Croucher Course, Hongkong

July 2025

Course 3: Multi-scale study of *Synechococcus* Pigment types: F…ean





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Dept Adaptation & Diversity in the Marine Environment (UMR 7144)
Ecology of Marine Plankton (EcoMaP) Team
Roscoff, France

Croucher course 3 outline

I. <u>Introduction on spectral niches</u>

II. Characterization on *Prochlorococcus* light-harvesting complexes

III. *Synechococcus* phycobilisomes and pigment diversity

IV. Biochemical and molecular characterization of CA4

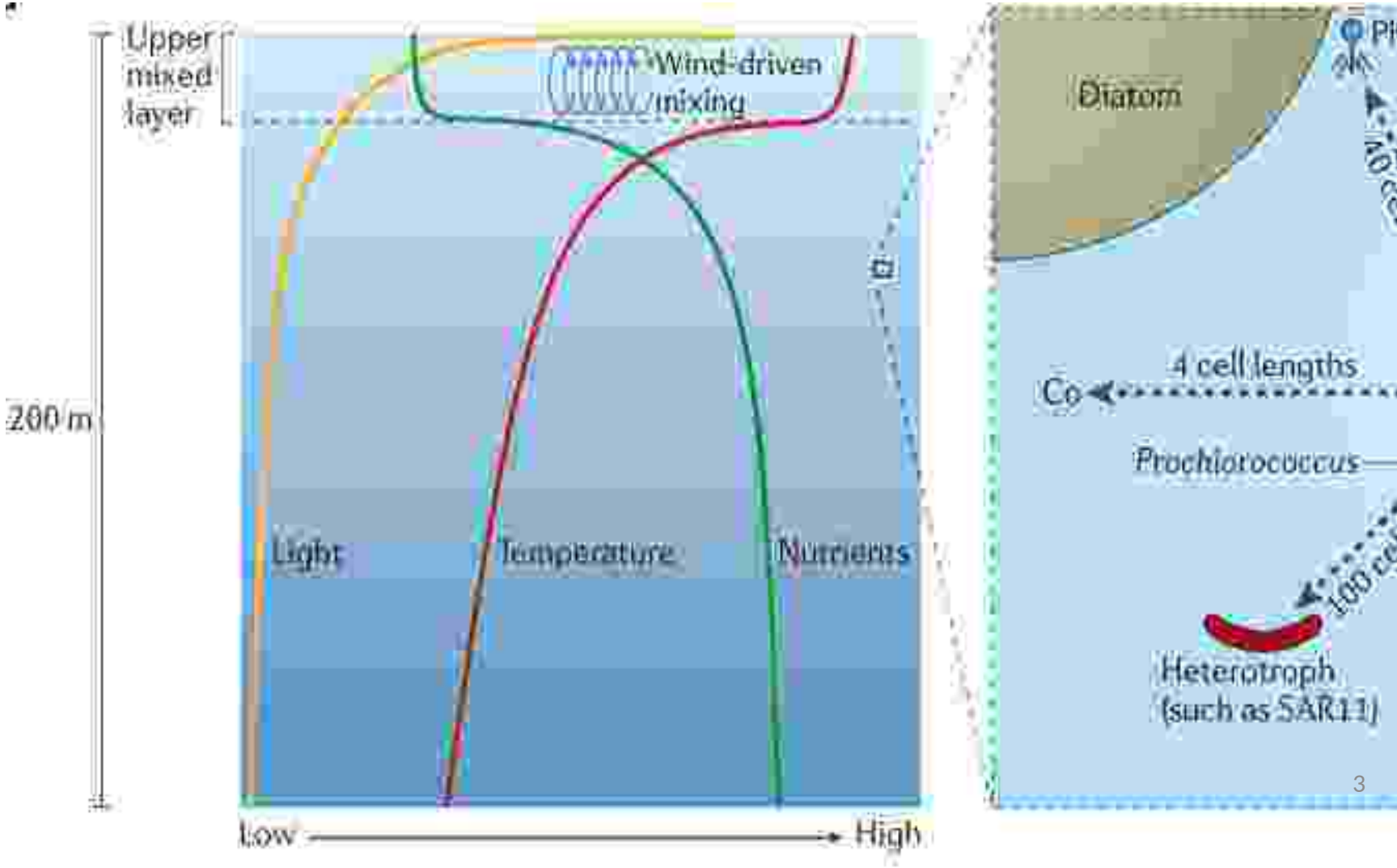
V. Evolution and diversity of pigment types

VI. Ecological distribution and modelling of pigment types



Solar energy is highly variable quantitatively and qualitatively

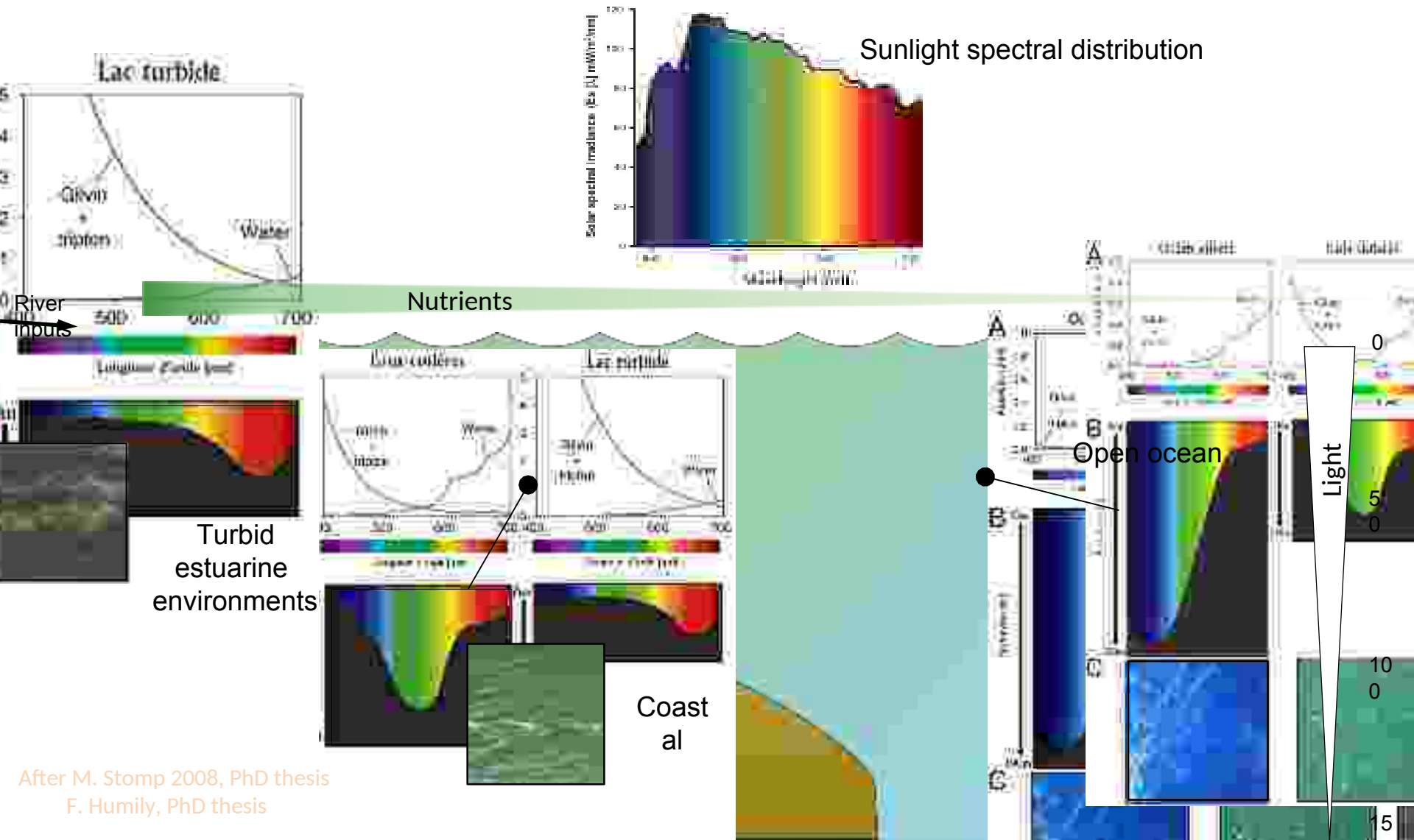
- Light quantity: exponential decrease with depth



Biller et al., 2015 Nature Rev Microbiol.

Solar energy is highly variable quantitatively and qualitatively

- Light quantity: exponential decrease with depth
- Light quality: varies with depth & along cost-offshore gradients



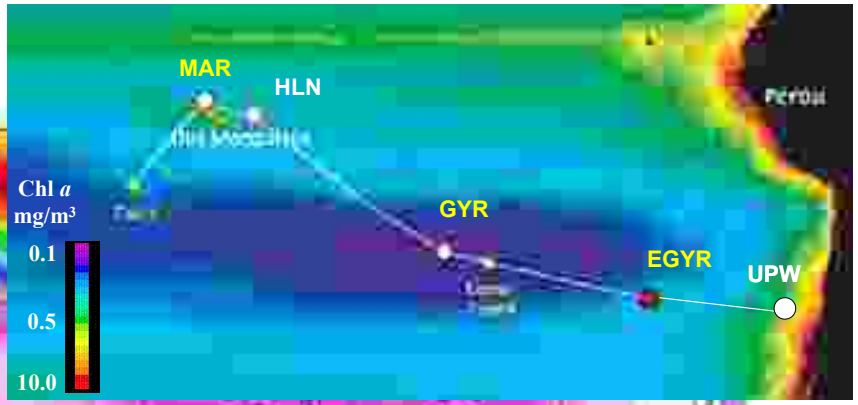
After M. Stomp 2008, PhD thesis
F. Humily, PhD thesis

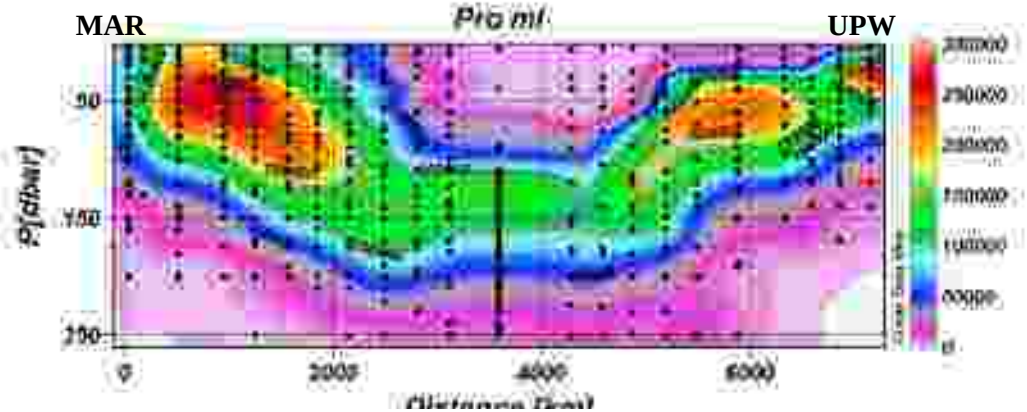
Violet waters also exist!

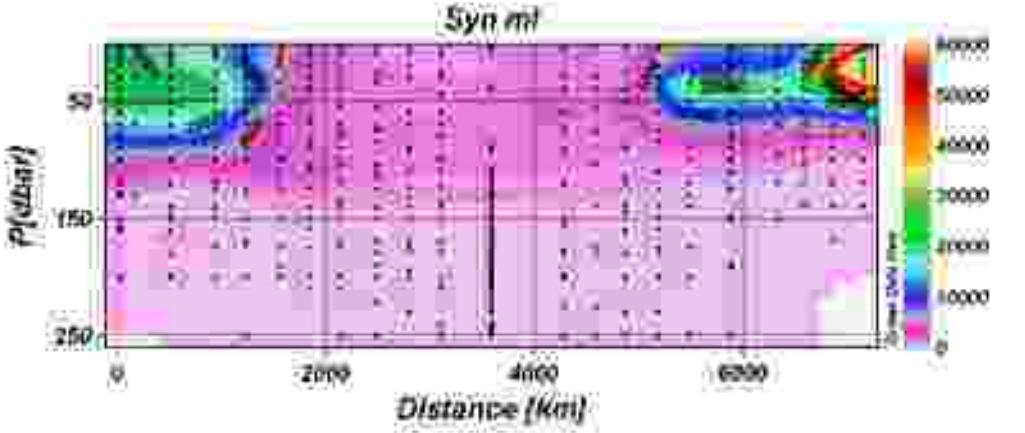
Biogeosciences, 4, 837–852, 2007

Contribution of picoplankton to the total particulate organic carbon concentration in the eastern South Pacific

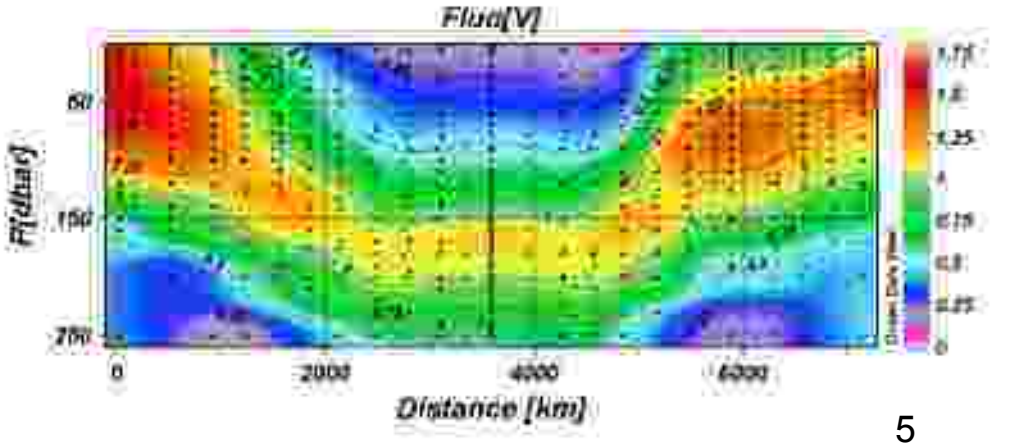
C. Grob[1,2,3], O. Ulloa[2], H. Claustre[3], Y. Huot[3], G. Alarcón[2], and D. Marie[4]









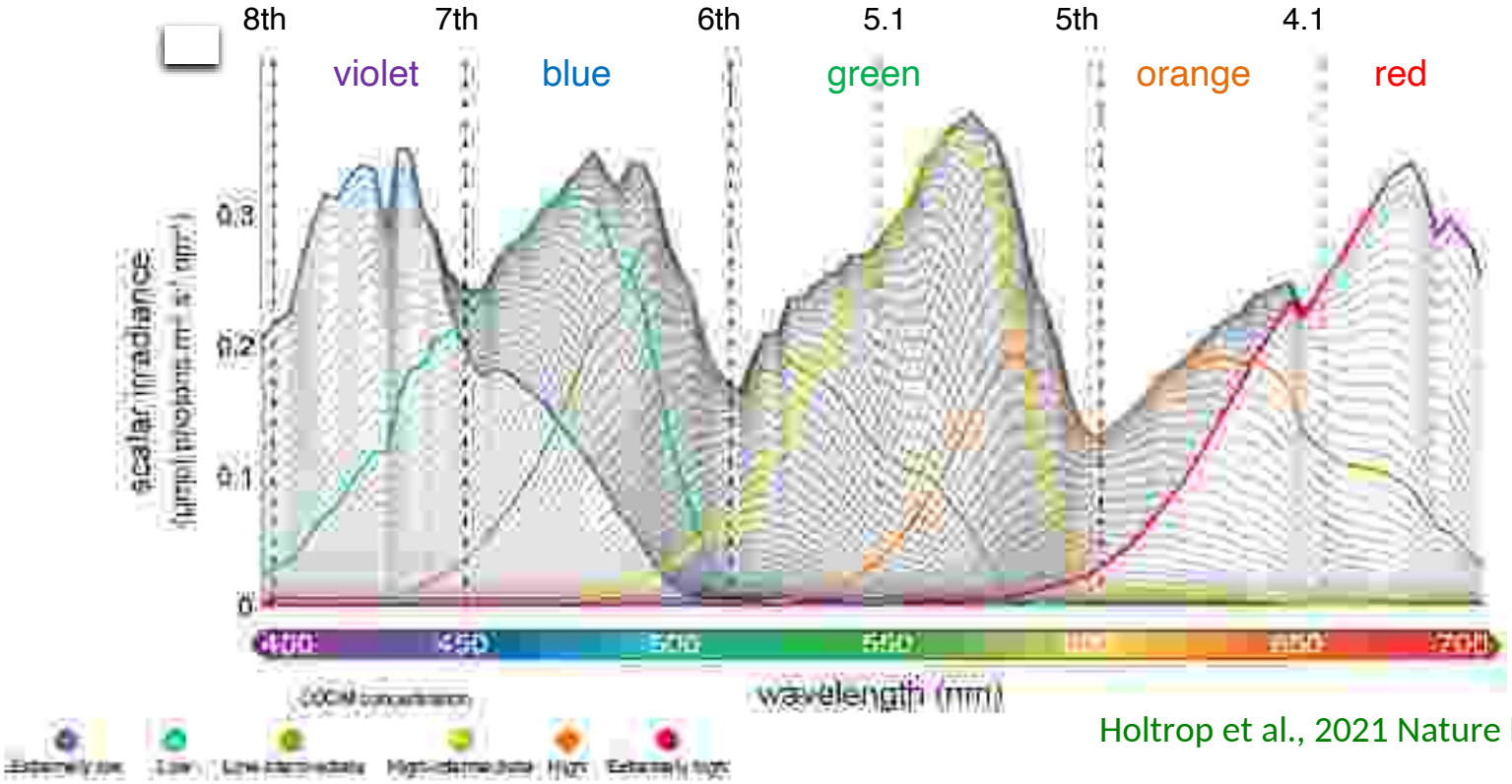


Solar energy is highly variable quantitatively and qualitatively

- Light quantity: exponential decrease with depth
- Light quality: varies with depth & along cost-offshore gradients

=> 5 spectral niches in the underwater light spectrum of aquatic environments, with valleys delineated by the vibration modes (harmonics) of the water molecule





Holtrop et al., 2021 Nature Ecol. Evol.

→ Thanks to the wide pigment diversification of phycobilisomes, *Synechococcus* has colonized all existing spectral niches, whereas *Prochlorococcus* is restricted to the violet and blue niches.

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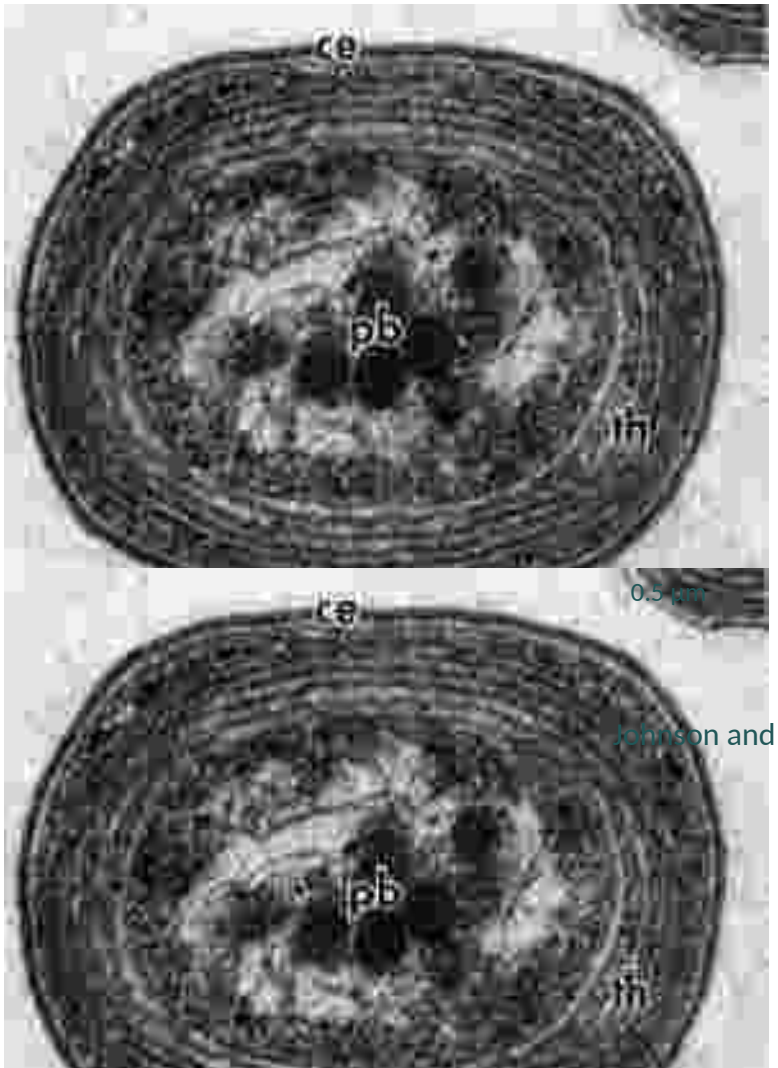
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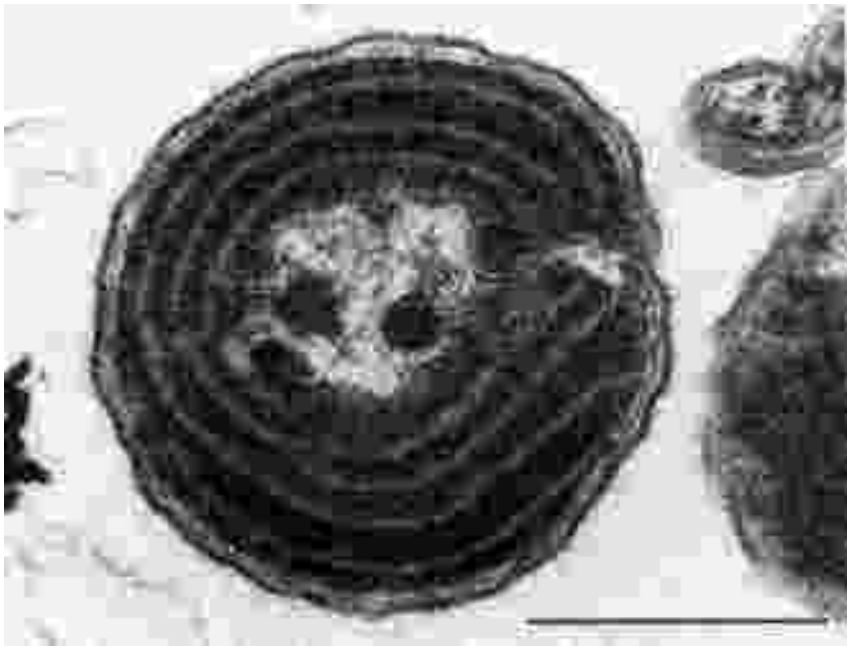


Why is the ultrastructure of *Prochlorococcus* and *Synechococcus* so different?

Prochlorococcus



Synechococcus



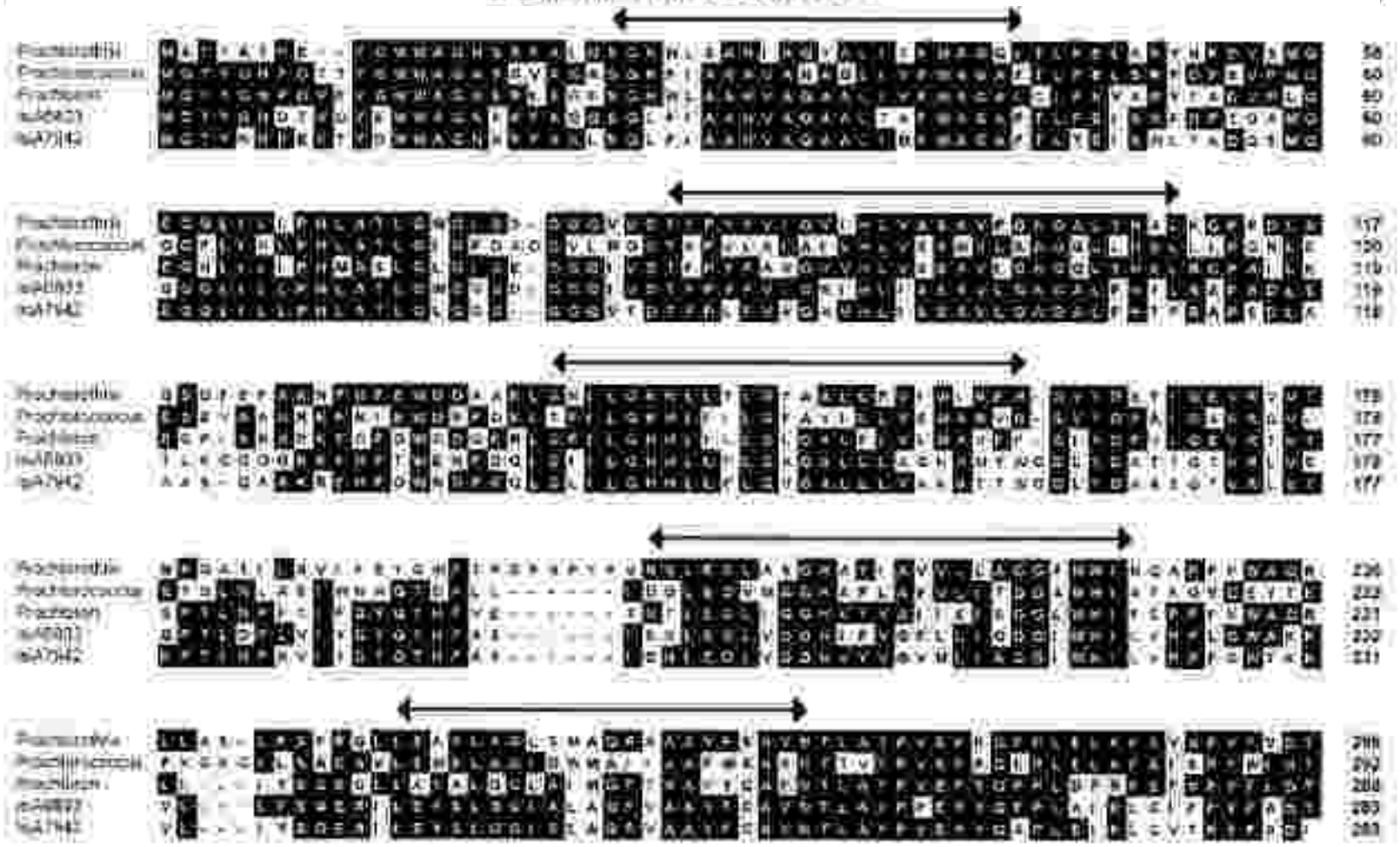
Johnson and Sieburth, 1979

Identification of *Prochlorococcus* Light-Harvesting Complexes

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 15244–15248, December 1996
Evolution

Independent evolution of the prochlorophyte and green plant chlorophyll *a*/*b* light-harvesting proteins

J. La Roche*†, G. W. M. van der Staay‡§, F. Partensky¶, A. Ducret‖, R. Aebersold‖, R. Li‖, S. S. Golden**, D. Larsen**, and R. R. Green‡‡



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Chl *a/b* (or div-Chl *a/b*) antenna dubbed « Pcb » for « prochlorophyte Chl *a/b* protein »

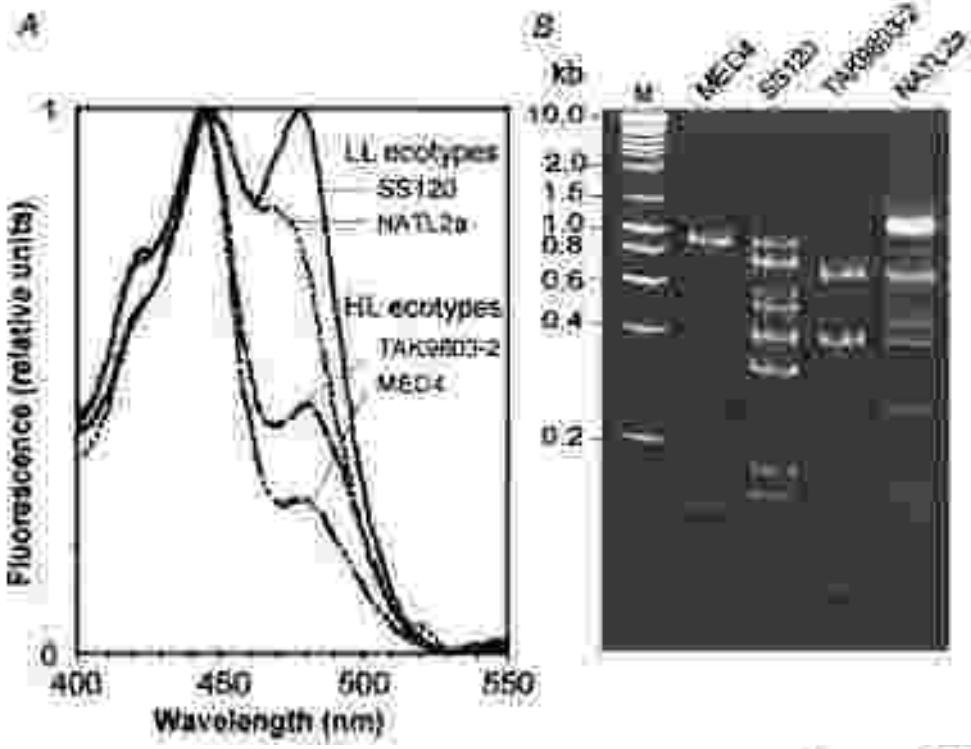
Pcb and IsiA are related to the PSII antenna PsbC (= CP43), and all have 6 transmembrane helices, while they are much more distantly related to plant or algal LHC that have 3 helices.

