Environmental Microbiology
Course 6

Cyanobacteria

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What are cyanobacteria?

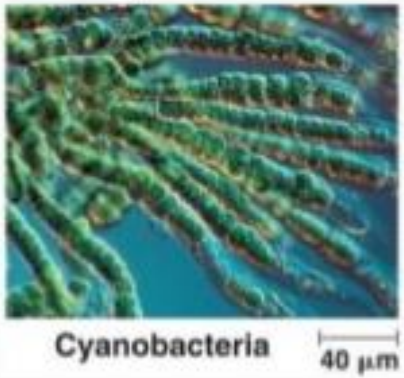
- Cyanobacteria, also called blue-green algae, are microscopic organisms found naturally in all types of water.
- **These single-celled organisms live in fresh, brackish (combined salt and fresh water), and marine water.**

What are cyanobacteria?

- **These organisms use sunlight to make their own food.**
- **In warm, nutrient-rich (high in phosphorus and nitrogen) environments, cyanobacteria can multiply quickly, creating blooms that spread across the water's surface.** The blooms might become visible.

Cyanobacteria

- Photosynthetic- contain chlorophyll pigment
- Found in ocean and on land
- Thick cell wall and no flagella
- Commonly called blue-green algae
- Considered the ancestors of present day chloroplasts
- Grow in colonies
- Can manufacture their own food through photosynthesis



Cyanobacteria

Cyanophyta (Blue-green algae)

- are prokaryotic bacteria; date to 3.5 BYA
- ~55% are blue-green
- ~15% are *never* blue-green; others may be green, olive, red, purple, black, or colorless
- Store food as glycogen (iodine negative)
- Never flagellate, but some can move (*oscillate* or glide)
- Reproduction
  - Sexual-not known
  - Vegetative-binary fission; fragmentation
  - Asexual
    - Akinete-germinates directly
    - Heterocyst-may divide either directly to a trichome or to endospores which 'germinate' to a trichome
- Presence of pseudovacuoles; gas-filled; affect buoyancy; may shade other species

Algae

Prokaryotic

Kingdom Monera (Bacteria)

- Cyanobacteria (“Blue-Green Algae”)

Eukaryotic

Kingdom Protista

- Dinophyta (“Dinoflagellates”)
- Raphidophyta (“Raphidophytes”)
- Bacillariophyta (“Diatoms”)
- Chrysophyta (“Golden Algae”)
- Chlorophyta (“Green Algae”)
- Phaeophyta (“Brown Algae”)
- Rhodophyta (“Red Algae”)

“Microalgae”

“Macroalgae”

Habit – success due to ability tolerate a wide range of conditions

- Marine – littoral and pelagic
- Fresh Water
- Hot Springs
- Terrestrial – soil flora

Why treat cyanobacteria together with the algae?

1. Oxygenic photosynthesis and pigments (chlorophyll a) as in algae and plants

$$CO_2 + 2H_2O \xrightarrow[\text{chlorophyll a}]{\text{light}} (CH_2O) + O_2 + H_2O$$

All other photosynthetic bacteria (e.g. green sulfurbacteria) have bacterial chlorophyll and anoxic photosynthesis

$$CO_2 + 2H_2S \xrightarrow[\text{Bacterial chlorophyll}]{\text{light}} (CH_2O) + 2S + H_2O$$

Why treat cyanobacteria together with the algae?

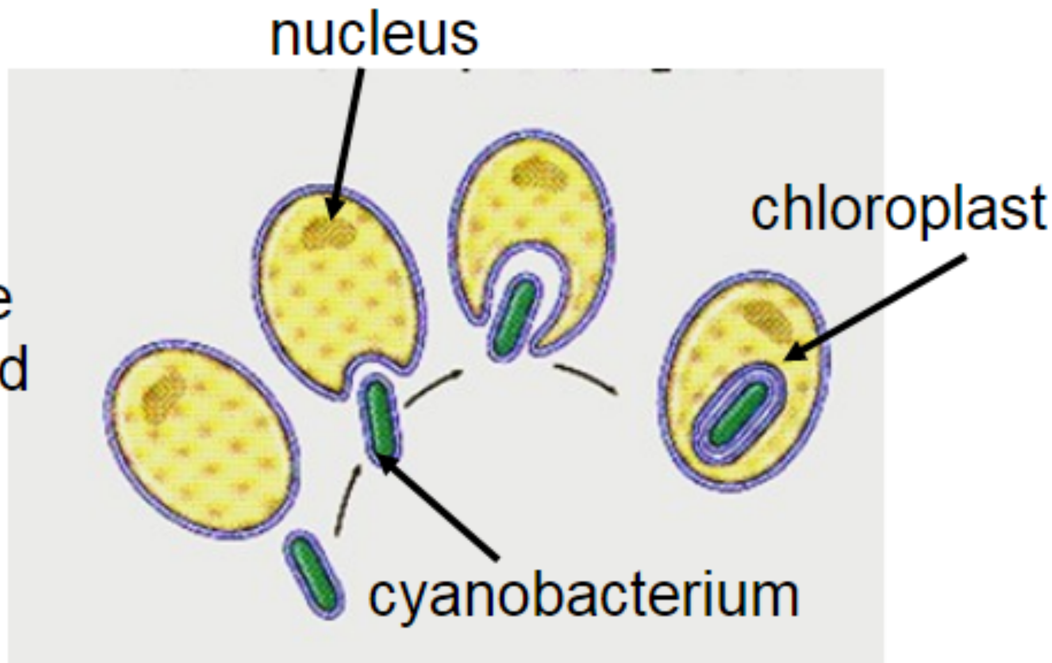
2. Cyanobacteria (blue greens) occur with algae in similar habitats, mainly aquatic environments.

Similar ecological function (as primary producers). Together with the algae they stand for ca. 40 % of global primary production

Why treat cyanobacteria together with the algae?

3. Algal (and plant) chloroplasts originated from primary endosymbiosis between heterotrophic eukaryote and a free living cyanobacterium

Cyanobacterium taken up by phagotrophic eukaryote and eventually transformed into a chloroplast



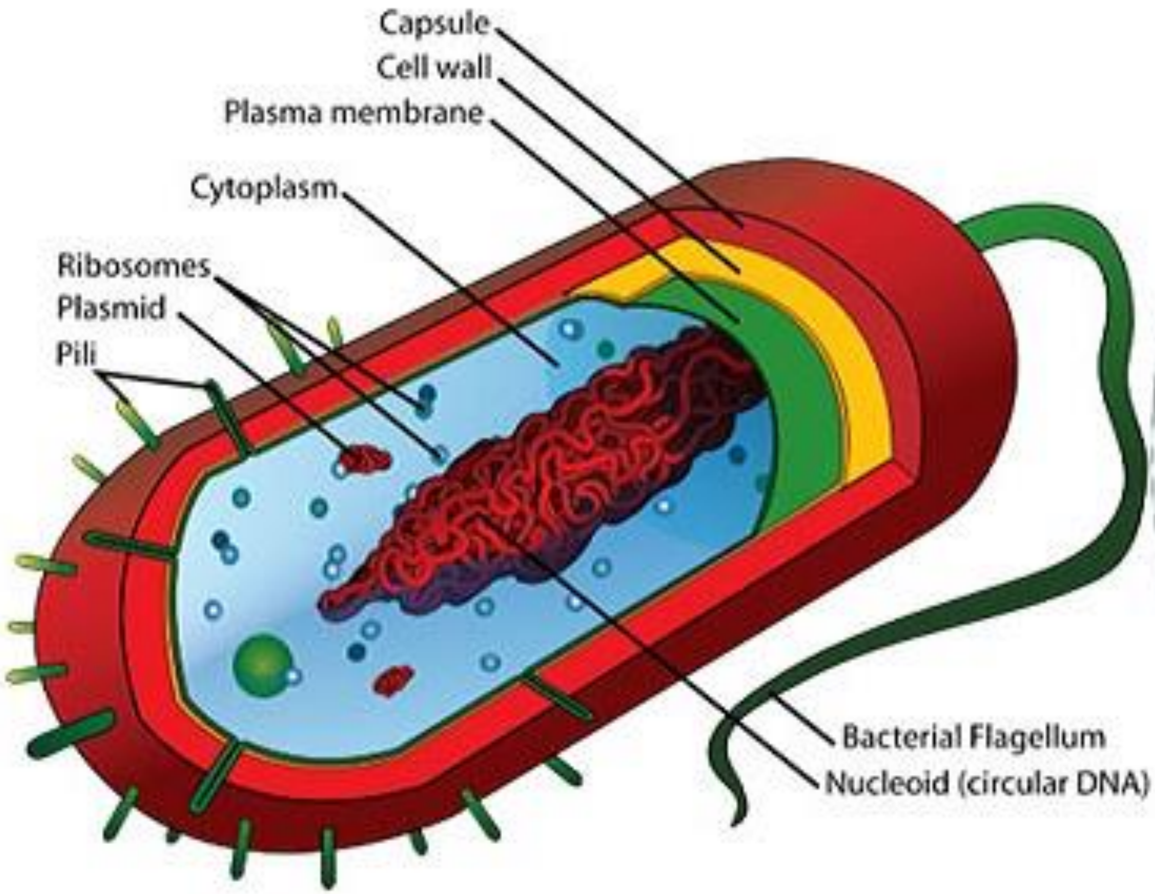
Differences between Bacteria and Cyanobacteria are given as follows:

Characters	Bacteria	Cyanobacteria
Size	Comparatively smaller.	Comparatively larger.
Distribution	Found every possible places in earth.	Only found in presence of sunlight and moisture.
Flagella	May bear flagella.	Flagella always absent.
Composition of cell wall	Glycolipids and peptidoglycan.	Cellulose and pectin.
Nutrition	May be autotrophic or heterotrophic.	Usually autotrophic.
Photosynthetic pigments	Photosynthetic pigment is bacteriochlorophyll.	Photosynthetic pigments is chlorophyll a.

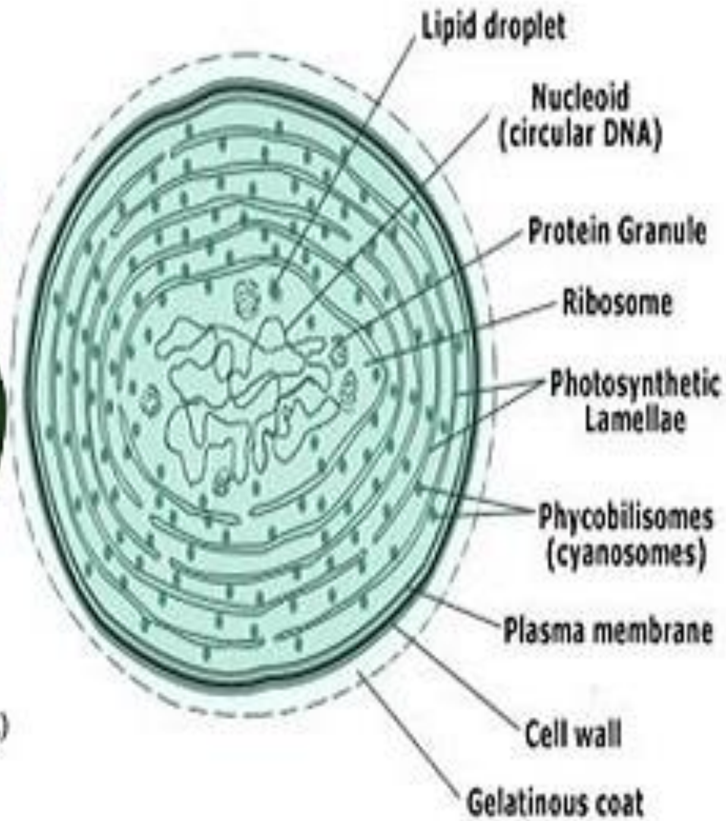
Accessory pigment	Absent	Accessory pigment like phycocyanin and phycoerythrin are present in dominating form.
Reserve food	Glycogen	Cyanophycean starch
Spore formation	Is endogenous.	Is not endogenous.
Hydrogen donor	During photosynthesis hydrogen donor is not water; as a result oxygen is not evolved. Thus photosynthesis is anoxygenic.	Hydrogen donor is water, oxygen is evolved. Process is oxygenic.
Locomotory organ	Flagella act as locomotory organ.	Lack flagella and other locomotory organ.
Heterocyst	Absent	Present.
Sexual reproduction	Takes place by conjugation, transformation and transduction.	Totally absent.

