



**HAL**  
open science

# Regulation of Carbon Metabolism by Environmental Conditions: A Perspective From Diatoms and Other Chromalveolates

Hélène Launay, Wenmin Huang, Stephen C. Maberly, Brigitte Gontero

► **To cite this version:**

Hélène Launay, Wenmin Huang, Stephen C. Maberly, Brigitte Gontero. Regulation of Carbon Metabolism by Environmental Conditions: A Perspective From Diatoms and Other Chromalveolates. *Frontiers in Plant Science*, 2020, 11, pp.1033. 10.3389/fpls.2020.01033 . hal-02903680

**HAL Id: hal-02903680**

**<https://hal.science/hal-02903680>**

Submitted on 21 Jul 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Regulation of carbon metabolism by environmental conditions: a perspective from  
2 diatoms and other chromalveolates

3

4 H  l  ne Launay<sup>1</sup>, Wenmin Huang<sup>1,2</sup>, Stephen C Maberly<sup>3</sup>, Brigitte Gontero<sup>1,\*</sup>

5

6 <sup>1</sup>Aix Marseille Univ CNRS, BIP UMR 7281, 31 Chemin Joseph Aiguier, 13402 Marseille  
7 Cedex 20, France

8

9 <sup>2</sup>Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden,  
10 Center of Plant Ecology, Core Botanical Gardens, Chinese Academy of Sciences,  
11 Wuhan 430074, China

12 <sup>3</sup>UK Centre for Ecology & Hydrology, Lake Ecosystems Group, Lancaster Environment  
13 Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP UK

14

15 \* **Correspondence:** Dr B. Gontero, CNRS-BIP, 31 Chemin Joseph Aiguier, 13 402  
16 Marseille Cedex 20 France, Tel.: (+33) 491164549, Fax : (+33) 491164689, E-mail :  
17 [bmeunier@imm.cnrs.fr](mailto:bmeunier@imm.cnrs.fr)

18

19

20 **Keywords:** Calvin cycle, CO<sub>2</sub> concentrating mechanism, *Phaeodactylum tricornutum*,  
21 Redox regulation, *Thalassiosira pseudonana*

22

23

24 **Abstract**

25 Diatoms belong to a major, diverse and species-rich eukaryotic clade, the Heterokonta,  
26 within the polyphyletic chromalveolates. They evolved as a result of secondary  
27 endosymbiosis with one or more Plantae ancestors, but their precise evolutionary  
28 history is enigmatic. Nevertheless, this has conferred them with unique structural and  
29 biochemical properties that have allowed them to flourish in a wide range of different  
30 environments and cope with highly variable conditions. We review the effect of pH,  
31 light and dark, and CO<sub>2</sub> concentration on the regulation of carbon uptake and  
32 assimilation. We discuss the regulation of the Calvin-Benson-Bassham cycle,  
33 glycolysis, lipid synthesis and carbohydrate synthesis at the level of gene transcripts  
34 (transcriptomics), proteins (proteomics) and enzyme activity. In contrast to  
35 Viridiplantae where redox regulation of metabolic enzymes is important, it appears to  
36 be less common in diatoms, based on the current evidence, but regulation at the  
37 transcriptional level seems to be widespread. The role of post-translational  
38 modifications such as phosphorylation, glutathionylation, etc., and of protein-protein  
39 interactions, has been overlooked and should be investigated further. Diatoms and  
40 other chromalveolates are understudied compared to the Viridiplantae, especially  
41 given their ecological importance, but we believe that the ever-growing number of  
42 sequenced genomes combined with proteomics, metabolomics, enzyme  
43 measurements and the application of novel techniques will provide a better  
44 understanding of how this important group of algae maintain their productivity under  
45 changing conditions.

46

47

## 48 INTRODUCTION

49 The chromalveolates are a polyphyletic eukaryote supergroup that includes many  
50 photosynthetic lineages including the cryptomonads, dinoflagellates, haptophytes and  
51 heterokonts (also called stramenopiles) (Keeling, 2009). The phylogeny of diatoms and  
52 their allied groups is complicated (Dorrell et al., 2017; Falciatore et al., 2020) and while  
53 the chromalveolates are not now regarded as a natural group we have retained the  
54 name here as a convenient and widely-used term. It has been estimated that over 50%  
55 of all formally described protists are chromalveolates (Cavalier-Smith, 2004; Cavalier-  
56 Smith and Chao, 2006). Within the diverse clade Heterokonta, diatoms  
57 (Bacillariophyceae) are photosynthetic microalgae that comprise between 30 000 and  
58 100 000 species (Mann and Vanormelingen, 2013). They evolved about 250 Myrs ago  
59 (Medlin, 2016), are found today in all aquatic environments, and contribute about 20%  
60 to global primary production (Falkowski et al., 1998). Like other heterokonts, diatoms  
61 originated via serial endosymbioses (Stiller et al., 2014) and their chloroplasts derive  
62 from a red and a green algal endosymbiosis and also contain genes from prokaryotes,  
63 their eukaryotic host, and genes acquired by horizontal transfer (Moustafa et al.,  
64 2009; Deschamps and Moreira, 2012; Dorrell et al., 2017). Consequently, diatom  
65 genomes are enriched in genes from different origins and this combination has gifted  
66 them with unique metabolic features. In addition to the metabolism needed to produce  
67 a silica cell wall (Hildebrand et al., 2018) a functioning urea cycle is present (Allen et  
68 al., 2011; Nonoyama et al., 2019). Diatoms have an Entner-Doudoroff glycolytic  
69 pathway (Fabris et al., 2012) but lack the oxidative pentose phosphate (OPP) pathway  
70 in their chloroplast (Wilhelm et al., 2006; Kroth et al., 2008; Gruber et al., 2009). Their  
71 principal storage compound is a polysaccharide, chrysolaminarin ( $\beta$ -1,3-glucan) that is  
72 located in the vacuole rather than the chloroplast (Huang et al., 2018). Diatoms also  
73 have a large diversity of the metalloenzyme carbonic anhydrase (CA) that interconverts  
74  $\text{CO}_2$  and  $\text{HCO}_3^-$ . They possess seven of the eight known CA sub-classes, some of  
75 which can make use of metal cations other than the canonical zinc (Jensen et al.,  
76 2019a; Alissa et al., 2020; Morel et al., 2020). In diatoms, both the large and the small  
77 subunits of ribulose biphosphate carboxylase-oxygenase (RuBisCO) are encoded by  
78 the chloroplast genome, in contrast to Viridiplantae where the small subunit is a nuclear  
79 encoded protein (Oudot-Le Secq et al., 2007). Moreover, most diatom plastid  
80 genomes, unlike those in Viridiplantae and the diatom *Seminavis robusta*, lack introns  
81 (Brembu et al., 2014). Also in diatoms, RuBisCO activation is mediated by the protein  
82 CbbX (Mueller-Cajar et al., 2011) that does not possess the cysteine residues found  
83 in RuBisCO activase (RCA) found in Viridiplantae, and thus cannot be redox regulated  
84 (Jensen et al., 2017). In addition, diatoms also have a pigment composition that  
85 substantially differs from plants (Kuczynska et al., 2015; Carreto and Catoggio,  
86 1976; Falkowski and Owens, 1980; Gilstad et al., 1993). The most important accessory  
87 pigments in diatoms are fucoxanthin and chlorophyll *c* rather than chlorophyll *b* in  
88 Viridiplantae (Green, 2011). Also, like all photosynthetic eukaryotes and  
89 cyanobacteria, they contain xanthophylls that are derived from  $\beta$  carotene but in  
90 contrast, lack the  $\alpha$ -carotene pathway. Diatoms are able to acclimate to a broad range  
91 of light irradiance and nutrient concentrations by adjusting their physiology and  
92 biochemical activity (Schoefs et al., 2017; Heydarizadeh et al., 2019). This requires a  
93 variety of mechanisms for balancing energy harvesting and light-energy consuming  
94 metabolic processes including carbon fixation (Wilhelm et al., 2006). In contrast to  
95 Viridiplantae, diatoms have a very low cyclic electron flow. To equilibrate the ratio of  
96 ATP to NADPH required for optimal photosynthesis, the chloroplast and the

97 mitochondrion, that are physically in contact, exchange these compounds (Bailleul et  
98 al., 2015) (Figure 1).

99 Regulation can act on, and be studied at, a cascade of different levels from DNA  
100 (genomics), mRNA (transcriptomics), proteins (proteomics and post-translational  
101 modifications, PTMs), metabolites (metabolomics) and enzyme activity, because each  
102 approach provides different types of information (Figure 1). Genomes are powerful  
103 resources to determine if a specific gene is present while transcriptomics detect under  
104 what conditions it is expressed. Fully annotated diatom genome sequences are only  
105 available for *Thalassiosira pseudonana*, a marine centric diatom and *Phaeodactylum*  
106 *tricornutum*, a marine or coastal pennate diatom (Armbrust et al., 2004; Bowler et al.,  
107 2008). Other sequences are also available for *Fragilariopsis cylindrus* (Mock et al.,  
108 2017) and *Pseudo-nitzschia multiseriata* ([https://genome.jgi.doe.gov/portal/pages/tree-](https://genome.jgi.doe.gov/portal/pages/tree-of-life.jsf)  
109 [of-life.jsf](https://genome.jgi.doe.gov/portal/pages/tree-of-life.jsf)) and there are further published genomes that are not yet publicly available  
110 (Tanaka et al., 2015; Traller et al., 2016; Basu et al., 2017; Villain et al., 2017; Ogura et  
111 al., 2018). Lauritano et al. (Lauritano et al., 2019), reviewed the current development  
112 of omics approaches on microalgae. Of the 26 eukaryotic microalgal genomes they  
113 listed, 15 were from chromalveolates and of these, 8 were from diatoms. However,  
114 regulation should also be analyzed at the level of enzyme activity and/ or metabolites  
115 (metabolomics), as these represent the final outcome of gene expression and activity  
116 (Prosser et al., 2014). The lifetime of an active enzyme, or of a metabolite, is related  
117 to its rate of synthesis and turnover. While the synthesis rates are on the order of 4–6  
118 amino acids per second for enzymes (Stein and Frydman, 2019) and several seconds  
119 for metabolites (Nikolaev and Lohse, 2006), their turnover rates can vary from a few  
120 seconds to extended periods respectively. Enzyme activity is also modulated by PTMs,  
121 such as acetylation, phosphorylation, methylation, glycosylation and  
122 formation/dissociation of disulfide bonds. These types of modulation are very fast (rate  
123 on the order of few per second), reversible, and are the most flexible regulatory  
124 responses at the protein level (Prabakaran et al., 2012). In diatom RuBisCO, a number  
125 of post-translational modifications of the large subunit are present, including 4-  
126 hydroxyproline,  $\beta$ -hydroxyleucine, hydroxylated and nitrosylated cysteine, mono- and  
127 dihydroxylated lysine, and trimethylated lysine (Valegard et al., 2018). Nevertheless,  
128 in order to understand the full scope of regulation by post-translational modifications  
129 (Grabsztunowicz et al., 2017) in chromalveolates, more studies are needed on PTM  
130 and proteomics. Regulation of gene expression is itself dependent on earlier response  
131 regulators (for example PTMs of histones and transcription factors) and on metabolite  
132 productions (for example cAMP). As a consequence, upon environmental changes,  
133 regulation of gene expression occurs over a longer timescale of several minutes to  
134 hours (Chauton et al., 2013).

135 Relationships between mRNA level and protein expression can be observed  
136 though this might be influenced by biological (e.g. properties of mRNA and proteins,  
137 cell cycle status) and by technical problems (accurate quantification of these two  
138 biological molecules) (Maier et al., 2009; Ponnala et al., 2014). Therefore there are  
139 discrepancies in the literature as regard to the extent of correlation between them. Net  
140 mRNA levels can be a major contributor to protein abundance, and for instance,  
141 positive relationship has been observed in yeast (Fournier et al., 2010), in the green  
142 alga *Chlamydomonas reinhardtii* (Castruita et al., 2011) and specifically in the diatom  
143 *T. pseudonana* (Clement et al., 2017b). Nevertheless, there are additional  
144 mechanisms, that control protein abundance including translational control and  
145 differential protein and mRNA degradation rates (Ponnala et al., 2014). However, since  
146 data on protein expression and activity are scarce, we have supplemented this type of

147 information with data on gene regulation as a first step to assess how diatoms respond  
148 to environmental change, even though there is not always a direct and positive  
149 relationship between mRNA level, protein expression and finally, metabolic activity  
150 (Figure 1).

151

## 152 **1. Regulation of photosynthesis by light and dark**

153 Changing light levels affect many processes, including cell division, and diatoms  
154 can acclimate efficiently to light variation by altering the expression of different cell  
155 cycle genes such as cyclins and cyclin-dependent kinases genes (Huysman et al.,  
156 2013). Here we focus on the best-studied effect of light, the regulation of  
157 photosynthesis although, there is much less information for diatoms than for the  
158 Viridiplantae (Jensen et al., 2017). In the Viridiplantae, that includes the Embryophyta,  
159 carbon fixation by the Calvin-Benson-Bassham (CBB) cycle is well known to be fine-  
160 tuned by dark-light transitions, involving regulation by pH (Werdan et al., 1975),  $Mg^{2+}$   
161 (Portis and Heldt, 1976), metabolite concentration (Anderson, 1973; Pupillo and  
162 Giulianipicari, 1975; Gardemann et al., 1983; Baalman et al., 1994) and primarily by  
163 the redox state of key enzymes (Buchanan et al., 1980; Schurmann and Jacquot,  
164 2000). Non-covalently bound 'small molecules' or metabolites also affect the rates of  
165 redox-interconversion of each redox-regulated enzyme in Viridiplantae and this fine-  
166 tuning regulation is well-described in a review from Knesting and Scheibe (2018).

