

Multicellular cyanobacteria,  
and the development of multicellular  
organisms in the planet

Mónica Vásquez

Laboratorio de Ecología Microbiana y Toxicología Ambiental



# Outline:

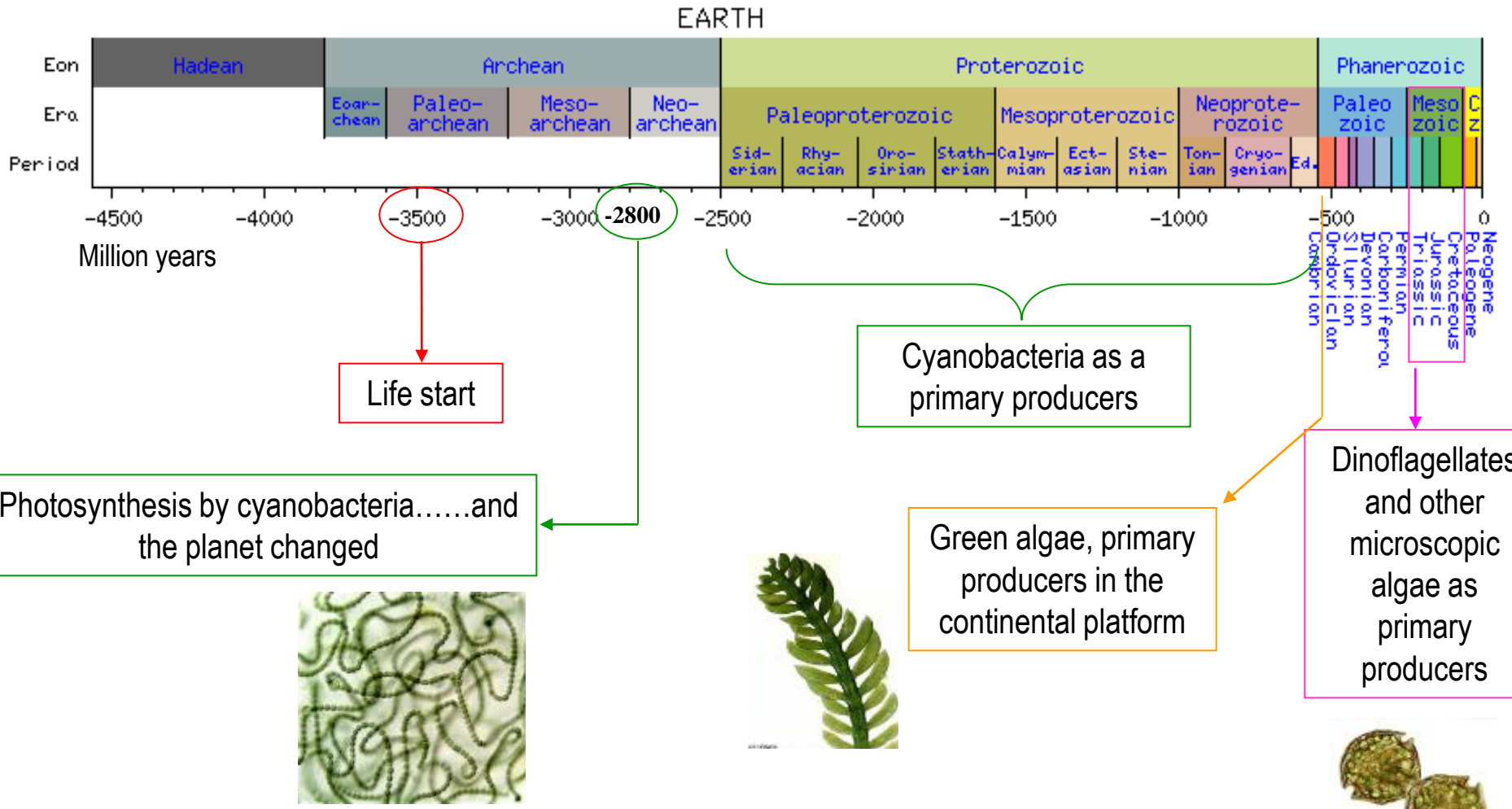
- Main role of cyanobacteria
- What do we know in cyanobacteria?
- Overview about cytoskeletal elements in bacteria
- Which is our model system?
- What do we know now with the whole genome analysis in our system?
- Preliminary results
- Next future

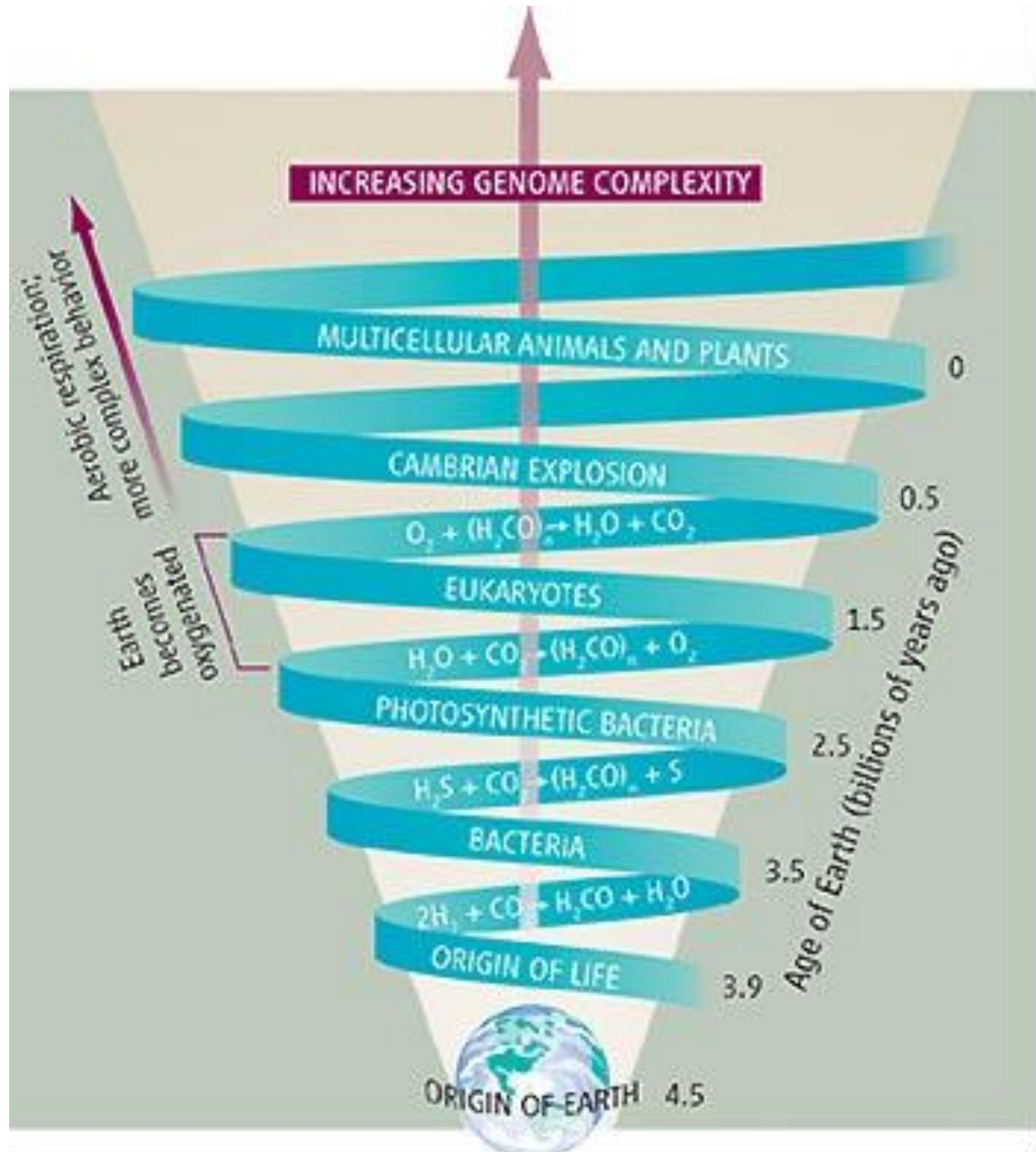
# What do we know about cyanobacteria?



# General characteristics of cyanobacteria

- Gram negative
- Symbionts or free living
- Photosynthesis
- Diazotrophy





# Oxygen sources

There are two sources for oxygen; biological and non-biological.

Non-biological: It is a process called **photodissociation**, in which ultraviolet rays break apart water molecules separating the oxygen from the hydrogen.

Studies have shown that the rate at which this could occur is the same rate at which hydrogen would escape the atmosphere .

**However, the rate at which this would occur is hardly sufficient to produce the amount of oxygen present on earth (Des Marais).**

# The only other solution is biological.

Organic life itself creates oxygen. Cyanobacteria was the first photosynthesizer, and thus the first producer of oxygen.

Life came before oxygen, because life is the cause of oxygen.

As science writer David Biello writes, **“Climate, volcanism, plate tectonics all played a key role in regulating the oxygen level during various time periods. Yet no one has come up with a rock-solid test to determine the precise oxygen content of the atmosphere at any given time from the geologic record. But one thing is clear—the origins of oxygen in Earth’s atmosphere derive from one thing: life”**

# **BUT**

## **Oxygen and life have a catch 22 relationship**

Catch 22 is situation in which an action has consequences which make impossible to pursue that action.

Oxygen is very harmful to life. At the same time oxygen is needed to provide the ozone layer which protects life from ultraviolet radiation (UVR) coming from the sun.

If Cyanobacteria came before oxygen, because it is the cause of oxygen, then Cyanobacteria would have had to develop several forms of protection to mitigate the damage from UVR: avoidance, scavenging, screening, repair, and programmed cell death.

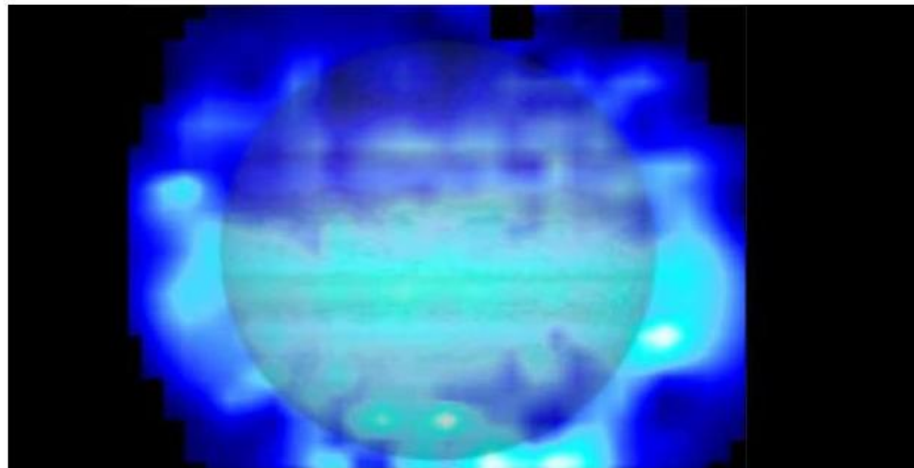
However, UVR damage is immediate and the time needed to “evolve” protection against it via natural selection, incredibly slow. So, UVR damage would occur before any such defense mechanisms could evolve.



## Nasa confirma que agua presente en Júpiter se originó de un cometa

De acuerdo a los expertos, el agua encontrada en su estratósfera no es originaria del planeta, sino que fue traída por un cometa que impactó con su superficie hace más de veinte años.

23/04/2013 - 17:15



LAS OPINIONES TIENEN UN PUNTO DE PARTIDA

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Los astrónomos finalmente han logrado encontrar una prueba directa de que **casi toda el agua presente en la estratósfera de Júpiter fue llevada allí por el cometa Shoemaker-Levy9**, el cual impactó al planeta en **1994**.

El descubrimiento, realizado con nuevos datos del **telescopio Herschel** de la Agencia Espacial Europea (ESA), reveló además la existencia de más agua en el hemisferio sur de Júpiter.



