CYAnobacterial platform Optimised for bioproduction

Further developments of the CYAO project results in the framework of circular economy

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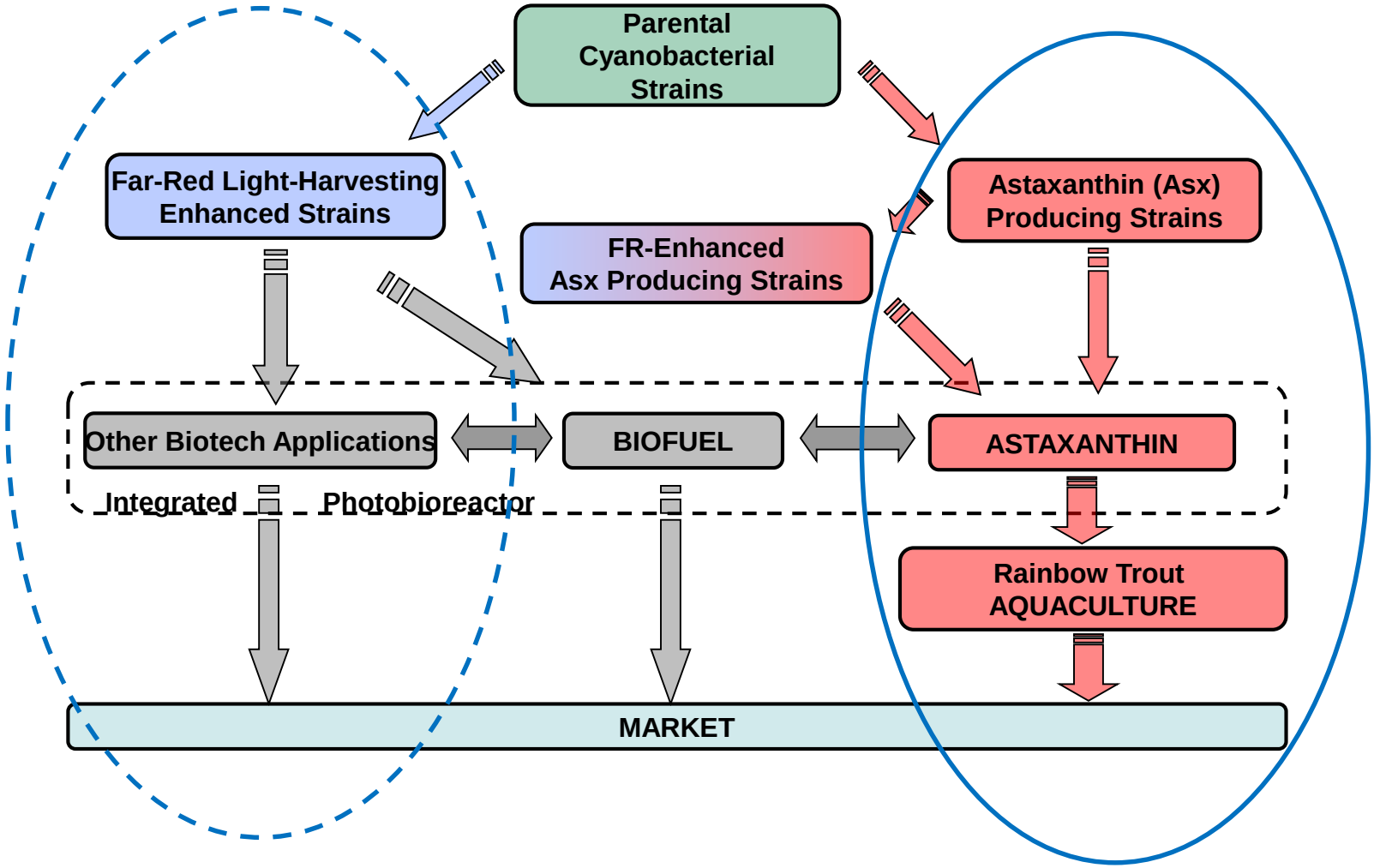
CYAO Closure Meeting

15 June 2021 – IRSA CNR, Verbania

Fondazione CARIPLO
Consiglio Nazionale delle Ricerche
ALGATECH

GOAL: expand and extend the light-harvesting potential of cyanobacterial cultures by controlling the synthesis of unconventional Chls (Chl*d* and Chl*f*), improving thereby their growth rate and fitness under PBR relevant conditions.

