated Hydrogenase/Bi directional/Reversible Hydrogenase*** encoded by ***hox gene,*** and are changed into molecules of Hydrogen (g) (H_2).

However 40% of this Hydrogen gas is recycled through Plastoquinone and Plastocyanin, by ***Uptake Hydrogenase*** enzyme encoded by ***hup gene***.

In this way the nitrogen fixation is done in nature by the Cyanobacteria and their Heterocyst.

The whole humanity is indebted to the kindness and greatness of these tiny microscopic life forms which not only paved way for the establishment of animal and Human life but also make a promising Biofertilizer for Plants.

Had Cyanobacteria not been there in nature, only God knows what would have been our fate?

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References:

1. **Bogorald, L. (1975).** Evolution of organelles and eukaryotic genomes. ***Science***. 188: 891.
2. **Bucher, T., Neupert, W., Sebald, W., and Werners, S., Eds. (1977):** Genetics and Biogenesis of Chloroplasts and Mitochondria. *North- Holland Publishing Company.* Amsterdam.
3. **Debajyoti Dutta, Debojyoti De, Surabhi Chaudhari and Sanjoy. K. Bhattacharya (2005):** Hydrogen production by Cyanobacteria. ***Microbial Cell Factories,*** 4:36
4. **DeRobertis E.D.P. and DeRobertis E.M.F. (1984):** *Cell and Molecular biology*. Holt-Saunders International Editions. Pp-278.
5. **F.E.Fritsch, (1944):** The Present day classification of algae**.** ***Botanical review***. Issue-**10**;pp-233-277
6. **Fay, P., W.D.P.Stewart, A.E.Walsby and G.E.Fogg (1968):**Is the Heterocyst the site of Nitrogen Fixation in Blue-Green algae? ***Nature***, London. **220:** 810- 812.
7. **Howarth DC. And Codd. GA (1985):** The Uptake and Production of molecular hydrogen by unicellular Cyanobacteria. ***General Microbiology*****; 131:** 1561-1569
8. **Lang, N. (1968):** The Fine Structure of Blue-Green Algae. ***Annual Review of Microbiology,*** **22:** 15-42.
9. **Mahler, H.R., and Raff, R.A. (1975):** The evolutionary origin of the mitochondrion, a non-symbiotic model. ***International review of cytology***. **43:** 2
10. **Margheri, M.C., Tredici M.R., Allotta G, Vagnoli L (1990):** Heterotrophic Metabolism and regulation of uptake hydrogenase activity in symbiotic Cyanobacteria. ***Developments in plant and soil sciences-bio nitrogen fixation***. Edited by: Polsinelli M, Materassi R, Vincenzini M. Dordrecht: Kluwer Academic Publications: pp. 481-486.
11. **Margulis, L. (1971):** Cell organelles such as mitochondria may have once been free-living organisms. ***Scientific American*****. 225:** 48.
12. **Saccone, C., and Quagliarello, E. (1975):** Biochemical Studies on mitochondria transcription and translation. ***International review of cytology***. **43:** 125
13. **Singh, V.P., and K.Trehan (1973):** Extra-cellular protein amino acids of blue-green algae I. The production of extra-cellular amino acids by Aulosira fertilissimia and Anacystis nidulans. ***Phykos.*** **12 (1-2):** 36
14. **Singh, V.P., and K.Trehan (1973):** Extra-cellular protein amino acids of blue- green algae II. Effect of nitrate and amino acids on liberation of amino acids by Aulosira fertilissimia. ***Phykos.*** **12 (1-2):** 42
15. **Stainer and Cohen-Bazire, (1977):** Photosynthetic Prokaryote; Cyanobacteria. ***Annual Review of Microbiology***. Issue-**31**; pp 225-274.
16. **Stewart, W.D.P. (1967):** Nitrogen Fixing Plants. ***Science***
17. **Tyagi, V.V.S. (1973):** Effect of some metabolic inhibitors on Heterocyst formation in blue-green algae *Anabaena doliolum*. ***Annals of Botany. 37:*** 361-368.
18. **Wolk, C.P. (1973):** ***Bacteriological Review*****. 37(1):** 32-101.