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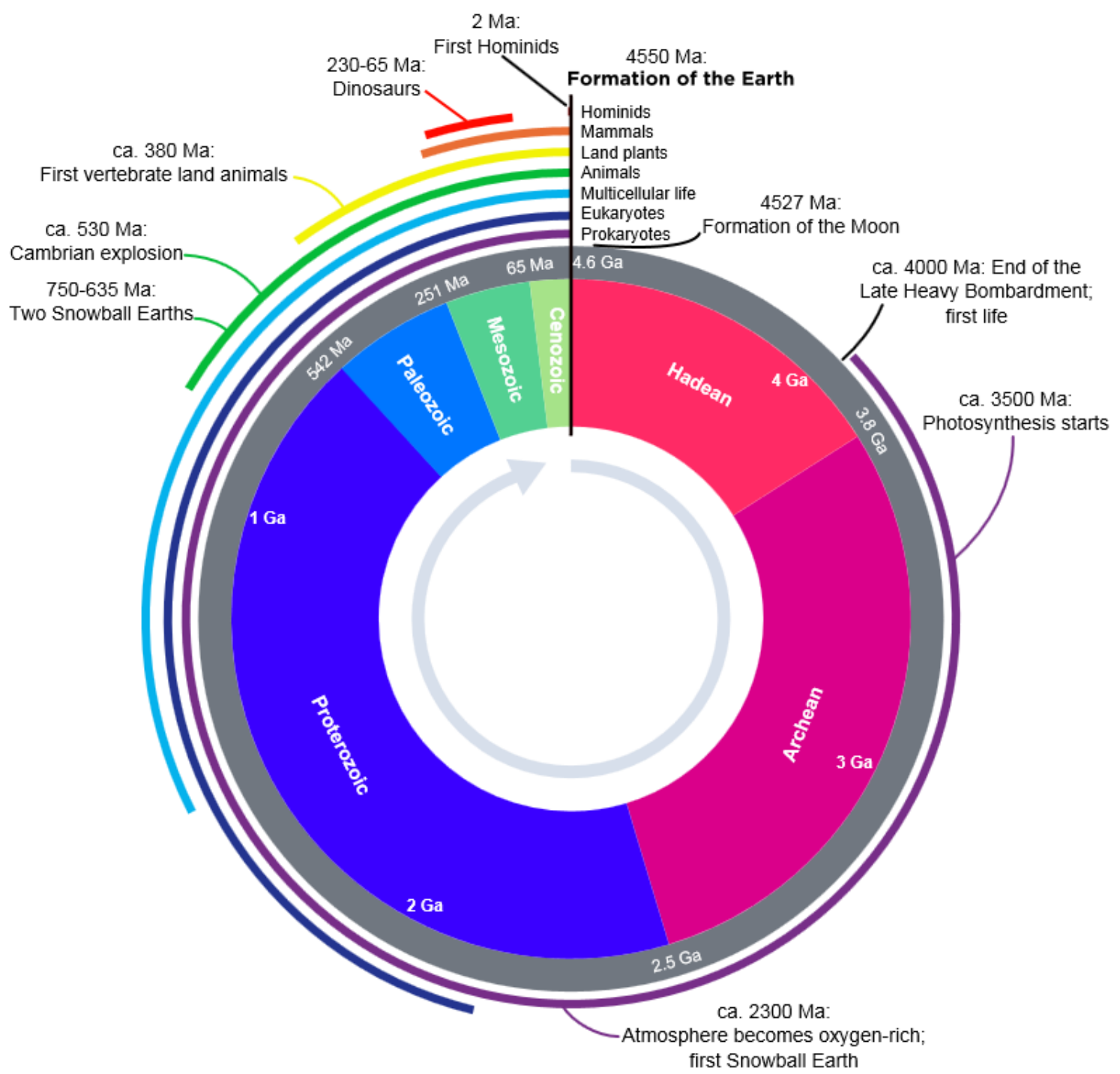
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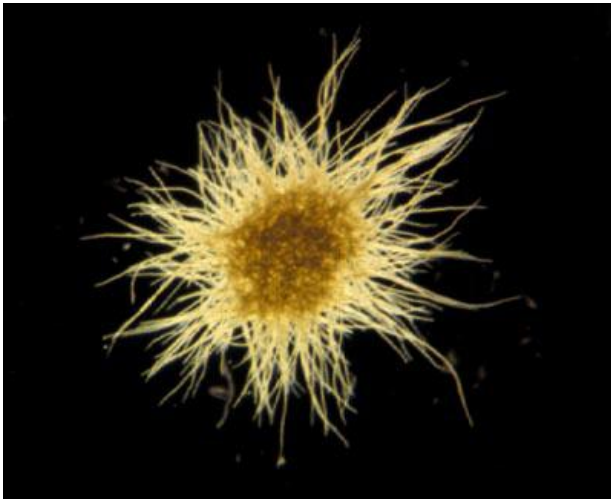
# Nitrogen fixation by cyanobacteria

Cyanobacteria inhabit nearly all illuminated environments on Earth and play **key roles** in the **carbon** and **nitrogen cycle** of the biosphere.

Several cyanobacterial strains are also capable of **diazotrophic growth**, an ability that may have been present in their last common ancestor in the Archaean.

Nitrogen fixation by cyanobacteria in coral reefs can fix twice the amount of nitrogen than on land—around **1.8 kg of nitrogen is fixed per hectare per day**.

The colonial marine cyanobacterium *Trichodesmium* is thought to fix nitrogen on such a scale that it accounts for almost half of the nitrogen-fixation in marine systems on a global scale.



[http://cmore.soest.hawaii.edu/education/kidskorner/images/Trichodesmium\\_microscope\\_400px.jpg](http://cmore.soest.hawaii.edu/education/kidskorner/images/Trichodesmium_microscope_400px.jpg)

# Characteristics of cyanobacteria

How do we can study the ancient mechanisms of multicellular formation. Which model?

- Genomic model

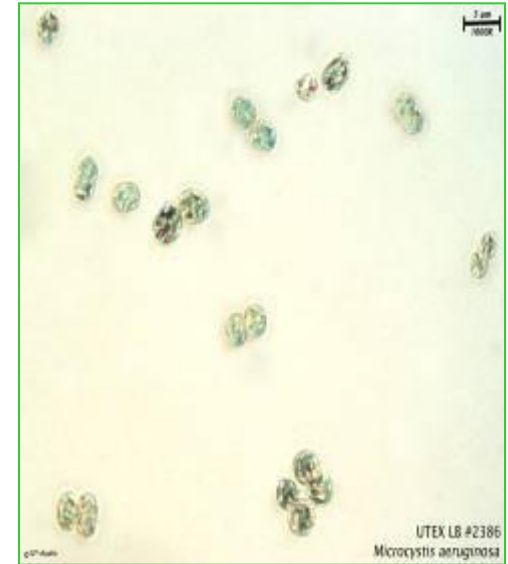
cyanobacteria DNA 1,7 – 9 Mpb

dinoflagellates or other eukaryotic microalgae DNA 3.000 – 215.000 Mpb

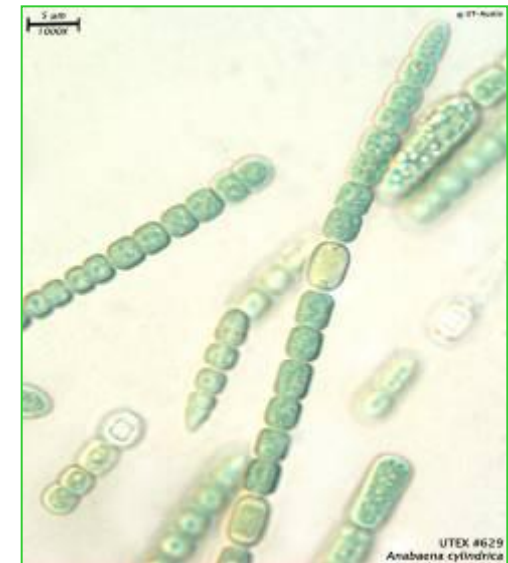
- Morphology

**Cell differentiation**

unicellular



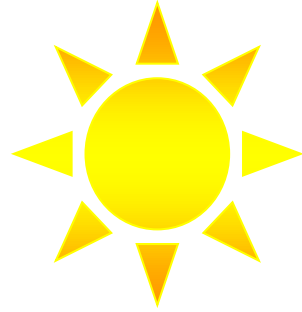
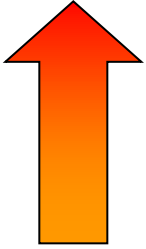
filamentous



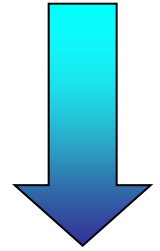


# Cyanobacterial Blooms

TEMPERATURE  
(21 °C – 27 °C)



NUTRIENTS



WIND



Chaohu Lake, China

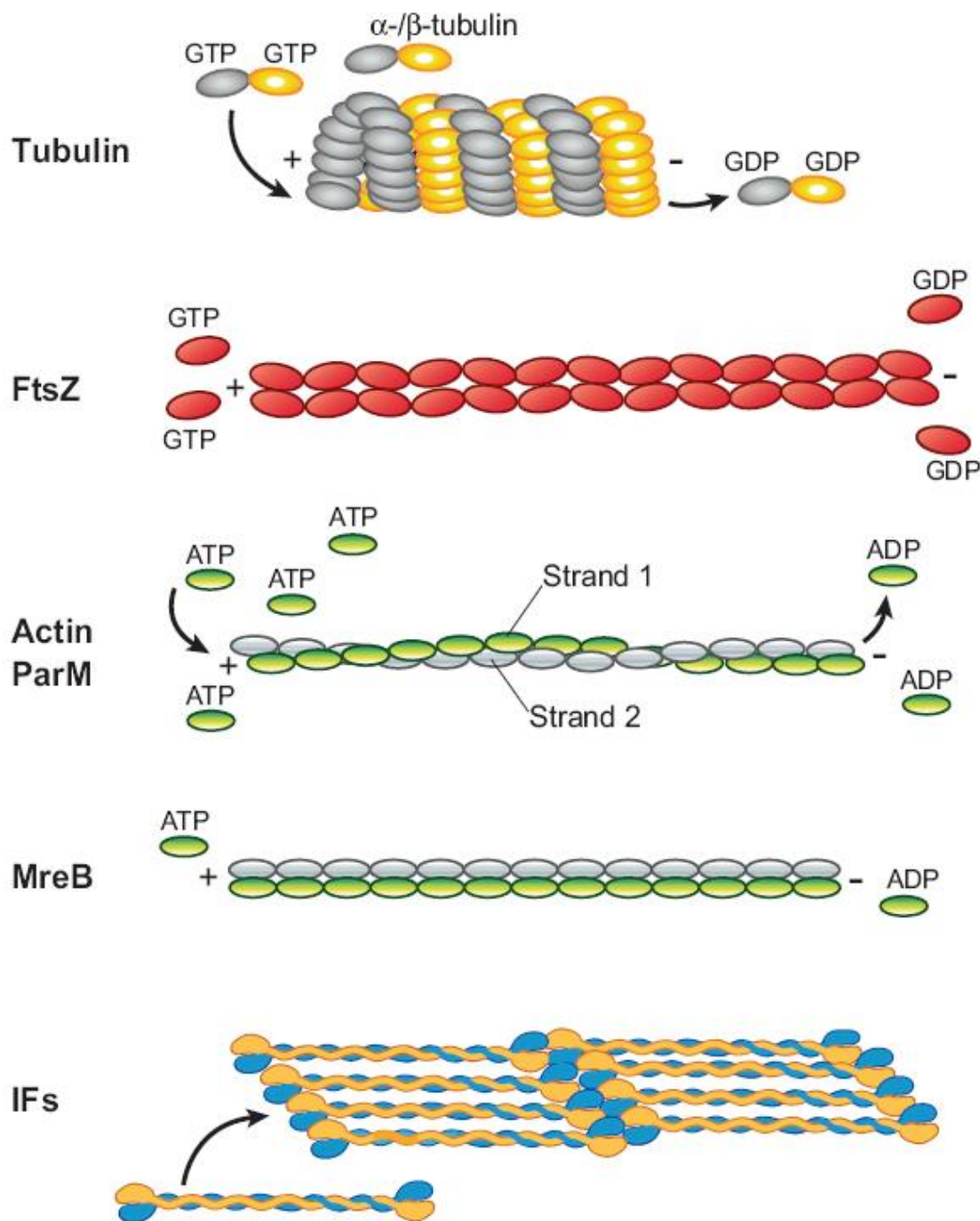
# **Why we focus on cyanobacteria to study multicellular development?**

**All cytoskeletal elements known from eukaryotic cells are also present in bacteria, where they perform vital tasks in many aspects of the physiology of the cell.**

# Which are the main elements?

Bacterial tubulin (**FtsZ**), actin (**MreB**), and intermediate filament (**IF**) **proteins** are key elements in:

- cell division
- chromosome and plasmid segregation
- maintenance of proper cell shape
- maintenance of cell polarity
- assembly of intracellular organelle-like structures.



Schematic drawing of cytoskeletal elements in eukaryotes (**tubulin, actin, and IFs**) and in bacteria (FtsZ, with putative protofilament structure, ParA, MreB, and IFs).

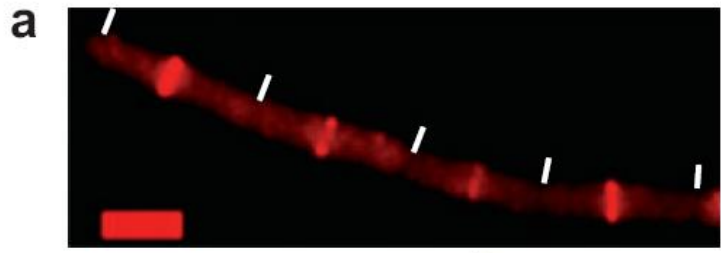
IFs, intermediate filaments. Both actin and MreB filaments (*green* and *gray*) are composed of identical subunits.

Figure 1

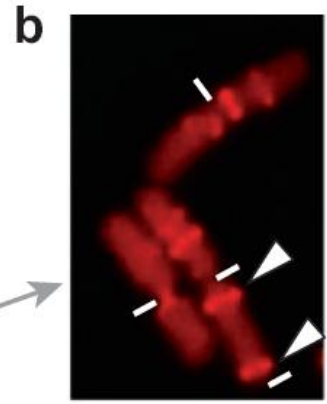


# Fluorescence microscopy of cytoskeletal elements in bacteria

(a) **FtsZ** forms a ring at the middle of the cell (*Bacillus subtilis* cells expressing FtsZ-CFP), initiating division.