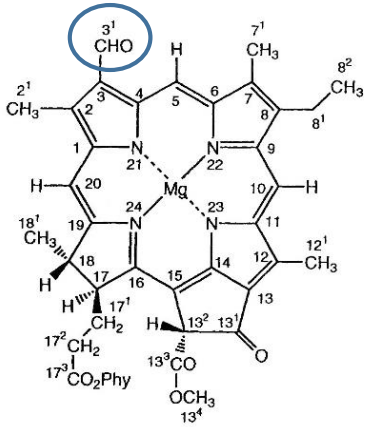


CHLOROPHYLL D, A GREEN PIGMENT OF RED ALGAE

Manning & Strain 1943 JBC Vol 151/1: 1-19

Chlorophyll *d* as a major pigment

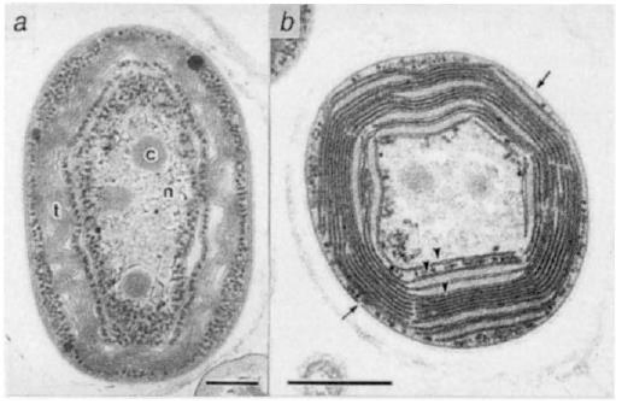
Miyashita et al. 1996 NATURE Vol 383

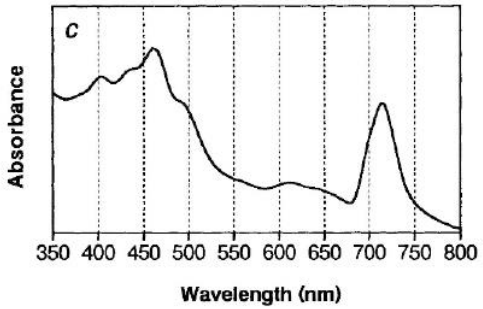


R2 => formyl group
(in Chl*a* and Chl*b*
R2 is a vinyl group
$CH=CH_2$)

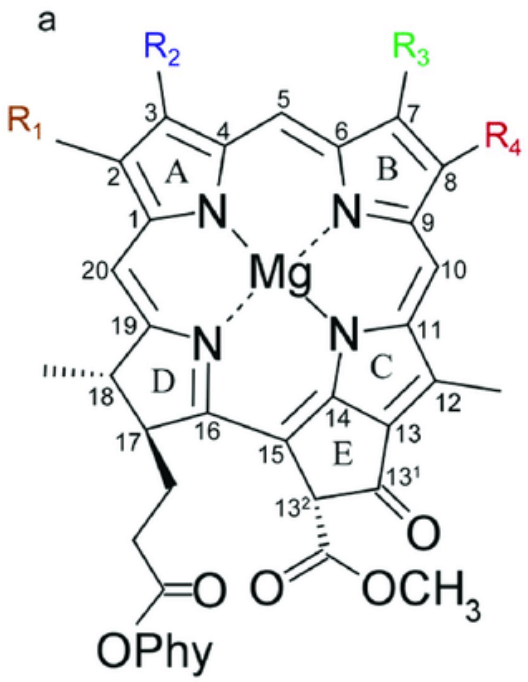
We have now isolated a previously undescribed oxygenic photosynthetic prokaryote containing chlorophyll *d* as a major green pigment: it has only a small amount of chlorophyll *a*.

We isolated the new organism from a suspension of algae squeezed out of *Lissoclinum patella*, a colonial ascidian, collected in 1993 from the marine coast of the Palau islands in the western Pacific Ocean. Cells are unicellular and spheroidal or ellipsoidal, 1.5–2.0 μm in diameter and 2.0–3.0 μm in length. They are photoautotrophs, and have evolved in the presence of oxygen. We used electron





Acaryochloris marina (~90-95% Chl*d*, constitutively)



