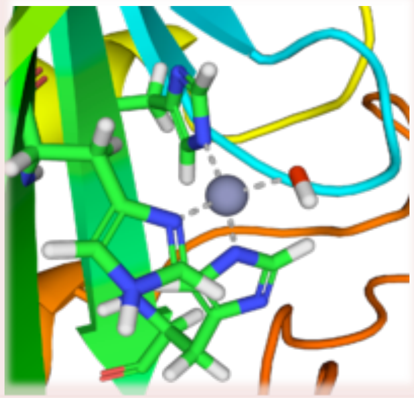
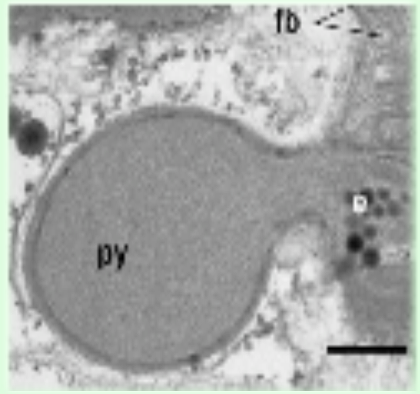
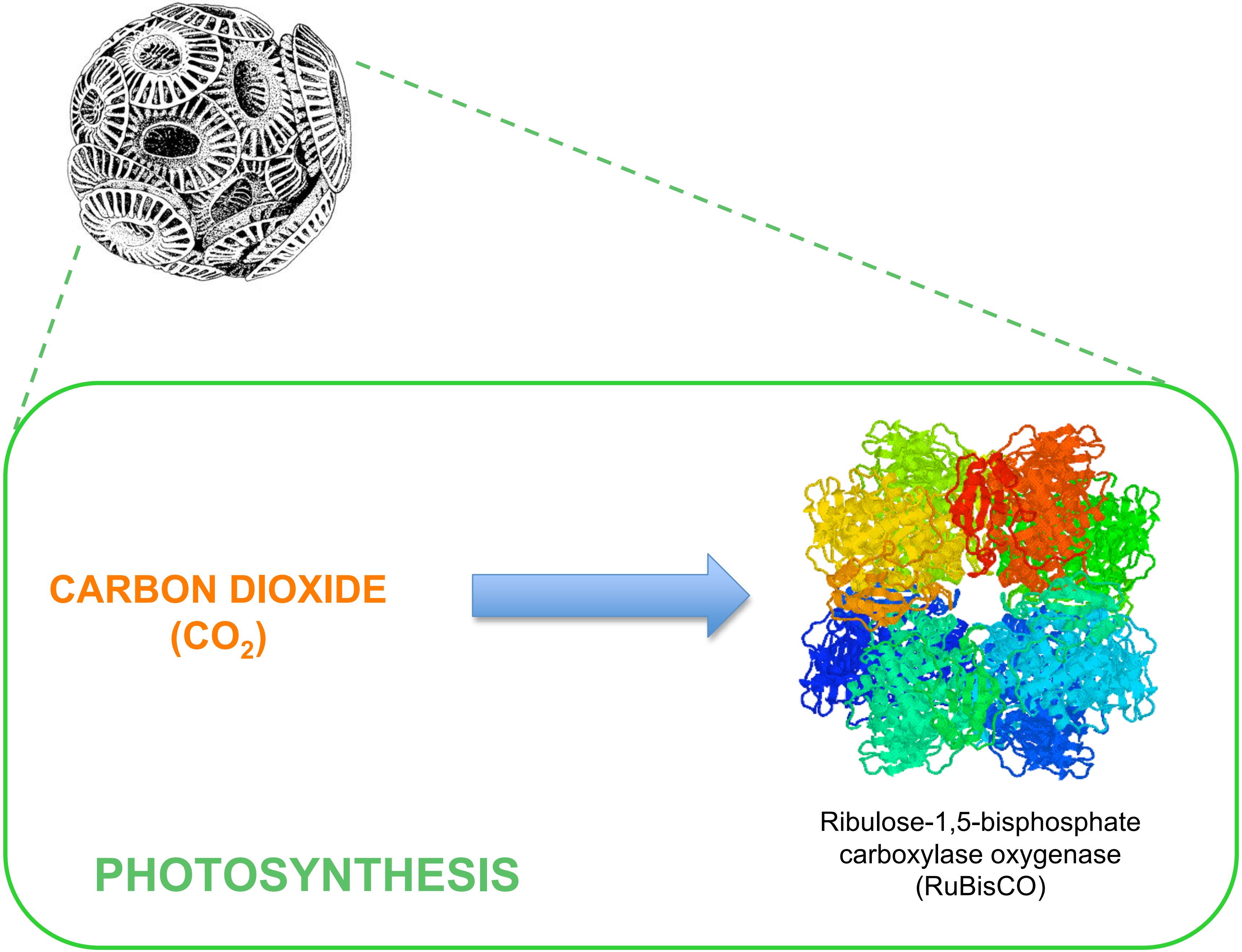
CARBON CONCENTRATING MECHANISMS & CO_2