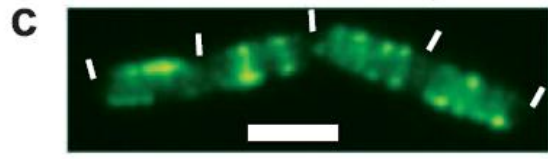


FtsZ-CFP  
2 μm



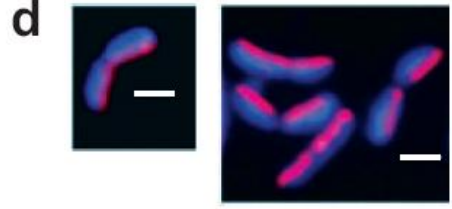
FtsZ-CFP

(b) **FtsZ** switches its position during differentiation; *B. subtilis* cells express FtsZ-CFP at the onset of sporulation. White arrowheads indicate two polar Z rings. Note the spiral forms of FtsZ in several cells.



YFP-MreB

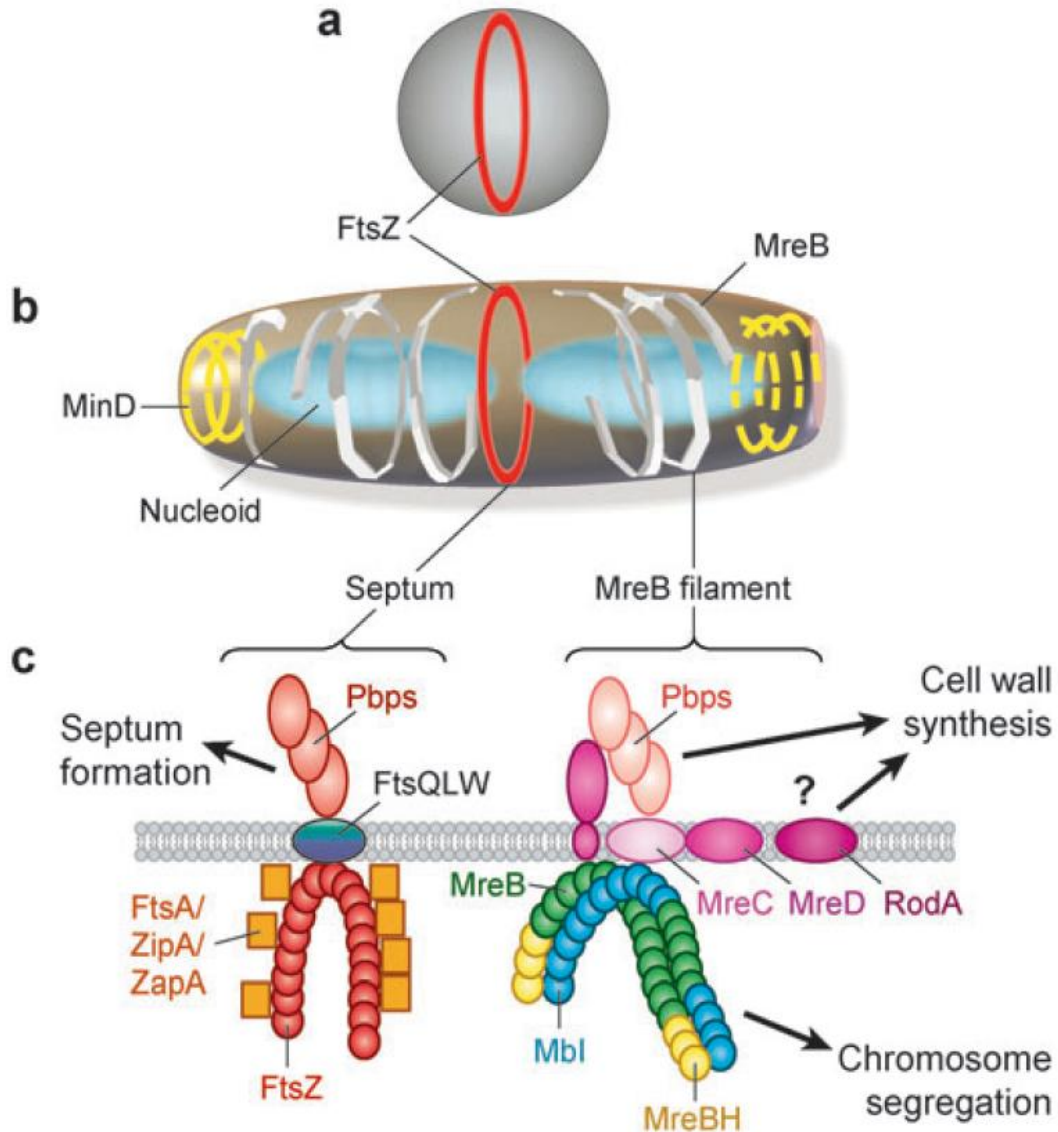
(c) **MreB** forms helical filaments underneath the cell membrane (*B. subtilis* cells expressing YFP-MreB).



Crescentin

(d) Crescentin localizes to the concave side of the bent *Caulobacter crescentus* cells (immunofluorescence with anti-crescentin antibodies; cells are stained with the blue DNA stain DAPI).

# Scheme of cytoskeletal elements in bacteria

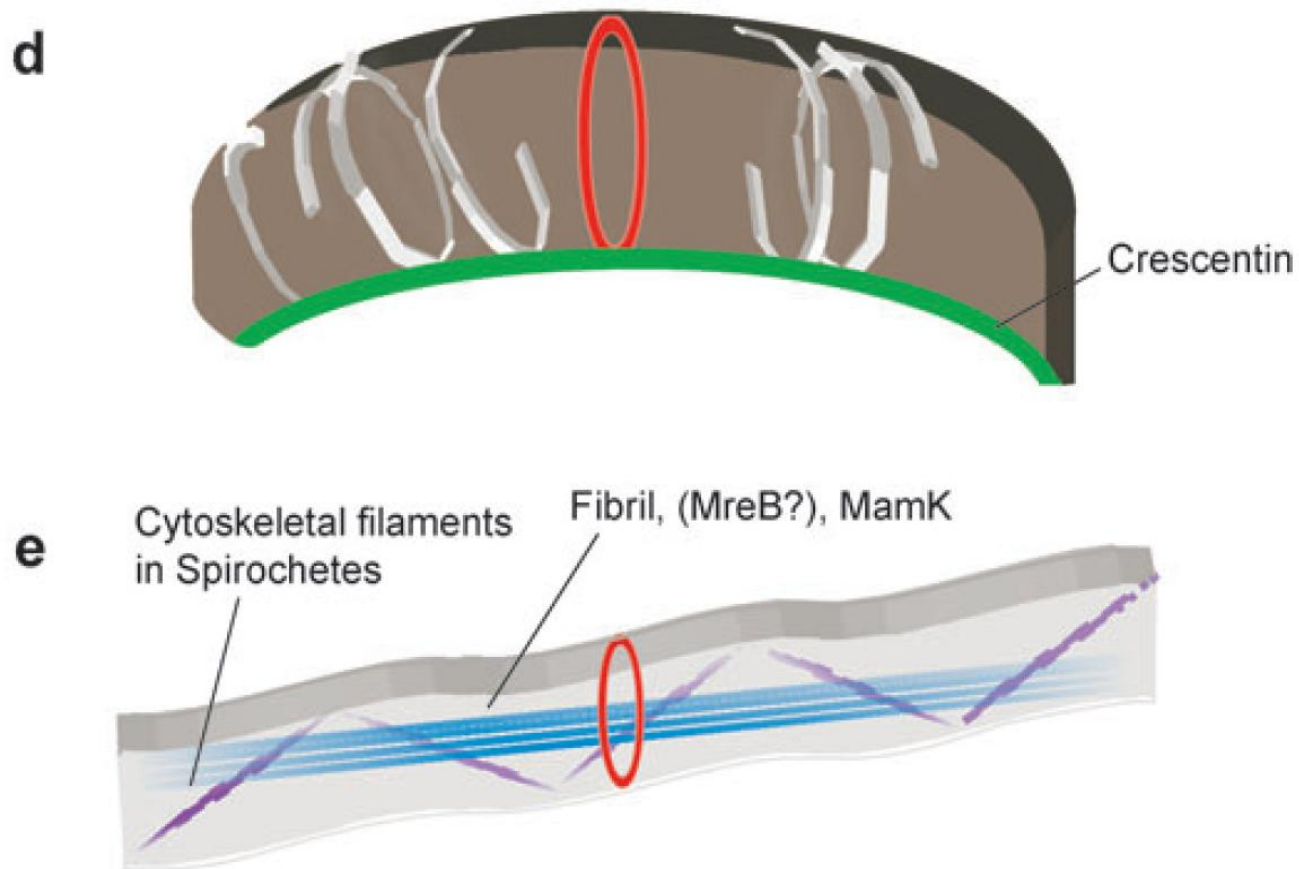


**(a) FtsZ** (and frequently also FtsA) forms a ring in the middle of coccid cells. In many cocci, division planes alternate in two or even three dimensions, giving rise to growth as tetrads or packets of cells, respectively.

**(b) FtsZ** forms a midcell ring in rod-shaped cells and recruits cytosolic division proteins and

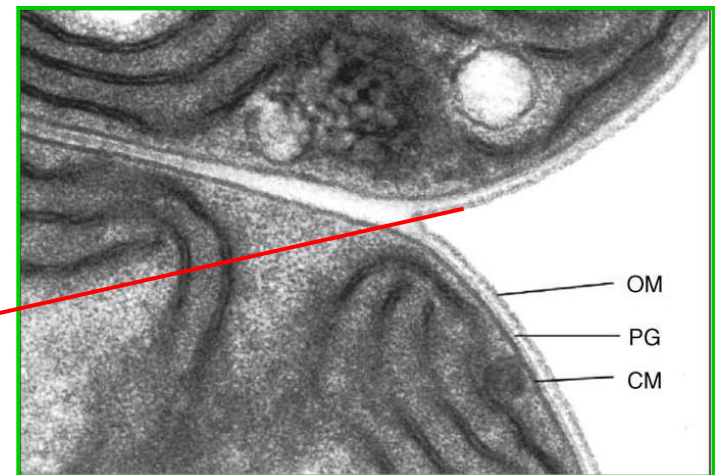
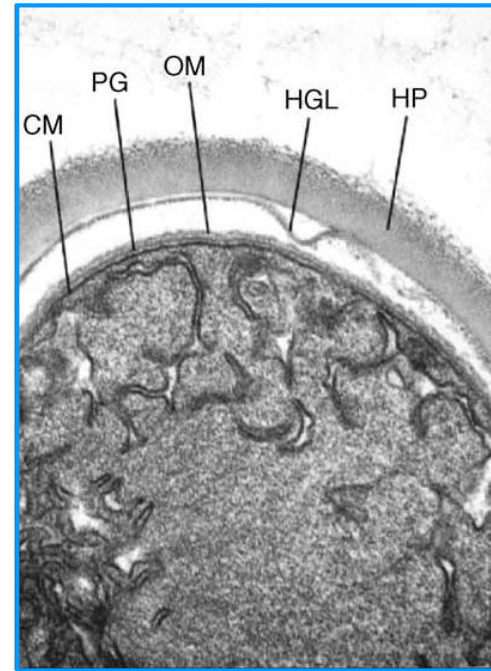
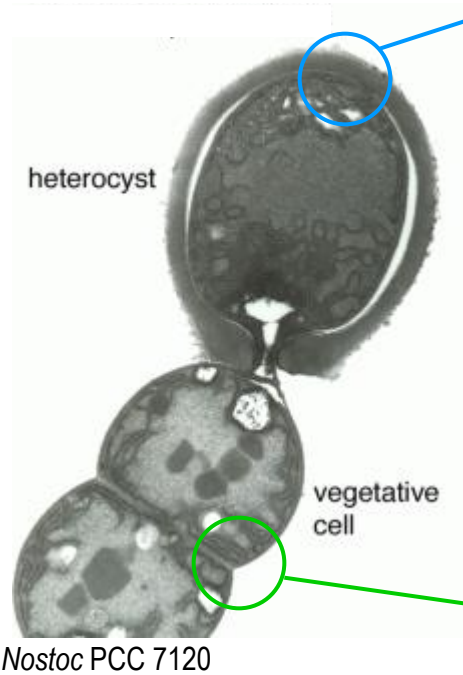
**(c)** membrane-bound division proteins, such that the division septum is synthesized by penicillin-binding proteins (Pbps). **MinD** forms spiral structures that are enriched at the cell poles, preventing assembly of Z rings. Nucleoids (which contain the chromosomes) prevent formation of Z rings, such that only the middle of the cell is competent for FtsZ polymerization after nucleoids have separated. MreB forms dynamic helical filaments that move underneath the cell membrane and affect chromosome segregation and maintenance of cell morphology.

**(c) MreB** proteins interact with membrane proteins (**MreC**) that affect cell morphology and in turn interact with **Pbps**.



(d) *C. crescentus* and (e) in spiral formed bacteria. Spirochetes contain cytoskeletal filaments along the long side of the cells, and fibril forms a ribbon-like structure along the short axis of cell wall-less *Spiroplasma* cells.