Differences between Bacteria and Cyanobacteria

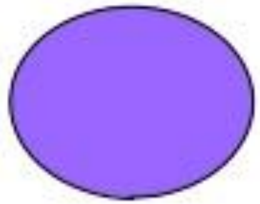
Bacteria



Cyanobacteria



Forms

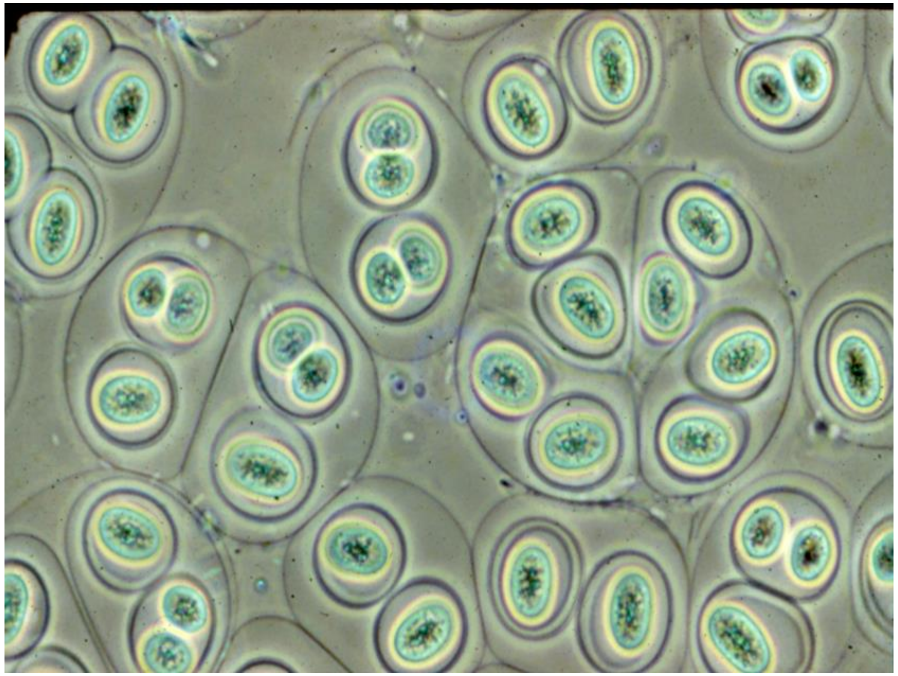


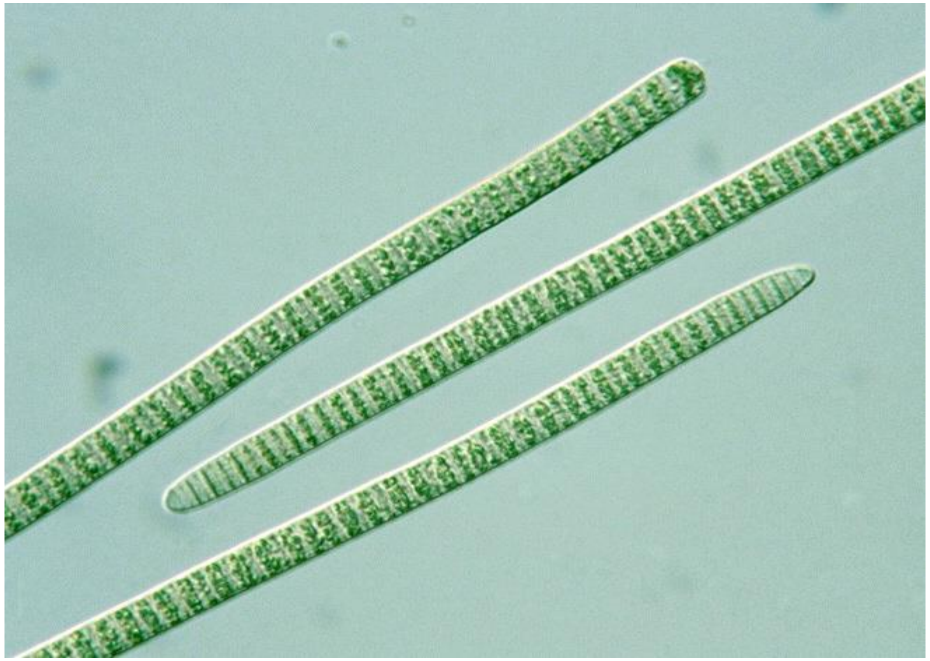
- **Unicell** – with mucilaginous envelope
- **Colonies** –

- **Filaments** – uniserate in a single row

- OR - multiserate – not TRUE branching when trichomes are > 1 in rows


Chroococcus

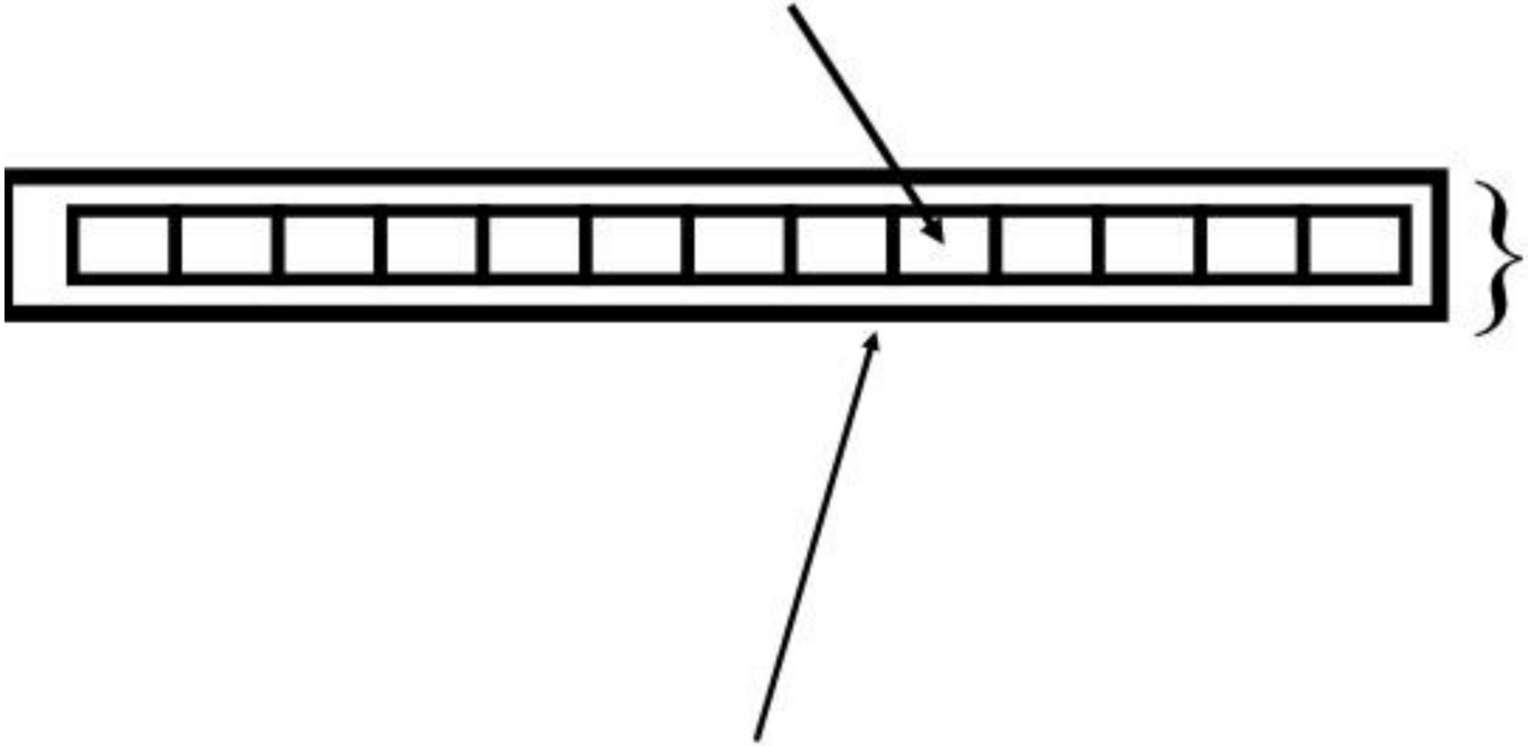

Gleocapsa


Oscillatoria


Nostoc

Features

Trichome – row of cells

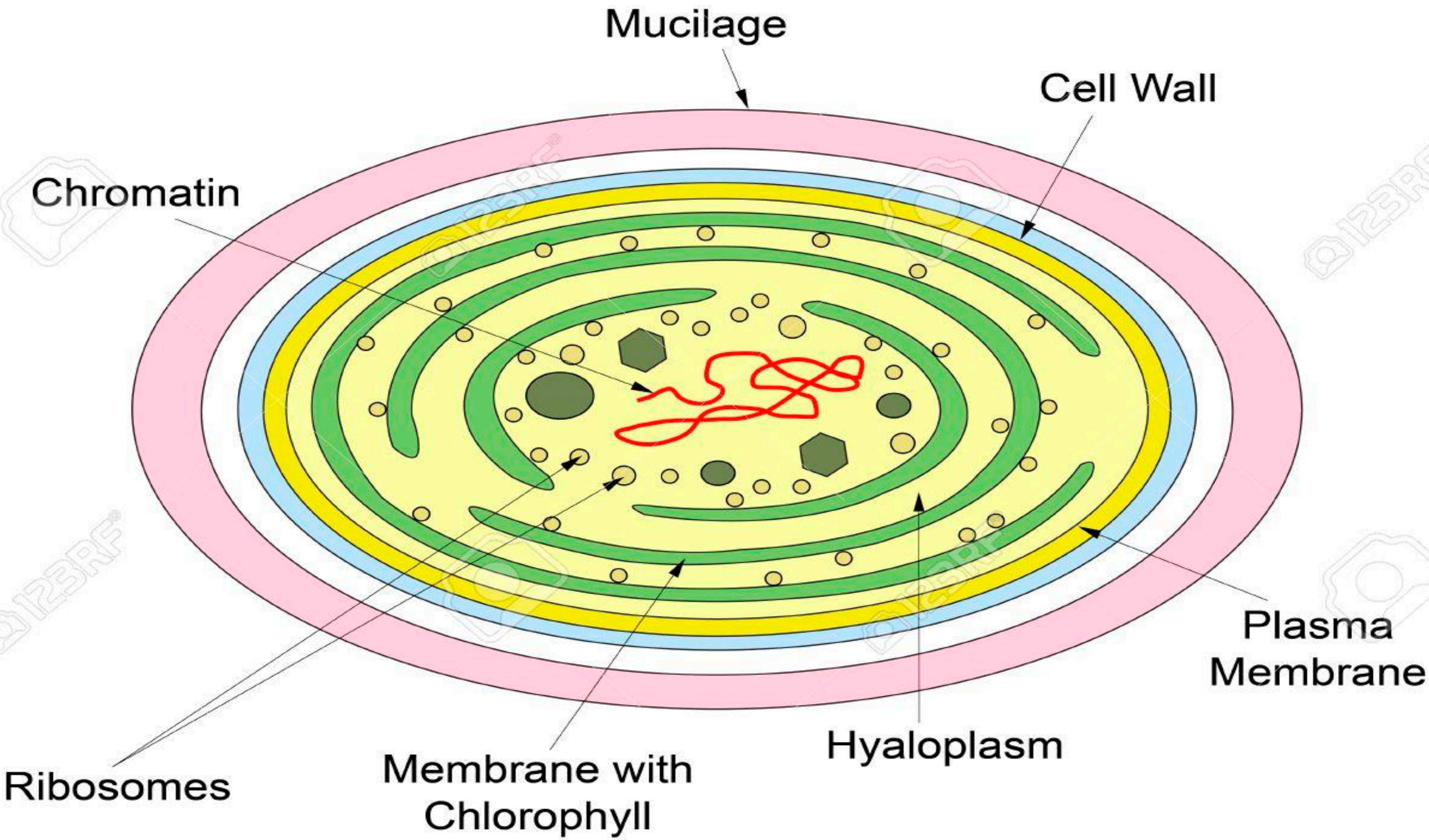


Filament

Mucilaginous sheath –

layer of mucilage outside of the cell wall.

Scheme of Cyanobacteria Cell



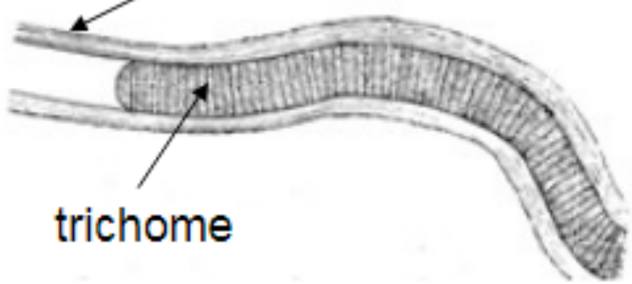
Cyanobacteria: general characteristics

Cell wall: as Gram-negative bacteria

central layer: murein (peptidoglykan)

Outside the cell wall a ± thick layer of polysaccharides and polypeptides may form a sheath in some filamentous forms. The sheath may be brownish in color, due to scytonemin (UV absorbing protecting pigment)

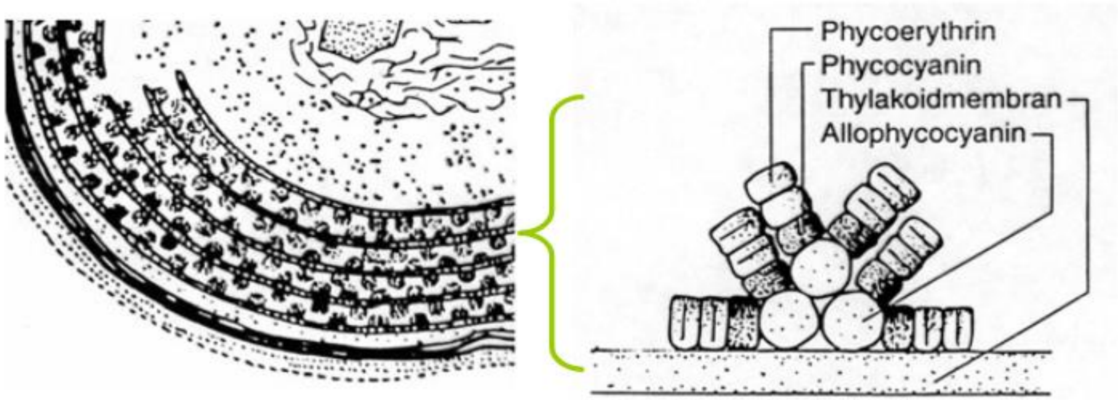
The cell row itself is called **trichome**

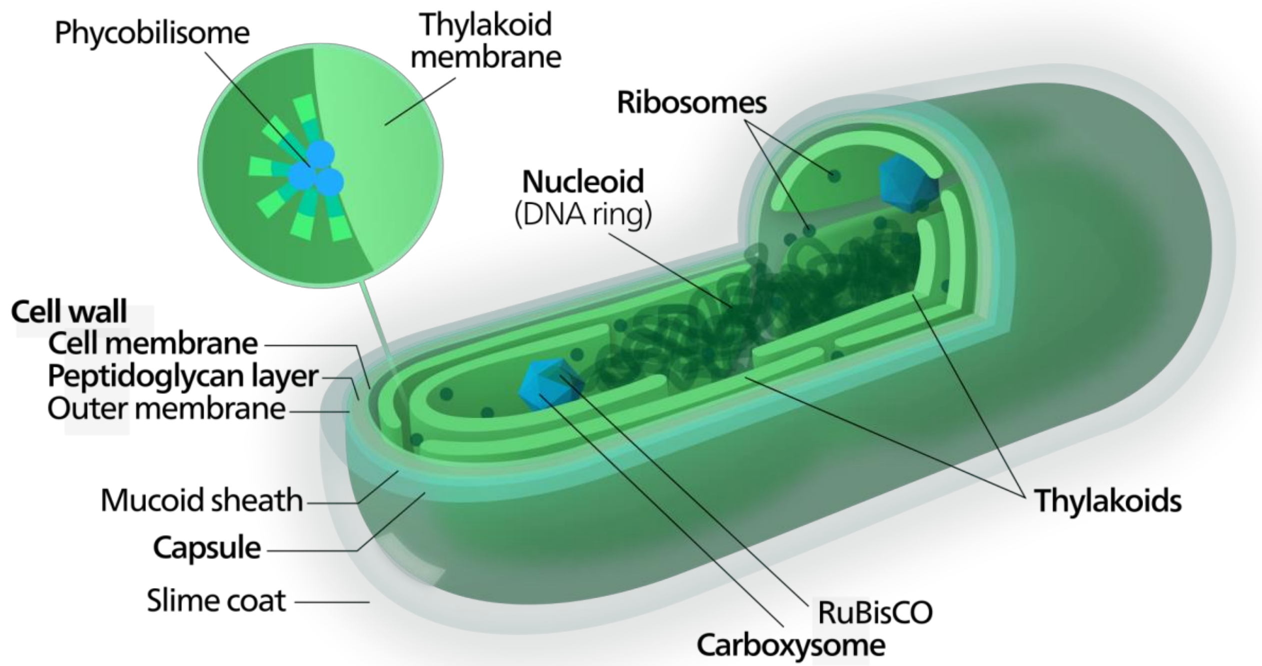


Cyanobacteria: general characteristics

Ultra structure:

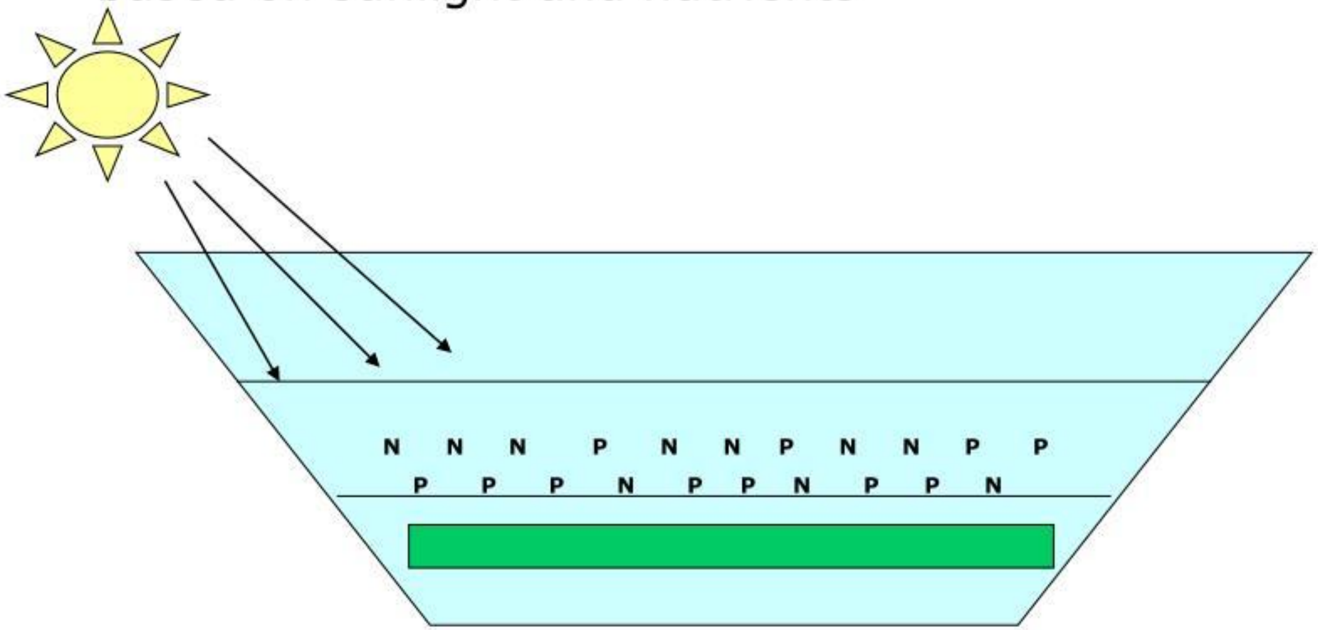
Thylakoids with phycobilisomes





Adaptations and Advantages

- Gas Vesicles
  - Some genera have gas vesicles to control buoyancy
  - Advantage-allows cyanobacteria to optimize growth based on sunlight and nutrients



Importance

1) First organisms to have 2 photosystems and to produce organic material and give off O_2 as a bi-product.

→Very important to the evolution of the earths' oxidizing atmosphere .

Cyanobacteria: general characteristics

Pigments:

chlorophyll a

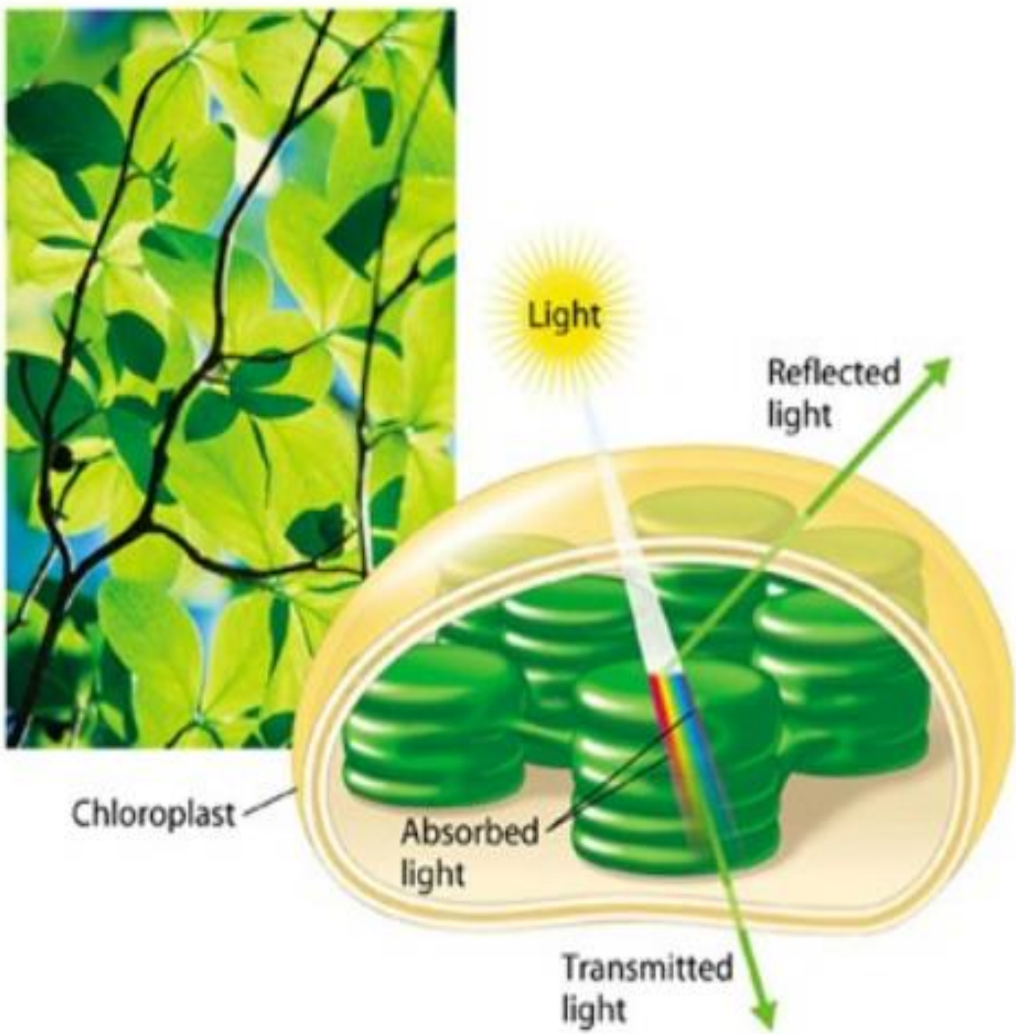
(three genera with chlorophyll a + b)

phycobilins → phycoerythrin, phycocyanin, allophycocyanin

carotenoids → A variety, some are specific, others as in algae and plants

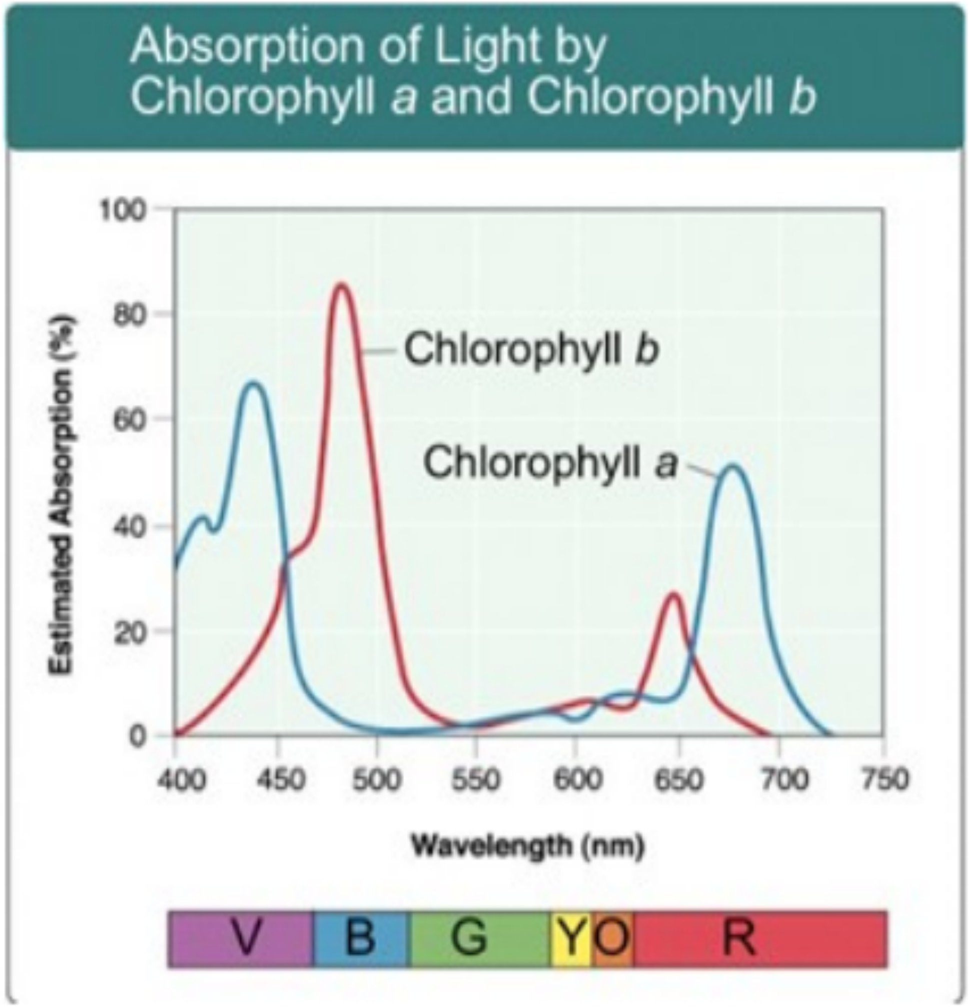
scytonemin - extra cellular, UV-shielding pigment

Visible radiation drives the light reactions



- Different pigments absorb light of different wavelengths.
- Chloroplasts contain several kinds of pigments (**chlorophyll *a* and *b*** as well as accessory pigments such as **carotenoids**).

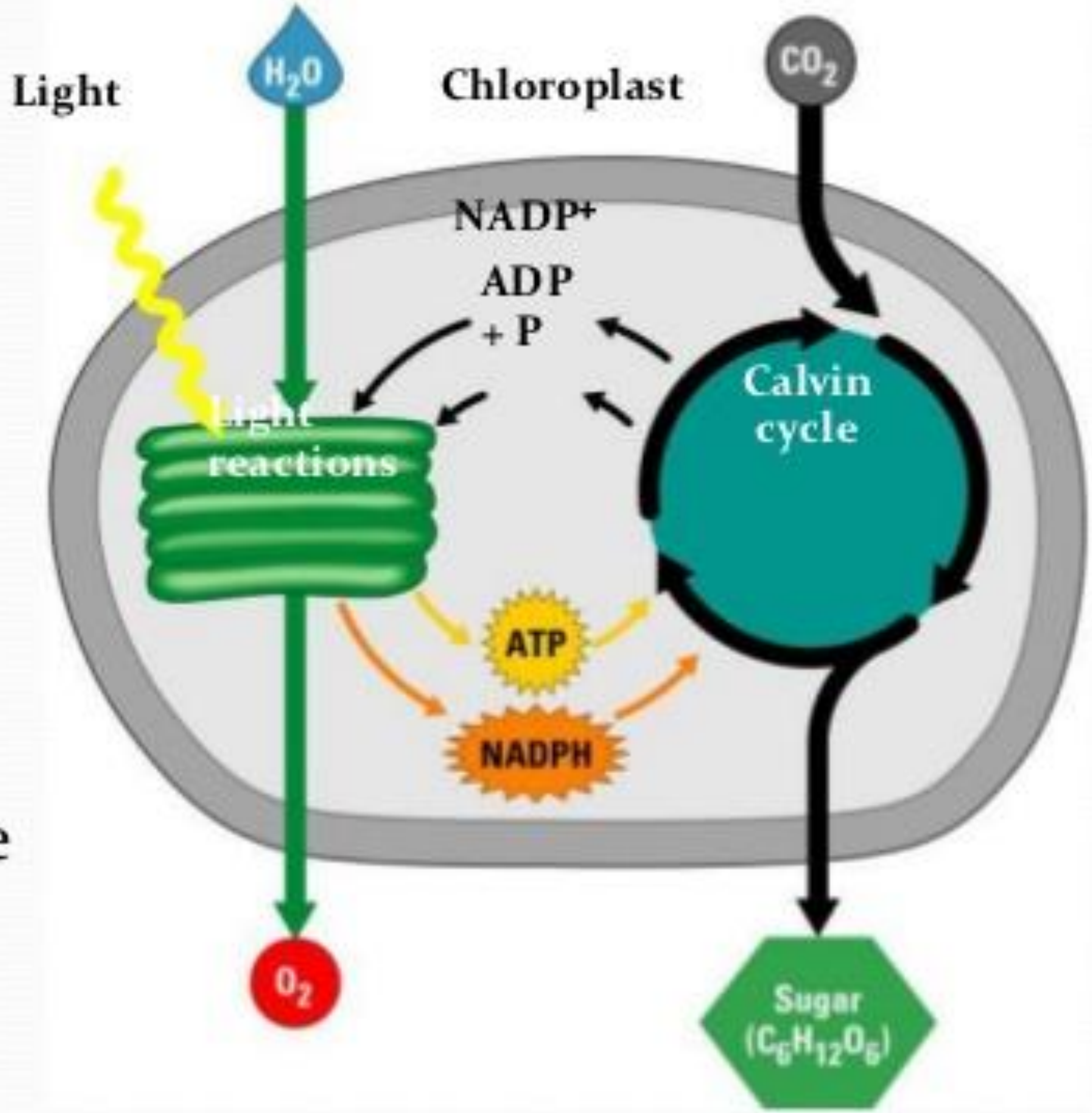
Light & Pigments



- **Chlorophyll a** absorbs mostly blue-violet and red light.
- **Chlorophyll b** absorbs mostly blue and orange light.
- <u>NOTE</u>: Chlorophyll absorbs light very well in the blue and red regions, but *not* the **green**. This is why plants are green. Colors that you see are wavelengths of light that are reflected back to your eye.