167

### 168 **1.1 Regulation by pH**

169 In chloroplasts from Embryophyta, dark-to-light transitions are accompanied by a shift  
170 of the chloroplast internal pH from 7 in the dark to 8 in the light (Werdan and Heldt,  
171 1973; Hauser et al., 1995). These changes directly regulate photosynthesis since many  
172 key chloroplastic enzymes have optimal activity at pH 8 and are much less active at  
173 pH 7 (reviewed in Gontero *et al.* (2006)). In diatoms, pH responses have mainly been  
174 studied for external/environmental, rather than internal, pH. External pH can affect  
175 growth rate, silicon metabolism and biomineralization of *Conticribra weissflogii*  
176 (formerly known as *Thalassiosira weissflogii*) as well as its intracellular/cytoplasmic pH  
177 homeostasis (Herve et al., 2012). For other photosynthetic organisms, it is not the  
178 extracellular pH, but the intracellular pH in the chloroplast that is the critical factor for  
179 regulation of carbon acquisition, transport capacity and other metabolic processes. To  
180 our knowledge, internal pH has only been measured for a small number of diatoms.  
181 For *P. tricornutum* and *Cyclotella* sp. the pH was around 7 in the dark and 7.5 in the  
182 light (Colman and Rotatore, 1995), and for *Navicula pelliculosa* it was 7.4 in the dark  
183 and 7.6 in the light (Colman and Rotatore, 1988). We found no published values for  
184 the pH within diatom chloroplasts stroma. One of the few studies of the effect of pH on  
185 enzyme activity in chromalveolates is for the chloroplastic glyceraldehyde-3-phosphate  
186 dehydrogenase (GAPDH) that catalyzes the reversible reduction and  
187 dephosphorylation of 1,3-bisphosphoglycerate to produce glyceraldehyde-3-  
188 phosphate and inorganic phosphate. Avilan et al. (Avilan et al., 2012) compared the  
189 optimal pH of GAPDH in the freshwater diatom, *Asterionella formosa*, the freshwater  
190 eustigmatophyte, *Pseudocharaciopsis ovalis* and the model green alga, *C. reinhardtii*.  
191 In *A. formosa*, GAPDH was still active at the pH occurring in the dark, assuming that  
192 the internal pH (pH 7) reflects the one in the chloroplast. This suggests that GAPDH is  
193 regulated by factors other than pH in this diatom, unlike in the green algal enzyme that  
194 was down-regulated at the pH that occurs in the dark. The response of GAPDH from  
195 the eustigmatophyte *P. ovalis* was similar to that of the green alga *C. reinhardtii*. We  
196 do not know the internal chloroplast pH for *P. ovalis* but if the dark-to-light pH transition

197 in this species is similar to that of *C. reinhardtii*, GAPDH could be partly regulated by  
198 pH under dark-light transitions. The different regulation of GAPDH by pH in the two  
199 heterokonts, *A. formosa* and *P. ovalis*, might be the result of the diverse evolutionary  
200 history of chromalveolates. Another example of regulation by pH is the luminal enzyme  
201 violaxanthin de-epoxidase (VDE, (Lavaud et al., 2012)) that is involved in dissipating  
202 excess light energy (Lohr and Wilhelm, 1999).

203 Beyond photosynthesis, the carbon metabolism of the marine diatom  
204 *Skeletonema costatum* is regulated by the pH of the growth medium (Taraldsvik and  
205 Myklestad, 2000). The content of the carbohydrate storage compound,  
206 chrysolaminarin ( $\beta$ -1,3-linked glucan) decreased from 7.1 mg L<sup>-1</sup> at pH 6.5 to 0.2 mg  
207 L<sup>-1</sup> at pH 9.4 and concomitantly, the total organic carbon as glucan also decreased  
208 from 60 to 10 %. The total amino acid content also decreased from 7.41 to 2.51  
209 fmol.cell<sup>-1</sup> when the pH of the growth medium increased (Taraldsvik and Myklestad,  
210 2000). It is unclear if these are direct effects on carbon and nitrogen metabolism of  
211 external or internal pH or indirect effects linked to the greater external concentration of  
212 CO<sub>2</sub> at pH 6.5 than at pH 9.4. Nevertheless, to relate these physiological responses to  
213 enzyme activity regulation, the authors report results from a Norwegian PhD thesis  
214 (Kirkvold, 1994) that showed that the specific activity of glutamine synthetase, a key  
215 enzyme in the metabolic pathway of glutamine and glutamate synthesis, also  
216 decreased with increasing pH when measured *in vitro*.

217 Studies on the effect of pH on activity should be expanded to more enzymes and  
218 the optimal pH compared to the internal pH in dark and light in order to determine if  
219 enzyme activity is regulated by internal pH. The difficulty of working with enzymes from  
220 diatoms and from chromalveolates in general, is probably responsible for the lack of  
221 data for this important group. For instance, in order to extract proteins from diatoms,  
222 litres of culture are required and it is not always easy to measure activity. Expressing  
223 recombinant diatom enzymes in heterologous systems is also challenging with many  
224 enzymes found in the insoluble fractions (B. Gontero, personal communication).  
225 Measurement of internal pH is also an experimental tour de force. Colman and  
226 Rotatore used the 5,5-dimethyl-2,4-oxazolidinadione distribution method that  
227 distributes between the medium and the cell as a function of their respective pH  
228 (Colman and Rotatore, 1988;1995). However, this method does not distinguish  
229 between the pH in the cytoplasm, chloroplast stroma or thylakoid lumen.

230

## 231 **1.2 Regulation by the redox state of cysteine residues**

232 The redox control of enzyme activity in the Viridiplantae is primarily mediated by small  
233 proteins, thioredoxins, that are oxidized in the dark and reduced in the light (Buchanan  
234 et al., 1980;Buchanan, 2017). This regulation avoids futile cycles between the CBB  
235 and the OPP pathway, since both occur within the chloroplast, with enzymes from the  
236 CBB being active in the light and those from OPP being active in the dark. In contrast,  
237 in diatom plastids the OPP is incomplete, and presumably lacking (Kroth et al., 2008),  
238 and accordingly the regulation of their metabolism is different (Jensen et al., 2017).  
239 Moreover, diatoms have a high stromal reductant pressure and in contrast to  
240 Viridiplantae, metabolic activity in long dark periods leads to an enhanced reduction  
241 state of the plastoquinone pool. In the dark, since the plastoquinone pool is reduced,  
242 it may regulate redox-sensitive enzymes as is the case for algal nitrate reductase  
243 (Giordano et al., 2005). This avoids reducing equivalents to accumulate maintaining

244 cellular redox poise (Wilhelm et al., 2014). In Viridiplantae in contrast, oxidizing  
245 conditions prevail in the dark, therefore suggesting that redox control may be different.  
246 Because of this unusual redox control, the redox regulation of diatom enzymes has  
247 been questioned (Wilhelm et al., 2006). However, diatoms possess many different  
248 thioredoxins, each encoded by a specific gene and located in different compartments,  
249 including the chloroplast. Most thioredoxins contain the regulatory cysteine residue in  
250 the conserved motif, WCGPC (Weber et al., 2009), thus they are likely to have specific  
251 regulation targets and some targets have been identified such as two CAs in *P.*  
252 *tricornutum* (Kikutani et al., 2012). The relatively few targets of thioredoxins currently  
253 identified in diatoms, contrasts with the 1188 targets found by combining qualitative  
254 and quantitative proteomic analyses in the *C. reinhardtii* thioredoxome (Perez-Perez  
255 et al., 2017).

256 Using a redox proteomics approach on *P. tricornutum*, Rosenwasser *et al.*  
257 identified the “redoxome”, or in other words the redox-sensitive proteins, and  
258 demonstrated its involvement in photosynthesis, photorespiration, lipid biosynthesis,  
259 and nitrogen metabolism (Rosenwasser et al., 2014). In that case however, the redox-  
260 sensitivity is a response to oxidative stress rather than light-dark transition even though  
261 reactive oxygen species are photo-induced, and increase in parallel to glutathione  
262 (GSH). GSH is a low-molecular-weight tripeptide that consists of cysteine (Cys),  
263 glutamic acid (Glu), and glycine (Gly) and is present in microorganisms, plants, and  
264 mammals (Zaffagnini et al., 2012). It can regulate protein activity by forming a mixed  
265 disulfide bridge between the thiol group of its Cys and an accessible free thiol on a  
266 protein, a process known as protein S-glutathionylation (Zaffagnini et al., 2012; Marri et  
267 al., 2014). This post-translational modification can protect specific Cys residues from  
268 irreversible oxidation but can also modulate protein activities (Zaffagnini et al.,  
269 2012; Marri et al., 2014; Thieulin-Pardo et al., 2015). In *T. pseudonana*, a diurnal redox-  
270 related pattern has been observed in which GSH accumulates in the light, and  
271 decreases upon darkness, (Dupont et al., 2004) (Figure 2). However, direct regulation  
272 of the enzyme activities by glutathionylation in diatoms, or in other chromalveolates, in  
273 contrast to Viridiplantae, has not yet been studied, to the best of our knowledge.

274 Although the effect of glutathionylation on enzymes has not been studied in  
275 diatoms, the effect of other reducing agents such as dithiothreitol has been  
276 investigated, though understudied as compared to other photosynthetic organisms  
277 such as Cyanobacteria, Chlorophyta, Rhodophyta and Embryophyta. The chloroplastic  
278 phosphoglycerate kinase belonging to the CBB cycle, catalyzes the ATP-Mg<sup>2+</sup>-  
279 dependent phosphorylation of 3-phosphoglycerate (3-PGA) to 1,3-  
280 bisphosphoglycerate, in a reversible reaction and was redox-regulated in *P.*  
281 *tricornutum* (Belen Bosco et al., 2012). However, in our hands, PGK was not redox-  
282 regulated, as was also the case in *T. pseudonana*, in *Navicula pelliculosa* grown with  
283 sea water and fresh water medium and in a freshwater diatom, *A. formosa* (Jensen et  
284 al., 2019b). In contrast to the Viridiplantae, two enzymes that are unique to the CBB  
285 were not redox regulated (Michels et al., 2005; Maberly et al., 2010; Jensen et al., 2017).  
286 These include, sedoheptulose 1,7-bisphosphatase that irreversibly catalyzes the  
287 dephosphorylation of sedoheptulose-1,7-bisphosphate producing sedoheptulose-7-  
288 phosphate, and phosphoribulokinase (PRK) that irreversibly catalyzes the ATP-Mg<sup>2+</sup>-  
289 dependent phosphorylation of ribulose-5-phosphate into ribulose-1,5-phosphate. The  
290 general lack or weak redox regulation of PRK in the chromalveolates (diatoms and



291 other groups) seems to be related to its sequence, where the connectivity between two  
292 regulatory cysteine residues is crucial (at position 16 and 55 in *C. reinhardtii* (Maberly  
293 et al., 2010)). In many photosynthetic organisms PRK can also be sequestered, and  
294 inactivated, in a PRK-GAPDH-CP12 complex, that has not yet been found in diatoms.  
295 The absence of the ternary complex with GAPDH in diatoms has been attributed to the  
296 absence of two cysteine residues on PRK (at position 243 and 249 numbered from the  
297 enzyme from *C. reinhardtii*) that are present in Cyanobacteria, Chlorophyta,  
298 Rhodophyta and Embryophyta where the complex has been identified (Thieulin-Pardo  
299 et al., 2015). In contrast however, a ferredoxin-NADP reductase (FNR)-GAPDH-CP12  
300 complex has been found in *A. formosa* (Mekhalfi et al., 2014). For the chloroplastic  
301 GAPDH, the regulation is more complex as discussed above in the pH regulation  
302 section, but in many diatoms, this enzyme seems to be redox regulated (Maberly et  
303 al., 2010; Mekhalfi et al., 2012; Mekhalfi et al., 2014; Jensen et al., 2019b).

304

### 305 **1.3 Direct light-dark control of gene expression**

306 Regulation at the transcriptional level by light-dark transitions, occurs in Viridiplantae,  
307 and also in diatoms (Sun et al., 2003; Fey et al., 2005). In *T. pseudonana*, after 12 h of  
308 light, 1859 genes were upregulated compared to cells exposed to 12 hours of dark,  
309 and inversely, after 12h of dark, 1326 genes were up-regulated compared to cells  
310 exposed to 12 h of light (Ashworth et al., 2013). Among the most highly expressed  
311 genes after 12 h of light were the ones encoding enzymes for cell division, DNA  
312 replication and repair, carbon metabolism and oxidative phosphorylation while after 12  
313 h of dark, the most highly expressed genes were those encoding ribosomal biogenesis,  
314 aminoacyl-tRNA and key photosynthetic enzymes (Figure 2). Some genes, such as  
315 that encoding RuBisCO, anticipates the diurnal cycle and is up-regulated before the  
316 onset of light. The dark-light expression pattern of genes was affected by growth phase  
317 (exponential vs stationary). In the stationary phase, the expression of only a few genes  
318 fluctuated under dark-light transitions (Ashworth et al., 2013). One of these genes  
319 encodes a putative pyruvate carboxylase suggesting a switch towards other types of  
320 metabolism such as gluconeogenesis and lipid biosynthesis. This might be explained  
321 by the hypothesis raised by Norici et al. in *S. marinoi* (Norici et al., 2011) who  
322 postulated that the diatom re-routes its metabolism towards lipid biosynthesis, because  
323 of the relatively high volume-based energy content of lipids in an organism in which  
324 size decreases with vegetative cell divisions, thus requiring carbon allocation into more  
325 energy-compact compounds.

326 In the light, more than 4500 transcripts were differentially expressed in *P.*  
327 *tricornutum*, including genes such as the one encoding for pyruvate transporter that  
328 had never been previously described in this organism (Chauton et al., 2013). This work  
329 shows that transcriptional regulation of carbohydrate and lipid metabolism occurs in  
330 diatoms (Figure 2). Indeed, the content of soluble glucans and lipids decreased in the  
331 dark and fatty acid biosynthesis genes were up-regulated within 30 minutes of a switch  
332 from dark to light. Fatty acid biosynthesis and the tricarboxylic acid (TCA) cycle are  
333 also tightly co-ordinated (Chauton et al., 2013). During the day, carbon skeletons are  
334 produced within the chloroplast while in the night these carbon-rich compounds are  
335 broken down in the mitochondria and the cytosol.

336 Interestingly, four carbon fixation enzymes were co-regulated in *P. tricornutum*:  
337 PGK, GAPDH, triose phosphate isomerase/GAPDH and PRK. Their mRNAs were all  
338 highest at the beginning of the light period (dawn) and lowest at the beginning of the  
339 dark period (dusk). Bai et al. (Bai et al., 2016) showed however that the expression of

340 PRK increased after four days of dark treatment using a proteomic approach. Similarly,  
341 the expression of phosphoribose isomerase, the enzyme that produces PRK substrate,  
342 as well as the expression of transaldolase and glyoxylase, increased after dark  
343 treatment.

344 Redox regulation of PRK is observed and well-studied in Viridiplantae, but in  
345 contrast in diatoms and other chromalveolates, as mentioned above, the activity of  
346 PRK was affected neither by oxidizing nor by reducing conditions (Maberly et al.,  
347 2010). Therefore, for enzymes that are not redox-regulated, regulation at the  
348 transcriptional level could allow a dark-light regulation of the CBB. The expression  
349 pattern of the gene of fructose-1,6-bisphosphatase (FBPase) that catalyzes the  
350 dephosphorylation of fructose-1,6-bisphosphate into fructose-6-phosphate and  
351 inorganic phosphate, was not induced to the same degree by light as other CBB  
352 enzymes. Since this enzyme can be redox regulated (Michels et al., 2005; Mekhalfi et  
353 al., 2012), its regulation could occur both at the level of activity and transcription.

354 Carbon acquisition is also stopped at night since in *P. tricornutum*, mRNA  
355 encoding for bicarbonate transporters of the Solute Carrier family 4 (SLC4) and for  
356 alpha-carbonic anhydrases, especially  $\alpha$ -CA-VII, were much less abundant in the dark  
357 than in the light (Chauton et al., 2013). Recently, in the same organism, the pattern of  
358 mRNA levels at a photon irradiance of 3, 300, 1000  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  differed at the  
359 lag, exponential and stationary phases of growth (Heydarizadeh et al., 2019).

