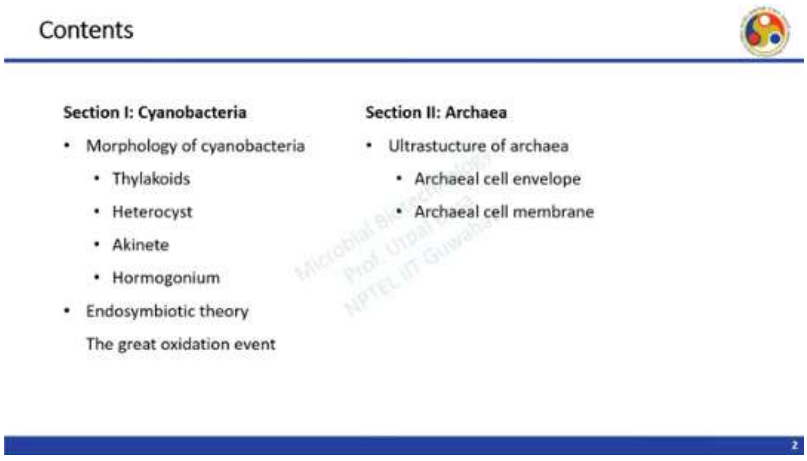
MICROBIAL BIOTECHNOLOGY

Prof. Utpal Bora
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati

Lecture07
Lec 7: Structure of cyanobacteria and archaea

Welcome to my course on microbial biotechnology. We are in Module 2, which deals with the structure and lifecycle of representative groups of viruses, prokaryotic, and eukaryotic microorganisms. In this lecture, number 3, we will discuss the structure of cyanobacteria and archaea. This is broadly divided into two sections: one dealing with cyanobacteria and the other with archaea. We'll start with the morphology of cyanobacteria, then also discuss the endosymbiotic theory and the Great Oxidation Event.

Under archaea, we'll discuss the ultrastructure of archaea, including the archaeal cell envelope and the archaeal cell membrane. So, let us begin with a discussion on cyanobacteria. Cyanobacteria, or blue-green algae, are gram-negative prokaryotic microorganisms that lack a nucleus or membrane-bound organelles. They perform oxygenic photosynthesis, deriving their blue-green color from pigments like carotenoids and chlorophyll. Cyanobacteria come in three forms: unicellular, filamentous without heterocysts, and filamentous with heterocysts, the latter aiding in nitrogen fixation.



Cyanobacteria

Cyanobacteria, or "blue-green algae," are Gram-negative, prokaryotic microorganisms that lack a nucleus or membrane-bound organelles.

They perform oxygenic photosynthesis, deriving their blue-green color from pigments like carotenoids and chlorophyll.

Cyanobacteria come in three forms: unicellular, filamentous without heterocysts, and filamentous with heterocysts, the latter aiding nitrogen fixation.

They can also grow heterotrophically, store phosphorus and nitrogen, and form colonies with various structures.

Cyanobacteria likely originated in freshwater or terrestrial environments and can produce gas vacuoles for buoyancy in water.

4

They can also grow heterotrophically, store phosphorus and nitrogen, and form colonies with various structures. Cyanobacteria likely originated in freshwater or terrestrial environments and can produce gas vacuoles for buoyancy in water. Cyanobacteria display a broad spectrum of morphological variations, as you can see in these pictures from A, B, C, D to H. In Figure A, you can see the unicellular Synechocystis. In B, Synechococcus elongatus. In the non-heterocystous forms, you can see Figure C, which is Arthrospira maxima; Figure D, Trichodesmium; and Figure E, Phormidium.

And then you have the false or non-branching heterocysts. For example, in F, you have Nostoc, and in G, you have Brasilonema, Brasilonema octogenarum. And then you have two branching heterocysts, which are number H, Stigonema. Let us look into the morphology of cyanobacteria. Cyanobacteria cells vary in size from under 1 micrometer in the case of picocyanobacteria to up to 100 micrometers in tropical

Cyanobacteria display a broad spectrum of morphological variations

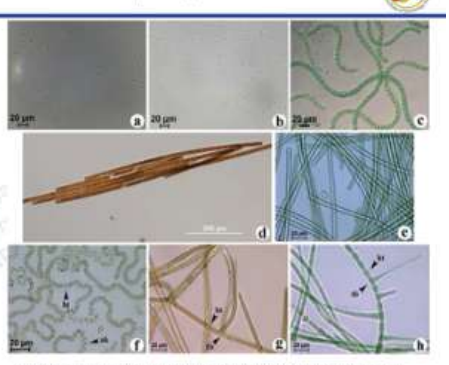
Fig. Morphological variations such as, unicellular, colonial, and multicellular filamentous forms.