Characterization of *Prochlorococcus* Light-Harvesting Complexes

PNAS | April 11, 2000 | vol. 97 | no. 8 | 4099

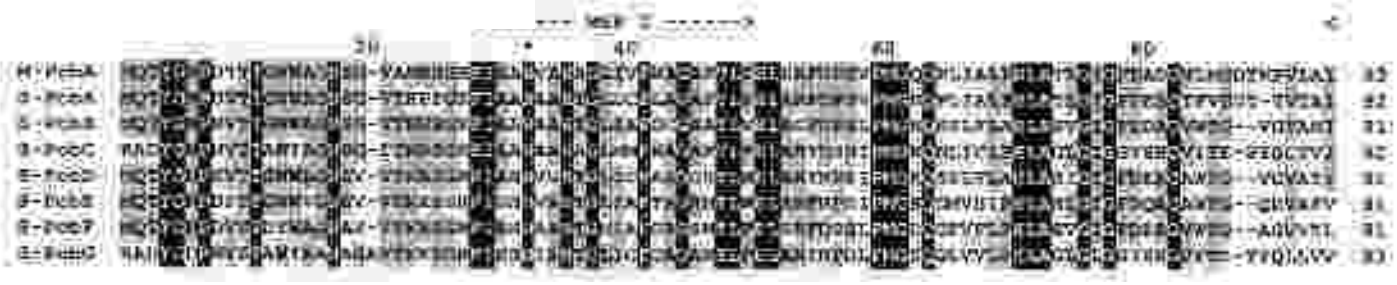
Multiplication of antenna genes as a major adaptation to low light in a marine prokaryote

Laurence Garczarek*, Wolfgang R. Hess†, Julia Holtzendorff*, Georg W. M. van der Staay*, and Frédéric Partensky*‡



The *Proc.* LLII strain SS120 possesses a gene family of 7 transcribed genes encoding different Pcb copies, some likely associated with PSI and others with PSII, while the HLI strain MED4 has a single *pcb* gene, which were successfully cloned and sequenced.

+ Indirect evidence from RFLP analysis that the the LLI strain NATL2a also has many *pcb* copies while the HLII strain TAK9803 has 2.



Ultrastructure of *Prochlorococcus* Light-Harvesting Complexes

Low-light-adapted *Prochlorococcus* species possess specific antenna for each photosystem

T. S. Bibby, I. Mary, J. Nield, F. Partensky & J. Barber

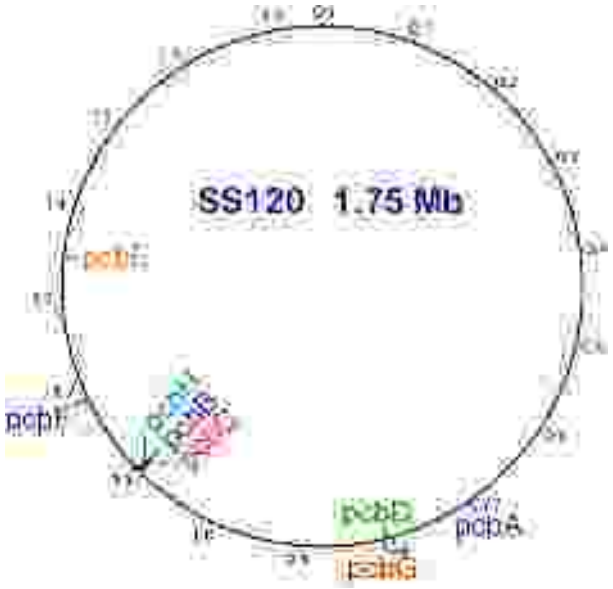


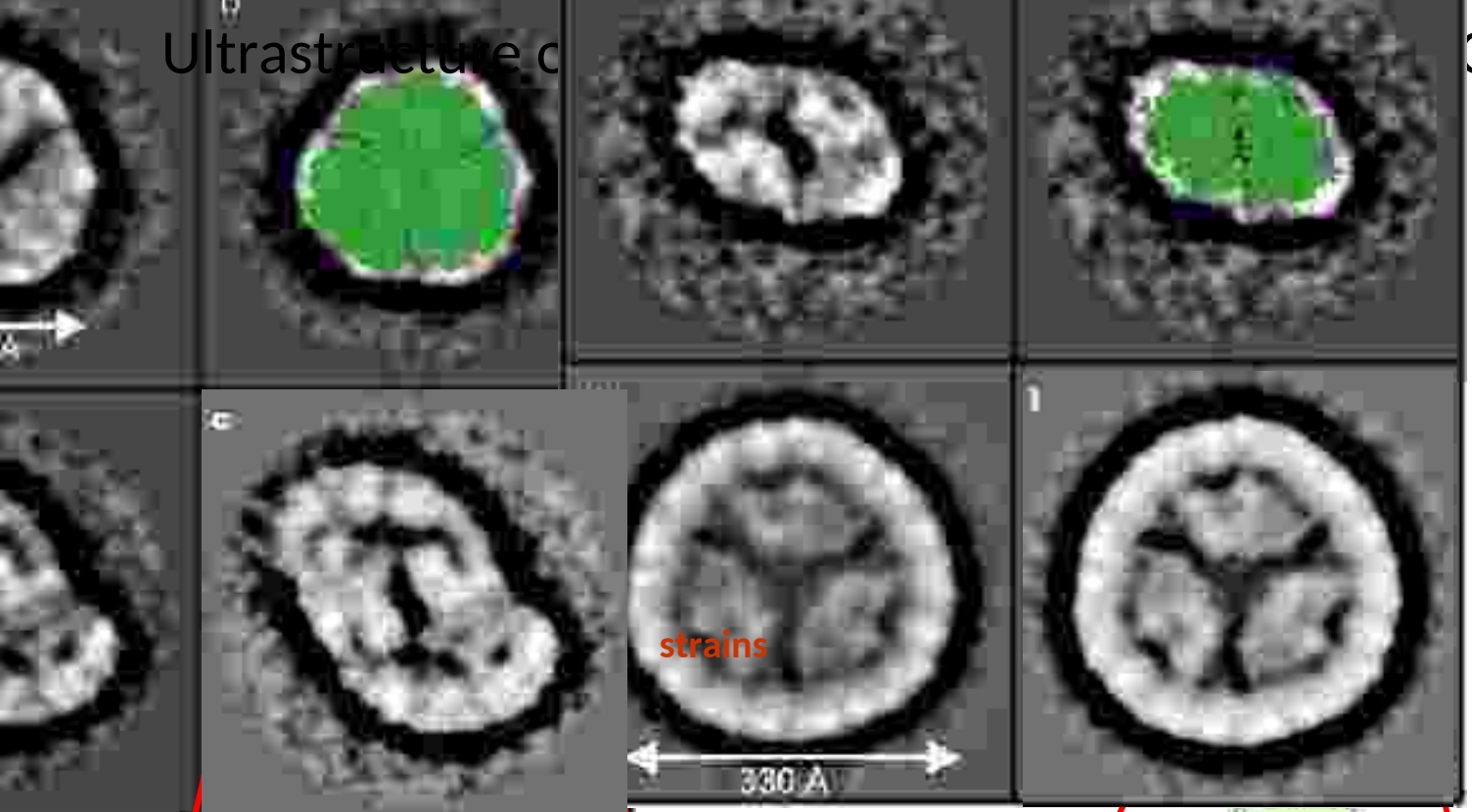
Table 1 Quantitative RT-PCR analyses of the expression of the indicated genes under +Fe and −Fe conditions

Prochlorococcus strain	Gene	Induction ratio (−Fe/+Fe)
MIT 9313	psaA	0.22 ± 0.08
	psbA	0.20 ± 0.02
	isiB	24.72 ± 12.90
SS120	psaA	0.35 ± 0.14
	psbA	0.49 ± 0.08
	isiB	12.13 ± 3.06
MED4	psaA	0.89 ± 0.12
	psbA	0.67 ± 0.20
	isiB	7.94 ± 1.33

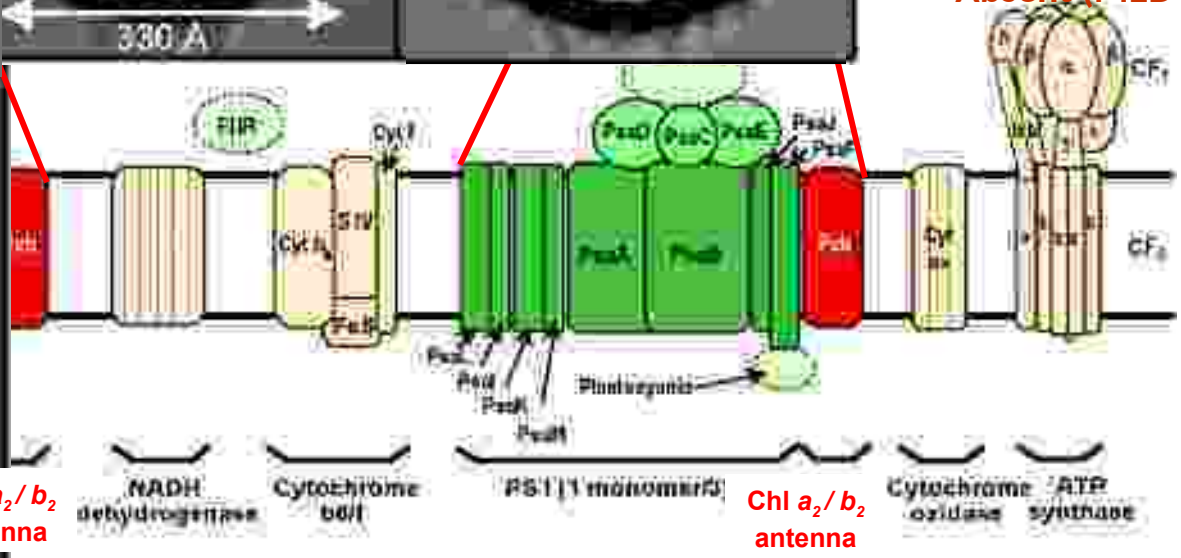
PSI-associated Pcb's

All PSII-associated Pcbs as well as the non-Fe inducible PSI-associated Pcb from SS120 have arisen from multiple events of gene duplication and divergence from an ancestral *isiA* gene.

Ultrastructure c Complexes



PSI antenna
- constitutive (SS120)
- inducible by -Fe (MIT9313)
- Absent (MED4)



Chl a_2/b_2 antenna

Chl a_2/b_2 antenna

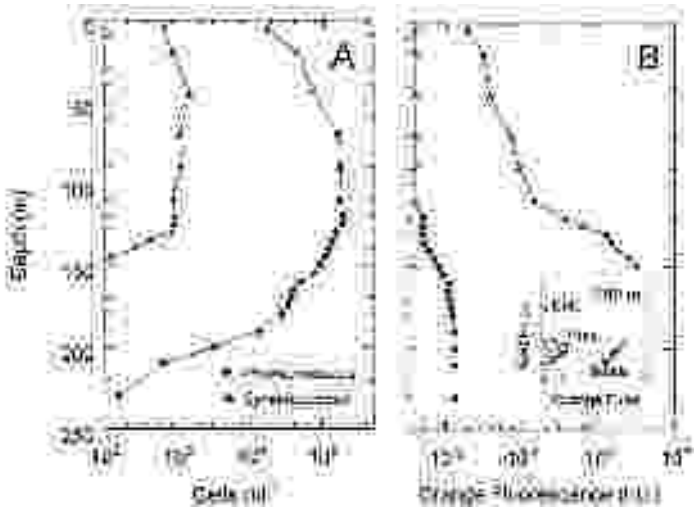
Chl a_2/b_2 antenna

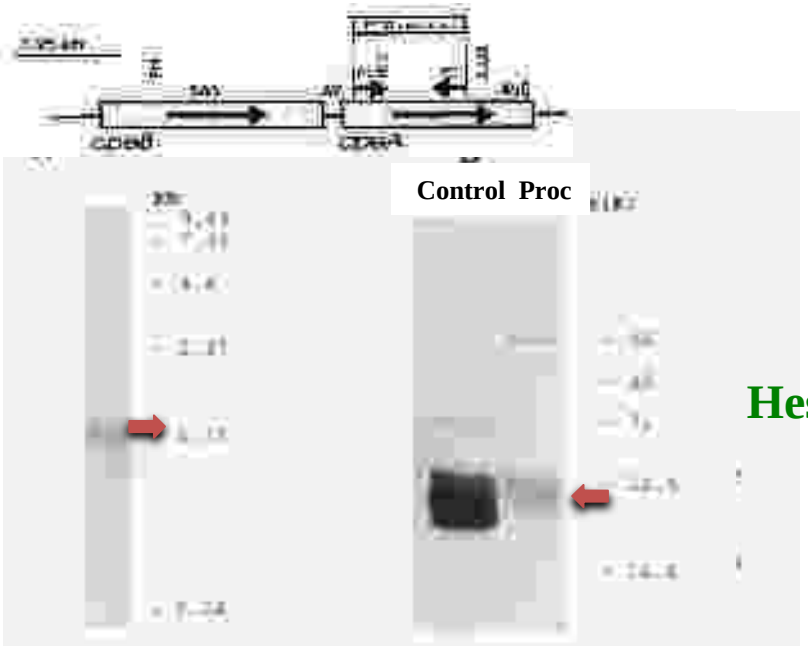
Bibby et al., 2003 *Nature*

Unveiling the presence of phycoerythrin in *Prochlorococcus*

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 11126–11130, October 1996

WOLFGANG R. HESS*†, FRÉDÉRIC PARTENSKY‡, GEORG W. M. VAN DER STAAY§, JOSÉ M. GARCIA-FERNANDEZ§, THOMAS BÖRNER*, AND DANIEL VAULOT§



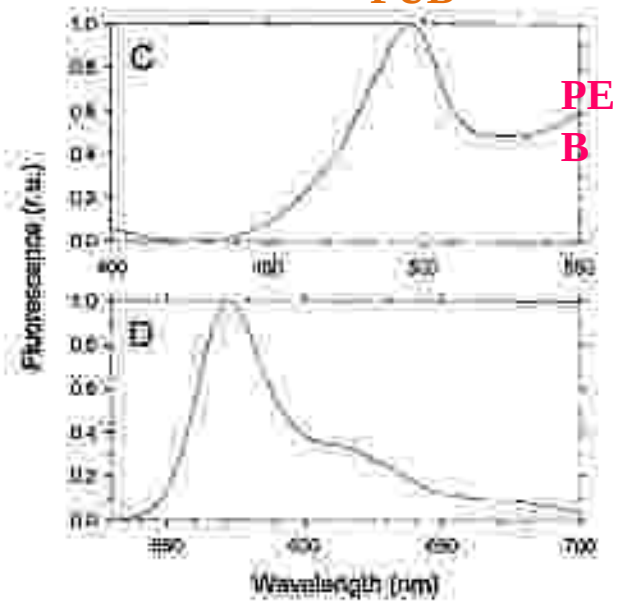


Hess et al., 1996

PUB

PE

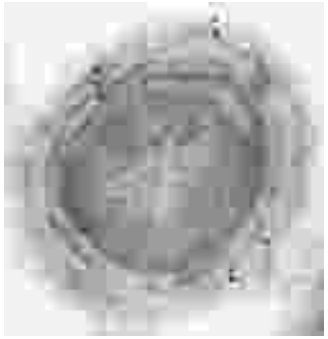
B



The *cpeBA* operon is expressed and translated into PE in SS120

PE contributes for only 2.6 % to light absorption at 495 nm.

=> Light sensor?



Plant Molecular Biology **40**: 507–521, 1999.

Wolfgang R. Hess[1,*], Claudia Steglich, Christiane Lichtlé[2] and Frédéric Partensky[3]



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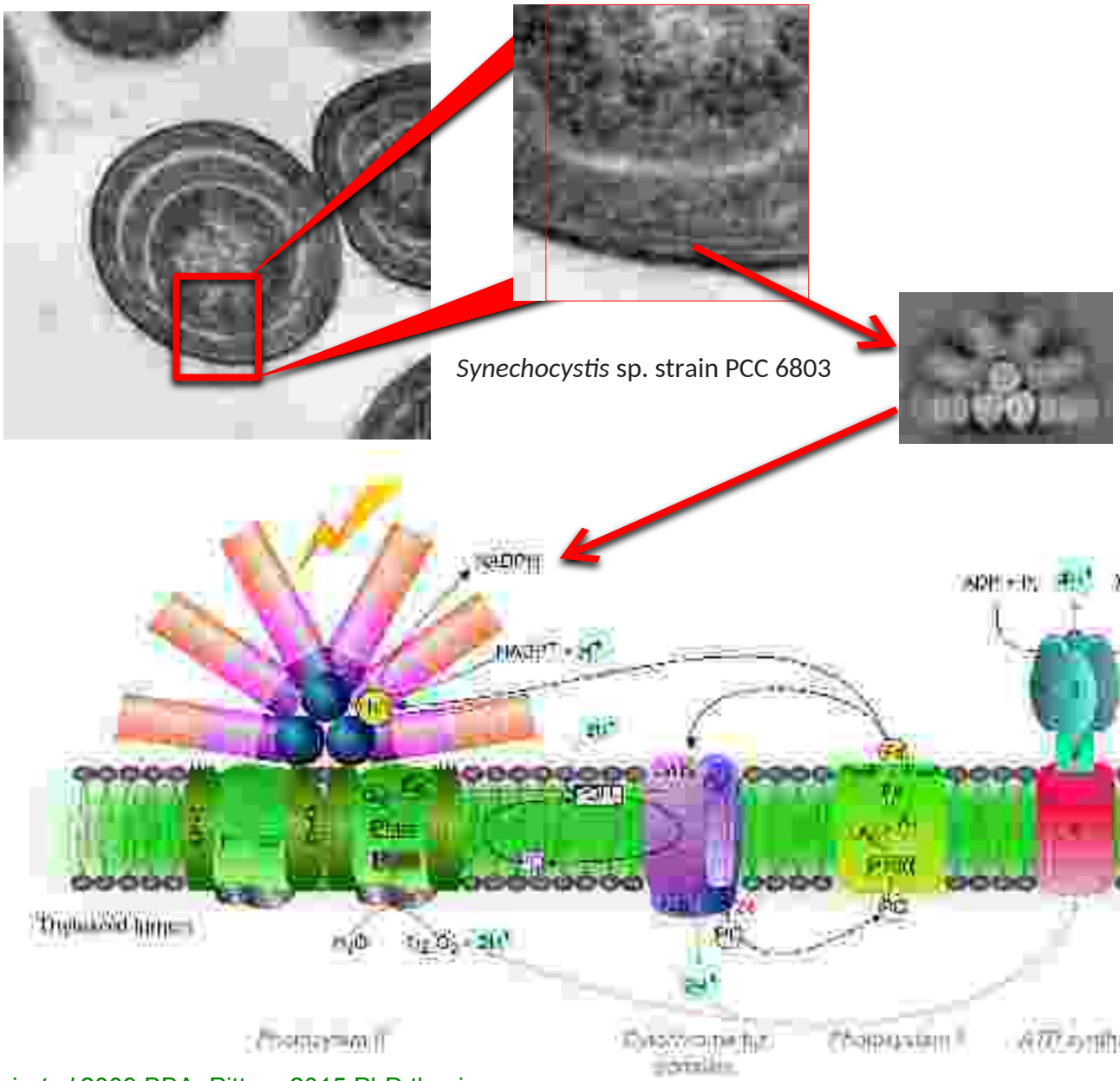
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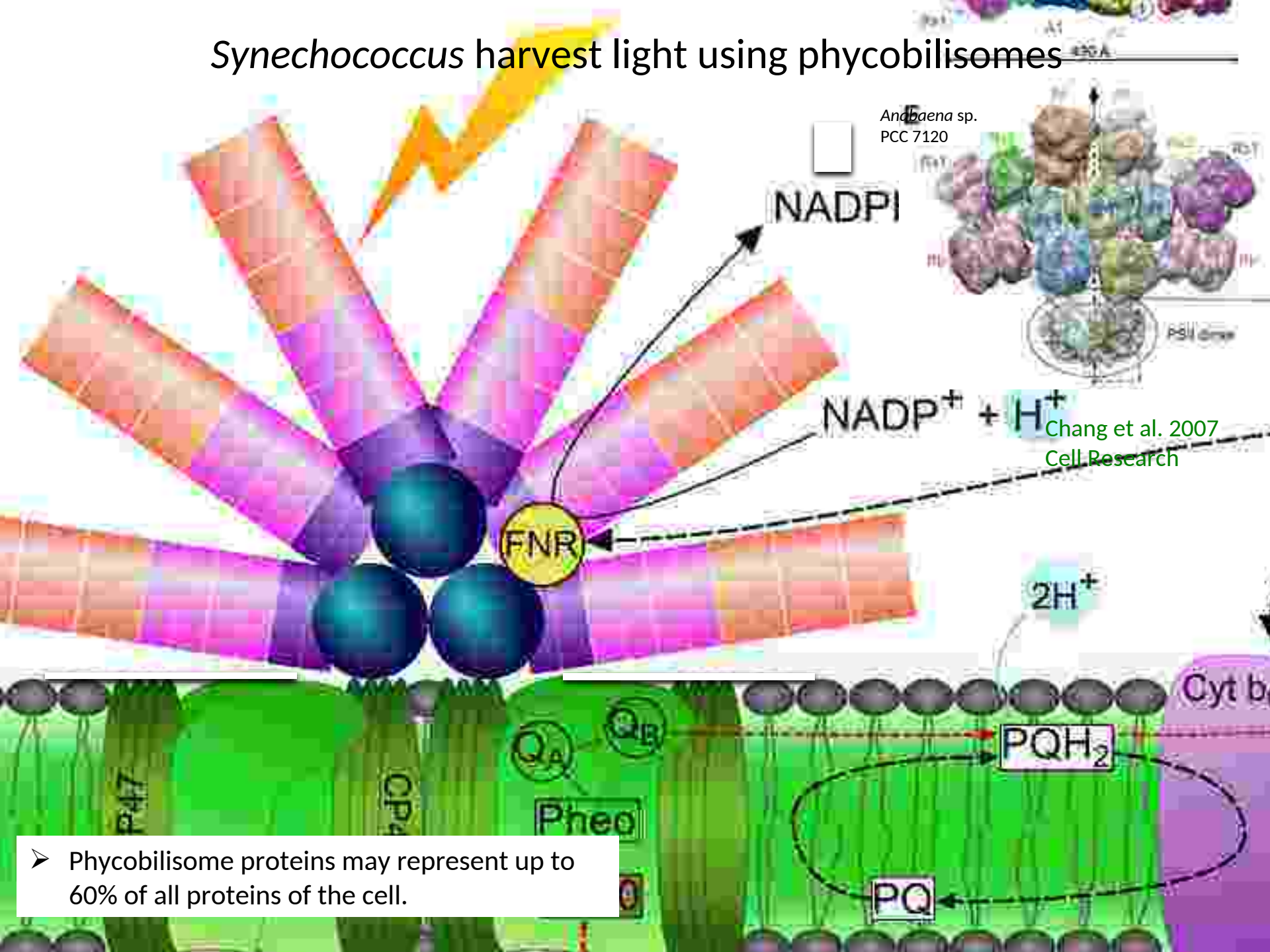
Synechococcus harvest light using phycobilisomes



Synechocystis sp. strain PCC 6803

Arteni *et al* 2009 BBA; Pittera 2015 PhD thesis

Synechococcus harvest light using phycobilisomes

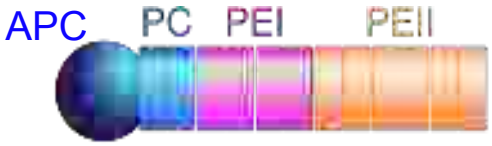


Chang et al. 2007
Cell Research

- Phycobilisome proteins may represent up to 60% of all proteins of the cell.

Phycobiliproteins : the phycobilisome building blocks

Model of *Synechococcus* phycobilisome rod



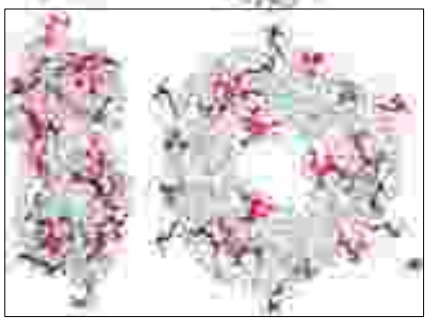
Each PBS rod consists of 3 to 6 hexamers depending on pigment type

Phycobiliproteins

APC	Allophycocyanin	2
PC	Phycocyanin	3
PE-I	Phycoerythrin-I	5
PE-II	Phycoerythrin-II	6

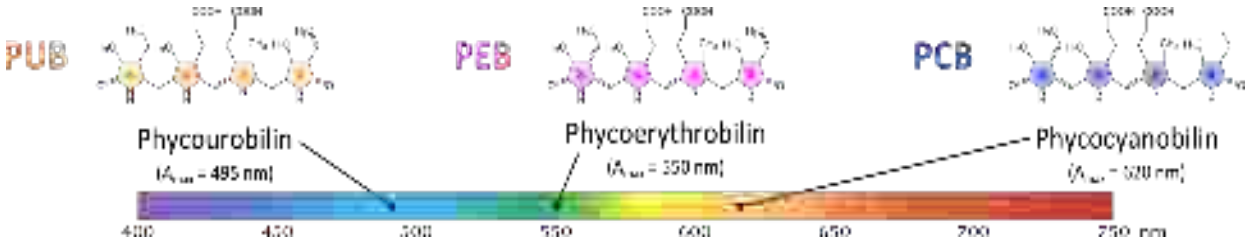
Phycobiliproteins covalently attach 2 to 6 chromophores (‘phycobilins’) per α-β monomer

Phycobiliproteins are made of two subunits (α and β) assembled into hexamers [$(\alpha\text{-}\beta)_6$]

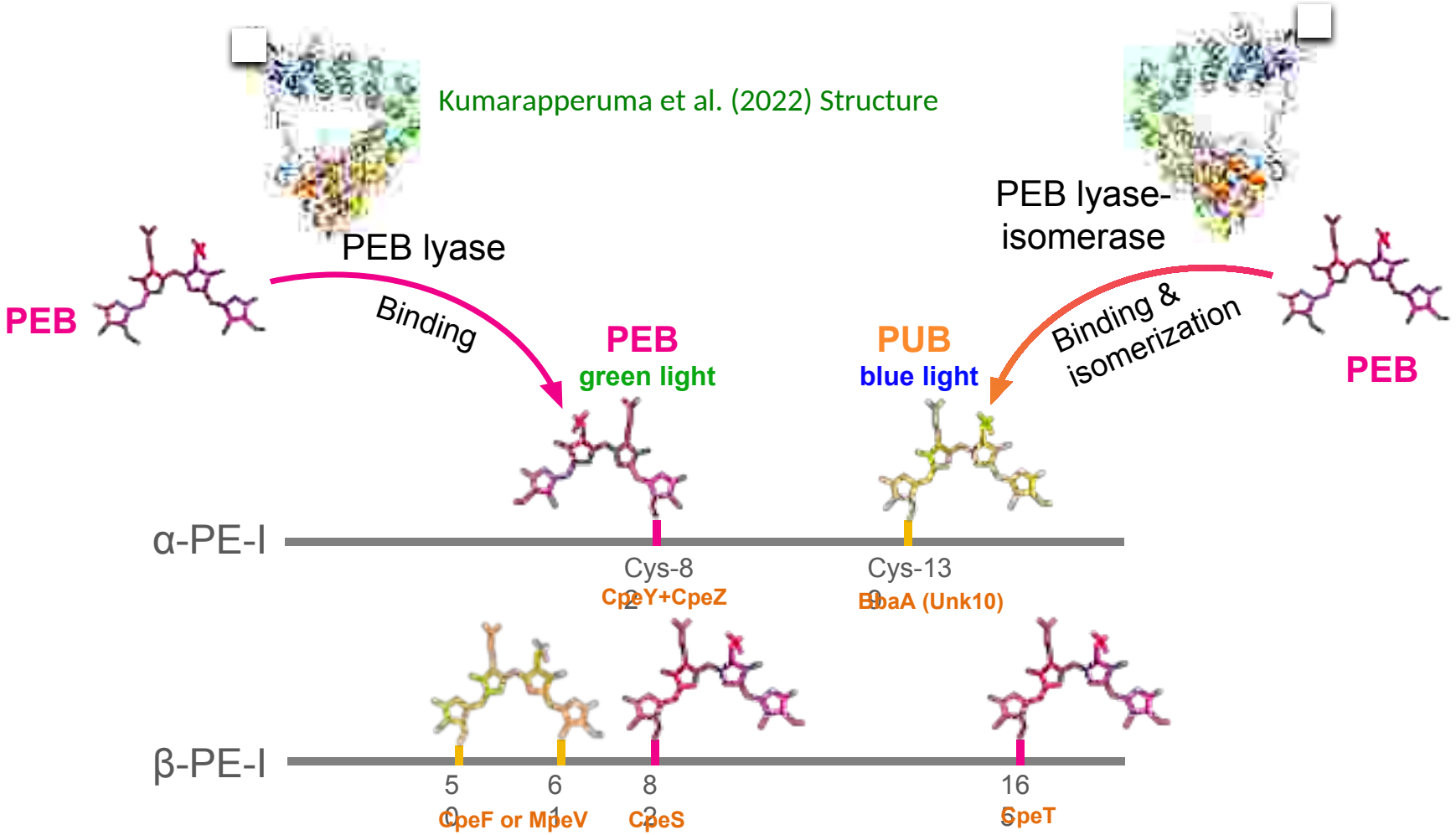


Side and top view of PE-I ’donut’-shaped hexamers

Phycobilins (isomers)



Covalent chromophore attachment requires phycobilin lyases



- Lyases act on α- or β-apoproteins <u>before</u> their assembly into α-β monomers then $(\alpha\text{-}\beta)_6$ hexamers, and once attached phycobilins cannot be removed enzymatically.
- Marine *Synechococcus* possess between 3 and 15 genes coding for putative lyases or lyases-isomerases, and most of them have been characterized in recent years

Biswas et al. 2011 JBC; Gasper et al. 2017 JBC; Kronfel et al. 2019 JBC; Carrigee et al. 2020 JBC, Kehoe et al. in prep.