360 Dark-treated *P. tricornutum* cells preferentially utilize carbon and nitrogen  
361 obtained from protein breakdown to increase lipid cell quotas at low cost (Bai et al.,  
362 2016). Long-term dark stress inhibited several key proteins involved in nitrogen  
363 assimilation and in the synthesis of the photosynthetic machinery. Simultaneously, key  
364 enzymes of glycolysis and the synthesis of fatty acids were induced apparently to  
365 assimilate the excess of C and N from protein breakdown. Uptake of other resources  
366 for growth are also light-regulated: transporters for uptake of phosphate and silica are  
367 higher in the light and nitrate and ammonium transporters are higher in the dark  
368 (Ashworth et al., 2013) (Figure 2). Data from the literature showing differential  
369 expression of proteins, transcripts and metabolites in light and dark are summarized in  
370 Figure 2.

371

#### 372 **1.4 Enzyme activity, metabolite concentration and carbon storage compounds**

373 Gene expression gives important clues on how light (quality, irradiance, duration)  
374 affects metabolism. However, it is also necessary to measure enzyme activity and  
375 metabolite concentration as these are the ultimate response to environmental change.  
376 For instance, an excess of light modifies lipid biosynthesis in the coastal marine diatom,  
377 *Skeletonema marinoi* (Norici et al., 2011). In *S. costatum*, carbohydrate increased with  
378 irradiance (Hitchcock, 1980) while lipids increased in *Chaetoceros calcitrans* (Harrison  
379 et al., 1990) and therefore the carbon allocation seems to be different and species-  
380 specific. Under different light-regimes, different species behave differently and the  
381 amount of essential fatty acids with growth irradiance is also species-specific. These  
382 examples illustrate that although enzyme activities have been measured, the  
383 mechanism underlying the change in activity is unknown. For example, PEPCK that  
384 converts oxaloacetate into PEP and  $\text{CO}_2$  in gluconeogenesis, increased 2.5-fold in  
385 cells of *S. marinoi* grown under low light (25  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) vs high light (250  
386  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ). The authors suggested that this enzyme might be involved in the  
387 conversion of lipid to carbohydrates especially under low light (Norici et al., 2011). In  
388 contrast, since energy demand for lipid synthesis is much higher than for carbohydrate  
389 synthesis (Raven et al., 2005; Subramanian et al., 2013) under excess light, lipids

390 represent a better sink for excess energy. As a consequence, lipid accumulation in  
391 high irradiance was observed in *S. marinoi*, although this is not always the case. In this  
392 species, other enzymes are probably not regulated by light since their activity remains  
393 unchanged. For instance, the activity of PEPC, that catalyzes the addition of  
394 bicarbonate ( $\text{HCO}_3^-$ ) to PEP to produce oxaloacetate, was similar for cells grown at  
395 low or high light. This enzyme is involved in C4 metabolism and in anaplerotic  
396 reactions. Similarly, the activity of glutamine synthetase that is involved in  
397 photorespiration did not change in cells grown at low or high light.

398

## 399 **2. Effect of CO<sub>2</sub>**

400 Like other algae, diatoms exhibit a range of responses to varying CO<sub>2</sub> concentration,  
401 including effects on photophysiology, rate of photosynthesis and growth, chemical and  
402 pigment composition, and community species composition, but there are large  
403 species- and context-specific variations in the magnitude and sign of response (Boelen  
404 et al., 2011;Torstensson et al., 2012;Gao and Campbell, 2014;Dutkiewicz et al.,  
405 2015;Bach and Taucher, 2019;Jensen et al., 2019b). At the ocean surface, the air-  
406 equilibrium concentration of CO<sub>2</sub> (Dickson, 2010) varies between 5 and 25  $\mu\text{M}$   
407 depending on temperature (Raven, 1994;Tortell, 2000;Kim et al., 2006;Matsuda et al.,  
408 2011;Maberly and Gontero, 2017). This CO<sub>2</sub> concentration is insufficient to saturate  
409 the carboxylating enzyme, RuBisCO (Young et al., 2016) and may not saturate rates  
410 of diatom growth or photosynthesis (Riebesell et al., 1993;Dutkiewicz et al., 2015). This  
411 is mitigated by CO<sub>2</sub>-concentrating mechanisms (CCMs) (Hopkinson et al., 2011) that  
412 are facultative and increase the concentration of CO<sub>2</sub> around RuBisCO, and are  
413 present in many phytoplankton. CCMs can involve biophysical or biochemical  
414 processes (Reinfelder et al., 2000;Reinfelder, 2011;Hopkinson et al., 2016) although  
415 the latter is controversial in diatoms (Clement et al., 2017a). The CCM regulation in  
416 diatoms is highly dependent on light as well as CO<sub>2</sub> concentration (Harada et al., 2006).  
417 However, the major determinant of the extent of CCM expression in *P. tricornutum* is  
418 CO<sub>2</sub> concentration, as it is in green algae (Matsuda and Kroth, 2014) and many  
419 components of CCM are suppressed under elevated CO<sub>2</sub> concentrations and induced  
420 at atmospheric levels or lower. The CO<sub>2</sub> concentration affects expression, and  
421 consequently the activity, of not only CCMs components but also that of enzymes from  
422 metabolic pathways such as the CBB cycle and glycolysis, though this is still  
423 understudied. Below we compile information on the regulation by CO<sub>2</sub> at the  
424 transcriptional and/or the enzyme activity levels of enzymes involved in the CCM and  
425 other metabolic pathways.

426

## 427 **2.1 CO<sub>2</sub>-concentrating mechanisms (CCMs)**

### 428 **2.1.1 Biochemical CCM**

429 In C4 metabolism, PEPC is the first carboxylating enzyme and traps bicarbonate into  
430 a C4 carbon compound. This compound is subsequently cleaved by a decarboxylase  
431 enzyme to provide a 3-carbon compound and CO<sub>2</sub>, near the active site of RuBisCO  
432 (Sage, 2004). As mentioned above, the presence of C4 or biochemical CCM in diatoms  
433 does not seem to be universal. For instance, there is evidence for it in *C. weissflogii*  
434 (Reinfelder et al., 2000;Reinfelder, 2011;Hopkinson et al., 2016) but it is absent in *P.*  
435 *tricornutum* (Haimovich-Dayana et al., 2013;Clement et al., 2017a;Ewe et al., 2018). In  
436 the eustigmatophyte *Nannochloropsis oceanica*, a novel type of C4-based CCM was  
437 proposed to occur when cells were shifted from high CO<sub>2</sub> (50 000 ppm) to low CO<sub>2</sub>

438 (100 ppm) (Wei et al., 2019). In this C4-based CCM, PEPC and PEPCK have been  
439 proposed to be involved in the primary inorganic carbon fixation steps in mitochondria,  
440 and not in chloroplasts. Subsequent decarboxylation of malate by a malic enzyme in  
441 the chloroplast, enriches CO<sub>2</sub> in the vicinity of RuBisCO (Figure 3). Transcripts levels  
442 of some C4 enzymes were not altered by CO<sub>2</sub> concentration and do not suggest a  
443 classic C4 metabolism, but activities of PEPC and malic enzyme increased under low  
444 CO<sub>2</sub> (Wei et al., 2019). More work is required to confirm this interesting possibility.

445

## 446 2.1.2 Biophysical CCM

447 In the genomes of *P. tricornutum* and *T. pseudonana*, nine and thirteen CA gene  
448 sequences have been found respectively (Tachibana et al., 2011; Samukawa et al.,  
449 2014). In *P. tricornutum*, the two chloroplastic pyrenoidal  $\beta$ -CAs PtCA1 and 2,  
450 responded to CO<sub>2</sub> (Satoh et al., 2001; Harada and Matsuda, 2005; Harada et al., 2005)  
451 and later this was confirmed by Tachibana *et al.* by semi-quantitative RT-PCR  
452 (Tachibana et al., 2011). The activation of both PtCA1 and PtCA2 under CO<sub>2</sub> limitation  
453 involves three *cis*-regulatory elements, TGACGT, ACGTCA, and TGACGC, at a region  
454 minus 86 to minus 42 upstream of the transcription start site. These elements, CCRE1  
455 to 3, are critical for the transcriptional response to ambient CO<sub>2</sub> via the level of the  
456 second messenger cAMP (Ohno et al., 2012; Tanaka et al., 2016). The sensing of CO<sub>2</sub>  
457 mediated by cAMP has been reported in cyanobacteria, fungi and mammals (Matsuda  
458 et al., 2011) and also in *T. pseudonana* (Hennon et al., 2015; Young and Morel, 2015).  
459 The transcriptional activation of PtCA2 in response to the decrease in CO<sub>2</sub>  
460 concentration was strongly light-dependent, such that either CO<sub>2</sub> or the absence of  
461 light can down-regulate the promoter. In contrast, CO<sub>2</sub> concentration and light have  
462 additive effects on the regulation of PtCA1 (Tanaka et al., 2016). It is worth remarking  
463 that both PtCA1 and PtCA2 were post translationally regulated by redox modifications  
464 via thioredoxins (Kikutani et al., 2012) (Figure 3).

465 Tachibana *et al.* also showed that three putative CA genes in *T. pseudonana*,  
466 CA-1, 3, and 7 ( $\alpha$ -CA,  $\zeta$ -CA and  $\delta$ -CA respectively) were induced by decreasing CO<sub>2</sub>,  
467 and function in CO<sub>2</sub>-limited environments (Tachibana et al., 2011). Similarly, in *C.*  
468 *weissflogii*, both CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> uptake increased in response to a CO<sub>2</sub> decrease and  
469 this was accompanied by an increase in both internal and external CA activity  
470 (Burkhardt et al., 2001). A recent proteomic study on *T. pseudonana* acclimated to low  
471 CO<sub>2</sub> (50 ppm) revealed a new uncharacterized protein, later called LCIP63, for “low-  
472 CO<sub>2</sub>-inducible protein of 63 kDa” that was up-regulated under low CO<sub>2</sub> (50 ppm) or at  
473 atmospheric CO<sub>2</sub> (400 ppm) (Clement et al., 2017a) but down-regulated when nitrogen,  
474 phosphorus or silicon were limiting conditions (Lin et al., 2017; Chen et al., 2018).  
475 LCIP63 was up-regulated in *T. pseudonana* growing at 300 ppm vs 1 000 ppm CO<sub>2</sub>  
476 (Valenzuela et al., 2018). Recently, this protein was identified as a new CA (designed  
477 as iota CA, (Jensen et al., 2019a)) that uses Mn<sup>2+</sup> as a co-factor instead of the more  
478 common divalent cation Zn<sup>2+</sup> (Tsuji et al., 2017; DiMario et al., 2018). The gene of iota  
479 CA is also present in bacterial genomes (Jensen et al., 2019a) and recently, the gene  
480 encoding this enzyme from the gram negative bacterium *Burkholderia territorii* was  
481 cloned and the purified recombinant protein showed a CA activity using Zn<sup>2+</sup> instead  
482 of Mn<sup>2+</sup> (Del Prete et al., 2020) indicating that the use of Mn<sup>2+</sup> as a co-factor could be  
483 a feature of diatoms.

484 In *N. oceanica*, transcriptomic, proteomic and metabolomic data are available  
485 for cells grown at high CO<sub>2</sub> (50 000 ppm) and low CO<sub>2</sub> (100 ppm); three of the CA  
486 transcripts were up-regulated ( $\beta$ -CA-2,  $\beta$ -CA-4 and  $\alpha$ -CA-5) under low CO<sub>2</sub> (Wei et al.,  
487 2019). In addition, the transcripts of bicarbonate transporters of the SLC4 family and  
488 several of the ABC transporter family were also more abundant at low CO<sub>2</sub>, indicating  
489 an active biophysical CCM in this organism. Similarly, in *P. tricornutum*, beside the  
490 regulation of numerous CAs by CO<sub>2</sub>, three out of seven SLC4 genes were induced by  
491 low CO<sub>2</sub> and were highly inhibited by the anion exchange inhibitor 4,4-  
492 diisothiocyanatostilbene 2,2-disulphonate (Nakajima et al., 2013). In *T. pseudonana*,  
493 genes homologous to those in *P. tricornutum* have been found (Matsuda et al., 2017),  
494 however their functionality at the protein level has not yet been studied. Two  
495 chloroplast transporters of the bestrophin family of anion channels that are permeable  
496 to HCO<sub>3</sub><sup>-</sup> were also more abundant at low CO<sub>2</sub> and may play a role in the biophysical  
497 CCM of this diatom (Kustka et al., 2014). These data are summarized in Figure 3.

498 Depending on future CO<sub>2</sub> emission scenarios, atmospheric levels of CO<sub>2</sub> are  
499 likely to reach 800 ppm by 2100 (IPCC, 2014; Gattuso et al., 2015). This relates to a  
500 temperature-dependent dissolved CO<sub>2</sub> concentration at the ocean surface of 25 to 50  
501  $\mu$ M. At this concentration, cAMP plays a crucial role in down-regulating CCM genes in  
502 *T. pseudonana*, in particular those encoding the chloroplastic  $\delta$ CA3, some transporters  
503 and some involved in photorespiration (e.g. glycolate oxidase). These photorespiration  
504 genes and CCM genes interestingly, belong to a single CO<sub>2</sub>-responsive cluster that  
505 shares the same upstream *cis*-regulatory sequences found in *P. tricornutum* that is  
506 also responsible for down-regulation of the  $\beta$ -CA gene upon increased CO<sub>2</sub> (Ohno et  
507 al., 2012). Similarly, genes involved in photosynthesis, the TCA cycle, oxidative  
508 phosphorylation and protein degradation were down-regulated, while in contrast other  
509 genes involved in signalling mechanisms were up-regulated at 800 ppm compared to  
510 400 ppm CO<sub>2</sub> (Hennon et al., 2015). Not all these genes contain the upstream  
511 regulatory region, though they were highly affected. This regulation is likely an indirect  
512 effect linked to the impact of high CO<sub>2</sub> on the genes of structural maintenance of  
513 chromosomes (SMC), transcription factors, and histones.

514 Sensing a change, either an increase or a decrease, in external CO<sub>2</sub>  
515 concentrations through cAMP seems to be a general rule of gene regulation rather  
516 than an exception even in diatoms (Young and Morel, 2015).