Unicellular:
(a) *Synechocystis* and (b) *Synechococcus elongates*

Non-heterocytous:
(c) *Arthrospira maxima*, (d) *Trichodesmium* and (e) *Phormidium*

False- or non-branching heterocytous:
(f) *Nostoc* and (g) *Brasilonema octagenarum*

True-branching heterocytous:
(h) *Stigonema*

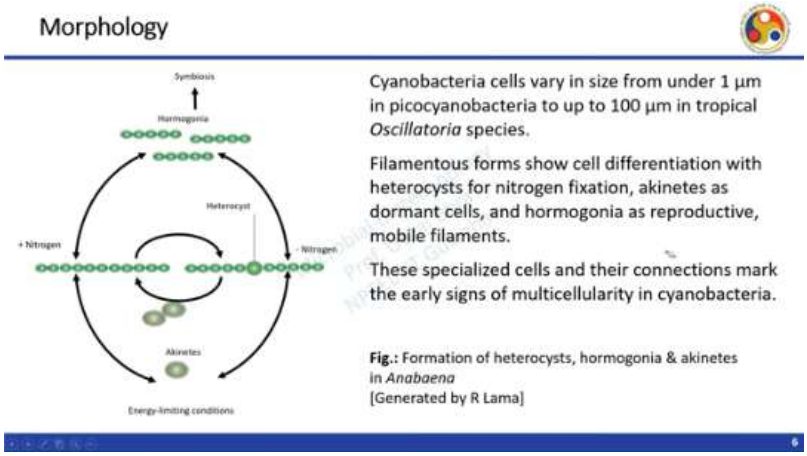


[**Authors:** Esteves-Ferreira *et al.* CC-BY-SA-4.0, via Wikimedia Commons]

5

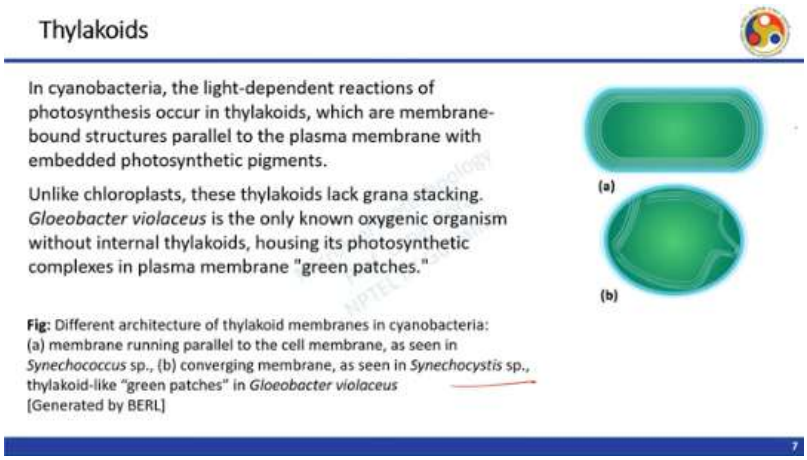
oscillatory species. Filamentous forms show cell differentiation with heterocysts for nitrogen fixation, akinetes as dormant cells, and hormogonia as reproductive mobile

filaments. These specialized cells and their connections mark the early signs of multicellularity in cyanobacteria. In this figure, you can see the formation of heterocysts, akinetes, and the hormogonium filaments in Anabaena thylakoids.

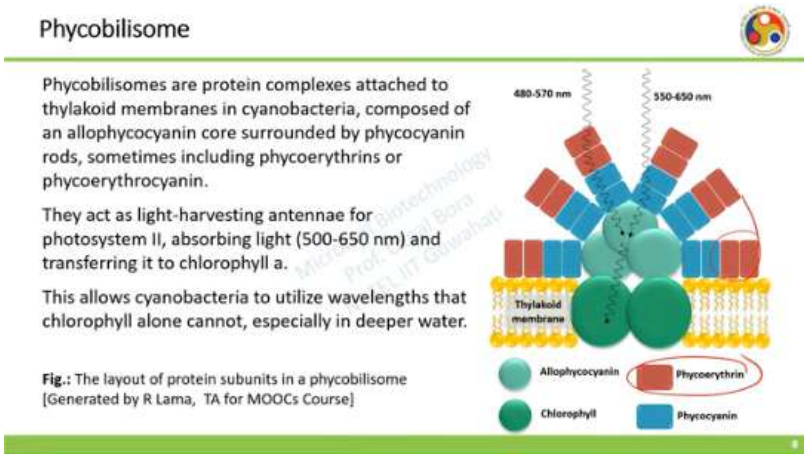


In cyanobacteria, the light-dependent reactions of photosynthesis occur in thylakoids, which are membrane-bound structures parallel to the plasma membrane with embedded photosynthetic pigments. Unlike chloroplasts, these thylakoids lack grana stacking. Gloeobacter Gloeobacter violaceus is the only known oxygenic organism without internal thylakoids, housing its photosynthetic complexes in plasma membrane green patches. In this figure, we can see a membrane running parallel to the cell membrane, as seen in Synechococcus.

And in B, we can see the converging membrane as seen in cyanococcystis species. So, these are two different variations of the thylakoid structure in cyanobacteria. Next comes the phycobilisome. Phycobilisomes are protein complexes attached to thylakoid membranes in cyanobacteria, composed of an allophycocyanin core surrounded by phycocyanin rods, sometimes including phycoerythrins or phycoerythrocyanin. They act as light-harvesting antennae for photosystem II.



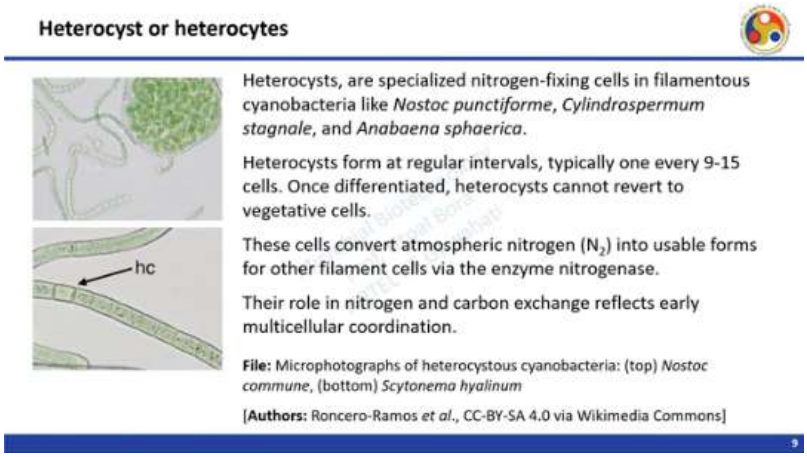
They absorb light around 500 to 650 nanometers and transfer it to chlorophyll A. This allows cyanobacteria to utilize wavelengths that chlorophyll alone cannot, especially in deeper water. So, you have this LO phycocyanin, and then we have here the chlorophyll. Then we have the phycoerythrin. In these Roth's repeat structures, and then it leads to other phycocyanin, and then you can see light of various wavelengths getting absorbed by these photo-harvesting systems. Heterocysts or heterocytes.



