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Regulation of the Calvin–Benson–Bassham cycle in the enigmatic diatoms: biochemical and evolutionary variations on an original theme

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In Plantae, the Calvin–Benson–Bassham (CBB) cycle is highly regulated and most of its enzymes have been thoroughly studied. Since diatoms arose as a result of secondary endosymbiosis with one or more Plantae ancestors, their precise evolutionary history is enigmatic and complex resulting in biochemical variations on the original CBB cycle theme. The Rubisco Michaelis constant for CO_2 is higher in diatoms than land plants and the nuclear-encoded Rubisco activase in Plantae is replaced by an analogous chloroplast-encoded CbbX (Calvin–Benson–Bassham protein X) in diatoms. In the CBB cycle reduction phase, phosphoglycerate kinase in diatoms is redox-regulated and similar to that in red algae; however, glyceraldehyde phosphate dehydrogenase (GAPDH) is not redox-regulated, unlike in Plantae. The phosphoribulokinase (PRK)-GAPDH-CP12 complex found in many photosynthetic organisms has not yet been found in diatoms, but a ferredoxin-NADP reductase (FNR)-GAPDH-CP12 complex has been found in one species. In the CBB cycle regeneration phase, sedoheptulose 1,7-bisphosphatase and PRK are not redox-regulated in diatoms, unlike in Plantae. Regulation at the transcriptional level seems to be important in diatoms. CBB cycle enzyme properties appear to be variable among diatoms, but this view relies on results from a few model species: a greater range of diatoms need to be studied to test this.

This article is part of the themed issue 'The peculiar carbon metabolism in diatoms'.

1. Introduction

Chloroplasts are pivotal to the most fundamental biochemical reactions upon which photosynthetic eukaryotes depend because they are the location of the Calvin–Benson–Bassham (CBB) cycle responsible for CO_2 assimilation [1]. This process uses ATP and NADPH produced in the thylakoids by the primary phase of photosynthesis, and involves 11 enzymes that catalyse 13 reactions. It comprises three main stages: a carboxylation step performed by ribulose-1,5-bisphosphate carboxylase-oxygenase; a reduction of phosphoglycerate performed by phosphoglycerate kinase (PGK) and NADP-dependent glyceraldehyde-3-phosphate dehydrogenase at the expense of ATP and NADPH; and a regeneration step that converts triose-phosphate into ribulose-1,5-bisphosphate involving a series of enzymes (from triose phosphate isomerase and fructose-1,6-bisphosphatase (FBPase) to phosphoribulokinase (PRK)).

The complexity and central role of the CBB cycle within a wider metabolic network, plus the variations in the supply of light, CO_2 and other resources, require the enzymes involved to be finely regulated. In the well-studied higher plants and green algae, the CBB cycle is active in the light and inactive in the dark and the main known regulatory mechanisms involve pH, Mg^{2+},

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redox-state, metabolites and protein–protein interactions [2,3]. One of the best known protein–protein interactions is transitory, linked to light-regulation and involves thioredoxin-mediated reversible reduction/oxidation of disulfide bonds/sulfhydryl group of cysteine residues [4]. In the light, thioredoxins (TRXs) are reduced by electrons from photosystem I and, in turn, reduce their target enzymes, while in the dark they are oxidized and then oxidize their targets. Diatoms have many different thioredoxins each encoded by a specific gene and located in different compartments, including the chloroplast; most contain the regulatory cysteine residues in the conserved motif, WCGPC [5]. Glutathionylation is another mechanism of redox regulation that can regulate CBB enzymes from photosynthetic organisms and some targets have been proposed [6], but little is known in diatoms and this is therefore not covered here. Another type of regulation found in higher plants, some algae and some cyanobacteria, involves the formation of multi-enzyme complexes that modify the regulatory and kinetic properties of the component enzymes [7,8]. Lastly, carbon fixation is also regulated at the transcriptional level [9,10].