517

## 518 **2.2 Enzymes from carbon metabolism**

519 Many related metabolic pathways are affected by CO<sub>2</sub> in addition to CCM expression.  
520 Gamma CA and the NADH-ubiquinone oxidoreductase complex (C1) are associated  
521 in many organisms (Cardol, 2011) and in *N. oceanica*, at very low [CO<sub>2</sub>] (100 ppm),  
522 the genes coding for these two mitochondrial enzymes were up-regulated (Figure 4).  
523 Since they can facilitate transport of CO<sub>2</sub> produced by the TCA cycle and  
524 photorespiration towards the chloroplast, in the form of bicarbonate, they contribute to  
525 what is called a basal CCM (Wei et al., 2019). At the enzyme activity level, *T.*  
526 *pseudonana* cells grown at high CO<sub>2</sub> concentration (20 000 ppm) vs atmospheric (400  
527 ppm) displayed higher NADPH-dependent GAPDH and FBPase activity (Figure 4)  
528 indicating that the CBB cycle could be affected even though PRK activity was unaltered  
529 (Clement et al., 2017b). The activity of pyruvate kinase, a glycolytic enzyme, was also  
530 strongly stimulated when cells were grown at 20 000 ppm (Figure 4) while two other  
531 glycolytic enzymes, NADH-dependent GAPDH and glucose-6-phosphate  
532 dehydrogenase (G6PDH) were unaffected (Clement et al., 2017b). In this diatom, a

533 model summarizing the effect of inorganic carbon limitation, based both on activities  
534 and protein expression profiles has been elaborated (Clement et al., 2017b). This  
535 model shows the remodelling of metabolism with a diversion of energy and resources  
536 towards carbon metabolism at high CO<sub>2</sub> and towards carbon capture at low CO<sub>2</sub>. An  
537 increase in  $\delta$ -CA gene expression, to capture as much CO<sub>2</sub> as possible, was also  
538 observed by Kutska *et al.* (Kustka et al., 2014) in *T. pseudonana*. Metabolism enzymes,  
539 as well as enzymes responsible for pigment synthesis and indirectly in light capture  
540 (Figure 4), were less abundant, while enzymes involved in reactive oxygen species  
541 (ROS) defence increased in order to avoid accumulation of ROS that might occur when  
542 energy is in excess. The increased activity of pyruvate kinase (Figure 4), observed in  
543 *T. pseudonana* seems to be shared by other diatoms, as it was observed in many  
544 diatoms from freshwater to seawater species grown under high CO<sub>2</sub> (20 000 ppm) vs  
545 atmospheric CO<sub>2</sub> (400 ppm) (Jensen et al., 2019b). In addition, a modification of gene  
546 expression has been described that allows synthesis of either PEP or pyruvate under  
547 carbon shortage, indicating that pyruvate is an important hub in these organisms  
548 (Heydarizadeh et al., 2017; Heydarizadeh et al., 2019) (Figure 4).

549

### 550 **3. Conclusions and future directions**

551 Physiological and genomic data are available for the response of some  
552 chromalveolates, especially diatoms, to light and CO<sub>2</sub>. They reveal the multitude and  
553 complexity of mechanisms that these organisms have evolved to cope with  
554 environmental variation. However, chromalveolates are still understudied compared to  
555 the Viridiplantae and more research is needed to unravel fully how this important group  
556 of algae maintain their productivity under changing conditions. There is a particular  
557 lack of information in diatoms on internal pH, especially in the chloroplast, on the  
558 identity of redox-regulated enzymes, on regulation by post-translational modification  
559 and on protein-protein interactions. There are existing and new methods that could be  
560 employed to tackle these knowledge gaps. For example, a range of pH-sensitive  
561 fluorescent probes are available to measure internal pH (Loiselle and Casey, 2010),  
562 although their low ability to penetrate the cell and organelle can be challenging.  
563 However, internal pH has also been measured successfully using the inorganic  
564 phosphate (<sup>31</sup>P) nuclear magnetic resonance frequency in other organisms such as  
565 fungi (Hesse et al., 2000) and the anammox bacterium, *Kuenenia stuttgartiensis* (van  
566 der Star et al., 2010) and this could be applied to chromalveolates.

567 There are a growing number of studies taking advantage of proteomics to study  
568 diatom responses to stress e.g. (Muhseen et al., 2015; Chen et al., 2018). Proteomics  
569 approaches have been used successfully in Viridiplantae to identify candidates for  
570 thioredoxin interactions (Montrichard et al., 2009; Perez-Perez et al., 2017). This could  
571 be extended to diatoms where there is a real challenge to assign specific targets to  
572 each of the many thioredoxins found in diatoms (Weber et al., 2009). Biotin-based  
573 proximity labelling approaches, coupled to quantitative proteomics, such as APEX  
574 BioID, are emerging tools for the study of protein-protein interactions (Santin et al.,  
575 2018; Beganton et al., 2019) that could be developed for chromalveolates. There is  
576 evidence for unusual PTMs involved in the regulation of RuBisCO from an arctic diatom  
577 (Valegard et al., 2018) and proteomics could also be a powerful approach to analyse  
578 these modifications. For instance, a phosphoproteomic analysis in *P. tricornutum*  
579 confirmed that phosphorylation occurs in many metabolic pathways (Chen et al.,  
580 2014).

581 Over-expressing or silencing a gene is starting to be applied to diatoms in order  
582 to determine their metabolic role (Hildebrand et al., 2017;Huang and Daboussi,  
583 2017;Falciatore et al., 2020). Recently, tailored TALEN endonucleases and the  
584 CRISPR/Cas9 system have been utilized in diatoms (Daboussi et al., 2014;Hopes et  
585 al., 2016;Nymark et al., 2016), allowing knockout strains with targeted genetic  
586 modifications to be produced. An overview of the genetic toolbox currently available  
587 for performing stable genetic modifications in diatoms is reviewed in Kroth *et al.* and  
588 Falciatore *et al.* (Kroth et al., 2018;Falciatore et al., 2020).

589 It is clear that the techniques mentioned above in combination with genome  
590 sequencing, “omics” and targeted approaches, will allow the biology of diatoms and  
591 chromalveolates to be understood better. However, since many responses seem to be  
592 species-specific, a wider range of species need to be studied, especially those from  
593 non-marine systems, to produce a more complete picture of the functioning in this  
594 supergroup with a mosaic of multi-lineage genomes.

## 595 **Author Contributions**

596

597 **All the authors participated in designing and writing the article.**

598

## 599 **Conflict of Interest Statement**

600 The authors declare that the research was conducted in the absence of any  
601 commercial or financial relationships that could be construed as a potential conflict of  
602 interest.

603

## 604 **Acknowledgments**

605 BG and HL were supported by the Centre National de la Recherche Scientifique  
606 (CNRS) and Aix-Marseille Université. BG is also supported by by the Agence Nationale  
607 de la Recherche (ANR-15-CE05-0021-03, SignauxBioNRJ). SCM was supported by  
608 the UK Centre for Ecology & Hydrology. WH was supported by a fellowship for a visiting  
609 scholar from the Chinese Academy of Sciences.

610

## 611 **References**

612

613 Alissa, S.A., Alghulikah, H.A., Alothman, Z.A., Osman, S.M., Del Prete, S., Capasso, C., Nocentini, A., and  
614 Supuran, C.T. (2020). Inhibition survey with phenolic compounds against the delta- and eta-  
615 class carbonic anhydrases from the marine diatom *thalassiosira weissflogii* and protozoan  
616 *Plasmodium falciparum*. *J. Enzym. Inhib. Med. Ch.* 35, 377-382.

617 Allen, A.E., Dupont, C.L., Obornik, M., Horak, A., Nunes-Nesi, A., Mccrow, J.P., Zheng, H., Johnson, D.A.,  
618 Hu, H., Fernie, A.R., and Bowler, C. (2011). Evolution and metabolic significance of the urea  
619 cycle in photosynthetic diatoms. *Nature* 473, 203-207.

620 Anderson, L.E. (1973). Regulation of pea leaf ribulose-5-phosphate kinase-activity. *Biochim. Biophys.*  
621 *Acta* 321, 484-488.

622 Armbrust, E.V., Berges, J.A., Bowler, C., Green, B.R., Martinez, D., Putnam, N.H., Zhou, S.G., Allen, A.E.,  
623 Apt, K.E., Bechner, M., Brzezinski, M.A., Chaal, B.K., Chiovitti, A., Davis, A.K., Demarest, M.S.,  
624 Detter, J.C., Glavina, T., Goodstein, D., Hadi, M.Z., Hellsten, U., Hildebrand, M., Jenkins, B.D.,  
625 Jurka, J., Kapitonov, V.V., Kroger, N., Lau, W.W.Y., Lane, T.W., Larimer, F.W., Lippmeier, J.C.,  
626 Lucas, S., Medina, M., Montsant, A., Obornik, M., Parker, M.S., Palenik, B., Pazour, G.J.,  
627 Richardson, P.M., Rynearson, T.A., Saito, M.A., Schwartz, D.C., Thamatrakoln, K., Valentin, K.,

628 Vardi, A., Wilkerson, F.P., and Rokhsar, D.S. (2004). The genome of the diatom *Thalassiosira*  
629 *pseudonana*: Ecology, evolution, and metabolism. *Science* 306, 79-86.

630 Ashworth, J., Coesel, S., Lee, A., Armbrust, E.V., Orellana, M.V., and Baliga, N.S. (2013). Genome-wide  
631 diel growth state transitions in the diatom *Thalassiosira pseudonana*. *Proc. Natl. Acad. Sci.*  
632 *U.S.A.* 110, 7518-7523.

633 Avilan, L., Maberly, S.C., Mekhalfi, M., Plateau, J., Puppo, C., and Gontero, B. (2012). Regulation of  
634 glyceraldehyde-3-phosphate dehydrogenase in the eustigmatophyte *Pseudocharaciopsis*  
635 *ovalis* is intermediate between a chlorophyte and a diatom. *Eur. J. Phycol.* 47, 207-215.

636 Baalman, E., Backhausen, J.E., Kitzmann, C., and Scheibe, R. (1994). Regulation of NADP-dependent  
637 glyceraldehyde-3-phosphate dehydrogenase-activity in spinach-chloroplasts. *Bot. Acta* 107,  
638 313-320.

639 Bach, L.T., and Taucher, J. (2019). CO<sub>2</sub> effects on diatoms: a synthesis of more than a decade of ocean  
640 acidification experiments with natural communities. *Ocean Sci.* 15, 1159-1175.

641 Bai, X., Song, H., Lavoie, M., Zhu, K., Su, Y., Ye, H., Chen, S., Fu, Z., and Qian, H. (2016). Proteomic  
642 analyses bring new insights into the effect of a dark stress on lipid biosynthesis in  
643 *Phaeodactylum tricornutum*. *Sci. Rep.* 6, 25494.

644 Bailleul, B., Berne, N., Murik, O., Petroutsos, D., Prihoda, J., Tanaka, A., Villanova, V., Bligny, R., Flori,  
645 S., Falconet, D., Krieger-Liszka, A., Santabarbara, S., Rappaport, F., Joliot, P., Tirichine, L.,  
646 Falkowski, P.G., Cardol, P., Bowler, C., and Finazzi, G. (2015). Energetic coupling between  
647 plastids and mitochondria drives CO<sub>2</sub> assimilation in diatoms. *Nature* 524, 366-369.

648 Basu, S., Patil, S., Mapleson, D., Russo, M.T., Vitale, L., Fevola, C., Maumus, F., Casotti, R., Mock, T.,  
649 Caccamo, M., Montresor, M., Sanges, R., and Ferrante, M.I. (2017). Finding a partner in the  
650 ocean: molecular and evolutionary bases of the response to sexual cues in a planktonic diatom.  
651 *New Phytol.* 215, 140-156.

652 Beganton, B., Coyaud, E., Mange, A., and Solassol, J. (2019). New approaches for protein-protein  
653 interaction study. *Med. Sci.* 35, 223-231.

654 Belen Bosco, M., Cristina Aleanzi, M., and Alvaro Iglesias, A. (2012). Plastidic phosphoglycerate kinase  
655 from *Phaeodactylum tricornutum*: On the critical role of cysteine residues for the enzyme  
656 function. *Protist* 163, 188-203.

657 Boelen, P., De Poll, W.H.V., Van Der Strate, H.J., Neven, I.A., Beardall, J., and Buma, A.G.J. (2011).  
658 Neither elevated nor reduced CO<sub>2</sub> affects the photophysiological performance of the marine  
659 Antarctic diatom *Chaetoceros brevis*. *J. Exp. Mar. Biol. Ecol.* 406, 38-45.

660 Bowler, C., Allen, A.E., Badger, J.H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C.,  
661 Maumus, F., Otiillar, R.P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde,  
662 M., Katinka, M., Mock, T., Valentin, K., Verret, F., Berges, J.A., Brownlee, C., Cadoret, J.P.,  
663 Chiovitti, A., Choi, C.J., Coesel, S., De Martino, A., Detter, J.C., Durkin, C., Falciatore, A., Fournet,  
664 J., Haruta, M., Huysman, M.J.J., Jenkins, B.D., Jiroutova, K., Jorgensen, R.E., Joubert, Y., Kaplan,  
665 A., Kroger, N., Kroth, P.G., La Roche, J., Lindquist, E., Lommer, M., Martin-Jezequel, V., Lopez,  
666 P.J., Lucas, S., Mangogna, M., Mcginnis, K., Medlin, L.K., Montsant, A., Oudot-Le Secq, M.P.,  
667 Napoli, C., Obornik, M., Parker, M.S., Petit, J.L., Porcel, B.M., Poulsen, N., Robison, M.,  
668 Rychlewski, L., Rynearson, T.A., Schmutz, J., Shapiro, H., Saut, M., Stanley, M., Sussman, M.R.,  
669 Taylor, A.R., Vardi, A., Von Dassow, P., Vyverman, W., Willis, A., Wyrwicz, L.S., Rokhsar, D.S.,  
670 Weissenbach, J., Armbrust, E.V., Green, B.R., Van De Peer, Y., and Grigoriev, I.V. (2008). The  
671 *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* 456, 239-  
672 244.

673 Brembu, T., Winge, P., Tooming-Klunderud, A., Nederbragt, A.J., Jakobsen, K.S., and Bones, A.M.  
674 (2014). The chloroplast genome of the diatom *Seminavis robusta*: New features introduced  
675 through multiple mechanisms of horizontal gene transfer. *Mar. Genom.* 16, 17-27.

676 Buchanan, B.B. (2017). The path to thioredoxin and redox regulation beyond chloroplasts. *Plant Cell*  
677 *Physiol.* 58, 1826-1832.



678 Buchanan, B.B., Lara, C., Delatorre, A., Jacquot, J.P., and Nishizawa, A.N. (1980). Thioredoxin and  
679 enzyme regulation. *Federation Proceedings* 39, 1973-1973.

680 Burkhardt, S., Amoroso, G., Riebesell, U., and Sultermeyer, D. (2001). CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> uptake in marine  
681 diatoms acclimated to different CO<sub>2</sub> concentrations. *Limnol. Oceanogr.* 46, 1378-1391.

682 Cardol, P. (2011). Mitochondrial NADH:ubiquinone oxidoreductase (complex I) in eukaryotes: a highly  
683 conserved subunit composition highlighted by mining of protein databases. *Biochim Biophys*  
684 *Acta* 1807, 1390-1397.

685 Carreto, J.I., and Catoggio, J.A. (1976). Variations in pigment contents of the diatom *Phaeodactylum*  
686 *tricornutum* during growth. *Mar. Biol.* 36, 105-112.

687 Castruita, M., Casero, D., Karpowicz, S.J., Kropat, J., Vieler, A., Hsieh, S.I., Yan, W., Cokus, S., Loo, J.A.,  
688 Benning, C., Pellegrini, M., and Merchant, S.S. (2011). Systems biology approach in  
689 *Chlamydomonas* reveals connections between copper nutrition and multiple metabolic steps.  
690 *Plant Cell* 23, 1273-1292.