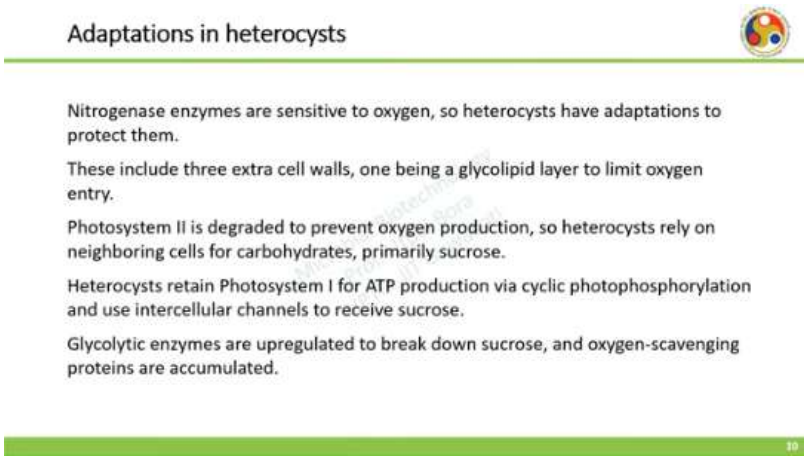
Heterocysts are specialized nitrogen-fixing cells in filamentous cyanobacteria like Nostoc punctiforme, Cylindrospermum stagneli, and Anabena sperica. These heterocysts form at regular intervals, typically one every 9 to 15 cells. So you can see here the heterocysts forming. Once differentiated, heterocysts cannot revert to vegetative cells. So this differentiation is unidirectional.

These cells convert atmospheric nitrogen into usable forms for other filament cells via the enzyme nitrogenase. Their role in nitrogen and carbon exchange reflects early multicellular

coordination. Let us discuss the adaptations in heterocysts. Nitrogenous enzymes are sensitive to oxygen. So, heterocysts have adaptations to protect them against oxygen injury.



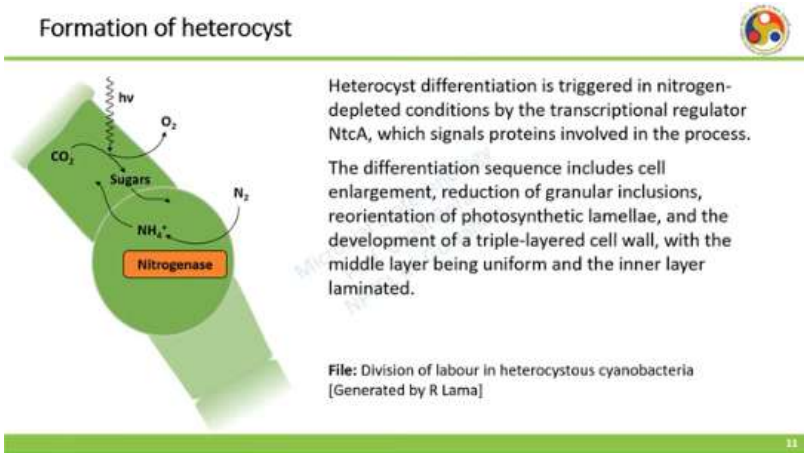
These include three extracellular layers, one being a glycolipid layer to limit oxygen entry. Photosystem II is degraded to prevent oxygen production. So you stop the source from within. Thus, heterocysts rely on neighboring cells for carbohydrates, primarily sucrose.



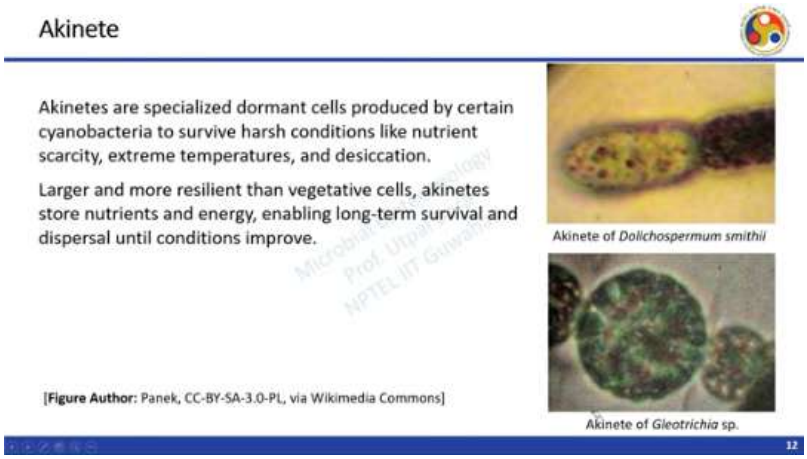
Heterocysts retain photosystem I for ATP production via cyclic photophosphorylation and use intracellular channels to retrieve sucrose. Glycolytic enzymes are regulated to break down sucrose, and oxygen-scavenging proteins are accumulated. Overall, all these mechanisms provide adaptations in heterocysts for protecting the nitrogenous enzyme, which is sensitive to oxygen. Here, you can see the nitrogenous enzyme and the transportation of sugar from neighboring cells. Heterocyst differentiation is triggered in nitrogen-depleted conditions.