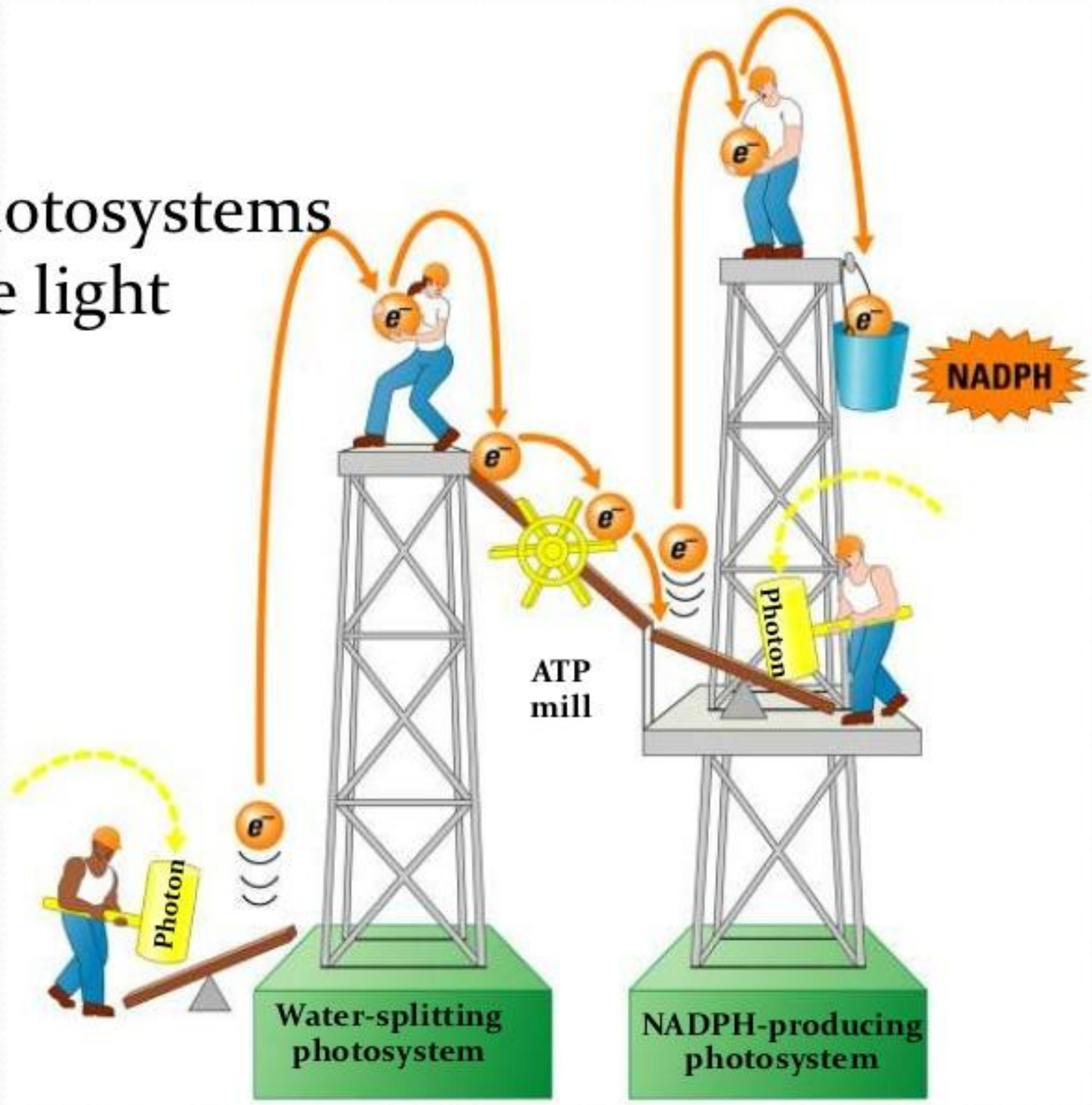
Photosynthesis occurs in 2 phases:

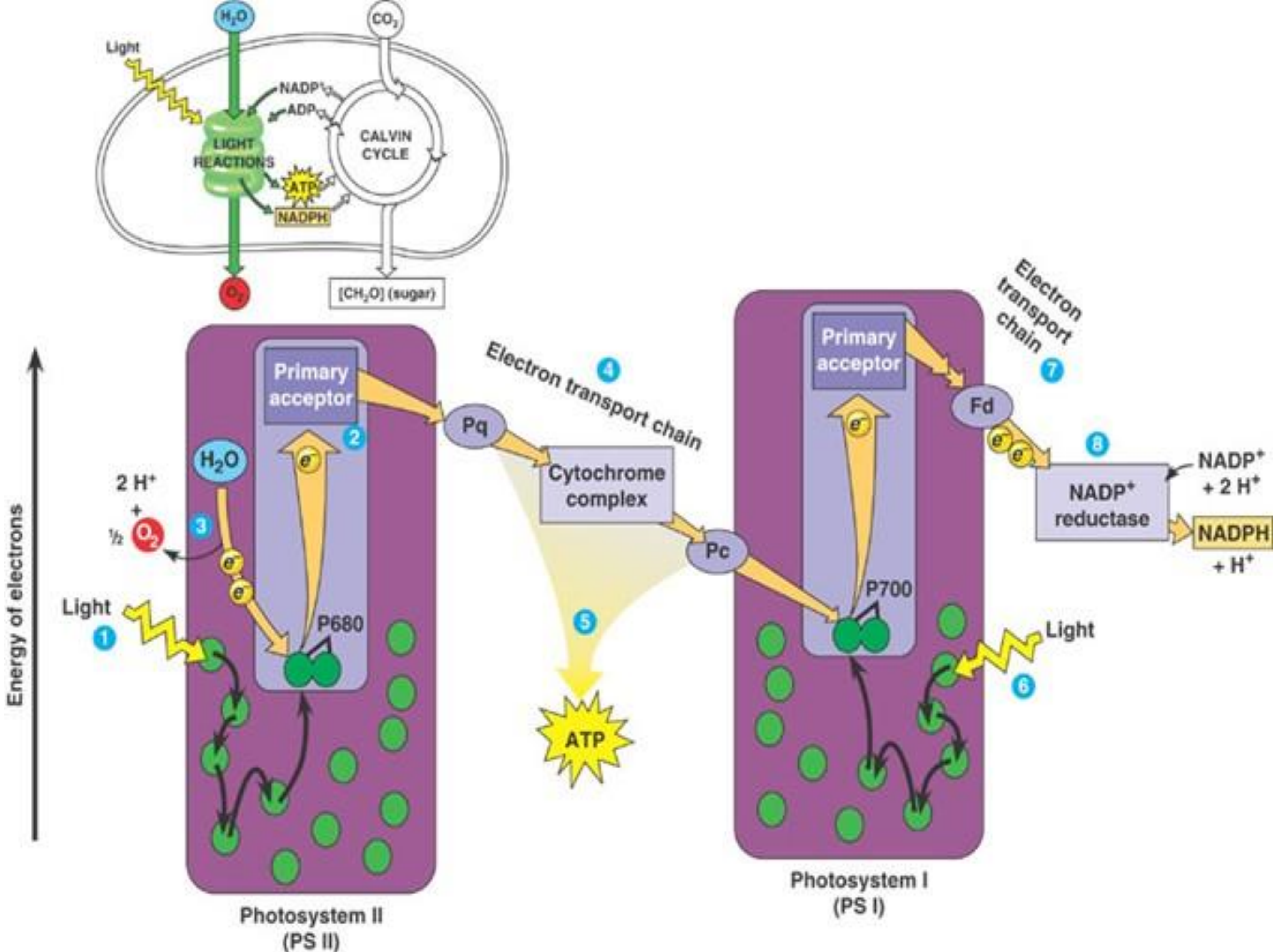
- The light reactions convert solar energy to chemical energy
  - Produce **ATP** & **NADPH**



- The Calvin cycle makes sugar from carbon dioxide
  - The **ATP** and **NADPH** are used to assemble sugars and other organic compounds

- Two types of photosystems cooperate in the light reactions

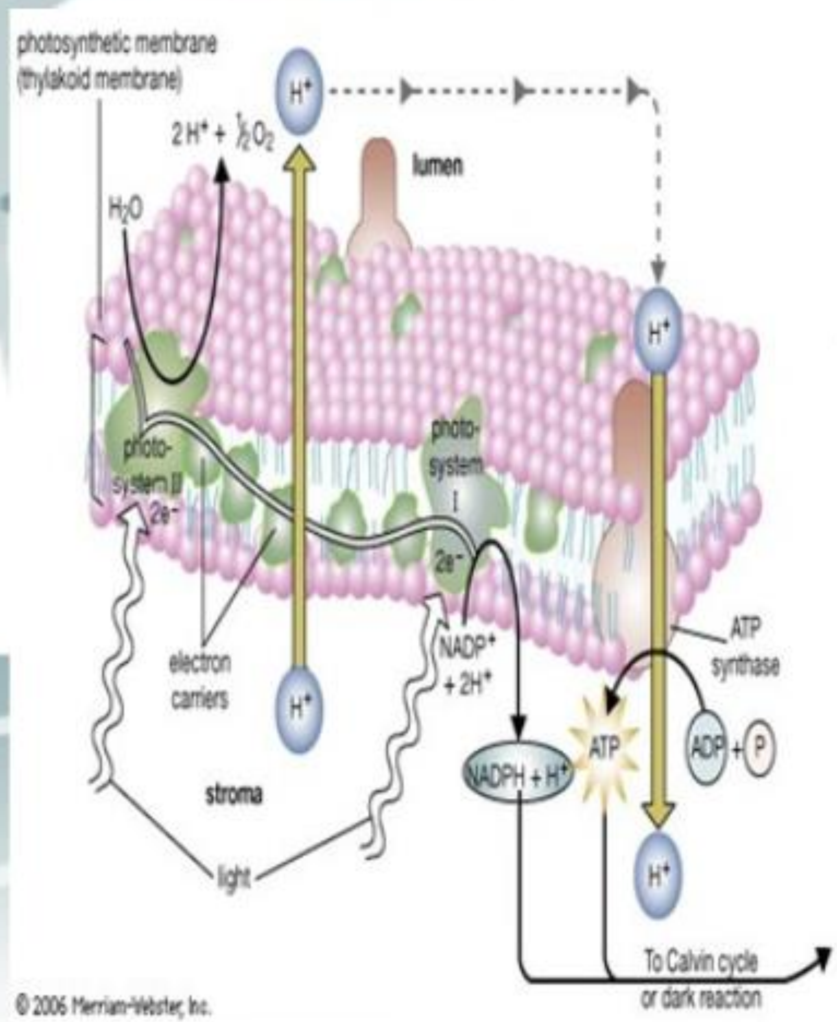




H2O
CO2
Light
NADP+
ADP
LIGHT REACTIONS
CALVIN CYCLE
ATP
NADPH
O2
[CH2O] (sugar)
Energy of electrons
Primary acceptor
Electron transport chain
Pq
Cytochrome complex
Pc
Primary acceptor
Electron transport chain
Fd
NADP+ reductase
NADP+ + 2 H+
NADPH + H+
H2O
2 H+ + ½ O2
e-
P680
P700
Light
Light
ATP
1
2
3
4
5
6
7
8
Photosystem II (PS II)
Photosystem I (PS I)