Diatoms (Bacillariophyceae) comprise 30 000–100 000 species [11], are ecologically widespread [12] and, despite having evolved relatively recently, around 250 million years ago [13], are major players in global biogeochemical cycles [14]. They originated via a secondary endosymbiosis and their chloroplasts derive from a red algal ancestor. Their pigments are chlorophyll *a*, *c* and fucoxanthin, rather than red algal chlorophyll *a* and phycoerythrin or green algal chlorophyll *a* and *b*, and photosynthesis products are stored as chrysolaminarin and lipid rather than red algal floridean starch or green algal starch. However, they have an enigmatic evolutionary history and also contain green algal, bacterial and animal-like genes [15–17]. Diatoms have a number of 'variations' on the original Plantae (Archaeplastida) theme including: the possession of a functioning urea cycle [18] and a Entner–Doudoroff glycolytic pathway [19], and lack the oxidative pentose phosphate (OPP) pathway in the chloroplast [20,21]. Since OPP produces ribose-5-phosphate used in the synthesis of nucleotides, its absence in the chloroplast requires pyrimidine and purine nucleotides that were synthesized in the cytosol to be transported into the chloroplast [22,23]. Because of all these differences, one might expect the CBB cycle in diatoms to be regulated differently from those in the Plantae.

2. Ribulose-1,5-bisphosphate carboxylase-oxygenase and its activase

The crux of the CBB cycle is the rate-limiting step catalysed by ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), the most abundant protein on Earth [24,25]. However, it has been shown that the levels of Rubisco have little impact on the control of carbon fixation and therefore this enzyme does not necessarily constrain photosynthesis under normal conditions [26]. Rubisco catalyses the carboxylation of ribulose-1,5-bisphosphate (RuBP) by CO_2 to give two molecules of 3-phosphoglycerate (PGA). The turnover number (k_c) of Rubisco, around 5 s^{-1}, is among the lowest for any enzyme and its catalytic efficiency (k_c/K_m) and affinity for CO_2 are low [27,28]. Furthermore, depending on the relative concentration of CO_2 and O_2 at the active site, and the specificity of the enzyme, Rubisco can catalyse an oxygenation reaction that reduces the rate of net photosynthesis and produces PGA and phosphoglycolate [29] that is metabolized through the photorespiratory pathway [30].

Crystal structures [31,32] show that the active site of Rubisco is formed by the interaction of the L subunits, in a head-to-tail arrangement with the C-terminus of one monomer next to the N-terminus of a second monomer to form two active sites per dimer; however, no structural information is available for Rubisco from diatoms. The CO_2/O_2 specificity factor Ω, (also called τ) is variable and for Rubisco from land plants is in the range 80–100 [33]. The specificity seems to have increased during evolution to compensate for the gradual atmosphere decrease in CO_2, and increase in O_2 [34]. As a result, the more recently evolved higher plants and diatoms, though having different forms of Rubisco (forms 1B and 1D respectively), have, in general, higher values of Ω (around 110) than cyanobacteria (around 47) and other algae (66 for green algae) [35,36] implying positive selection of diatom and land plant Rubisco characteristics [37]. By contrast, the K_m for CO_2 of diatom Rubisco is highly variable (from 23 to 60 µM [38]) and greater than that found in land plants (around 10 µM [38]).

In aquatic systems, CO_2 diffusion is low and the CO_2 concentration is often below the relatively high $K_m[CO_2]$ of diatom Rubisco; therefore, a CO_2-concentrating mechanism (CCM) is beneficial [38–41]. Different forms of CCMs exist involving bicarbonate transport, and, contentiously, C4 metabolism [42]. In both energy-requiring processes, intra- or extracellular carbonic anhydrases (CA) are involved to maintain chemical equilibrium between CO_2 and bicarbonate. In diatoms, the location and number of CAs are variable [43] and CAs are redox-regulated by TRX in *Phaeodactylum tricornutum* [44]. To our knowledge, this has not been reported in other photoautotrophs and whether or not it occurs in other diatoms is currently unknown.