by the transcriptional regulatory NTC-A, which signals a protein involved in the process. The differentiation sequence includes cell enlargement, reduction of granular inclusions,

reorientation of photosynthetic lamellae, and development of a triple-layered cell wall, with the middle layer being uniform and the inner layer laminated. So, this picture shows the division of labor in heterocystous cyanobacteria. Akinete. Akinetes are specialized dormant cells produced by certain cyanobacteria to survive harsh conditions like nutrient scarcity, extreme temperatures, and desiccation.



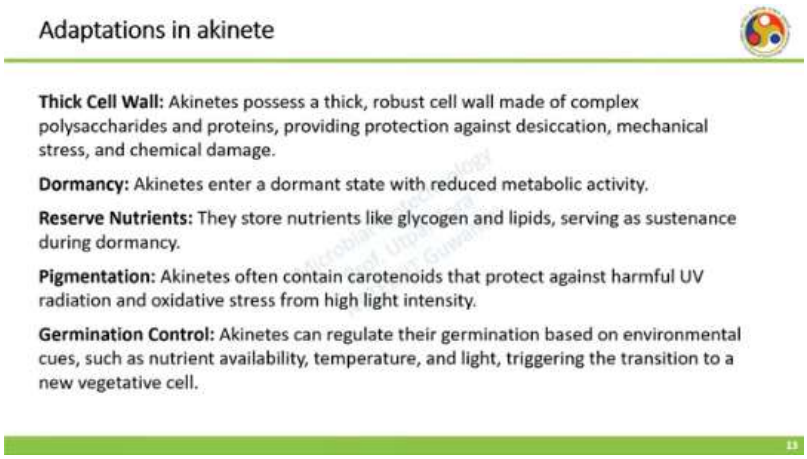
You can see here the akinete of Nostoc cospermum and the akinete of Gloeotrichia in the bottom picture. Larger and more resilient than vegetative cells, these akinetes store nutrients and energy, enabling long-term survival and dispersal until conditions improve. What are the adaptations in akinetes? They have various mechanisms of adaptation, the first being the development of a thick cell wall. Akinetes possess a thick, robust cell wall made of complex polysaccharides and proteins, providing protection against desiccation, the loss of water, mechanical stress, and chemical damage.



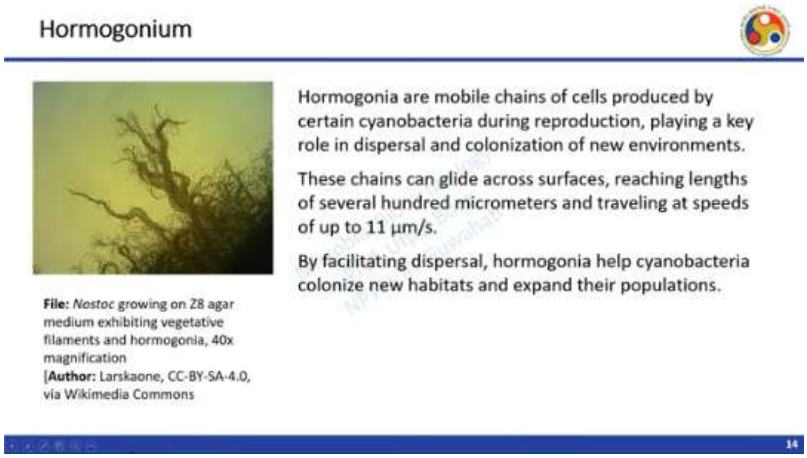
Another mechanism is that of dormancy. The akinetes enter a dormant state with reduced metabolic activity. And they have nutrient reserves. They store nutrients like glycogen and

lipids, serving as sustenance during dormancy. And they have pigmentation, containing carotenoids that protect against harmful UV radiation and oxidative stress from high light intensity.

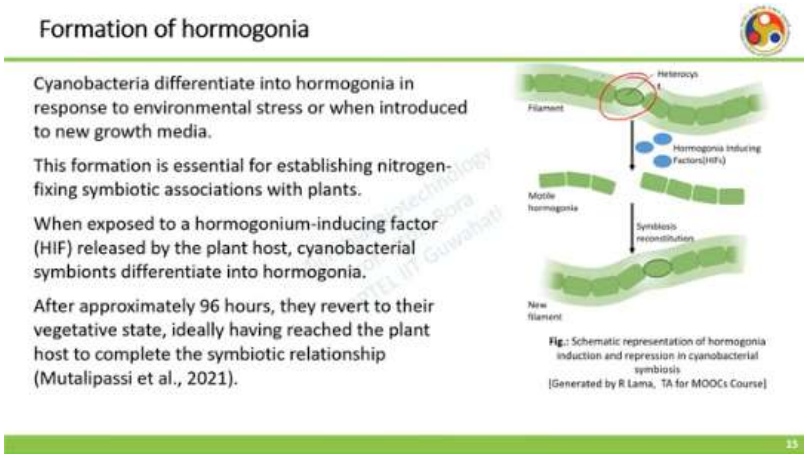
And they have germination control. Triggering the transition to a new vegetative cell. Let us now discuss the Hormogonium. Hormogonia are mobile chains of cells produced by certain cyanobacteria during reproduction, playing a key role in dispersal and colonization of new environments. Here we can see Nostoc growing on Z8 agar medium exhibiting vegetative filaments and hormogonia under 40X magnification.