- Chl*b* and Chl*d* both contain a formyl side-chain

- putative Chl*d* synthase => could belong to the CAO superfamily

	R_1	R_2	R_3	R_4
chlorophyll *a*	CH_3	$CH{=}CH_2$	CH_3	$CH_2\text{-}CH_3$
chlorophyll *b*	CH_3	$CH{=}CH_2$	CHO	$CH_2\text{-}CH_3$
chlorophyll *d*	CH_3	CHO	CH_3	$CH_2\text{-}CH_3$
chlorophyll *f*	CHO	$CH{=}CH_2$	CH_3	$CH_2\text{-}CH_3$
8-vinyl chlorophyll *a*	CH_3	$CH{=}CH_2$	CH_3	$CH{=}CH_2$

(taken from Loughlin et al. 2014 SCIENTIFIC REPORTS Vol 4: 6069)

Niche adaptation and genome expansion in the chlorophyll *d*-producing cyanobacterium *Acaryochloris marina*

Swingley et al. 2008 PNAS Vol 105/6: 2005

- Five putative CAO-like proteins are encoded by *A. marina*

- Most of these fall into orthologous clusters with other hypothetical cyanobacterial proteins and only one, **AM15665**, does not have any significant homologs

US 20090203070A1

(19) **United States**

(12) **Patent Application Publication**

Devroe et al.

(10) **Pub. No.: US 2009/0203070 A1**

(43) **Pub. Date: Aug. 13, 2009**

(54) **HYPERPHOTOSYNTHETIC ORGANISMS**

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(21) Appl. No.: **12/268,406**

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C12N 1/13	(2006.01)

(52) **U.S. Cl.** **435/69.1**; 435/252.33; 435/257.2

(57) **ABSTRACT**

The present disclosure identifies pathways and mechanisms to confer improved industrial fitness on engineered organisms. It also discloses engineered organisms having improved industrial fitness. Synthetic biologic engineering modules are disclosed that provide for light capture, carbon dioxide fixation, NADH production, NADPH production, thermotolerance, pH tolerance, flue gas tolerance, salt tolerance, nutrient independence and near infrared absorbance. The disclosed engineered organisms can include one or more of these modules. Also provided are methods of using the engineered organism to produce carbon-based products of interest, biomass or pharmaceutical agents.

[0325] The protein corresponding to locus tag AM1_5665 (protein id ABW30612.1) [*Acaryochloris marina* MBIC11017] (FIG. 6B) was noted by Swingley et al., PNAS 105:2005 (2008) as being a likely oxygenase but not having significant homology with known examples. This could mean it is especially significant in Chl d formation.

AM1_5665

A. marina «putative» Chl *d* synthase gene

SCIENTIFIC REPORTS

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OPEN

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A novel species of the marine cyanobacterium *Acaryochloris* with a unique pigment content and lifestyle

Frédéric Partensky[1], Christophe Six[1], Morgane Ratin[1], Laurence Garczarek[1], Daniel Vaulot[1], Ian Probert[2], Alexandra Calteau[3], Priscillia Gourvil[2], Dominique Marie[1], Théophile Grébert[1], Christiane Bouchier[4], Sophie Le Panse[2], Martin Gachenot[2], Francisco Rodríguez[5] & José L. Garrido[6]

Here we describe RCC1774, the type strain of a new species that is phylogenetically related to the *Acaryochloris* genus, but which possesses Chl *a* as the major photopigment as well as Chl *b*, zeaxanthin, β,ε-carotene and PC as main accessory pigments. This is the first time that this suite of pigments is reported for a member of the *Acaryochloris* genus and for cyanobacteria at large. This novel species, that we propose to call *Acaryochloris thomasi* sp. nov. in honour of its isolator, Jean-Claude Thomas, is therefore the fourth 'green oxyphotobacterium' ever described, but the only one which possesses substantial amounts of PC. This discovery should thus provide interesting novel insights into the evolution of pigment synthesis in cyanobacteria.

tree; Fig. 1), when such genomes will be available. In any case, the genomic comparison of RCC1774 and Chl *d*-producing *Acaryochloris* spp. should help discover valid candidate(s) for Chl *d* synthase(s). In this context, although it has been suggested that *A. marina* MBIC11017 AM1_5665 might have this function[44], the presence of a close homolog in RCC1774 (C1752_00555) somewhat invalidates this hypothesis, while two other proposed candidates (AM1_5023 and AM1_5798) have only distant homologs in RCC1774 and are therefore more likely to be involved in Chl *d* synthesis.