691 Cavalier-Smith, T. (2004). "Chromalveolate diversity and cell megaevolution: interplay of membranes,  
692 genomes and cytoskeleton," in *Organelles, genomes and eukaryote phylogeny: an*  
693 *evolutionary synthesis in the age of genomics*, eds. R.P. Hirt & D.S. Horner. CRC press), 75-108.

694 Cavalier-Smith, T., and Chao, E.E.Y. (2006). Phylogeny and megasystematics of phagotrophic  
695 heterokonts (kingdom Chromista). *J. Mol. Evol.* 62, 388-420.

696 Chauton, M.S., Winge, P., Brembu, T., Vadstein, O., and Bones, A.M. (2013). Gene regulation of carbon  
697 fixation, storage, and utilization in the diatom *Phaeodactylum tricornutum* acclimated to  
698 light/dark cycles. *Plant Physiol.* 161, 1034-1048.

699 Chen, X.-H., Li, Y.-Y., Zhang, H., Liu, J.-L., Xie, Z.-X., Lin, L., and Wang, D.-Z. (2018). Quantitative  
700 proteomics reveals common and specific responses of a marine diatom *Thalassiosira*  
701 *pseudonana* to different macronutrient deficiencies. *Front. Microbiol.* 9.

702 Chen, Z., Yang, M.K., Li, C.Y., Wang, Y., Zhang, J., Wang, D.B., Zhang, X.E., and Ge, F. (2014).  
703 Phosphoproteomic analysis provides novel insights into stress responses in *Phaeodactylum*  
704 *tricornutum*, a model diatom. *J. Proteome. Res.* 13, 2511-2523.

705 Clement, R., Jensen, E., Prioretti, L., Maberly, S.C., and Gontero, B. (2017a). Diversity of CO<sub>2</sub>-  
706 concentrating mechanisms and responses to CO<sub>2</sub> concentration in marine and freshwater  
707 diatoms. *J. Exp. Bot.* 68, 3925-3935.

708 Clement, R., Lignon, S., Mansuelle, P., Jensen, E., Pophillat, M., Lebrun, R., Denis, Y., Puppo, C., Maberly,  
709 S.C., and Gontero, B. (2017b). Responses of the marine diatom *Thalassiosira pseudonana* to  
710 changes in CO<sub>2</sub> concentration: a proteomic approach. *Sci. Rep.* 7.

711 Colman, B., and Rotatore, C. (1988). Uptake and accumulation of inorganic carbon by a fresh-water  
712 diatom. *J. Exp. Bot.* 39, 1025-1032.

713 Colman, B., and Rotatore, C. (1995). Photosynthetic inorganic carbon uptake and accumulation in two  
714 marine diatoms. *Plant Cell Environ.* 18, 919-924.

715 Daboussi, F., Leduc, S., Marechal, A., Dubois, G., Guyot, V., Perez-Michaut, C., Amato, A., Falciatore, A.,  
716 Juillerat, A., Beurdeley, M., Voytas, D.F., Cavarec, L., and Duchateau, P. (2014). Genome  
717 engineering empowers the diatom *Phaeodactylum tricornutum* for biotechnology. *Nat.*  
718 *Commun.* 5, 3831.

719 Del Prete, S., Nocentini, A., Supuran, C.T., and Capasso, C. (2020). Bacterial iota-carbonic anhydrase: a  
720 new active class of carbonic anhydrase identified in the genome of the Gram-negative  
721 bacterium *Burkholderia territorii*. *J Enzyme Inhib Med Chem* 35, 1060-1068.

722 Deschamps, P., and Moreira, D. (2012). Reevaluating the green contribution to diatom genomes.  
723 *Genome Biol. Evol.* 4, 795-800.

724 Dickson, A.G. (2010). "The carbon dioxide system in seawater: equilibrium chemistry and  
725 measurements," in *Guide to best practices for ocean acidification research and data reporting*,  
726 eds. U. Riebesell, V.J. Fabry, L. Hansson & J.P. Gattuso. (Luxembourg: Publications Office of  
727 the European Union), 17-40.

728 Dimario, R.J., Machingura, M.C., Waldrop, G.L., and Moroney, J.V. (2018). The many types of carbonic  
729 anhydrases in photosynthetic organisms. *Plant Sci.* 268, 11-17.

730 Dorrell, R.G., Gile, G., McCallum, G., Meheust, R., Bapteste, E.P., Klinger, C.M., Brillet-Gueguen, L.,  
731 Freeman, K.D., Richter, D.J., and Bowler, C. (2017). Chimeric origins of ochrophytes and  
732 haptophytes revealed through an ancient plastid proteome. *Elife* 6.

733 Dupont, C.L., Goepfert, T.J., Lo, P., Wei, L.P., and Ahnerz, B.A. (2004). Diurnal cycling of glutathione in  
734 marine phytoplankton: field and culture studies. *Limnol. Oceanogr.* 49, 991-996.

735 Dutkiewicz, S., Morris, J.J., Follows, M.J., Scott, J., Levitan, O., Dyhrman, S.T., and Berman-Frank, I.  
736 (2015). Impact of ocean acidification on the structure of future phytoplankton communities.  
737 *Nat. Clim. Change* 5, 1002-1006.

738 Ewe, D., Tachibana, M., Kikutani, S., Gruber, A., Bartulos, C.R., Konert, G., Kaplan, A., Matsuda, Y., and  
739 Kroth, P.G. (2018). The intracellular distribution of inorganic carbon fixing enzymes does not  
740 support the presence of a C<sub>4</sub> pathway in the diatom *Phaeodactylum tricorutum*. *Photosynth.*  
741 *Res.* 137, 263-280.

742 Fabris, M., Matthijs, M., Rombauts, S., Vyverman, W., Goossens, A., and Baart, G.J.E. (2012). The  
743 metabolic blueprint of *Phaeodactylum tricorutum* reveals a eukaryotic Entner-Doudoroff  
744 glycolytic pathway. *Plant J.* 70, 1004-1014.

745 Falciatore, A., Jaubert, M., Bouly, J.-P., Bailleul, B., and Mock, T. (2020). Diatom molecular research  
746 comes of age: model species for studying phytoplankton biology and diversity. *Plant Cell* 32,  
747 547-572.

748 Falkowski, P.G., Barber, R.T., and Smetacek, V. (1998). Biogeochemical controls and feedbacks on  
749 ocean primary production. *Science* 281, 200-206.

750 Falkowski, P.G., and Owens, T.G. (1980). Light-shade adaptation - 2 strategies in marine-  
751 phytoplankton. *Plant Physiol.* 66, 592-595.

752 Fey, V., Wagner, R., Brautigam, K., and Pfannschmidt, T. (2005). Photosynthetic redox control of  
753 nuclear gene expression. *J. Exp. Bot.* 56, 1491-1498.

754 Fournier, M.L., Paulson, A., Pavelka, N., Mosley, A.L., Gaudenz, K., Bradford, W.D., Glynn, E., Li, H.,  
755 Sardu, M.E., Fleharty, B., Seidel, C., Florens, L., and Washburn, M.P. (2010). Delayed  
756 correlation of mRNA and protein expression in rapamycin-treated cells and a role for Ggc1 in  
757 cellular sensitivity to rapamycin. *Mol. Cell. Proteomics* 9, 271-284.

758 Gao, K., and Campbell, D.A. (2014). Photophysiological responses of marine diatoms to elevated CO<sub>2</sub>  
759 and decreased pH: a review. *Funct. Plant Biol.* 41, 449-459.

760 Gardemann, A., Stitt, M., and Heldt, H.W. (1983). Control of CO<sub>2</sub> fixation. Regulation of spinach  
761 ribulose-5-phosphate kinase by stromal metabolite levels. *BBA-Bioenergetics* 722, 51-60.

762 Gattuso, J.P., Magnan, A., Billé, R., Cheung, W.W.L., Howes, E.L., Joos, F., Allemand, D., Bopp, L., Cooley,  
763 S.R., Eakin, C.M., Hoegh-Guldberg, O., Kelly, R.P., Pörtner, H.O., Rogers, A.D., Baxter, J.M.,  
764 Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., Sumaila, U.R., Treyer, S., and Turley, C.  
765 (2015). Contrasting futures for ocean and society from different anthropogenic CO<sub>2</sub> emissions  
766 scenarios. *Science* 349, aac4722.

767 Gilstad, M., Johnsen, G., and Sakshaug, E. (1993). Photosynthetic parameters, pigment composition  
768 and respiration rates of the marine diatom *Skeletonema-costatum* grown in continuous light  
769 and a 12/12 h light-dark cycle. *J. Plankton Res.* 15, 939-951.

770 Giordano, M., Chen, Y.B., Koblizek, M., and Falkowski, P.G. (2005). Regulation of nitrate reductase in  
771 *Chlamydomonas reinhardtii* by the redox state of the plastoquinone pool. *Eur. J. Phycol.* 40,  
772 345-352.

773

774

775 Gontero, B., Avilan, L., and Lebreton, S. (2006). "Control of carbon fixation in chloroplasts," in *Annual*  
776 *plant reviews*, eds. W.C. Plaxton & M.T. Mcmanus.), 187-218.

777 Grabsztunowicz, M., Koskela, M.M., and Mulo, P. (2017). Post-translational modifications in regulation  
778 of chloroplast function: recent advances. *Front. Plant Sci.* 8, 1-12.

779 Green, B.R. (2011). After the primary endosymbiosis: an update on the chromalveolate hypothesis and  
780 the origins of algae with Chl *c*. *Photosynth. Res.* 107, 103-115.

781 Grouneva, I., Jakob, T., Wilhelm, C., and Goss, R. (2006). Influence of ascorbate and pH on the activity  
782 of the diatom xanthophyll cycle-enzyme diadinoxanthin de-epoxidase. *Physiol. Plantarum* 126,  
783 205-211.

784 Gruber, A., Weber, T., Bártulos, C.R., Vugrinec, S., and Kroth, P.G. (2009). Intracellular distribution of  
785 the reductive and oxidative pentose phosphate pathways in two diatoms. *J. Basic Microb.* 49,  
786 58-72.

787 Haimovich-Dayana, M., Garfinkel, N., Ewe, D., Marcus, Y., Gruber, A., Wagner, H., Kroth, P.G., and  
788 Kaplan, A. (2013). The role of C<sub>4</sub> metabolism in the marine diatom *Phaeodactylum tricornutum*.  
789 *New Phytol.* 197, 177-185.

790 Harada, H., and Matsuda, Y. (2005). Identification and characterization of a new carbonic anhydrase in  
791 the marine diatom *Phaeodactylum tricornutum*. *Can. J. Bot.* 83, 909-916.

792 Harada, H., Nakajima, K., Sakaue, K., and Matsuda, Y. (2006). CO<sub>2</sub> sensing at ocean surface mediated  
793 by cAMP in a marine diatom. *Plant Physiol.* 142, 1318-1328.

794 Harada, H., Nakatsuma, D., Ishida, M., and Matsuda, Y. (2005). Regulation of the expression of  
795 intracellular beta-carbonic anhydrase in response to CO<sub>2</sub> and light in the marine diatom  
796 *Phaeodactylum tricornutum*. *Plant Physiol.* 139, 1041-1050.

797 Harrison, P.J., Thompson, P.A., and Calderwood, G.S. (1990). Effects of nutrient and light limitation on  
798 the biochemical composition of phytoplankton. *J. Appl. Phycol.* 2, 45-56.

799 Hauser, M., Eichelmann, H., Oja, V., Heber, U., and Laisk, A. (1995). Stimulation by light of rapid pH  
800 regulation in the chloroplast stroma *in vivo* as indicated by CO<sub>2</sub> solubilization in leaves. *Plant*  
801 *Physiology* 108, 1059.

802 Hennon, G.M.M., Ashworth, J., Groussman, R.D., Berthiaume, C., Morales, R.L., Baliga, N.S., Orellana,  
803 M.V., and Armbrust, E.V. (2015). Diatom acclimation to elevated CO<sub>2</sub> via cAMP signalling and  
804 coordinated gene expression. *Nat. Clim. Change* 5, 761-765.

805 Herve, V., Derr, J., Douady, S., Quinet, M., Moisan, L., and Lopez, P.J. (2012). Multiparametric analyses  
806 reveal the pH-dependence of silicon biomineralization in diatoms. *PLoS One* 7, e46722.

807 Hesse, S.J.A., Ruijter, G.J.G., Dijkema, C., and Visser, J. (2000). Measurement of intracellular  
808 (compartmental) pH by <sup>31</sup>P NMR in *Aspergillus niger*. *J. Biotechnol.* 77 5-15.

809 Heydarizadeh, P., Boureba, W., Zahedi, M., Huang, B., Moreau, B., Lukomska, E., Couzinet-Mossion, A.,  
810 Wielgosz-Collin, G., Martin-Jezequel, V., Bougaran, G., Marchand, J., and Schoefs, B. (2017).  
811 Response of CO<sub>2</sub>-starved diatom *Phaeodactylum tricornutum* to light intensity transition.  
812 *Philos. Trans. R. Soc. B Biol. Sci.* 372, 20160396.

813 Heydarizadeh, P., Veidl, B., Huang, B., Lukomska, E., Wielgosz-Collin, G., Couzinet-Mossion, A.,  
814 Bougaran, G., Marchand, J., and Schoefs, B. (2019). Carbon orientation in the diatom  
815 *Phaeodactylum tricornutum*: the effects of carbon limitation and photon flux density. *Front.*  
816 *Plant Sci.* 10.

817 Hildebrand, M., Lerch, S.J.L., and Shrestha, R.P. (2018). Understanding diatom cell wall silicification—  
818 Moving forward. *Front. in Mar. Sci.* 5.

819 Hildebrand, M., Manandhar-Shrestha, K., and Abbriano, R. (2017). Effects of chrysolaminarin synthase  
820 knockdown in the diatom *Thalassiosira pseudonana*: Implications of reduced carbohydrate  
821 storage relative to green algae. *Algal Res.* 23, 66-77.

822 Hitchcock, G.L. (1980). Diel variation in chlorophyll *a*, carbohydrate and protein content of the marine  
823 diatom *Skeletonema costatum*. *Mar. Biol.* 57, 271-278.

824 Hopes, A., Nekrasov, V., Kamoun, S., and Mock, T. (2016). Editing of the urease gene by CRISPR-Cas in  
825 the diatom *Thalassiosira pseudonana*. *Plant Methods* 12, 49.

826 Hopkinson, B.M., Dupont, C.L., Allen, A.E., and Morel, F.M.M. (2011). Efficiency of the CO<sub>2</sub>-  
827 concentrating mechanism of diatoms. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3830-3837.

828 Hopkinson, B.M., Dupont, C.L., and Matsuda, Y. (2016). The physiology and genetics of CO<sub>2</sub>  
829 concentrating mechanisms in model diatoms. *Curr. Opin. Plant Biol.* 31, 51-57.