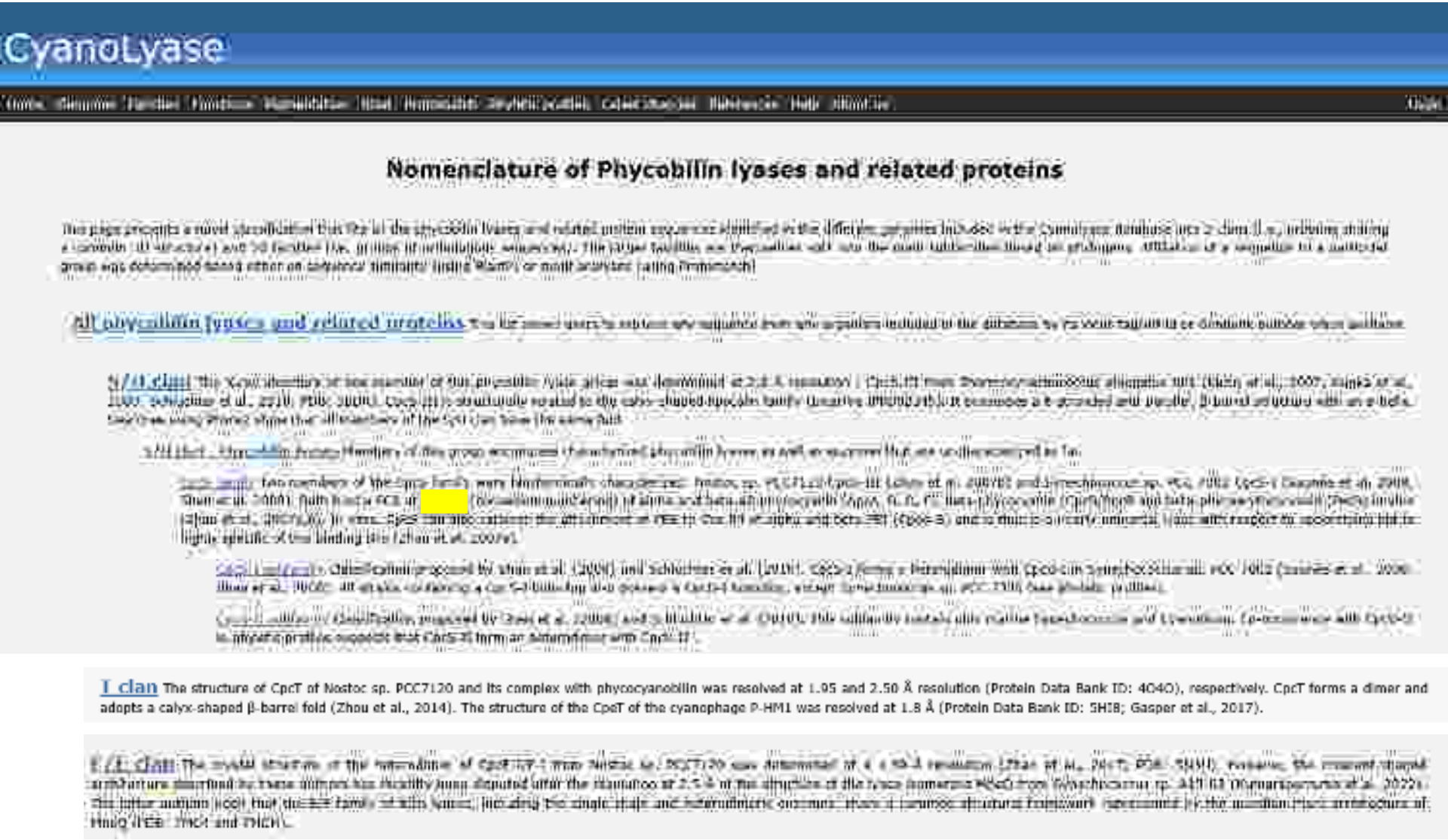
Covalent chromophore attachment requires phycobilin lyases



- Cyanolyase is a manually curated database devoted to cyanobacterial lyases (nomenclature, assignment by Blast search, protein motifs, phyletic profiles, references, etc.).

Bretaudeau et al. 2012 Nucl. Acids Res.

Covalent chromophore attachment requires phycobilin lyases



- Phycobilin lyases comprises three mains superfamilies or “clans” (S/U, T and E/F) generally acting on a specific binding site (e.g. Cys-82 ‘consensus’ site for members of clan S).

Bretaudeau et al. 2012 Nucl. Acids Res.

Assembly of PBS hexamers into PBS rods requires linker proteins

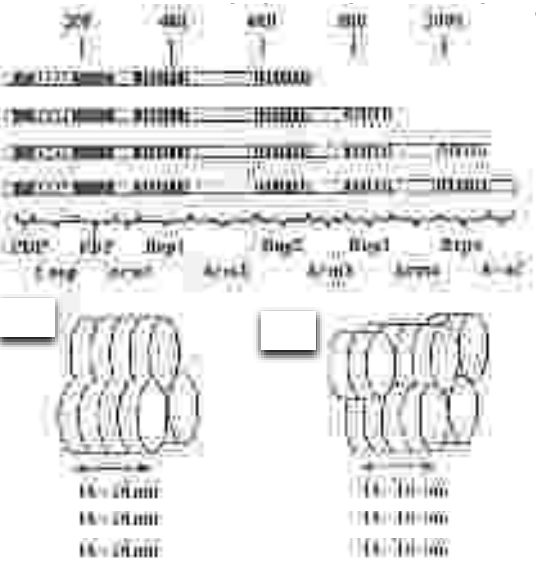
- Marine *Synechococcus* strains contain between 6 and 11 genes coding for linker-like proteins: we assume that there are up to 6 hexamers per rod (3 in *Synechocystis*).



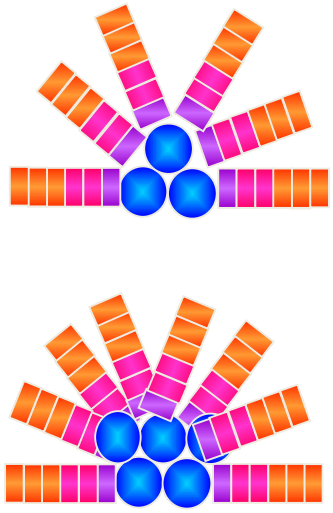
Six et al. 2007 Genome Biol.

Grébert et al. 2022 Genome Biol. Evol.

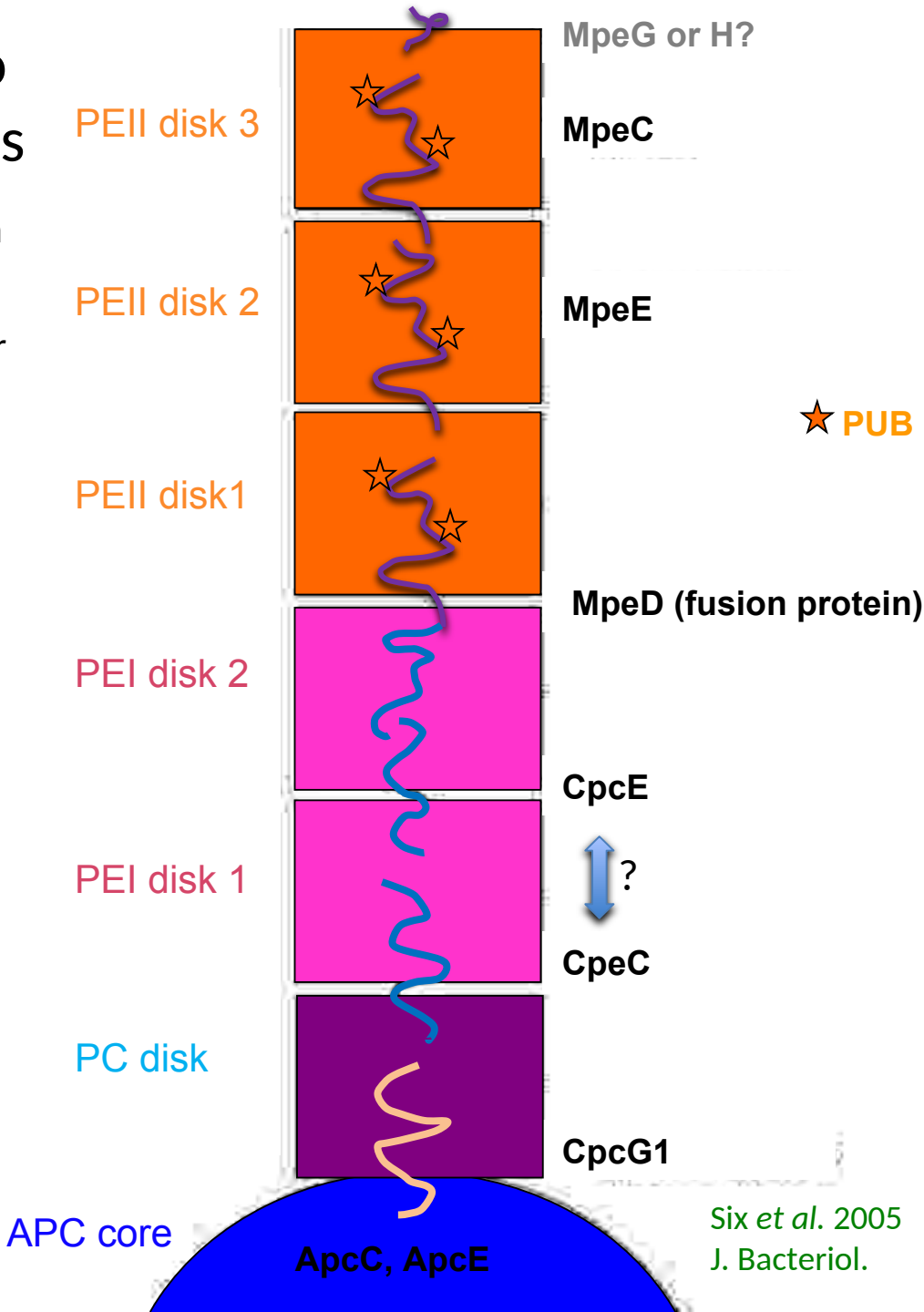
- The numbers of repeat (REP) domains in ApcE defines the number of cylinders in the PBS of rods (6 or 8).



Sidler 1987



Six et al. 2007



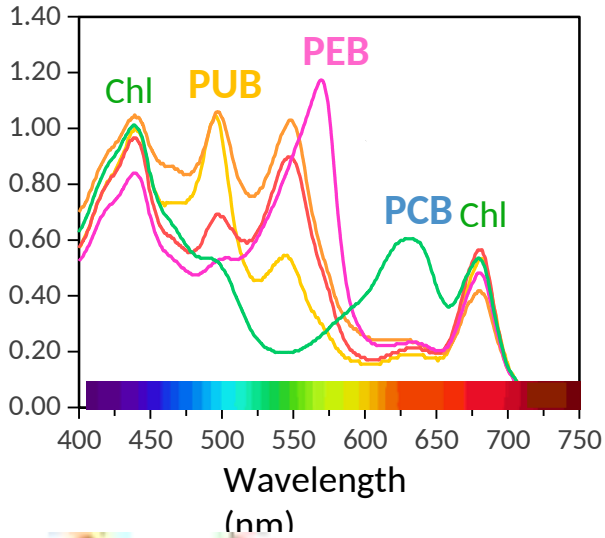
Six *et al.* 2005 J. Bacteriol.

Synechococcus Pigment Types

- Marine *Synechococcus* have the most diverse pigment content of all phytoplanktonic groups

PC Phycocyanin
PE-I Phycoerythrin I
PE-II Phycoerythrin II







PC

PT 1



PC+PE-I

PT 2A/B



PC+PE-I+PE-II

PT 3

Subtype 3a
Low PUB:PEB
GL specialists

Subtype 3b
Intermediate PUB:PEB
= 3d having lost CA4 ability

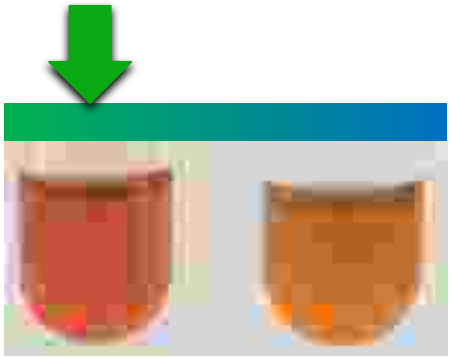
Subtype 3c/f
High PUB:PEB
BL specialists

Subtypes 3dA/B
Variable PUB:PEB
Type IV chromatic acclimation (**CA4**)

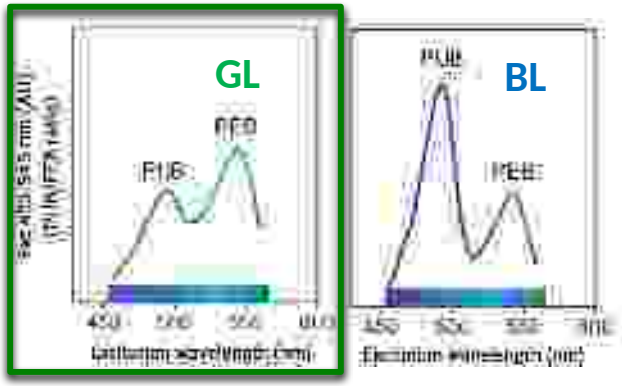
Six et al. 2007 Genome Biol.; Humily et al. 2013 Plos One; Grébert et al. 2018 PNAS

Type IV chromatic acclimation (CA4) provides cells the ability to absorb either **Blue** or **Green** light with high efficiency

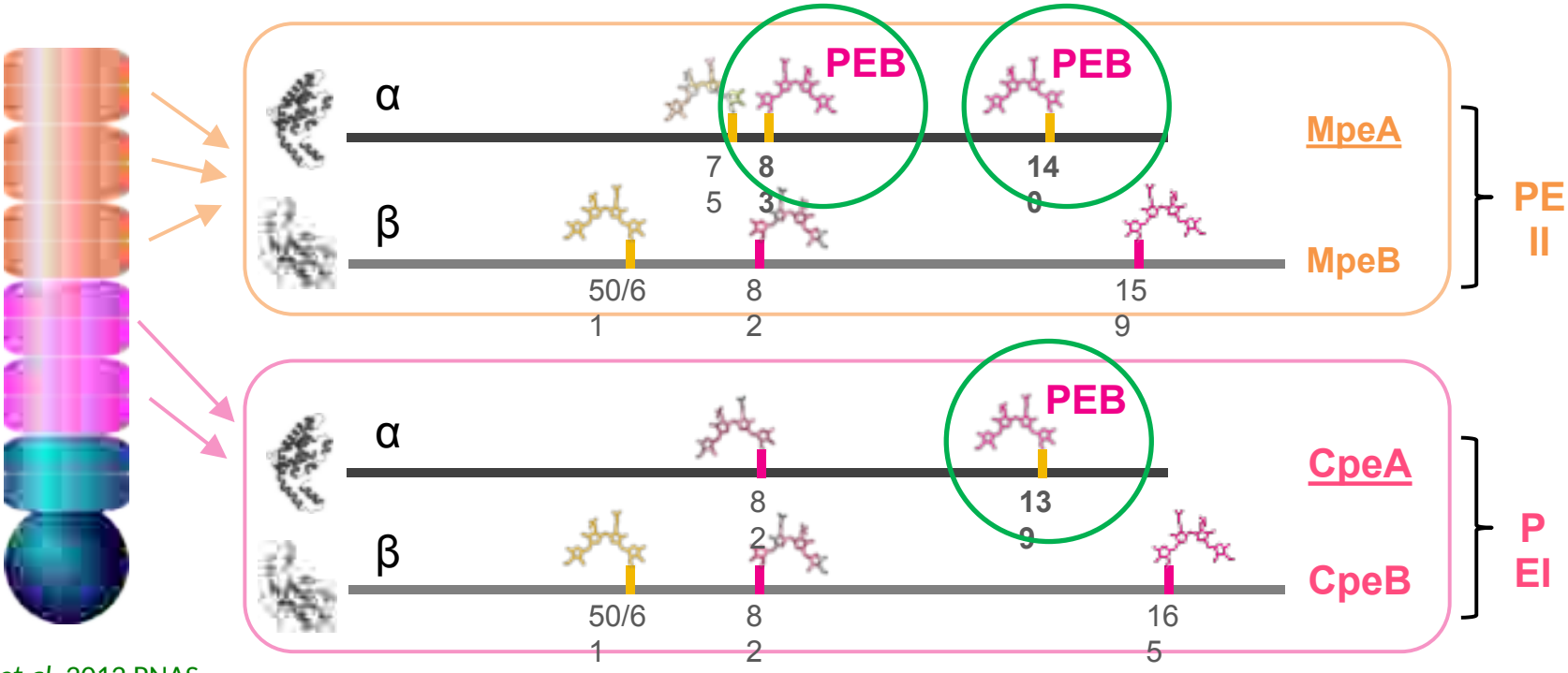
Green Light (**GL**)



Blue Light (**BL**)



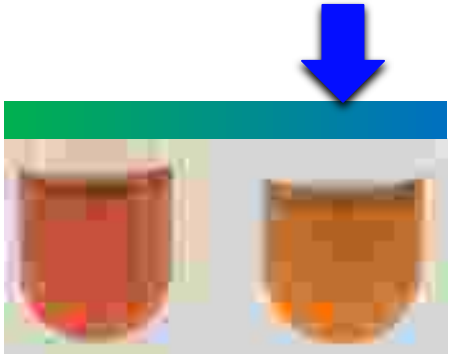
$Exc_{495:550}$ fluorescence excitation ratio, a proxy for PUB:PEB ratio: 0.6 in GL ⇔ 1.6 in BL



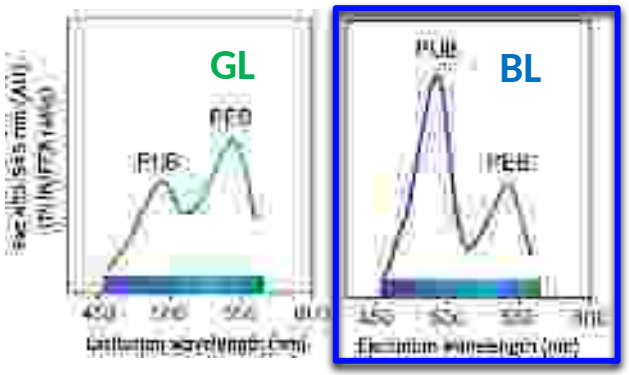
Shukla *et al*. 2012 PNAS

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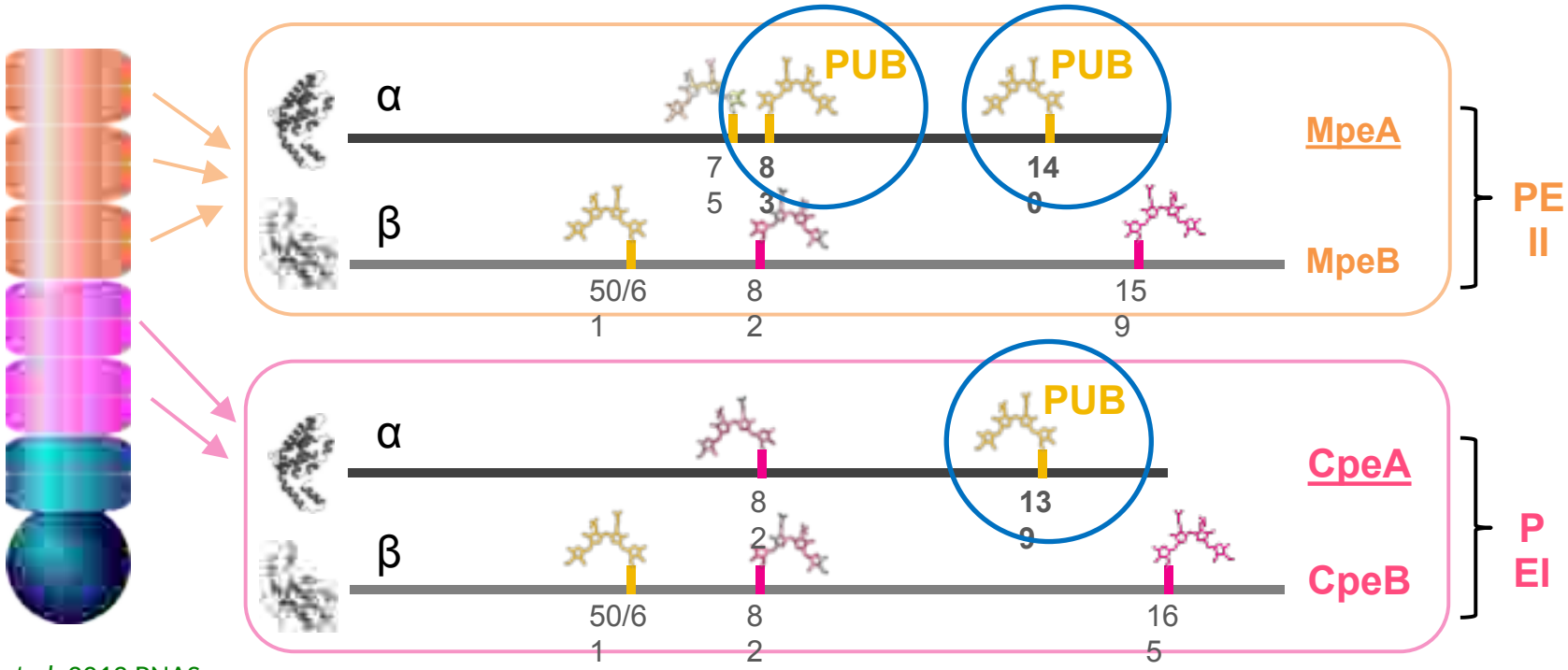
Green Light (**GL**)



Blue Light (**BL**)



$Exc_{495:550}$ fluorescence excitation ratio, a proxy for PUB:PEB ratio: 0.6 in GL ⇔ 1.6 in BL



Shukla *et al*. 2012 PNAS

Chromatic acclimation is a common and diverse process in Cyanobacteria



B. Palenik, 2001

T.W.Engelmann, 1902

Sanfilippo et al. (2019) Annu. Rev Microbiol.

- CA3 is the first discovered and best studied type of CA
- CA4 is the sole chromatic acclimation process known so far to occur between BL and GL

The phycobilisome (PBS) rod region

- Phycobiliprotein α & β-subunits
- Phycobilin lyases
- Phycobilin synthesis
- Linkers
- Regulator
- Unknowns





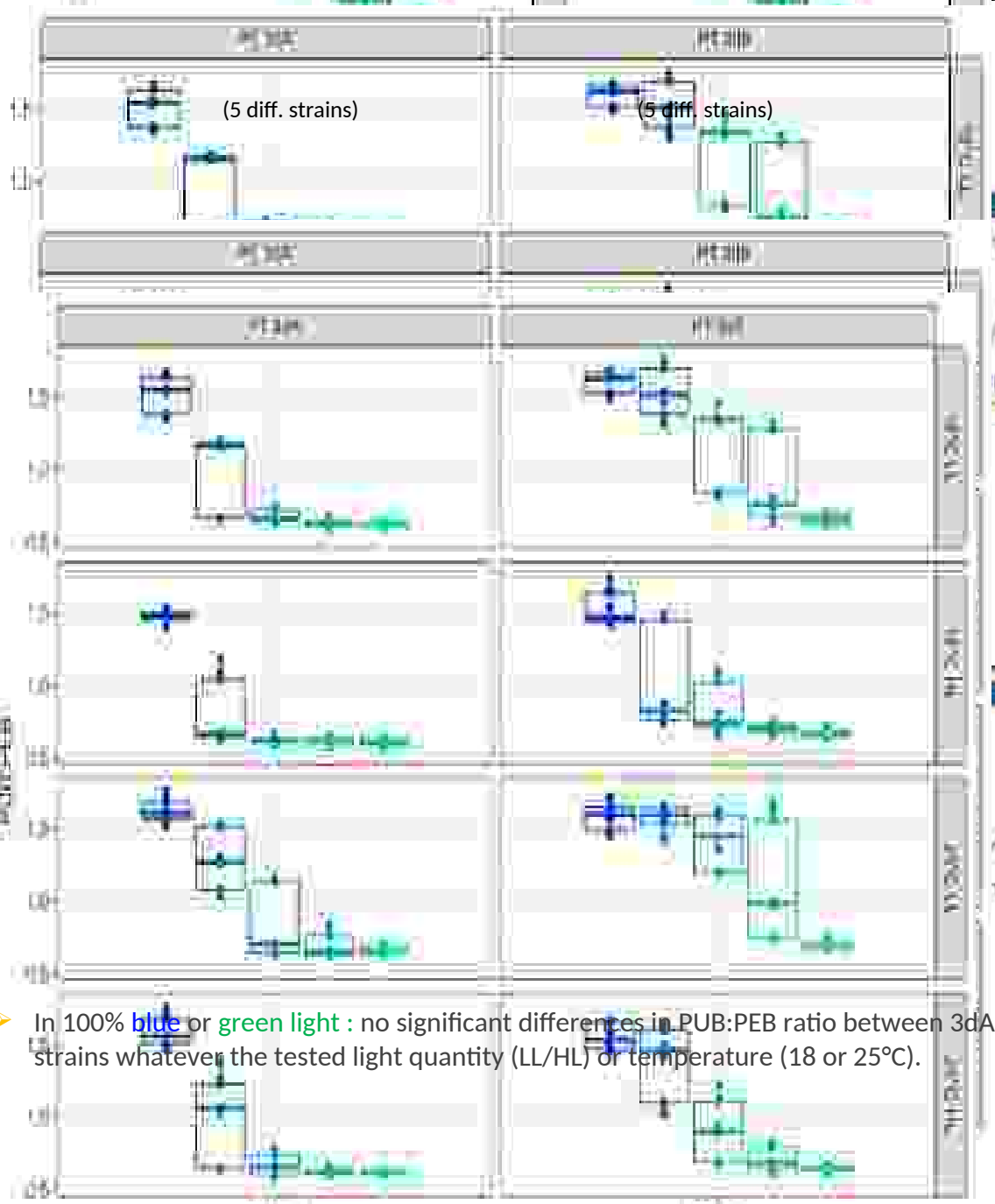


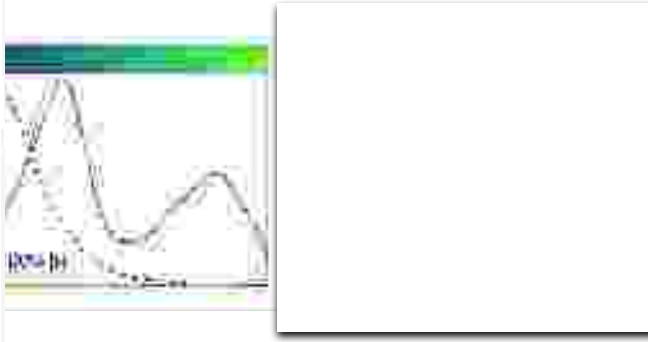
C

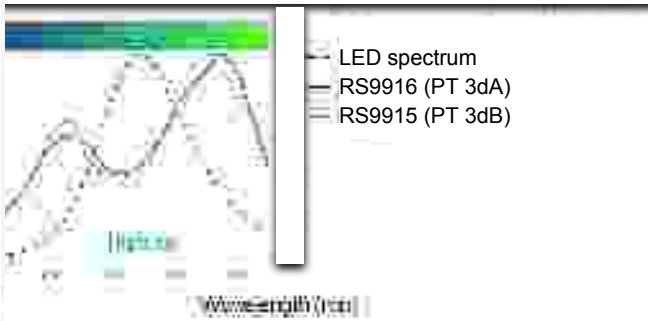
- Specialized genomic region gathering genes for the synthesis of PBS rods
- Gene content and organization specific to each PT
- Chromatic acclimaters possess a CA4 genomic island occurring in two configurations: CA4-A and CA4-B
- CA4-A island anywhere in the genome / CA4-B island exclusively found in the middle of PBS rod region