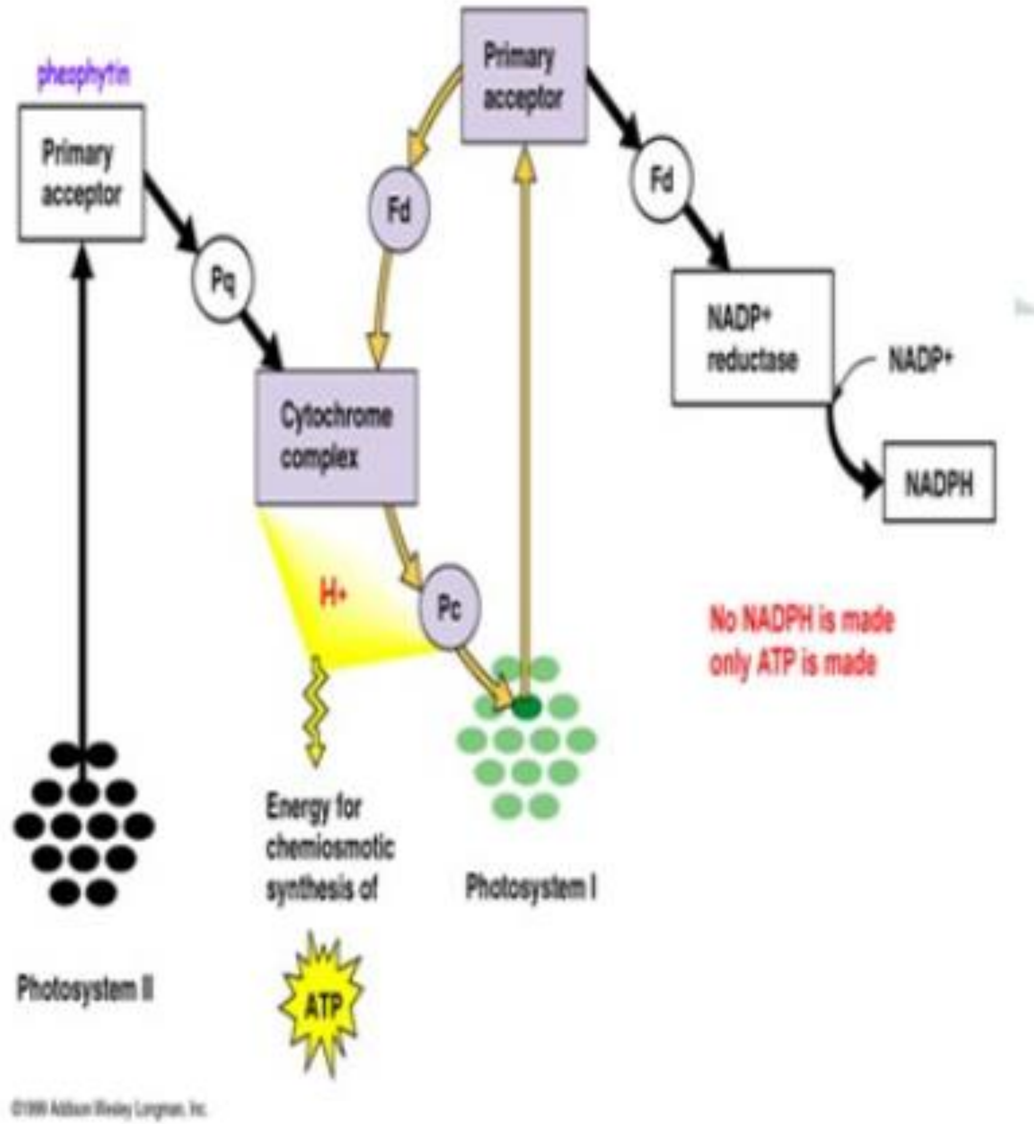
Photosystem II

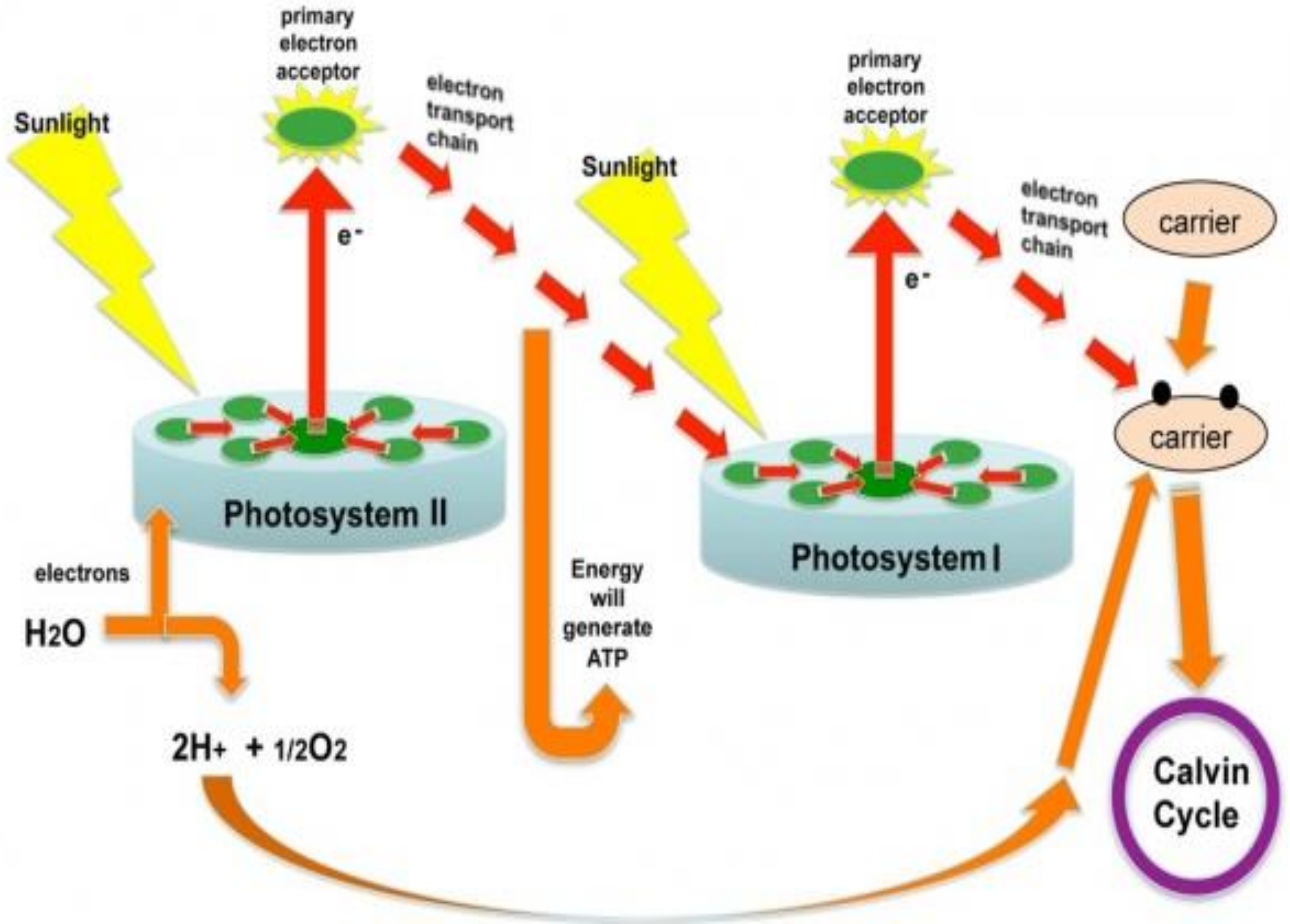
- water is split
- oxygen atom combines with oxygen from another split water forming molecular oxygen-O_2
- each excited electron passes from photosystem II to photosystem I via electron transport chain



Photosystem I

- primary electron acceptor captures an excited electron
- excited electrons are passed through short electron transport chain to $NADP^+$ reducing it to NADPH
- $NADP^+$ is final electron acceptor
- electrons are stored in high state of potential energy in NADPH molecule
- NADPH, ATP and O_2 are products of light reactions





Sunlight
primary electron acceptor
e-
electron transport chain
Sunlight
primary electron acceptor
e-
electron transport chain
carrier
carrier
Photosystem II
Photosystem I
electrons
H2O
Energy will generate ATP
2H+ + 1/2O2
Calvin Cycle

Calvin Cycle

- Calvin Cycle (light-independent) occur in the stroma:
  - **Carbon fixation**
  - Carbon dioxide is “fixed” into the sugar glucose.
  - **ATP** and **NADPH** molecules created during the light reactions power the production of this glucose.

Importance

2) Many – fix or convert atmospheric nitrogen into usable forms through Nitrogen Fixation when other forms are unavailable.

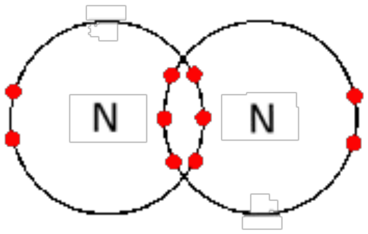
IMPORTANT because atmospheric N_2 is unavailable to most living organisms because breaking the triple bond is difficult

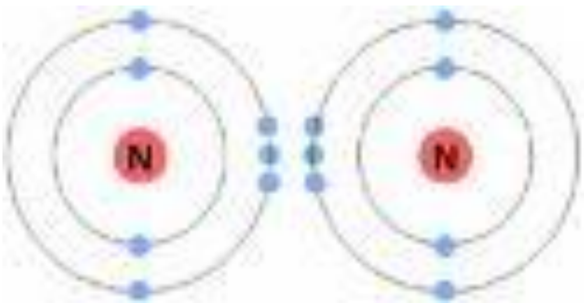
$$N \equiv N$$

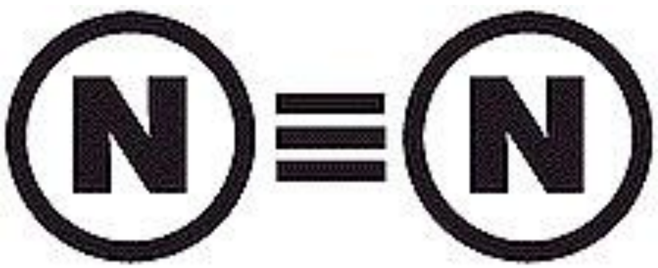
Nitrogen

- Nitrogen (N) is an essential component of DNA, RNA, and proteins, the building blocks of life.
- All organisms require nitrogen to live and grow.
- The majority (78%) of the Earth's atmosphere is N_2.

Nitrogen's triple bond





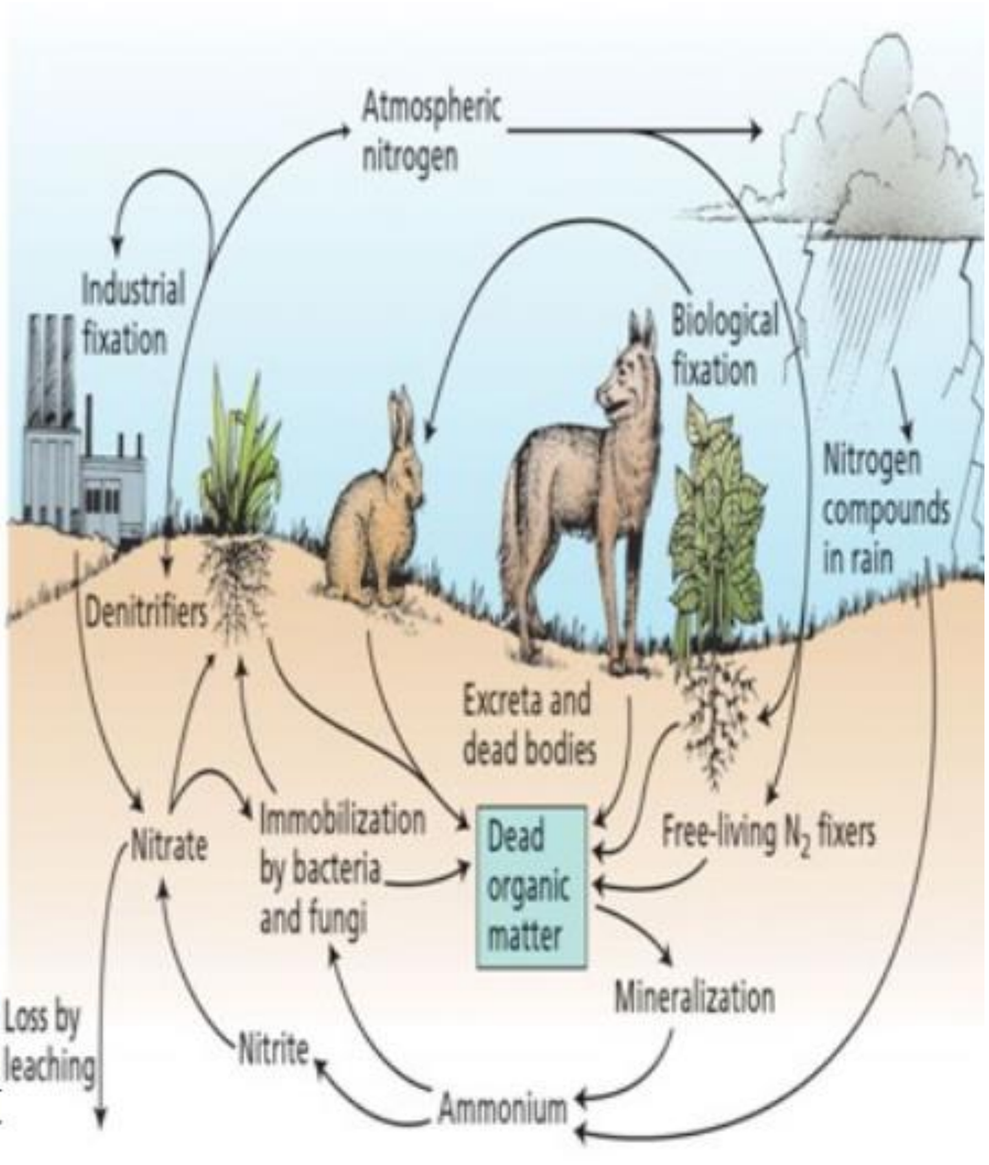


- Although the majority of the air we breathe is N_2, most of the nitrogen in the atmosphere is **unavailable** for use by organisms.

- This is because the strong **triple bond** between the N atoms in N_2 molecules makes it relatively inert (like a noble gas).

NITROGEN IN THE ENVIRONMENT

- Present in many forms
- 78% of atmosphere is N_2
  - Most of this is NOT available to living organisms
- Getting N_2 for the atmosphere requires breaking the triple bond between N_2 gas to produce:
- Ammonia (NH_3)
- Nitrate (NO_3^-)
- So, N_2 has to be ***fixed*** from the atmosphere so plants can use it



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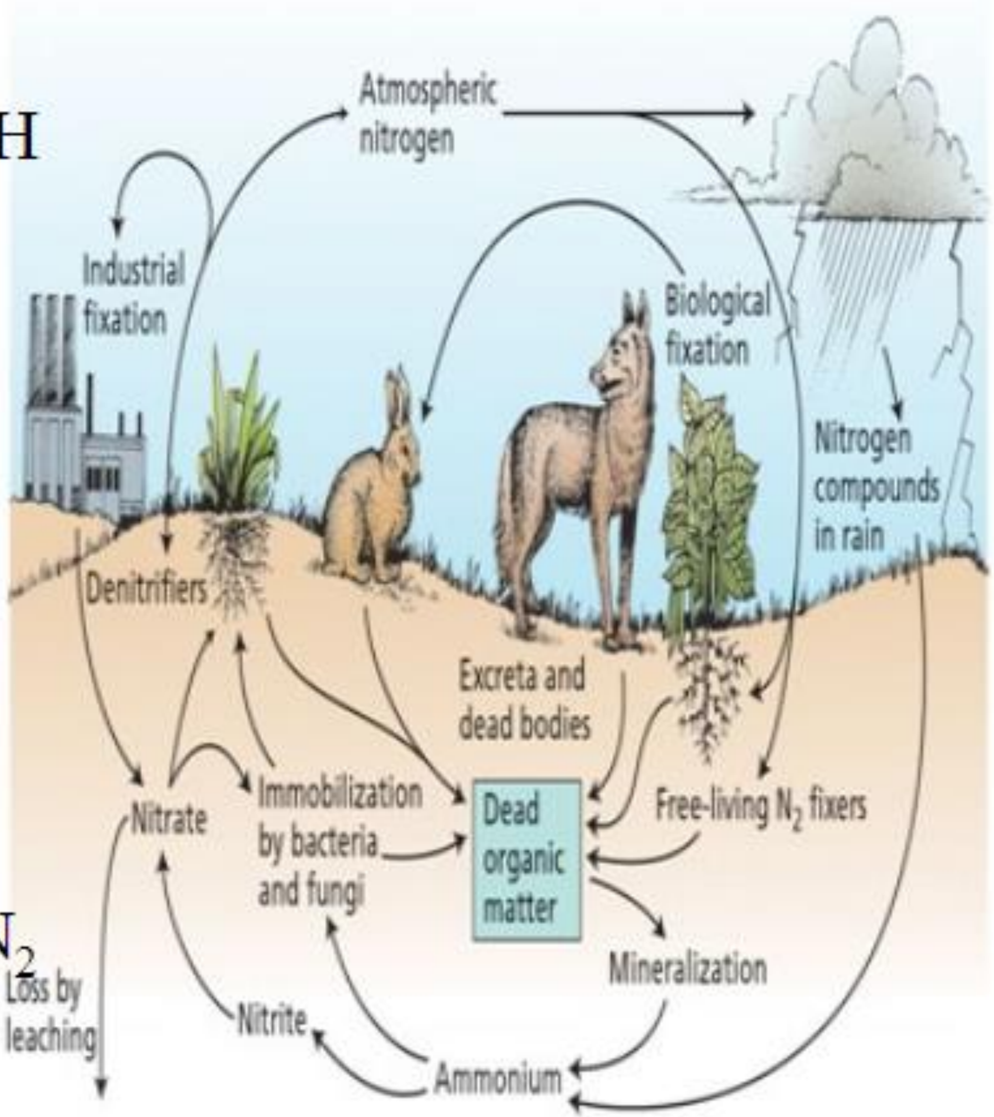
NITROGEN IN THE ENVIRONMENT

