CO_2 Acquisition In Cyanobacteria: Some Things Do Change – Evolution & diversity

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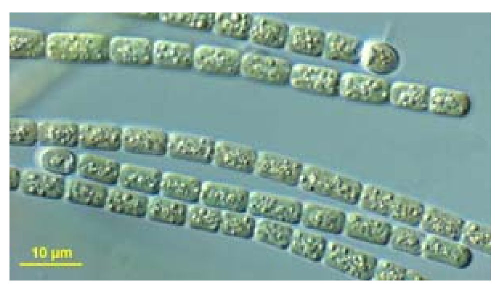
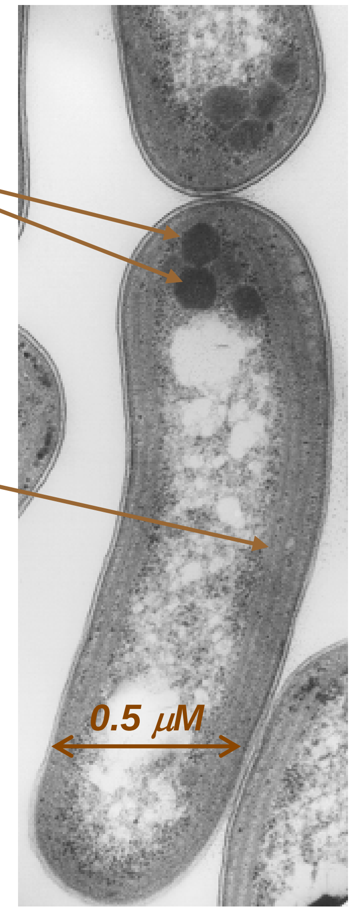




Theory of natural selection

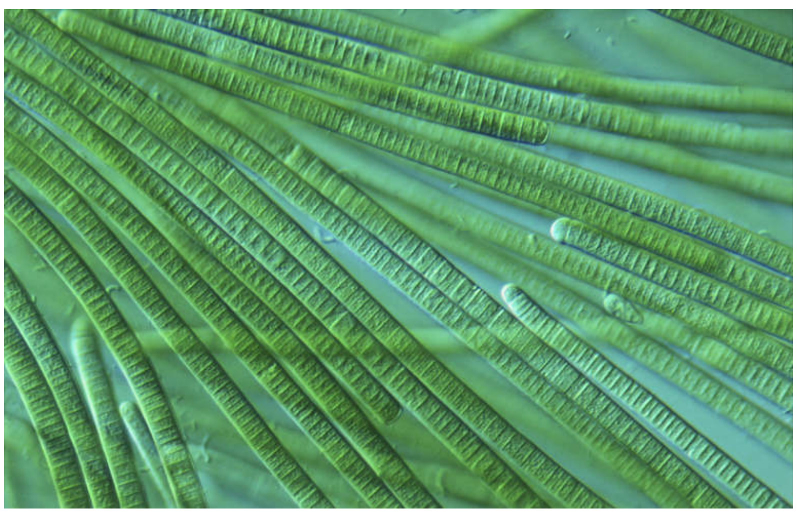
- Wallace & Darwin provided the basic framework for understanding the evolution and diversity in living things; these ideas apply very well to cyanobacteria.
- Darwin did not know about DNA, genes & direct transfer of traits, had he done so, he would have certainly suggested rapid periods of evolution by "horizontal gene transfer".
- HGT appears to have played a significant role in cyanobacterial & bacterial evolution, particularly in CO_2 acquisition processes.

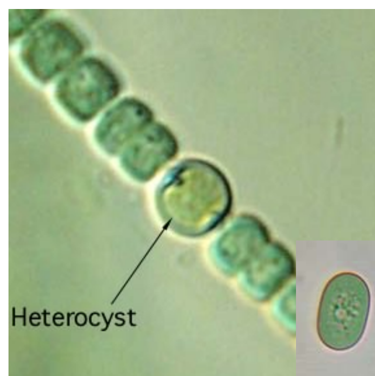
Evolution & diversity of CO_2 acquisition in cyanobacteria: Areas covered

- What are cyanobacteria?
- The problems of surviving in aquatic environments.
- When did the cyanobacterial lineage begin ?
- Evolutionary pressures on Rubisco (primary CO_2 fixing enzyme)
- Evolution of the cyanobacterial CO_2 concentrating mechanism.
- Attributes and diversity of cyanobacterial CCMs.
- When did the CCM evolve and why did C_3 plants “miss out”?

What are cyanobacteria ?

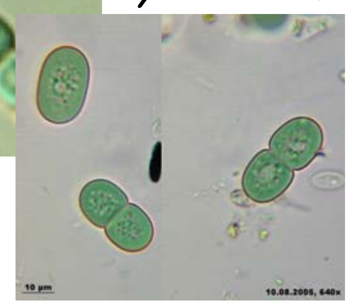
- *Also known as Blue-Green algae, the cyanobacteria are an ancient linage of photosynthetic bacteria that utilise chlorophyll for light harvesting.*
- *They inhabit an amazing diversity of ecological niches from marine, freshwater, polar, desert crusts, plant or lichen symbioses, soda lakes, hypersaline lakes, hot springs, etc; some are primary nitrogen-fixers.*
- *Several clades, but essentially filamentous or unicellular (single-celled).*
- *They evolve Oxygen and use Rubisco to fix CO_2 into sugars.*



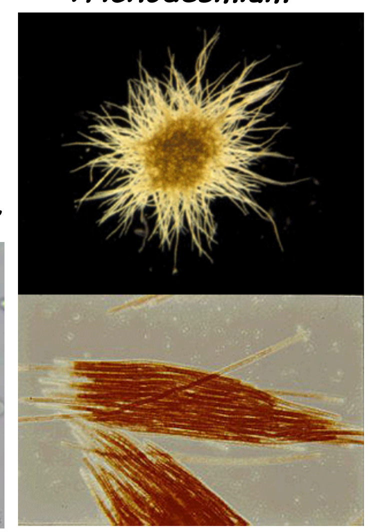


Nostoc

Synechococcus



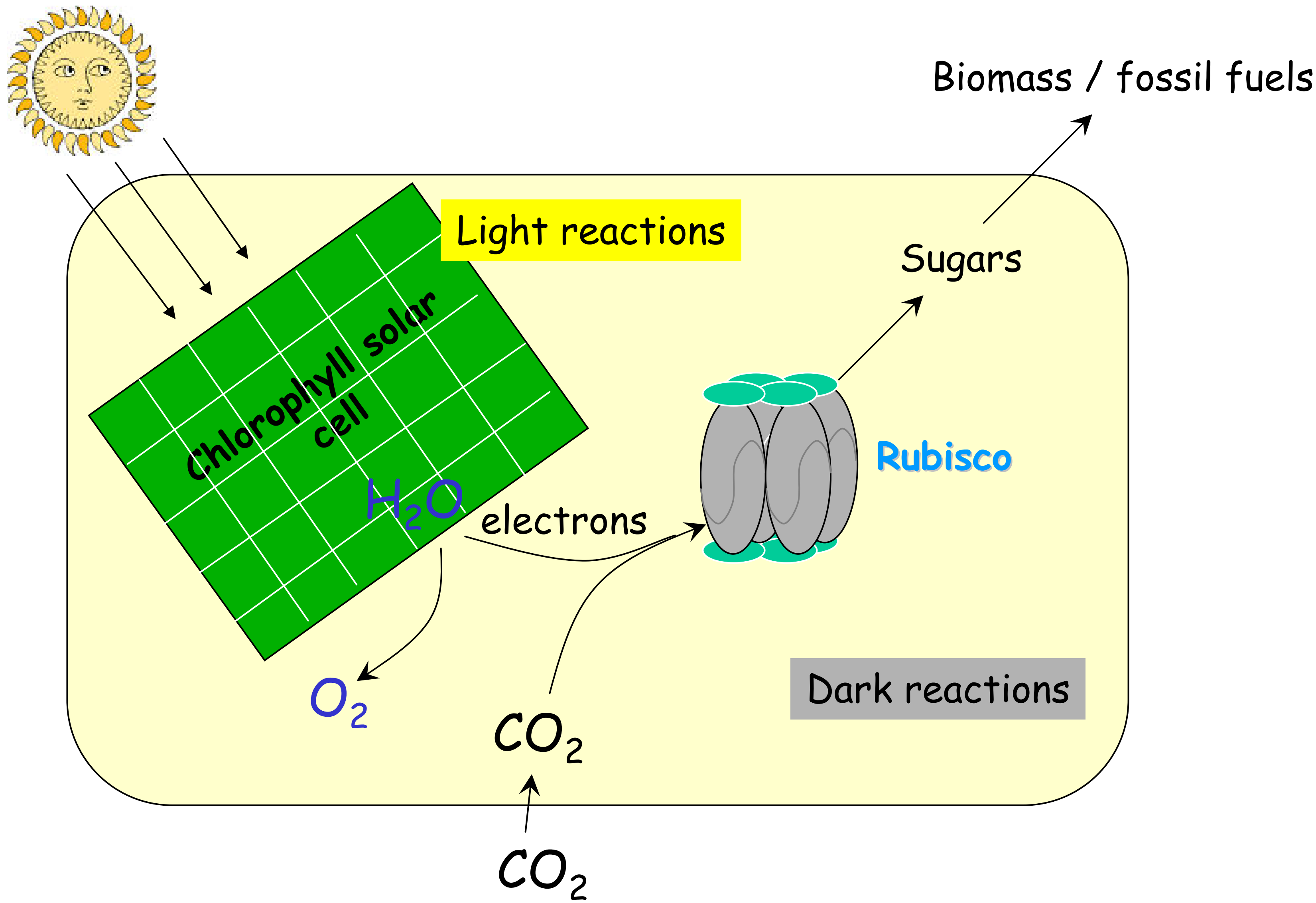
Trichodesmium



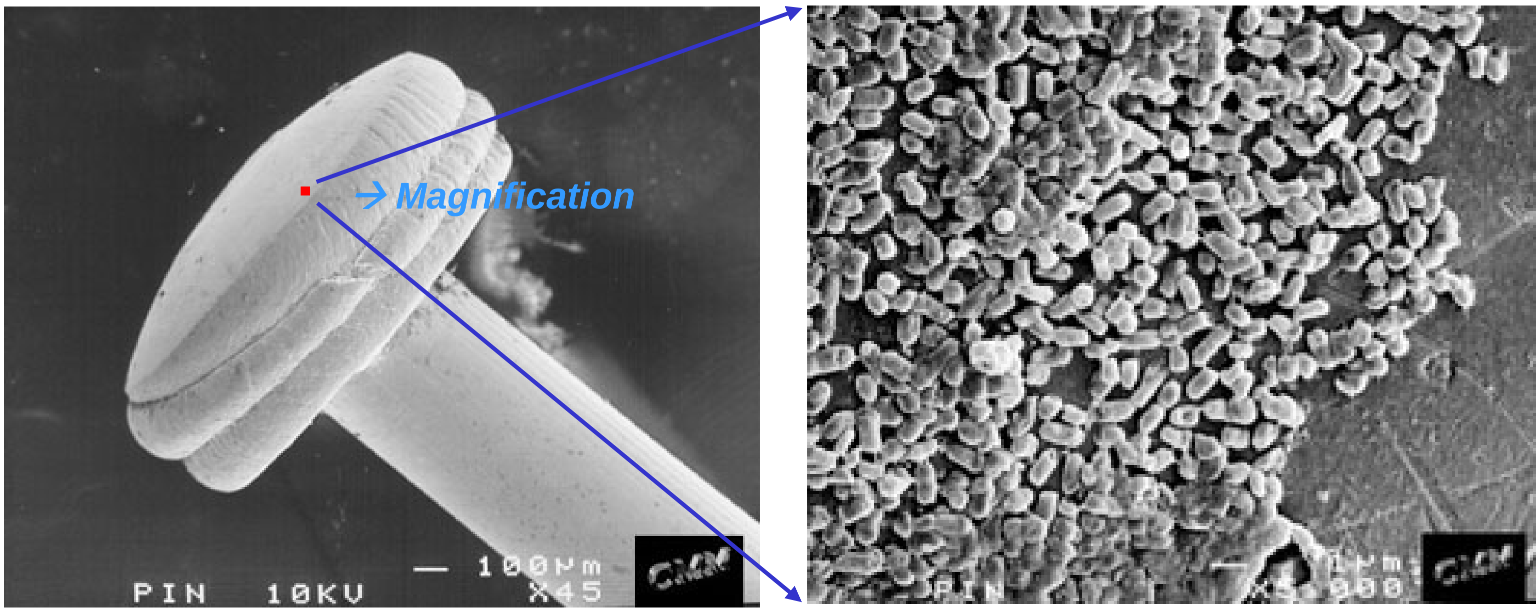
Some cyanobacteria are also opportunists when N & P are in good supply

Algae outbreak hampers Olympic sailing preparations, Qingdao, China (www.abc.net.au)

The two sides of photosynthesis



How many cyanobacterial cells will fit on the head of pin?