830 Huang, W., and Daboussi, F. (2017). Genetic and metabolic engineering in diatoms. *Philos. Trans. R.*  
831 *Soc. B Biol. Sci.* 372, 20160411.

832 Huang, W., Haferkamp, I., Lepetit, B., Molchanova, M., Hou, S., Jeblick, W., Río Bártulos, C., and Kroth,  
833 P.G. (2018). Reduced vacuolar  $\beta$ -1,3-glucan synthesis affects carbohydrate metabolism as well  
834 as plastid homeostasis and structure in *Phaeodactylum tricorutum*. *Proc. Natl. Acad. Sci.*  
835 *U.S.A.* 115, 4791-4796.

836 Huysman, M.J.J., Fortunato, A.E., Matthijs, M., Costa, B.S., Vanderhaeghen, R., Van Den Daele, H.,  
837 Sachse, M., Inze, D., Bowler, C., Kroth, P.G., Wilhelm, C., Falciatore, A., Vyverman, W., and De  
838 Veylder, L. (2013). AUREOCHROME1a-mediated induction of the diatom-specific cyclin dsCYC2  
839 controls the onset of cell division in diatoms (*Phaeodactylum tricorutum*). *Plant Cell* 25, 215-  
840 228.

841 IPCC (2014). Climate Change. Synthesis Report. Contribution of Working Groups I, II and III to the Fifth  
842 Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team,  
843 R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

844 Jakob, T., Goss, R., and Wilhelm, C. (2001). Unusual pH-dependence of diadinoxanthin de-epoxidase  
845 activation causes chlororespiratory induced accumulation of diatoxanthin in the diatom  
846 *Phaeodactylum tricorutum*. *J. Plant Physiol.* 158, 383-390.

847 Jensen, E., Clement, R., Maberly, S.C., and Gontero, B. (2017). Regulation of the Calvin-Benson-  
848 Bassham cycle in the enigmatic diatoms: biochemical and evolutionary variations on an original  
849 theme. *Philos. Trans. R. Soc. B Biol. Sci.* 372, 20160401.

850 Jensen, E.L., Clement, R., Kosta, A., Maberly, S.C., and Gontero, B. (2019a). A new widespread subclass  
851 of carbonic anhydrase in marine phytoplankton. *ISME J.* 13, 2094-2106.

852 Jensen, E.L., Maberly, S.C., and Gontero, B. (2020). Insights on the functions and ecophysiological  
853 relevance of the diverse carbonic anhydrases in microalgae. *Int. J. Mol. Sci.* 21, 2922.

854 Jensen, E.L., Yanguéz, K., Carriere, F., and Gontero, B. (2019b). Storage compound accumulation in  
855 diatoms as response to elevated CO<sub>2</sub> concentration. *Biology* 9, 5.

856 Keeling, P.J. (2009). Chromalveolates and the evolution of plastids by secondary endosymbiosis. *J.*  
857 *Eukaryot. Microbiol.* 56, 1-8.

858 Kikutani, S., Tanaka, R., Yamazaki, Y., Hara, S., Hisabori, T., Kroth, P.G., and Matsuda, Y. (2012). Redox  
859 regulation of carbonic anhydrases via thioredoxin in chloroplast of the marine diatom  
860 *Phaeodactylum tricorutum*. *J. Biol. Chem.* 287, 20689-20700.

861 Kim, J.-M., Lee, K., Shin, K., Kang, J.-H., Lee, H.-W., Kim, M., Jang, P.-G., and Jang, M.-C. (2006). The  
862 effect of seawater CO<sub>2</sub> concentration on growth of a natural phytoplankton assemblage in a  
863 controlled mesocosm experiment. *Limnol. Oceanogr.* 51, 1629-1636.

864 Knesting, J., and Scheibe, R. (2018). Small molecules govern thiol redox switches. *Trends Plant Sci.* 23,  
865 769-782.

866 Kojadinovic-Sirinelli, M., Villain, A., Puppo, C., Sing, S.F., Prioretti, L., Hubert, P., Gregori, G., Zhang, Y.Z.,  
867 Sassi, J.F., Claverie, J.M., Blanc, G., and Gontero, B. (2018). Exploring the microbiome of the  
868 "star" freshwater diatom *Asterionella formosa* in a laboratory context. *Environ. Microbiol.* 20,  
869 3601-3615.

870 Kroth, P.G., Bones, A.M., Daboussi, F., Ferrante, M.I., Jaubert, M., Kolot, M., Nymark, M., Rio Bártulos,  
871 C., Ritter, A., Russo, M.T., Serif, M., Winge, P., and Falciatore, A. (2018). Genome editing in  
872 diatoms: achievements and goals. *Plant Cell. Rep.* 37, 1401-1408.

873 Kroth, P.G., Chiovitti, A., Gruber, A., Martin-Jezequel, V., Mock, T., Parker, M.S., Stanley, M.S., Kaplan,  
874 A., Caron, L., Weber, T., Maheswari, U., Armbrust, E.V., and Bowler, C. (2008). A model for  
875 carbohydrate metabolism in the diatom *Phaeodactylum tricorutum* deduced from  
876 comparative whole genome analysis. *PLoS One* 3, e1426.

877 Kuczynska, P., Jemiola-Rzeminska, M., and Strzalka, K. (2015). Photosynthetic pigments in diatoms.  
878 *Mar. Drugs* 13, 5847-5881.

879 Kustka, A.B., Milligan, A.J., Zheng, H., New, A.M., Gates, C., Bidle, K.D., and Reinfelder, J.R. (2014). Low  
880 CO<sub>2</sub> results in a rearrangement of carbon metabolism to support C<sub>4</sub> photosynthetic carbon  
881 assimilation in *Thalassiosira pseudonana*. *New Phytol.* 204, 507-520.

882 Lauritano, C., Ferrante, M.I., and Rogato, A. (2019). Marine natural products from microalgae: an -  
883 omics overview. *Mar. Drugs* 17, 269.

884 Lavaud, J., Materna, A.C., Sturm, S., Vugrinec, S., and Kroth, P.G. (2012). Silencing of the violaxanthin  
885 de-epoxidase gene in the diatom *Phaeodactylum tricornutum* reduces diatoxanthin synthesis  
886 and non-photochemical quenching. *PLoS One* 7, e36806.

887 Lin, Q., Liang, J.R., Huang, Q.Q., Luo, C.S., Anderson, D.M., Bowler, C., Chen, C.P., Li, X.S., and Gao, Y.H.  
888 (2017). Differential cellular responses associated with oxidative stress and cell fate decision  
889 under nitrate and phosphate limitations in *Thalassiosira pseudonana*: Comparative  
890 proteomics. *PLoS One* 12, e0184849.

891 Lohr, M., and Wilhelm, C. (1999). Algae displaying the diadinoxanthin cycle also possess the  
892 violaxanthin cycle. *Proc. Natl. Acad. Sci. U.S.A.* 96, 8784-8789.

893 Loisel, F.B., and Casey, J.R. (2010). "Measurement of intracellular pH," in *Membrane transporters in*  
894 *drug discovery and development: methods and protocols*, ed. Q. Yan. (Totowa, NJ: Humana  
895 Press), 311-331.

896 Maberly, S.C., Courcelle, C., Groben, R., and Gontero, B. (2010). Phylogenetically-based variation in the  
897 regulation of the Calvin cycle enzymes, phosphoribulokinase and glyceraldehyde-3-phosphate  
898 dehydrogenase, in algae. *J. Exp. Bot.* 61, 735-745.

899 Maberly, S.C., and Gontero, B. (2017). Ecological imperatives for aquatic CO<sub>2</sub>-concentrating  
900 mechanisms. *J. Exp. Bot.* 68, 3797-3814.

901 Maier, T., Güell, M., and Serrano, L. (2009). Correlation of mRNA and protein in complex biological  
902 samples. *FEBS Letters* 583, 3966-3973.

903 Mann, D.G., and Vanormelingen, P. (2013). An inordinate fondness? The number, distributions, and  
904 origins of diatom species. *J. Eukaryot. Microbiol.* 60, 414-420.

905 Marri, L., Thieulin-Pardo, G., Lebrun, R., Puppo, R., Zaffagnini, M., Trost, P., Gontero, B., and Sparla, F.  
906 (2014). CP12-mediated protection of Calvin-Benson cycle enzymes from oxidative stress.  
907 *Biochimie* 97, 228-237.

908 Matsuda, Y., Hopkinson, B.M., Nakajima, K., Dupont, C.L., and Tsuji, Y. (2017). Mechanisms of carbon  
909 dioxide acquisition and CO<sub>2</sub> sensing in marine diatoms: a gateway to carbon metabolism.  
910 *Philos. Trans. R. Soc. B Biol. Sci.* 372, 20160403.

911 Matsuda, Y., and Kroth, P.G. (2014). "Carbon fixation in diatoms," in *The structural basis of biological*  
912 *energy generation. Advances in photosynthesis and respiration (including bioenergy and*  
913 *related processes)*, ed. M.F. Hohmann-Marriott. (Dordrecht: Springer Netherlands), 335-362.

914 Matsuda, Y., Nakajima, K., and Tachibana, M. (2011). Recent progresses on the genetic basis of the  
915 regulation of CO<sub>2</sub> acquisition systems in response to CO<sub>2</sub> concentration. *Photosynth. Res.* 109,  
916 191-203.

917 Medlin, L.K. (2016). Evolution of the diatoms: major steps in their evolution and a review of the  
918 supporting molecular and morphological evidence. *Phycologia* 55, 79-103.

919 Mekhalfi, M., Avilan, L., Lebrun, R., Botbol, H., and Gontero, B. (2012). Consequences of the presence  
920 of 24-epibrassinolide, on cultures of a diatom, *Asterionella formosa*. *Biochimie* 94, 1213-1220.

921 Mekhalfi, M., Puppo, C., Avilan, L., Lebrun, R., Mansuelle, P., Maberly, S.C., and Gontero, B. (2014).  
922 Glyceraldehyde-3-phosphate dehydrogenase is regulated by ferredoxin-NADP reductase in the  
923 diatom *Asterionella formosa*. *New Phytol.* 203, 414-423.

924 Michels, A.K., Wedel, N., and Kroth, P.G. (2005). Diatom plastids possess a phosphoribulokinase with  
925 an altered regulation and no oxidative pentose phosphate pathway. *Plant Physiol.* 137, 911-  
926 920.

927 Milligan, A.J., Mioni, C.E., and Morel, F.M.M. (2009). Response of cell surface pH to pCO<sub>2</sub> and iron  
928 limitation in the marine diatom *Thalassiosira weissflogii*. *Mar. Chem.* 114, 31-36.

929 Mock, T., Otilar, R.P., Strauss, J., McMullan, M., Paajanen, P., Schmutz, J., Salamov, A., Sanges, R.,  
 930 Toseland, A., Ward, B.J., Allen, A.E., Dupont, C.L., Frickenhaus, S., Maumus, F., Veluchamy, A.,  
 931 Wu, T., Barry, K.W., Falciatore, A., Ferrante, M.I., Fortunato, A.E., Glockner, G., Gruber, A.,  
 932 Hipkin, R., Janech, M.G., Kroth, P.G., Leese, F., Lindquist, E.A., Lyon, B.R., Martin, J., Mayer, C.,  
 933 Parker, M., Quesneville, H., Raymond, J.A., Uhlig, C., Valas, R.E., Valentin, K.U., Worden, A.Z.,  
 934 Armbrust, E.V., Clark, M.D., Bowler, C., Green, B.R., Moulton, V., Van Oosterhout, C., and  
 935 Grigoriev, I.V. (2017). Evolutionary genomics of the cold-adapted diatom *Fragilariopsis*  
 936 *cylindrus*. *Nature* 541, 536-540.

937 Montrichard, F., Alkhalfioui, F., Yano, H., Vensel, W.H., Hurkman, W.J., and Buchanan, B.B. (2009).  
 938 Thioredoxin targets in plants: the first 30 years. *J. Proteomics* 72, 452-474.

939 Morel, F.M.M., Lam, P.J., and Saito, M.A. (2020). Trace metal substitution in marine phytoplankton.  
 940 *Annu. Rev. Earth Pl. Sc.* 48, null.

941 Moustafa, A., Beszteri, B., Maier, U.G., Bowler, C., Valentin, K., and Bhattacharya, D. (2009). Genomic  
 942 footprints of a cryptic plastid endosymbiosis in diatoms. *Science* 324, 1724-1726.

943 Mueller-Cajar, O., Stotz, M., Wendler, P., Hartl, F.U., Bracher, A., and Hayer-Hartl, M. (2011). Structure  
 944 and function of the AAA<sup>+</sup> protein CbbX, a red-type Rubisco activase. *Nature* 479, 194-199.

945 Muhseen, Z.T., Xiong, Q., Chen, Z., and Ge, F. (2015). Proteomics studies on stress responses in  
 946 diatoms. *Proteomics* 15, 3943-3953.

947 Nakajima, K., Tanaka, A., and Matsuda, Y. (2013). SLC4 family transporters in a marine diatom directly  
 948 pump bicarbonate from seawater. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1767-1772.

949 Nikolaev, V.O., and Lohse, M.J. (2006). Monitoring of cAMP synthesis and degradation in living cells.  
 950 *Physiology* 21, 86-92.

951 Nonoyama, T., Kazamia, E., Nawaly, H., Gao, X., Tsuji, Y., Matsuda, Y., Bowler, C., Tanaka, T., and  
 952 Dorrell, R.G. (2019). Metabolic innovations underpinning the origin and diversification of the  
 953 diatom chloroplast. *Biomolecules* 9, 322.

954 Norici, A., Bazzoni, A.M., Pugnetti, A., Raven, J.A., and Giordano, M. (2011). Impact of irradiance on the  
 955 C allocation in the coastal marine diatom *Skeletonema marinoi* Sarno and Zingone. *Plant Cell*  
 956 *Environ.* 34, 1666-1677.

957 Nymark, M., Sharma, A.K., Sparstad, T., Bones, A.M., and Winge, P. (2016). A CRISPR/Cas9 system  
 958 adapted for gene editing in marine algae. *Sci. Rep.* 6, 24951.

959 Ogura, A., Akizuki, Y., Imoda, H., Mineta, K., Gojobori, T., and Nagai, S. (2018). Comparative genome  
 960 and transcriptome analysis of diatom, *Skeletonema costatum*, reveals evolution of genes for  
 961 harmful algal bloom. *BMC Genomics* 19, 765.

962 Ohno, N., Inoue, T., Yamashiki, R., Nakajima, K., Kitahara, Y., Ishibashi, M., and Matsuda, Y. (2012). CO<sub>2</sub>-  
 963 cAMP-responsive cis-elements targeted by a transcription factor with CREB/ATF-like basic  
 964 zipper domain in the marine diatom *Phaeodactylum tricornutum*. *Plant Physiol.* 158, 499-513.

965 Oudot-Le Secq, M.P., Grimwood, J., Shapiro, H., Armbrust, E.V., Bowler, C., and Green, B.R. (2007).  
 966 Chloroplast genomes of the diatoms *Phaeodactylum tricornutum* and *Thalassiosira*  
 967 *pseudonana*: comparison with other plastid genomes of the red lineage. *Mol. Genet. Genomics*  
 968 277, 427-439.