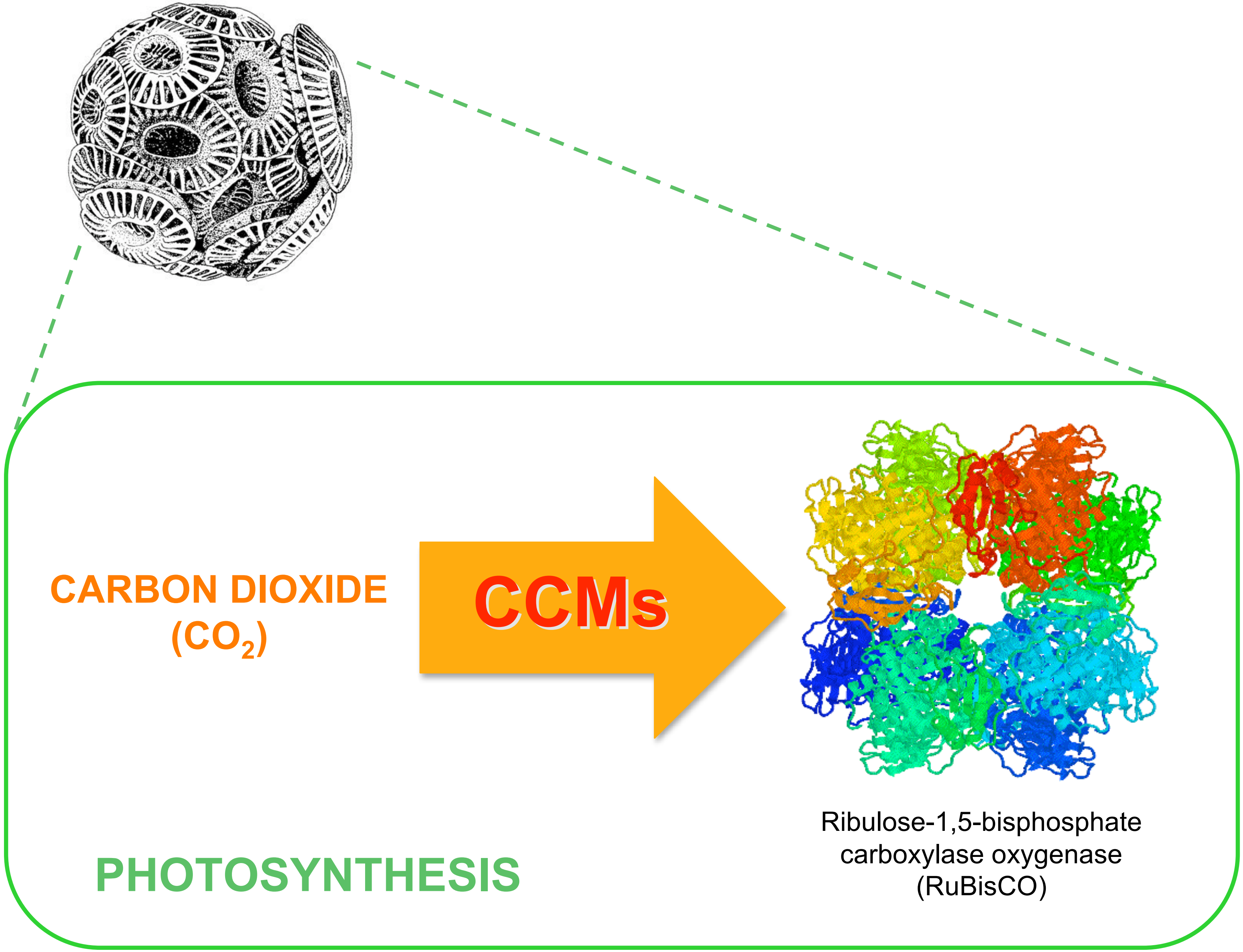




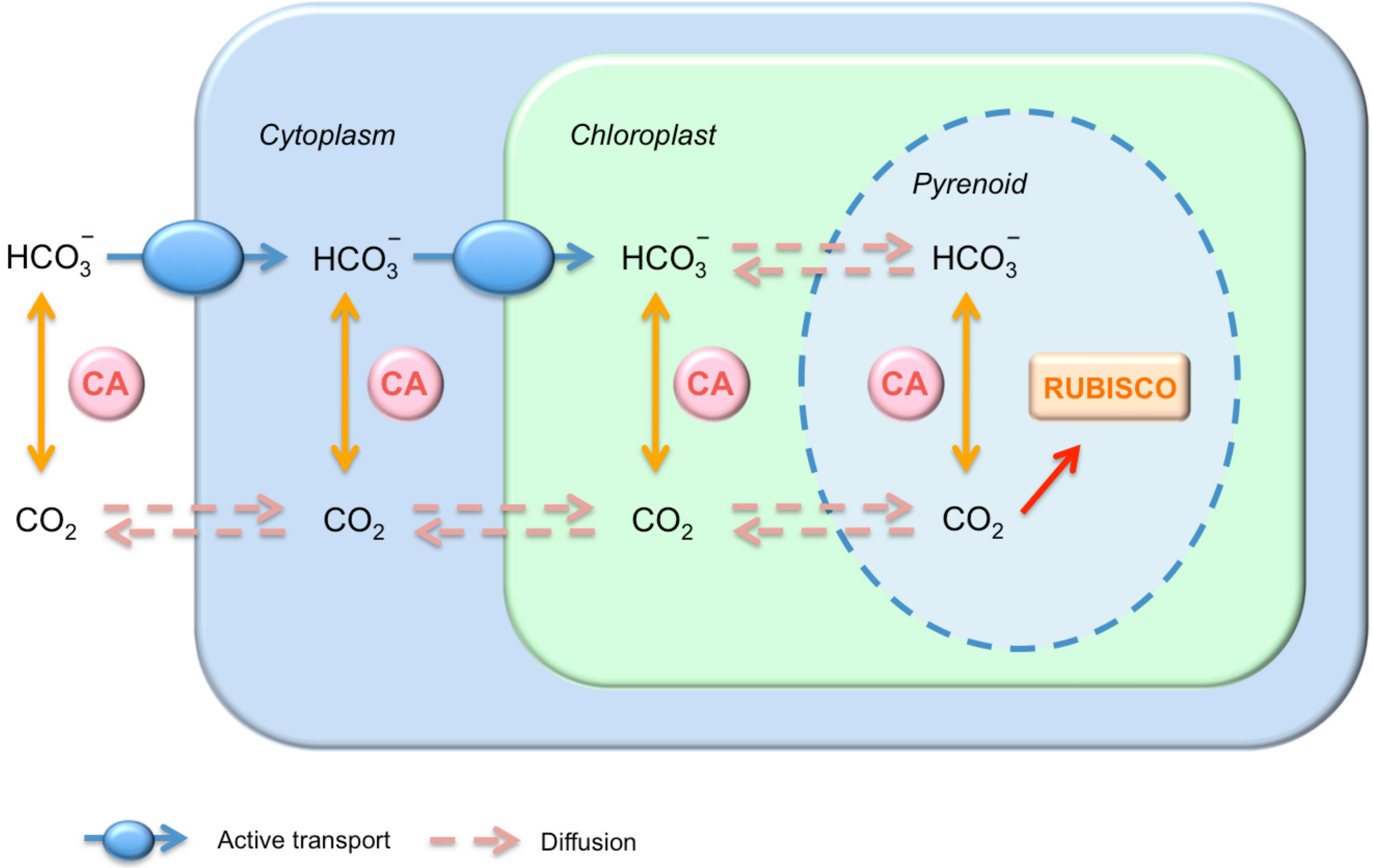
ROS RICKABY & RENEE LEE
UNIVERSITY OF OXFORD

UKOA Annual Science Meeting Ÿ University of Exeter Ÿ 16 – 18 April 2012


CARBON DIOXIDE
(CO_2)
PHOTOSYNTHESIS
Ribulose-1,5-bisphosphate
carboxylase oxygenase
(RuBisCO)