Scanning EM, The Centre for Microscopy and Microanalysis

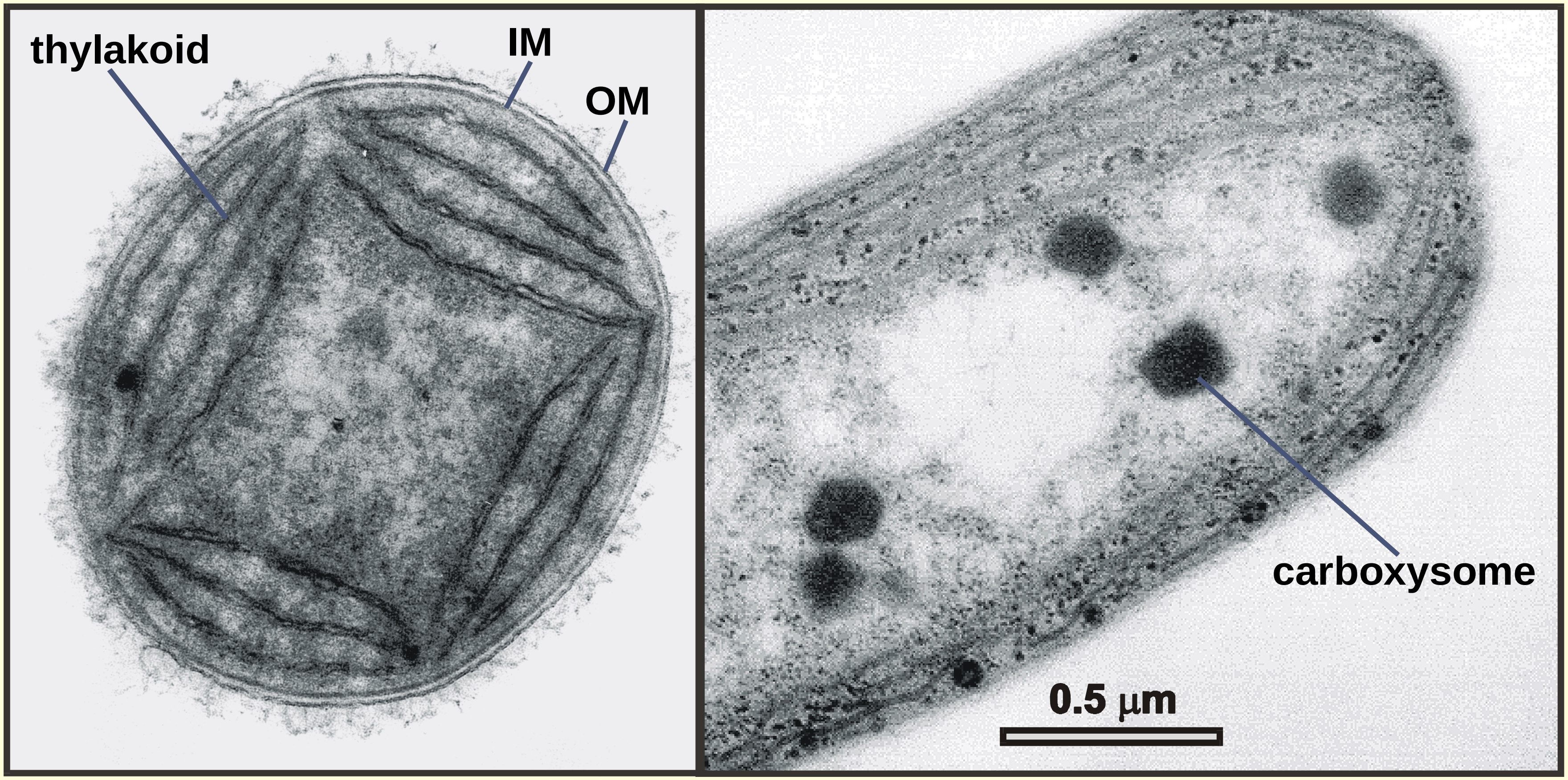
2.8 x 0.8 μM

Synechococcus

For freshwater Synechococcus species, about 27,000 cells should fit on the head of pin (carefully packed).

- *Deep sea Synechococcus ~50,000.*
- *Filamentous strains 5-10,000 cells/pinhead*
- *(3-5 million carboxysomes per pinhead)*

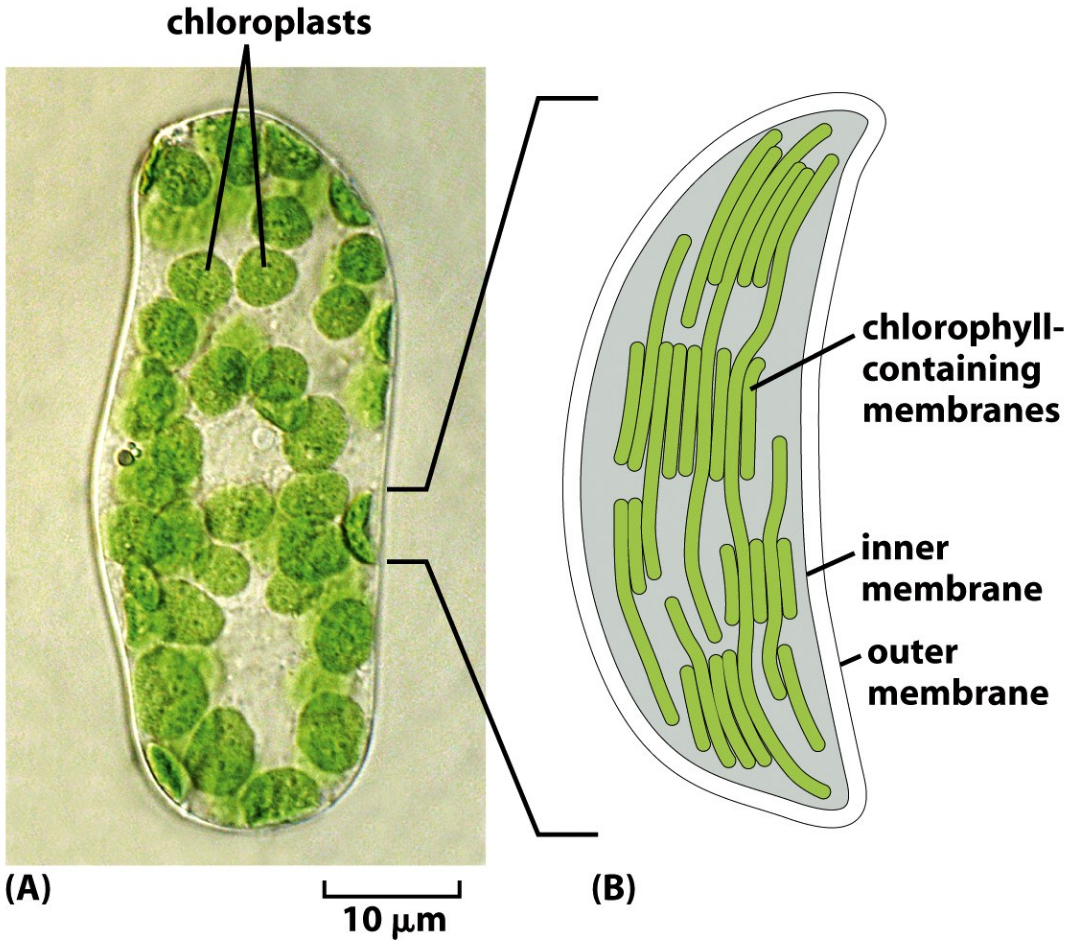
Ultrastructure of Synechococcus sp. PCC7002 (a halo-estuarine species)



D_Price/7002em.cdr

Electron-micrographs of ultra-thin sections embedded in epoxy resin & doped with heavy metals.

A cyanobacterial ancestor was the progenitor of the modern day chloroplast



The plant chloroplast is the essential primary production factory for sugars & starch, and ultimately biomass.

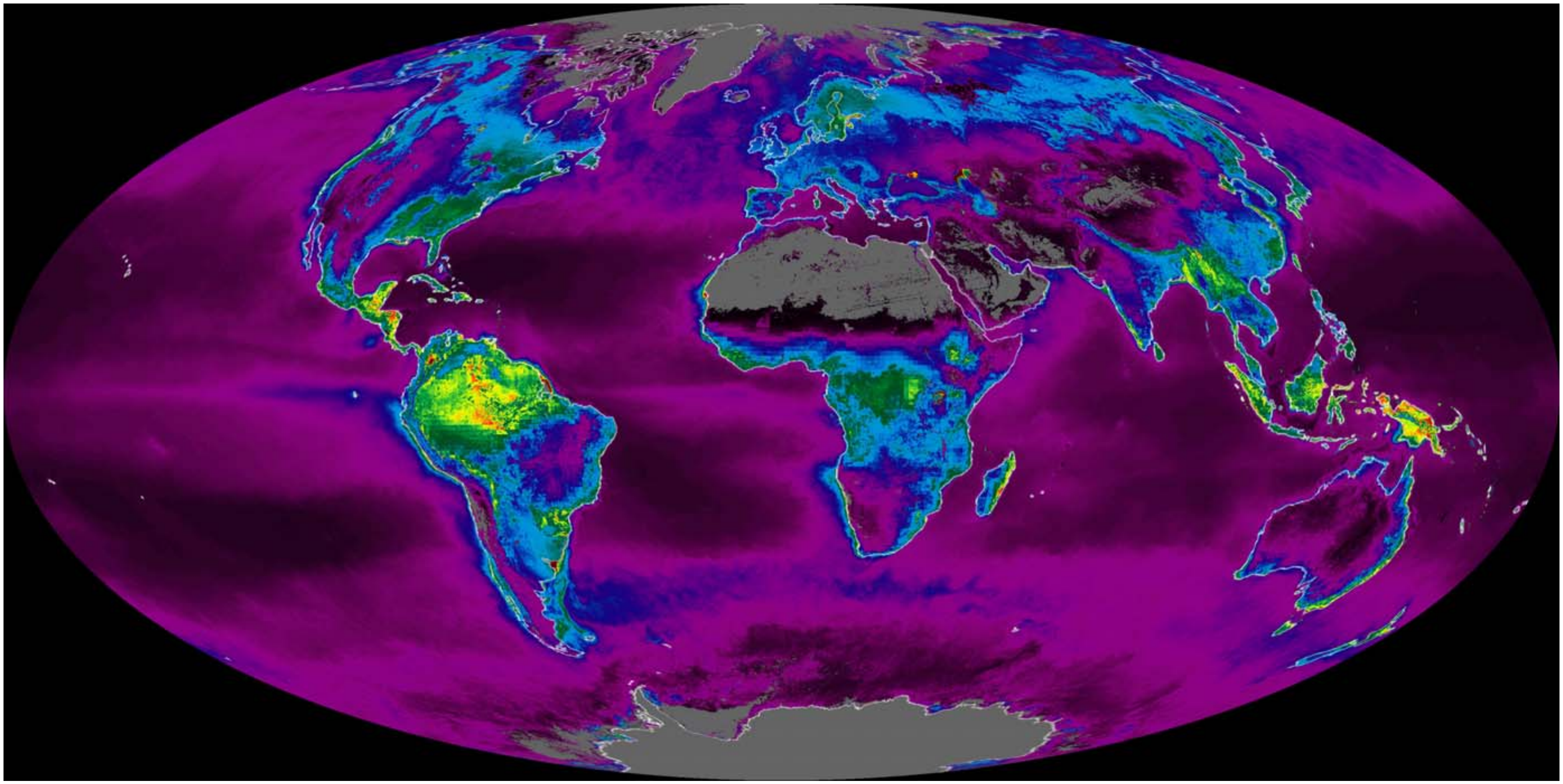
Terrestrial crops & ecosystems would be unsustainable without this ancient endosymbiont.

Molecular Biology of the Cell, Fifth Edition, Garland Science 2008

Almost 50% of global primary productivity is oceanic*

** C Field, et al. Science **282**, 237 (1998)*

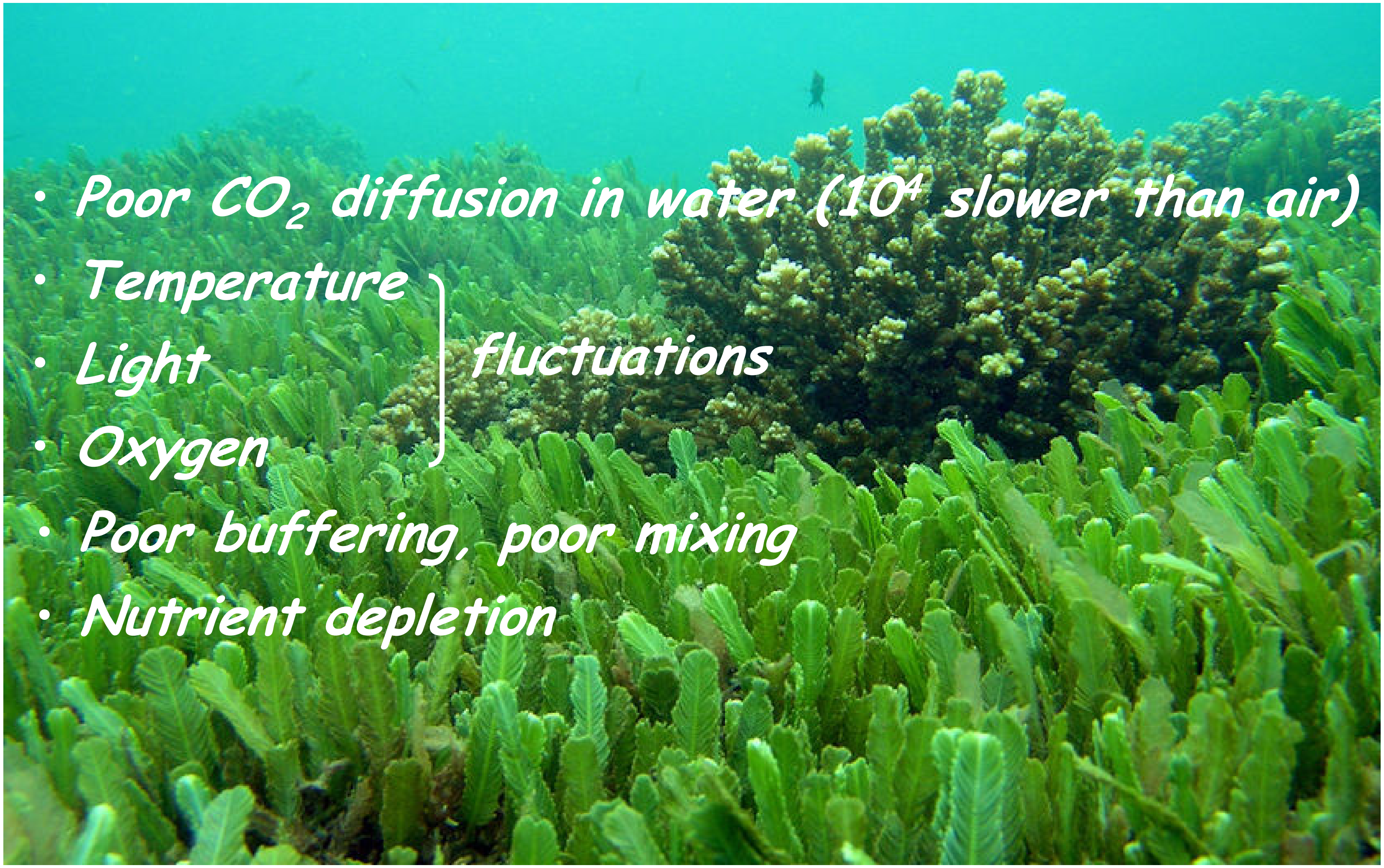
2002 Net Primary Production



http://earthobservatory.nasa.gov/Newsroom/NPP/npp.html

cyanobacteria, diatoms,
macro-algae, seagrass, etc.
→ cyanobacteria ~ 25% of global productivity. → marine foodweb/fisheries.