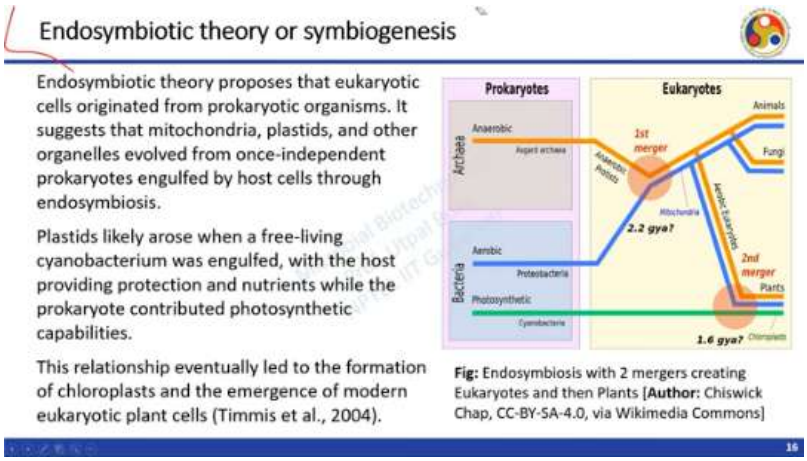
These chains can glide across surfaces, reaching lengths of several hundred micrometers and traveling at speeds of up to 11 micrometers per second. By facilitating dispersal, hormogonia help cyanobacteria colonize new habitats and expand their populations. How hormogonia are formed. So, here is a schematic representation of hormogonia induction and repression in cyanobacterial symbiosis. So, here you can see the filament with a heterocyst.



Then, due to hormogonia-inducing factors, there will be the formation of motile hormogonia. And then we have symbiosis reconstitution, which will give rise to new filaments. The cyanobacteria differentiate into hormogonia in response to environmental stress or when introduced to new growth media. This formation is essential for establishing nitrogen-fixing symbiotic associations with plants. When exposed to a hormogonium-inducing factor released by a plant host, cyanobacterial symbionts differentiate into hormogonia.



After approximately 96 hours, they revert to their vegetative state. Ideally, having reached the plant host to complete the symbiotic relationship. The endosymbiotic theory or symbiogenesis. Endosymbiotic theory proposes that eukaryotic cells originated from prokaryotic organisms. It suggests that mitochondria, plastids, and other organelles evolved from independent prokaryotes engulfed by host cells through endosymbiosis.

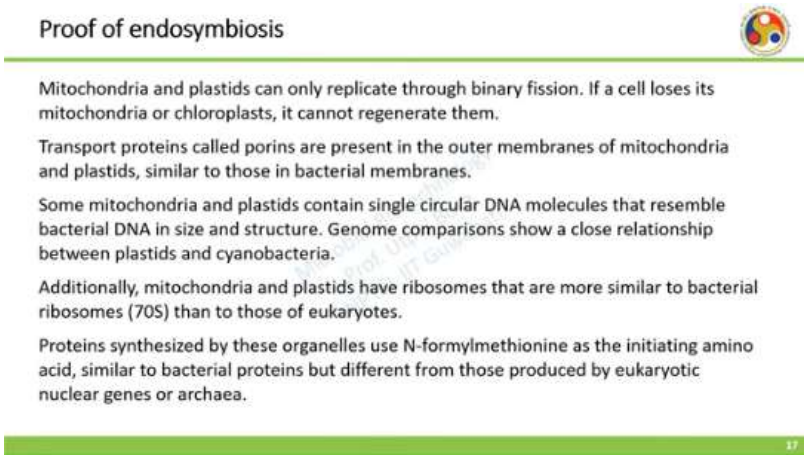


Plastids likely arose when a free-living cyanobacterium was engulfed by the host, providing protection and nutrients while the prokaryote contributed photosynthetic

capability, which was a win-win situation for both systems. This relationship eventually led to the formation of chloroplasts and the emergence of modern eukaryotic plant cells. So, here in this figure, you can see endosymbiosis with two mergers creating eukaryotes and then plants. So, here the prokaryotes include archaea. And then bacteria, the first being anaerobic and the second being aerobic.

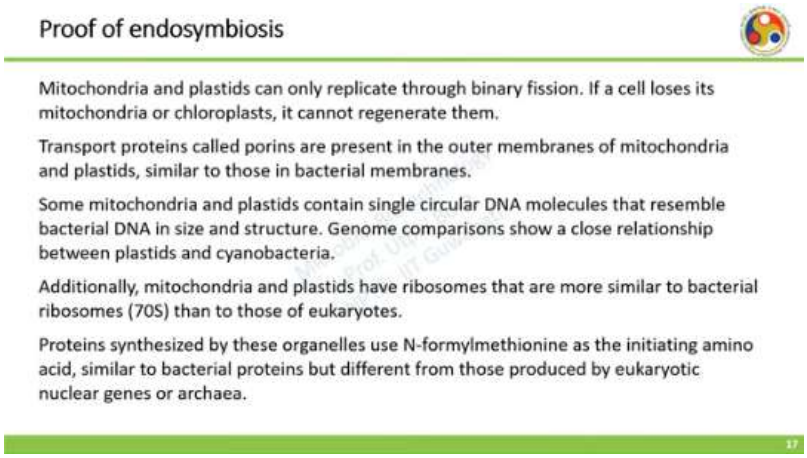
The first merger you can see is of the anaerobic protists, so here the mitochondria is being mobilized into the eukaryotic system, and this mitochondria is part of the animals as well as the fungi. Then this comes from the proteobacteria, as you can see over here. Then you have the cyanobacteria, which was photosynthetic, and got merged into the plant cells, and the plant cells also have the mitochondria. So, this is in brief the endosymbiotic theory or symbiogenesis, and this is how the eukaryotes probably acquired these independently existing microbes, and during evolution, they became organelles inside them.