Six *et al.* 2007 Genome Biol; Larsson *et al.* 2014 ISME J; Humily *et al.* 2013 PLoS One ; Sanfilippo *et al.* 2016 PNAS

ypic differences



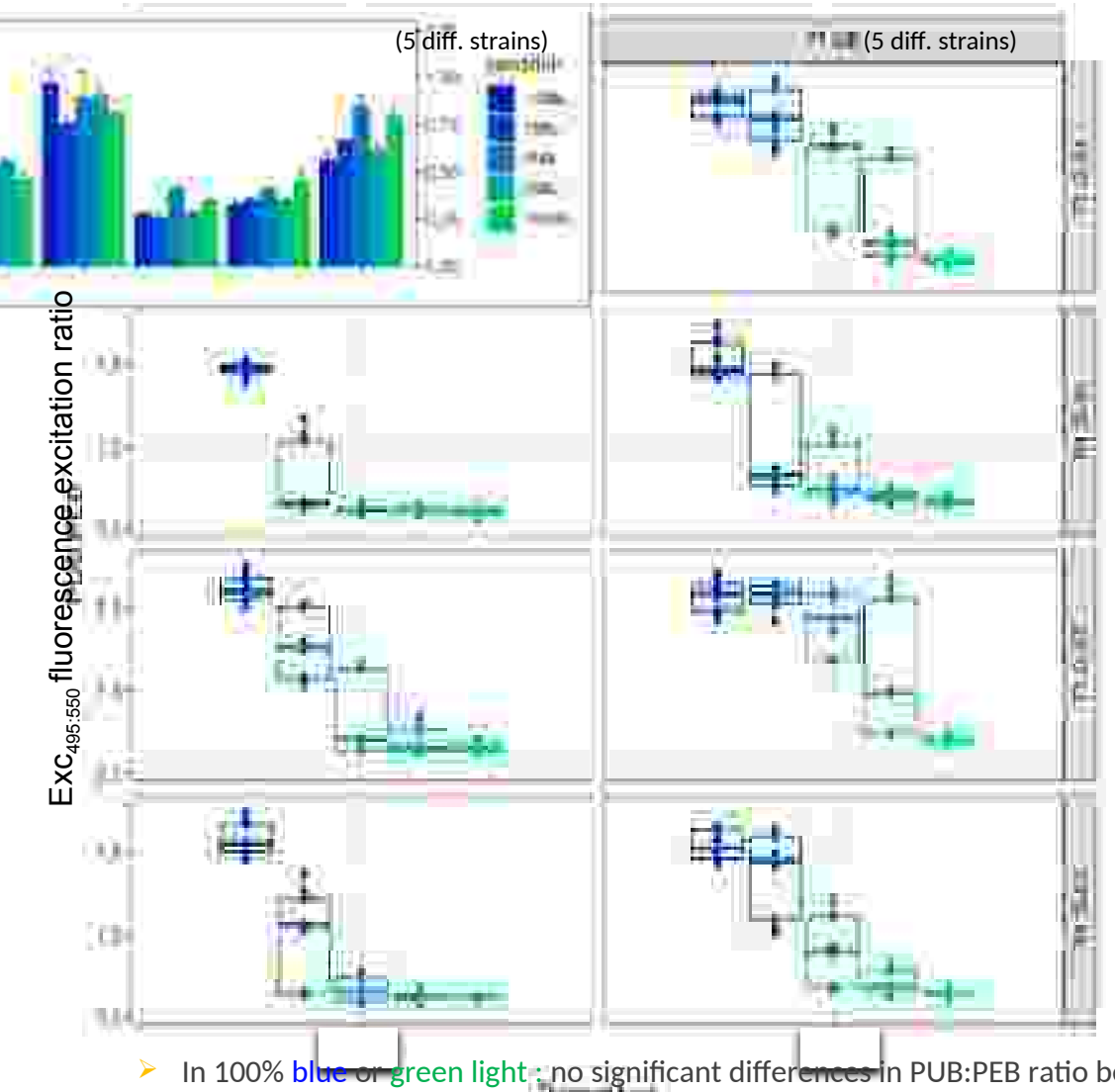


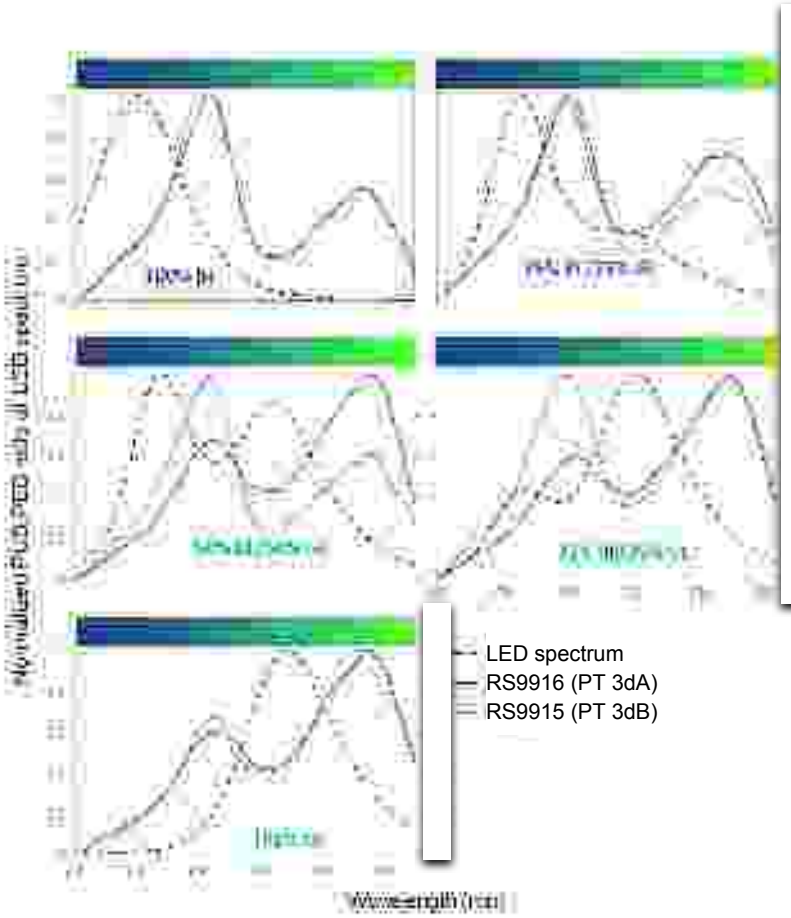


Dufour *et al.* Front. Microbiol. 2024

- In 100% blue or green light : no significant differences in PUB:PEB ratio between 3dA and 3dB strains whatever the tested light quantity (LL/HL) or temperature (18 or 25°C).

Pigment types 3dA and 3dB differ by subtle phenotypic differences

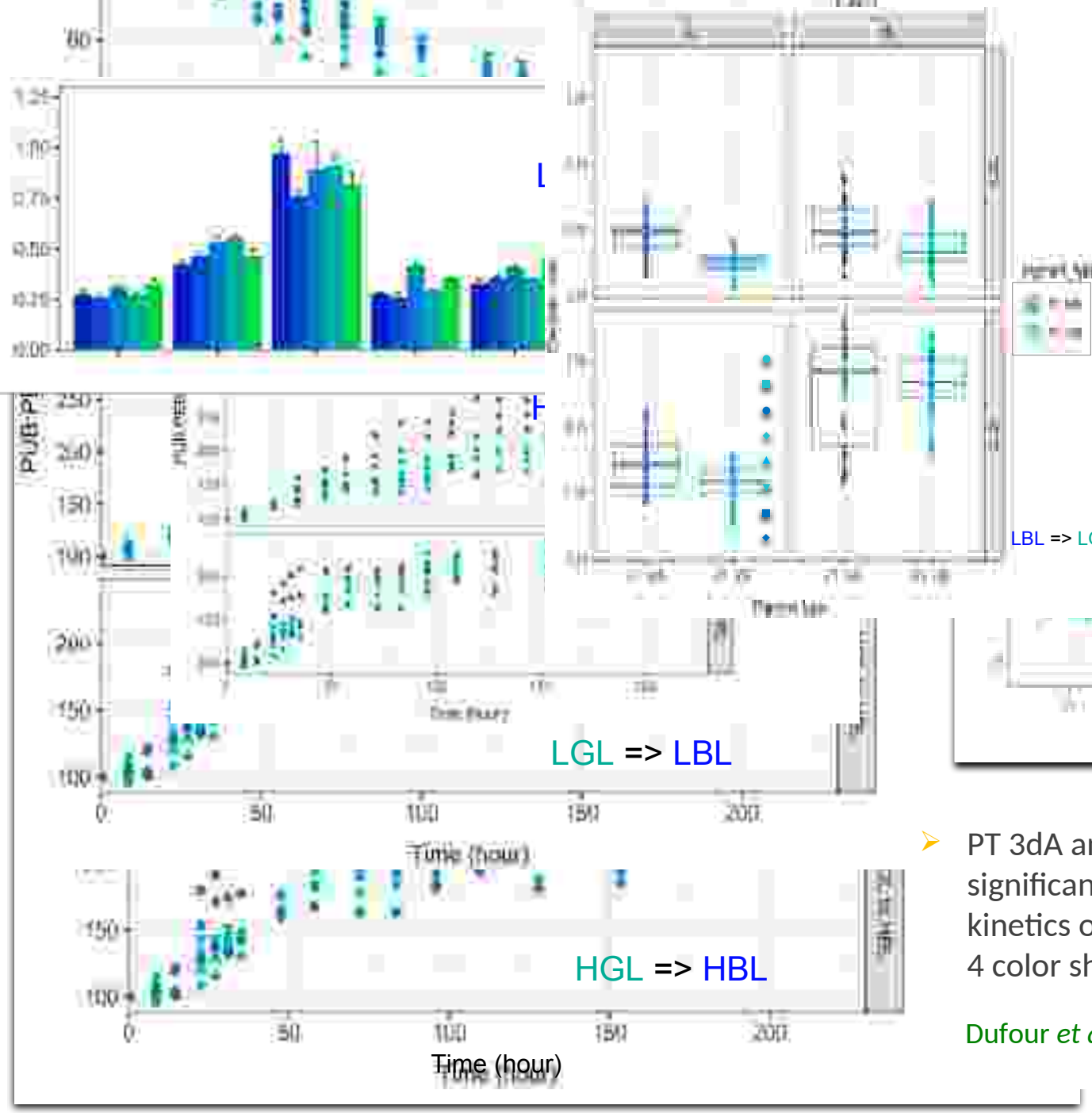


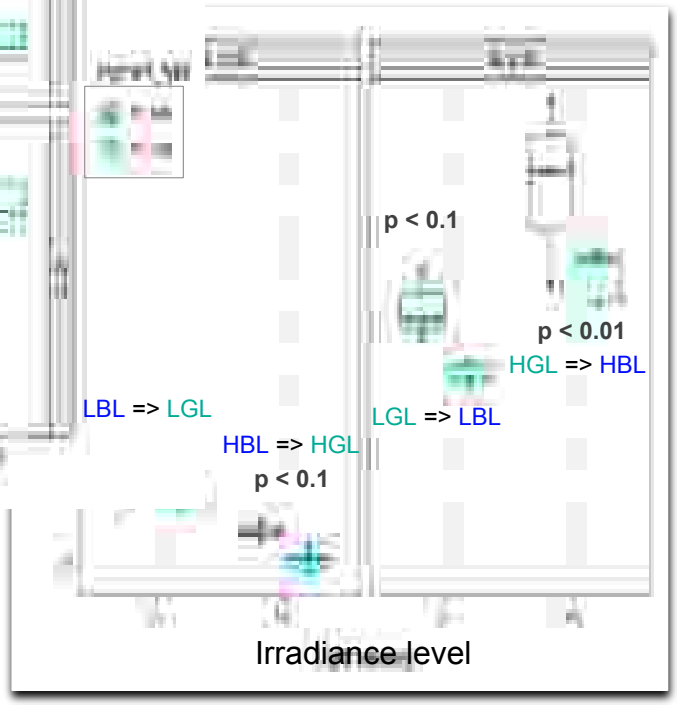


Dufour *et al.* Front. Microbiol. 2024

- In 100% blue or green light : no significant differences in PUB:PEB ratio between 3dA and 3dB strains whatever the tested light quantity (LL/HL) or temperature (18 or 25°C).
- In intermediate blue-green mixes, PT 3dA cells preferentially display a low PUB:PEB and PT 3dB cells a high PUB:PEB, suggesting they need a different proportion of BL and GL to trigger the CA4 process.

typic differences





- PT 3dA and 3dB strains exhibit significantly different acclimation kinetics of PUB:PEB ratio, in 3 out the 4 color shifts tested.

Dufour *et al*. Front. Microbiol. 2024

Croucher course 3 outline

I. Introduction

II. Characterization on *Prochlorococcus* light-harvesting complexes

III. *Synechococcus* phycobilisomes and pigment diversity

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VI. Ecological distribution and modelling of pigment types



Pipeline used by the core ‘CA4 team’ to characterize genes involved in CA4



D.M. Kehoe Lab
(Indiana Univ.)

Whole cells
(WT/mutant)



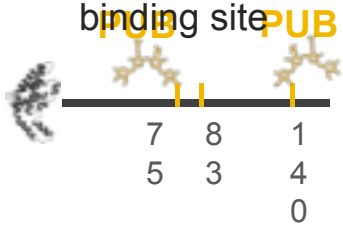
Phycobilisomes



Phycobiliprotein subunits



Chromophore and binding site



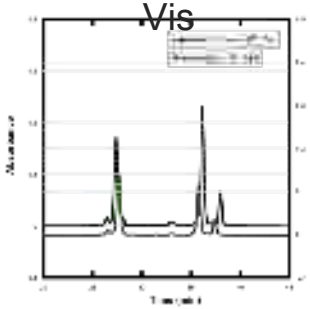
Cultures



Soluble proteome/ sucrose gradient



HPLC/UV-Vis



LC/UV-Vis/MS-MS



Jon Karty/Jon Trinidad
Mass Spec Facility (IU)

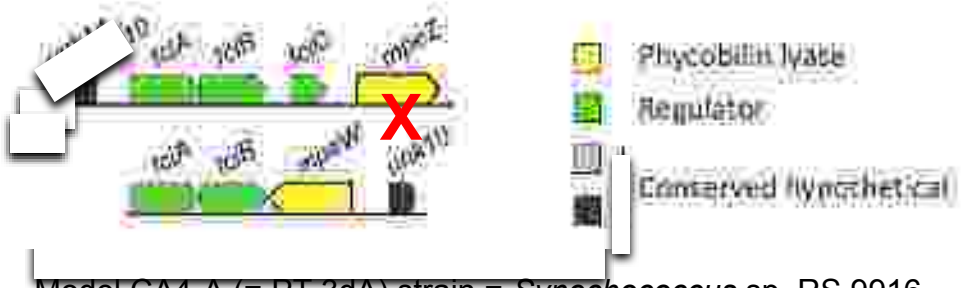
Station Biologique Roscoff

F. Partensky & L. Garczarek Lab
Also :
Comparative genomics,
Comparative physiology, etc.



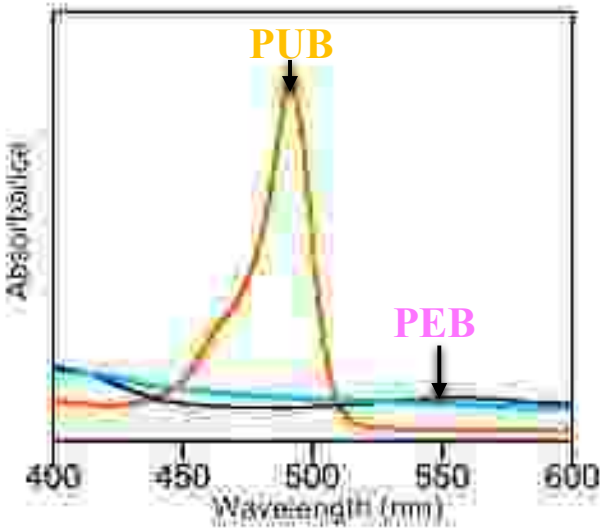
W.M. Schluchter Lab
(Univ. New orleans)
Also:
Heterologous expression
Targeted mutagenesis

MpeZ is a PEB lyase-isomerase that attaches PUB to MpeA Cys-83 in blue light



Model CA4-A (= PT 3dA) strain = *Synechococcus* sp. RS 9916

Expression in *Escherichia coli*



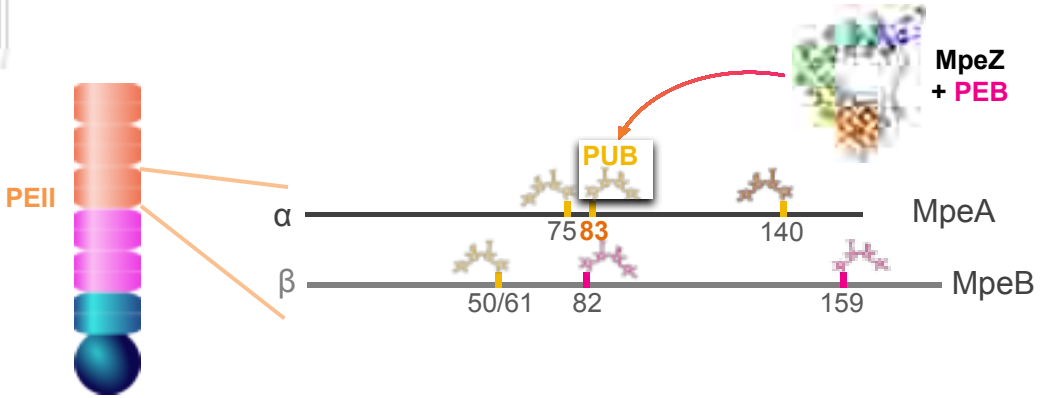
Chromophore attachment in WT versus *mpeZ* mutant:

Strain	MpeA Cysteine position	Bilin in GL	Bilin in RL
WT	75 83 140	PUB PEB PEB	PUB PEB PEB
mpeZ-	75 83 140	PUB PEB PEB	PUB PEB PEB

MpeZ is *necessary* to attach PEB at MpeA Cys-83

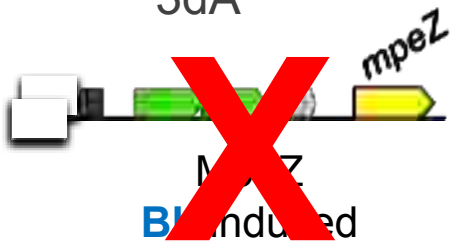
Slide courtesy of D. Kehoe

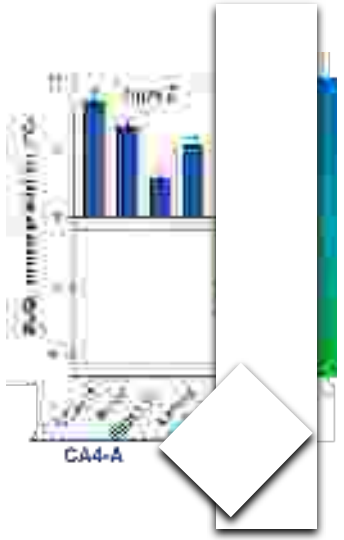
MpeZ is a lyase-isomerase *sufficient* to attach PEB at MpeA Cys-83 and isomerize it to PUB



Shukla *et al*. PNAS (2012)

Molecular differences between CA4-A and CA4-B processes

	PT 3dA	PT 3dB
CA4 island	 **BL** induced **PUB** on Cys-83 MpeA	 **GL** induced **PEB** on Cys-83 MpeA
PBS rod region	 MpeY constitutively expressed **PEB** on Cys-83 MpeA	 MpeQ constitutively expressed **PUB** on Cys-83 MpeA



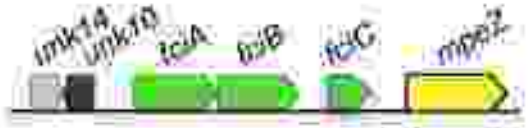
Shukla *et al.* 2012 PNAS; Sanfilippo *et al.* 2019 PNAS

Grébert *et al.* 2021 PNAS

- Both CA4-A and CA4-B involve the competition between a PEB lyase and a lyase-isomerase **but** they display opposite mechanisms.
- Basal state (in absence of CA4) = GL for PT 3dA (PUB/PEB=0.6) and BL for PT 3dB (1.6)

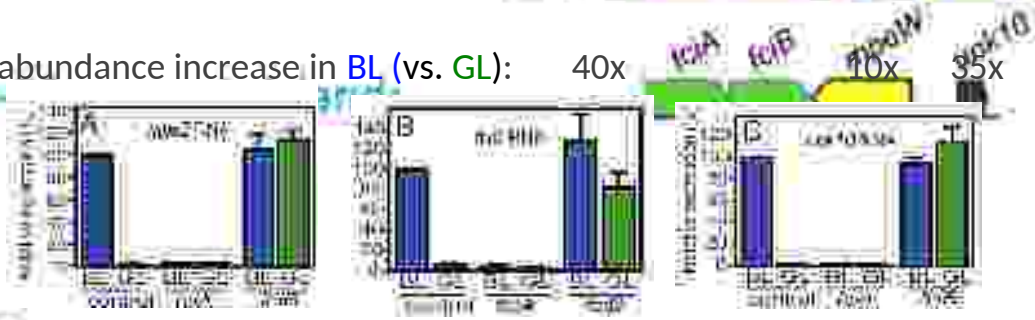
  => PT 3dA ancestors = GL specialists / PT 3dB ancestors = BL specialists.

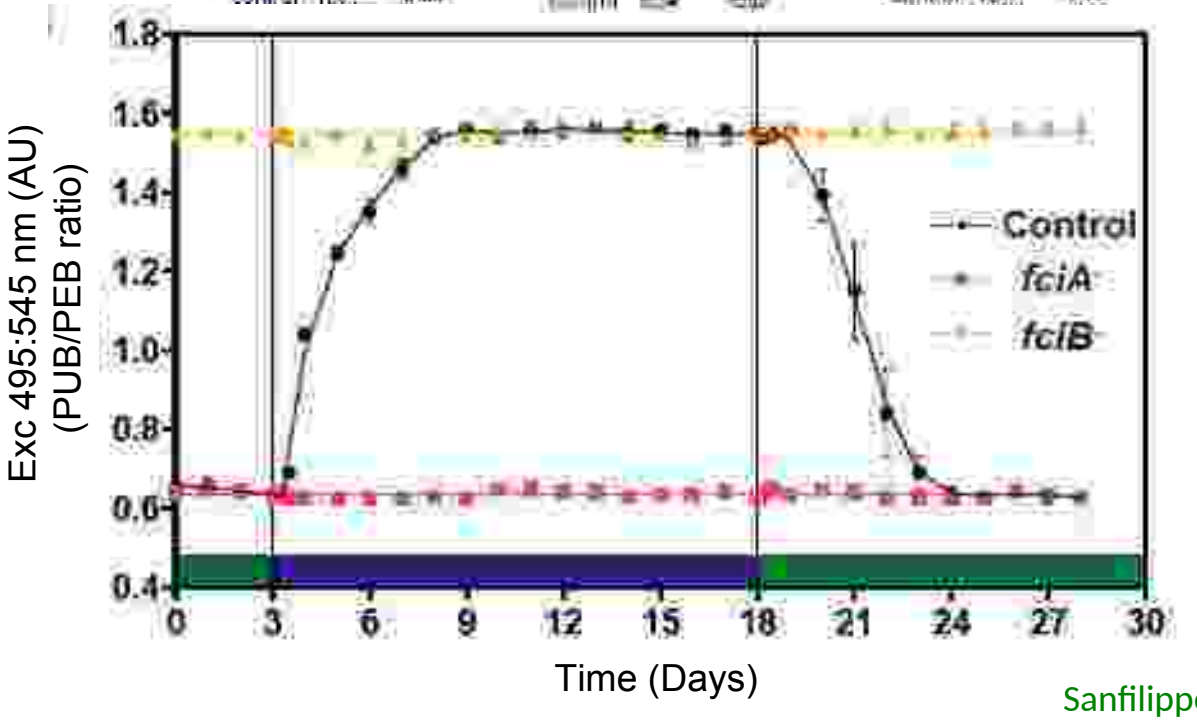
"CA4-A" genomic island:

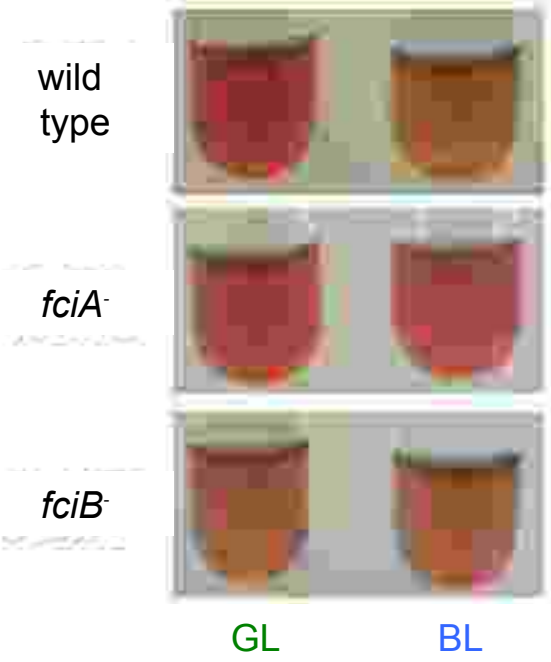




RNA abundance increase in BL (vs. GL): 40x 10x 35x





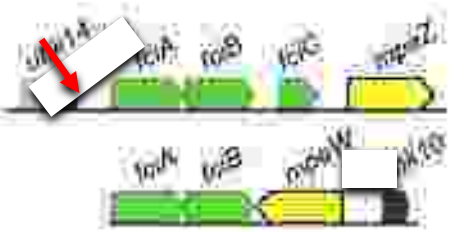


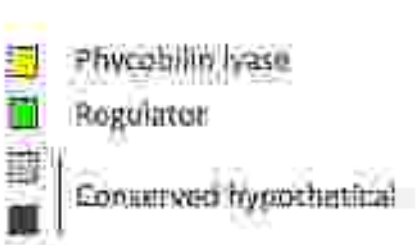
Sanfilippo *et al.*, 2016 PNAS

- FciA is an **activator** and FciB a **repressor** of the CA4 process (in PT 3dA strains…)

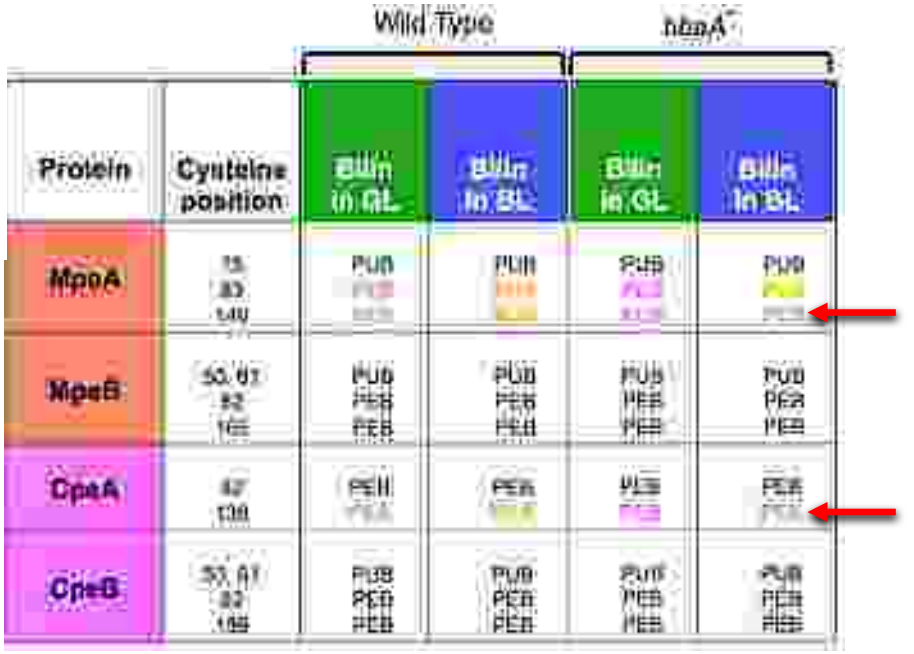
Slide courtesy of D. Kehoe

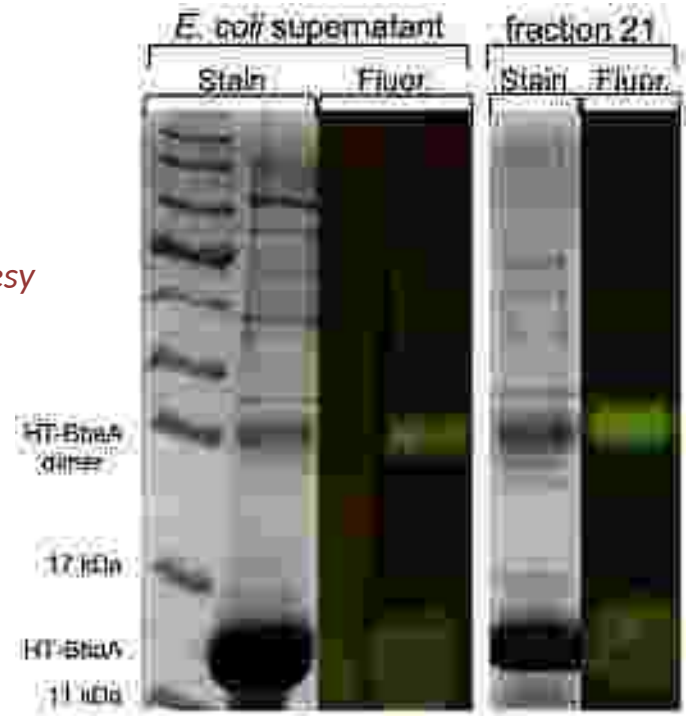
Unk10, a Nif11 family member is required for addition of PUB to MpeA Cys-140 and CpeA Cys-139 in blue light





=> Unk10 = BbaA (Blue-harvesting bilin addition)





Slide courtesy of D. Kehoe

BbaA monomer model



BbaA dimer/chromophore model



⇒ BbaA is a small **novel lyase** likely organized as a dimer

⇒ BbaA also occurs **in PUB-containing non-*Syn* cyanobacteria** (e.g., *Crocosphaera*)

Chen et al., submitted soon to PNAS

Croucher course 3 outline

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II. Characterization on *Prochlorococcus* light-harvesting complexes