Photosynth Res (2015) 125:255–265
DOI 10.1007/s11120-015-0082-4

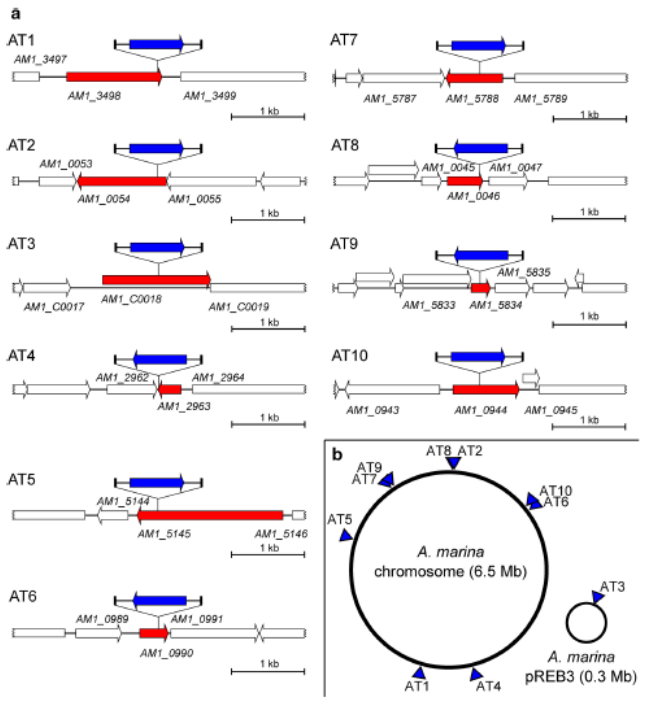
REGULAR PAPER

Identification of a phenotype

Establishment of the forward genetic analysis of the chlorophyll *d*-dominated cyanobacterium *Acaryochloris marina* MBIC 11017 by applying in vivo transposon mutagenesis system

Kazuyuki Watabe · Mamoru Mimuro · Tohru Tsuchiya

In the present study, we succeeded in transposon mutagenesis and functional complementation in *A. marina*. This achievement is the first case of forward genetic analysis of *A. marina* and is a major breakthrough of molecular genetic analysis of *A. marina*. Thus, our system could contribute to our understanding of the adaptation to far-red light in *A. marina*, especially the identification of the gene responsible for Chl *d* biosynthesis. Moreover, this study strongly suggests that our technique could be applied to a wide range of cyanobacteria. Therefore, if transformation isolated in the future. In particular, our transposon mutagenesis and functional complementation system will be applied to the investigation of the adaptation mechanism to far-red light, including Chl *d* biosynthesis.



Generation and screening of the (transposon-tagged) mutants

Genetic basis of the phenotype

frontiers in Microbiology

REVIEW
published: 09 November 2016
doi: 10.3389/fmicb.2016.01809

Generation
(by chemicals/radiation treatment)
and screening of *A. marina*
mutants

2015). As a likely consequence, all tested cyanobacteria were found to be more radiation resistant than *E. coli*. It is also important to study DNA recombination and repair in cyanobacteria for biotechnological purposes, since many recombinant strains appeared to be genetically unstable. They somehow managed to inactivate the (newly-introduced) heterologous genes of industrial interest. Thus, a better understanding of DNA recombination and repair in cyanobacteria may lead to increasing the genetic stability of biotechnologically important strains, an important industrial goal.

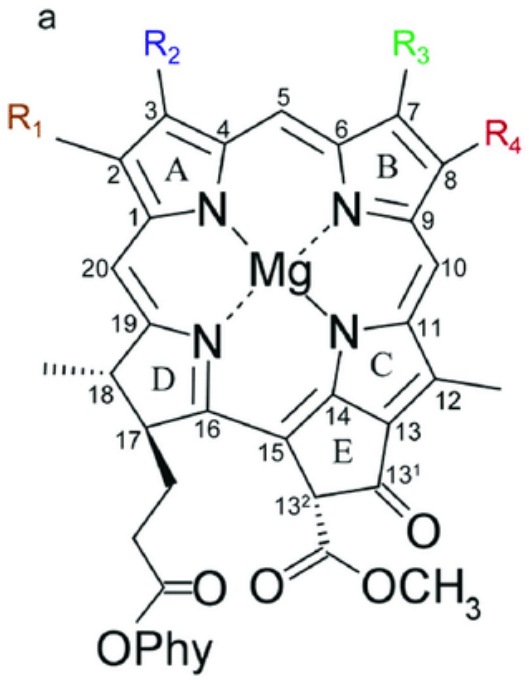
Comparative Genomics of DNA Recombination and Repair in Cyanobacteria: Biotechnological Implications