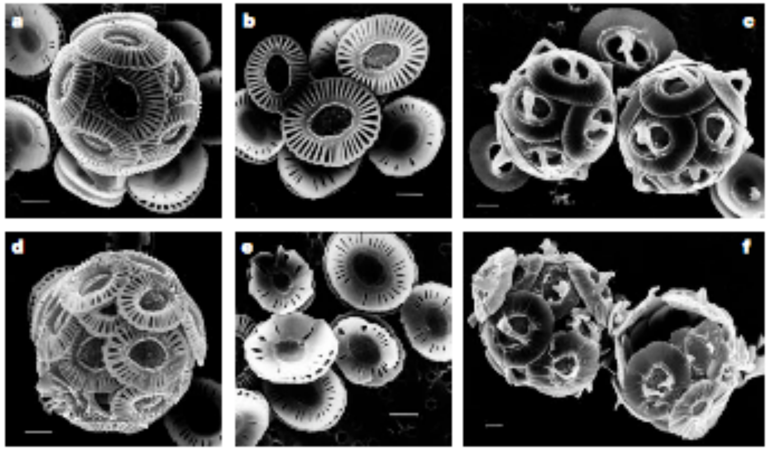

CARBON DIOXIDE
(CO_2)
CCMs
Ribulose-1,5-bisphosphate
carboxylase oxygenase
(RuBisCO)
PHOTOSYNTHESIS

CARBON CONCENTRATING MECHANISMS (CCMs)

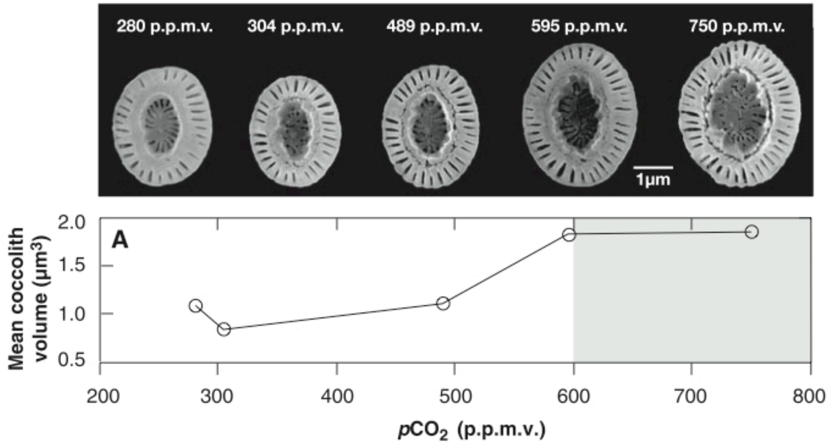


Adapted from Hopkinson et al. (2011) & Giordano et al. (2005)

STRAIN SPECIFIC RESPONSES TO pCO_2



Riebesell et al. (2000)



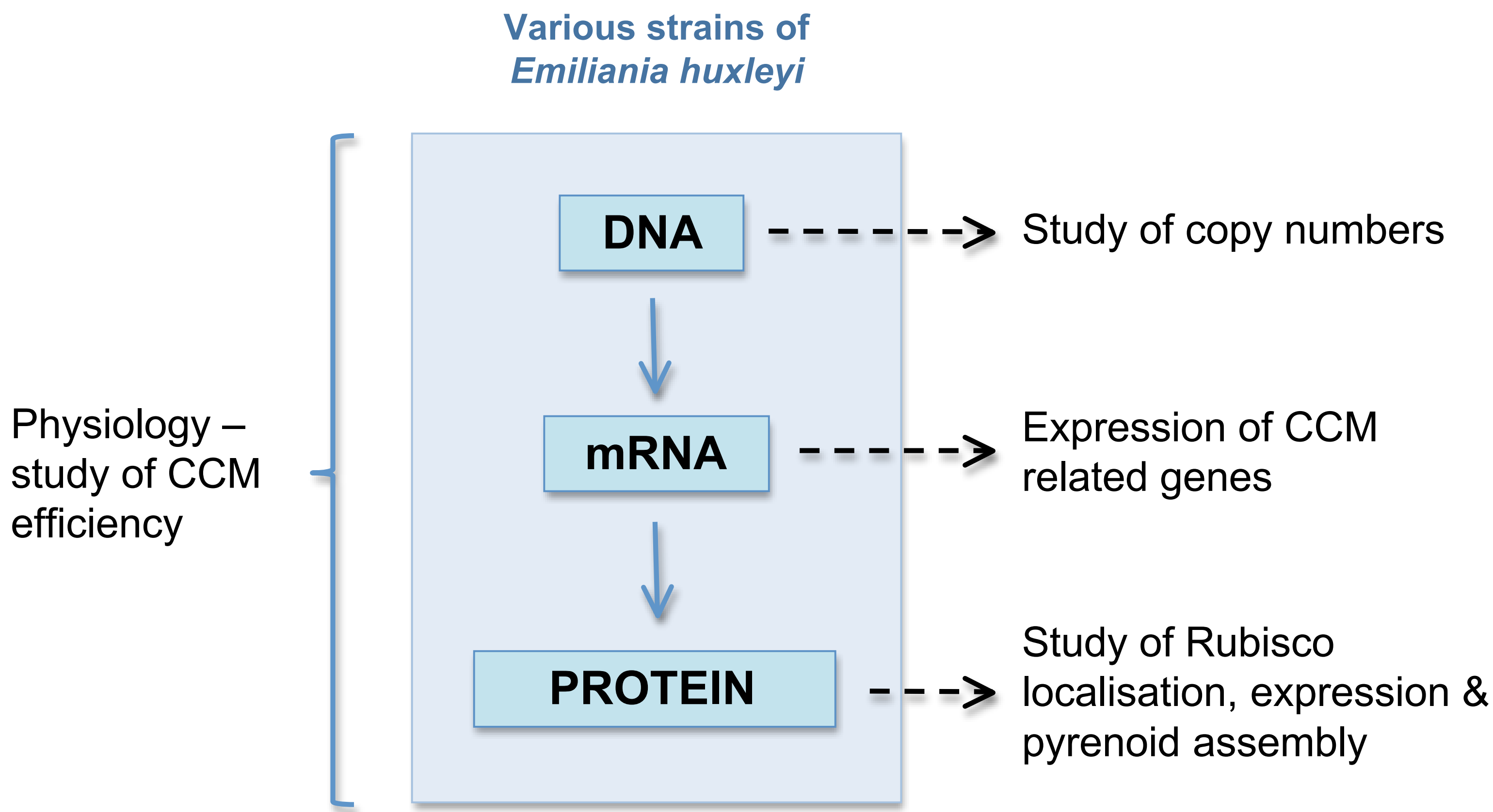
Iglesias-Rodriguez et al. (2008)