III. *Synechococcus* phycobilisomes and pigment diversity

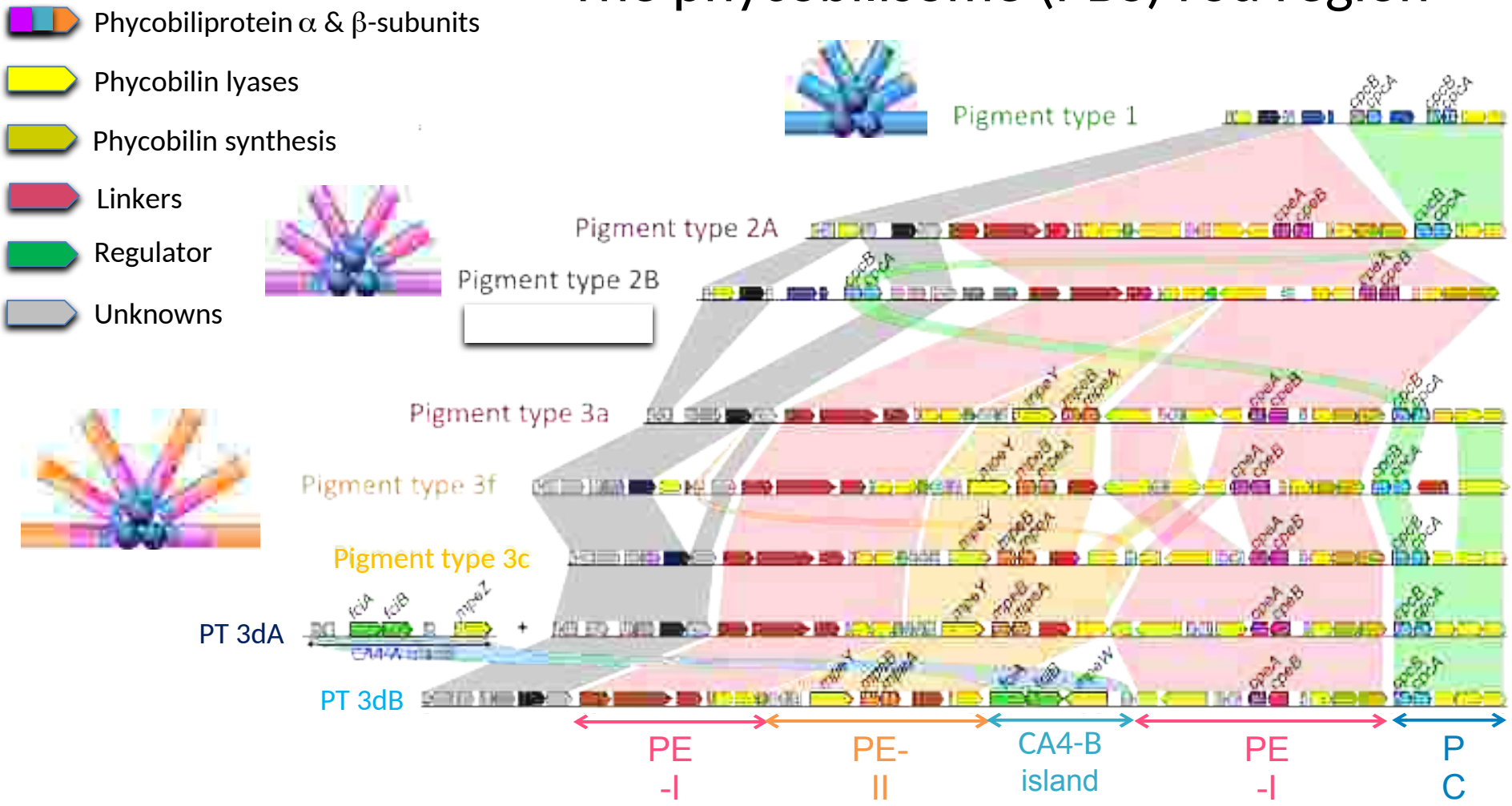
IV. Biochemical and molecular characterization of CA4

V. Evolution and diversity of pigment types

VI. Ecological distribution and modelling of pigment types



The phycobilisome (PBS) rod region



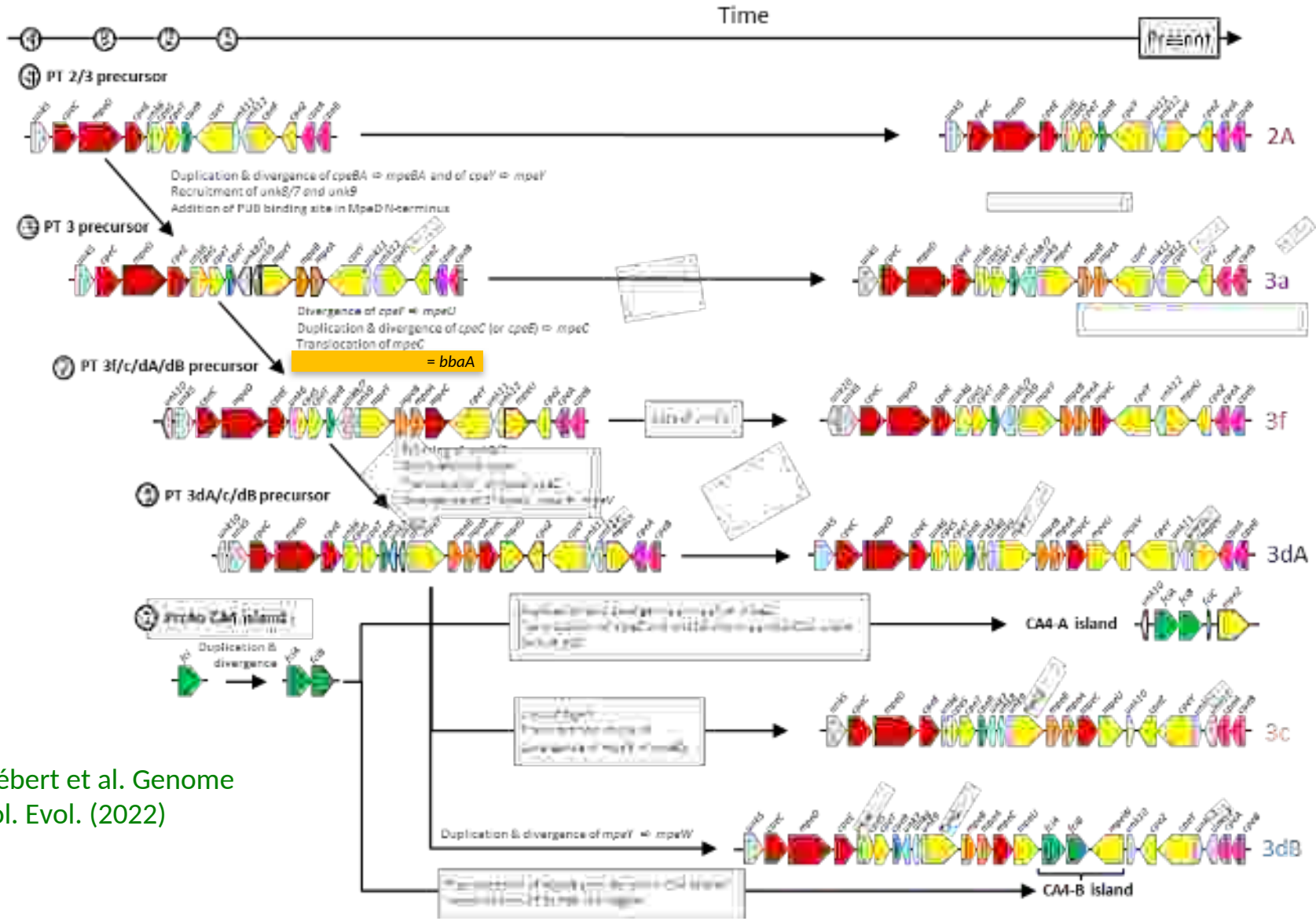
- Three main pigment types (PT 1, 2, 3) and 6 subtypes in genomes from cultured marine *Synechococcus* strains.

  Q1: Which one came first? 1, 2B, 2A, …

  Q2: Is there any yet unknown pigment type diversity in field populations?

Six *et al.* 2007 Genome Biol; Larsson *et al.* 2014 ISME J; Humily *et al.* 2013 PLoS One ; Sanfilippo *et al.* 2016 PNAS

Possible evolutionary scenario for the occurrence of the different PBS rod regions



Grébert et al. Genome Biol. Evol. (2022)

⇒ Parsimonious evolutionary scenario globally congruent with individual phylogenies of genes of the PBS rod region.

A targeted metagenomic approach to retrieve PBS rod regions from field populations



Q2: Is there any yet unknown pigment type diversity in natural *Syn* populations ?

Natural samples

Prefiltration/Concentration

Cell sorting by flow cytometry

Whole Genome Amplification
(from 2,500 cells)

Fosmid libraries

Screening
(hybridization and PCR)

Fosmid sequencing

PBS regions from the field



- Large inserts (25-45kbp) compatible with the size of the PBS rod region

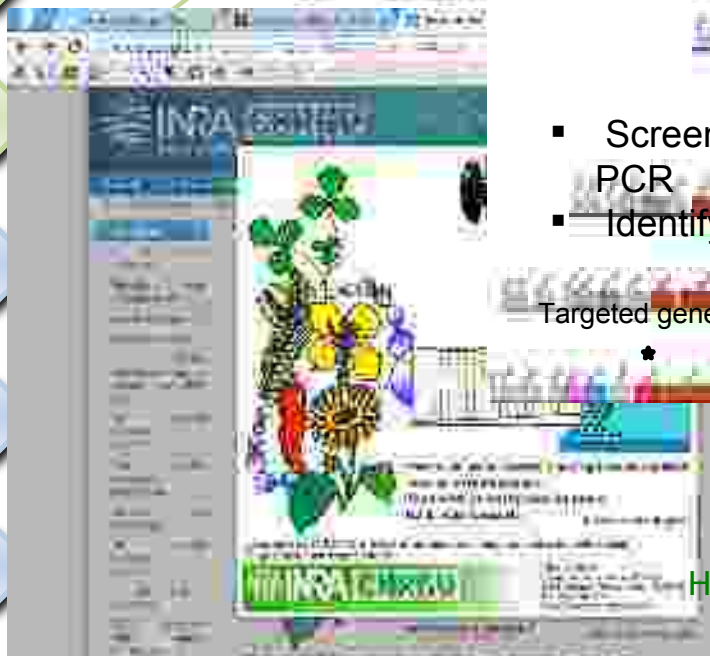


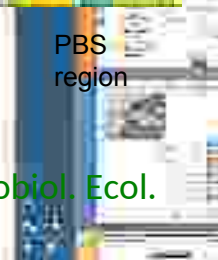




- Screening by hybridization and PCR
- Identify clones with PBS region

Targeted genes for screening: *cpeC*, *mpeBA*, *cpeU*, *cpcBA*





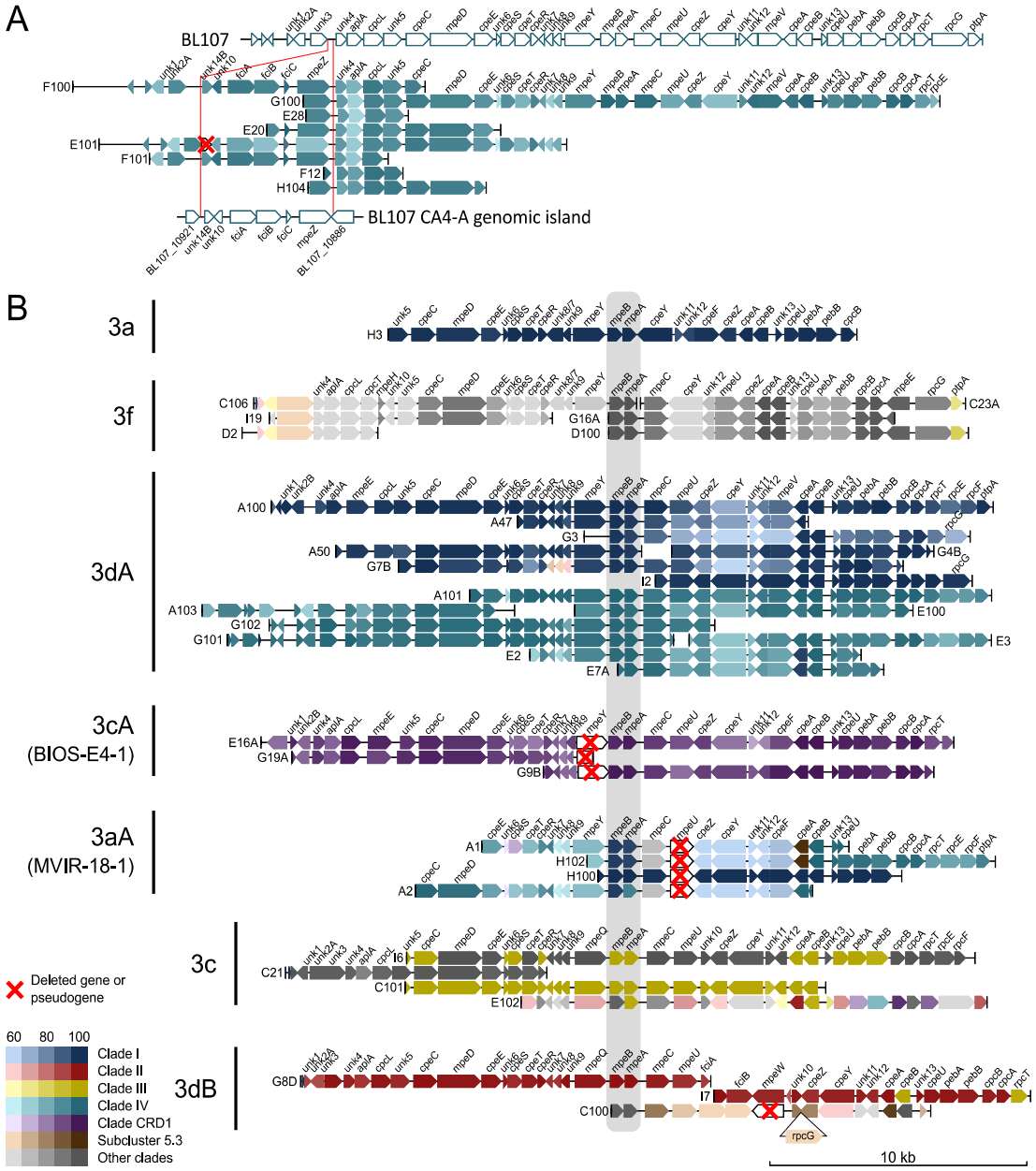
Humily et al. 2014. FEMS Microbiol. Ecol.

Diversity of the PBS rod region in field *Syn* populations

Q2: Is there any yet unknown pigment type diversity in natural *Syn* populations?

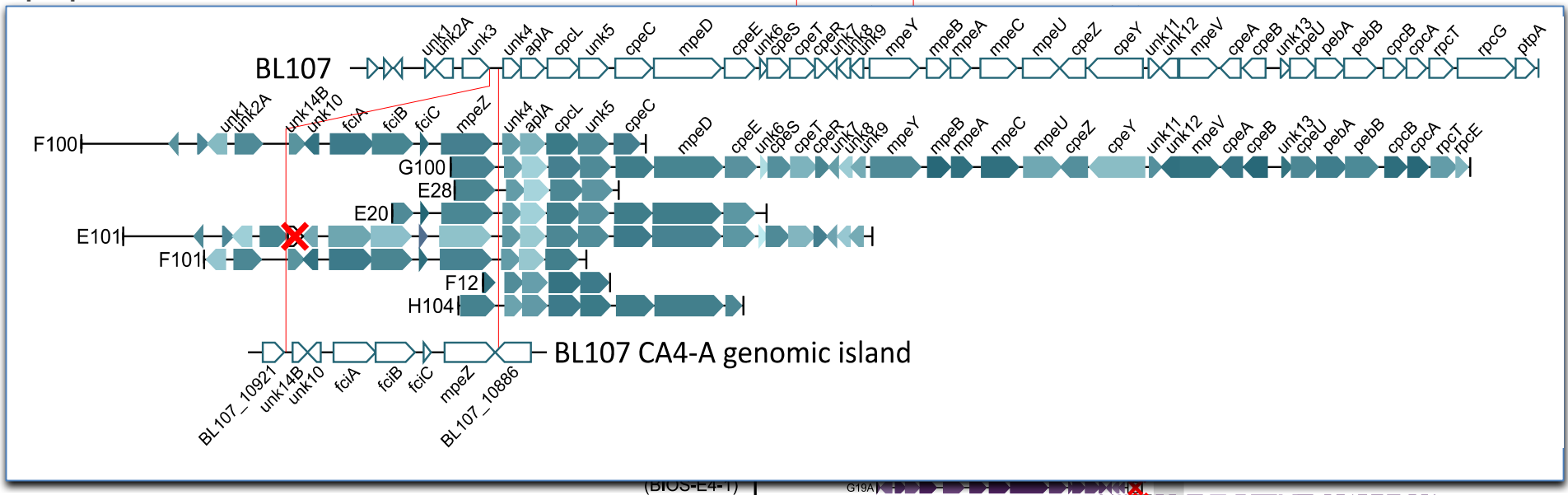


Grébert et al. Genome Biol. Evol. (2022)



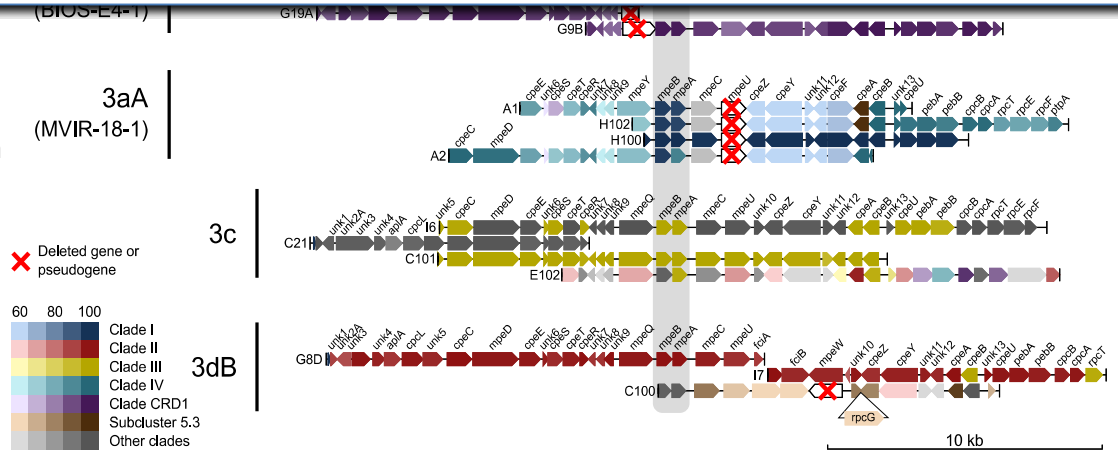
Diversity of the PBS rod region in field *Syn* populations

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- Novelties:

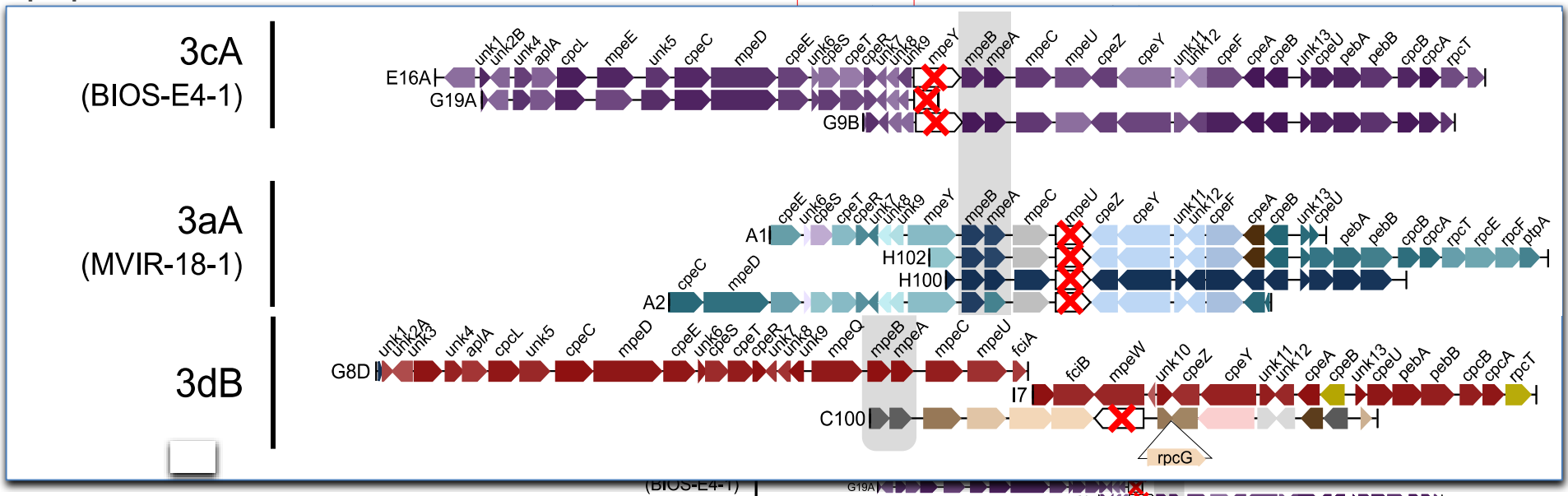
- PT 3dA with the CA4-A island within the PBS rod region



Grébert et al. Genome Biol. Evol. (2022)

Diversity of the PBS rod region in field *Syn* populations

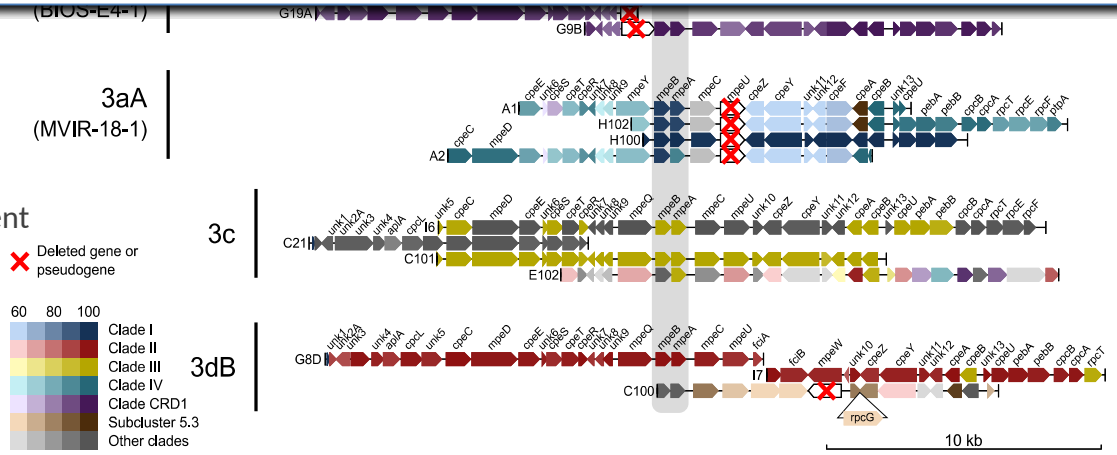
Q2: Is there any yet unknown pigment type diversity in field *Syn* populations?



- Novelties:

- PT 3dA with the CA4-A island <u>within</u> the PBS rod region

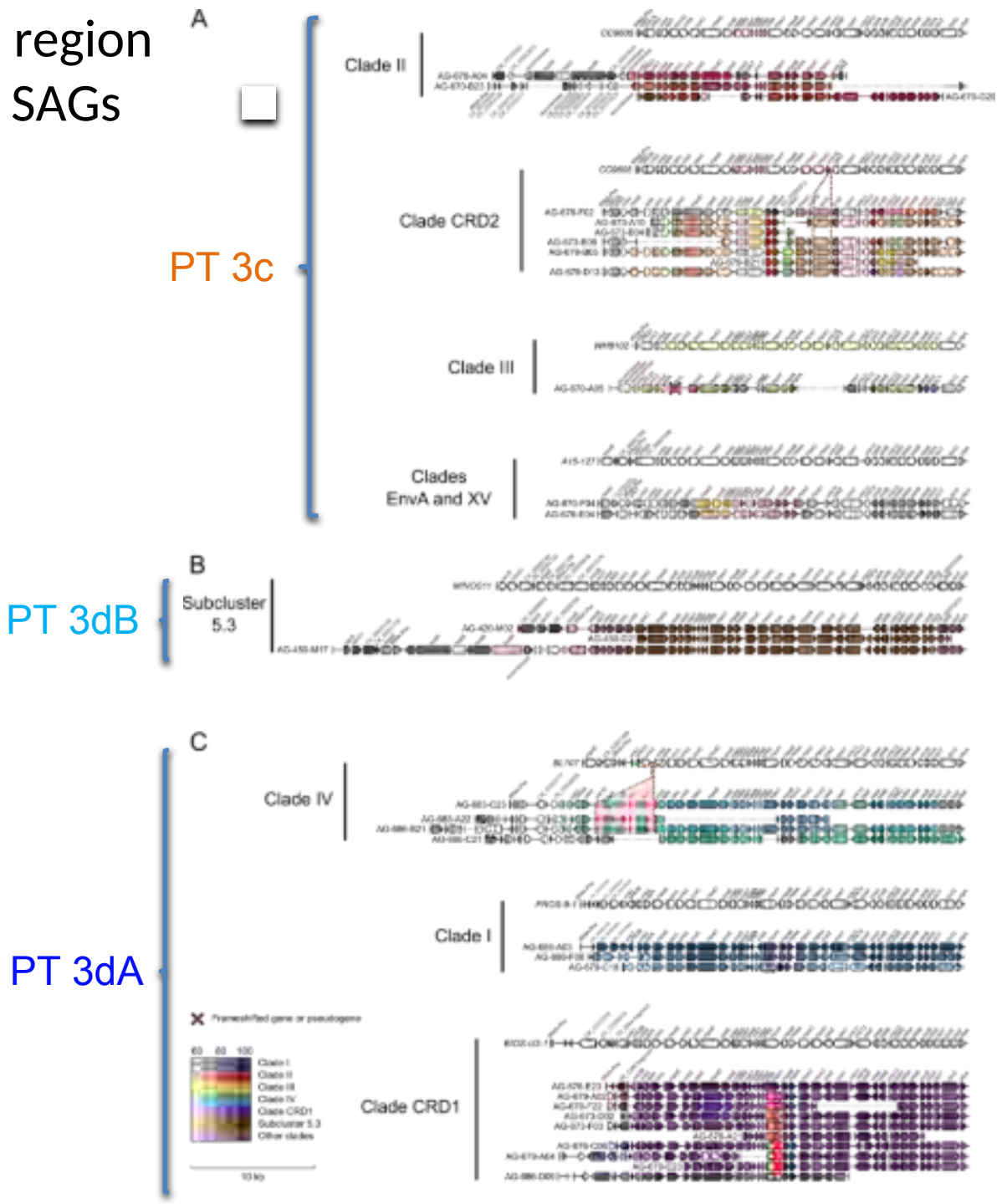
- Presence of <u>natural</u> lyase KO mutants in the environment either known from isolates or yet unknown (*mpeW*)



Grébert et al. Genome Biol. Evol. (2022)

Diversity of the PBS Rod region in field *Syn* populations: SAGs

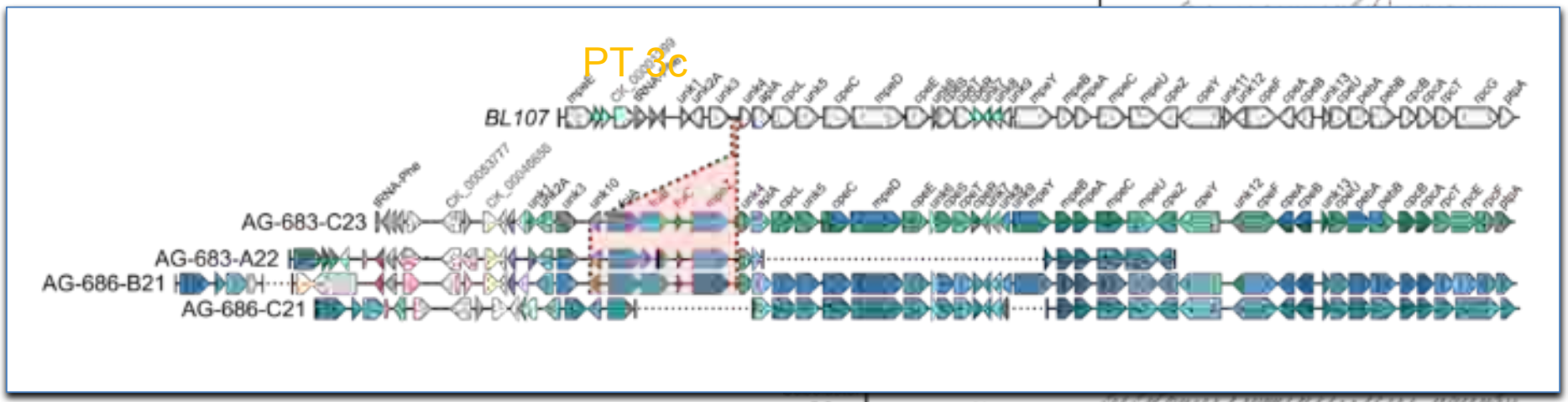
(Berube et al. 2018 Sci Data)



Grébert et al. Genome Biol Evol (2022)