Corinne Cassier-Chauvat, Théo Veaudor and Franck Chauvat *

Institute for Integrative Biology of the Cell, CEA, Centre Nationnal de la Recherche Scientifique (CNRS), Universite Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette Cedex, France

The cyanobacterium *A. marina* MBIC11017 possesses the most complete, and complex, set of DNA repair genes: *alkB* (two copies), *dinB* (rare in cyanobacteria), *lexA*, *mutL*, *mutM*, *mutS* (two copies), *mutT*, *mutY*, *ogt* (three copies), *phr*, *radA*, *recA* (seven copies, four of them located on plasmids), *recD* (three copies, including two plasmidic copies), *recF*, *recG*, *recJ* (two copies), *recN*, *recO*, *recQ* (two copies), *recR*, *ruvABC*, *ssb* (two copies), *sulA*, *umuC* (three copies including two plasmid copies), *umuD* (four copies including two plasmid copies), *uvrABCD* and *xerC* (eight copies, including six on plasmids). However,

Nature comes to the rescue: **Chl*f***



R1 => formyl group

(in Chl*a* and Chl*b*

R2 is a methyl group CH_3)

	R_1	R_2	R_3	R_4
chlorophyll *a*	CH_3	$CH{=}CH_2$	CH_3	$CH_2{-}CH_3$
chlorophyll *b*	CH_3	$CH{=}CH_2$	CHO	$CH_2{-}CH_3$
chlorophyll *d*	CH_3	CHO	CH_3	$CH_2{-}CH_3$
chlorophyll *f*	CHO	$CH{=}CH_2$	CH_3	$CH_2{-}CH_3$
8-vinyl chlorophyll *a*	CH_3	$CH{=}CH_2$	CH_3	$CH{=}CH_2$

(taken from Loughlin et al. 2014 SCIENTIFIC REPORTS Vol 4: 6069)

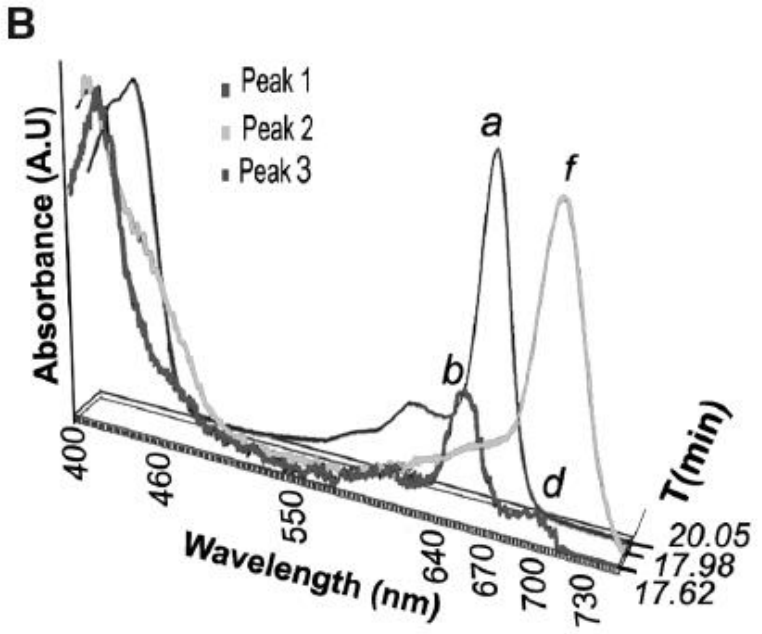
Science

A Red-Shifted Chlorophyll

Min Chen, Martin Schliep, Robert D. Willows, Zheng-Li Cai, Brett A. Neilan and Hugo Scheer

Science **329** (5997), 1318-1319.
DOI: 10.1126/science.1191127originally published online August 19, 2010

The morphological features of stromatolites provide a unique environment for specific but diverse cyanobacterial communities (*7*). We cultured a sample from Hamelin pool under near-infrared light (720 nm) (*8*). Analysis of a methanolic extract of stromatolites from Shark Bay, Western Australia, by high-performance liquid chromatography (HPLC) revealed a complex mixture of chlorophylls (Fig. 1A): In addition to a detectable amount of Chl a (peak 3) and bacteriochlorophyll a (peak B), there were trace amounts of Chl d and a new pigment, Chl f (peak 2 in Fig. 1A). The optical