969 Perez-Perez, M.E., Mauries, A., Maes, A., Tourasse, N.J., Hamon, M., Lemaire, S.D., and Marchand, C.H.  
 970 (2017). The deep thioredoxome in *Chlamydomonas reinhardtii*: new insights into redox  
 971 regulation. *Mol. Plant* 10, 1107-1125.

972 Ponnala, L., Wang, Y., Sun, Q., and Van Wijk, K.J. (2014). Correlation of mRNA and protein abundance  
 973 in the developing maize leaf. *Plant Journal* 78, 424-440.

974 Portis, A.R., and Heldt, H.W. (1976). Light-dependent changes of Mg<sup>2+</sup> concentration in stroma in  
 975 relation to Mg<sup>2+</sup> dependency of CO<sub>2</sub> fixation in intact chloroplasts. *BBA-Bioenergetics* 449, 434-  
 976 446.

977 Prabakaran, S., Lippens, G., Steen, H., and Gunawardena, J. (2012). Post-translational modification:  
 978 nature's escape from genetic imprisonment and the basis for dynamic information encoding.  
 979 *Wires Syst. Biol. Med.* 4, 565-583.

980 Prosser, G.A., Larrouy-Maumus, G., and De Carvalho, L.P. (2014). Metabolomic strategies for the  
981 identification of new enzyme functions and metabolic pathways. *EMBO Rep.* 15, 657-669.

982 Pupillo, P., and Giulianipiccoli, G. (1975). The reversible depolymerization of spinach chloroplast  
983 glyceraldehyde-phosphate dehydrogenase-interaction with nucleotides and dithiothreitol.  
984 *Eur. J. Biochem.* 51, 475-482.

985 Raven, J., Brown, K., Mackay, M., Beardall, J., Giordano, M., Granum, E., Leegood, R., Kilminster, K.,  
986 and Walker, D. (2005). "Iron, nitrogen, phosphorus and zinc cycling and consequences for  
987 primary productivity in the oceans," in *Micro-organisms and earth systems-advances in  
988 geomicrobiology*, eds. G. Gadd, K. Semple & H. Lappin-Scott. Cambridge University Press,  
989 Cambridge, UK), 247-272.

990 Raven, J.A. (1994). Carbon fixation and carbon availability in marine phytoplankton. *Photosynth. Res.*  
991 39, 259-273.

992 Reinfelder, J.R. (2011). "Carbon concentrating mechanisms in eukaryotic marine phytoplankton," in  
993 *Annu. Rev. Mar. Sci.*, eds. C.A. Carlson & S.J. Giovannoni.), 291-315.

994 Reinfelder, J.R., Kraepiel, A.M.L., and Morel, F.M.M. (2000). Unicellular C<sub>4</sub> photosynthesis in a marine  
995 diatom. *Nature* 407, 996-999.

996 Riebesell, U., Wolfgladrow, D.A., and Smetacek, V. (1993). Carbon dioxide limitation of marine  
997 phytoplankton growth rates. *Nature* 361, 249-251.

998 Rosenwasser, S., Graff Van Creveld, S., Schatz, D., Malitsky, S., Tzfadia, O., Aharoni, A., Levin, Y.,  
999 Gabashvili, A., Feldmesser, E., and Vardi, A. (2014). Mapping the diatom redox-sensitive  
1000 proteome provides insight into response to nitrogen stress in the marine environment. *Proc.*  
1001 *Natl. Acad. Sci. U.S.A.* 111, 2740-2745.

1002 Sage, R.F. (2004). The evolution of C<sub>4</sub> photosynthesis. *New Phytol.* 161, 341-370.

1003 Samukawa, M., Shen, C., Hopkinson, B.M., and Matsuda, Y. (2014). Localization of putative carbonic  
1004 anhydrases in the marine diatom, *Thalassiosira pseudonana*. *Photosynth Res* 121, 235-249.

1005 Santin, Y.G., Doan, T., Lebrun, R., Espinosa, L., Journet, L., and Cascales, E. (2018). In vivo TssA proximity  
1006 labelling during type VI secretion biogenesis reveals TagA as a protein that stops and holds the  
1007 sheath. *Nat. Microbiol.* 3, 1304-1313.

1008 Satoh, D., Hiraoka, Y., Colman, B., and Matsuda, Y. (2001). Physiological and molecular biological  
1009 characterization of intracellular carbonic anhydrase from the marine diatom *Phaeodactylum*  
1010 *tricornutum*. *Plant Physiol.* 126, 1459-1470.

1011 Schoefs, B., Hu, H., and Kroth, P.G. (2017). The peculiar carbon metabolism in diatoms. *Philos. Trans.*  
1012 *R. Soc. B Biol. Sci.* 372, 20160405.

1013 Schurmann, P., and Jacquot, J.P. (2000). Plant thioredoxin systems revisited. *Annu. Rev. Plant Physiol.*  
1014 *Plant Mol. Biol.* 51, 371-400.

1015 Stein, K.C., and Frydman, J. (2019). The stop-and-go traffic regulating protein biogenesis: how  
1016 translation kinetics controls proteostasis. *J. Biol. Chem.* 294, 2076-2084.

1017 Stiller, J.W., Schreiber, J., Yue, J., Guo, H., Ding, Q., and Huang, J. (2014). The evolution of  
1018 photosynthesis in chromist algae through serial endosymbioses. *Nat. Commun.* 5, 5764.

1019 Subramanian, S., Barry, A.N., Pieris, S., and Sayre, R.T. (2013). Comparative energetics and kinetics of  
1020 autotrophic lipid and starch metabolism in chlorophytic microalgae: implications for biomass  
1021 and biofuel production. *Biotechnol. Biofuels* 6, 150.

1022 Sun, N., Ma, L.G., Pan, D.Y., Zhao, H.Y., and Deng, X.W. (2003). Evaluation of light regulatory potential  
1023 of Calvin cycle steps based on large-scale gene expression profiling data. *Plant Mol. Biol.* 53,  
1024 467-478.

1025 Tachibana, M., Allen, A.E., Kikutani, S., Endo, Y., Bowler, C., and Matsuda, Y. (2011). Localization of  
1026 putative carbonic anhydrases in two marine diatoms, *Phaeodactylum tricornutum* and  
1027 *Thalassiosira pseudonana*. *Photosynth. Res.* 109, 205-221.

1028 Tanaka, A., Ohno, N., Nakajima, K., and Matsuda, Y. (2016). Light and CO<sub>2</sub>/cAMP signal cross talk on  
1029 the promoter elements of chloroplastic beta-carbonic anhydrase genes in the marine diatom  
1030 *Phaeodactylum tricornutum*. *Plant Physiol.* 170, 1105-1116.

1031 Tanaka, T., Maeda, Y., Veluchamy, A., Tanaka, M., Abida, H., Marechal, E., Bowler, C., Muto, M.,  
1032 Sunaga, Y., Tanaka, M., Yoshino, T., Taniguchi, T., Fukuda, Y., Nemoto, M., Matsumoto, M.,  
1033 Wong, P.S., Aburatani, S., and Fujibuchi, W. (2015). Oil accumulation by the oleaginous diatom  
1034 *Fistulifera solaris* as revealed by the genome and transcriptome. *Plant Cell* 27, 162-176.

1035 Taraldsvik, M., and Myklestad, S.M. (2000). The effect of pH on growth rate, biochemical composition  
1036 and extracellular carbohydrate production of the marine diatom *Skeletonema costatum*. *Eur.*  
1037 *J. Phycol.* 35, 189-194.

1038 Thieulin-Pardo, G., Remy, T., Lignon, S., Lebrun, R., and Gontero, B. (2015). Phosphoribulokinase from  
1039 *Chlamydomonas reinhardtii*: a Benson-Calvin cycle enzyme enslaved to its cysteine residues.  
1040 *Mol. Biosyst.* 11, 1134-1145.

1041 Torstensson, A., Chierici, M., and Wulff, A. (2012). The influence of increased temperature and carbon  
1042 dioxide levels on the benthic/sea ice diatom *Navicula directa*. *Polar Biol.* 35, 205-214.

1043 Tortell, P.D. (2000). Evolutionary and ecological perspectives on carbon acquisition in phytoplankton.  
1044 *Limnol. Oceanogr.* 45, 744-750.

1045 Traller, J.C., Cokus, S.J., Lopez, D.A., Gaidarenko, O., Smith, S.R., Mccrow, J.P., Gallaher, S.D., Podell, S.,  
1046 Thompson, M., Cook, O., Morselli, M., Jaroszewicz, A., Allen, E.E., Allen, A.E., Merchant, S.S.,  
1047 Pellegrini, M., and Hildebrand, M. (2016). Genome and methylome of the oleaginous diatom  
1048 *Cyclotella cryptica* reveal genetic flexibility toward a high lipid phenotype. *Biotechnol. Biofuels*  
1049 9, 258.

1050 Tsuji, Y., Nakajima, K., and Matsuda, Y. (2017). Molecular aspects of the biophysical CO<sub>2</sub>-concentrating  
1051 mechanism and its regulation in marine diatoms. *J. Exp. Bot.* 68, 3763-3772.

1052 Valegard, K., Andralojc, P.J., Haslam, R.P., Pearce, F.G., Eriksen, G.K., Madgwick, P.J., Kristoffersen, A.K.,  
1053 Van Lun, M., Klein, U., Eilertsen, H.C., Parry, M.a.J., and Andersson, I. (2018). Structural and  
1054 functional analyses of Rubisco from arctic diatom species reveal unusual posttranslational  
1055 modifications. *J. Biol. Chem.* 293, 13033-13043.

1056 Valenzuela, J.J., Garcia De Lomana, A.L., Lee, A., Armbrust, E.V., Orellana, M.V., and Baliga, N.S. (2018).  
1057 Ocean acidification conditions increase resilience of marine diatoms. *Nat. Commun.* 9, 2328.

1058 Van Der Star, W.R., Dijkema, C., De Waard, P., Picioreanu, C., Strous, M., and Van Loosdrecht, M.C.  
1059 (2010). An intracellular pH gradient in the anammox bacterium *Kuenenia stuttgartiensis* as  
1060 evaluated by <sup>31</sup>P NMR. *Appl. Microbiol. Biot.* 86, 311-317.

1061 Villain, A., Kojadinovic, M., Puppo, C., Prioretti, L., Hubert, P., Zhang, Y., Gregori, G., Roulet, A., Roques,  
1062 C., Claverie, J.-M., Gontero, B., and Blanc, G. (2017). Complete mitochondrial genome  
1063 sequence of the freshwater diatom *Asterionella formosa*. *Mitochondrial DNA B* 2, 97-98.

1064 Weber, T., Gruber, A., and Kroth, P.G. (2009). The presence and localization of thioredoxins in diatoms,  
1065 unicellular algae of secondary endosymbiotic origin. *Mol. Plant* 2, 468-477.

1066 Wei, L., El Hajjami, M., Shen, C., You, W., Lu, Y., Li, J., Jing, X., Hu, Q., Zhou, W., Poetsch, A., and Xu, J.  
1067 (2019). Transcriptomic and proteomic responses to very low CO<sub>2</sub> suggest multiple carbon  
1068 concentrating mechanisms in *Nannochloropsis oceanica*. *Biotechnol. Biofuels* 12, 168.

1069 Werdan, K., and Heldt, H.W. (1973). Measurement of pH value in stroma and thylakoid space of intact  
1070 chloroplasts. *Hoppe Seylers Z. Physiol. Chem.* 354, 223-224.

1071 Werdan, K., Heldt, H.W., and Milovancev, M. (1975). The role of pH in regulation of carbon fixation in  
1072 chloroplast stroma. Studies on CO<sub>2</sub> fixation in light and dark. *BBA-Bioenergetics* 396, 276-292.

1073 Wilhelm, C., Buechel, C., Fisahn, J., Goss, R., Jakob, T., Laroche, J., Lavaud, J., Lohr, M., Riebesell, U.,  
1074 Stehfest, K., Valentin, K., and Kroth, P.G. (2006). The regulation of carbon and nutrient  
1075 assimilation in diatoms is significantly different from green algae. *Protist* 157, 91-124.

1076 Young, J.N., Heureux, A.M.C., Sharwood, R.E., Rickaby, R.E.M., Morel, F.M.M., and Whitney, S.M.  
1077 (2016). Large variation in the Rubisco kinetics of diatoms reveals diversity among their carbon-  
1078 concentrating mechanisms. *J. Exp. Bot.* 67, 3445-3456.

1079 Young, J.N., and Morel, F.M.M. (2015). The CO<sub>2</sub> switch in diatoms. *Nat. Clim. Change* 5, 722-723.

1080 Zaffagnini, M., Bedhomme, M., Groni, H., Marchand, C.H., Puppo, C., Gontero, B., Cassier-Chauvat, C.,  
1081 Decottignies, P., and Lemaire, S.D. (2012). Glutathionylation in the photosynthetic model



1082 organism *Chlamydomonas reinhardtii*: a proteomic survey. *Mol. Cell. Proteomics* 11,  
1083 M111.014142.

1084  
1085 **Figure legends**

1086  
1087 **Figure 1: Schematic of the different levels of regulation in diatoms.** Transcription  
1088 of the genome by mRNA polymerase (yellow circle) converts nuclear DNA (blue) and  
1089 chloroplast DNA (grey) into mRNA (yellow). Translation by the ribosome (purple)  
1090 converts mRNA into protein (brown). Regulation can also be modulated by enzyme  
1091 activity (blue stars), post-translational modification (green diamonds) and small  
1092 molecules such as co-factors or metabolites (green carbon skeletons). The transport  
1093 of molecules between the cytoplasm and the chloroplast is represented by a green  
1094 double-headed arrow across cylinders. ATP synthesized in the mitochondrion can be  
1095 transported into the chloroplast (brown arrow), while the reducing power (e.g. NADPH,  
1096 represented by e<sup>-</sup>) of the chloroplast is transported to the mitochondrion (red arrow)  
1097 (Bailleul et al., 2015). PPS: Periplasmic Space, CER: Chloroplast Endoplasmic  
1098 Reticulum, PPC: Periplastidial Compartment, Mit: Mitochondrion, Chlp: Chloroplast,  
1099 Cyt: Cytoplasm, PTM: Post-Translational Modification, Nuc: Nucleus.