Regulation of Rubisco matches the CO_2 fixation rate to the photosynthetic electron transport rate, helping to adjust the concentration of chloroplast metabolites to the demand by photosynthesis [45]. All *bona fide* Rubisco must first be activated or carbamylated at a lysine residue (e.g. Lys-201 of the spinach enzyme [46]) by a non-substrate CO_2. Carbamate formation is favoured by alkaline pH and high concentrations of Mg^{2+} in the stroma. Not only Rubisco is regulated by these changes in Plantae, but also PRK, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the two phosphatases from the CBB cycle [3]. In plants, Rubisco has been extensively studied and can form multi-enzyme complexes with other CBB enzymes (for review see [3]). Rubisco is also regulated by Rubisco activase (RCA) [47]. RCA binds to inactive Rubisco and upon ATP hydrolysis, changes the conformation of the enzyme, thereby generating a highly active form of Rubisco. RCA thus works along with pH and Mg^{2+} to match the activity of Rubisco to the supply of resources. RCA is a member of the AAA^+ (*A*TPase *a*ssociated with diverse cellular *a*ctivities) protein superfamily [47,48]. RCA can be redox regulated at its C-terminus, which contains two cysteine residues (Cys392 and Cys411) that form a disulfide bridge under oxidized conditions, producing a conformational change that blocks ATP interaction with RCA [49]. Surprisingly, no homologue of RCA is known for red type Rubisco. Recently however, a crystal structure of a protein that plays the activase role for the red type Rubisco,

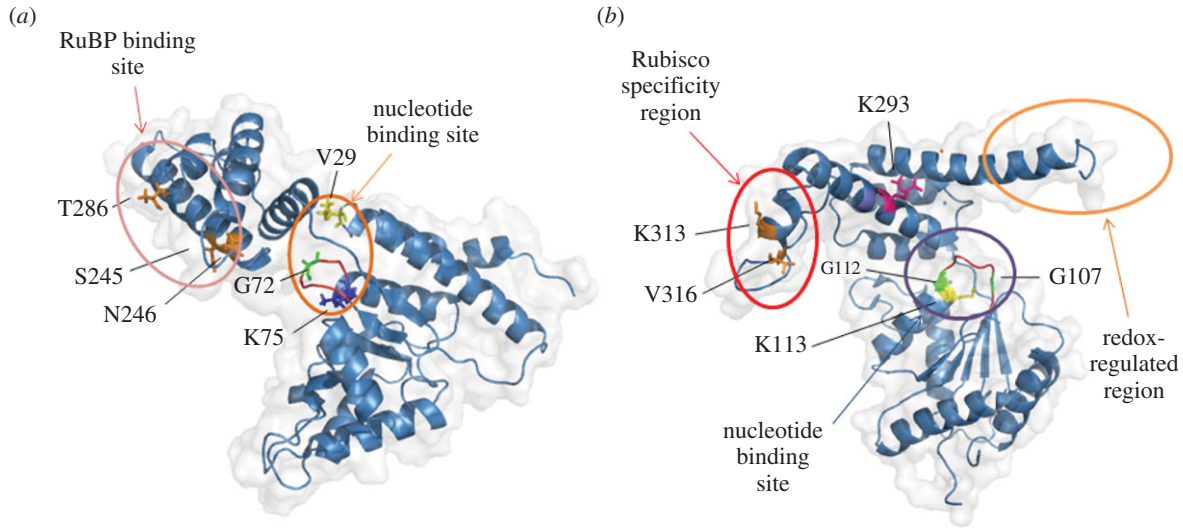

Figure 1. Comparison of CbbX and RCA. CbbX monomer from *P. tricornutum* (*a*) is compared with RCA from *A. thaliana* (PDB: 4W5 W; (*b*)). Important residues are identified and highlighted. Residues are numbered according to *P. tricornutum* and *A. thaliana* respective sequences. The 'P-loop' (Walker-A) is also shown (in red) in both models. In CbbX, key amino acids for RuBP and nucleotide binding sites were predicted according to the *R. sphaeroides* model [50,51]. Residues K313 and V316 in RCA are involved in RCA-Rubisco specificity [49,52]. Residues involved in RCA ATP binding site—K113 [52,53], G112, G107 [54]—and ATP hydrolysis—K293 [52]—are shown. A redox-regulated region (see [49]) is also identified, although part of the C-terminus is missing in the representation. *Phaeodactylum tricornutum* CbbX was modelled using SWISS MODEL webtool and *R. sphaeroides* CbbX crystal structure (PDB: 3SYL) as template. Ribbon representations were produced with PyMol software.