# Cellular differentiation in filamentous cyanobacteria



External membrane, continuous a long the filament



## FraC keep the filament integrity

*Nostoc* PCC 7120

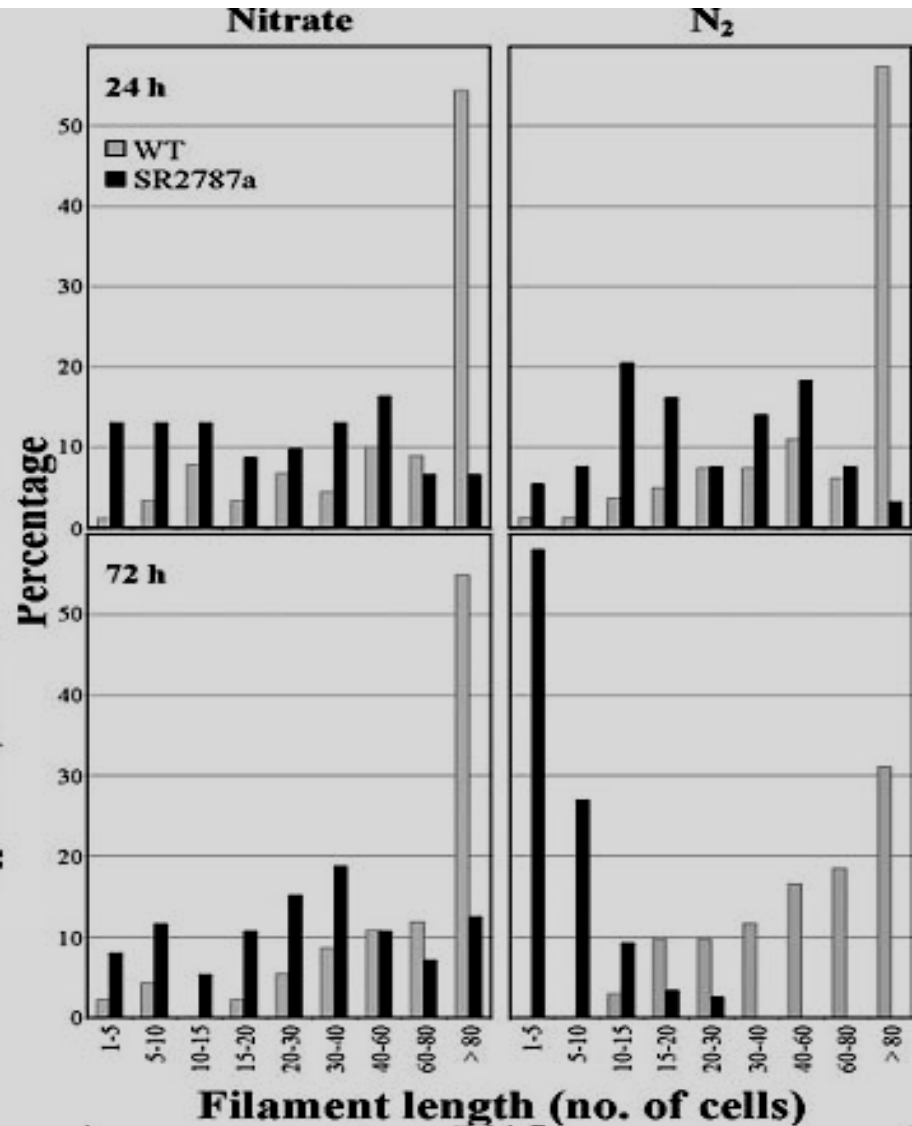
(Bauer *et al*, 1995)

$\Delta fraC$



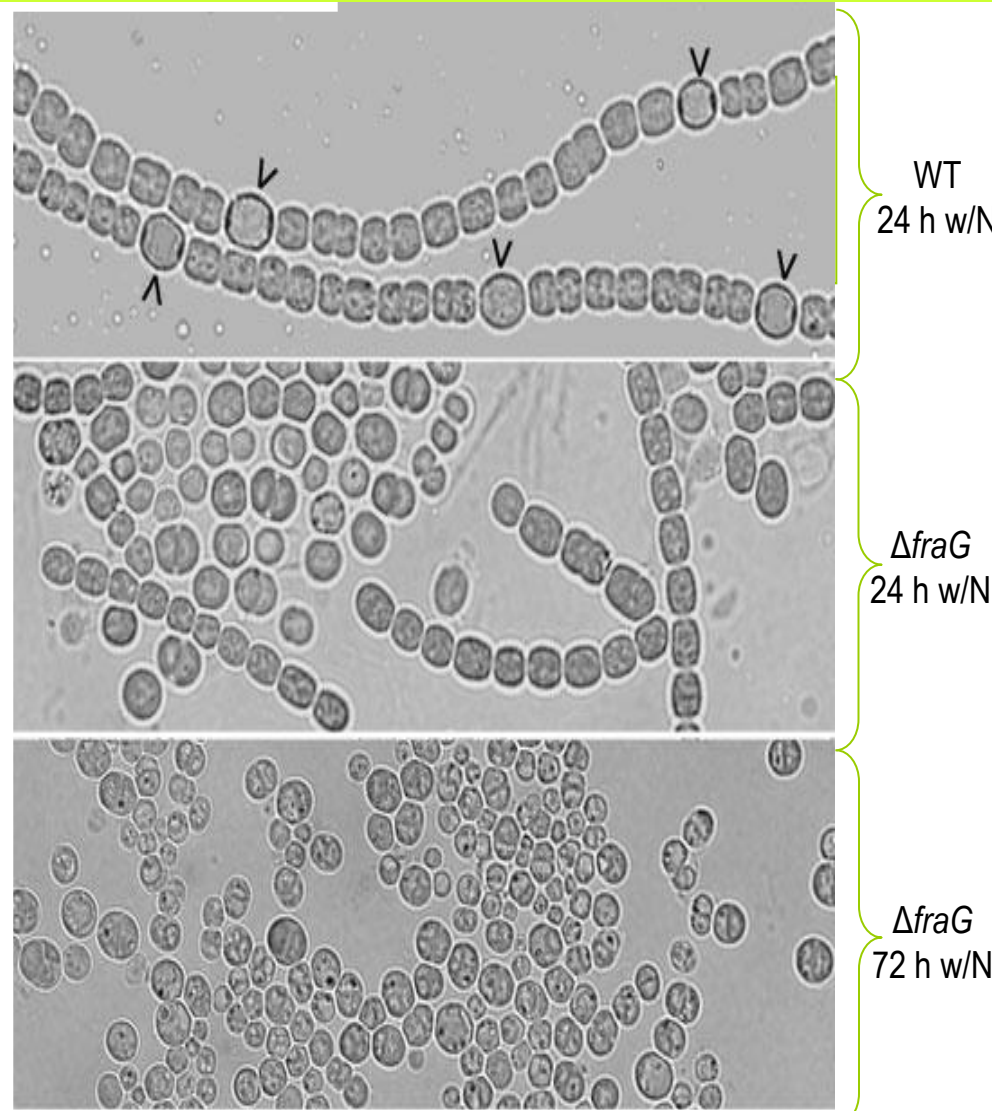
- FraC (179 aa), hydrophobic regions (3 transmembrane domains).
- $\Delta fraC$  present a small filament surrounded by polysaccharide.
- $\Delta fraC$  form heterocysts (Nitrogen fixation)

# FraG keep the filament integrity



*Nostoc* PCC 7120

(Flores *et al*, 2007)



(Nayar *et al*, 2007)

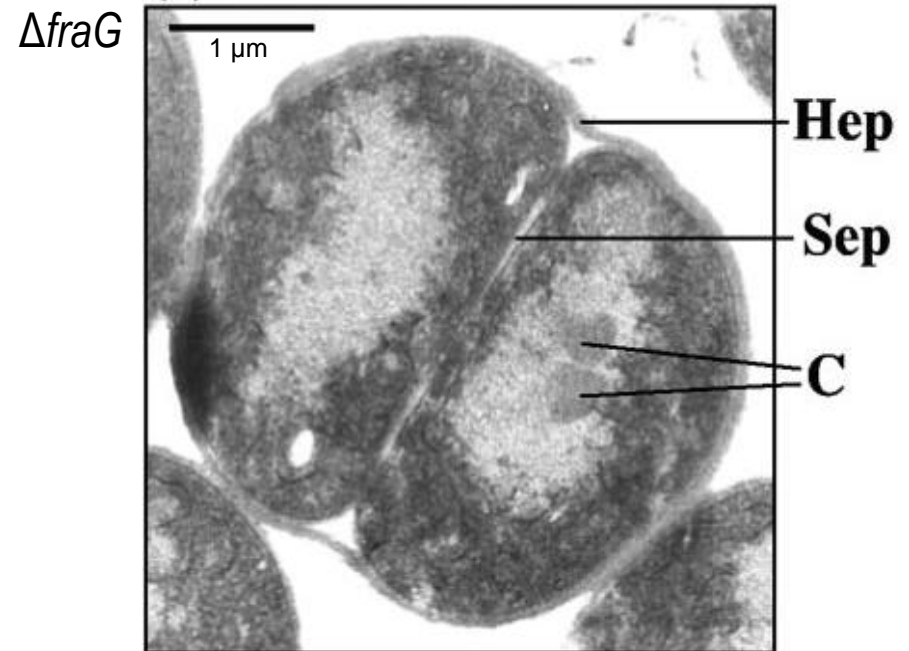
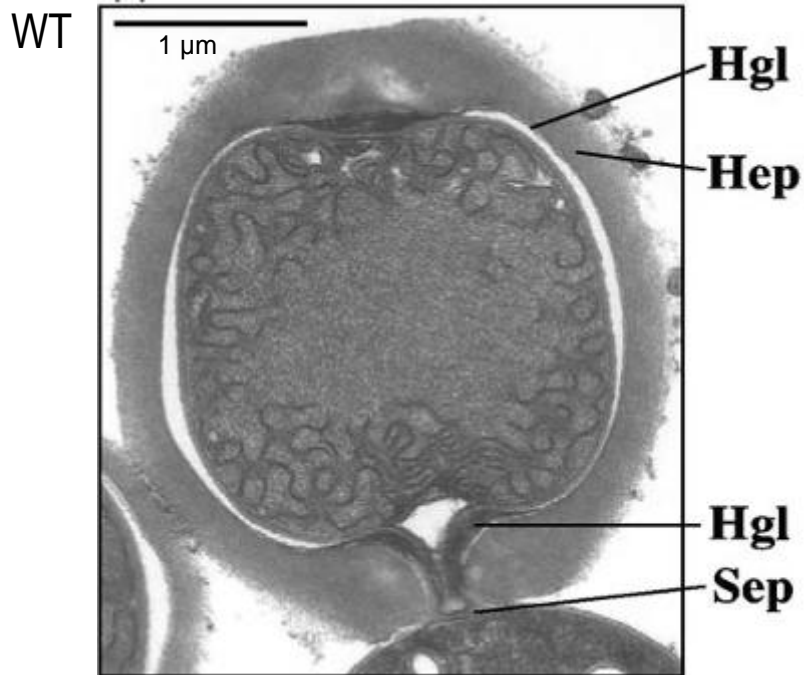
Flores E, Pernil R, Muro-Pastor AM, Mariscal V, Maldener I, Lechno-Yossef S, Fan Q, Wolk CP, Herrero A. J Bacteriol. 2007.

Nayar AS, Yamaura H, Rajagopalan R, Risser DD, Callahan SM. Microbiology. 2007.

# *fraG* necessary for heterocyst development

*Nostoc* PCC 7120

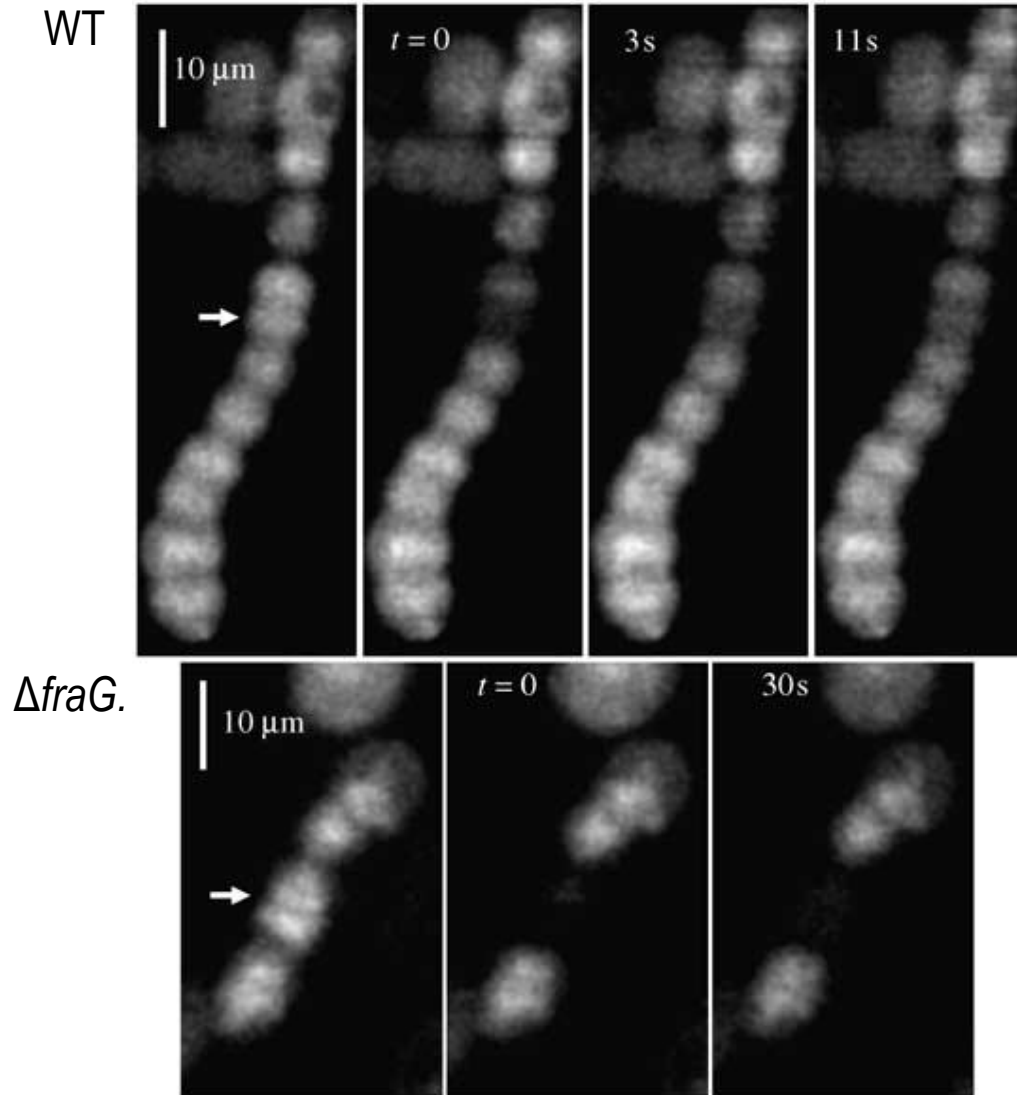
(Flores *et al*, 2007)