Limitations on CO_2-fixation for cyanobacteria, algae & aquatic plants



- Poor CO_2 diffusion in water (10^4 slower than air)
- Temperature
- Light
- Oxygen

fluctuations

- Poor buffering, poor mixing
- Nutrient depletion

Inorganic carbon (Ci) species in aquatic environments: a good strategy is to utilise HCO_3^- for photosynthesis

CO_2

air

water

(10 -12 μM)

(CO_2 + OH^- ⇔ HCO_3^-)
Carbonic anhydrase

$$H_2O + CO_2 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow CO_3^{2-} + H^+$$

$pK_a \sim 6.3$ $pK_a \sim 10.3$

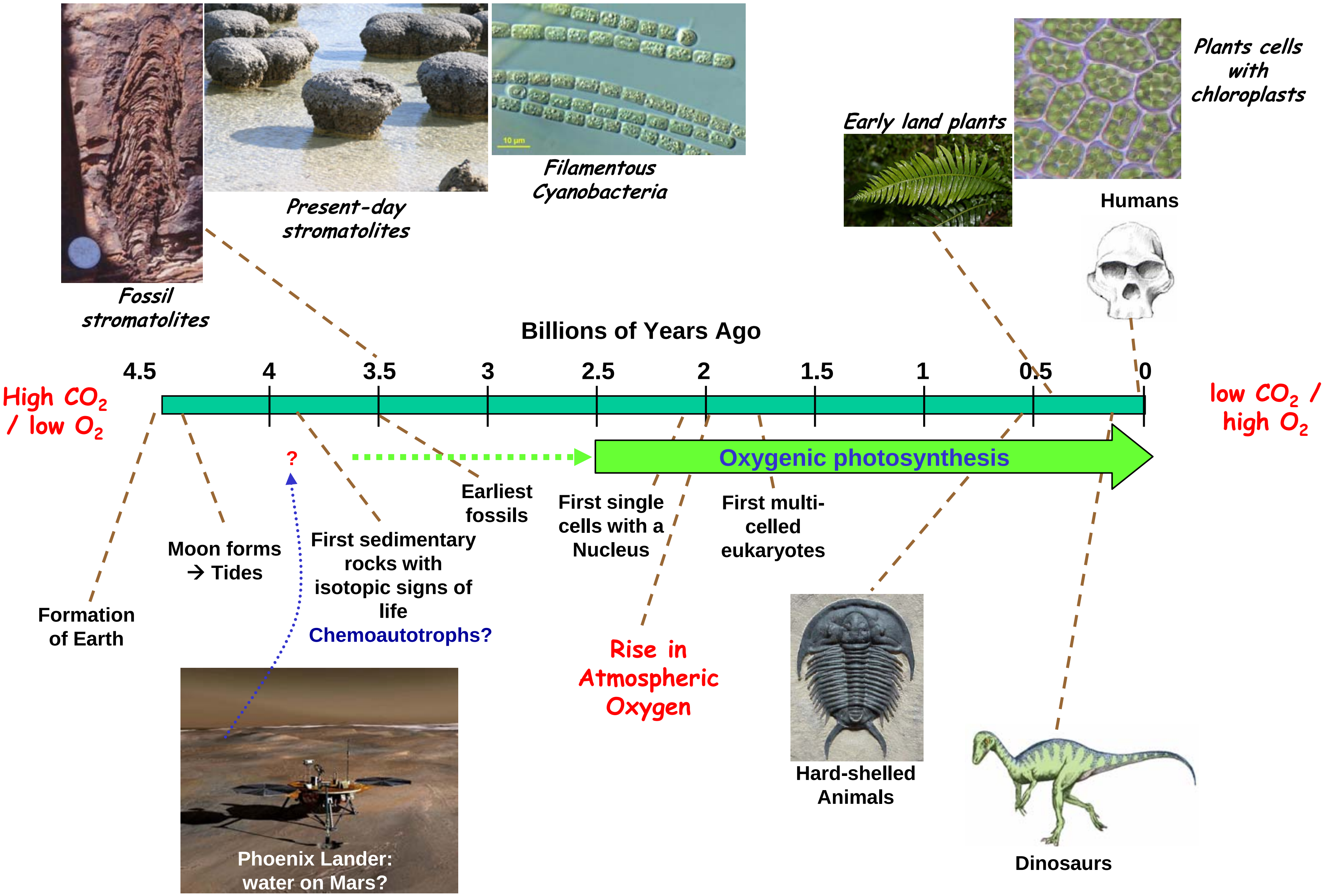
- *CO_2 diffusion in water is 10^4 slower than in air (slow supply rate).*
- *CO_2 supply rate impacted by temperature, pH, demand, mixing, etc.*
- *CO_2 ⇔ HCO_3^- interconversion is slow at pH 8 ($t_{1/2}$ ~ 15 sec).*
- *At high pH, CO_2 can be trapped as HCO_3^- or $CO_3^=$*
  eg. at pH 8 the CO_2^{air}: C_i^{aq} ratio is ~ 35.

When did the cyanobacterial lineage begin ?

Living fossils

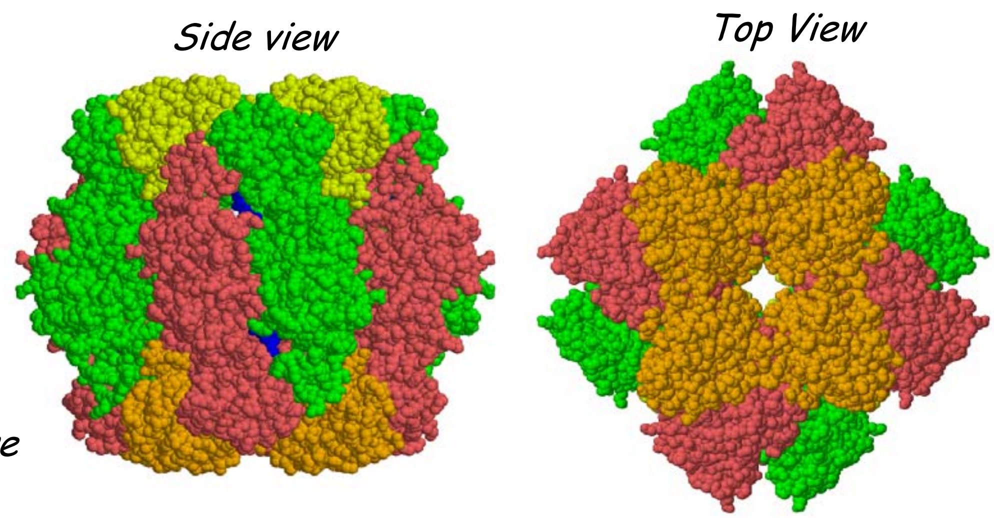
Present-day stromatolites, Shark Bay, WA (low tide)

A condensed view of cyanobacterial evolution



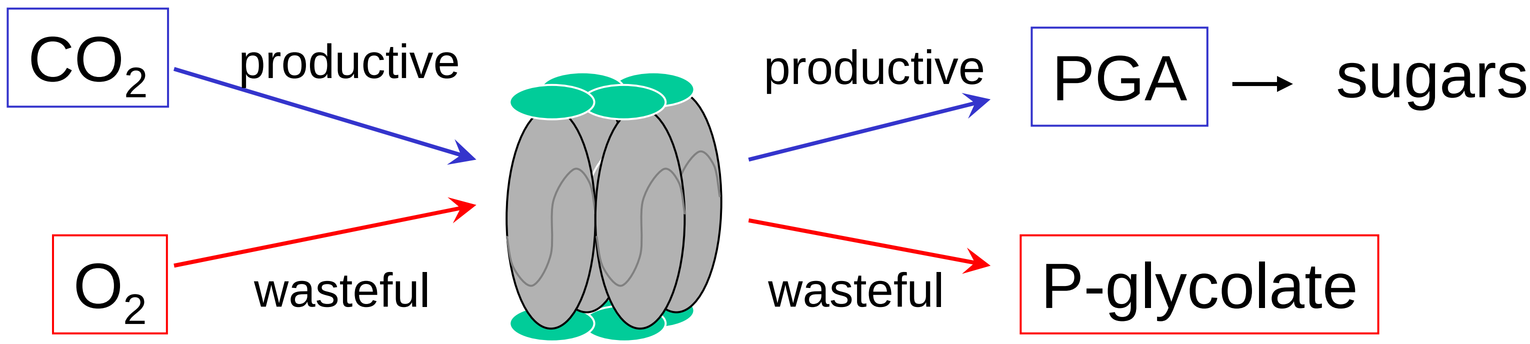
Limitations imposed by the CO_2-fixing enzyme, Rubisco

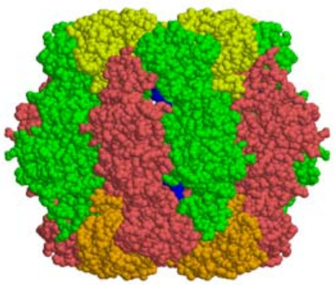
- ***Rubisco, Ribulose bisphosphate carboxylase-oxygenase, is a relatively inefficient enzyme.***
- ***But it evolved when atmospheric conditions differed greatly form modern atmospheric conditions.***



Rubisco L_8S_8 crystal structure

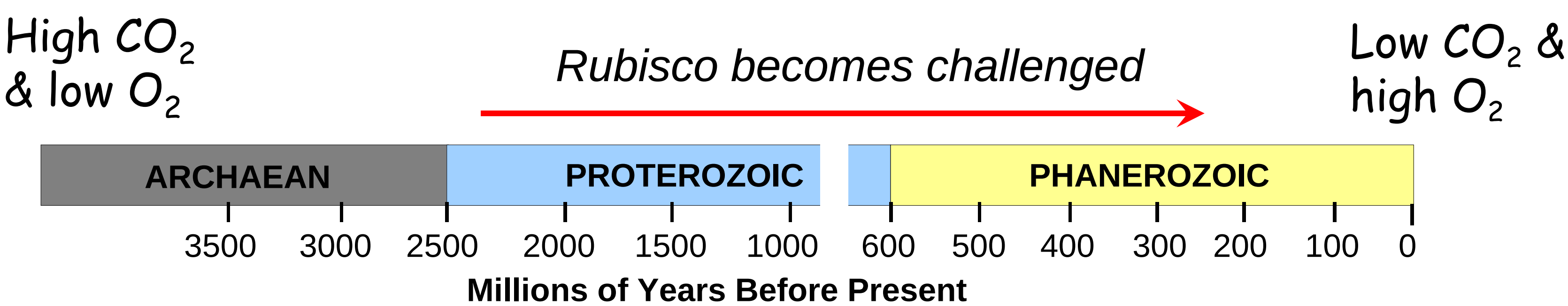
The problems for Rubisco



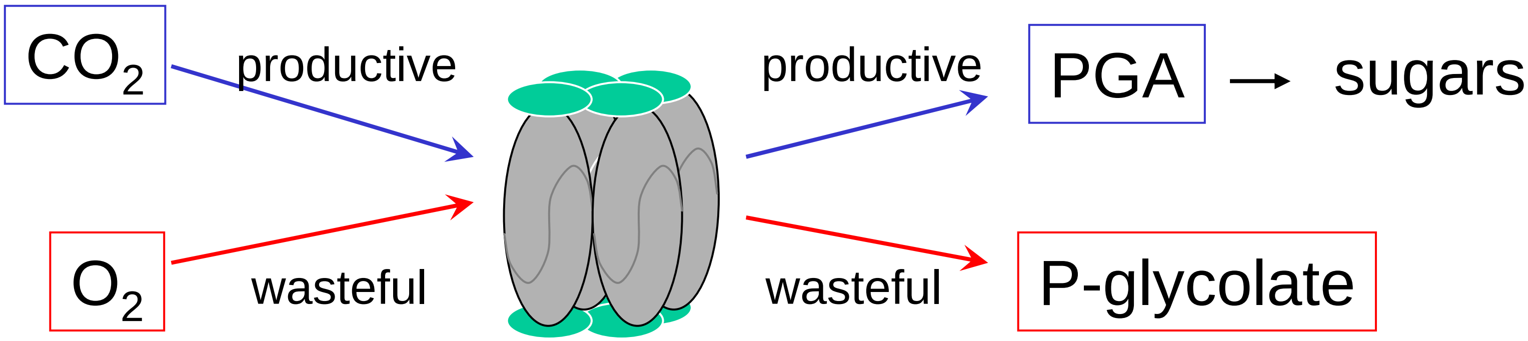


Problems for Rubisco

- *Low affinity for CO_2*
- *Oxygen as an alternate substrate*
- *Problems increase with temperature*