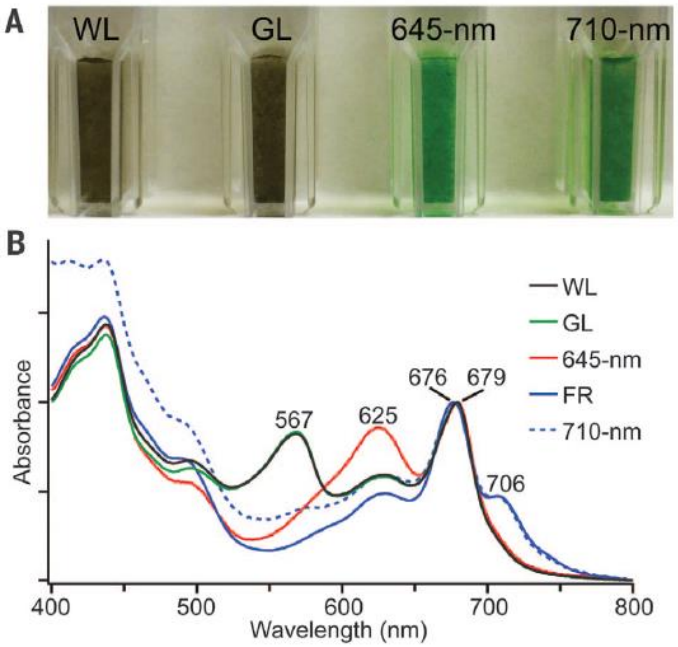


Chl*f* (and Chl*d*) is produced as a MINOR pigment in several cyanobacteria strains

The synthesis of Chl*f*/*d* is part of a COMPLEX ACCLIMATION PROCESS:
FaRLiP (far-red light photoacclimation)

Leptolyngbya sp. strain JSC-1

Fig. 2. JSC-1 cells have enhanced absorption at 700 to 750 nm when grown in far-red light. (**A**) Appearance of cells grown in WL, GL, 645-nm light, and 710-nm light. (**B**) Absorption spectra of strain JSC-1 cells grown in WL (black line), GL (green line), 645-nm light (red line), FR (solid blue line), and 710-nm light (dotted blue line).



Science

Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light

Fei Gan, Shuyi Zhang, Nathan C. Rockwell, Shelley S. Martin, J. Clark Lagarias and Donald A. Bryant

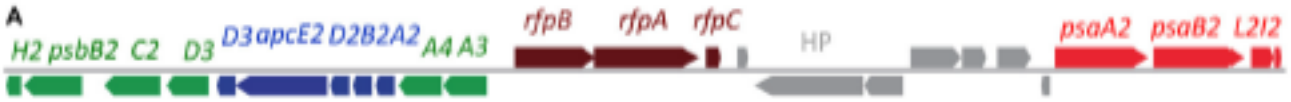
Science **345** (6202), 1312-1317.
DOI: 10.1126/science.1256963originally published online August 21, 2014

frontiers in Microbiology

ORIGINAL RESEARCH
published: 25 November 2015
doi: 10.3389/fmicb.2015.01303

RfpA, RfpB, and RfpC are the Master Control Elements of Far-Red Light Photoacclimation (FaRLiP)