# *fraG* is also necessary for diffusion of soluble compounds among the cells of the filament

*Nostoc* PCC 7120

(Mullineaux *et al*, 2008)



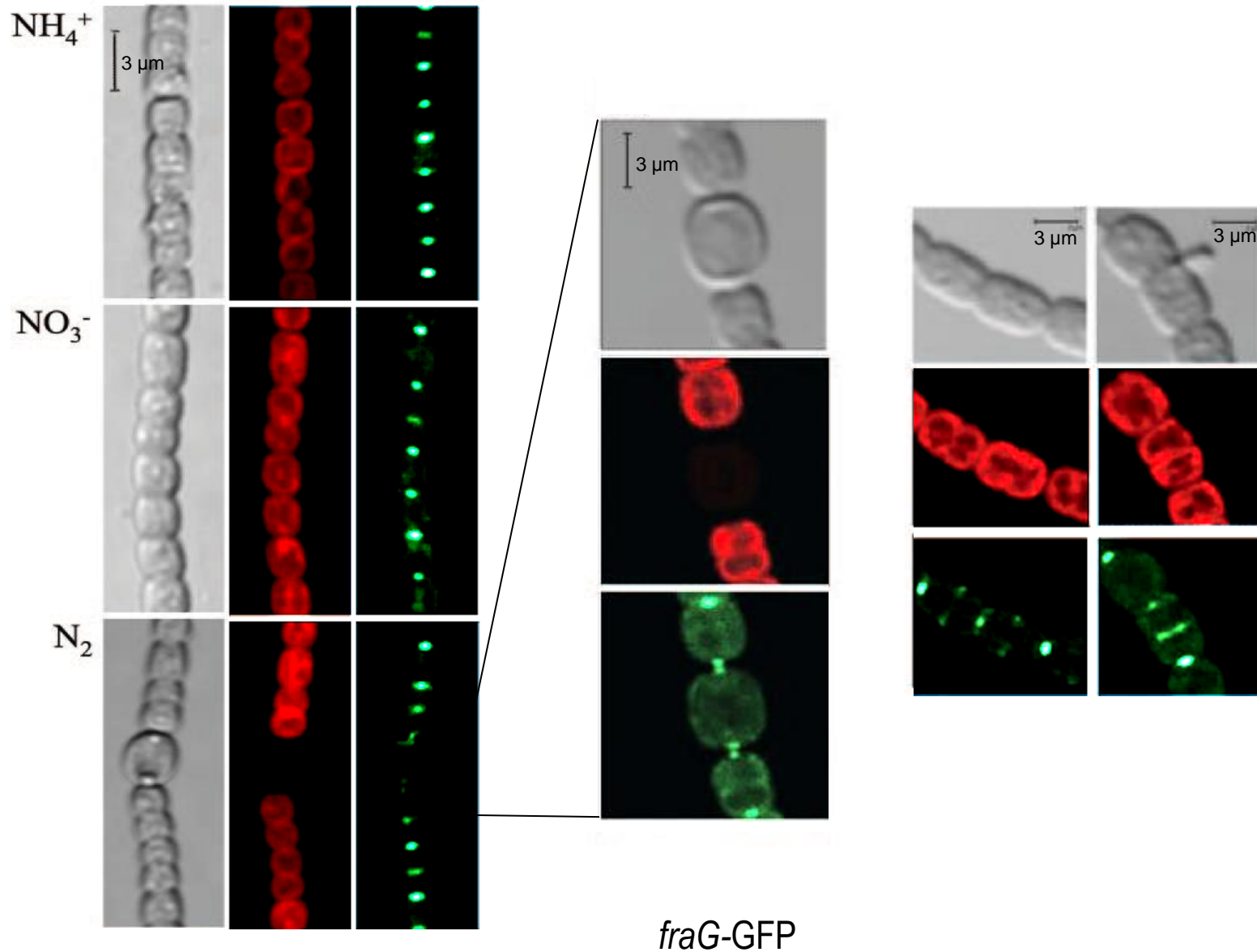
FRAP(Fluorescence recovery after photobleaching ): Calcein in vegetative cells



# Subcellular localization of FraG

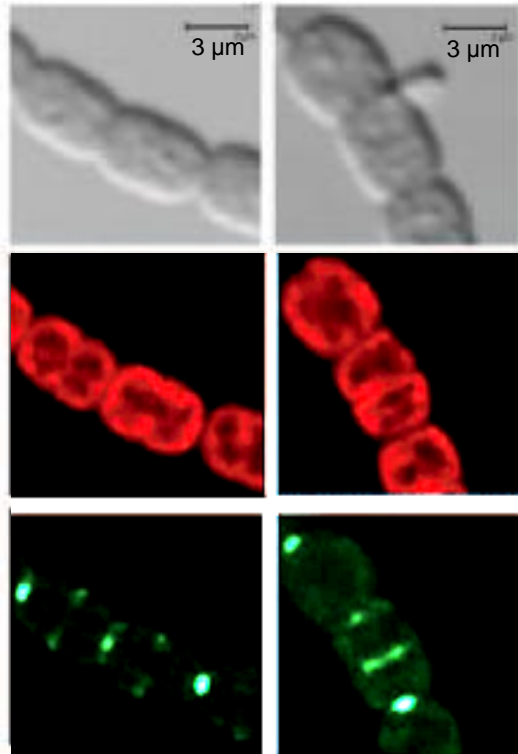
*Nostoc* PCC 7120

(Flores *et al*, 2007)

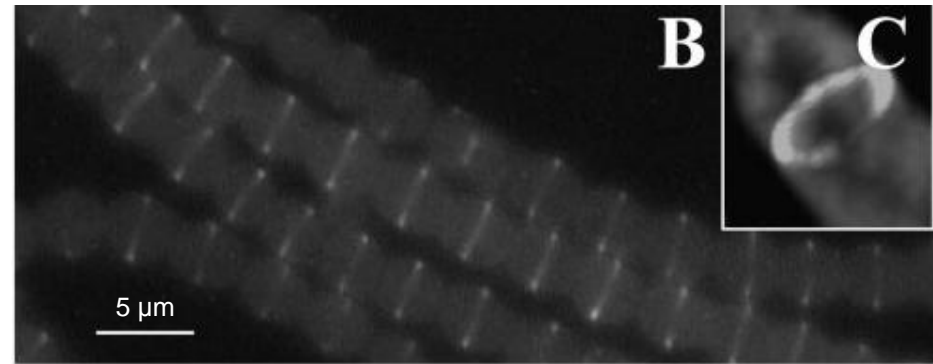


# FraG and FtsZ form the Z-ring during cellular division

*Nostoc* PCC 7120



*fraG*-GFP (Flores *et al*, 2007)



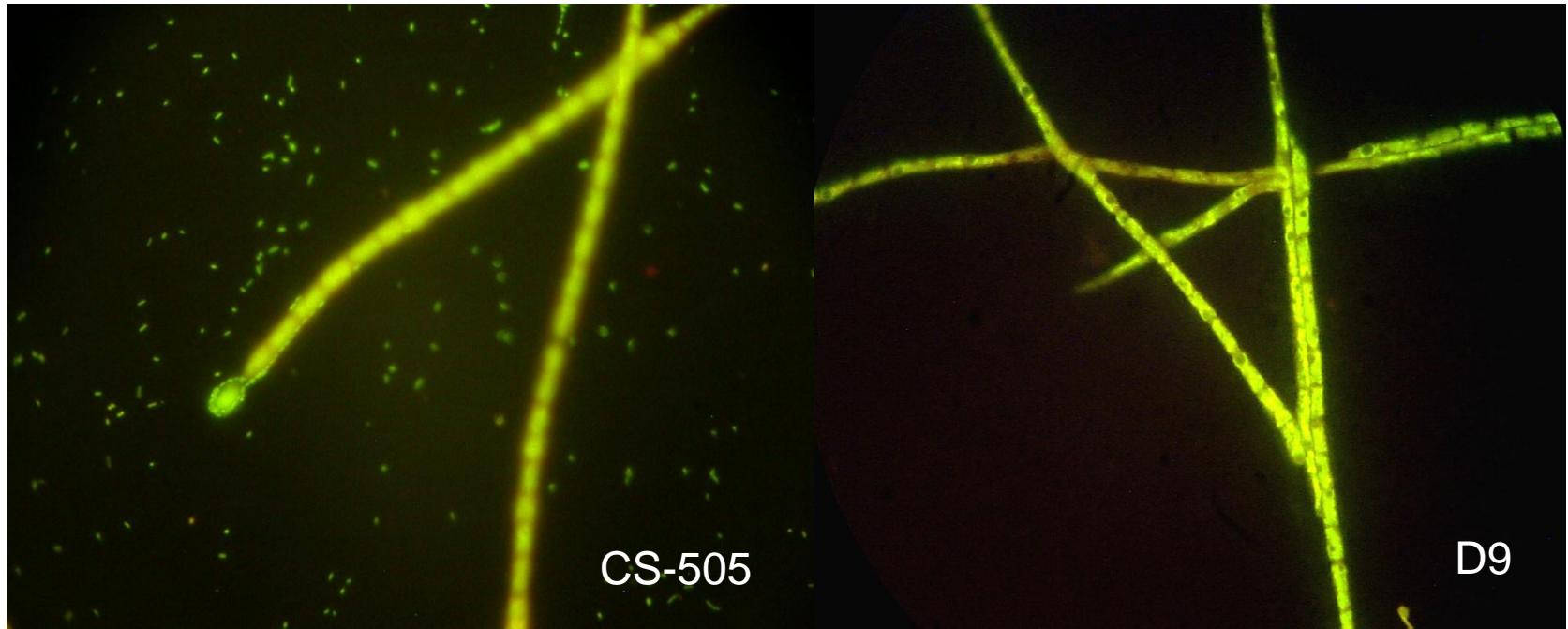
*ftsZ*-GFP

(Sakr *et al*, 2005)

Flores E, Pernil R, Muro-Pastor AM, Mariscal V, Maldener I, Lechno-Yossef S, Fan Q, Wolk CP, Herrero A. *J Bacteriol.* 2007.

Sakr S, Jeanjean R, Zhang C, Arcondeguy T. *Journal of Bacteriology.* 2006.

# Our system:



CS-505

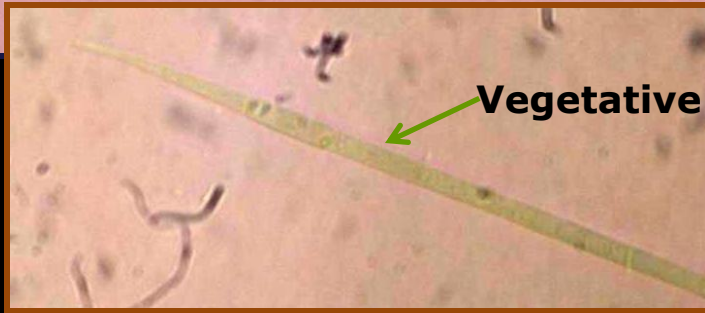
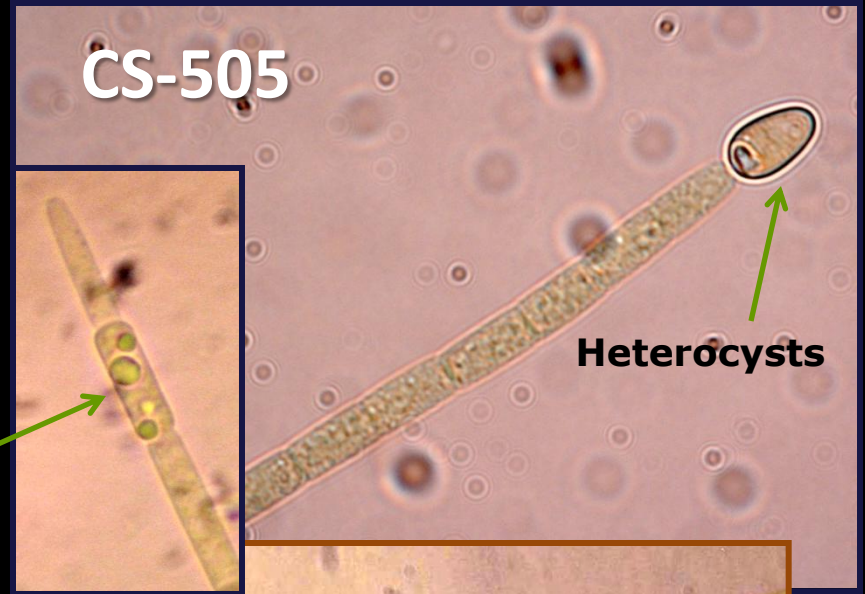
D9