CbbX (Calvin–Benson–Bassham protein X), was described in *Rhodobacter sphaeroides* [50]. CbbX also shows the typical AAA^+ hexameric conformation and has functional analogy with RCA despite their low sequence homology (18%). Using BLAST software we found CbbX homologues in all the stramenopiles (more than 100) that we could check, and also showed that the CbbX gene is encoded in the chloroplast genome, unlike the RCA gene which is located in the nuclear genome. Using CbbX red-type Rubisco from *R. sphaeroides* (3ZUH.pdb) as a template, we found that the structures of CbbX in *Thalassiosira pseudonana*, *P. tricornutum* and *Asterionella formosa* were similar (data not shown). Some slight differences were found between *Arabidopsis thaliana* RCA and *P. tricornutum* CbbX that are both regulated through their C-terminus. *Phaeodactylum tricornutum*. CbbX (figure 1*a*), unlike RCA, does not have cysteine residues that could provide redox sensitivity; however, it is regulated upon RuBP binding at its C-terminus [50]. The presence of six amino acid residues within a long flexible C-terminal tail found in red type Rubisco from *R. sphaeroides*, and also present in diatoms (figure 2*a*), seems to be necessary for fully activating the CbbX ATPase activity [50]. We hypothesize that the allosteric regulation of the CbbX by RuBP found in *R. sphaeroides* will also be present in the diatoms and will regulate Rubisco.

3. Phosphoglycerate kinase

PGK is involved in several pathways, such as glycolysis and gluconeogenesis, and phosphorylates 3-phosphoglycerate (PGA) with ATP, producing 1,3-biphosphoglycerate (BPGA). Different isoforms are located in different compartments [55]. In higher plants, PGK is thought to have a cyanobacterial origin which, after subsequent gene duplication, gave rise to the chloroplastic and cytosolic enzymes [56,57]. Green algae also have a cyanobacterial-derived PGK. Previously, it was thought that green algae only had a chloroplastic form of PGK [58], but subsequently many cytosolic PGK sequences have been found in green algae [59,60]. In diatoms, the number of PGK isoenzymes and their subcellular location is also variable [22]. A comparative analysis of the sequenced genomes of the diatoms, *P. tricornutum*, *T. pseudonana* and *Fragilariopsis cylindrus* [61] showed that PGK isoforms in the cytosol, mitochondria and chloroplast are different, and that more than one isoform can occur within the same subcellular compartment. The variation in PGK isoforms among diatom species is greater in the cytosol than in the mitochondria and chloroplasts, indicating that cytosolic PGK genes were less conserved during diatom diversification. In comparison, diatom chloroplastic PGK is more conserved and more closely related to the red algae from which they derive [62].

Chloroplastic PGK can be redox-regulated by TRXs and specifically the f isoform [63]. In *Chlamydomonas reinhardtii*, and in the cyanobacterium *Synechocystis* sp. PCC6803, TRX-mediated regulation of PGK involves the formation/dissociation of a disulfide bond between Cys265 and Cys99 [63], and Cys352 and Cys378 (numbering from diatom PGK [58,64]), respectively. However, PGK might not be regulated in all green algae, land plants or cyanobacteria because some species lack critical cysteine residues. In contrast to other CBB enzymes, PGK is regulated in at least some diatoms. In *P. tricornutum*, chloroplastic PGK is a target of TRX and can be inactivated by oxidation [65]. This might be a consequence of sulfenic acid formation on the Cys80 (–SOH) and subsequent disulfide bond formation between Cys61 and Cys99. These three cysteine residues are also present in *T. pseudonana* and *F. cylindrus*, suggesting a common mechanism among diatoms, but they are absent in other stramenopiles (figure 2*b*).