- This occurs naturally by:-
  Lightning :
  - **8%:** splits H_2O: the free O and H attack N_2 – forms HNO_3 (nitric acid) which fall to ground with rain
- *Photochemical reactions* :
  - **2%:** photochemical reactions between NO gas and O_3 to give HNO_3
- *Nitrogen fixation* :
  - **90%:** biological – bacteria fix N_2 to ammonium (NH_4^+)



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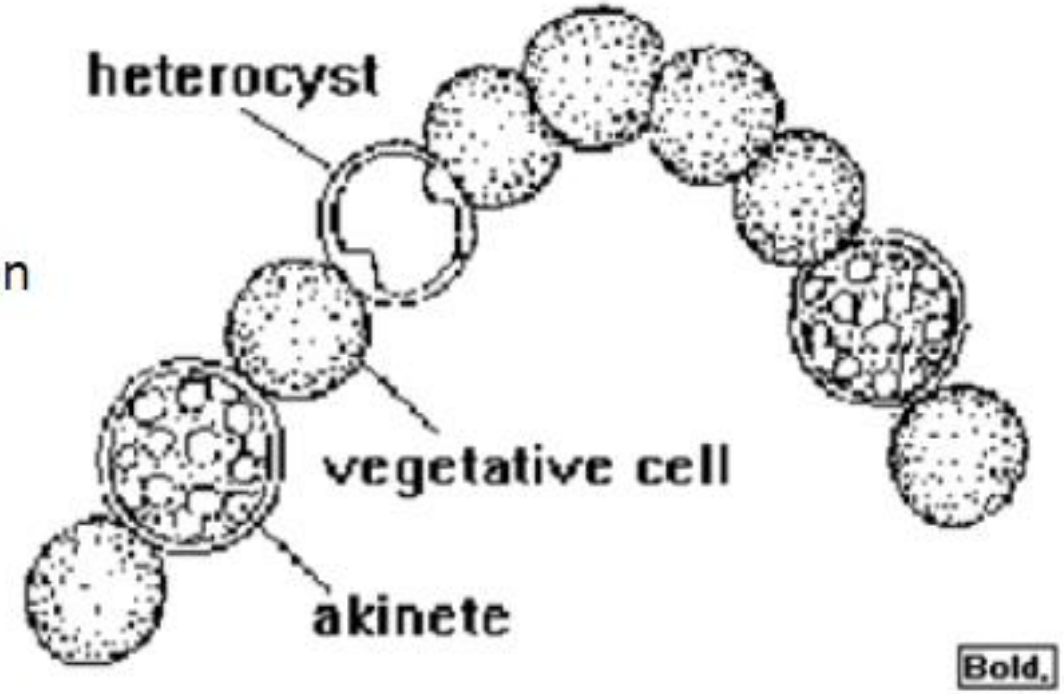
Nitrogen Fixation Requires Anaerobic Conditions

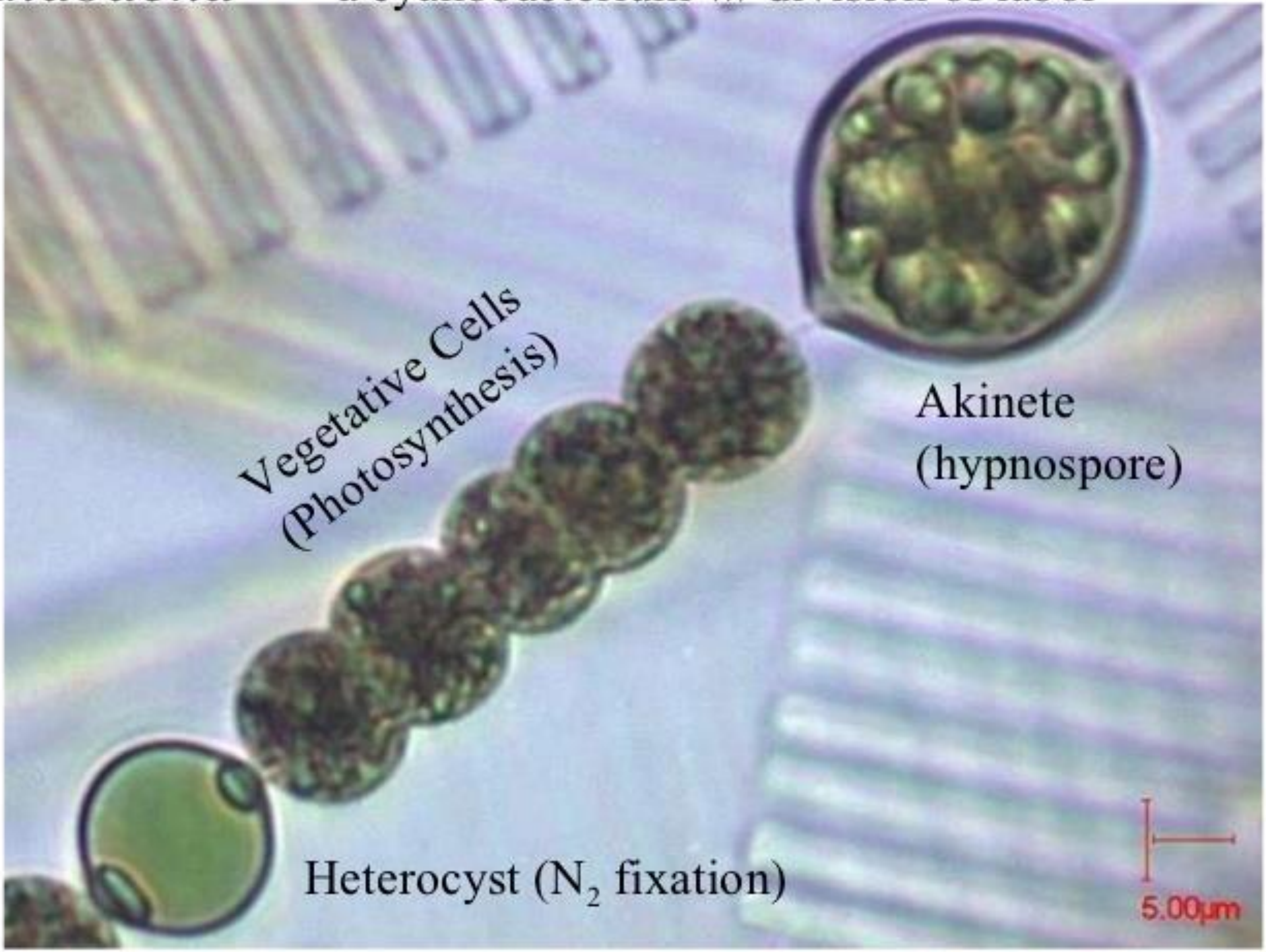
- In cyanobacteria, anaerobic conditions are created in specialized cells called ***heterocysts***
  - ***These are thick-walled cells which lack photosystem II—the oxygen producing photosystem of chloroplasts***
- Cyanobacteria can fix nitrogen under anarobic conditions such as those that occur in flooded fields
  - In Asian countries, nitrogen fixing cyanobacteria of both the heterocyst and non-heterocyst types are the major means of maintaining an adequate nitrogen supply in rice fields
    - ***They fix nitrogen when the fields are flooded, and die as the fields dry, releasing the fixed nitrogen into the soil***

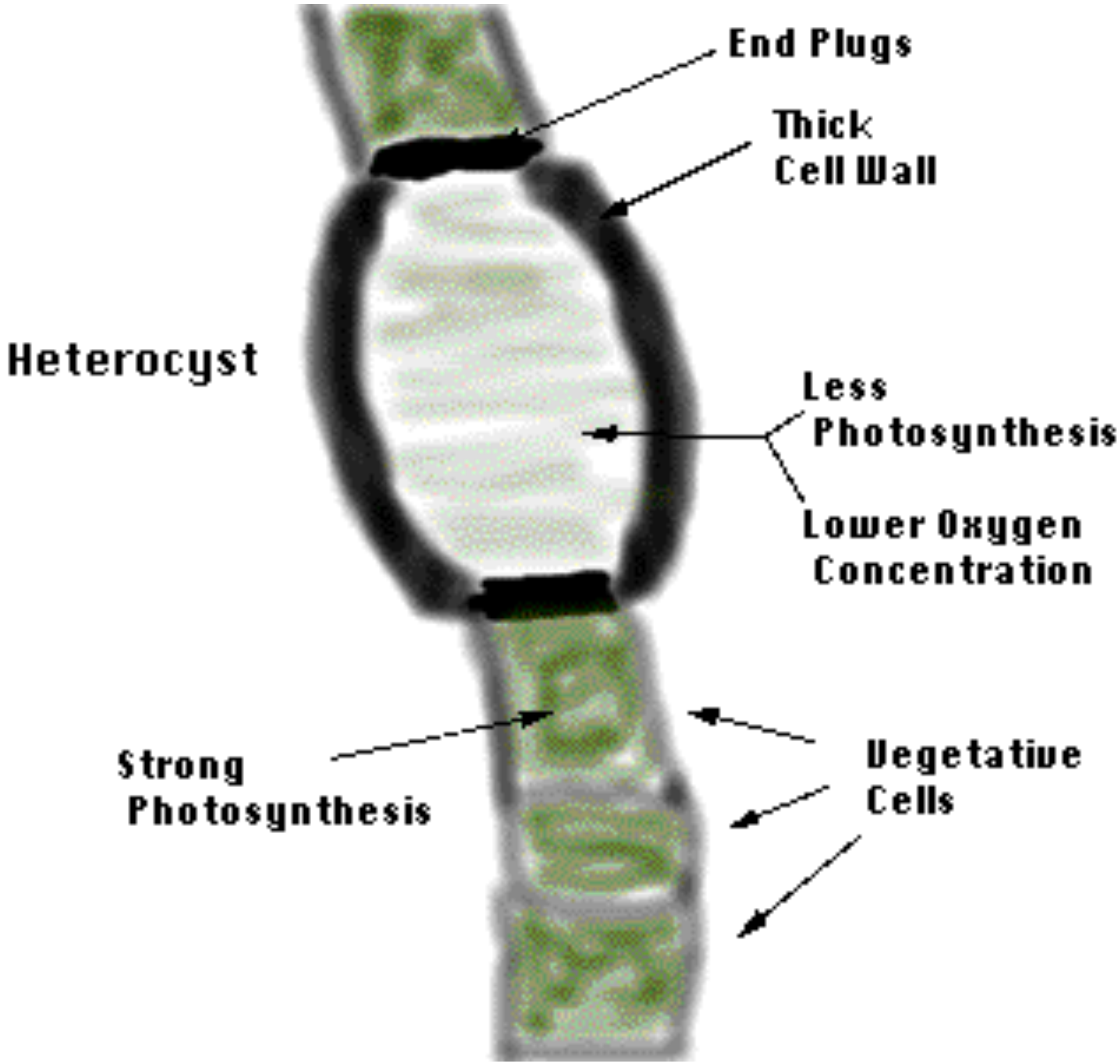
Anabaena (Cyanobacteria) 1

- Vegetative cell
  - Primary metabolic function (photosynthesis)
- Heterocyst
  - Nitrogen fixation
- Akinete
  - Dormant spore forming cell



Anabaena --a cyanobacterium w/ division of labor




End Plugs
Thick Cell Wall
Heterocyst
Less Photosynthesis
Lower Oxygen Concentration
Strong Photosynthesis
Vegetative Cells

Heterocyst

- Larger than vegetative cells
- Hollow looking
- Thick walled – doesn't allow atmospheric gas to enter.
- Photosynthetically inactive
- No CO_2 fixation or O_2 evolution
- Formation of heterocysts triggered by [molybdenum] and and low [nitrogen]

Nitrogen Fixation

- ONLY cyanobacteria and prokaryotic bacteria can FIX nitrogen.
- Of these two only CYANOBACTERIA evolve OXYGEN during photosynthesis
- Important because nitrogenase (enzyme involved in fixing nitrogen) is INACTIVATED by O_2.