*Cylindrospermopsis raciborskii*

*Raphidiopsis brookii*

*Raphidiopsis brooki* D9

*Cylindrospermopsis raciborskii*



Origin: Brasil

PSP Toxins: **STX, dcSTX, GTX2, GTX3, dcGTX2, dcGTX3**



Origin: Australia

Toxins: **Cylindrospermopsin (CYN) and deoxy-cylindrospermopsin (doCYN)**



# The first part of the story



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Systematic and Applied Microbiology 32 (2009) 37–48

SYSTEMATIC AND  
APPLIED MICROBIOLOGY

[www.elsevier.de/syapm](http://www.elsevier.de/syapm)

## Toxicity phenotype does not correlate with phylogeny of *Cylindrospermopsis raciborskii* strains

Karina Stucken<sup>a,b</sup>, Alejandro A. Murillo<sup>a,b</sup>, Katia Soto-Liebe<sup>a,b</sup>,  
Juan J. Fuentes-Valdés<sup>a,b</sup>, Marco A. Méndez<sup>c</sup>, Mónica Vásquez<sup>a,b,\*</sup>

<sup>a</sup>*Laboratorio de Ecología Microbiana y Toxicología Ambiental, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, 8331010 Santiago, Chile*

<sup>b</sup>*Millennium Nucleus on Microbial Ecology and Environmental Microbiology and Biotechnology, Alameda 340, 6513492 Santiago, Chile*

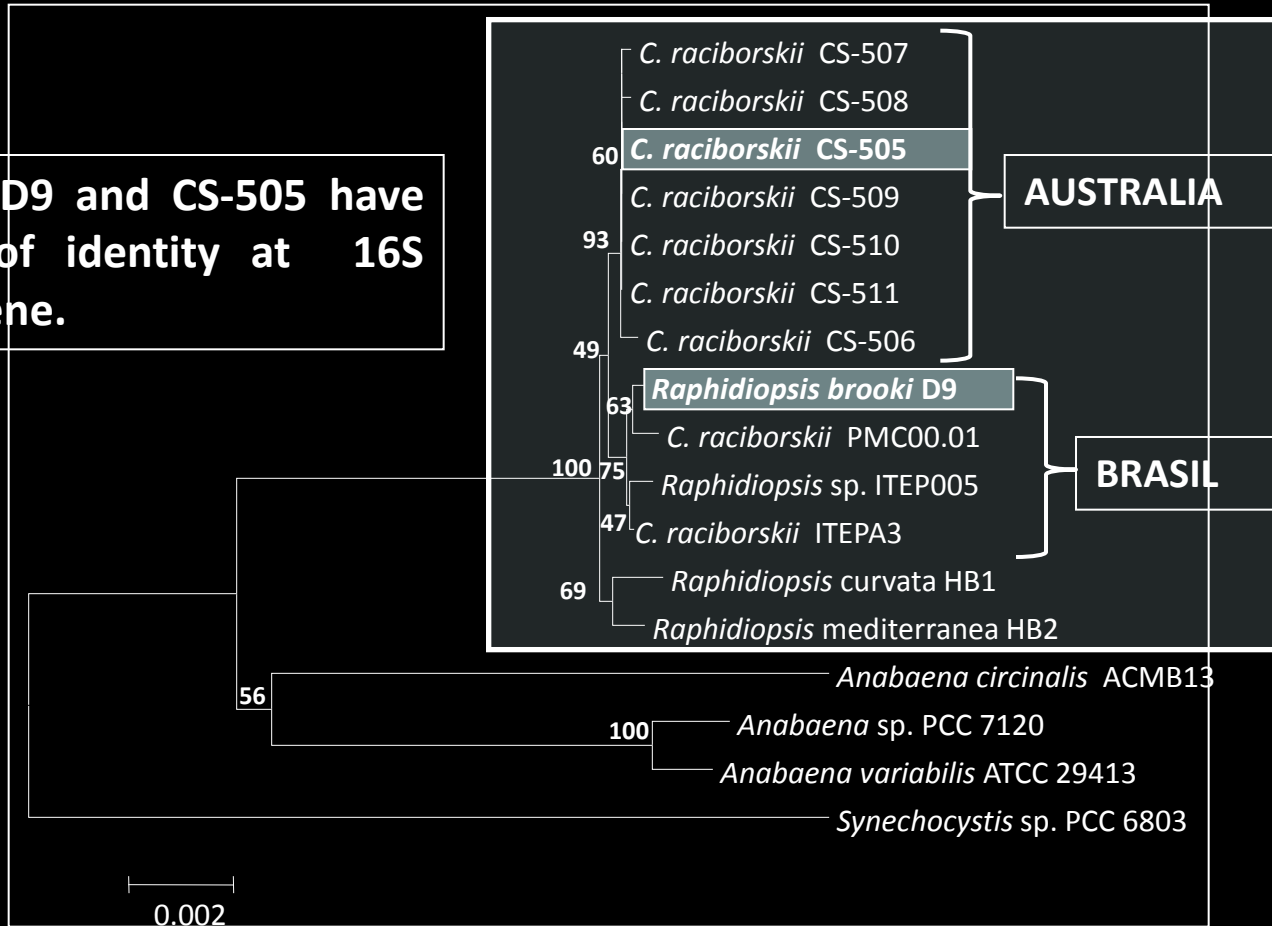
<sup>c</sup>*Laboratorio de Genómica Evolutiva, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile*





*Cylindrospermopsis raciborskii* strains and *Raphidiopsis brooki* D9 form a monophyletic cluster

Strains D9 and CS-505 have 99.5% of identity at 16S rRNA gene.



# “Annotation team”



Karina Stucken



Alejandro Murillo



Alvaro Muñoz

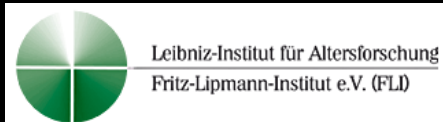


Juan José Fuentes



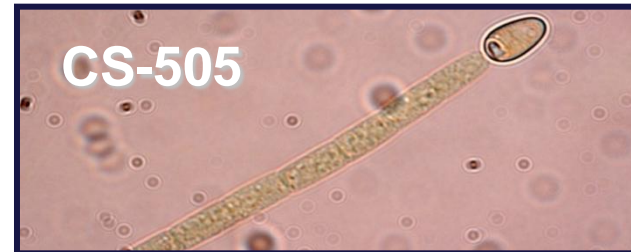
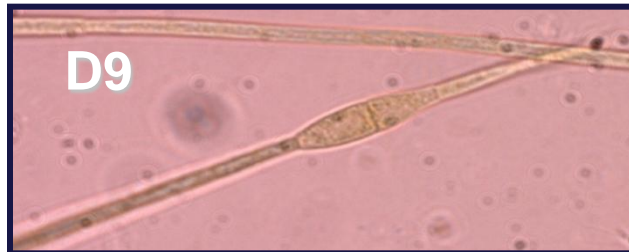
Secuenciación y anotación automática

Gernot Glöeckner



# Genome generalities

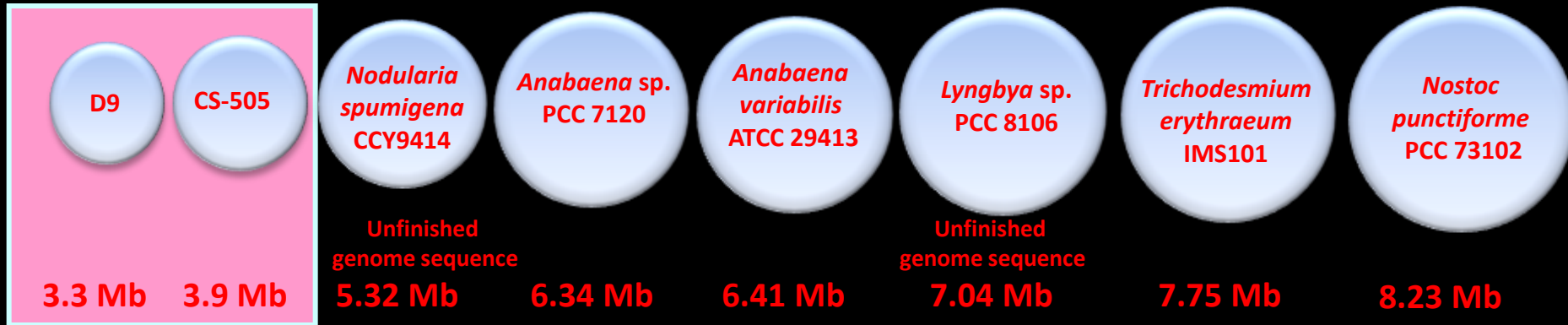
Whole genome 454 sequencing was used for sequencing the genomes of D9 and CS-505.



Genome size	3.3 Mb	3.9 Mb
contigs >3.5 kb	33	94
sequencing depth	27X	35X
G+C content %	40	40.2
Genes	3088	3968
CDS	3010	3452
rRNA	9	9
tRNA	42	42
Shared CDS	2627	2627
Transposases	7	55
Phage integrases	-	2
Repeated regions	53	406
repeats (% of total)	1.7	6.3
Plasmids	?	?



# Filamentous cyanobacteria genome sizes

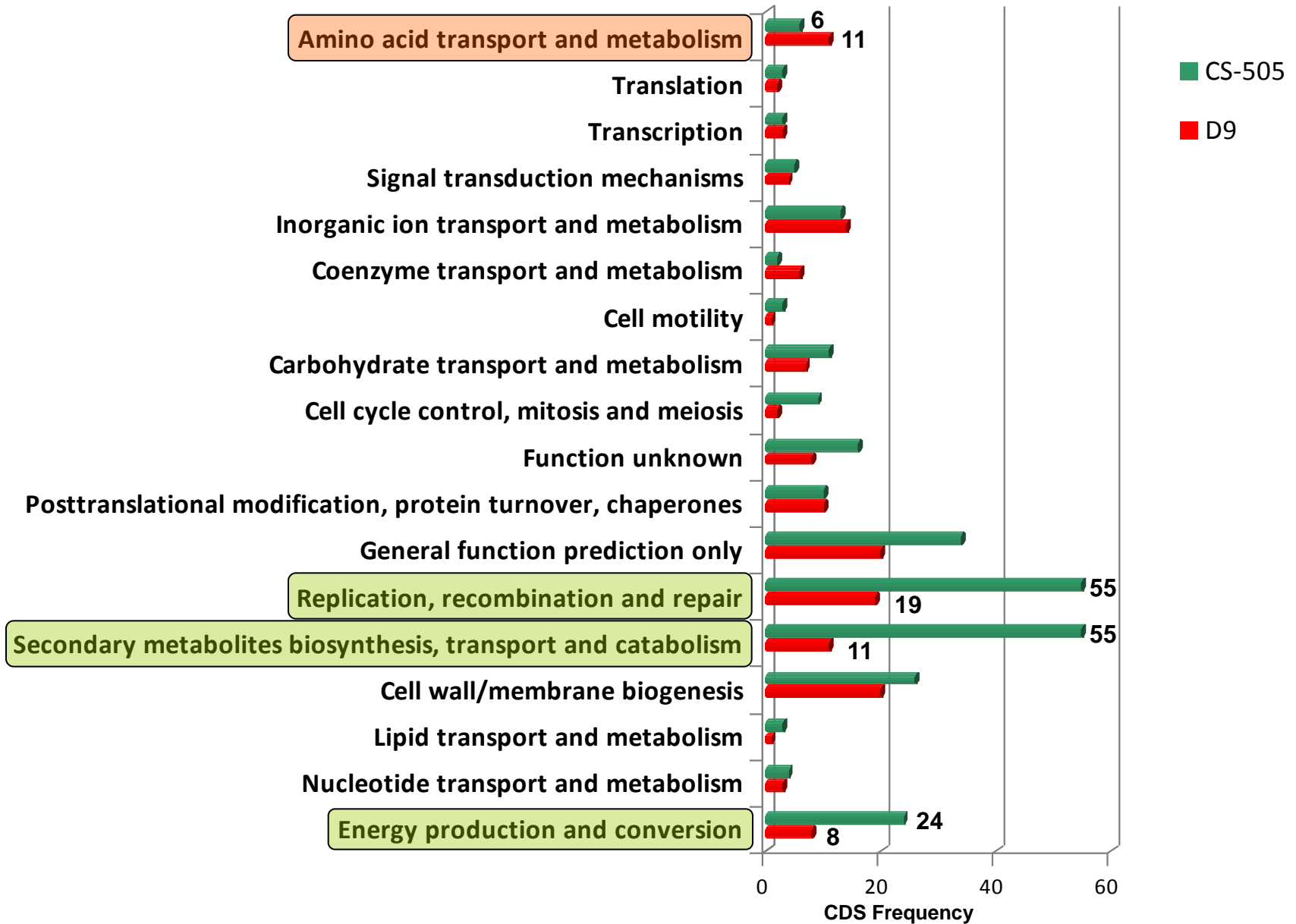


**Soon in PLoS ONE!**

D9 and CS-505 would contain the minimal set of genes essential for the growth as a multicellular organism



# COG categories unique CDS



# COG categories unique CDS

Amino acid transport and metabolism

Translation

Transcription

Signal transduction mechanisms

Inorganic ion transport and metabolism

Coenzyme transport and metabolism

Cell motility

Carbohydrate transport and metabolism

Cell cycle control, mitosis and meiosis

Function unknown

Posttranslational modification, protein turnover, chaperones

General function prediction only

Replication, recombination and repair

Secondary metabolites biosynthesis, transport and catabolism

Cell wall/membrane biogenesis

Lipid transport and metabolism

Nucleotide transport and metabolism

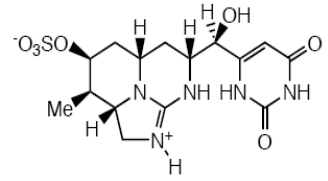
Energy production and conversion

Transposases

- Novel secondary metabolites pathway
- Heterocyst glycolipids
- **Cylindrospermopsin gene cluster**