4. Glyceraldehyde 3-phosphate dehydrogenase and CP12

GAPDH exists as three main forms in higher plants and algae, two cytosolic forms involved in gluconeogenesis and

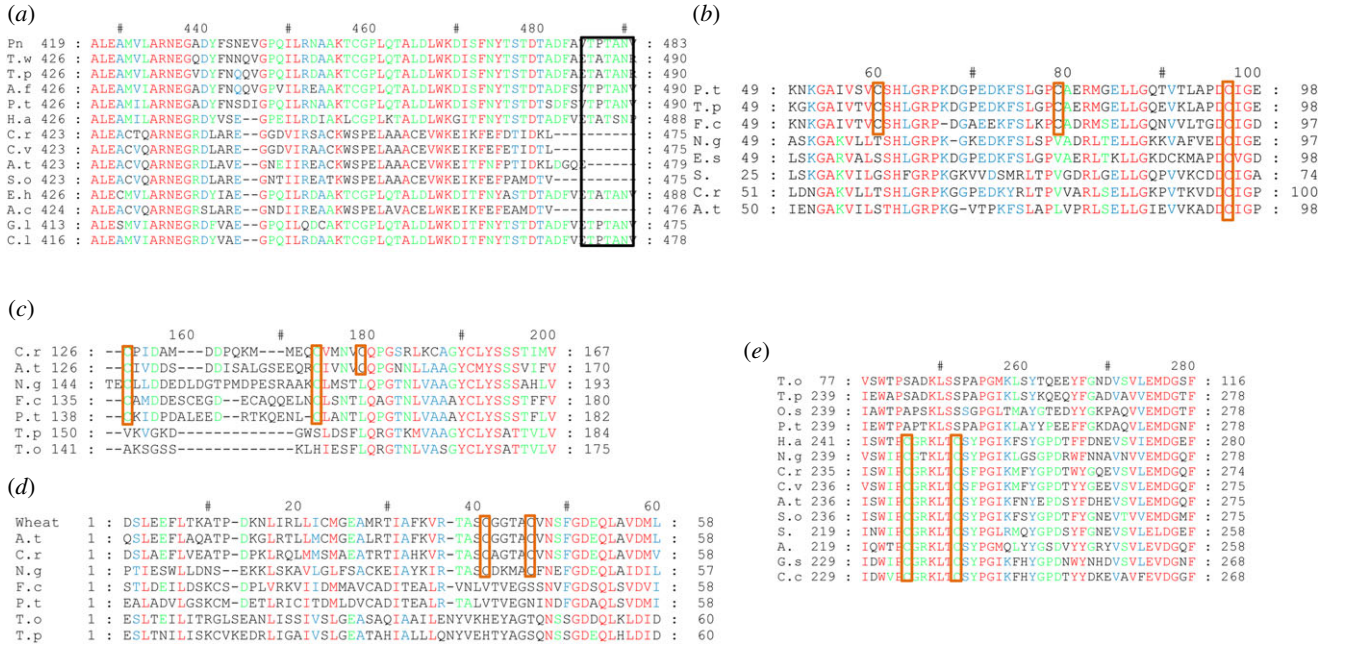

Figure 2. Alignment of Calvin cycle enzymes. (*a*) Rubisco C-terminal region alignment. Black rectangle shows C-terminal extension; (*b*) PGK; (*c*) FBPase; (*d*) SBPase; (*e*) PRK. Species used are the following: Green algae: C.r: *Chlamydomonas reinhardtii*, V.c: *Volvox carteri*, C.v: *Chlorella vulgaris*; Diatoms: Pn: *Pseudo-nitzchia* sp., T.w: *Thalassiosira weisflogii*, T.p: *Thalassiosira pseudonana*, T.o: *Thalassiosira oceanica*, P.t: *Phaeodactylum tricornutum*, A.f: *Asterionella formosa*, O.s: *Odontella sinensis*; F.c: *Fragilariopsis cylindrus*; Coccolithophores: E.h: *Emiliania huxleyi*; Other stramenopiles: H.a: *Heterosigma akashiwo*, N.g: *Nannochloropsis gaditana*; Rhodophytes: G.l: *Gloiocladia laciniata*, C.l: *Chondria littoralis*; Cyanobacteria: S.: *Synechococcus* sp. PCC 7336, S. sp: *Synechocystis* sp., A.c: *Anabaena cylindrica*, A.: *Anabaena* sp.; Land plants: A.t: *Arabidopsis thaliana*, S.o: *Spinacia oleracea*, B.n: *Brassica napus*, P.m: *Prunus mume*, S.l: *Solanum lycopersicum*. Redox-regulated cysteine residues are highlighted by an orange rectangle. Alignments were performed with ClustalW, through MEGA6 software, and processed by GenDoc software.