Mechanisms to Separate Nitrogenase from Oxygen

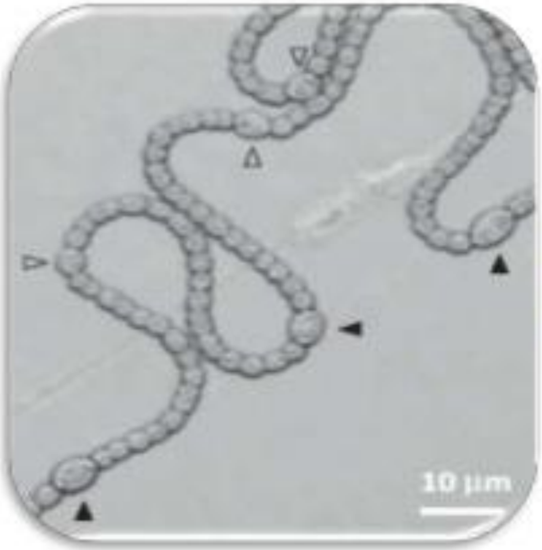
- Heterocyst (spatial)

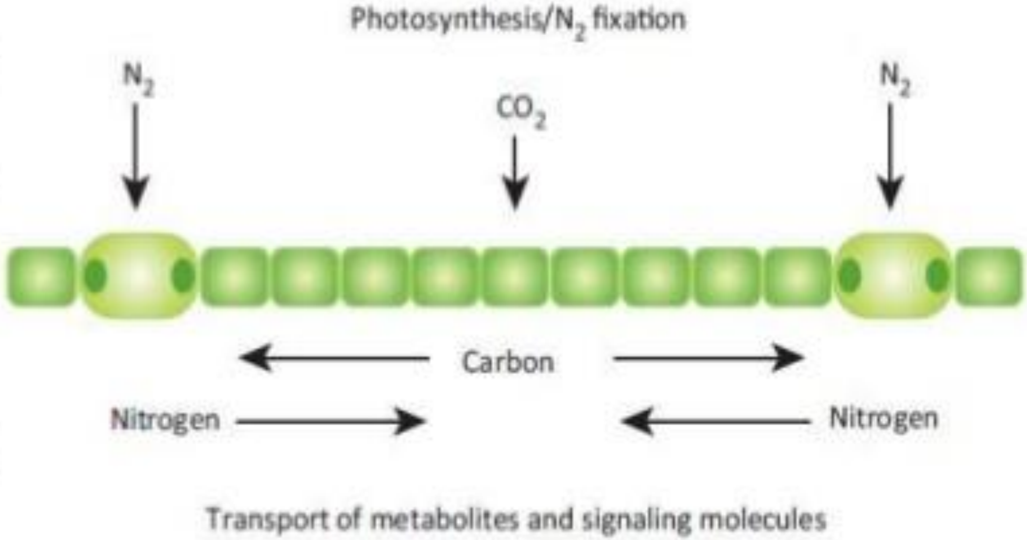
OR

- Fix Nitrogen in the DARK but not LIGHT – found in non-heterocystic cyanobacteria (temporal)

HETEROCYST IN CYANOBACTERIA

- In the presence of a source of combined nitrogen such as nitrate or ammonium, *Anabaena* grows as long filaments containing hundreds of photosynthetic vegetative cells.
- In the absence of combined nitrogen, it produces heterocysts every ten to twenty vegetative cells along filaments.





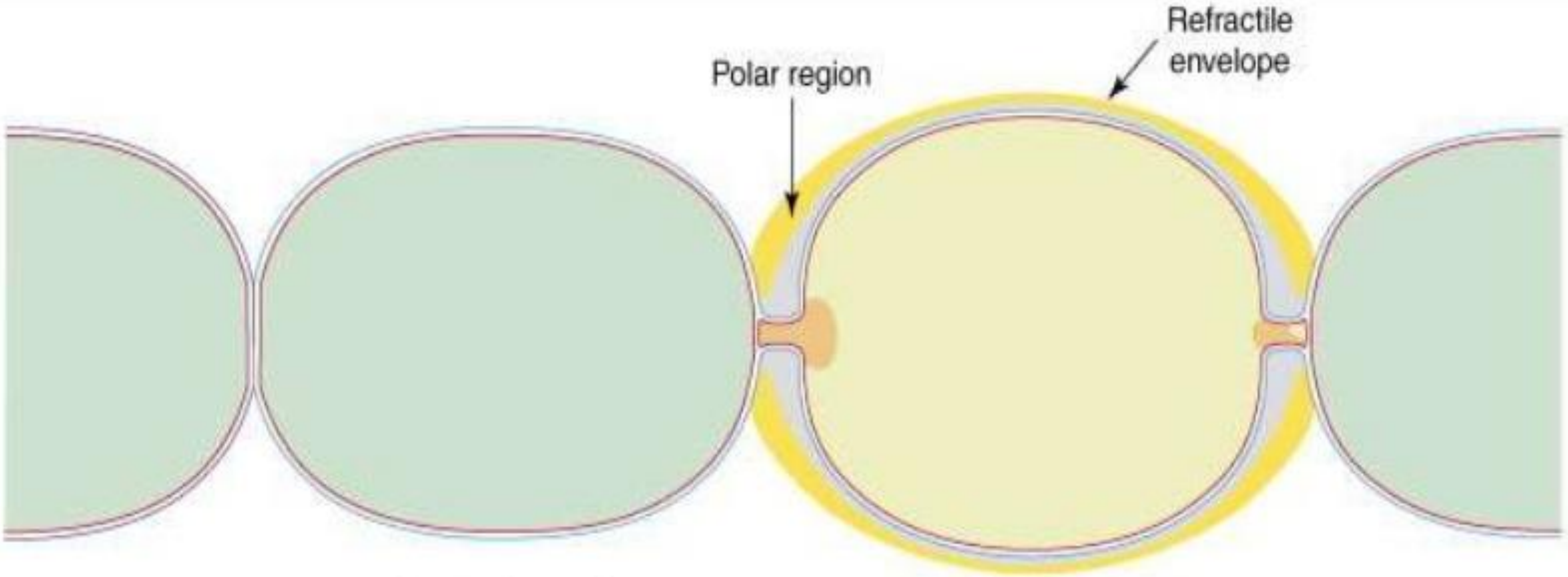
HETEROCYST IN ANABAENA

- Oxygen-producing photosystem PSII is dismantled during differentiation and heterocysts devlopement.

- Morphological changes include the deposition of two additional envelope layers around the heterocyst: an inner "laminated" layer composed of two heterocyst specific glycolipids (HGL)and an outer polysaccharide layer (HEP).

- Heterocysts and vegetative cells are mutually interdependent. Because they lack photosystem II and carbon fixation, heterocysts are dependent on vegetative cells for a source of reductant and carbon, which is probably partially supplied as sucrose.

HETEROCYST IN ANABAENA



Vegetative cell

Photolysis of water

$2H_2O \rightarrow 4H^+ + 4e^- + O_2$

Carbon dioxide fixation

$CO_2 + 4H^+ + 4e^- \rightarrow [CH_2O] + H_2O$

Heterocyst

Nitrogen fixation

$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$

Hydrogen uptake

$2H_2 + O_2 \rightarrow 2H_2O$

Both cell types

Carbohydrate oxidation

$[CH_2O] + H_2O \rightarrow CO_2 + 4H^+ + 4e^-$

Respiration

$4H^+ + 4e^- + O_2 \rightarrow 2H_2O$

TRENDS in Microbiology

Akinetes

AKINETES

- **Akinetes**
  Most filamentous cyanobacteria develop perennating (dormant structures) in adverse condition. These structures are larger than the vegetative cells, are equipped with thick walls, and are called **akinetes** . When favourable conditions return, they germinate and produce new filaments.

Advantage for Cyanobacteria

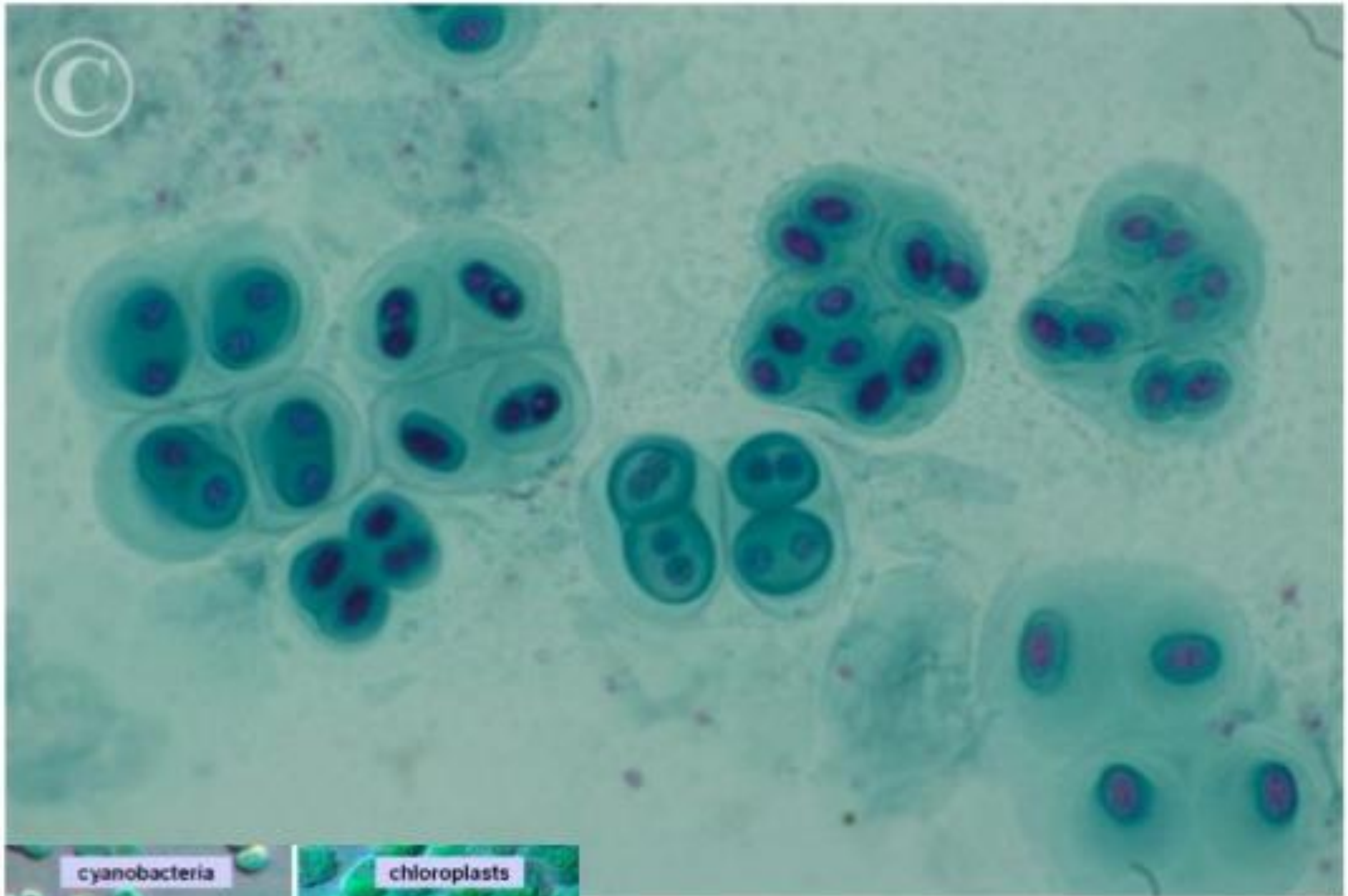
- **Can live in fluctuating environments of aerobic and anaerobic with light present.**
- Cyanobacteria can fix gaseous nitrogen, and are efficient at storing phosphorus. Buoyancy of this species varies due to the changing size of their internal pockets of gas. These alga cells can migrate in calm waters in response to nutrient or light gradients. Akinetes are capable of living in sediments for months and even years and then "seed" a water body. Optimal conditions for cyanobacteria growth are high temperature long sunny days, high levels of phosphorus and nitrogen, and calm winds which allow the cells to migrate to the surface. Reproduction takes place through trichome fragmentation, the splitting of the chain of cells, and is promoted by photosynthesis.

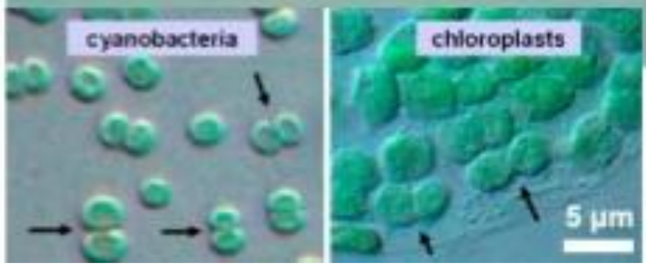
Asexual Reproduction

1. Binary Fission
2. Hormogonia formation
3. Akinete formation
4. Fragmentation
5. Endospore
6. Exospore

Binary fission

Cyanobacterial fission



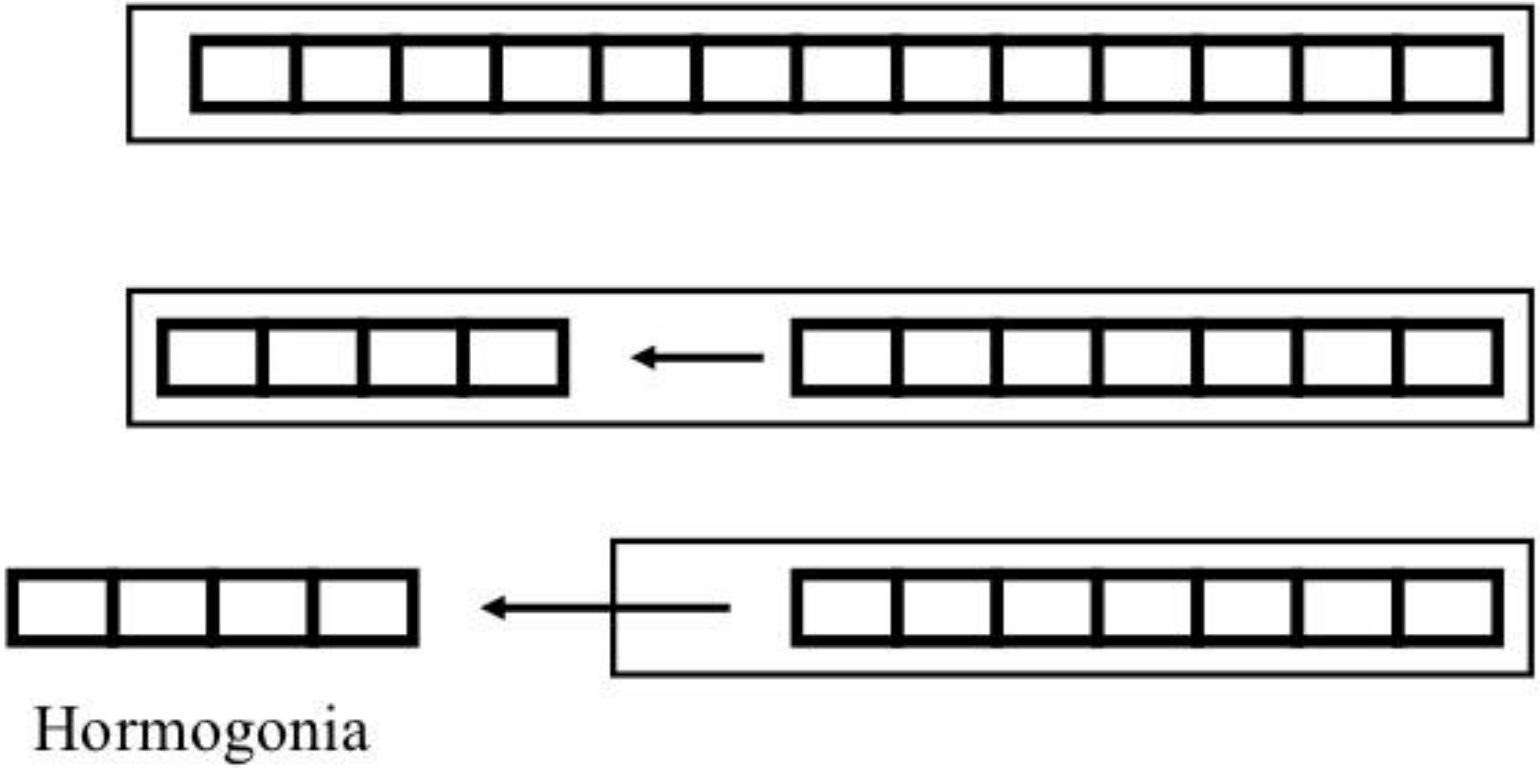


Binary fission of cyanobacteria

Gloeocapsa sp., algae. (Light micrograph of Gloeocapsa sp., a type of blue-green algae or cyanobacteria. They reproduce solely by fission, a form of asexual division in which two identical algae are produced.