Now what are the proof of this kind of theories or endosymbiosis? Mitochondrial plastes can only replicate to binary fission. If a cell loses its mitochondrial chloroplast, it cannot regenerate them. So these are two independent systems within the cell or eukaryotic cells. But the cells cannot produce them as such.



Transport proteins called porins are present in the outer membrane of a mitochondrion plastic similar to those in bacterial membranes. So, which gives some indication that they were derived from these prokaryotic organisms. They are basically the prokaryotic organisms which have developed a symbiotic relationship with these eukaryotic cells. Some mitochondrion plastics contain single circular DNA molecules that resemble bacterial DNA in size and structure. Genome comparisons show a close relationship between plastids and cyanobacteria.

Additionally, mitochondria and plastids have ribosomes that are more similar to bacterial ribosomes than to those of eukaryotes. Proteins synthesized by these organelles use N-formylmethionine as the initiating amino acid similar to bacterial proteins but different from those produced by eukaryotic nucleogens or archaea. The Great Oxidation Event The Great Oxidation Event marks a significant period in art history about 2.4 billion years ago, characterized by a substantial increase in atmospheric oxygen. Early photosynthetic organisms like cyanobacteria, developed the ability to perform oxygenic photosynthesis, producing molecular oxygen as a byproduct.



As these organisms proliferated, oxygen accumulated in the atmosphere, saturating oceans and lands and triggering extensive chemical reactions and environmental changes. This rise in oxygen altered the atmosphere's composition, leading to the formation of an ozone layer that protected the planet's surface from harmful ultraviolet radiation. Additionally, oxygen facilitated the evolution of aerobic organisms, enabling more efficient energy metabolism through cellular respiration. Let us now discuss the archaea. Archaea were initially mistaken for bacteria due to their similar size and shape and were recognized as a separate domain in the three-domain classification alongside bacteria and eukarya.

The Great Oxidation Event

The Great Oxidation Event marks a significant period in Earth's history, about 2.4 billion years ago, characterized by a substantial increase in atmospheric oxygen.

Early photosynthetic organisms like cyanobacteria developed the ability to perform oxygenic photosynthesis, producing molecular oxygen (O_2) as a byproduct.

As these organisms proliferated, oxygen accumulated in the atmosphere, saturating oceans and land and triggering extensive chemical reactions and environmental changes.

This rise in oxygen altered the atmosphere's composition, leading to the formation of an ozone layer that protected the planet's surface from harmful ultraviolet radiation.

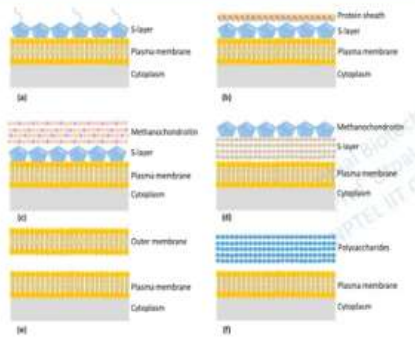
Additionally, oxygen facilitated the evolution of aerobic organisms, enabling more efficient energy metabolism through cellular respiration (Cardona, Murray & Rutherford, 2015).

18

They have genes and metabolic pathways that are more closely related to eukaryotes. The unique biochemistry of archaea includes ether-linked lipids in their cell membranes and diverse energy sources such as organic compounds, ammonia, metal ions, and hydrogen gas. As unicellular prokaryotes with circular genomes lacking spliceosomal introns, archaea exhibit co-located genes. The DNA replication, transcription, and translation mechanisms are more similar to those of eukaryotes than bacteria. Distinct features include membranes made of isoprenoid chains, a motility structure called the archaellum, and specialized metabolism like methanogenesis.

Let us look into the structure of the archaeal cell envelope. Unlike bacteria, archaea do not have a uniformly distributed cell wall. However, different groups of archaea share various cell wall components that are structurally similar to those found in bacteria. The physical properties of these components enable many archaeal species to thrive in extreme environments. In this figure, we can see different types of archaeal cell envelope structures from A to F. For example, in A, we have the S-layer, which is also present in B and C.

Archaeal cell envelope



Unlike bacteria, archaea do not have a uniformly distributed cell wall type.

However, different groups of archaea share various cell wall components that are structurally similar to those found in bacteria.