Study	Strain	Growth	PIC production	POC production	PIC:POC ratio
Feng et *al.* 2008	$CCMP371^{C}$	—	\	—	\
Iglesias-Rodriguez et *al.* 2008	$NZEH_{R}$	\	/	/	—
Langer et *al.* 2009	$RCC1212_{B}^{O}$	—	\	—	\
	$RCC1216_{R}^{O}$	—	\	—	\
	$RCC1238_{A}^{C}$	—	—	⌒	—
	$RCC1256_{A}^{C}$	\	⌒	⌒	—
Riebesell et *al.* 2000	$PLYB92/11_{A}^{C}$	—	\	/	\
Sciandra et *al.* 2003	TW1				
Shi et *al.* 2009	$NZEH_{R}$	—	/	/	\
This study	$RCC1256_{A}^{C}$	\	\	—	\
	$NZEH_{R}$	—	\		\

Hoppe et al. (2011)

CCMs IN COCCOLITHOPHORES

- Not as well characterised as cyanobacteria or *Chlamydomonas reinhardtii*



CARBONIC ANHYDRASE: AN INTRODUCTION

$$CO_2 + H_2O \xleftrightarrow{\textit{Carbonic anhydrase}} HCO_3^- + H^+$$

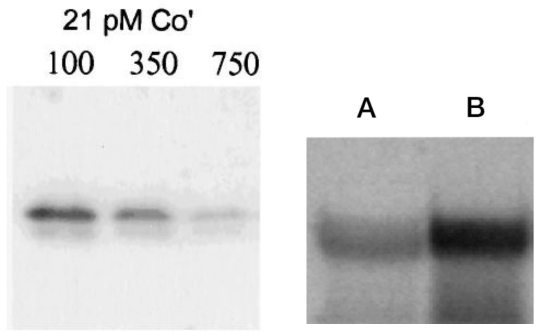
CARBONIC ANHYDRASE

- α *Meldrum & Roughton (1933)*
- β *Hewett-Emmett et al. (1984)*
- γ *Alber & Ferry (1994)*
- δ *Roberts et al. (1997)*
- ζ *Lane et al. (2005)*

- Several classes of CA – a result of convergent evolution
- Localised to various subcellular compartments
- Metalloenzyme with diverse physiological roles
- Fundamental role in carbon concentration