Two evolutionary choices to solve the problems



Problems for Rubisco

- *Low affinity for CO_2*
- *Oxygen as an alternate substrate*
- *Problems increase with temperature*

protein evolution ***Turbo-charging***

Evolve a better Rubisco

- *Higher affinity for CO_2*
- *More discerning for CO_2*
- *But, lower catalytic efficiency*

& higher N requirements

Develop a CO_2 Concentrating Mechanism (CCM)

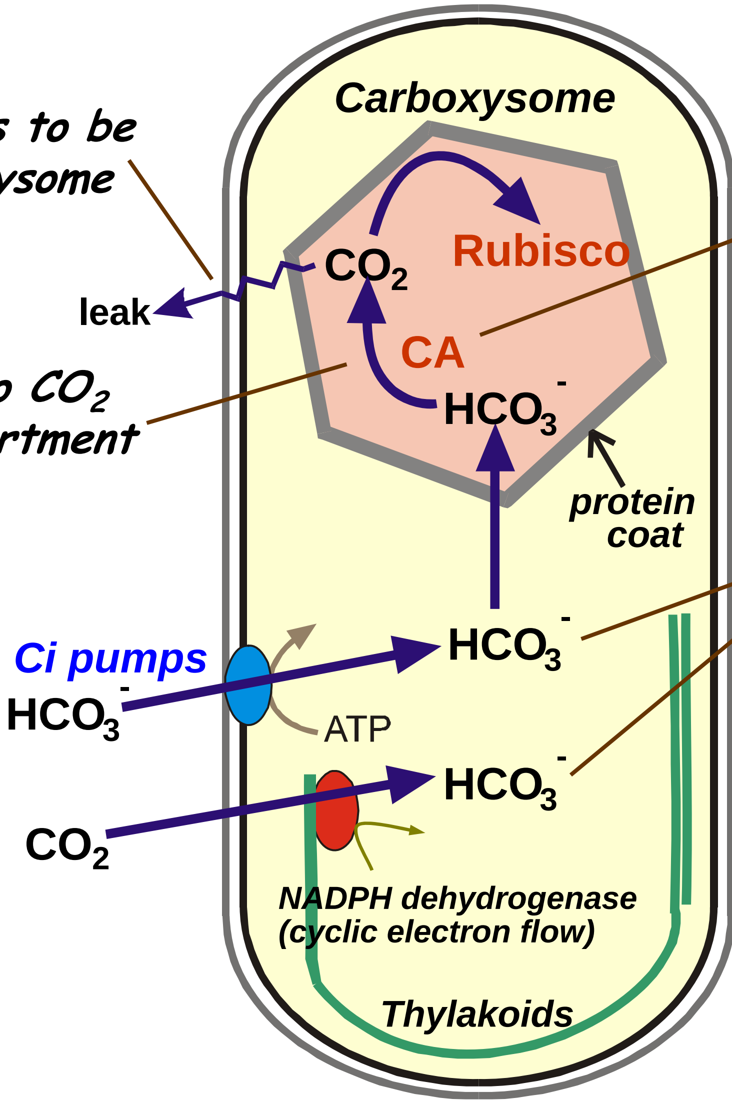
- *Cyanobacteria*
- *Algae; Aquatic plants*
- *Land plants*

The CO_2-concentrating mechanism (CCM) in freshwater/oceanic cyanobacteria

CO_2 leakage needs to be minimised (carboxysome shell & CO_2 pump)

HCO_3^- converted to CO_2 inside micro-compartment containing Rubisco

Active uptake: multiple systems



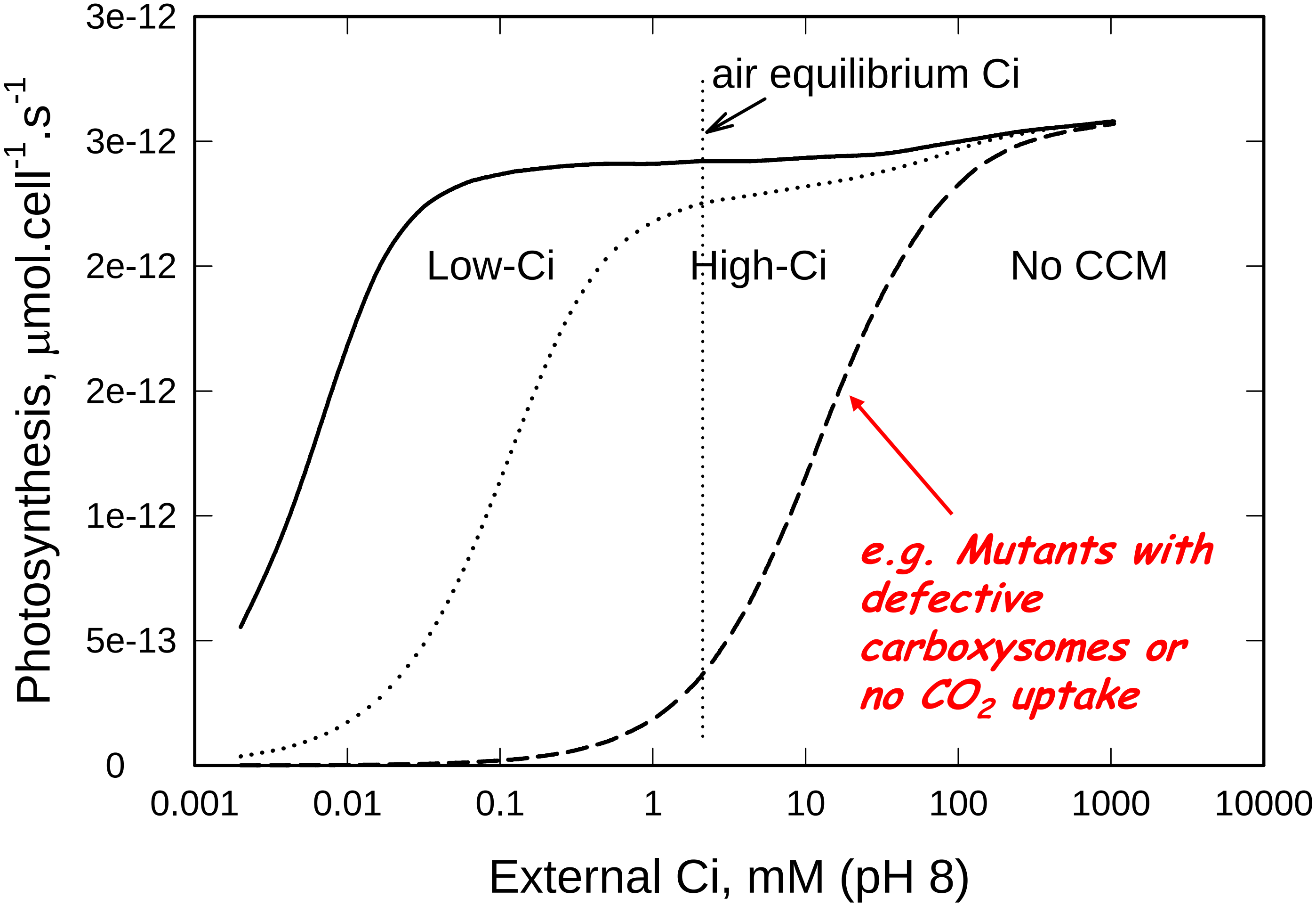
specifically localised carbonic anhydrase

Ci delivered as HCO_3^- – up to 1000x accumulation (chemical disequilibria)

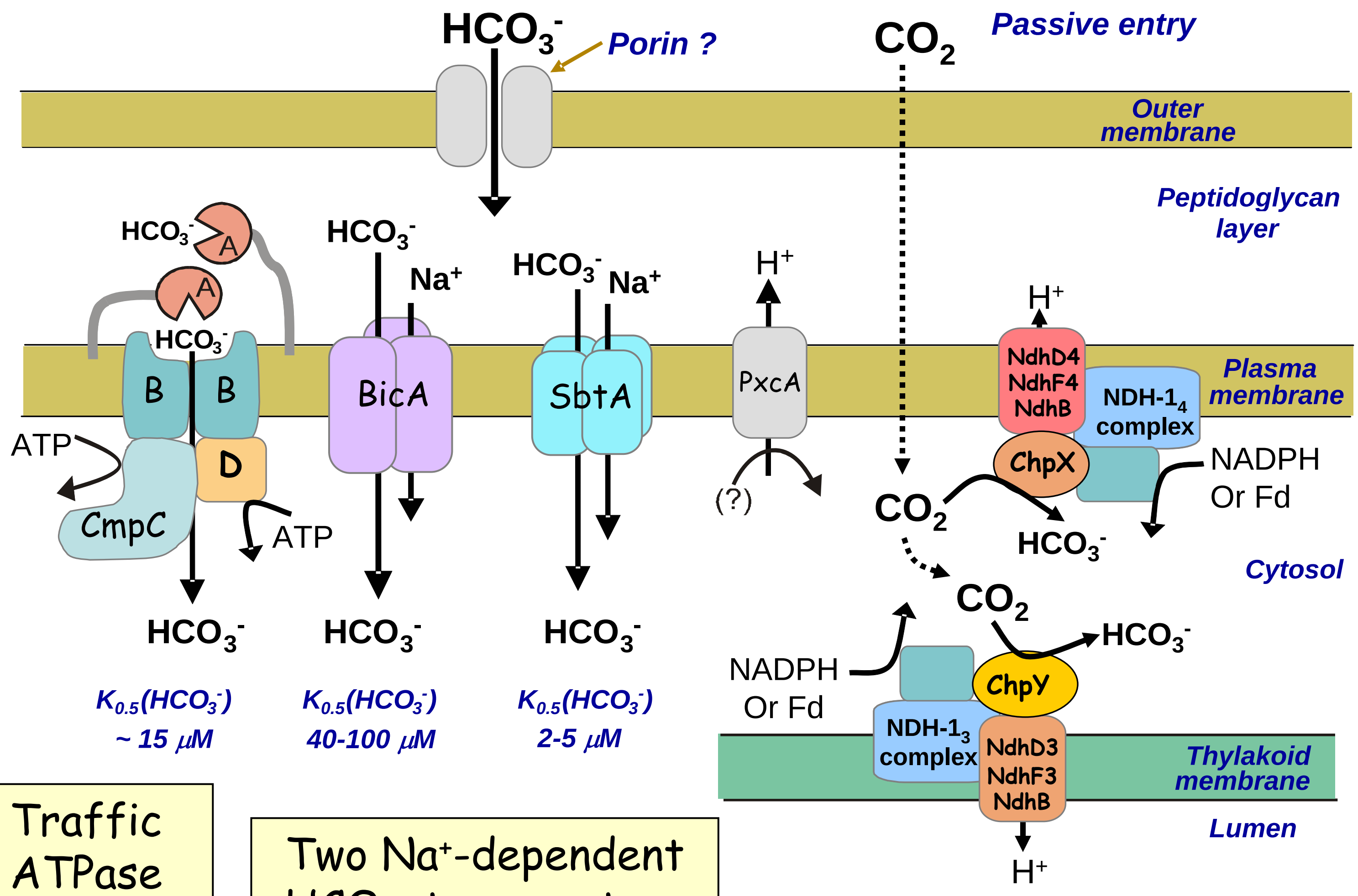
Outcomes: Rubisco operates at catalytic maximum → nitrogen-use efficiency & very low photorespiration → better ecological fitness.

How effective is the cyanobacterial CCM?

