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► **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

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

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20 **Keywords:** Calvin cycle, CO₂ 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 CO₂ 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 (β -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 CO_2 and 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 β carotene but in
90 contrast, lack the α -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, β -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 (β -1,3-linked glucan) decreased from 7.1 mg L⁻¹ at pH 6.5 to 0.2 mg
207 L⁻¹ 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⁻¹ 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 CO₂ 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-Mg²⁺-
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-Mg²⁺-
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 α -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 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 (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 CO₂**

400 Like other algae, diatoms exhibit a range of responses to varying CO₂ 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 CO₂ (Dickson, 2010) varies between 5 and 25 μM
407 depending on temperature (Raven, 1994;Tortell, 2000;Kim et al., 2006;Matsuda et al.,
408 2011;Maberly and Gontero, 2017). This CO₂ 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 CO₂-concentrating mechanisms (CCMs) (Hopkinson et al., 2011) that
412 are facultative and increase the concentration of CO₂ 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 CO₂ concentration (Harada et al., 2006).
417 However, the major determinant of the extent of CCM expression in *P. tricornutum* is
418 CO₂ concentration, as it is in green algae (Matsuda and Kroth, 2014) and many
419 components of CCM are suppressed under elevated CO₂ concentrations and induced
420 at atmospheric levels or lower. The CO₂ 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 CO₂ 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 CO₂-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 CO₂, 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 CO₂ (50 000 ppm) to low CO₂

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 CO₂ in the vicinity of RuBisCO (Figure 3). Transcripts levels
442 of some C4 enzymes were not altered by CO₂ concentration and do not suggest a
443 classic C4 metabolism, but activities of PEPC and malic enzyme increased under low
444 CO₂ (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 β -CAs PtCA1 and 2,
450 responded to CO₂ (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 CO₂ 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 CO₂ via the level of the
456 second messenger cAMP (Ohno et al., 2012; Tanaka et al., 2016). The sensing of CO₂
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 CO₂
460 concentration was strongly light-dependent, such that either CO₂ or the absence of
461 light can down-regulate the promoter. In contrast, CO₂ 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 (α -CA, ζ -CA and δ -CA respectively) were induced by decreasing CO₂,
467 and function in CO₂-limited environments (Tachibana et al., 2011). Similarly, in *C.*
468 *weissflogii*, both CO₂ and HCO₃⁻ uptake increased in response to a CO₂ 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 CO₂ (50 ppm) revealed a new uncharacterized protein, later called LCIP63, for “low-
472 CO₂-inducible protein of 63 kDa” that was up-regulated under low CO₂ (50 ppm) or at
473 atmospheric CO₂ (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 CO₂
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 Mn²⁺ as a co-factor instead of the more
478 common divalent cation Zn²⁺ (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 Zn²⁺ instead
482 of Mn²⁺ (Del Prete et al., 2020) indicating that the use of Mn²⁺ 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 CO₂ (50 000 ppm) and low CO₂ (100 ppm); three of the CA
486 transcripts were up-regulated (β -CA-2, β -CA-4 and α -CA-5) under low CO₂ (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 CO₂, indicating
489 an active biophysical CCM in this organism. Similarly, in *P. tricornutum*, beside the
490 regulation of numerous CAs by CO₂, three out of seven SLC4 genes were induced by
491 low CO₂ 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 HCO₃⁻ were also more abundant at low CO₂ 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 CO₂ emission scenarios, atmospheric levels of CO₂ are
499 likely to reach 800 ppm by 2100 (IPCC, 2014; Gattuso et al., 2015). This relates to a
500 temperature-dependent dissolved CO₂ concentration at the ocean surface of 25 to 50
501 μ M. At this concentration, cAMP plays a crucial role in down-regulating CCM genes in
502 *T. pseudonana*, in particular those encoding the chloroplastic δ 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 CO₂-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 β -CA gene upon increased CO₂ (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 CO₂ (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 CO₂ 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 CO₂
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 CO₂ 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 [CO₂] (100 ppm),
522 the genes coding for these two mitochondrial enzymes were up-regulated (Figure 4).
523 Since they can facilitate transport of CO₂ 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 CO₂ 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 CO₂ and towards carbon capture at low CO₂. An
537 increase in δ -CA gene expression, to capture as much CO₂ 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 CO₂ (20 000 ppm) vs
545 atmospheric CO₂ (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 CO₂. 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 (³¹P) 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

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612

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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⁻) 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.