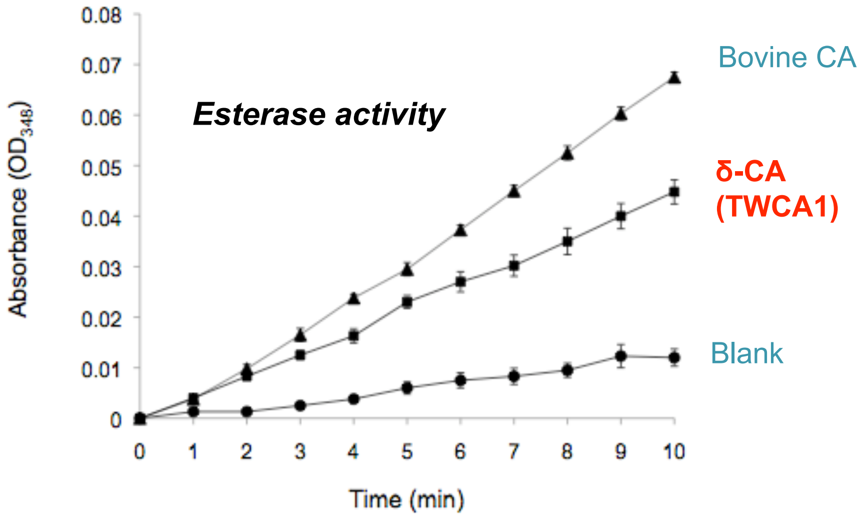
δ-CARBONIC ANHYDRASE

- Least studied carbonic anhydrase
- Found predominantly in marine phytoplankton
- δ-CA is induced by low CO_2
- *In vitro* enzymatic characterization has been unsuccessful to date (Roberts *et al.* 1997; Soto *et al.* 2006; Lapointe *et al.* 2008)



Lane & Morel (2000)

δ-CA is a functional carbonic anhydrase



By overexpressing TWCA1 in a pTWIN2 expression vector system (& subsequent purification), we demonstrated that this protein is a catalytically active δ-CA with both esterase & CO_2 hydration activity

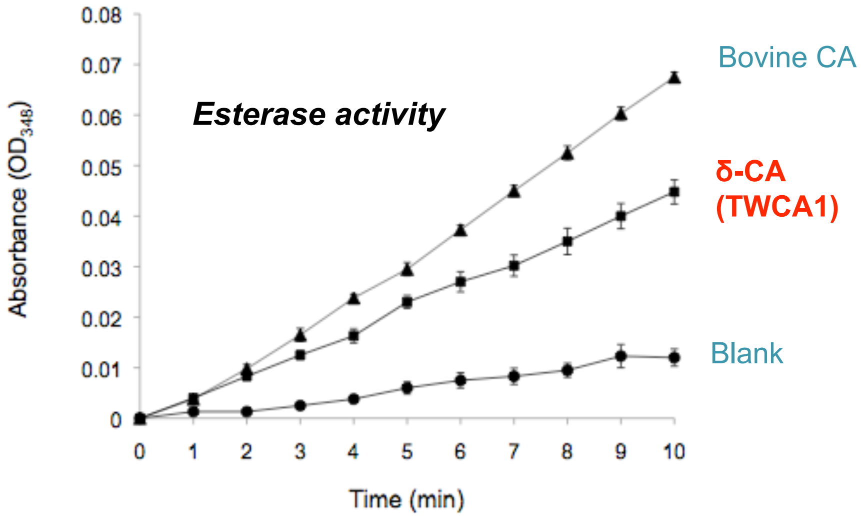
Sample	**CO_2 hydration** Specific activity (WAU mg^{-1})	**Esterase activity** Specific activity (U mg^{-1})
TWCA1	425 ± 9 (4)	635 ± 45 (4)
Bovine CA	1970 ± 98 (4)	1090 ± 63 (4)
Boiled TWCA1	0	0

Manuscript in revision, Journal of Phycology

δ-CARBONIC ANHYDRASE

Different CA expression in various strains of *E. huxleyi* = **difference in CCM efficiency** & adaptation to an ever-changing CO_2 environment??

δ-CA is a functional carbonic anhydrase



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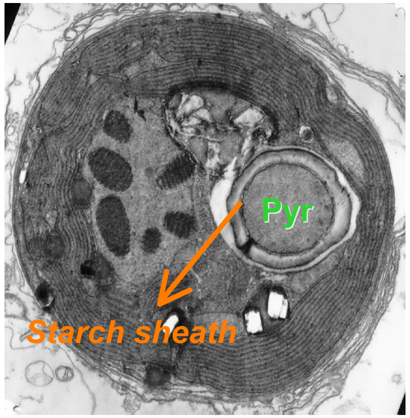
Manuscript in revision, Journal of Phycology

PYRENOIDS: AN INTRODUCTION

- Present in nearly all unicellular algae & many macroscopic species (both freshwater & marine)