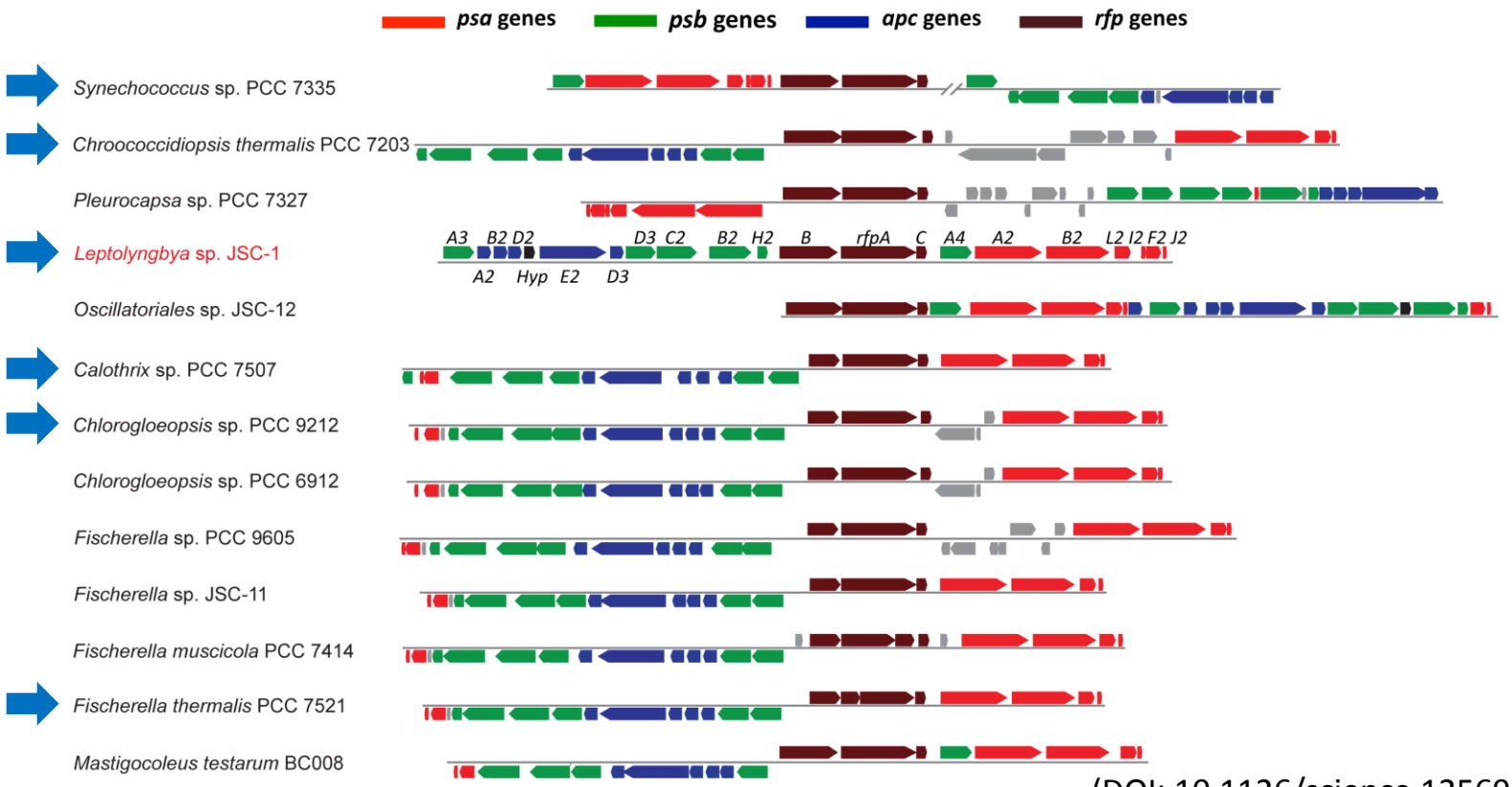
Chi Zhao[1], Fei Gan[1†], Gaozhong Shen[1] and Donald A. Bryant[1,2]*



RfpA: red/far-red photoreceptor (histidine kinase domain)
RfpC: response regulator (transfers a phosphate from RfpA to RfpB)
RfpB: response regulator (DNA-binding domain) activates transcription

Additionally, the cells synthesize both chlorophyll (Chl) *f* and Chl *d*. Using biparental mating from *Escherichia coli*, we constructed null mutants of three genes, *rfpA*, *rfpB*, and *rfpC*, in the cyanobacteria *Chlorogloeopsis fritschii* PCC 9212 and *Chroococcidiopsis thermalis* PCC 7203. The resulting mutants were no longer able to modify their photosynthetic apparatus to absorb FRL, were no longer able to synthesize Chl *f*, inappropriately synthesized Chl *d* in white light, and were unable to transcribe genes of the FaRLiP gene cluster. We conclude that RfpA, RfpB, and RfpC constitute a FRL-activated signal transduction cascade that is the master control switch for the FaRLiP response. FRL is proposed to activate (or inactivate) the histidine kinase activity

During FaRLiP several subunits of the photosynthetic apparatus are replaced by divergent protein variants encoded from a large gene cluster in the genome



(DOI: 10.1126/science.1256963)

> 15 species of cyanobacteria contain the FaRLiP gene cluster

➡ experimentally confirmed to grow photoautotrophically and to synthesize Chl*f*/*d* in Far Red light

Science

Light-dependent chlorophyll f synthase is a highly divergent paralog of PsbA of photosystem II

Ming-Yang Ho, Gaozhong Shen, Daniel P. Canniffe, Chi Zhao and Donald A. Bryant

Science **353** (6302), aaf9178.
DOI: 10.1126/science.aaf9178originally published online July 7, 2016

Transcription and phylogenetic profiling suggested that the gene(s) responsible for this activity were in the conserved FaRLiP gene cluster. This led us to focus on *psbA4*, a divergent member of the *psbA* gene family encoding so-called "super-rogue" PsbA, a paralog to the D1 core subunit of PSII. We used reverse genetics and heterologous expression to identify the Chl f synthase of two cyanobacteria capable of FaRLiP: *Chlorogloeopsis fritschii* PCC 9212 and *Synechococcus* sp. PCC 7335.