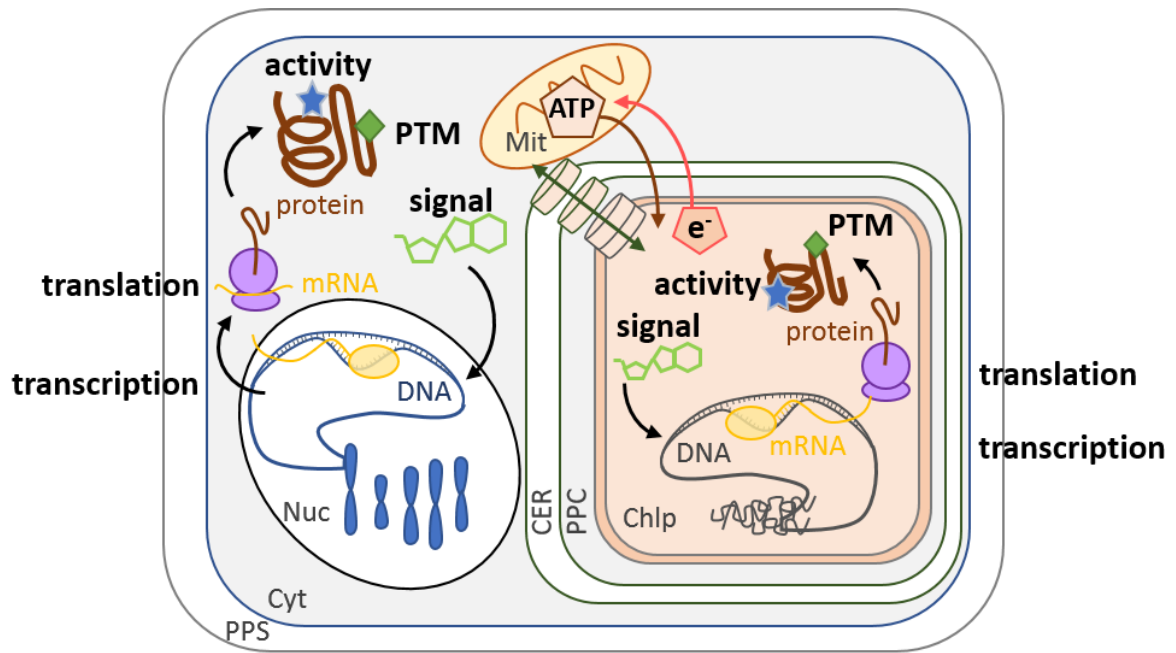
1120
1121 **Figure 3: Location of the CCM components in different diatom species.** The
1122 carbonic anhydrases (circles) and bicarbonate transporters (arrows) are shown for:
1123 *N. oceanica* (Wei et al., 2019) (magenta), *T. pseudonana* (Samukawa et al.,
1124 2014; Jensen et al., 2020) (purple) and *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* β -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 [CO₂].** 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 [CO₂] signalling molecule that regulates gene expression (Hennon et al.,
1140 2015; Ohno et al., 2012; Young and Morel, 2015). High CO₂ corresponds to 20 000 to
1141 50 000 ppm but in Hennon et al (2015) it is 800 ppm and low CO₂ 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.