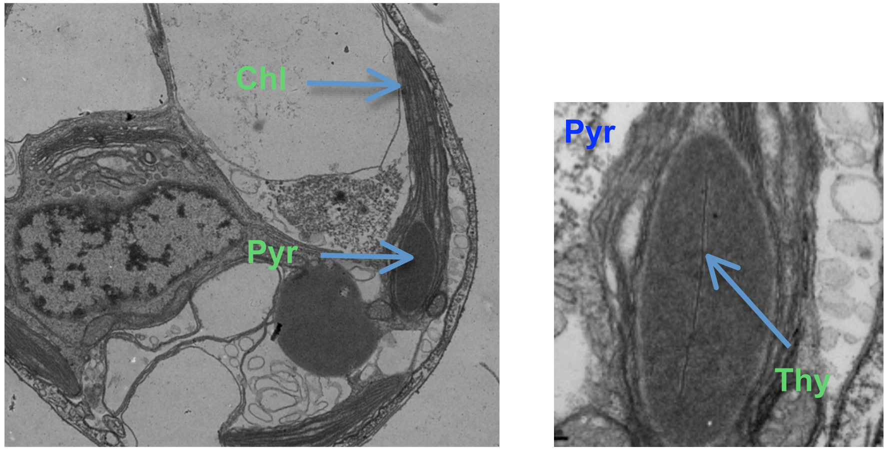
A protein complex, located in the stroma of the chloroplast

Often surrounded by a sheath of carbohydrate in green algae

Until the 1980s, pyrenoid thought to be site of starch synthesis, which was discredited after mutant studies

- Holdsworth (1971) successfully isolated pyrenoids from green algae & showed that it was composed of up to 90% Rubisco
- Pyrenoid acts as a diffusion barrier, minimising leakage of CO_2 from the chloroplast, ensuring CO_2 saturation of Rubisco
- *C. reinhardtii* insertional mutants (lacking a pyrenoid) have been shown to grow poorly on low levels of CO_2 (Ma et al. 2011)

PYRENOIDS: PRESENT STUDY



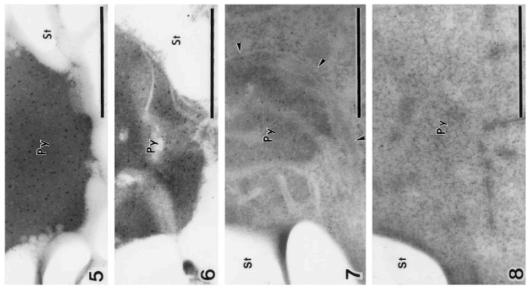
Transmission electron microscopy (TEM) of Helicosphaera carteri

QUESTIONS:

- Distribution of pyrenoids across various species/strains of haptophytes
- Does the pyrenoid ultrastructure (thylakoid membrane) vary between species/strains and to what extent?

In situ localisation of Rubisco

- In Chlamydomonas, the amount and localisation of Rubisco in the stroma varies with growth conditions (Borkhsenious et al. 1998) & strains (Morita et al. 1999)
- Is there a variation between closely related haptophytes or strains of *E. huxleyi*?



Morita et al. (1999)

5. *Cd. macrostellata*
6. *Cd. radiata*
7. *Cd. Insignis*
8. *Cd. bipapillata*

Strains	CO_2 condition	O_2 evolution rate (μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$)	$K_{0.5}(CO_2)$ (μM)	Ci pool (μM)
Cd. mutabilis UTEX 578	L	122.7 ± 33.2	9.7 ± 0.9	252 ± 57
	H	124.3 ± 35.5	7.8 ± 1.5	–
Cd. radiata UTEX 966	L	92.8 ± 18.9	2.9 ± 1.7	231 ± 91
	H	107.0 ± 16.3	3.4 ± 0.3	–
Cd. augustae UTEX 1969	L	132.3 ± 31.4	0.1 ± 0.02	–
	H	129.0 ± 32.7	0.2 ± 0.2	–
Cd. macrostellata SAG 72.81	L	110.8 ± 32.2	1.2 ± 0.1	–
	H	109.4 ± 32.0	1.0 ± 0.1	–
Cd. bipapillata SAG 11–47	L	168.0 ± 26.2	11.0 ± 3.5	24 ± 8
	H	136.7 ± 44.5	19.8 ± 6.5	–
Cr. insignis NIES-447	L	76.7 ± 8.1	2.2 ± 0.5	31 ± 11
	H	80.6 ± 27.8	17.8 ± 1.8	–

Work in progress by Maeve Eason-Hubbard (Graduate student)