glycolysis, and a chloroplastic form involved in the CBB cycle. GAPDH isolated from chloroplasts can use either NAD(H) or NADP(H) to catalyse the reversible reduction and dephosphorylation of 1,3-bisphosphoglycerate (BPGA) to produce glyceraldehyde-3-phosphate and inorganic phosphate. In vascular plants, GAPDH exists either as a heterotetramer of two A subunits and two B subunits (A_2B_2), or as a homotetramer of four A subunits (A_4) [66]. In red and green algae, only the A subunit exists. The A and B subunits are very similar, except that the B subunit has a C-terminal extension of 30 amino acid residues that contains two cysteine residues homologous to the CP12 C-terminus [67]. Diatoms, like most stramenopiles, have a chloroplastic GAPDH comprising four C1 subunits, similar to the cytosolic form in all studied species [68,69] which, like the A subunit of GAPDH, does not have a CP12-homologous region at its C-terminus.

CP12 is a chloroplastic intrinsically disordered protein, involved in GAPDH redox regulation and in the formation of a GAPDH-CP12-PRK complex that has been found in land plants [70], green and red algae [71–73] and the cyanobacterium *Synechococcus elongatus* [74]. The complex forms in oxidizing conditions (i.e. during the night), with inactive enzymes, and dissociates in reducing conditions (i.e. during the day) through TRX reduction, releasing active enzymes [70,75]. CP12 sequences usually contain four cysteine residues. Two of them (Cys23 and Cys31) are important for the association of CP12 with PRK, whereas the two other cysteine residues (Cys 66 and Cys75) are important for GAPDH binding [72,76,77]. A highly conserved domain ($W_{XX}VEE_{XXXXX}H$) is also present, is located in the middle of CP12 sequence and is involved in the formation of the CP12-PRK complex, but not the CP12-GAPDH complex [78].The CP12 sequences in diatoms differ from those from land plants, cyanobacteria and green algae to the extent that it has been suggested that CP12 is absent in diatoms [17]. A protein (ID: XP_002288136.1) found in *T. pseudonana* has 35 per cent identity with *C. reinhardtii* CP12 and has the ($VAWD_XVEEL_XAA_XSHK$) sequence and the two N-terminal cysteine residues. In *P. tricornutum*, a CP12-like protein is present that has a 38% percent identity with the green algal CP12 and a TSPEARVAWDAVEEM sequence, but lacks the four cysteine residues. Interestingly, in diatoms, though the presence of the complex mentioned above has not been shown, another complex involving GAPDH, a CP12-like protein and ferredoxin-NADP reductase (FNR) has been described in the freshwater diatom *A. formosa* [79]. In this diatom, the redox regulation of GAPDH was dependent on the presence of the CP12-like protein, as when GAPDH was dissociated from this protein, the redox regulation was absent. Another regulation of GAPDH by NADP(H) occurs when GAPDH interacts with the CP12-like protein in the presence of FNR [79]. GAPDH regulation in diatoms, however, seems to vary from one species to another [80] and more work is required on this enzyme as well as on the CP12-like proteins in these organisms.

5. Fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase

FBPase and sedoheptulose-1,7-bisphosphatase (SBPase) irreversibly catalyse the dephosphorylation of fructose-1,6-bisphosphate (FBP) and sedoheptulose-1,7-bisphosphate (SBP) producing fructose-6-phosphate and sedoheptulose-7-phosphate, respectively [81,82]. SBPase is unique to the

CBB cycle, while FBPase is involved in several pathways. Chloroplastic FBPases of red and green algae, and plants are not of cyanobacterial origin but instead are believed to have evolved from cytosolic forms through gene duplication [83,84]. Similarly, because cyanobacteria lack SBPase and instead use a FBPase that can hydrolyse both FBP and SBP, it is likely that the plastid SBPase did not originate from cyanobacteria [85]. While FBPase involved in gluconeogenesis is regulated by fructose-2,6-bisphosphate and AMP, similarly to the mammalian enzyme [86], the chloroplastic FBPase, like SBPase, is regulated by light via TRXs [87]. Both phosphatases are also regulated by light-induced changes in stromal Mg^{2+} and pH [88]. Chloroplastic FBPases in Plantae bear three conserved cysteine residues at positions 155, 174 and 179 (numbered from spinach enzyme) that are responsible for redox regulation. These residues are absent in cytosolic enzymes that are consequently redox-insensitive [89]. In diatoms, the regulatory cysteine residues, Cys153, Cys174 and Cys179 (numbering from green algae and higher plants) are absent in the chloroplastic FBPases from *Thalassiosira oceanica* and *T. pseudonana*, while only Cys153 and Cys174 are present in *P. tricornutum*, *F. cylindrus* and in the eustigmatophyte *Nannochloropsis gaditana* (figure 2*c*). For *Odontella sinensis*, it has been mentioned that FBPase and SBPase are redox-regulated, but no data were presented [90] and unfortunately no sequences are available for these enzymes from this species. Whether or not FBPase is redox-regulated in diatoms requires further investigation.

SBPases from Plantae bear two regulatory cysteine residues, Cys41 and Cys46 (numbering from green algae and higher plants), and are highly redox-regulated. Based on this high regulation, on antisense RNA techniques, and on modelling studies in land plants, it seems that SBPase, even more than FBPase, plays an important role in the control of carbon flux through the CBB cycle in land plants [26]. The SBPases from diatoms lack these regulatory cysteine residues (figure 2*d*), suggesting that they are unlikely to be redox-regulated, and they are not regulated at the transcript level [91], suggesting that this enzyme plays a less strategic role in diatoms than in Plantae.

6. Phosphoribulokinase

PRK is unique to the CBB cycle, and catalyses the ATP-Mg^{2+}-dependent phosphorylation of ribulose-5-phosphate (Ru5P), thus regenerating RuBP, the Rubisco substrate. PRKs from different organisms differ in their catalytic and regulatory properties and their oligomerization state. In anoxygenic photosynthetic bacteria such as *R. sphaeroides*, PRK is octameric, inhibited by AMP, allosterically activated by NADH [92] and its structure has been solved at a resolution of 2.6 Å [93]. In some well-studied oxygenic phototrophs, PRK is dimeric and regulated by redox-sensitive cysteine residues and/or inhibitory GAPDH-CP12-PRK complex formation [73]. The activity of PRK from the diatom *O. sinensis* is not affected by light or dark treatment [90]. Moreover, PRK from the freshwater diatom *A. formosa* is insensitive to a reducing agent, dithiothreitol, that mimicks *in vitro* the action of TRX *in vivo* [94]. It is currently unknown if the absence of the OPP pathway from the chloroplast is functionally linked (how and why) to the lack of redox-regulation of PRK [20,90]. A survey of PRK activities in species from many phylogenetic groups showed that not only the presence of the two regulatory cysteine residues, but also the distance between them, determined whether or not the enzyme was redox-regulated. For example, PRKs from the Chlorophyta all have 38 residues between the two cysteine residues at positions 16 and 55 (numbering from the spinach and green algal enzymes), and enzymes from this group are redox-regulated [80]. In contrast, in diatoms the distance between the two regulatory cysteine residues is five amino acid residues longer than in land plants and green algae, and apparently this insertion is sufficient to prevent PRK redox-regulation.