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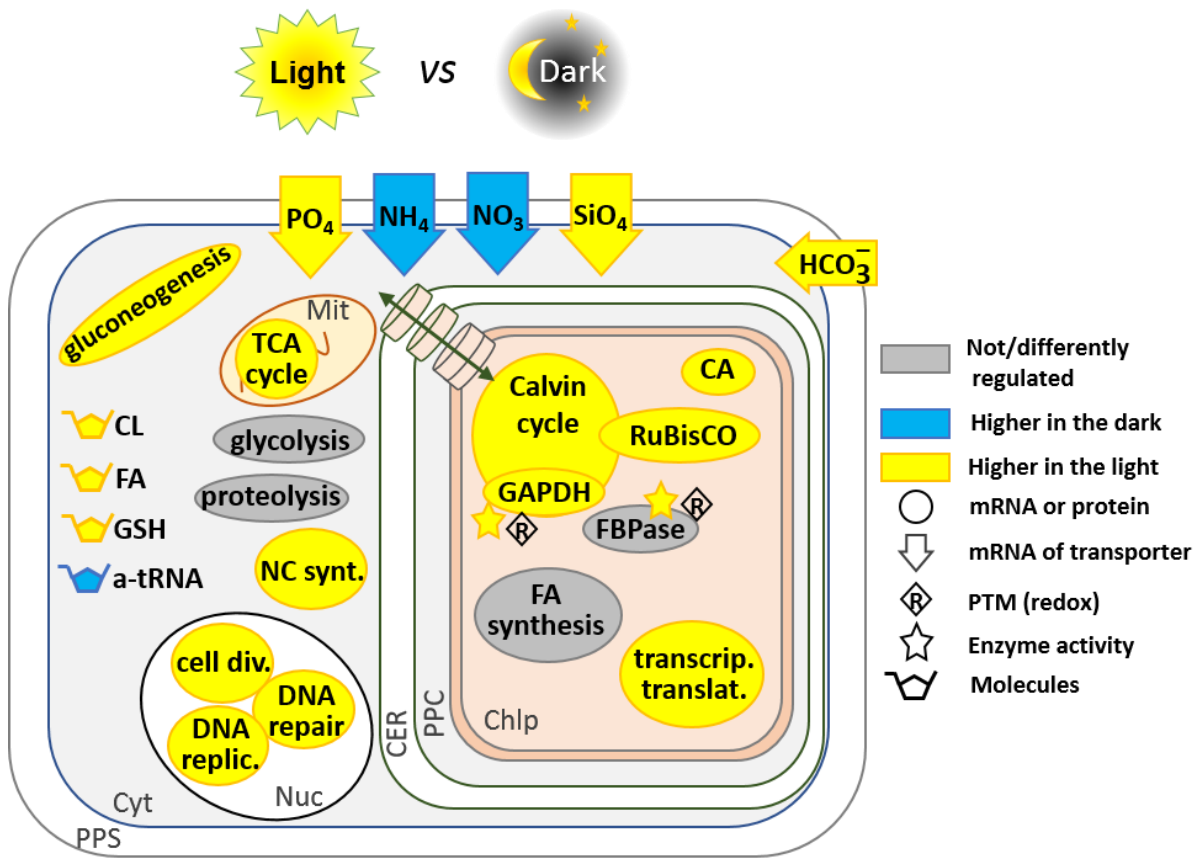