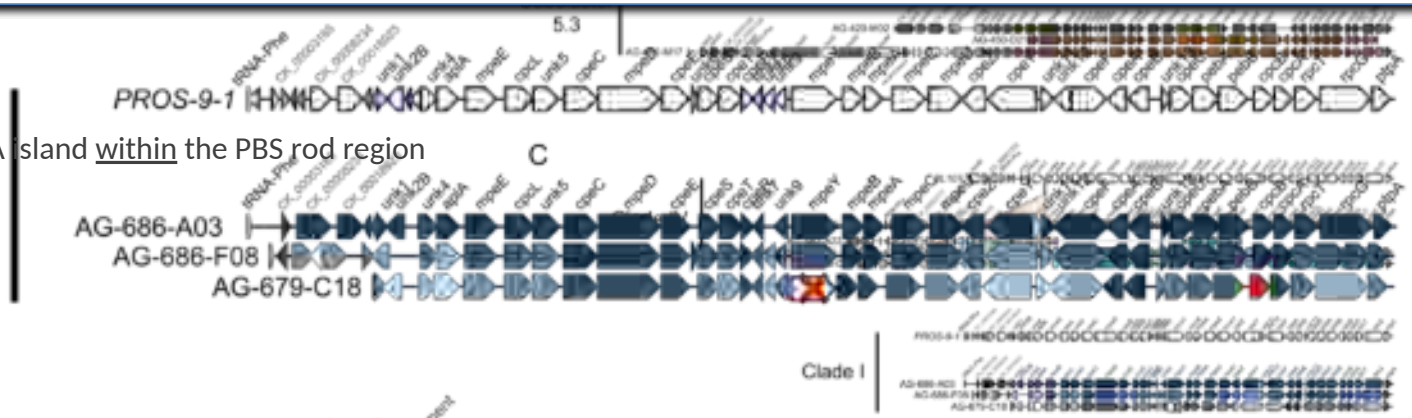
Diversity of the PBS Rod region in field *Syn* populations: SAGs

(Berube et al. 2018 Sci Data)

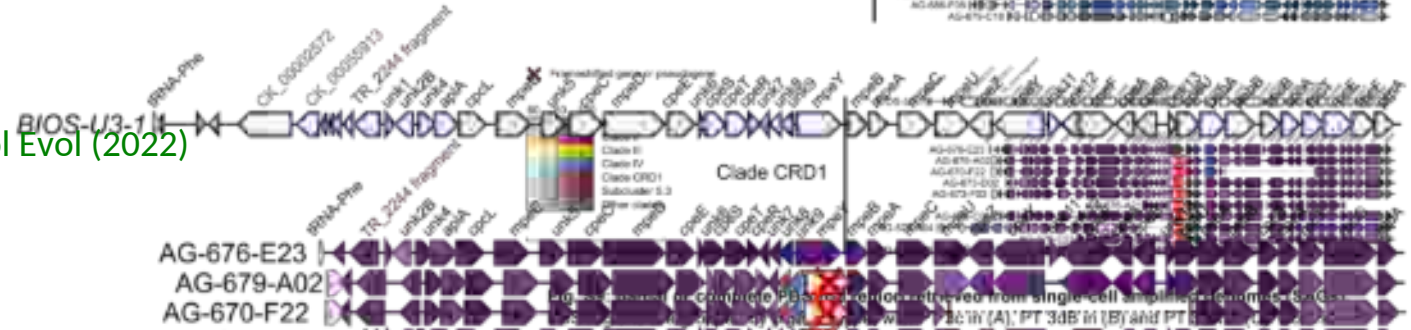


- Novelties:

- **More** PT 3dA with the CA4-A island <u>within</u> the PBS rod region



Clade I



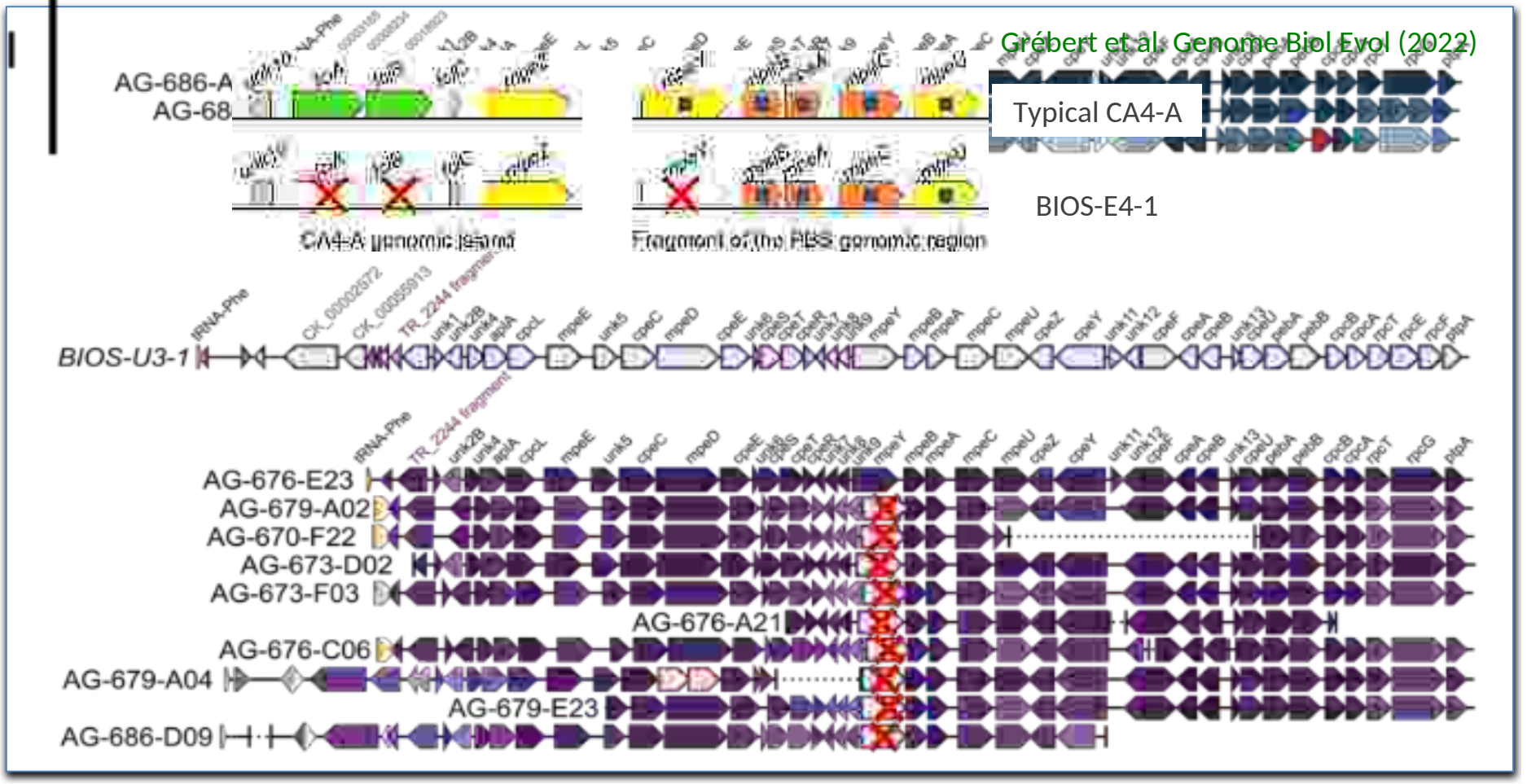
Clade CRD1

Grébert et al. Genome Biol Evol (2022)

Diversity of the PBS Rod region in field *Syn* populations: SAGs

(Berube et al. 2018 Sci Data)





- More natural lyase KO mutants in the environment notably ***mpeY*-less mutants**...
  Yet SAGs allow one to also retrieve the **CA4-A island** and this lacks *cpcA* and *cpcB* =>this is natural Kos (formerly PT 3dA) from low-Fe regions turned into BL specialists (=PT 3cA)!!



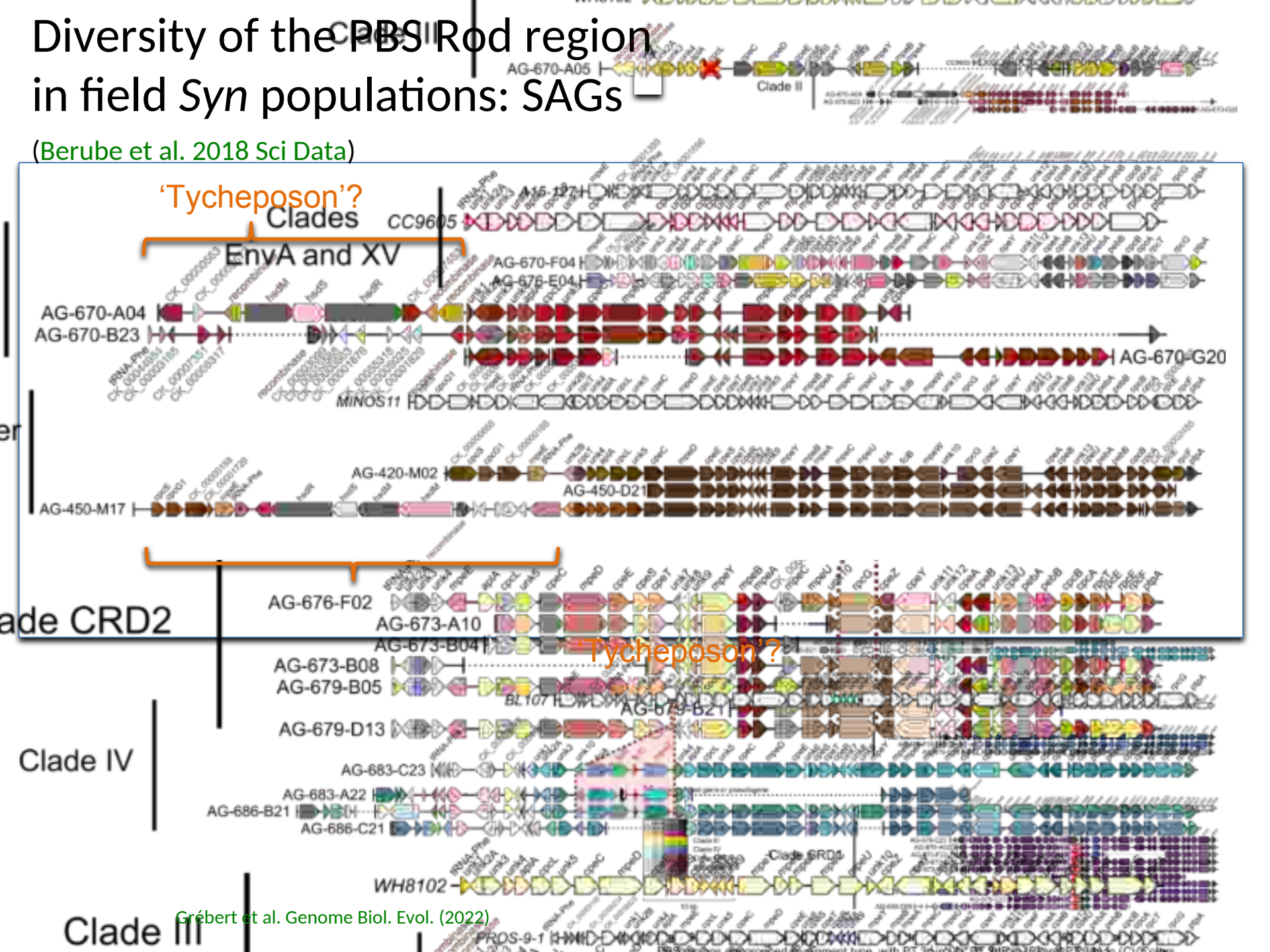
region retrieved from single-cell amplified genomes (SAGs).

ype, with PT 3c in (*A*), PT 3dB in (*B*) and PT 3dA in (*C*). Colors

rain giving the best blastp hit within the given pigment type

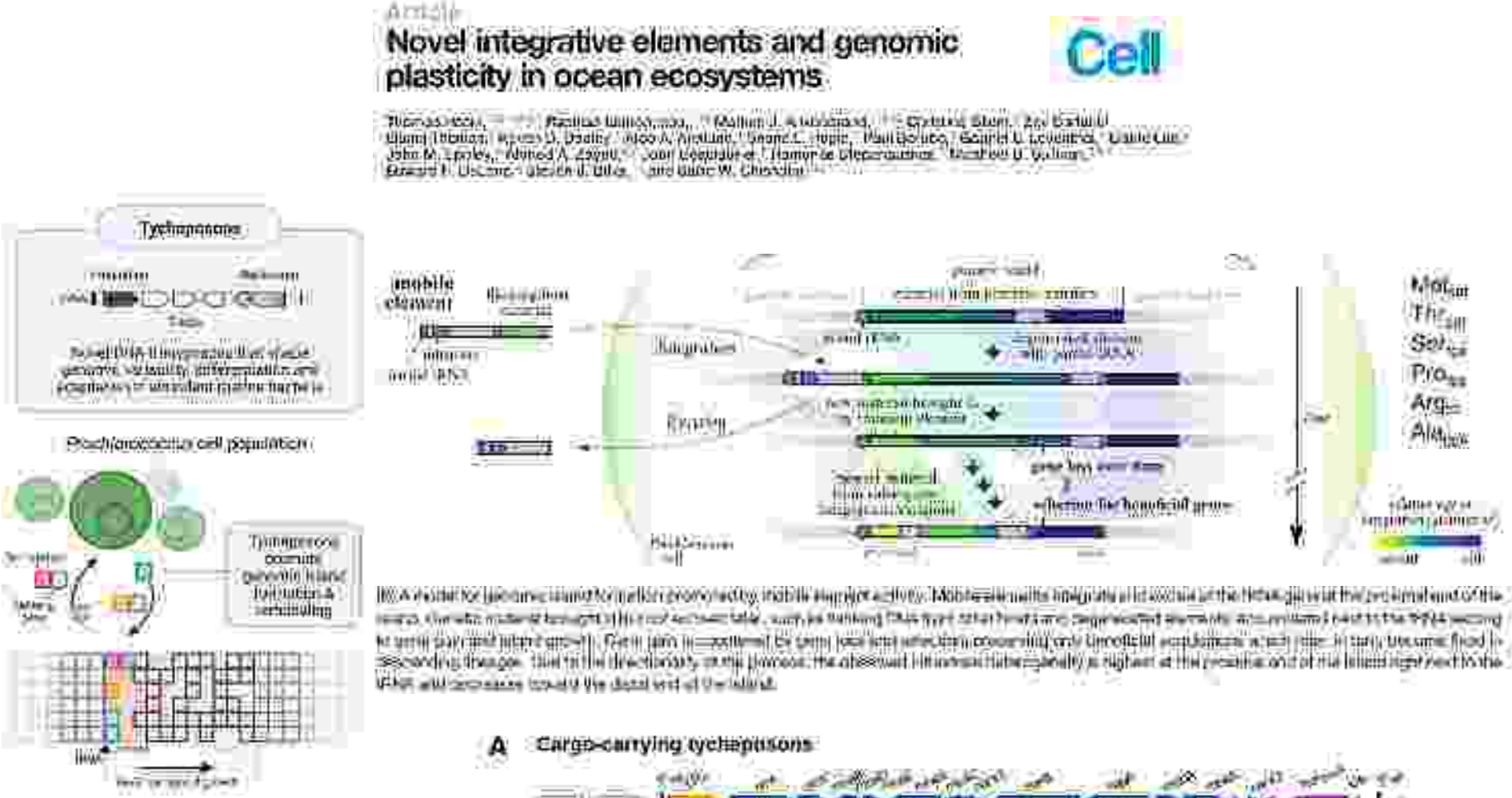
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Grébert et al. Genome Biol. Evol. (2022)

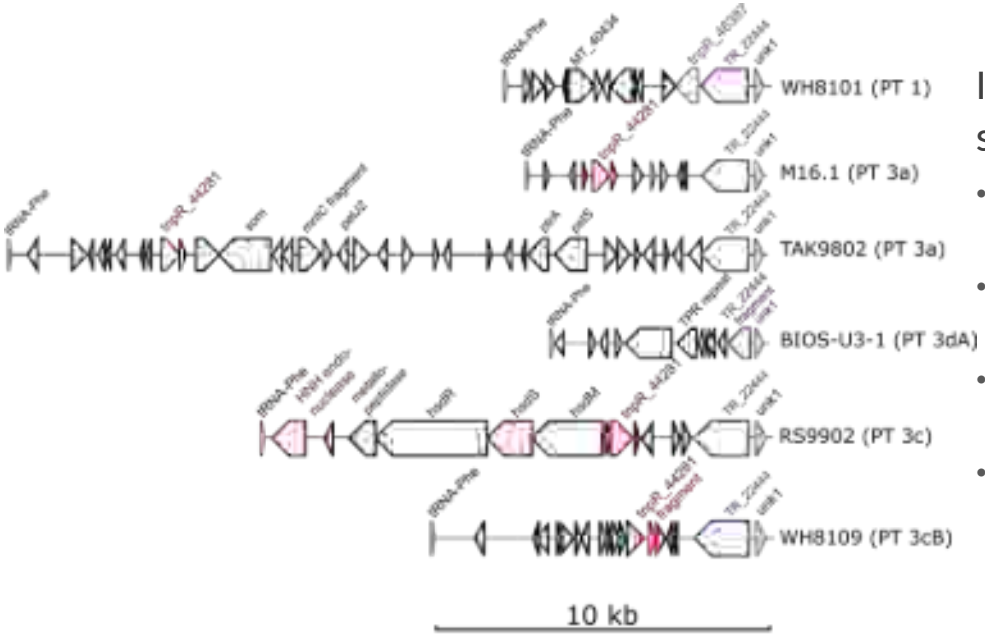
Tycheposons in *Prochlorococcus*



Examples of cargo modules with roles in nutrient assimilation
=> **Tycheposons have a key role in niche adaptation**

Hackl et al. Cell (2023)

Tycheposons also exist in *Synechococcus* upstream the PBS region



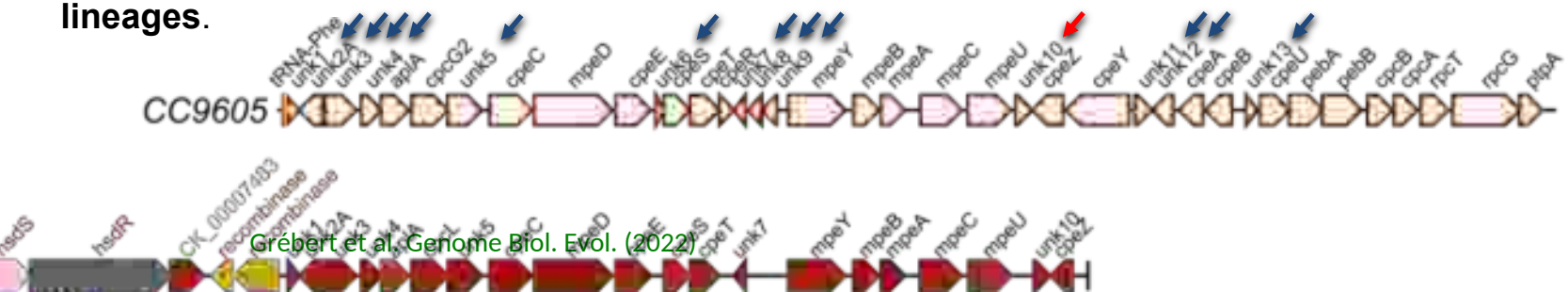
Grébert et al. Fig. 2

In both *Syn* SAGs and WGS, some PBS rod regions start with a tycheposon-like region

- Like all PBS rod regions, those with a tycheposon always start with tRNA-Phe-GAA (≠ *Prochlorococcus*)
- Frequent presence of a tyrosine recombinase (complete or fragmentary) immediately upstream *unk1*
- Presence of other mobile elements (transposon resolvase, other recombinases, restriction-modification system, etc.)
- Many short hypothetical or conserved hypothetical genes and some complete functional genes

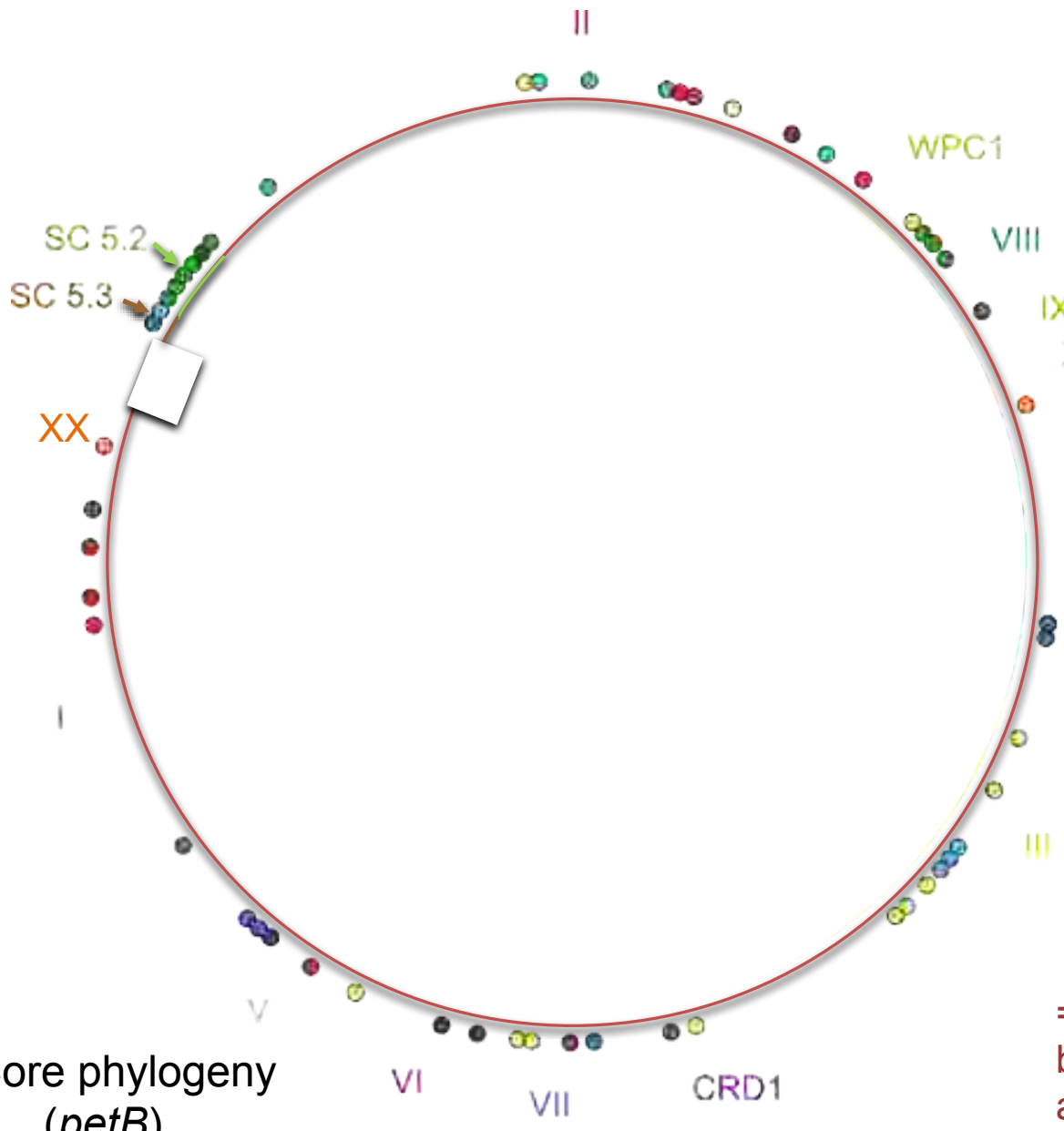
Tycheposons are likely important in the variability of the gene content of the PBS rod region and thus in adaptation to the different **spectral niches**.

They may also have played a key role in the acquisition of the **short unknown genes (*unk1-13*)** located in the PBS rod region and/or in the **maintenance of pigment diversity in the different lineages**.



Grébert et al. Genome Biol. Evol. (2022)

Evolution of *Syn PTs*: core phylogeny vs. pigment type



Core phylogeny (*petB*)

Pigment types

- PT
- PT
- PT
- PT
- PT
- PT
- 3dB

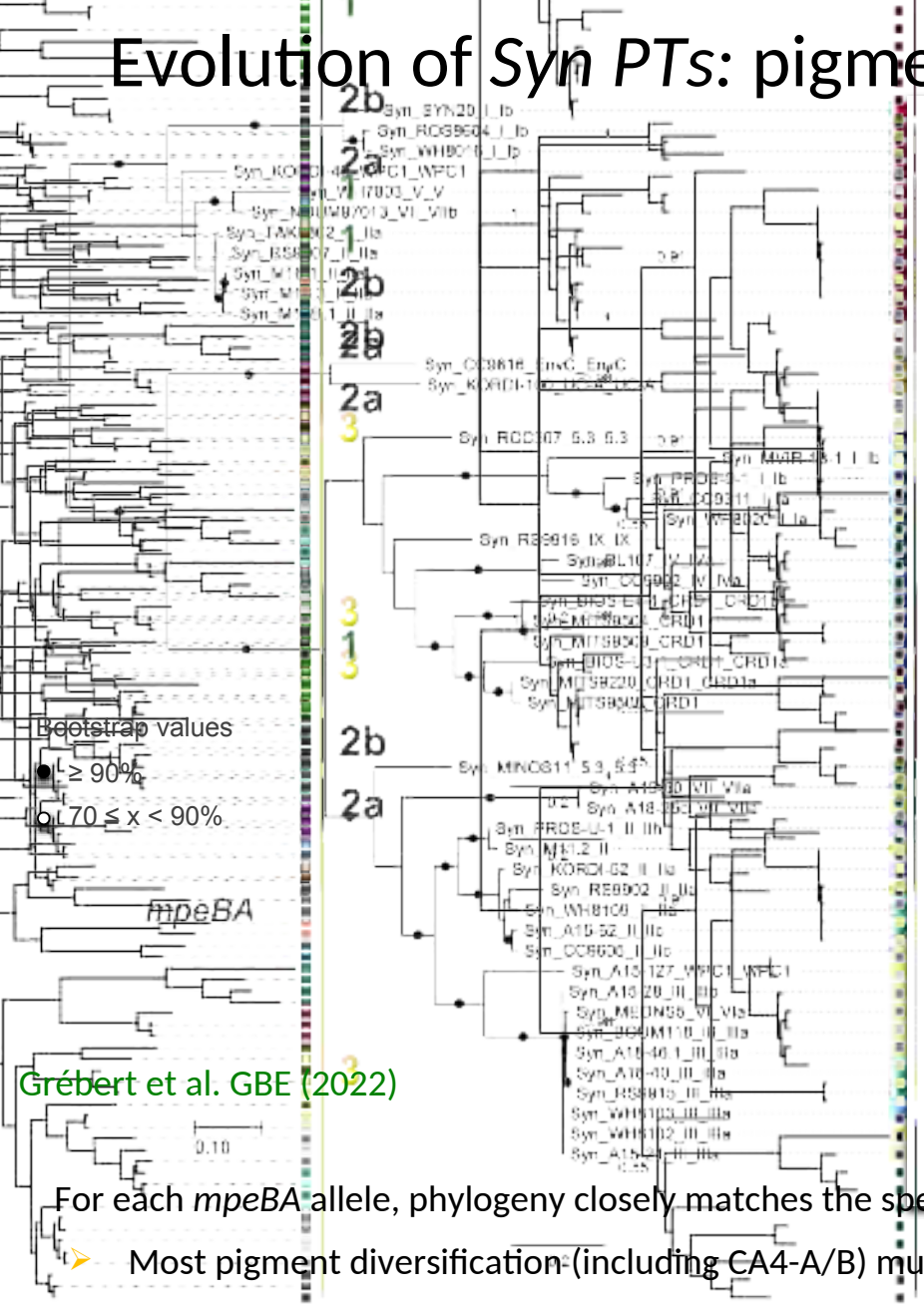
SC 5.1: all PTs
SC 5.2: only PT1 and PT2
SC 5.3: PT 3dA-B (+PT 2)

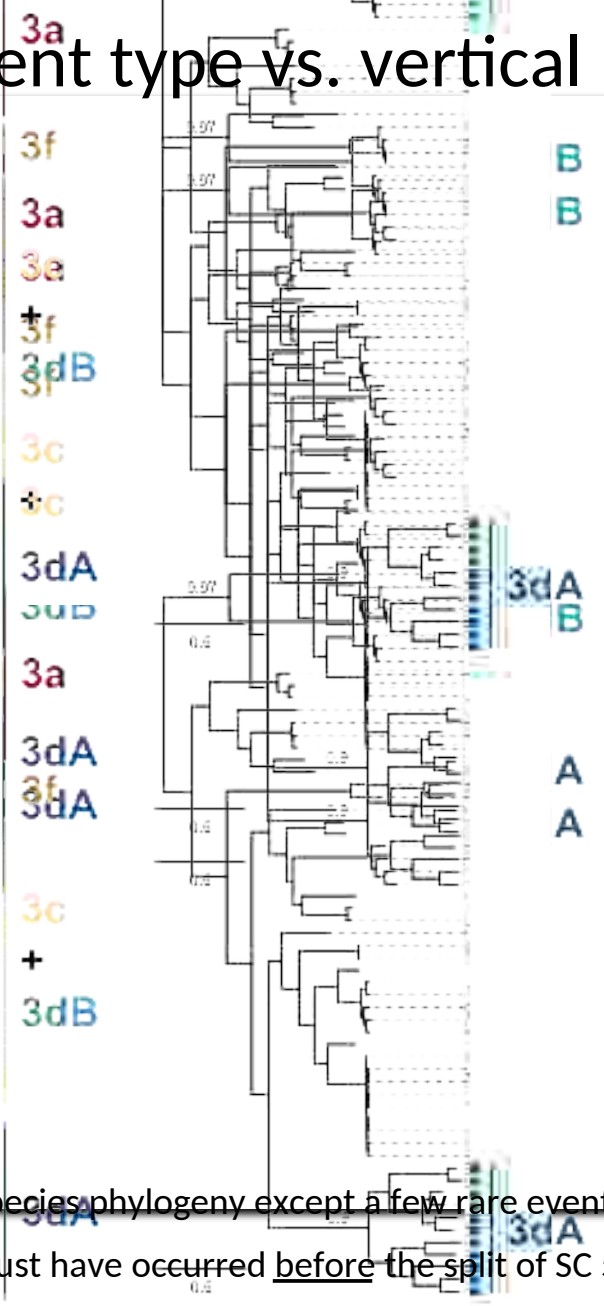
A given clade can contain several different PTs

A same PT can be found in different clades

=> At first look, no congruence between vertical phylogeny (*petB*) and pigment type, but…

Evolution of *Syn PTs*: pigment type vs. vertical phylogeny





Grébert et al. GBE (2022)

For each *mpeBA* allele, phylogeny closely matches the species phylogeny except a few rare events of HGT (e.g. MEDNS5)

- Most pigment diversification (including CA4-A/B) must have occurred before the split of SC 5.1 and 5.3

- Pigment diversity maintained during the diversification of SC 5.1 lineages by **incomplete lineage sorting (ILS)**, a population-scale mechanism in which an ancestral polymorphism is NOT sorted during a speciation event.