Plot of Ci response for low and high-Ci cells



The suite of Ci-uptake systems in cyanobacteria



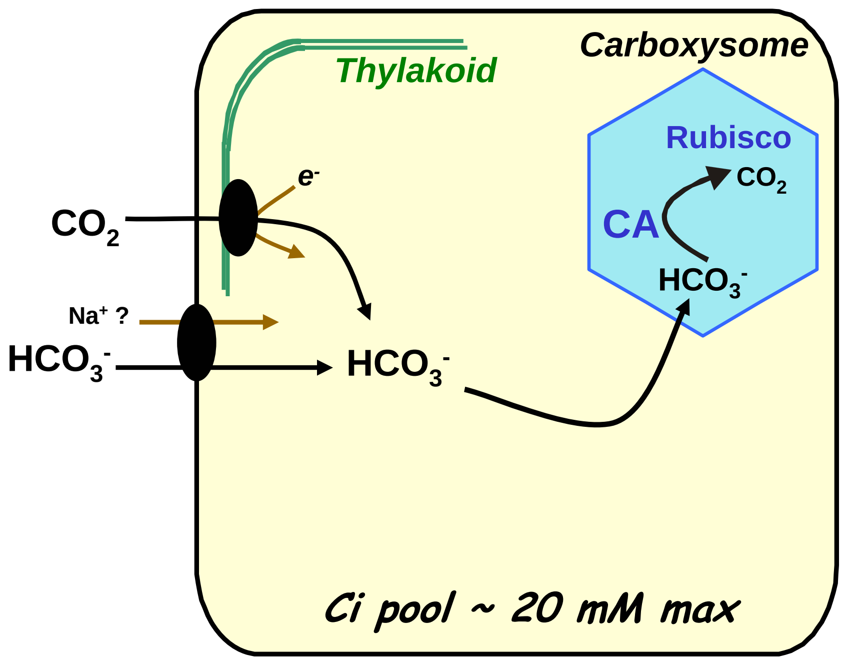
Traffic ATPase (BCT1)

Two Na^+-dependent HCO_3^- transporters

Two CO_2 uptake systems

The cyanobacterial CCM has two extreme states:
Needed to evolve genetic systems for fine control of expression

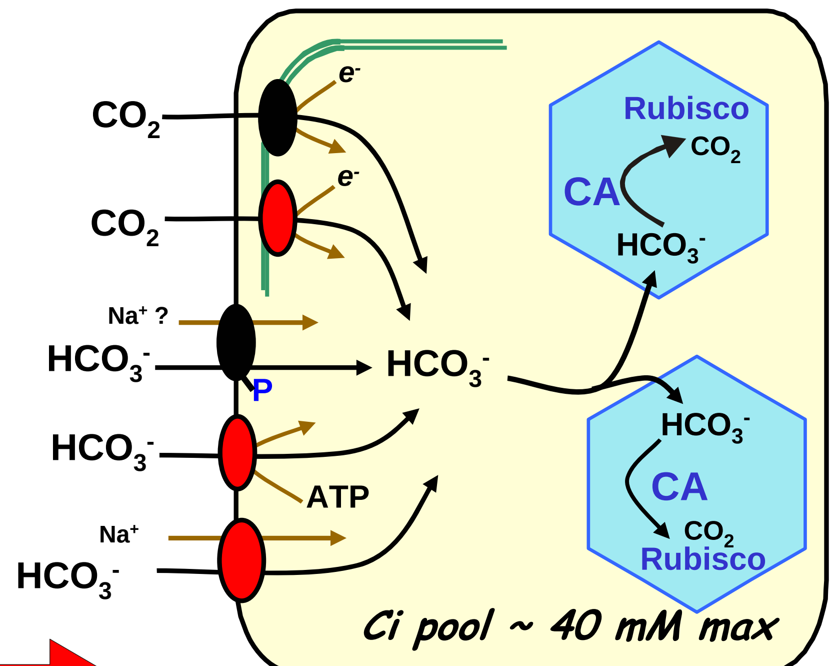
Constitutive:
lower affinity state
(Ci excess eg. 5% CO_2 in air)



Net transport affinity
$K_{0.5}$ (Ci) ~ 200 μM

Low CO_2 induction

Inducible:
higher affinity state
(Ci deficiency eg. 20 ppm CO_2)



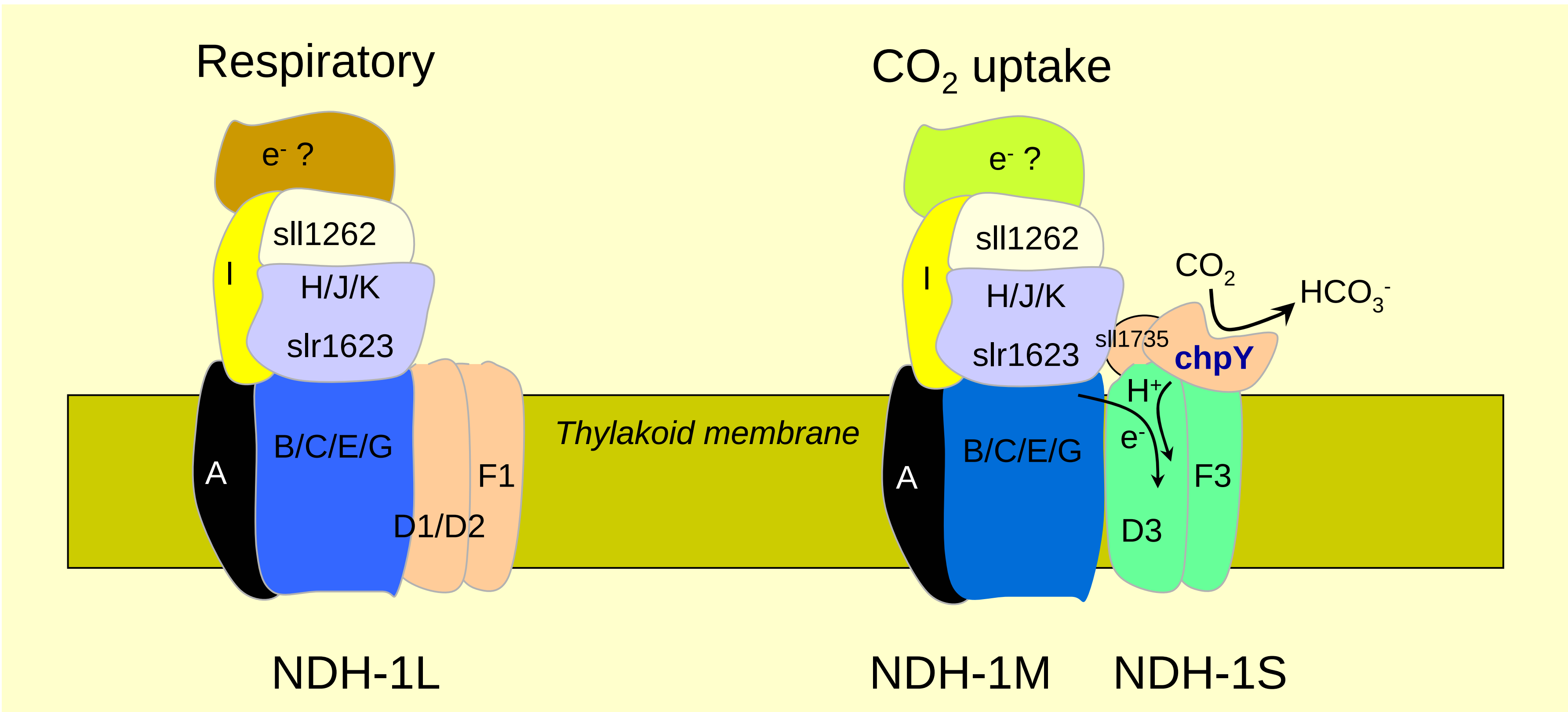
Net transport affinity
$K_{0.5}$ (Ci) ~ 10-15 μM

Ci Transporters probably evolved from existing nutrient transporters or complexes

Uptake System	CCM Function	Closest homologue	Homologue Function
CmpABCD (BCT1)	High affinity HCO_3^- uptake; Traffic ATPase	NrtABCD - NRT1 bacterial ABC transporter	High affinity nitrate/nitrite uptake
BicA	Medium affinity HCO_3^- uptake; Na^+-dependent	SulP family (present in Eukaryotes & prokaryotes)	Proton dependent sulphate uptake
SbtA	High affinity HCO_3^- uptake; Na^+-dependent	Sodium symporter family (prokaryotes)	Major facilitator superfamily (MFS) transporter
Ndh-I_3	Low affinity CO_2 uptake; Constitutive; ChpX unique protein	NADPH dehydrogenase respiratory complex	Respiratory complex
Ndh-I_4	High affinity CO_2 uptake; Low-CO_2 inducible; ChpY (related to ChpX)	NADPH dehydrogenase respiratory complex	Respiratory complex

Two types of NDH-1 complexes in thylakoids

NDH-1 = NAD(P)H dehydrogenase (Plastoquinone oxidoreductase)



14+ proteins

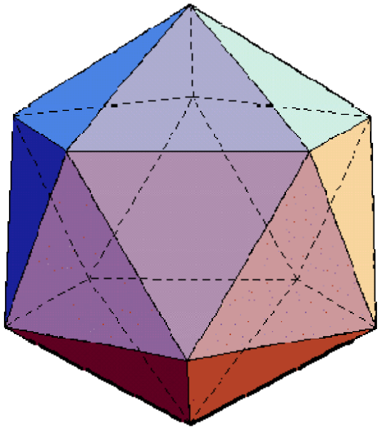
15+ proteins

Herranen et al., 2004. Plant Physiology 134: 470-481

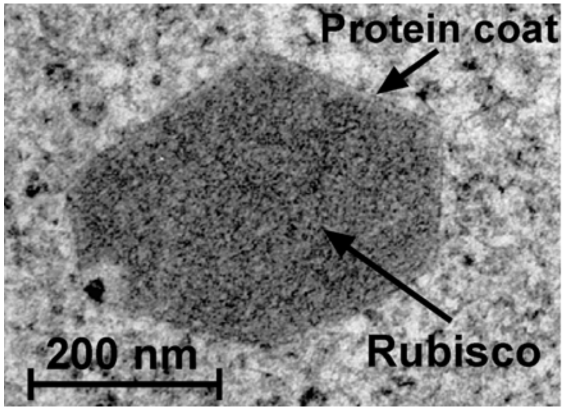
Prommeenate et al., 2004. J Biol Chem 279: 28165-28173

Carboxysome function & protein make-up

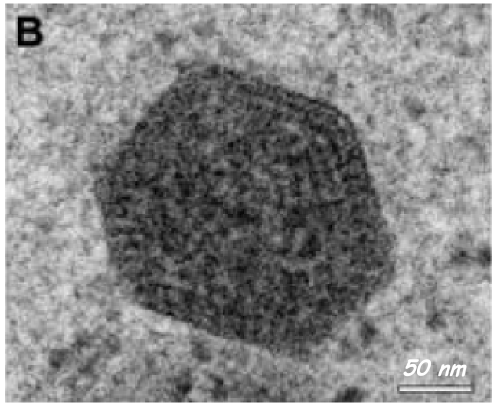
- *Carboxysomes are micro-compartments (mini-organelles) that are essential for efficient CO_2 fixation in cyanobacteria.*
- *Rubisco is packaged into the polyhedral structure*



Icosahedron (20 identical facets)



Anabaena carboxysome



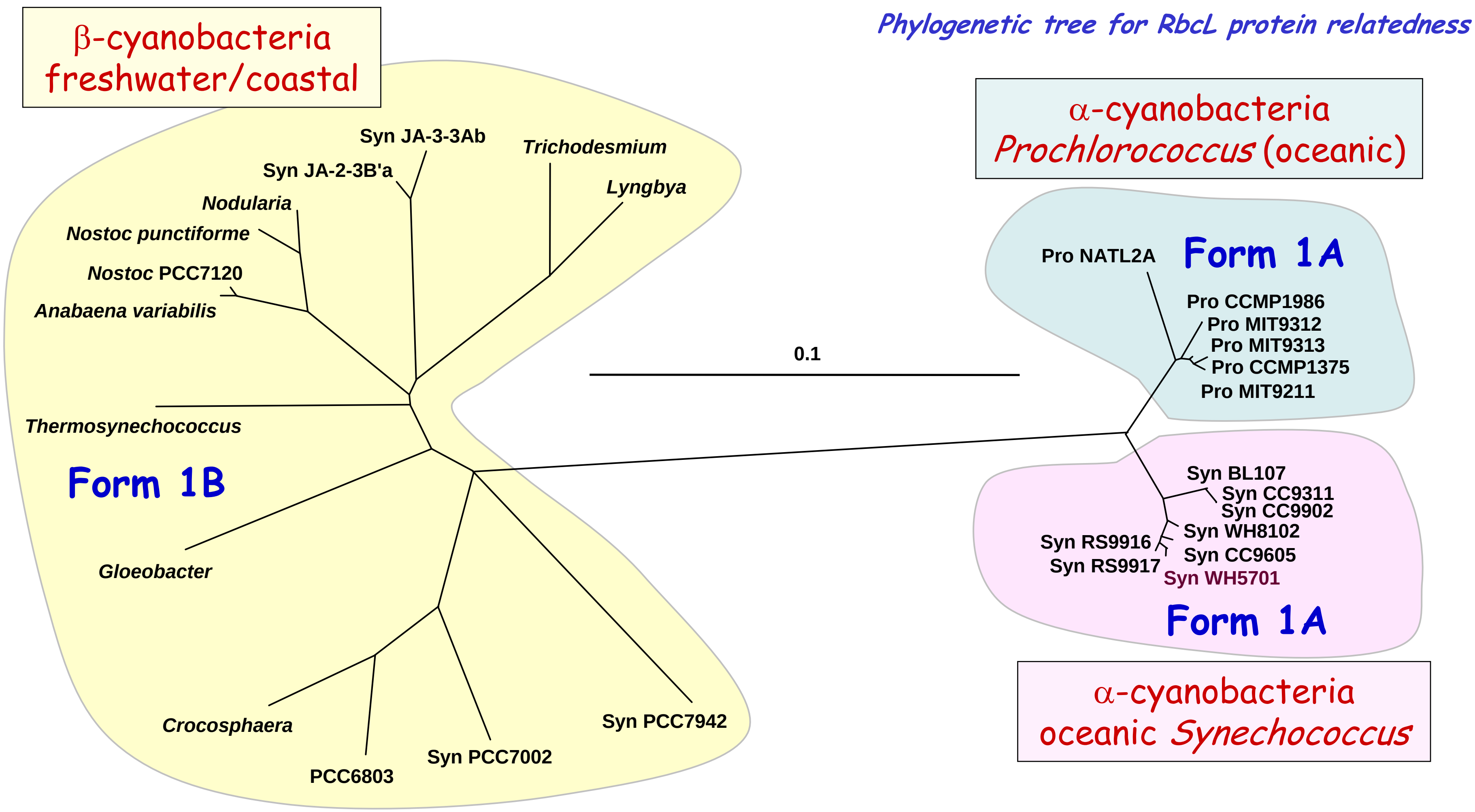
PCC6803 carboxysome

(Kerfeld et al Science 2005)

On the basis of Rubisco & carboxysome types the cyanobacteria divide into two basic groups