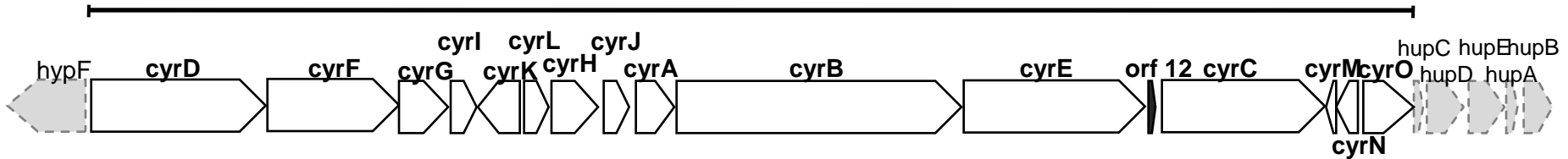
Nitrogen fixation genes

# Cylindrospermopsin gene cluster



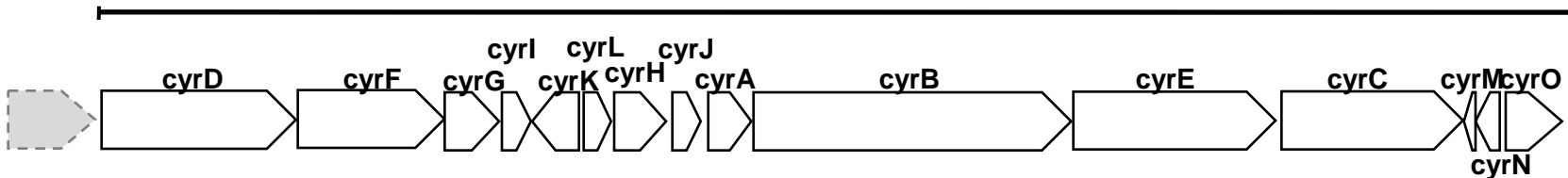
Cylindrospermopsin

41.6 kb



## *C. raciborskii* AWT205

41.6 kb



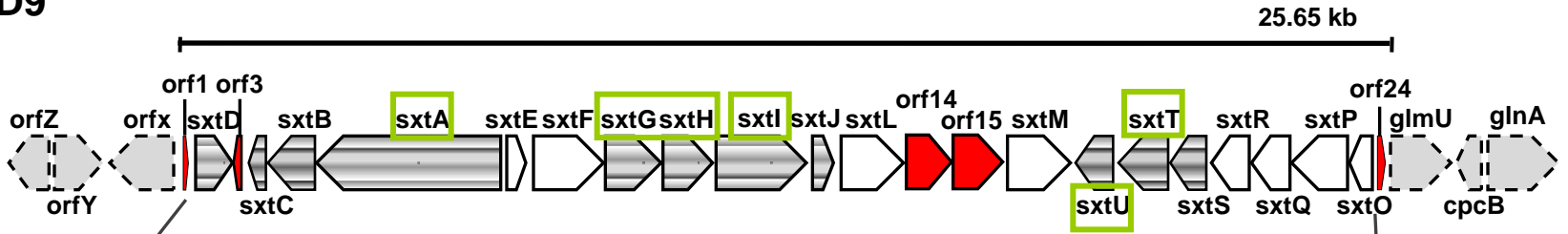
Comparison of the cylindrospermopsin gene cluster of strain CS-505 with the *cyr* gene cluster described in *C. raciborskii* AWT205 (Mihali et al., 2008).

**orf 12 encodes for a transposase fragment, the only different gene found within both CYN clusters**

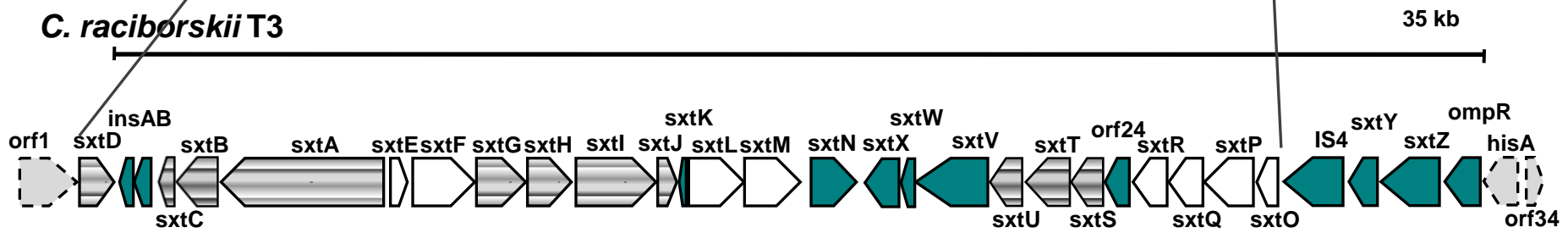
**The hydrogenase gene cluster is under the regulation of NtcA → Nitrogen regulation of CYN biosynthesis genes?**

# Saxitoxin gene cluster




D9



*C. raciborskii* T3

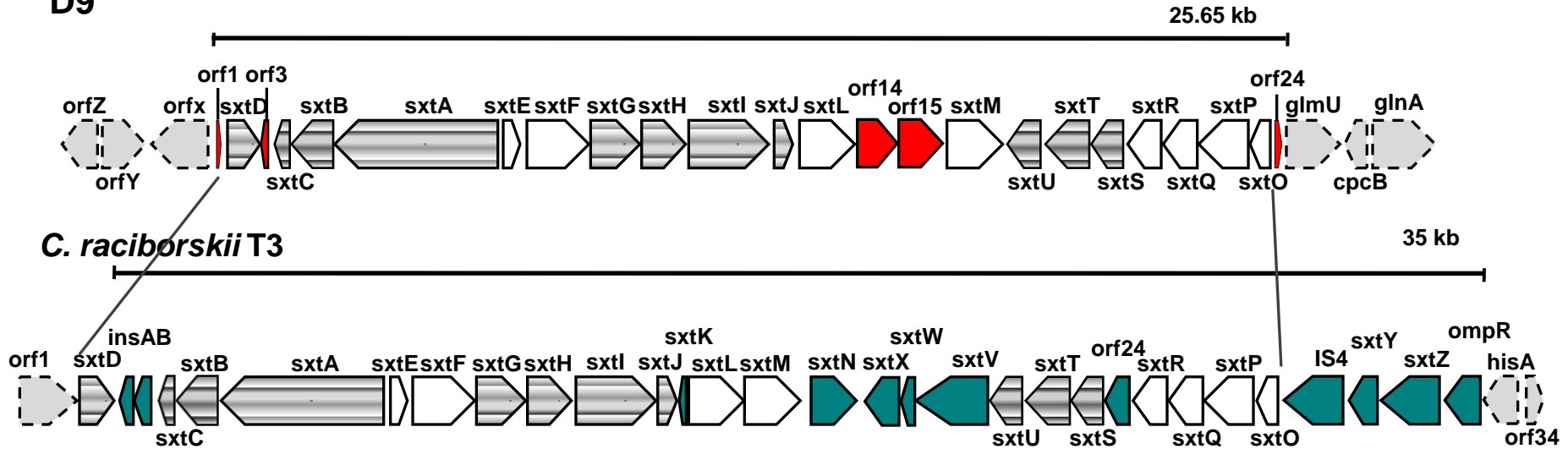


Comparison of the saxitoxin gene cluster of strain D9 with the *sxt* gene cluster described in *C. raciborskii* T3 (Kellmann et al., 2008).

-  Genes involved in the biosynthesis of STX (100% identity between D9 and T3)
-  Genes unique in D9
-  Genes unique in T3

# Saxitoxin gene cluster

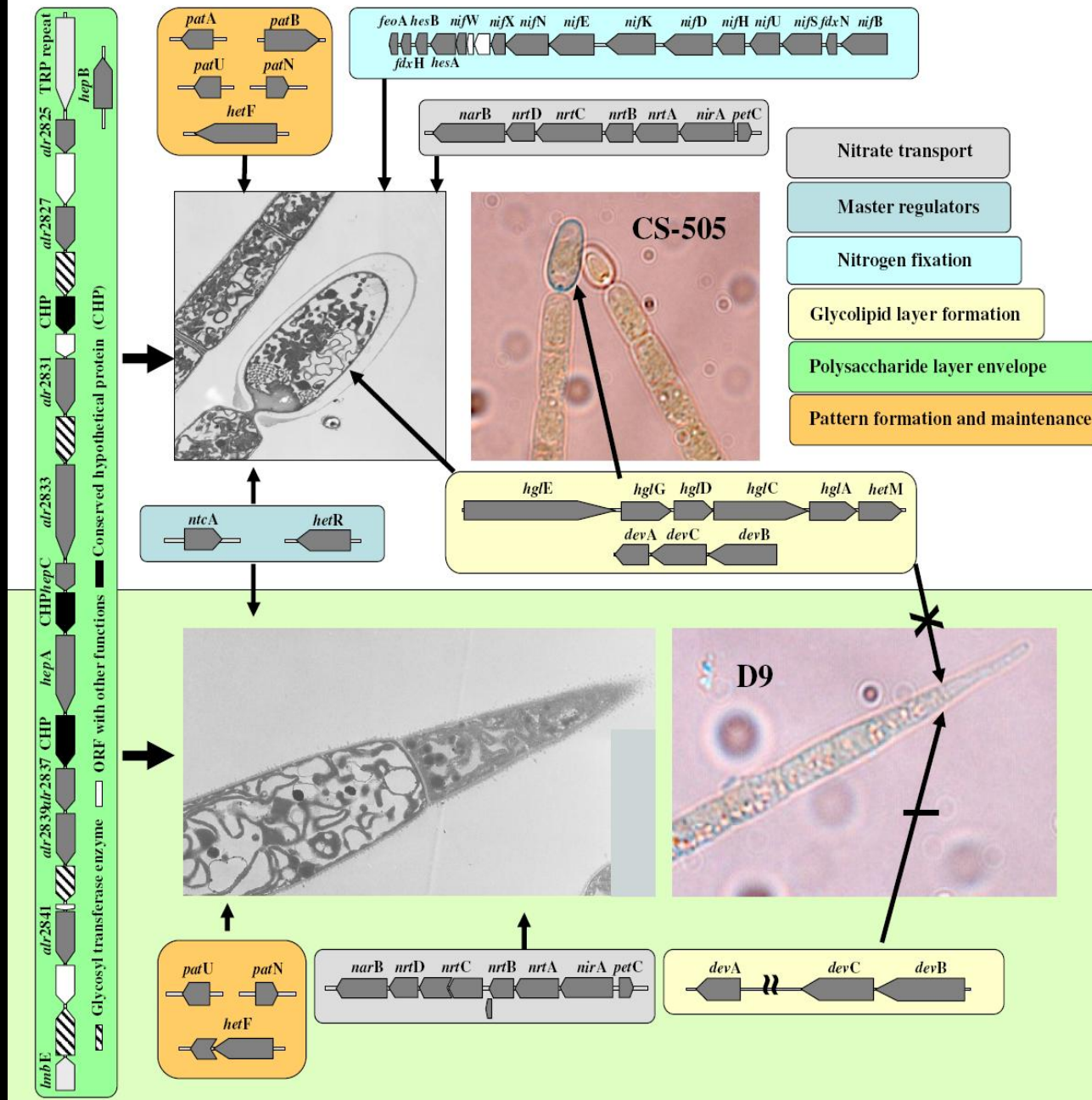
D9



orf1  
orf3  
orf24 } Hypothetical proteins

**orf14 gene product contains a sulfotransferase (ST) conserved signature**  
**orf15 encodes for a protein with similarity to the bacterial ring-hydroxylating dioxygenase**

*cpcB* encodes for Allophycocyanin beta-subunit, ApcB  
*glnA* encodes for glutamine synthase, GlnA } *cpcB* and *glnA* are regulated by NtcA



**Figure 1.** Overview of the main genes and gene clusters involved in Nitrogen metabolism and heterocyst development in strains CS-505 and D9. The different processes are shown in color rectangles. Pictures in the left side are electronic microscopies showing the heterocyst of CS-505 and the apical differentiated cell of D9. Pictures at the right side are light microscopies showing alcian blue staining of the heterocyst polysaccharide.