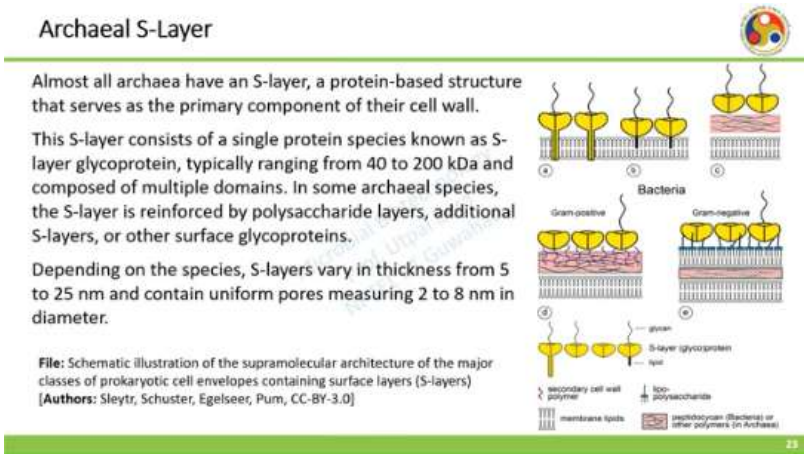
The physical properties of these components enable many archaeal species to thrive in extreme environments.

Fig: Different types of Archaeal cell envelope structures [Generated by R Lama]

22

D, and then we have, in certain cases, for example, a protein seed in B. Then we have this plasma membrane present all throughout. Then, in C, we have this methanocondroitin, and in F, we have the polysaccharides. And, in certain cases, in the case of E, we can see outer membranes. So, this shows that the cell envelope structures of archaea are very diverse. Now, let us try to discuss some of these in a little bit more detail. The archaeal S-layer.

Almost all archaea have an S-layer, a protein-based structure that serves as the primary component of their cell wall. This S-layer consists of a single protein species known as S-layer glycoprotein, typically ranging from 40 to 200 kilodaltons. Composed of multiple domains in some archaeal species, the S-layer is reinforced by polysaccharide layers, additional S-layers, or other surface glycoproteins. Depending on the species, S-layers vary in thickness from 5 to 25 nanometers and contain uniform pores measuring 2 to 8 nanometers in diameter. In this figure, we can see the schematic illustration of the supramolecular architecture of the major classes of prokaryotic cell envelopes containing the surface S-layers.



Pseudomurein. Pseudomurein is named for its similarity to bacterial peptidoglycan. It consists of linear chains of alternating amino sugars and N-acetylglucosamine and N-acetylmuramic acid. These are linked by beta-1,3 glycosidic bonds. Each chain is connected to a short chain of amino acids, typically glutamic acid, alanine, or lysine, and these peptide chains are cross-linked to enhance the cell wall's strength. Pseudomurein is found in the cell wall of organisms within the Methanobacteriales and Methanopyrales orders.

Pseudomurein

Pseudomurein, named for its similarity to bacterial peptidoglycan, consists of linear chains of alternating amino sugars: N-acetylglucosamine (GlcNAc or NAG) and N-acetyltalosaminuronic acid (NAcTalNA or NAT), linked by β-(1,3)-glycosidic bonds. Each NAT is connected to a short chain of L-amino acids (typically glutamic acid, alanine, or lysine), and these peptide chains are cross-linked to enhance the cell wall's strength.

Pseudomurein is found in the cell walls of organisms within the Methanobacteriales and Methanopyrales orders.

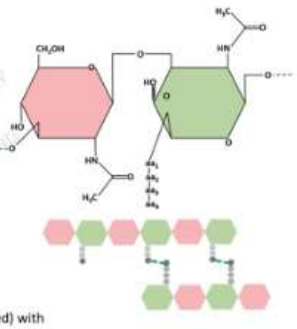


Fig: Finer structure pseudomurein, showing NAT (light green) and NAG (red) with amino acid chain (grey) and cross-linkage (dark green) [Generated by R Lama]

Methanocondroitin. Methanocondroitin is composed of proteoglycans with repeating trimeric units that include one glucuronic acid and two N-acetylgalactosamine molecules. These galactose units are linked by beta-1,4-glycosidic bonds, while glucuronic acid connects to galactose via a beta-1,3-glycosidic bond. These are found exclusively in aggregated cells of Methanosarcina. Methanocondroitin resembles eukaryotic chondroitin surfaces, which is reflected in its name. Other types of cell walls.

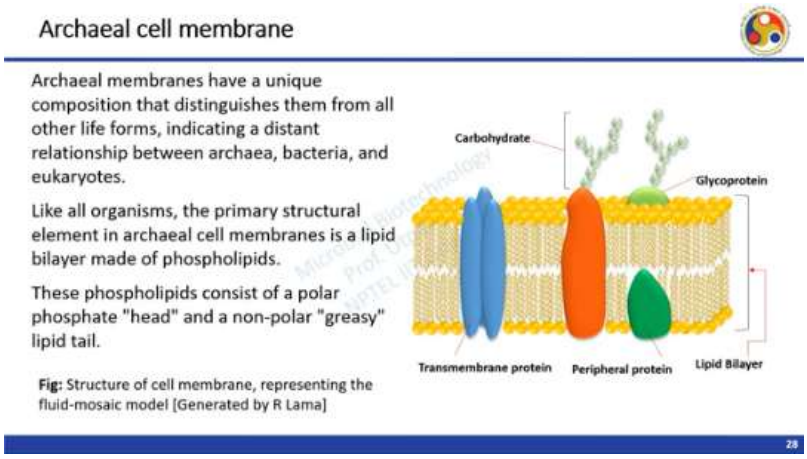
Some archaea, like Ignicoccus, SM1, Eukaryon, and Arman, possess an additional lipid membrane outside the cellulosic membrane. Methanospirillum species have an outer proteinaceous sheath over the S-layer, with protein subunits arranged in a paracrystalline structure resembling stacked hoops. This layer can be separated under reducing conditions. Other species may have various polysaccharides, including methanocondroitin and pseudomurein, along with glutaminyl glycan in the halophilic genus Neptunococcus. Then we have lipoglycan in thermoacidophilic Thermoplasma species, like Ferroplasma acidophilum and Thermoplasma acidophilum.