- ***α-cyanobacteria (mostly deep oceanic forms); Form 1A.***
- ***β-cyanobacteria (freshwater and coastal species); Form 1B.***
- ***each have distinctive characteristics.***

Two groups of cyanobacteria based on Rubisco & carboxysome types



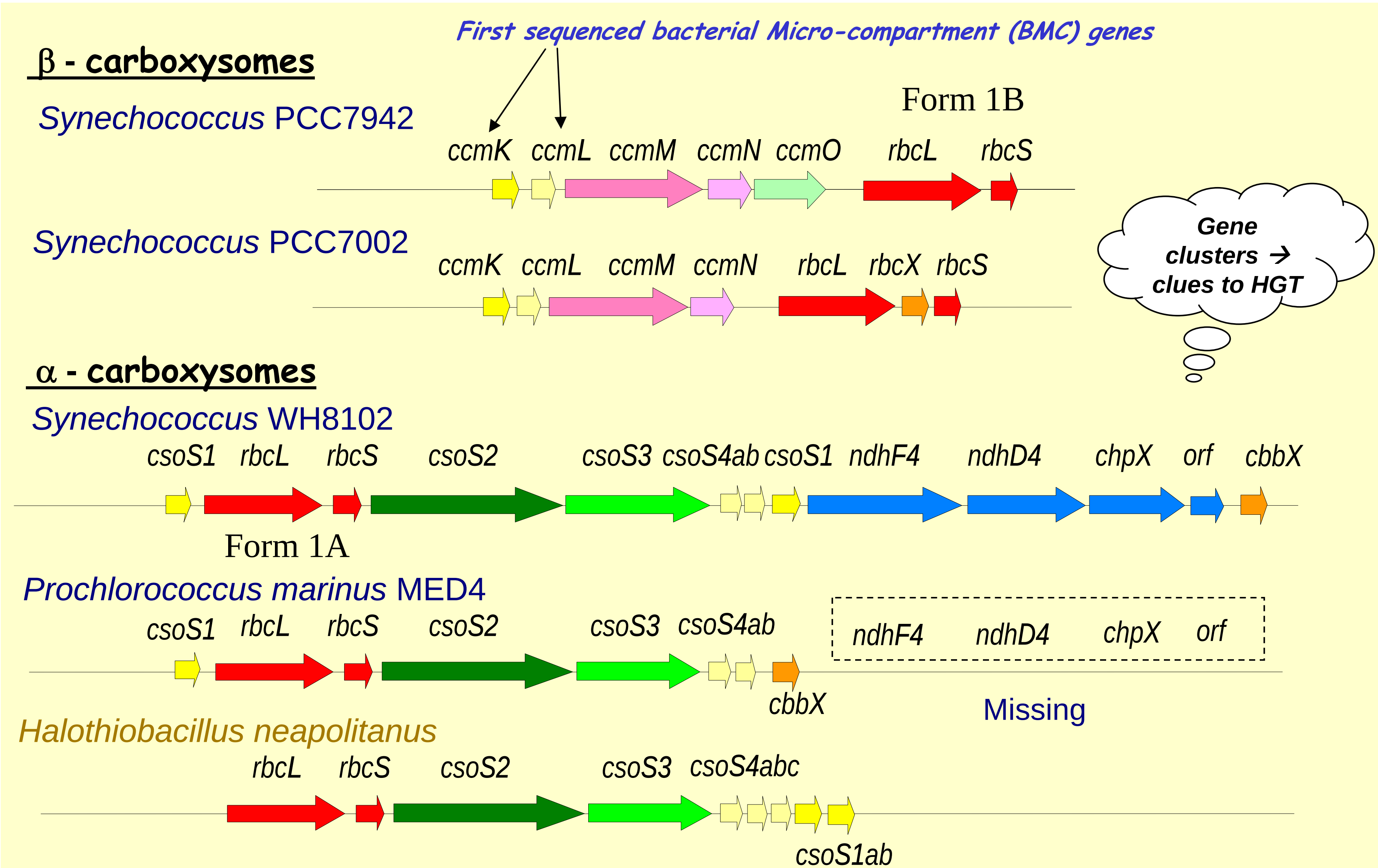
Form 1B Rubisco = β-cyanobacteria
(β-carboxysomes)

ccmKLMNO genes, *ccaA*

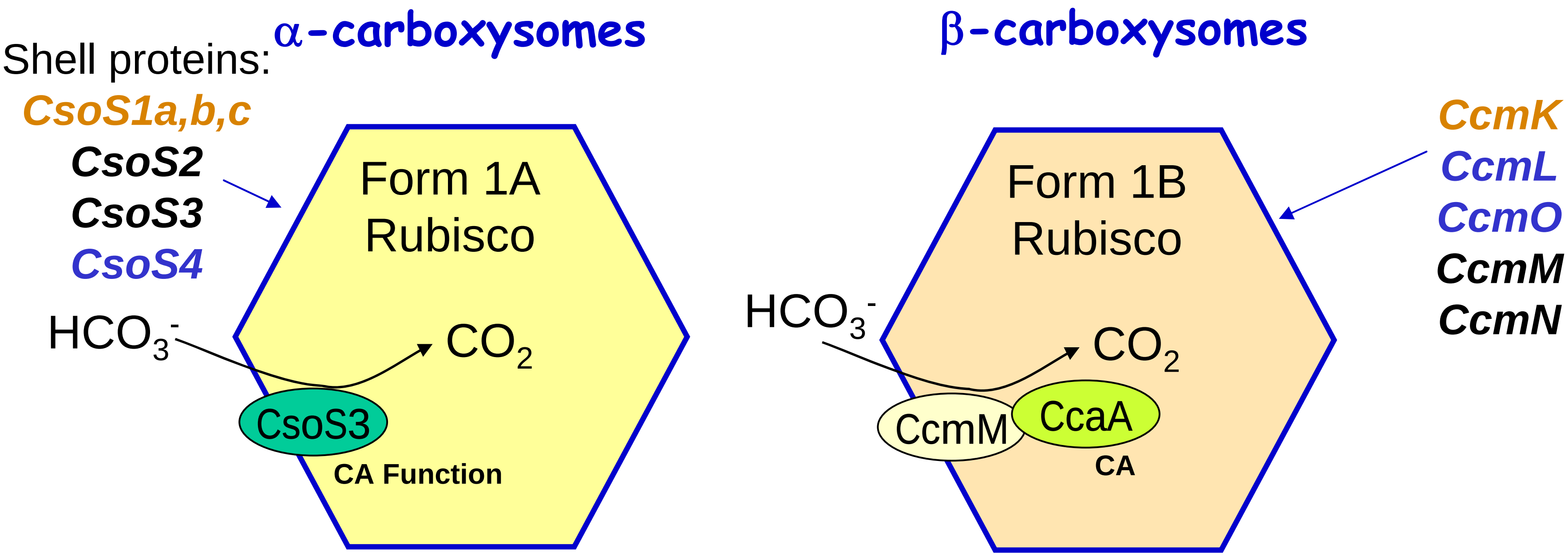
Form 1A Rubisco = α-cyanobacteria
(α-carboxysomes)

cso genes

Two types of carboxysomes in cyanobacteria



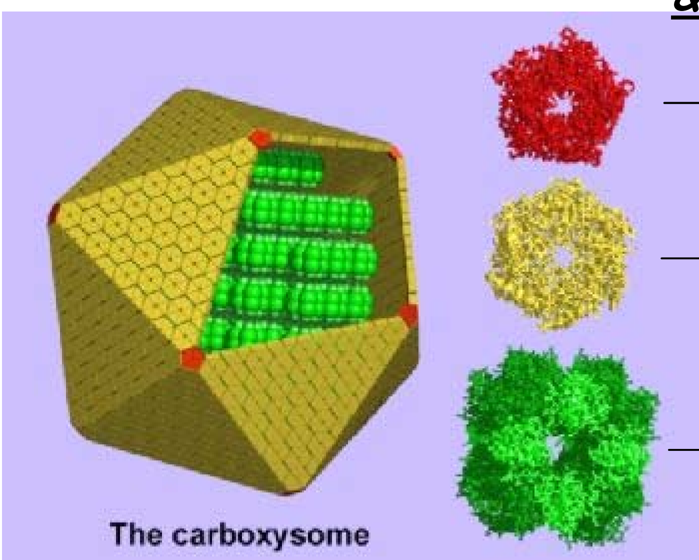
Two Carboxysome types have arisen by parallel evolution



- *Some small proteins are related to each other, and have "bacterial micro-compartment" domain signatures.*
- *CsoS2, CsoS3, CcmM & CcmN have no significant sequence homologies – but there appear to be some structural equivalents.*
- *Specific CA types vary, but the shell protein CsoS3 is a CA, and CcmM has a gamma-CA N-terminus region & CcaA is bound.*

Two carboxysome types in cyanobacteria: some proteins are related

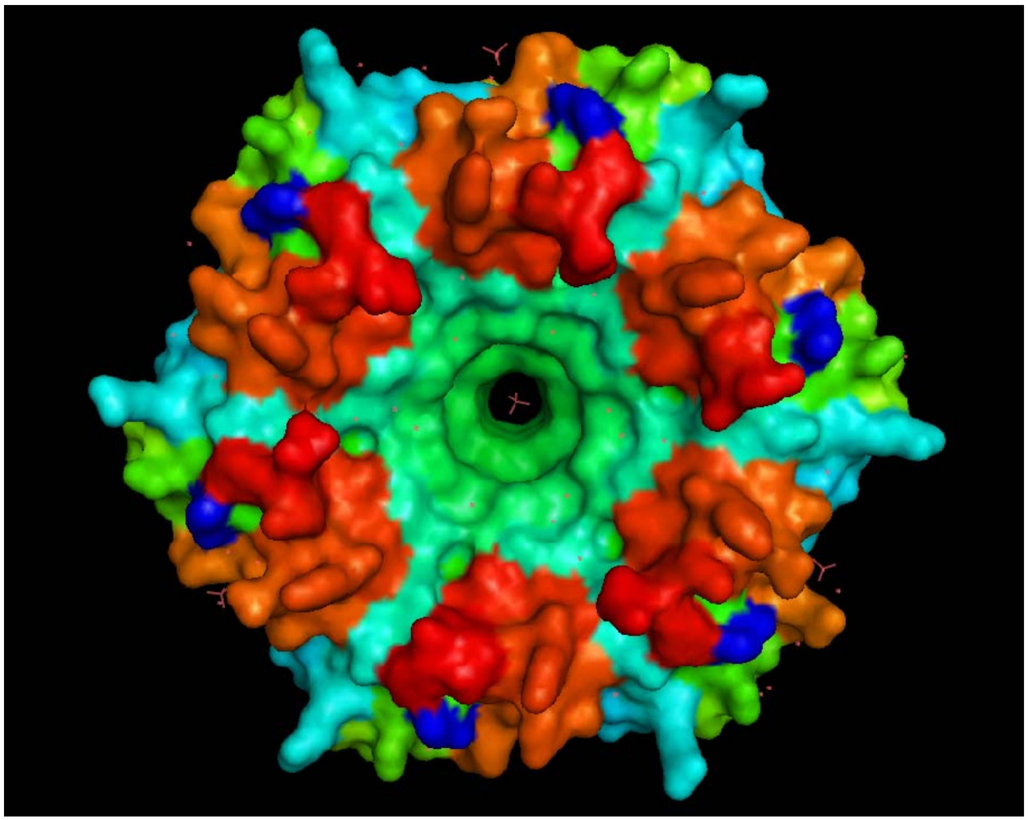
- ***Some proteins are related at an amino acid sequence and structural level.***
- ***prime evidence of HGT***



α-cyanobacteria & sulphur bacteria	β-cyanobacteria	Salmonella enterosome*
CsoS4ab	CcmL	PduK, N & T
CsoS1abc	CcmK, CcmO	PduA, PduJ
Form-1A Rubisco	Form-1B Rubisco	NA

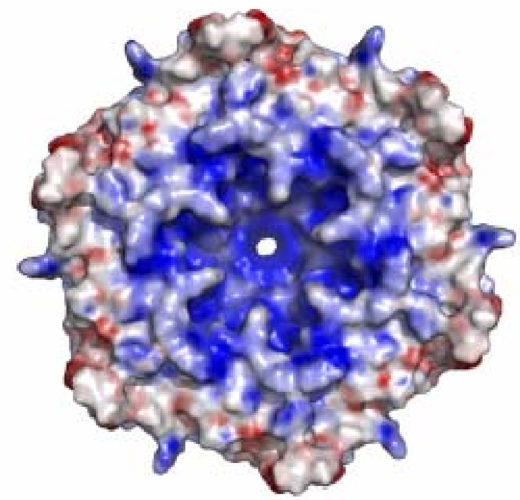
* 1,2-propanediol utilization (pdu)

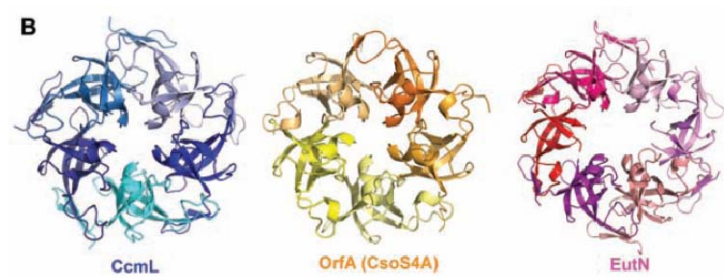
The small shell proteins have similar folded structures



Crystal structure of ccmK1 (hexameric)