Asexual Reproduction

Hormogonia – short piece of trichome found in filaments. It detaches from parent filament and glides away



Hormogonia

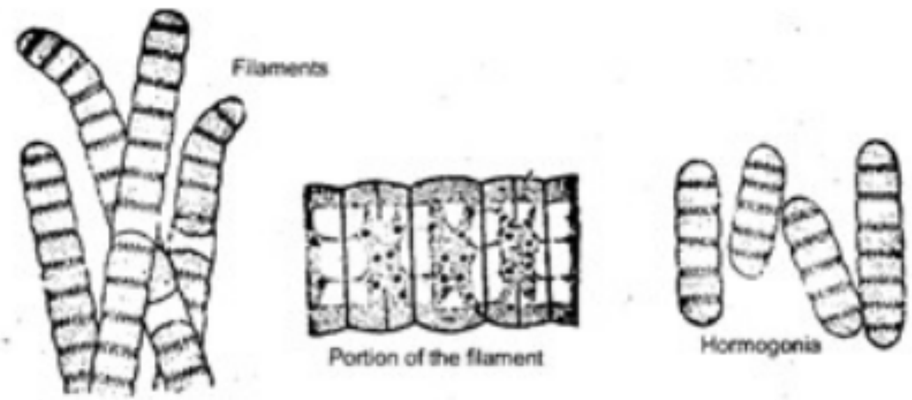


Fig. 2.13. Oscillatoria shows hormogonia for reproduction.

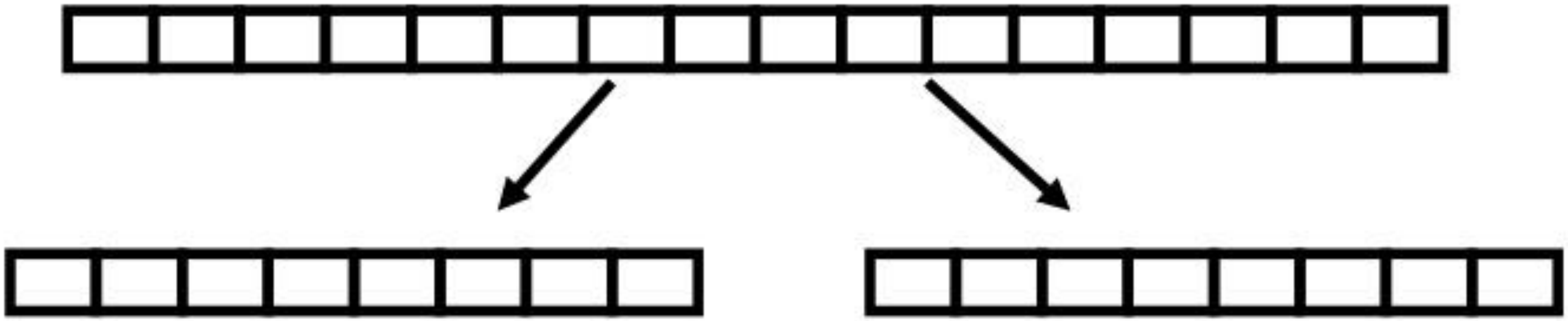
Q. 17. Draw a well labelled diagram to show E.M. of a cyanobacterial cell.

HORMOGONIA

- **Hormogonia**
  All filamentous Cyanobacterium reproduce by fragmentation of their filaments (trichomes) at more or less regular intervals to form short pieces each consisting of 5-15 cells. These short pieces of filaments are called **hormogonia.** The latter show gliding motility and develop into new full-fledged filaments

Asexual Reproduction

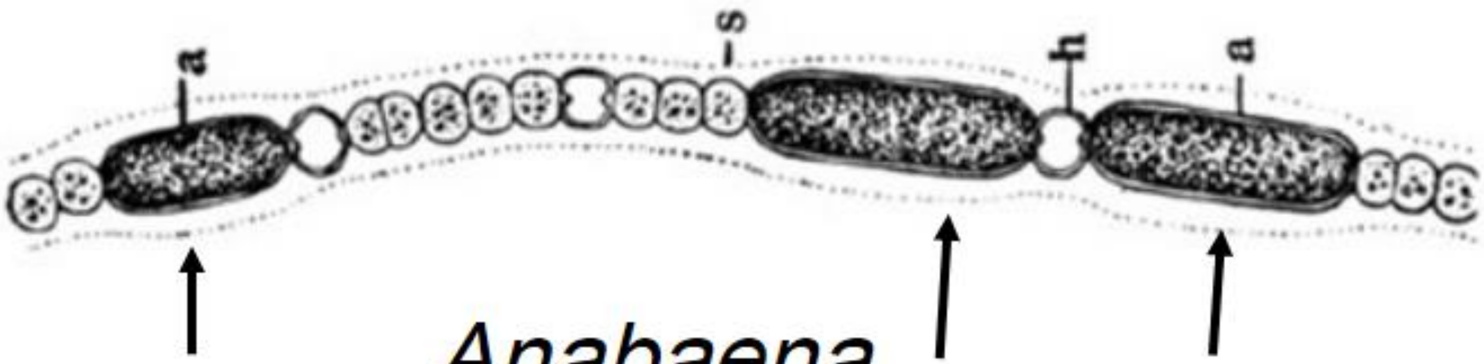
Fragmentation - fragmentation



Cyanobacteria: systematic characters

Vegetative reproduction:

<u>Akinetes</u>: resting cells with thick cell walls and enriched with storage products



Anabaena

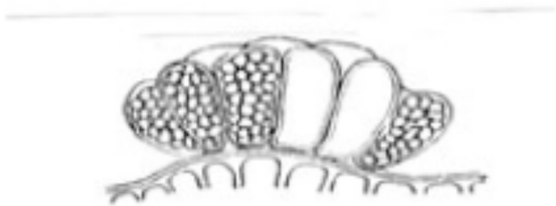
Akinetes may survive for years in darkness and under dry conditions

Cyanobacteria: systematic characters

Asexual reproduction:

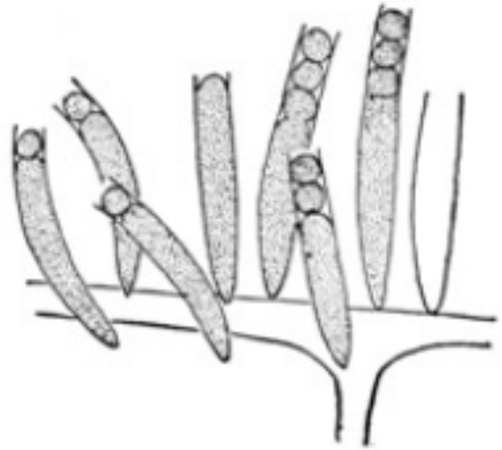
Formation of spores, only in some unicellular forms

endospores
(= baeocytes)



Dermocarpa

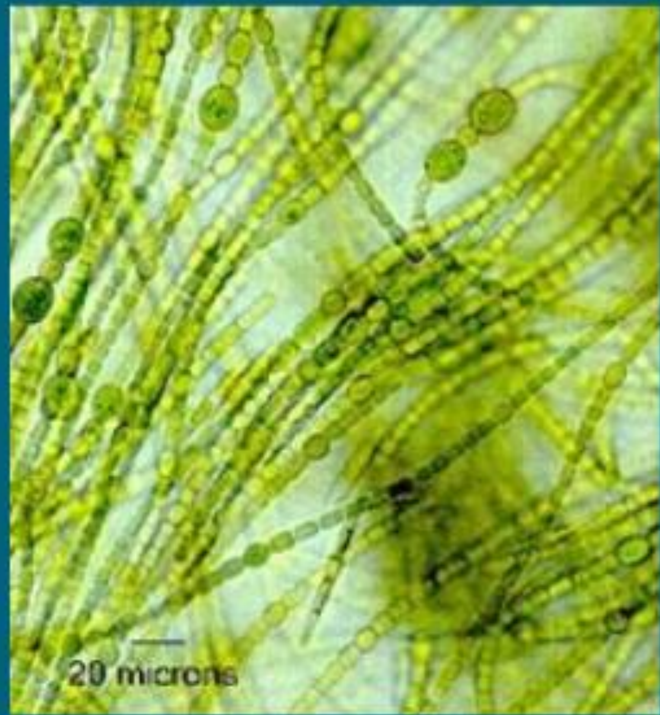
exospores



Chamaesiphon

Why They Thrive

- Nitrogen fixation – use atmospheric nitrogen
- Colony formation inhibits predation
- Carbon, phosphorus, nitrogen storage mechanisms
- Gas vesicles for vertical migration
- Akinete formation ("spores")
- Toxin production



Growth Requirements

- Sunlight
- Warm water (hot summer days)
- Calm, stagnant conditions
- Phosphorus

Potentially Harmful Algal Blooms

- When conditions are right, algae may form blooms, scums, or masses

What is a harmful algal bloom (HAB)?

• excessive growth of cyanobacteria aka blue-green algae – WHO, DOW and USACE recognize 100,000 cells/mL as cautionary level

• can potentially produce toxins capable of causing illness or irritation - sometimes death - in pets, livestock, and humans

• caused by a combination of environmental factors including abundant nutrients (N and P) warmer temperatures, lentic waters and sunlight

• nutrient sources include agricultural runoff, lawn fertilizers, sewage effluent and P weathered from the limestone bedrock here in the Bluegrass

Cyanobacterial Harmful Algal Blooms (CyanoHABs)

- Influential factors
  - Increased water temperature (> 25C)
  - Light intensity
  - Long hydraulic retention time (>1 month)
  - Droughts (change in water chemistry)
  - Nutrient enrichment
  - Opportunistic changes in habitat, esp. competing bacteria or phytoplankton (e.g., Asiatic mussels in Great Lakes)
  - Contaminants from effluent & storm water
  - Agricultural run-off (especially N and P)
- Growth varies temporally- hourly to daily to seasonally & longer
- Challenging to predict spatially
  - Influenced by geology, topography, weather, etc.
  - Concentration may be stratified and subject to rapid change (e.g., by wind)

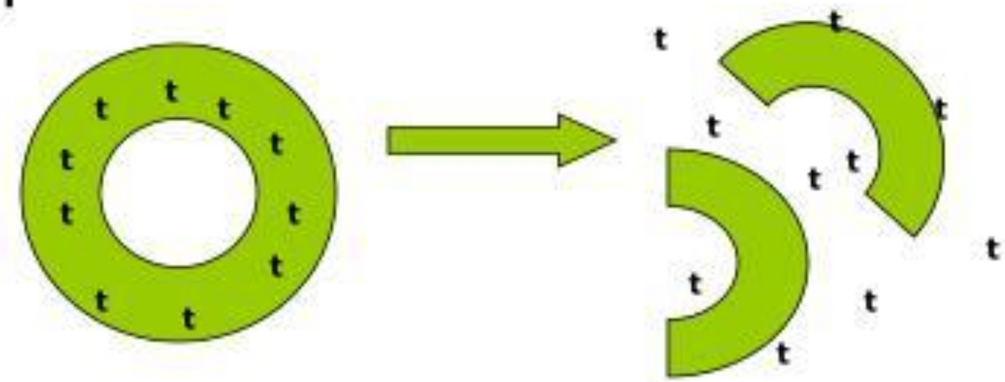
Microcystis sp. bloom

Problems with Blooms

- Unsightly
- Taste Problems: algae can lead to bad tastes in the water including bitter, fishy, and sweet.
- Odor Problems: algae can cause water to smell fishy, grassy, like geraniums, or musty.
- Fish Kills
- Toxins

Toxins

- Cyanobacteria produce biotoxins
  - Biotoxins are any toxins produced by a living organism (plant, fungi, animal, bacterium)
- The toxins produced by cyanobacteria are collectively referred to as cyanotoxins
- Toxins are stored in cells and released upon cell lysis or death



Toxins

Common Cyanotoxins in New Hampshire

Toxin	Target Organ	Cyanobacteria
Microcystin (Hepatotoxin)	Liver	*Microcystis* *Oscillatoria* *Anabaena*
Anatoxin (Nuerotoxin)	Nerve synapses	*Anabaena* *Oscillatoria*
Saxitoxin (Neurotoxin)	Nerve axons	*Aphanizomenon* *Anabaena*
Dermatotoxin (Aplysiatoxin)	Skin	*Lyngbya* *Oscillatoria*

Toxins

- Lethal dose (LD-50) of microcystin = 25 - 150 μg/kg of body weight **(0.025 - 0.150 mg/kg)**
- Compared with some of the most venomous snakes in the world

Common Name	LD-50 (mg/kg)
Coastal Taipan	0.009
Field's Horned Viper	**0.02**
Many Banded Krait	0.08
Indian Krait	0.089
Beaked Sea Snake	0.107

Note: this comparison based on route of exposure (intraperitoneal). LD-50 can differ among different exposure routes

Toxins

Human Effects

ACUTE

- Gastrointestinal effects
  - Nausea, vomiting, diarrhea
- Hepatic illness (liver)
- Dermatitis
- Ear and mouth irritation

CHRONIC

- Liver damage
- Tumor growth