Using a conjugation-based DNA transfer system (*14*), we constructed null mutants for *psbA4* genes in two cyanobacteria capable of FaRLiP (*8*): *C. fritschii* PCC 9212 and *Synechococcus* sp. PCC 7335 (fig. S3). Neither *psbA4* mutant was able to synthesize Chl f when the mutant cells were grown in FRL. The characteristic long-wavelength

with Chl f. Heterologous expression of the *psbA4* gene from *C. fritschii* PCC 9212 in the model non-FaRLiP cyanobacterium *Synechococcus* sp. PCC 7002 led to the synthesis of Chl f. These results showed that *psbA4* (renamed *chlF*) encodes the Chl f synthase. Growth experiments using intervals of FRL and darkness showed that Chl f synthesis is light-dependent, which implies that ChlF is a photo-oxidoreductase that oxidizes Chl a (or Chlide a) instead of water.

Hp 1:

- psbA4 (renamed ChlF) encodes for Chlf synthase

- homodimers of ChlF constitute “specialised” RC that oxidise Chl*a* to Chl*f*

Chlorophyll *f* synthesis by a super-rogue photosystem II complex

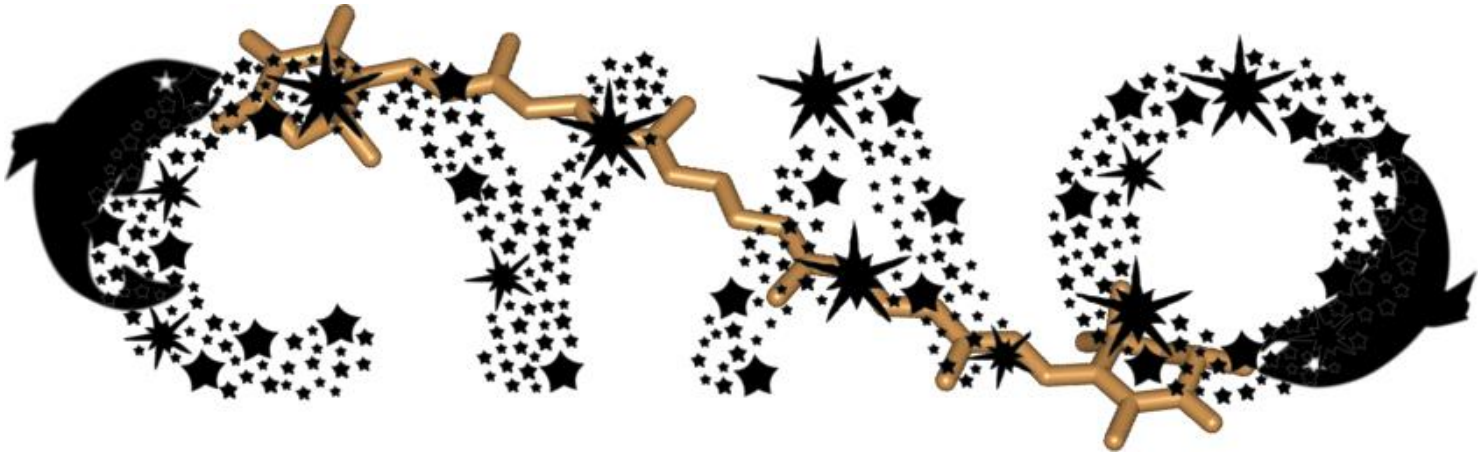
Trinugroho et al. 2020 NATURE PLANTS Vol 6: 238

Hp 2:

- ChlF can replace D1 in the RC forming “modified” RC (heterodimers of ChlF/D2) substitute) capable of synthesising Chl*f*

- These new class of PSII compexes are termed “super-rogue”

still… the complexity of Chl*d* and Chl*f* synthesis remains unknown



Thanks!