CsoS1

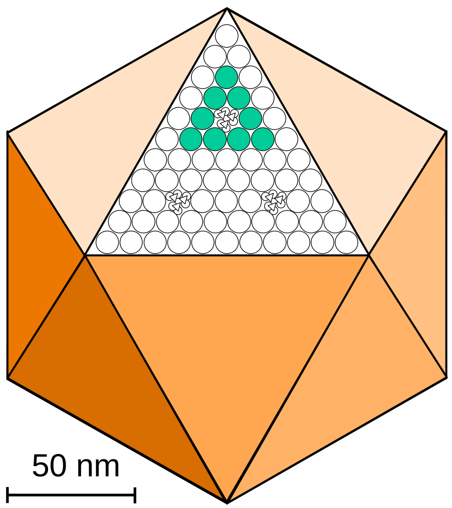




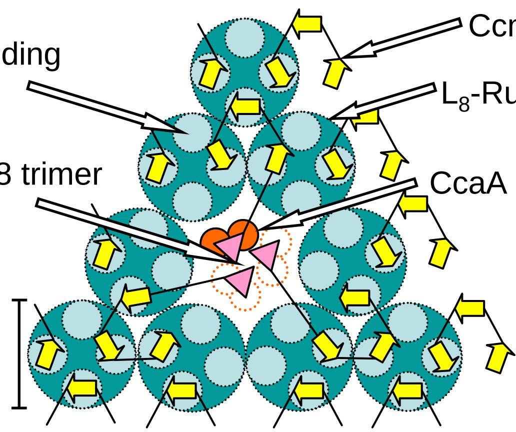
ccmL homologues (pentameric)

Work of S Kerfeld & T Yeates, Berkeley

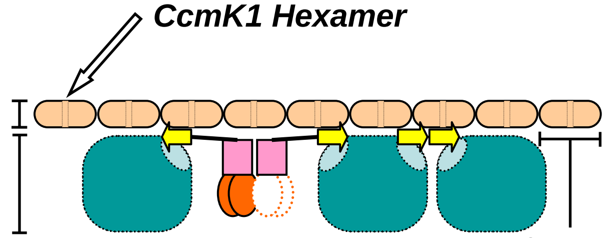
A Model for carboxysome shell formation based on CcmM-Rubisco complexes:



Icosahedral carboxysome



CcmM58 trimer nucleus

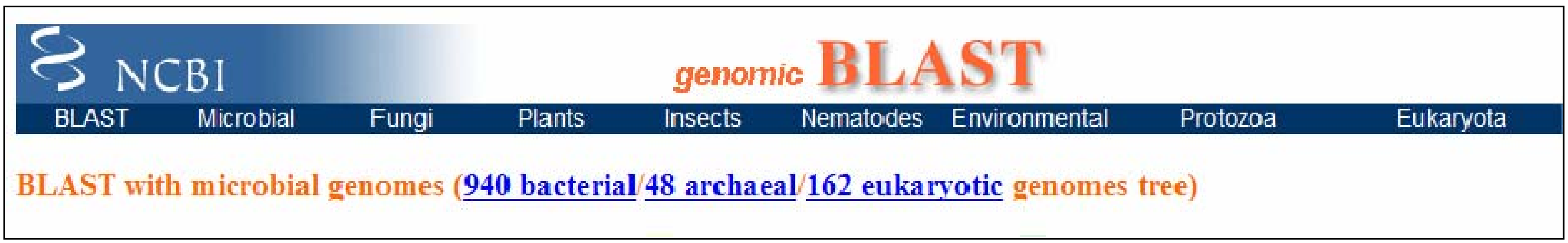


Cross-sectional view

Proposed that each CcmM58-trimer-nuclei can associate with CccM35 units to form a 7-unit core as the basis for each facet.

Long et al., J. Biol Chem, 2007

CCM Diversity
(Resources: 42 fully sequenced genomes)



Diversity of Ci-transporters in cyanobacteria

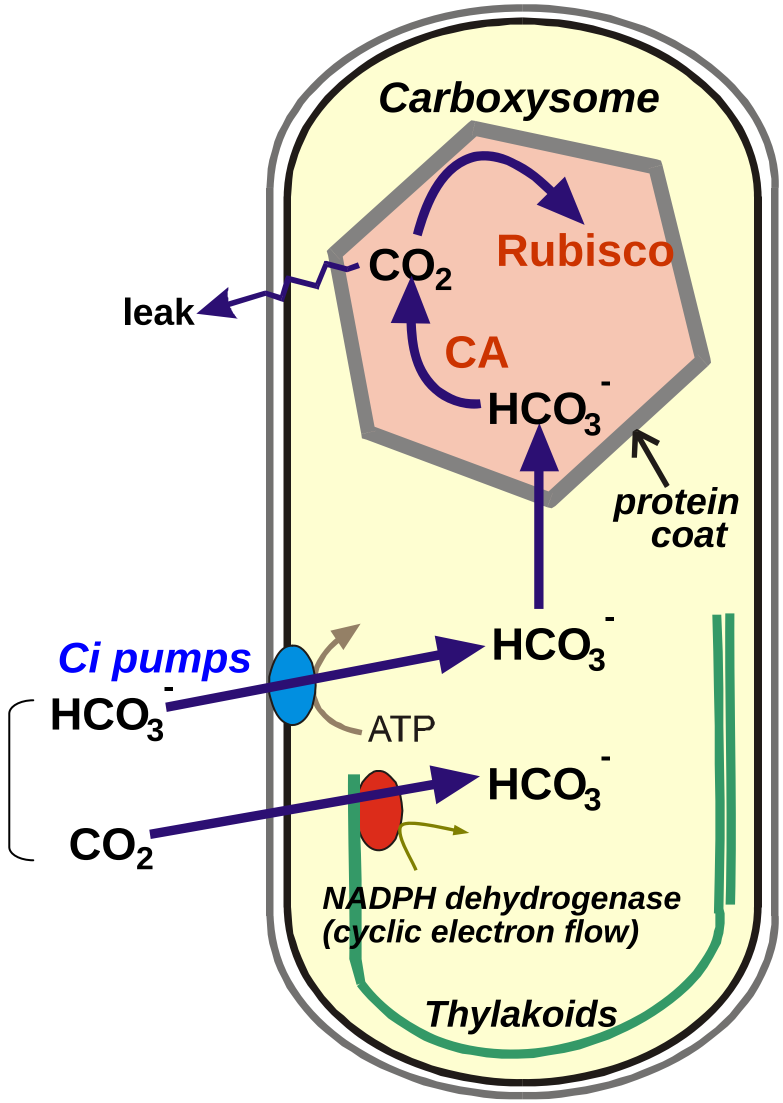
Habitat	Species	Bicarbonate uptake			CO_2 uptake	
		BCT1	SbtA	BicA	Ndh-1_4	Ndh-1_3
freshwater	*Synechocystis* PCC6803	Medium	High ☑	High ☑	Medium ☑	Medium ☑
freshwater	*Synechococcus* PCC7942	Medium ☑	High ☑	Low	Medium ☑	Medium ☑
freshwater	*Nostoc* PCC7120	Medium	High	High	Medium	Medium
freshwater	*Anabaena variabilis*	Medium	High	High	Medium	Medium
freshwater	*Nostoc punctiforme*	Medium	Low	Low	Medium	Medium
hot springs	*Thermosynechococcus*	Medium	-	Low	Medium	Medium
terrestrial mats	*Gloeobacter violaceus*	Medium	-	-	Medium	Medium
coastal	*Synechococcus* PCC7002	-	High ☑	High ☑	Medium ☑	Medium ☑
coastal	*Synechococcus* CC9902	-	-	High	Medium	-
coastal	*Trichodesmium erythraeum*	-	-	High	Medium	-
coastal	*Crocosphaera watsonii*	-	High	High	Medium	Low
oceanic	*Synechococcus* WH8102	-	-	High ☑	Medium	-
oceanic	*Synechococcus* CC9605	-	-	High	Medium	-
oceanic	*Synechococcus* CC9311	-	Low	High	Medium	-
oceanic	*Prochlorococcus* MED4	-	Low	Medium	-	-
oceanic	*Prochlorococcus* MIT9313	-	Low	Medium	-	-
oceanic	*Prochlorococcus* MIT9312	-	Low	Medium	-	-
oceanic	*Prochlorococcus* SS120	-	Low	Medium	-	-

Homology
- High
- Medium
- Low

☑ *Verified transport activity*

No CO_2 uptake genes

When did cyanobacterial CCMs evolve?

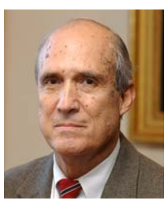


Evolution of Cyanobacterial and Algal CCMs

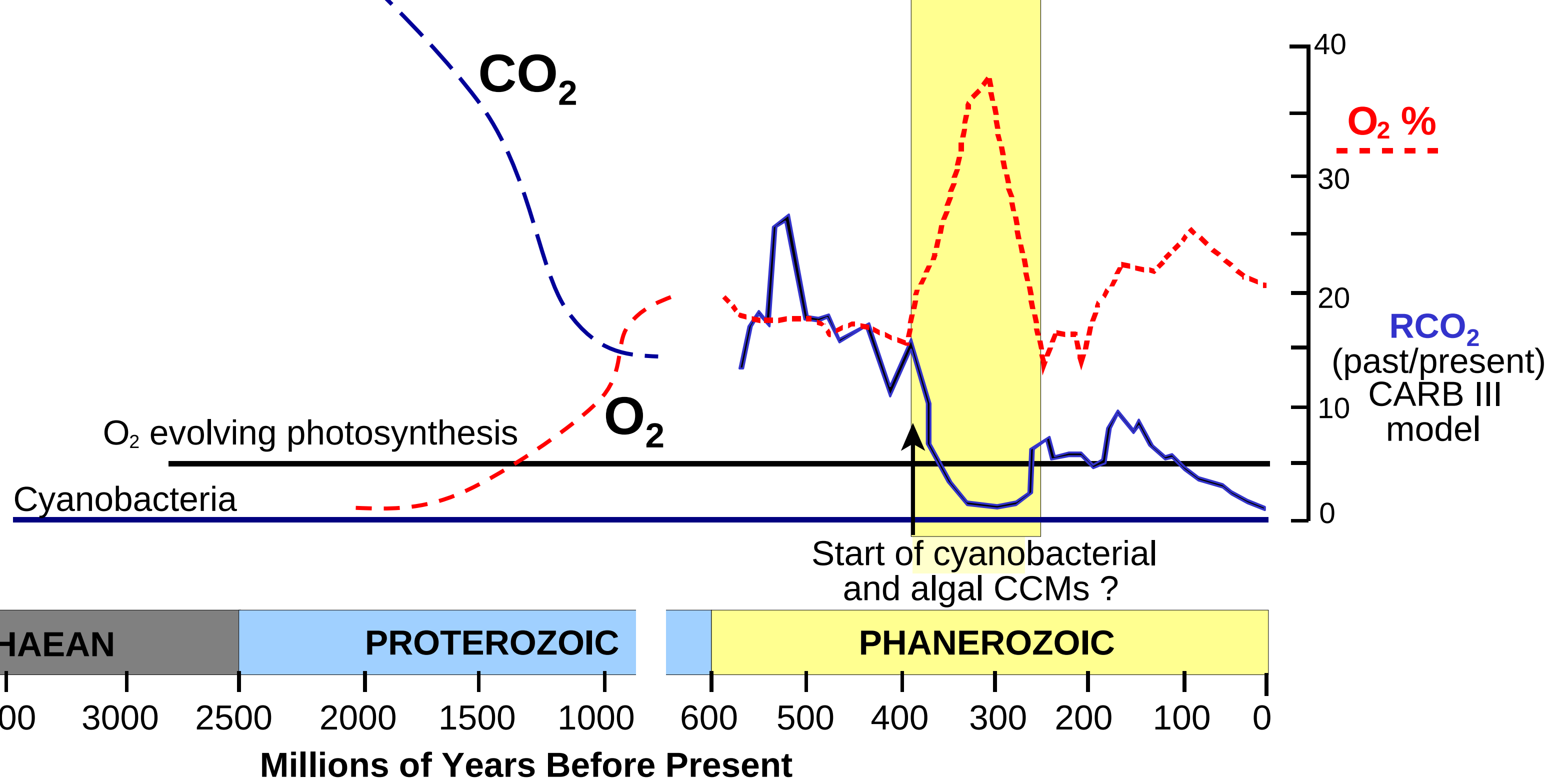
***Crunch-time*: A marked period during the Carboniferous when a sharp decline in CO_2 levels occurred along with a sharp rise in O_2 levels.**