Croucher course 3 outline

I. Introduction

II. Characterization on *Prochlorococcus* light-harvesting complexes

III. *Synechococcus* phycobilisomes and pigment diversity

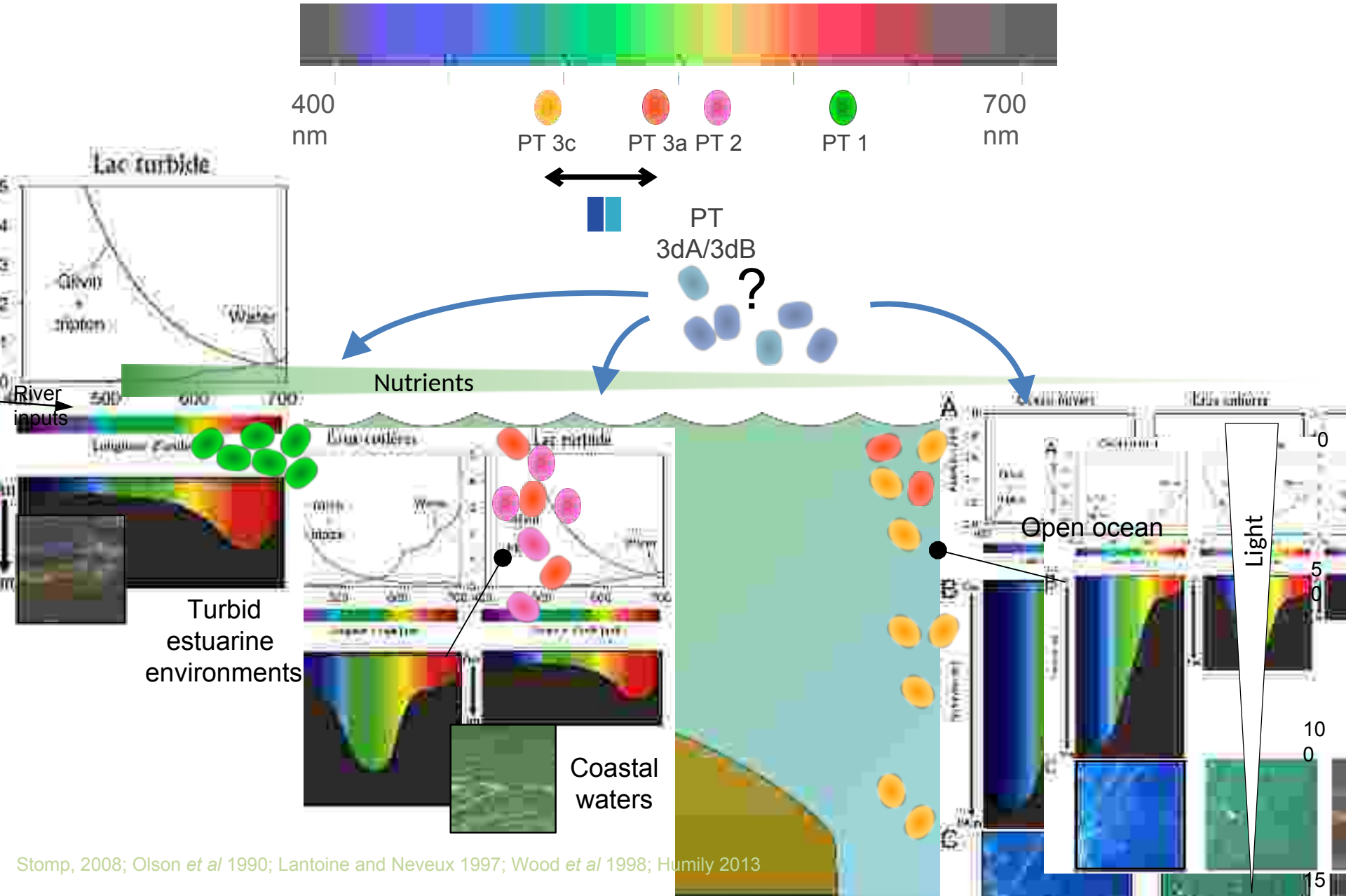
IV. Biochemical and molecular characterization of CA4

V. Evolution and diversity of pigment types

VI. Ecological distribution and modelling of pigment types

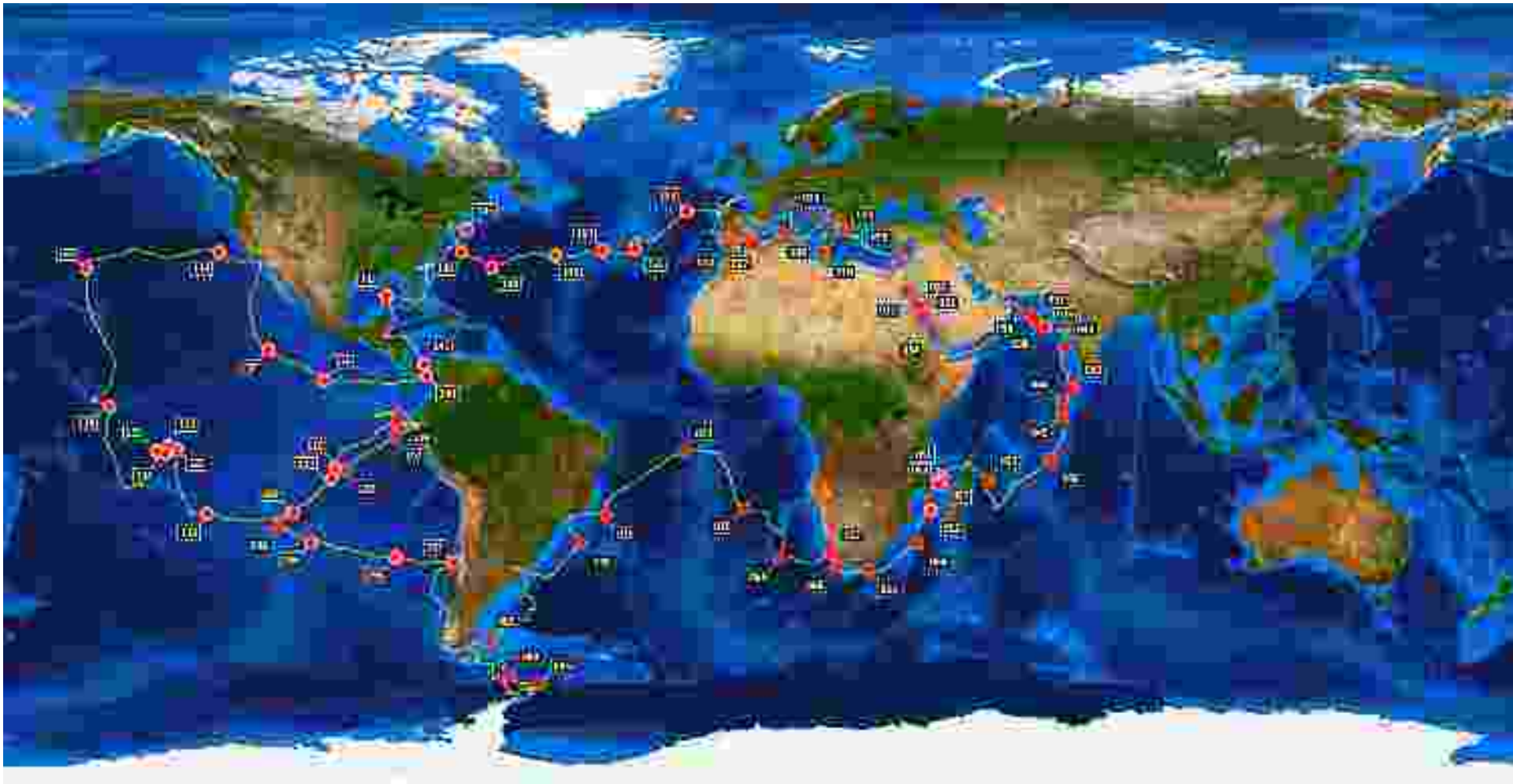


Ecological significance of chromatic acclimation?



Stomp, 2008; Olson *et al* 1990; Lantoine and Neveux 1997; Wood *et al* 1998; Humily 2013

The *Tara Oceans* expedition 2009-2013

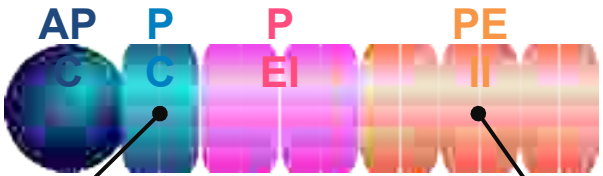


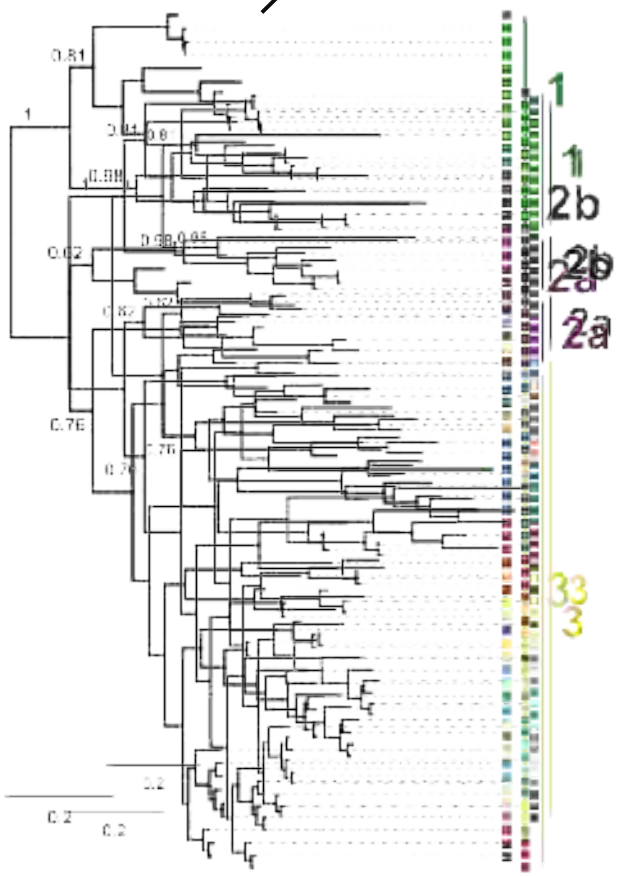
- 66 sampling stations covering all major oceanic basins
- 109 metagenomes from the bacterial size fraction (0.22-1.6/3 µm), surface and mesopelagic

Dataset used for studying the global distribution of *Synechococcus* pigment types

TARA OCEANS

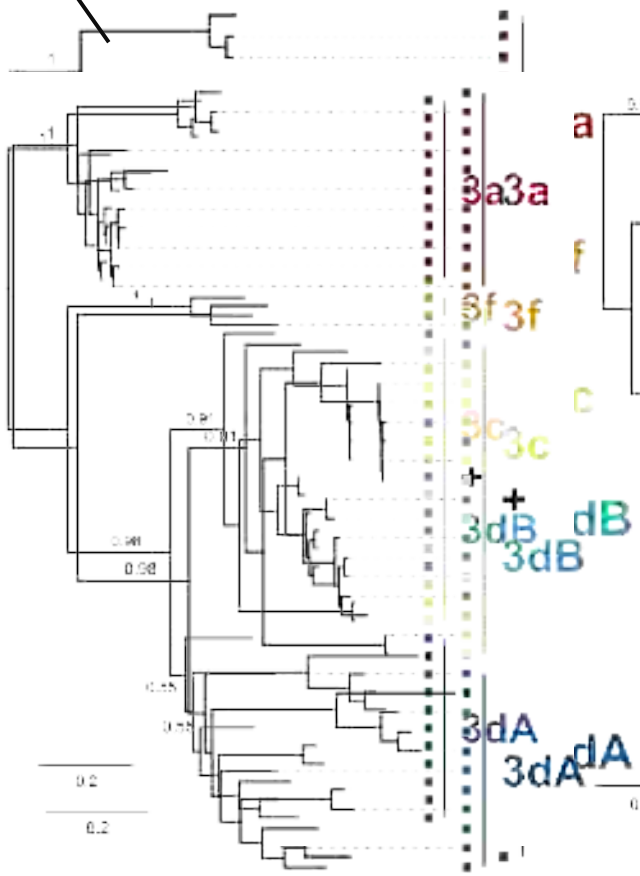
Choice of relevant markers to discriminate all pigment types



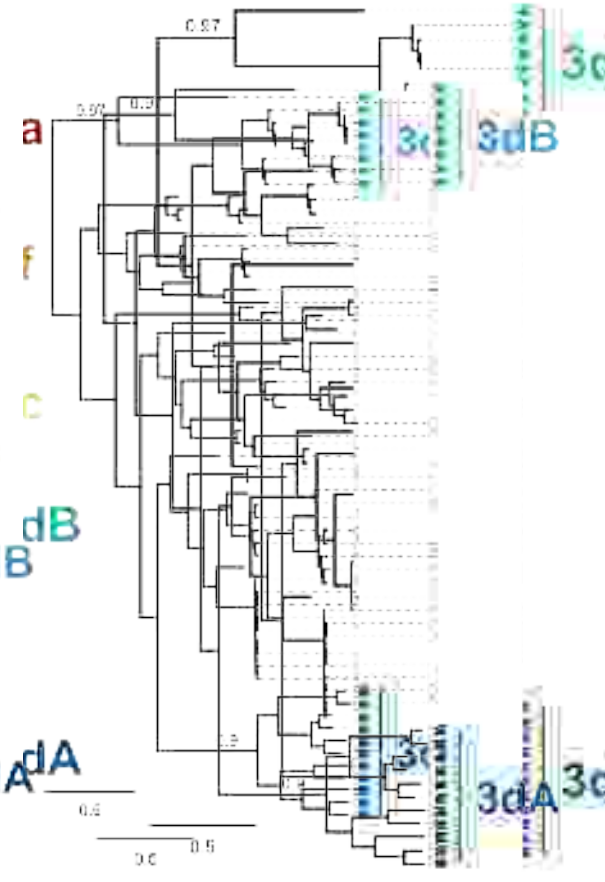


cpcBA





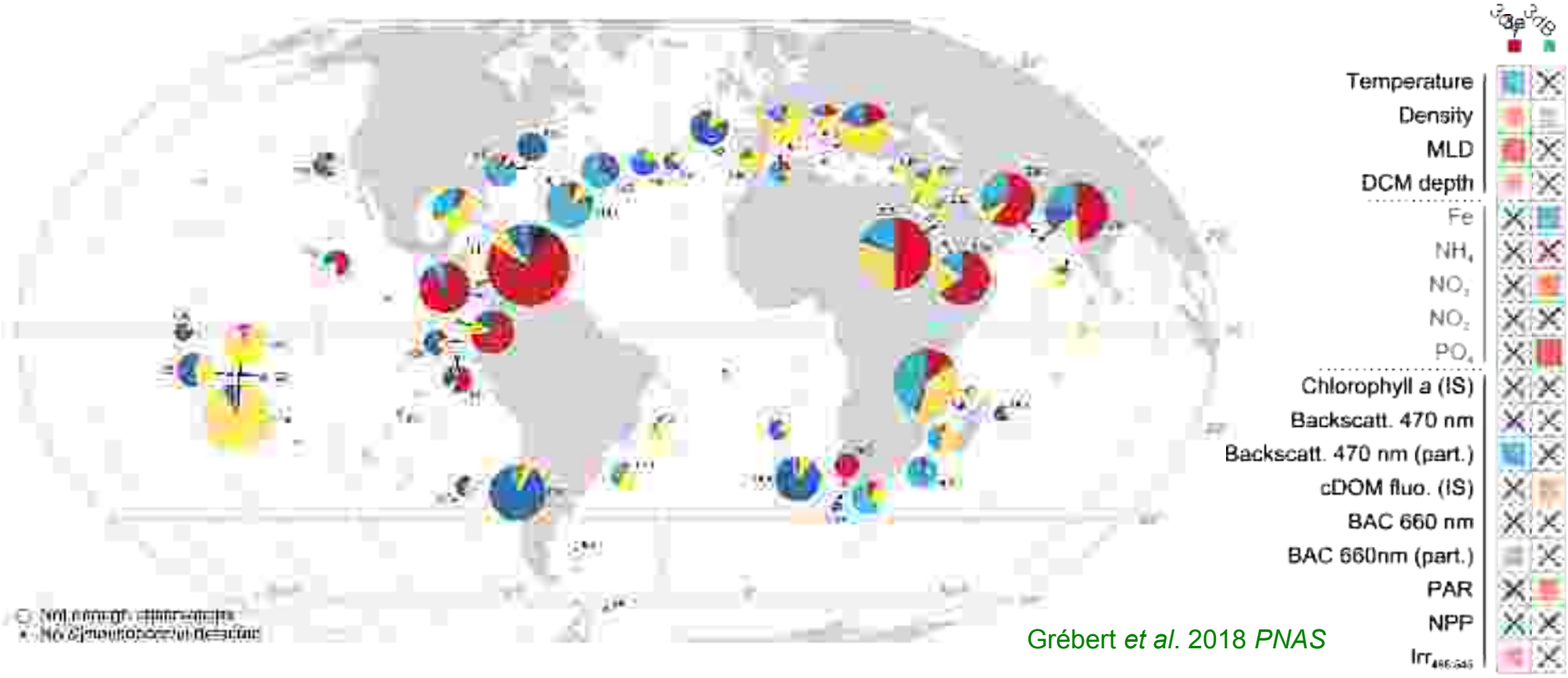
mpeBA



mpeW-Y-Q-Z

=> A combination of three different marker genes is necessary to predict the main pigment types

Global distribution of *Synechococcus* pigment types

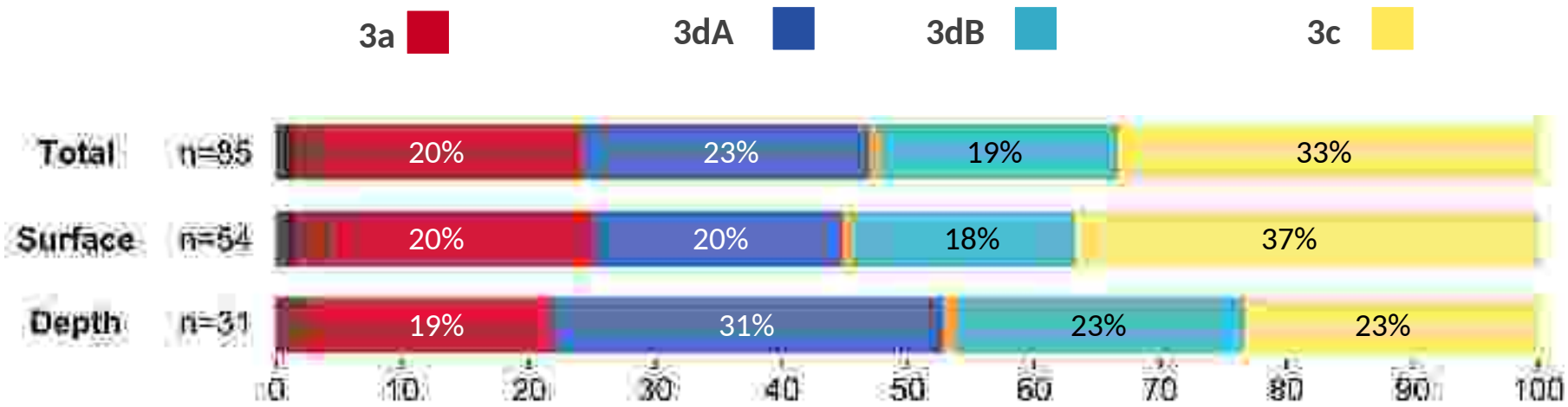


Grébert *et al*. 2018 *PNAS*

- PT 3 is by far the dominant PT in pelagic areas (PT 1 and 2A/2B virtually absent)

3a	3c	3dA	3dB
High temperature	Low nutrients, iron-rich High temperature	High nutrients Low temperature	Low nutrients (N, P) oligotrophic waters
Green, particle-rich waters	Clear waters Low Chlorophyll *a*	Productive, phytoplankton rich waters	Low light

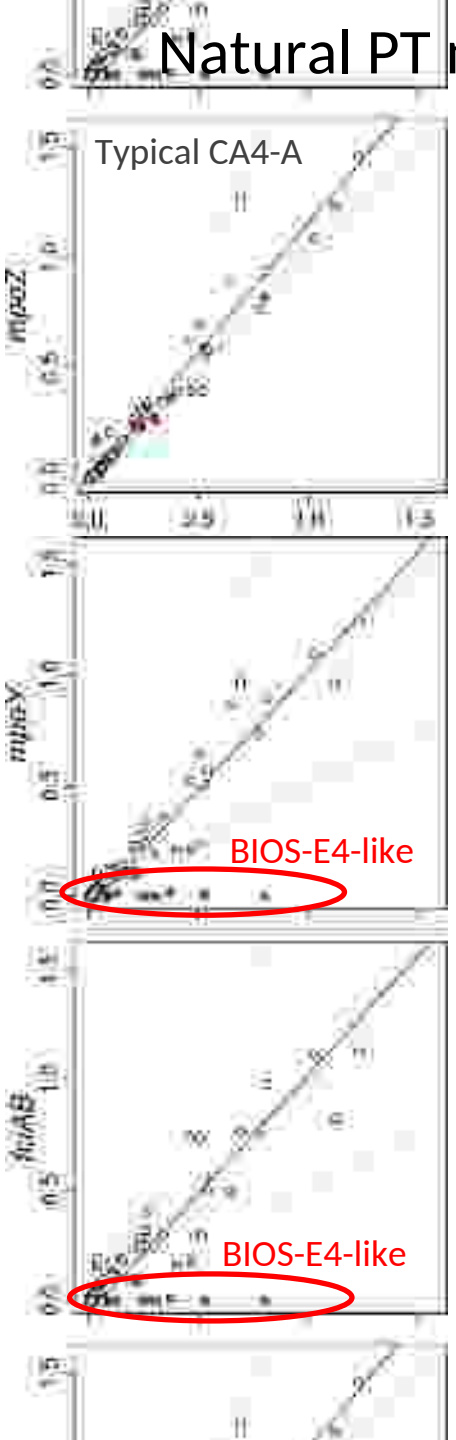
Global abundance of *Synechococcus* pigment types



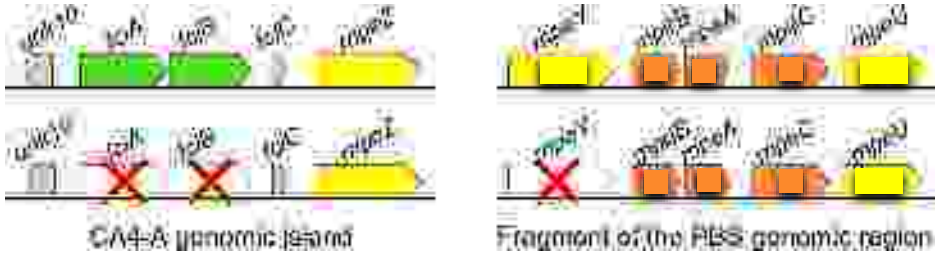
Grébert et al. 2018 *PNAS*

- Unexpected abundance of CA4, which constitutes the dominant pigment type, especially at depth

Natural PT mutants occur in field *Synechococcus* populations



- Recruitment of additional marker genes allows detecting populations of natural mutants



Typical CA4-A => PT 3dA

BL phenotype => PT 3cA (BIOS-E4-1)

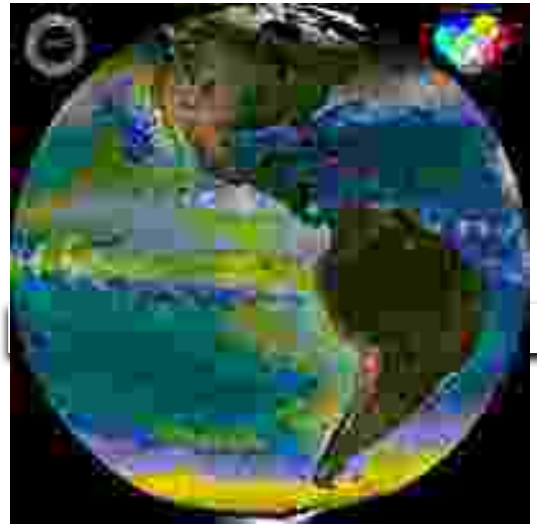
- Typical CA4-A populations: *mpeBA* = *mpeY* = *mpeZ* = *fciAB*
- BIOS-E4-1-like populations: *mpeBA* = *mpeZ* >> *fciAB* = *mpeY*

- BIOS-E4-1-like populations (PT 3cA) constitute up to 15% of the global Syn population with a PT 3dA genotype along the Tara Oceans transect.

- This natural mutation could constitute an adaptation to Fe-poor, blue environments by inactivating CA4, useless in these permanently blue waters.

Grébert *et al.* 2018 *PNAS*

Modelling the spatial distribution of *Synechococcus* PTs using the Darwin Model



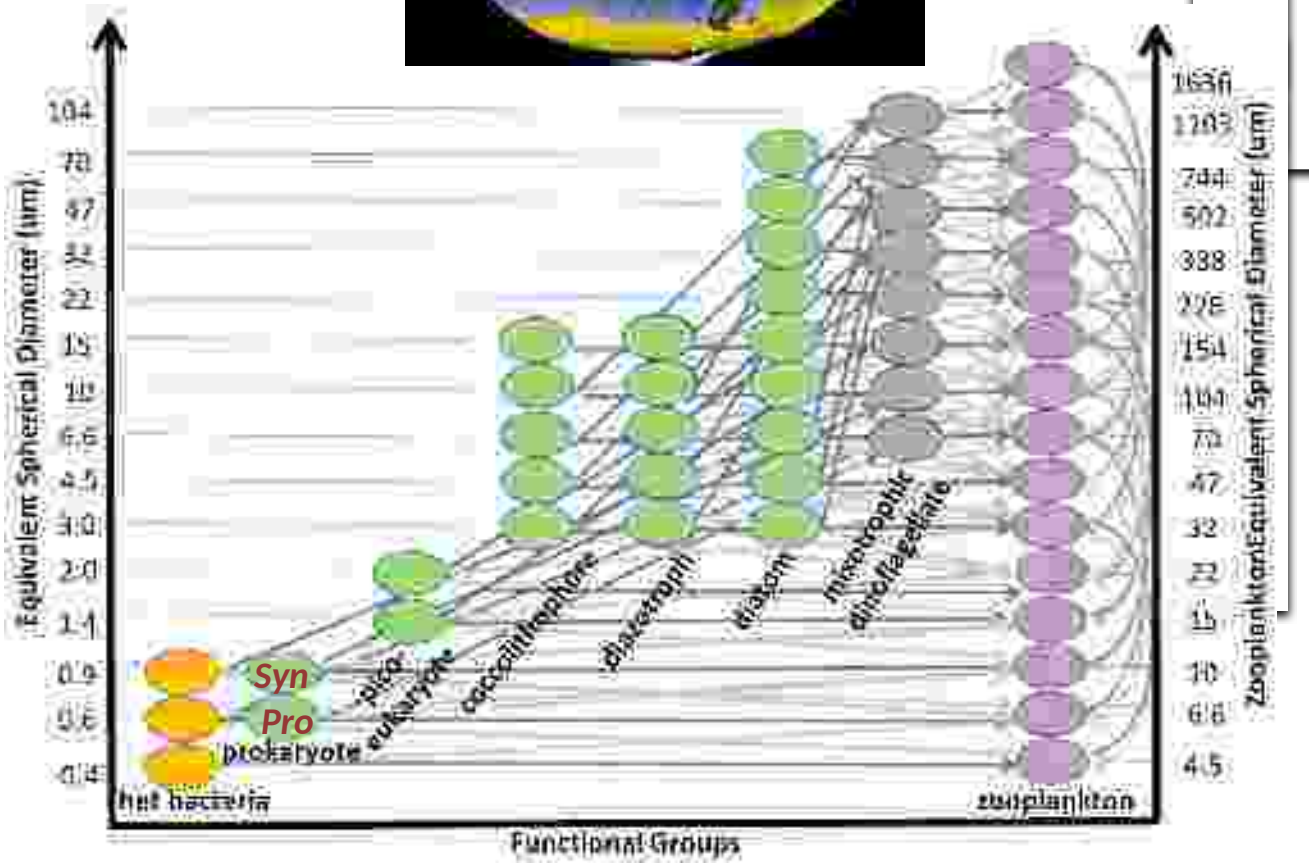




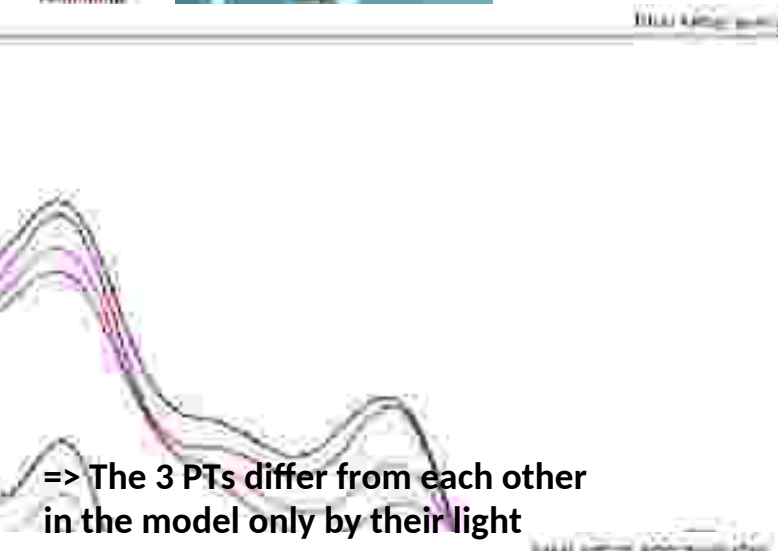
M. Follows **S. Dutkiewicz**
and many others...