# Exclusive Genes from filamentous cyanobacteria

## The Smallest Known Genomes of Multicellular and Toxic Cyanobacteria: Comparison, Minimal Gene Sets for Linked Traits and the Evolutionary Implications

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Hetz	alr0099	Filament integrity (Merino-Puerto <i>et al</i> , 2010)
FraD	alr2393	
hypothetical protein	all1765	
hypothetical protein	all2320	
hypothetical protein	all1729	
hypothetical protein	all2344	
hypothetical protein	alr0202	
hypothetical protein	alr4863	

ment



# Exclusive Genes from filamentous cyanobacteria

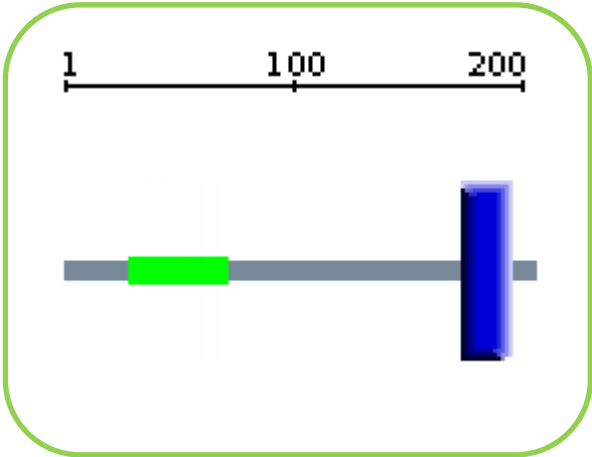


**Protein**


**Locus**

hypothetical protein	all2320
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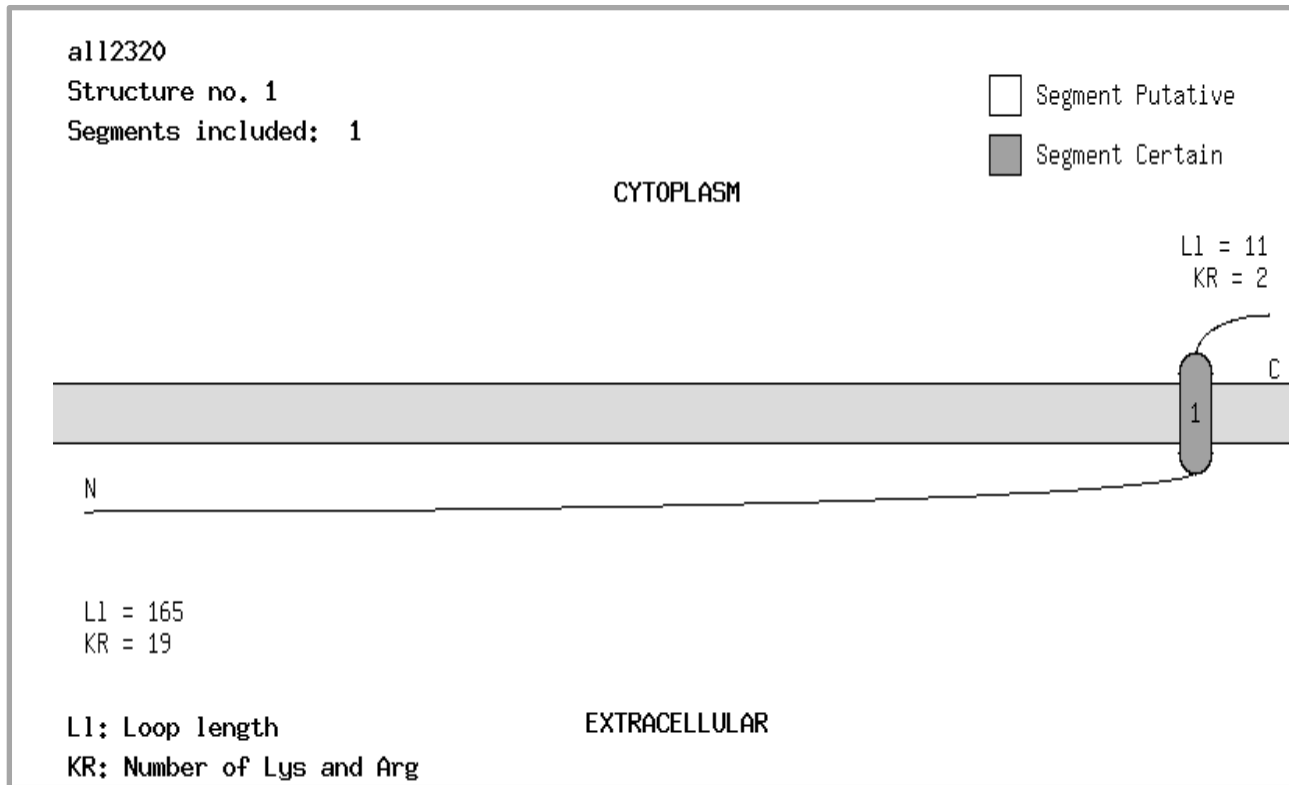
**All2320**



 Transmembrane domain

 Coiled coil region

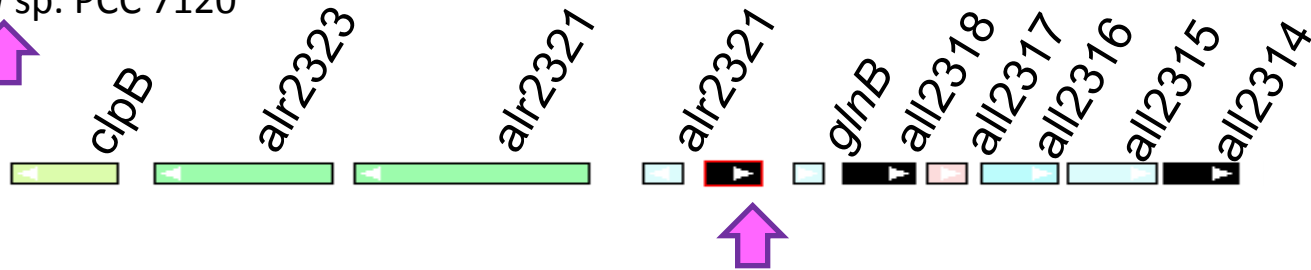
# Localization in the cell



# Gene context

The Cyanobacteria Genome Browser

*Anabaena* sp. PCC 7120  
all2320

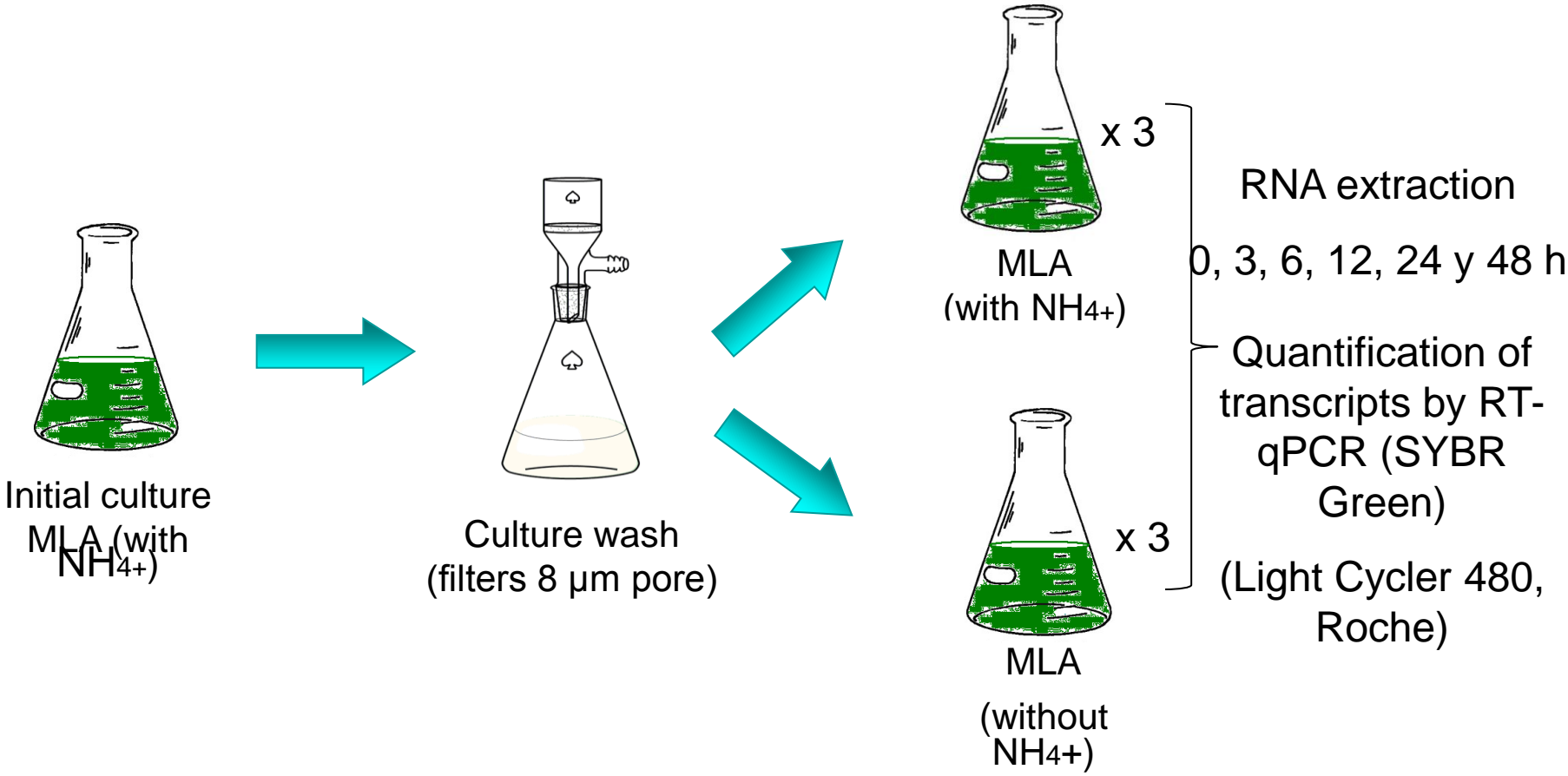


***glnB***: nitrogen regulatory protein PII (overexpression under nitrogen deprivation)  
***clpB***: endopeptidase ATP-binding protein.

***all2320***

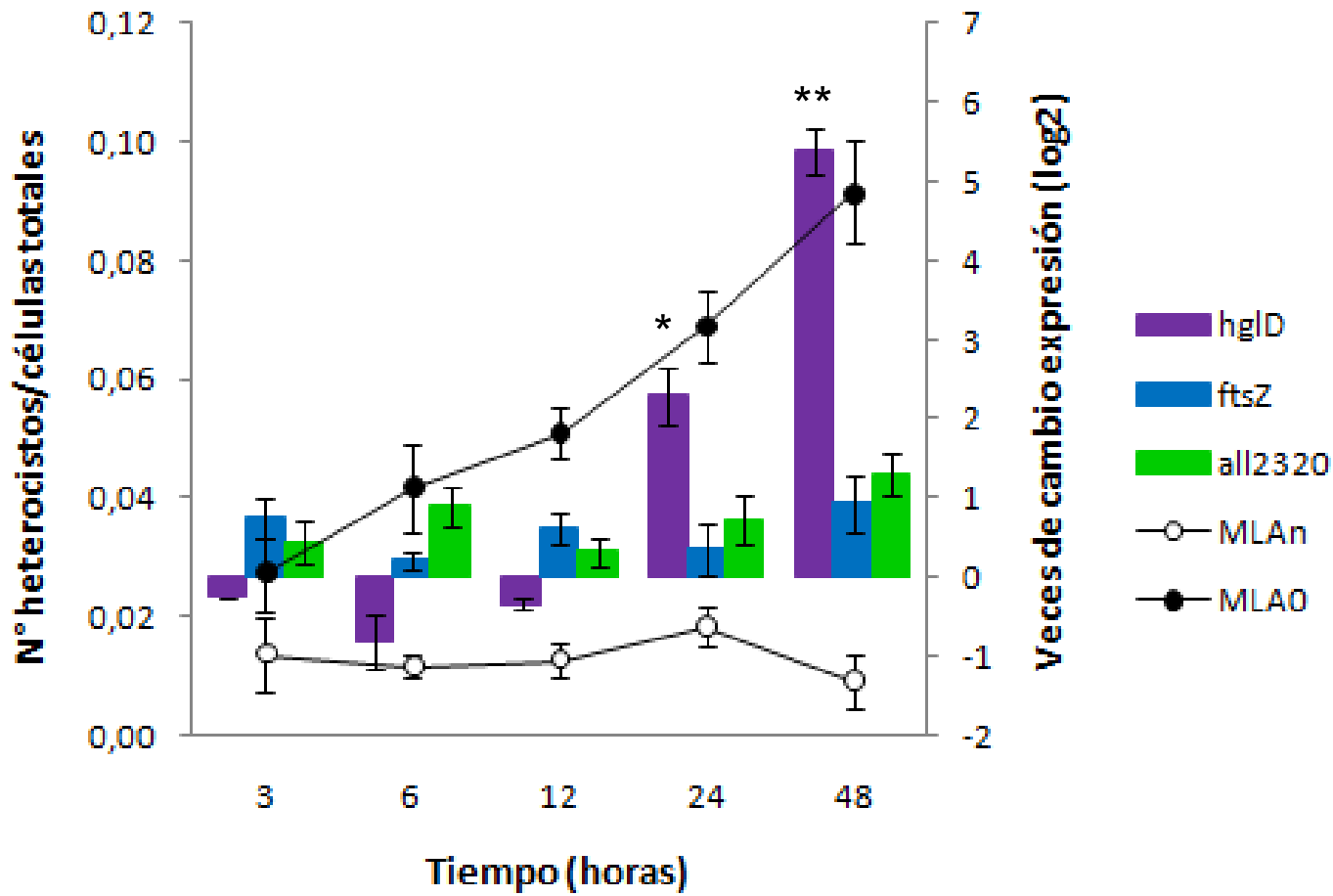
**Objective: Gene expression of *all2320* in presence and absence of fixed nitrogen in *Anabaena* sp. PCC 7120.**

**qPCR**



Direct light, Temperature 25 °C

# all2320 gene expression



**hglD:** heterocyst glycolipid synthase gene.

**ftsZ:** cell division gene.

all2320 expression is constitutive in presence and absence of fixed nitrogen in the media.



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**Thank you!**

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Toxicology Lab**