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1151 Figure 1

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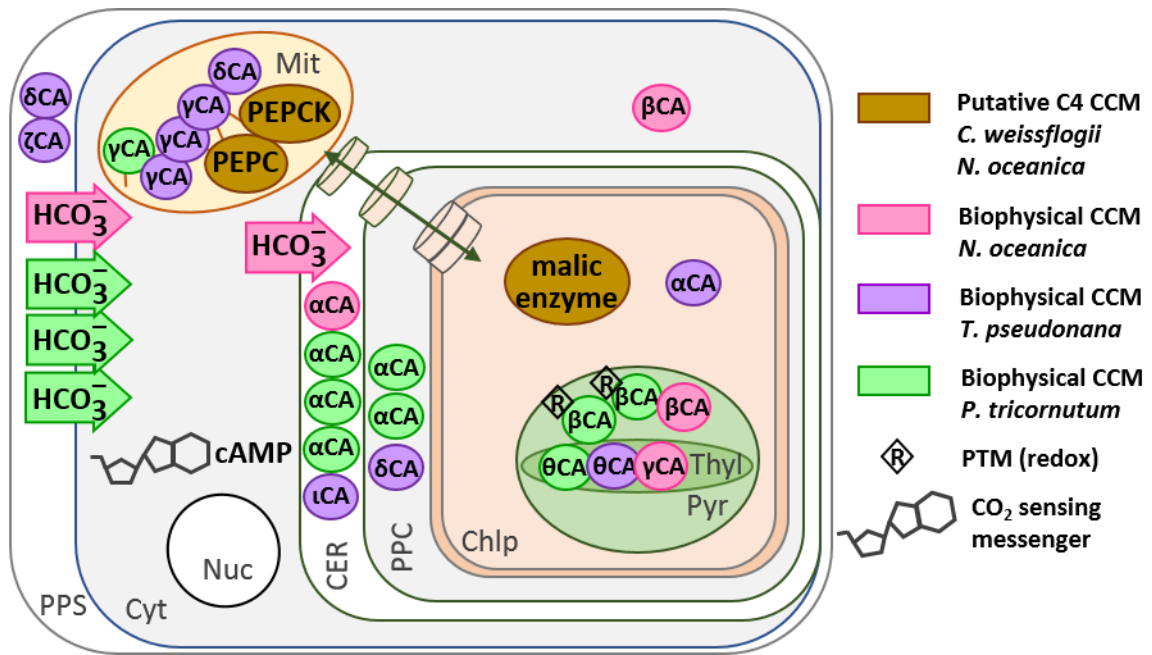


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1155 Figure 2

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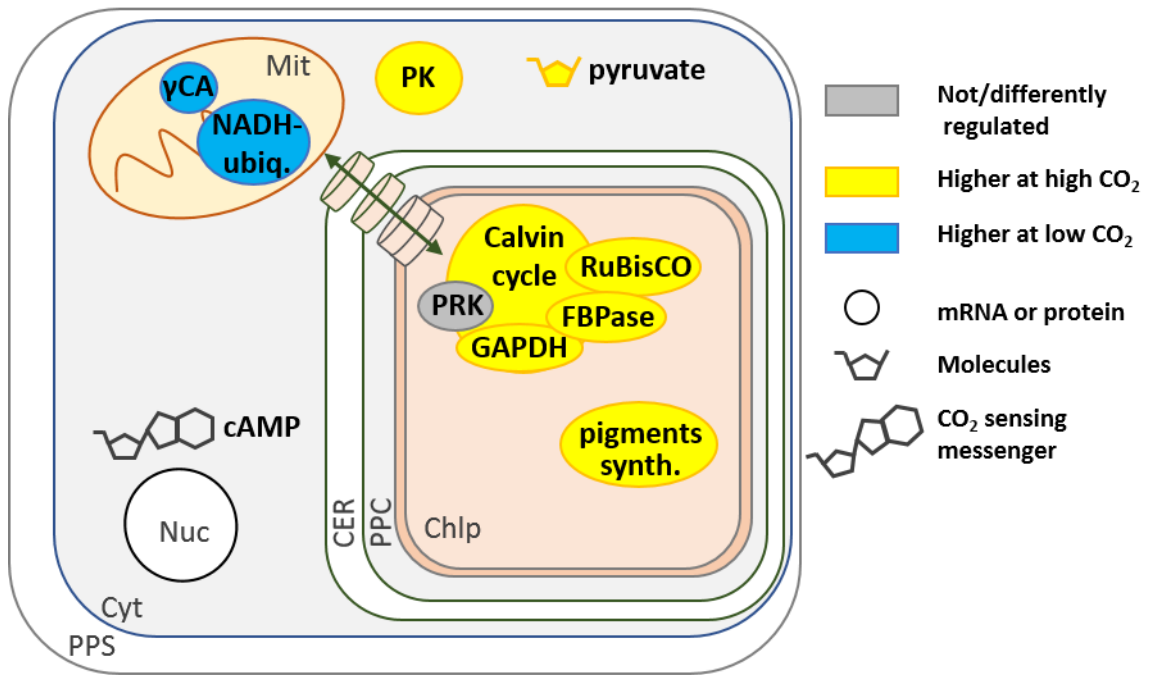
1157

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1159 Figure 3

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High vs Low [CO₂]



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1162

1163 Figure 4