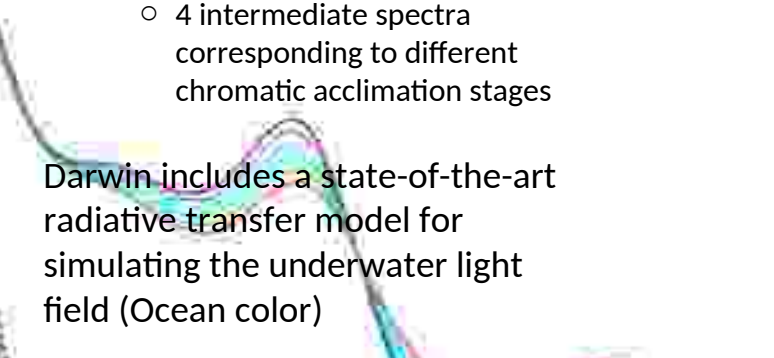
Modeling Marine Microbes

The Darwin Project is an initiative to advance the development and application of novel models of marine microbes and microbial communities, identifying the relationships of individuals and communities to their environment, connecting cellular-scale processes to global microbial community structure.



Modelling the spatial distribution of the three main *Synechococcus* PTs





=> The 3 PTs differ from each other in the model only by their light absorption spectra

- Green light specialist : 1 spectrum
- Blue light specialist: 1 spectrum
- Chromatic acclimater: 6 spectra
    - Full green light
    - Full blue light
    - 4 intermediate spectra corresponding to different chromatic acclimation stages

Darwin includes a state-of-the-art radiative transfer model for simulating the underwater light field (Ocean color)

Modelling the spatial distribution of the three main *Synechococcus* PTs

GL specialists

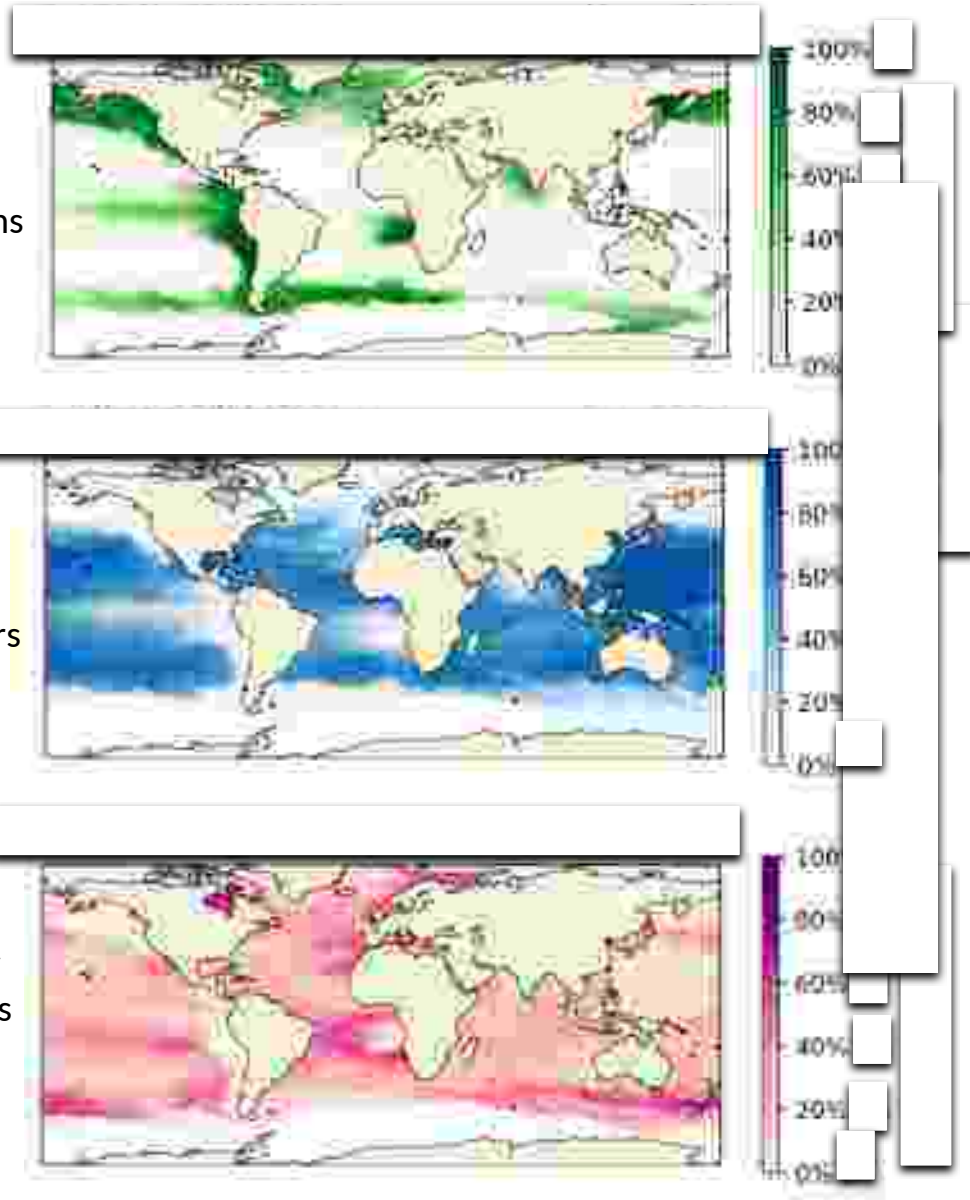
- Eastern ocean margins & subpolar waters

BL specialists

- Warm open ocean waters

Chromatic acclimaters

- Ubiquitous and particularly abundant in transition zones

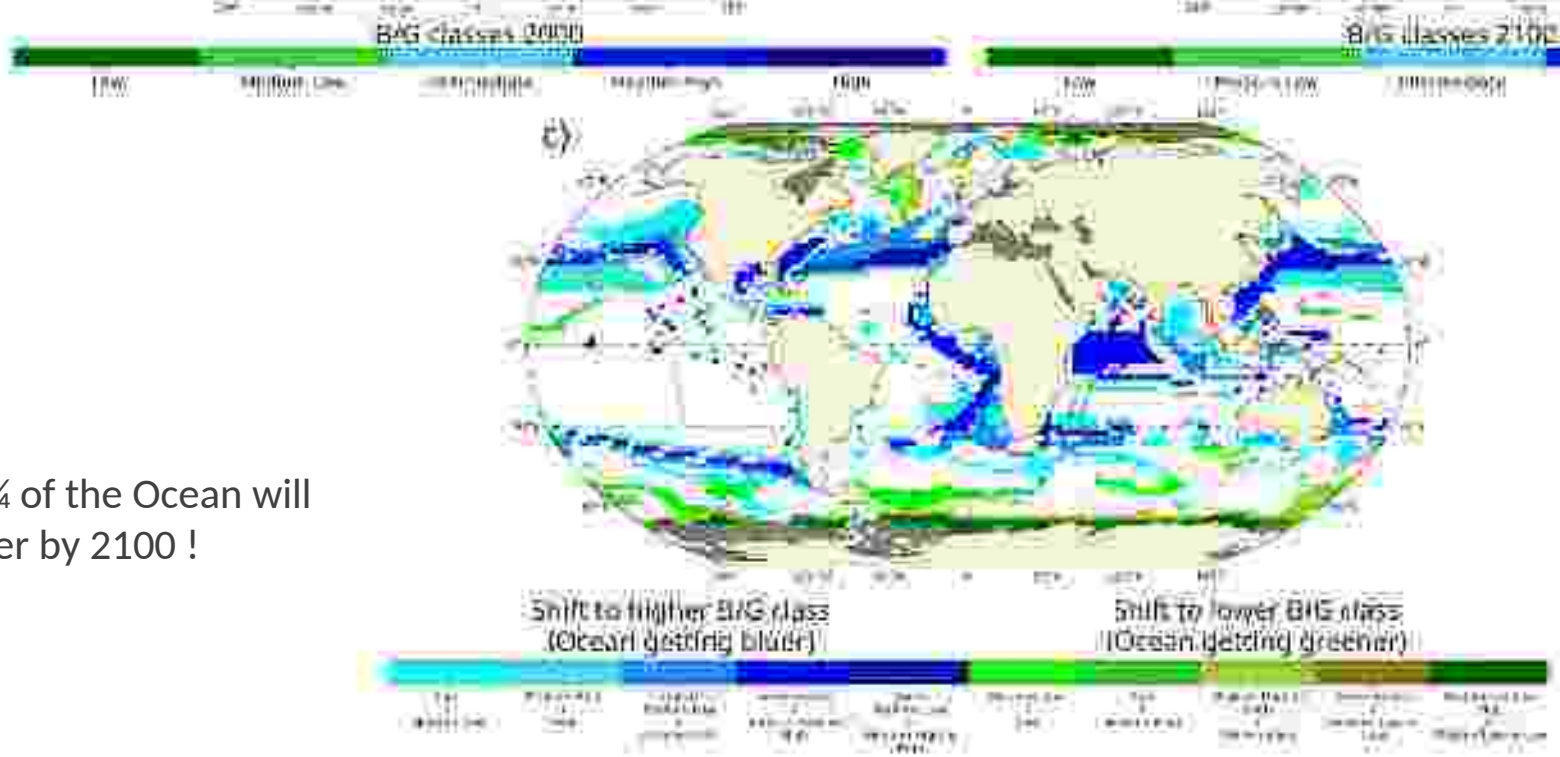


- How will these distributions evolve in future?

Mattei *et al*. 2025 Sci. Adv.

Modelling

- More than ¼ of the Ocean will become bluer by 2100 !



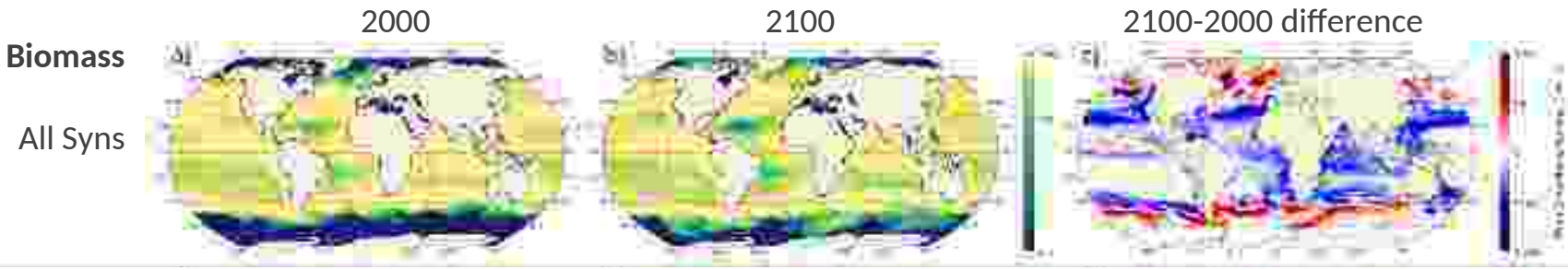
Change direction	Class	% Ocean Area	Tot % Ocean Area
Same class	Low	13.39	
	Medium Low	18.28	
	Intermediate	6.81	66.84%
	Medium High	11.49	
	High	20.76	
Bluer	Low -> Medium Low	5.15	
	Medium High -> High	6.88	
	[illegible]	5.55	26.58%
	Intermediate -> Medium High or High	8.14	
	Low or Medium Low -> Medium High or High	0.44	
Greener	Medium High or High -> Intermediate	0.32	
	[illegible]	0.01	
	Medium High or High -> Medium Low or Low	1.06	6.58%
	Medium Low -> Low	2.46	
	High -> Medium High	0.59	

Mattei *et al*. Submitted to Nature Comm.

Modelling the *future* spatial distribution of the main *Synechococcus* PTs

=> Like most other phytoplankton species, the global *Syn.* population is predicted to decrease by ca. 4 % by 2100 compared to 2000.

=> Within Syn, BS ↑ from 68% to 71%, GS → from 10 to 11% while CA ↓ from 22 to 18%, with a total biomass loss of 23%



- Climate change reduces the fitness advantage of CA4!

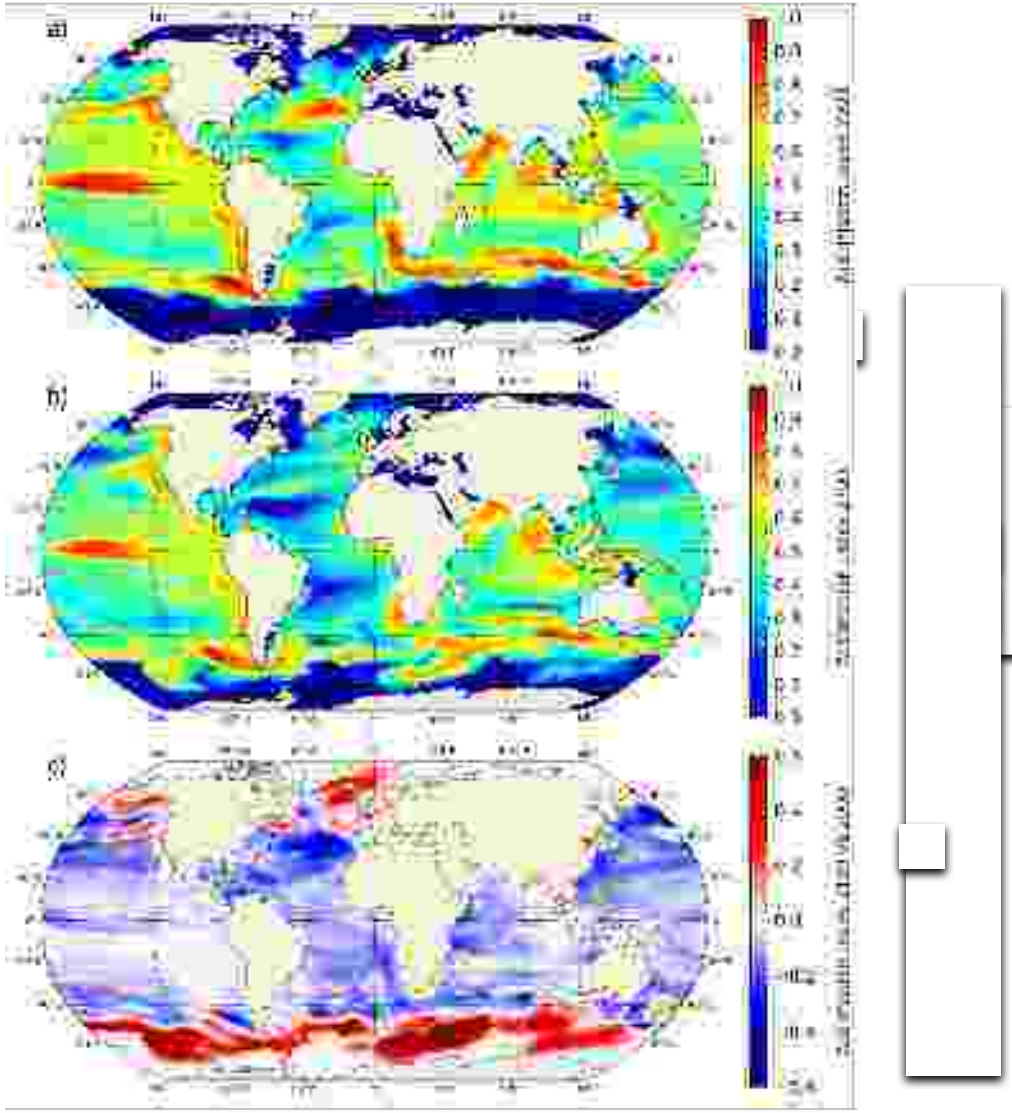
Mattei *et al*. Submitted to Nature Comm.

Modelling the *future* spatial distribution of the main *Synechococcus* PTs

Variations in the '**acclimation index**', a proxy indicating the extent to which the CA4 strategy was exploited in the simulated ocean:

- High index values = coexistence of multiple acclimation states for CA cells, driven by a highly variable underwater light field
- Low index values = fewer dominant acclimation states, reflecting a more stable light environment.

=> The reduction in the fitness advantage of CA4 is due to a lower variability of ocean color driven by climate change.



Mattei *et al*. Submitted to Nature Com.

Take Home Messages

- Most PBS rod genes are localized in a specialized region (the PBS rod region) whose gene content and organization is specific of a given pigment type.

- PT 1 (PC only) and PT 2 (PC+PEI) cells are restricted to estuaries or low salinity environments, while PT 3 (PC+PEI+PEII) dominate in the open ocean.

- CA4 is conferred by a small specific genomic island, which occurred twice during Evolution, a first time to transform former GL specialists and a second time former BL specialists into CA4-capable strains (a.k.a 'blue-green light generalists')

- Chromatic acclimaters constitute about half of all *Synechococcus* cells in oceanic areas, with PTs 3dA (CA4-A island) and 3dB (CA4-B island) cells being about equally abundant and with complementary distributions

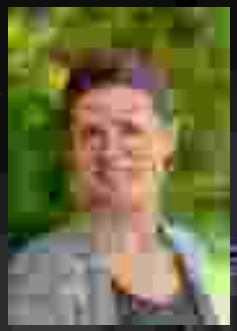
- Chromatic acclimaters are particularly abundant in transition zones but the fitness advantage of CA4 will decrease in the future ocean because the latter will become bluer and less variable.

Acknowledgements

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David Kehoe lab



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Théophile Grébert



Louison Dufour

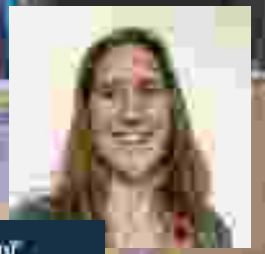


Wendy Schluchter lab



THE UNIVERSITY of NEW ORLEANS

Anna Hickman



University of Southampton



CNRS • SORBONNE UNIVERSITÉ

Station Biologique de Roscoff







Francesco Mattei

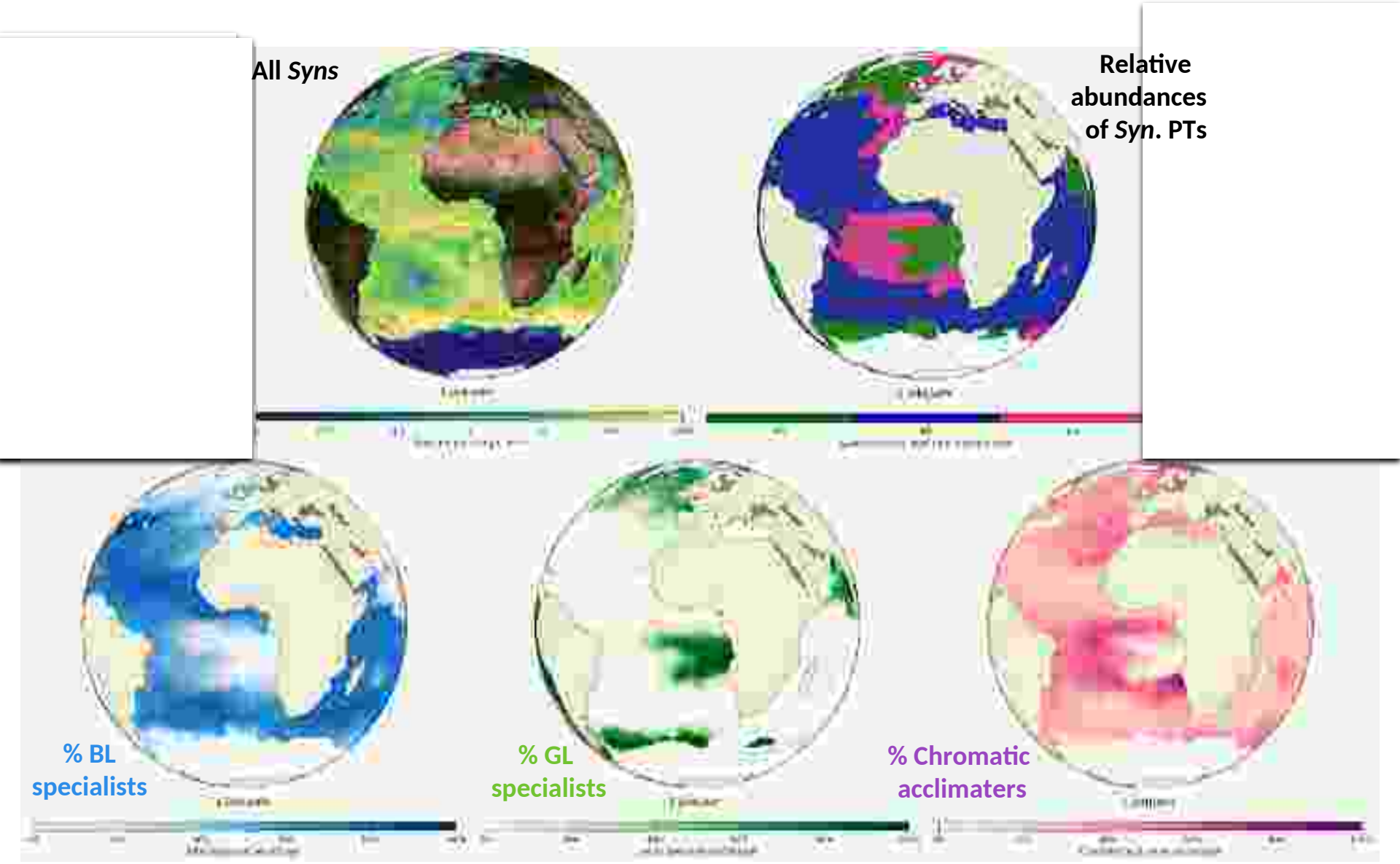


LOV

ANR EFFICACY

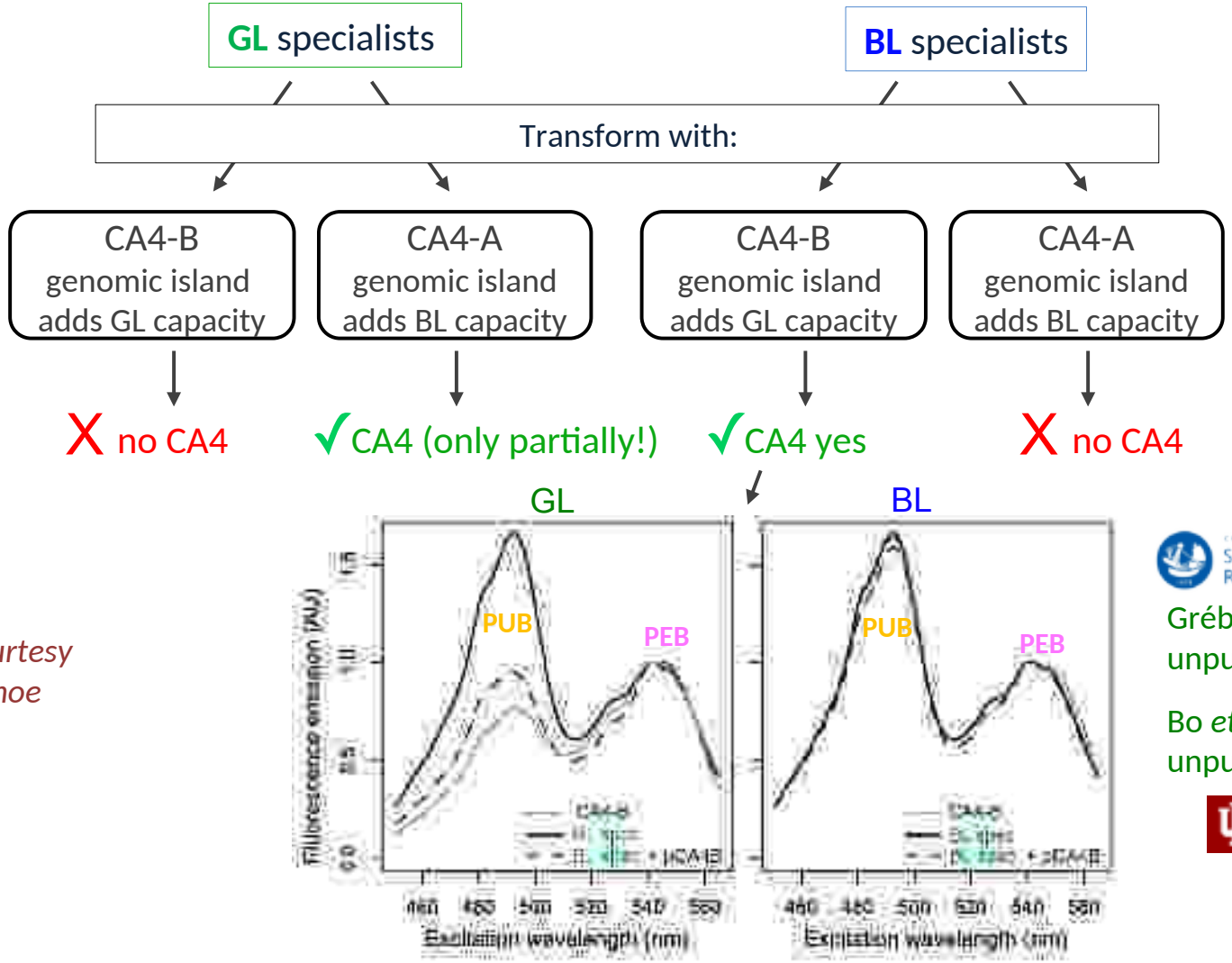
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Questions?



Mattei *et al*. 2025 Sci. Adv.

Each CA4 genomic island is sufficient to convert a different type of specialist



Slide courtesy of D. Kehoe

Station Biologique Roscoff

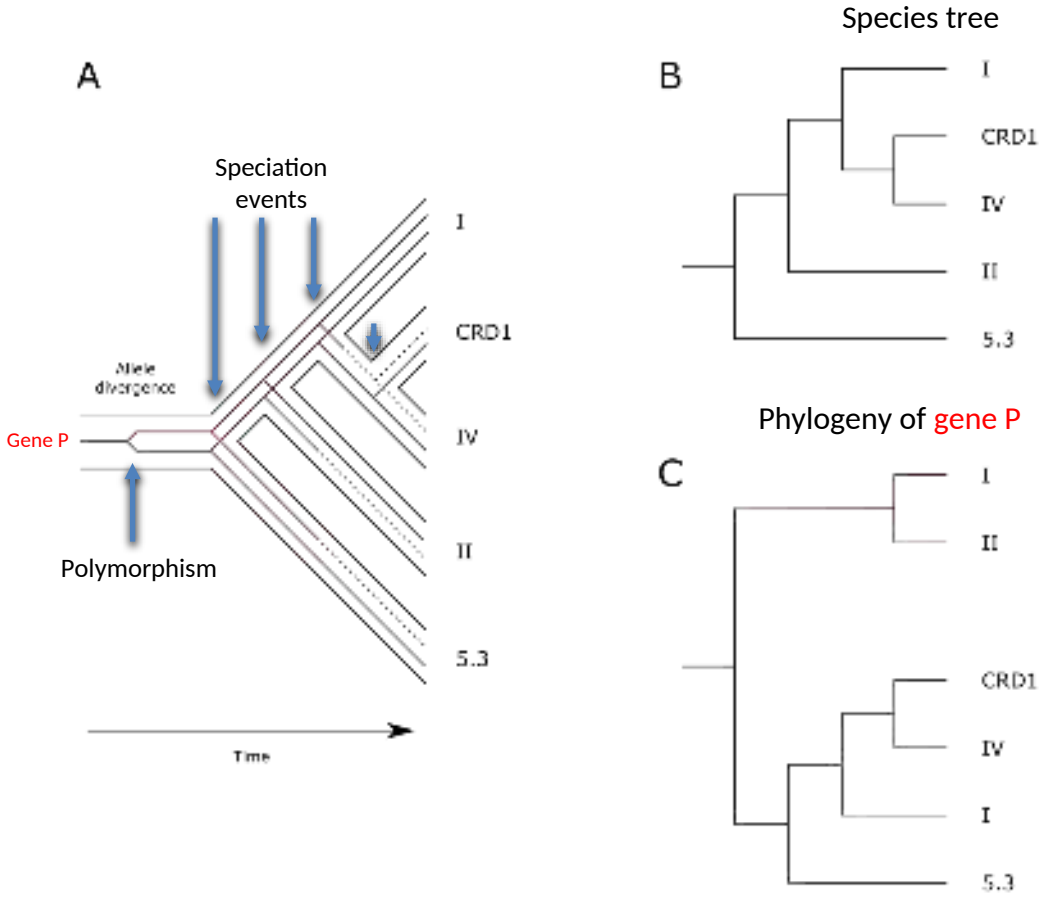
Grébert *et al.* unpublished

Bo *et al.* unpublished

CA4 genomic islands are *sufficient* to confer the CA4 phenotype

This must occur through different but related mechanisms

Incomplete Lineage Sorting (ILS)



ILS is a population-scale mechanism in which an ancestral polymorphism is not sorted during a speciation event. The consequence is that the gene tree is discordant with the species tree.
=> No other mechanism can reasonably explain the observed gene trees.

ILS is *not* a process that generates diversity, but a mechanism that can explain incongruences between gene and species phylogenies. In contrast, tycheposons do generate diversity.