Other types of cell wall

Some archaea, like *Ignicoccus*, SM1 Euryarchaeon, and ARMAN, possess an additional lipid membrane outside the cytoplasmic membrane (Klingl, Pickl, & Flechsler, 2019).

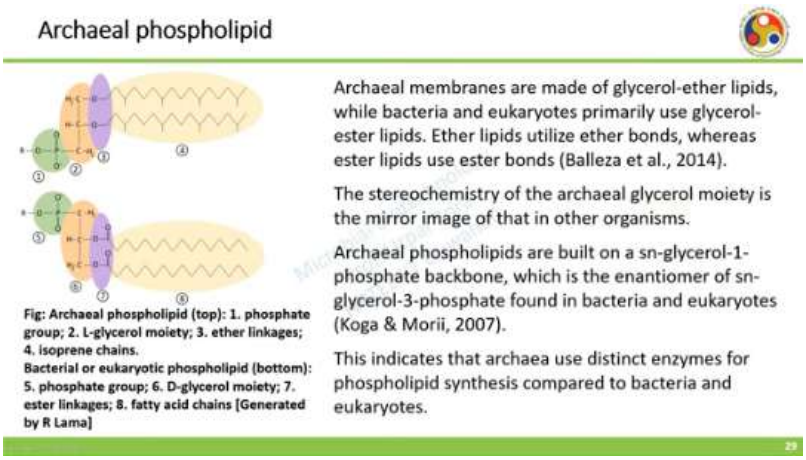
Methanospirillum species have an outer proteinaceous sheath over the S-layer, with protein subunits arranged in a paracrystalline structure resembling stacked hoops; this layer can be separated under reducing conditions.

Then we have heteropolysaccharides in Helicoccus morhuae, composed of sulfated units of various sugars, including glucosamine, galactosamine, glucosaminuronic acid, glucose, galactose, and aminos. The archaeal cell membrane. Archaeal membranes have a unique composition that distinguishes them from all other life forms, indicating a distinct relationship between archaea, bacteria, and eukaryotes. So, here we can see a glycoprotein and some carbohydrate chains over here, then there is a peripheral protein and a transmembrane protein. All these are embedded in the lipid bilayer on either side.

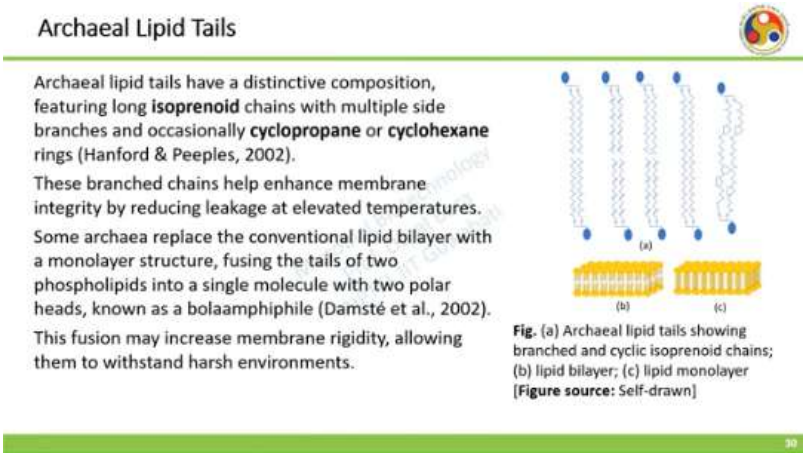


Like all organisms, the primary structural element in archaeal membranes is a lipid bilayer, as I have already mentioned, which is made of phospholipids. These phospholipids consist of a polar phosphate head and a non-polar greasy lipid tail. Archaeal membranes are made of glycerol ether lipids, while bacteria and eukaryotes primarily use glycerol ester lipids. Ether lipids utilize ether bonds, whereas ester lipids use ester bonds.

The stereochemistry of the archaeal glycerol moiety is the mirror image of that in other organisms. Archaeal lipids are built on an sn-glycerol-1-phosphate backbone, which is the enantiomer of sn-glycerol-3-phosphate found in bacteria and eukaryotes. This indicates that archaea use distinct enzymes for phospholipid synthesis compared to bacteria and eukaryotes. So, in this figure, we can see the archaeal phospholipid: one, the phosphate group; two, the L-glycerol moiety; three, the ether linkages; and four, the isoprene chains. And then, in the bacterial or eukaryotic phospholipid in the bottom figure, we can see number five, the phosphate group; the D-glycerol moiety, number six; the ester linkage, number seven; and the fatty acid chain, number eight.



Let us look into the structure of the archaeal lipid tails. Archaeal lipid tails have a distinctive composition, featuring long isoprenoid chains with multiple side branches and occasionally cyclopropane or cyclohexane rings. These branched chains help enhance membrane integrity by reducing leakage at elevated temperatures. So, in this figure, you can see the alkyl lipid tails showing branched and cyclic isoprenoid chains in figure A, and you can see the lipid bilayer in figure B and the lipid monolayer in figure C. Some archaea replace the conventional lipid bilayer with a monolayer structure, fusing the tails of two phospholipids into a single molecule with two polar heads, known as bolaamphiphiles.



This fusion may increase membrane rigidity, allowing them to withstand harsh environments. So, with this, we come to the end of this lecture, which was focused on archaea and cyanobacteria.