COPY NUMBERS

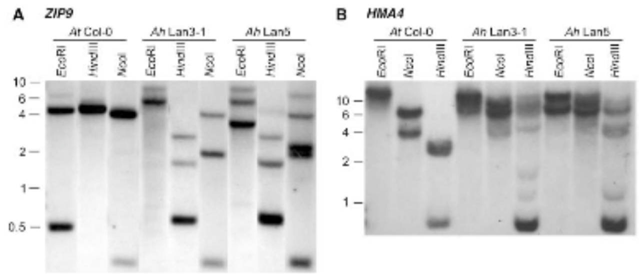
- Various diseases (e.g. cancer), pathogenicity/toxicity & tolerance in a variety of environmental conditions are caused by gene copy number variants
- High transcript levels may be attributed to the presence of several gene copies in the genome

AN EXAMPLE :

LETTERS

Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of *HMA4*

Marc Hanikenne[1†*], Ina N. Talke[1†*], Michael J. Haydon[1†], Christa Lanz[2], Andrea Nolte[1†], Patrick Motte[3,4], Juergen Kroymann[5], Detlef Weigel[2] & Ute Krämer[1†]



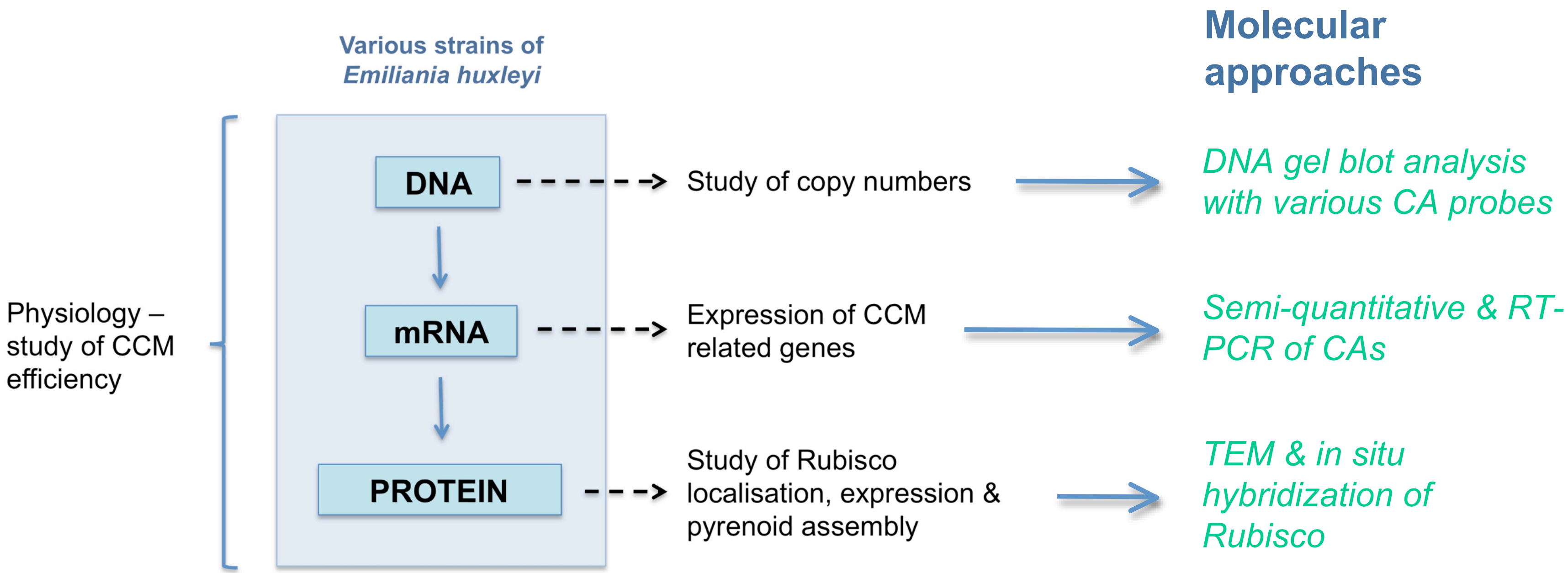
Talke et al. (2006)

Metal tolerance

Genes involved in conferring metal tolerance are shown to have a higher copy number (ZIP9, HMA4) in *A. halleri* (metal accumulator) vs. *A. thaliana* (non-accumulator) using DNA Gel-Blot Analysis

Different CA copy number in various strains of *E. huxleyi* = **difference in CCM efficiency** ??

SUMMARY & FUTURE WORK



- Improve knowledge of cell physiology (CCM & photosynthesis)
- Explain the variation among strains of *Emiliania huxleyi*
- Understand how species adapt to an ever-changing marine environment (OA)