1100  
1101 **Figure 2: Regulation of pathways by light and dark.** This schematic includes  
1102 regulatory pathways from *P. tricornutum* (Chauton et al., 2013; Bai et al., 2016), and *T.*  
1103 *pseudonana* (Ashworth et al., 2013). Where up-regulation of the mRNA transcription  
1104 preceded the start of the photoperiod, we have represented it as being higher in the  
1105 light, this is the case for RuBisCO expression in *T. pseudonana* (Ashworth et al., 2013).  
1106 Glycolysis and proteolysis pathways are up-regulated in the light in *T. pseudonana*,  
1107 but down-regulated in *P. tricornutum*. In contrast, fatty acid synthesis is down-regulated  
1108 in *T. pseudonana* but up-regulated in *P. tricornutum*. Fatty acid content increased after  
1109 3 h of light in *P. tricornutum* (Chauton et al., 2013), we have thus represented the  
1110 molecule as being higher in the light. FBPase gene expression is not regulated, but its  
1111 activity is redox-regulated (Michels et al., 2005; Mekhalfi et al., 2012). Redox regulation  
1112 of enzyme activity has been shown on the isolated proteins *in vitro*, and by analogy  
1113 with the Viridiplantae lineage, this could be related to light-to-dark transitions. The  
1114 transport of molecules between the cytoplasm and the chloroplast is represented by a  
1115 green double-headed arrow across cylinders. CL: Chrysolaminarin, FA: Fatty Acid,  
1116 GSH: Glutathione, a-tRNA: aminoacyl-transfer RNA, CA: Carbonic Anhydrase, NC  
1117 synt.: Nucleotide synthesis, cell div.: Cell division, DNA replic.: DNA replication, PPS:  
1118 Periplasmic Space, CER: Chloroplast Endoplasmic Reticulum, PPC: PeriPlastidial  
1119 Compartment, Mit: Mitochondrion, Chlp: Chloroplast, Cyt: Cytoplasm, Nuc: Nucleus.

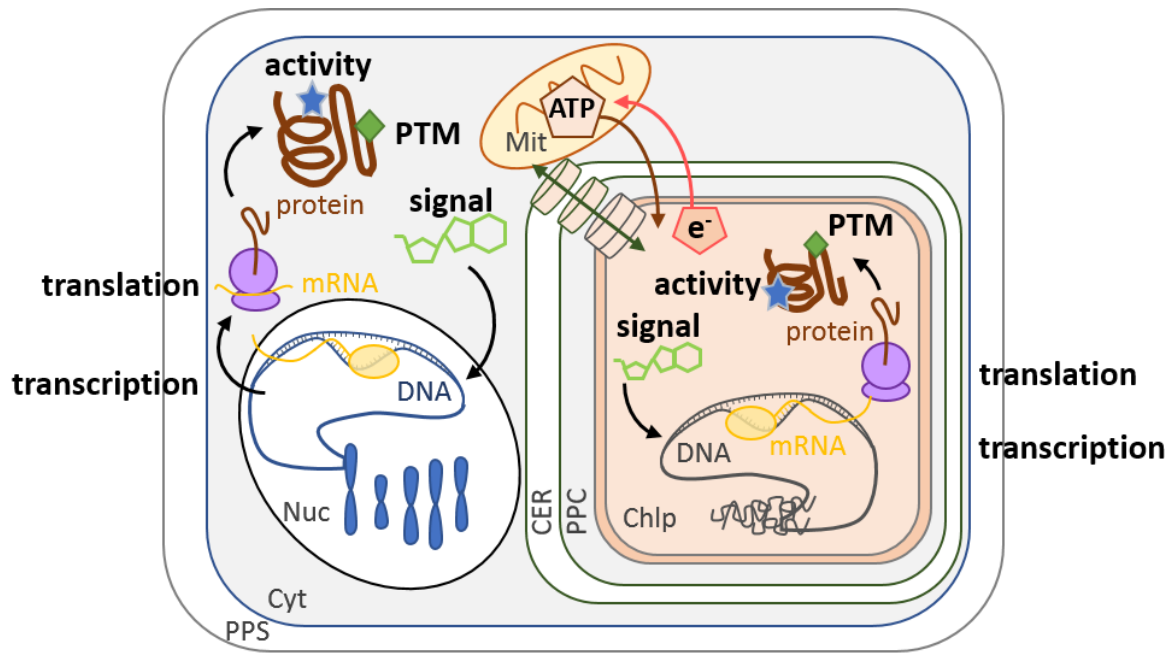
1120  
1121 **Figure 3: Location of the CCM components in different diatom species.** The  
1122 carbonic anhydrases (circles) and bicarbonate transporters (arrows) are shown for:  
1123 *N. oceanica* (Wei et al., 2019) (magenta), *T. pseudonana* (Samukawa et al.,  
1124 2014; Jensen et al., 2020) (purple) and *P. tricornutum* (Jensen et al., 2020) (green).  
1125 The putative C4-CCM components proposed for *N. oceanica* (Wei et al., 2019) and  
1126 *C. weissflogii* (Ohno et al., 2012; Reinfelder et al., 2000; Hopkinson et al.,  
1127 2016; Tanaka et al., 2016). The transport of molecules between the cytoplasm and

1128 the chloroplast is represented by a green double-headed arrow across cylinders.  
1129 Redox regulation of CA activity has been shown on the isolated *P. tricornutum*  $\beta$ -CA  
1130 *in-vitro*. PEPC: Phosphoenolpyruvate Carboxylase, PEPCK: Phosphoenolpyruvate  
1131 Carboxykinase, CA: Carbonic Anhydrase, PPS: Periplasmic Space, CER:  
1132 Chloroplast Endoplasmic Reticulum, PPC: PeriPlastidial Compartment, Mit:  
1133 Mitochondrion, Chlp: Chloroplast, Cyt: Cytoplasm, Nuc: Nucleus. Figure adapted  
1134 from Jensen *et al.* (Jensen et al., 2020).

1135

1136 **Figure 4: Regulation of pathways by [CO<sub>2</sub>].** This schematic includes regulatory  
1137 pathways from different species, including *N. oceanica* (Wei et al., 2019) and *T.*  
1138 *pseudonana* (Clement et al., 2017b; Jensen et al., 2019b). cyclic AMP (cAMP) is a  
1139 general [CO<sub>2</sub>] signalling molecule that regulates gene expression (Hennon et al.,  
1140 2015; Ohno et al., 2012; Young and Morel, 2015). High CO<sub>2</sub> corresponds to 20 000 to  
1141 50 000 ppm but in Hennon et al (2015) it is 800 ppm and low CO<sub>2</sub> is 50 to 400 ppm.  
1142 The transport of molecules between the cytoplasm and the chloroplast is represented  
1143 by a green double-headed arrow across cylinders. NADH ubiq.: NADH-ubiquinone  
1144 oxidoreductase complex, PK: Pyruvate Kinase, pigments synth.: pigments synthesis,  
1145 CA: Carbonic Anhydrase. PPS: Periplasmic Space, CER: Chloroplast Endoplasmic  
1146 Reticulum, PPC: PeriPlastidial Compartment, Mit: Mitochondrion, Chlp: Chloroplast,  
1147 Cyt: Cytoplasm, Nuc: Nucleus.

1148

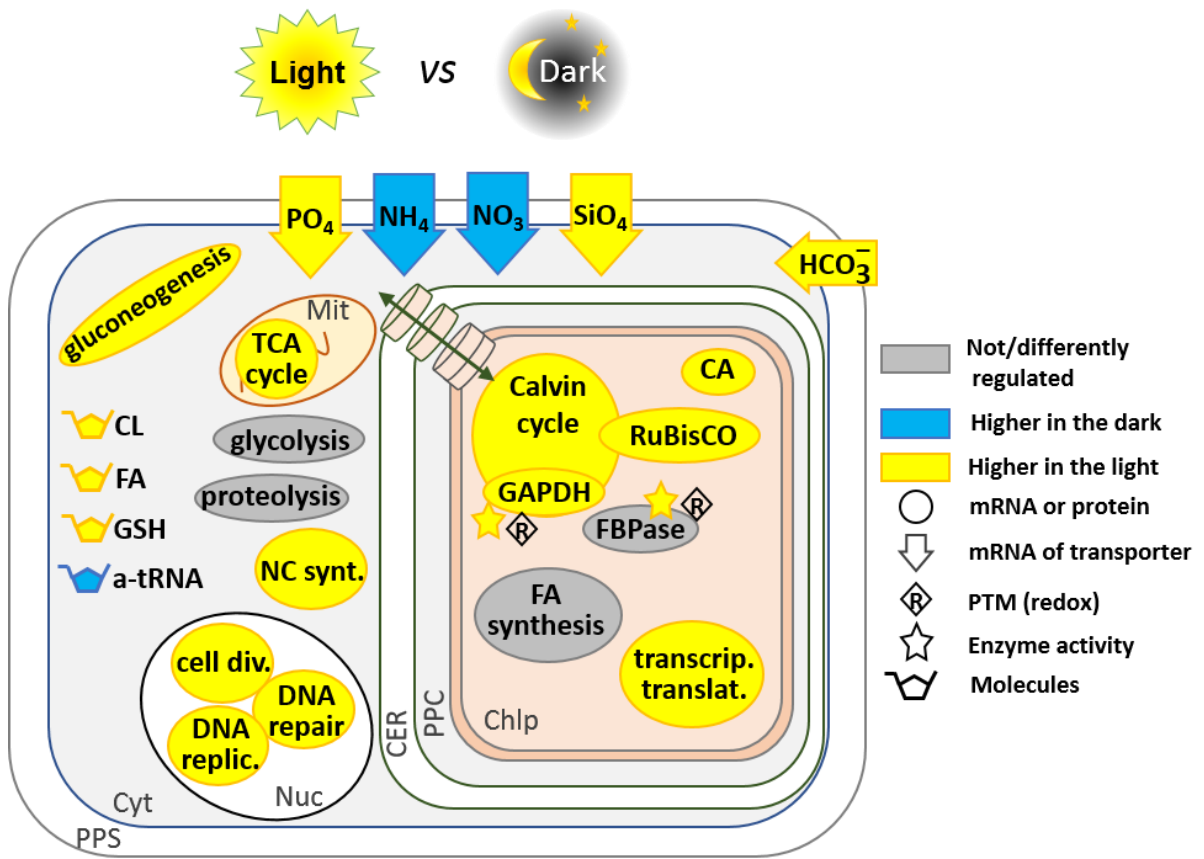


1149

1150

1151 Figure 1

1152

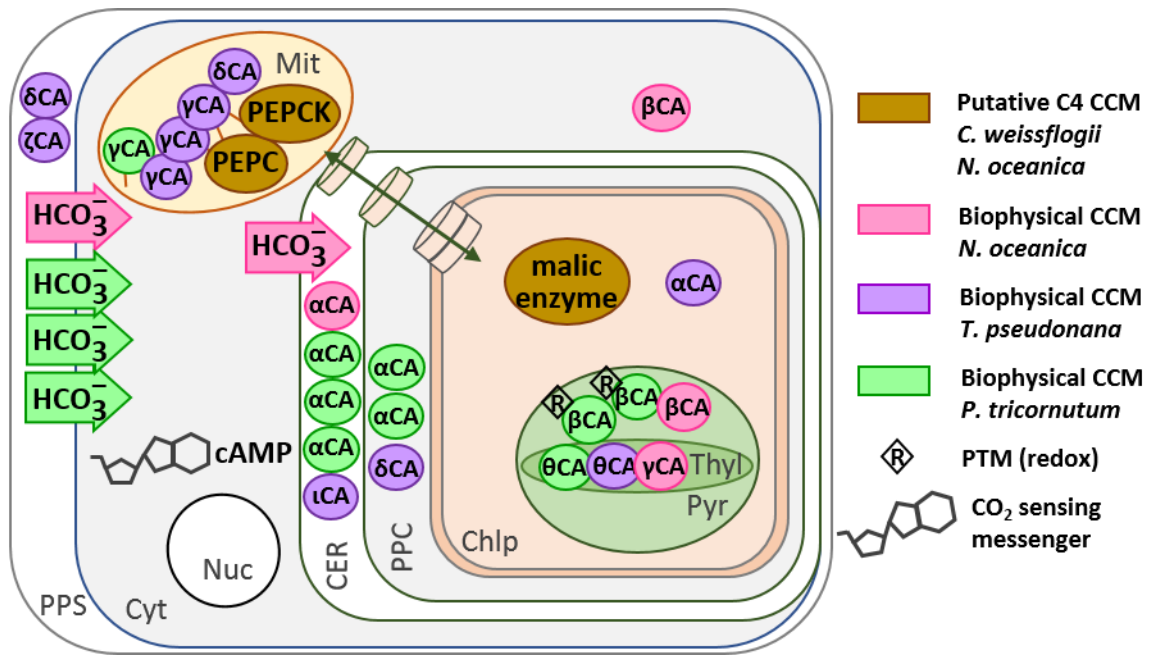


1153

1154

1155 Figure 2

1156



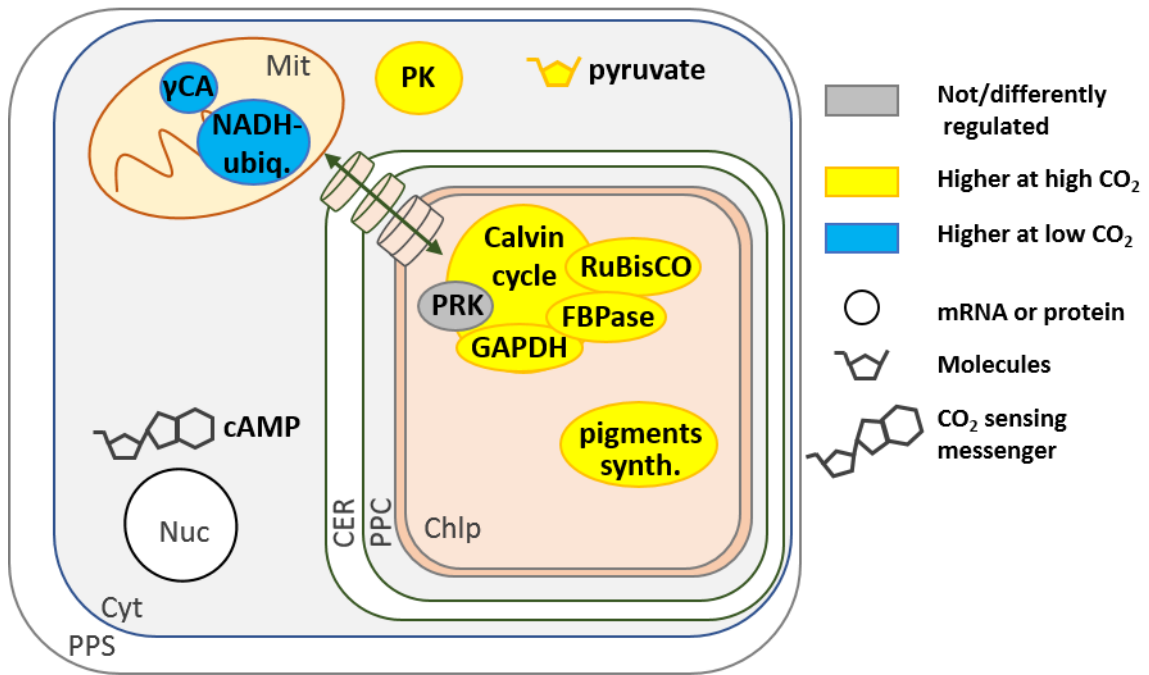
1157

1158

1159 Figure 3

1160

# High vs Low [CO<sub>2</sub>]



1161

1162

1163 Figure 4