→ Maximum selection pressure for CCMs

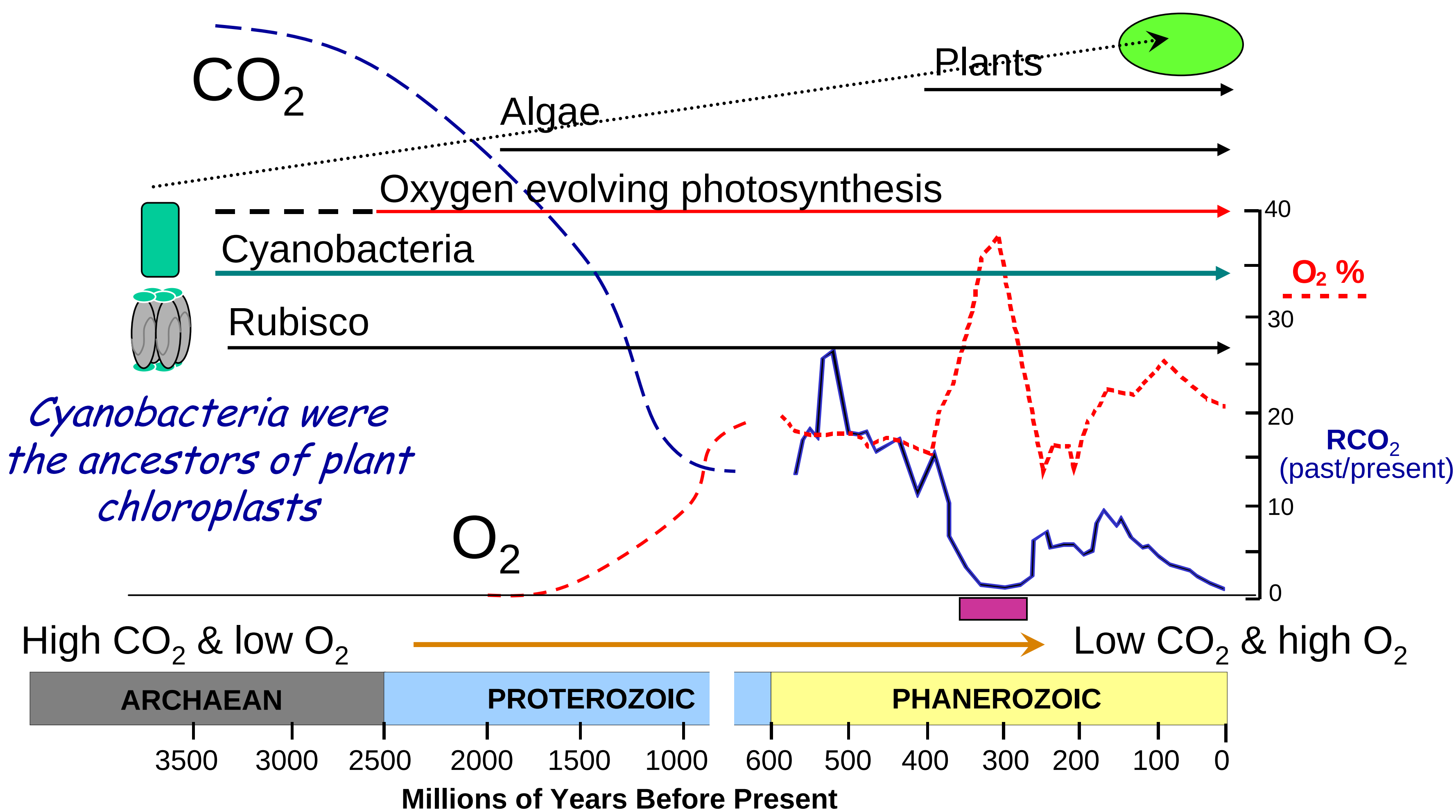
John Raven, U. Dundee



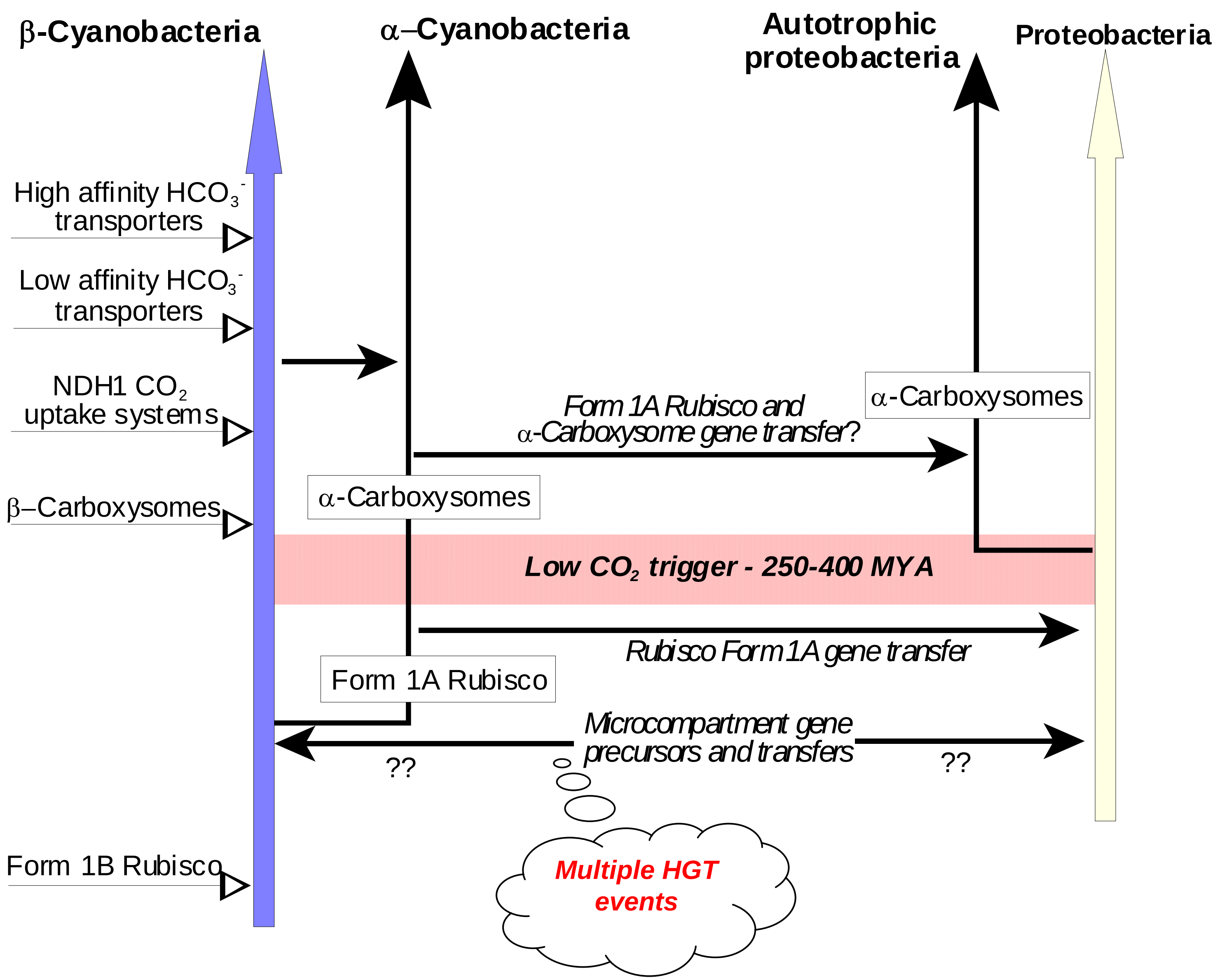
Bob Berner, Yale



Evolution of photosynthesis & atmospheric side-effects



The potential path of CCM evolution in cyanobacteria



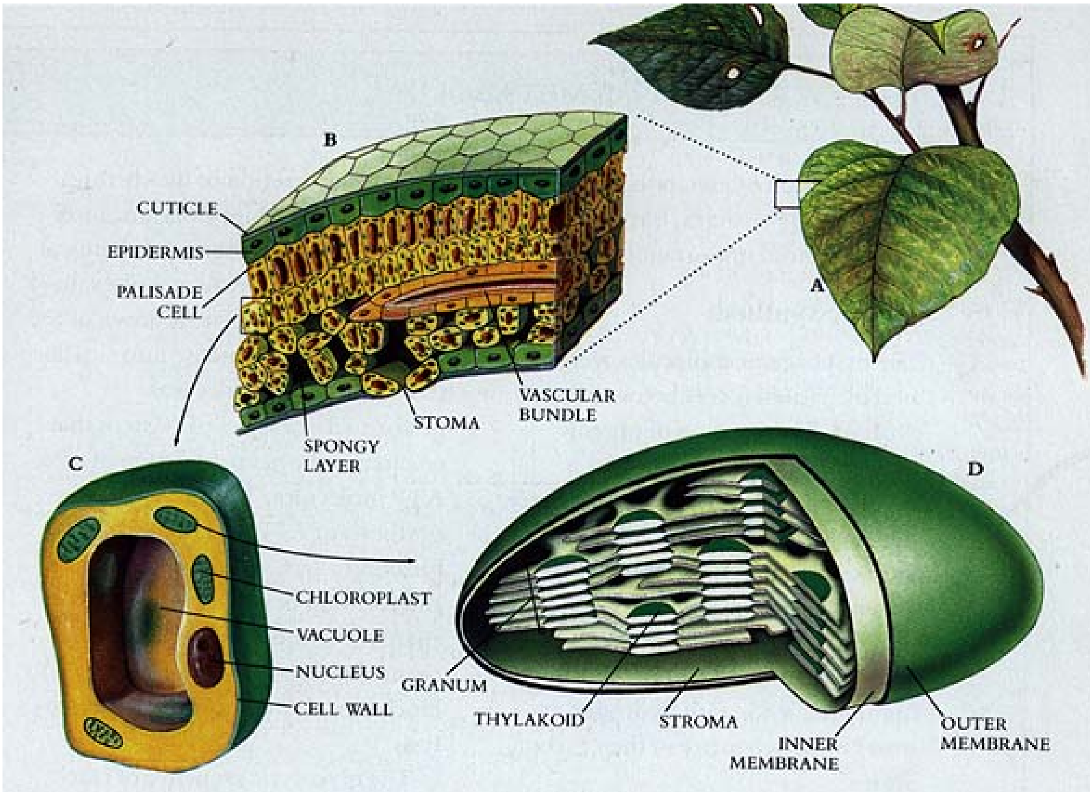
Transferring parts of the cyanobacterial CCM to C_3 chloroplasts?? The goal of improving water and nitrogen-use efficiencies

wheat

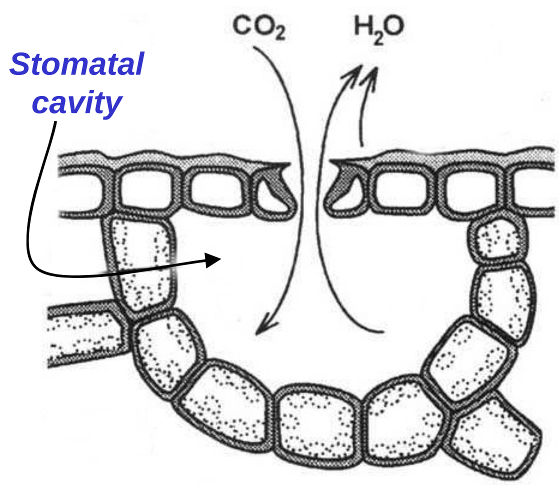
canola

Most key crop plants do NOT have a CCM (known as C_3 plants)

Prospects for Genetic Engineering



Stomata on a leaf surface



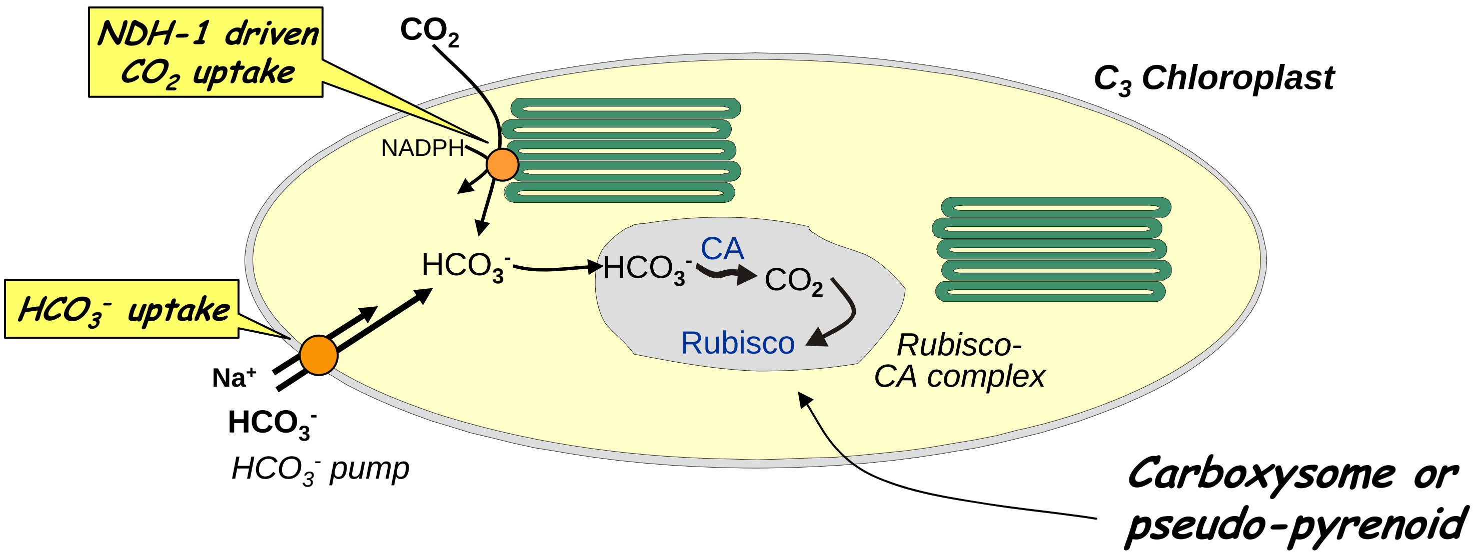
- C_3 crops plants lose around 500 water molecules for every CO_2 gained by passive diffusion via stomata (high water cost).

- A large fraction of cellular nitrogen is devoted to the key carboxylase, Rubisco (a large nitrogen cost).

- But, C_4 plants such as maize and sugar cane have a complex CCM and use ~ half the water cf. C_3 plants

- Introduction of parts of the cyanobacterial CCM into C_3 crop plants could be expected to improve water and nitrogen-use efficiency.

Placing a basal form of the CCM in C_3 chloroplasts



Goal: improved water & Nitrogen-use efficiencies

(akin to the "C4 rice" concept, but wheat and barley are more obvious targets in semi-arid agriculture).