In the green alga *C. reinhardtii*, the arginine residue at position 64 in PRK is important both for the binding of Ru5P [95] and for the formation of the supramolecular complex with GAPDH [96]. Recently, it has been shown that the formation of a disulfide bridge between Cys243 and Cys249 in *C. reinhardtii* is essential for the formation of the ternary complex involving PRK-CP12-GAPDH [97]. These cysteine residues are highly conserved and located at the same position among diverse photosynthetic organisms such as in *A. thaliana* and *S. elongatus* where the complex is also present, but are absent in PRK from diatoms (figure 2*e*). This suggests that diatom PRK is not able to form a complex with CP12 and GAPDH, and hitherto this complex has not been found in any diatoms. To conclude, PRK in diatoms does not seem to be regulated by redox process or by protein–protein interaction upon light/dark transition but could be regulated at the transcript level as shown in *P. tricornutum* [91] and possibly furthermore by other mechanisms yet to be discovered.

7. Transcriptional regulation of Calvin–Benson–Bassham enzymes

Transcriptional regulation of CBB enzymes could be an important adaptive response of photosynthetic organisms to environmental variation [98], but relatively little is known about this. In land plants, CBB genes are targets for gene regulation under light variation [10] and abiotic stresses [99]. Moreover, upstream regulatory motifs for 12 CBB genes [10] and transcription factors (TF) have been identified [98,100], most of them belonging to the bZIP family. However, in diatoms, we were not able to find any homologues of the TFs [100] although some proteins containing the bZIP motif were present in their genome (data not shown). In *C. reinhardtii*, a TF (CIA5) involved in CCM-related gene expression, is thought to be a negative regulator of some CBB genes, but the mechanism is not yet clear [101]. However, this TF seems to be exclusive to green algae because no homologues were found in other organisms.

In *A. thaliana*, the expression of the genes for CP12s, PRK and GAPDH is tissue-specific [102] and, more interestingly, is coordinated, consistent with the formation of a ternary complex between the three proteins [103]. In *P. tricornutum*, the genes encoding PRK, and the chloroplastic but not the cytosolic GAPDH, are regulated by light/dark transition [91]. This suggests that the mechanisms of gene expression of the two GAPDH homologues are probably different. In *C. reinhardtii*, the SBPase gene *csbp* is light-regulated by its 1.4 kb upstream region [104]. In contrast, in *P. tricornutum*, as mentioned above, both the SBPase gene and protein are unaffected by light or dark, a characteristic that might be shared with other diatoms. However, triose phosphate isomerase, FBPase and fructose-bisphosphate aldolase are

highly upregulated after 24–48 h under high light in this species [105].

Further evidence that CBB enzymes from diatoms are regulated at the gene level derives from nutrient starvation experiments. A comparison of the transcriptomes of different diatoms showed that CBB genes were generally downregulated under nitrate deprivation [106]. Similarly, in N-deprived *P. tricornutum*, most CBB genes were downregulated while OPP pathway genes were upregulated [107]. In silicon-starved *T. pseudonana*, *rbcR (*or *ycf30),* a gene encoding for a Rubisco transcriptional regulator, was downregulated while lipid biosynthesis genes were upregulated [108]. In the red alga, *Cyanidioschyzon merolae*, *rbcR* upregulates the *rbcLS-cbbx* operon that contains the *rbcLS* gene, encoding for the Rubisco large and small subunit, during dark-to-light transition [109]. This operon is present in red algae, raphidophytes and α-proteobacteria, while the *rbcLS-cbbx* operon is absent in diatoms and coccolithophores. Therefore, *cbbx* and *rbcLS* are not under the control of the same promoter. However, all organisms, including diatoms, have *rbcR* and *rbcLS* that could be controlled by this regulator. In *P. tricornutum*, the *rbcLS* gene is highly upregulated during the first 6 h of a 12 h photoperiod, although its expression was not correlated with CO_2 fixation [110]. Similarly, a lag between the maximum transcript level (at 09.00) and the maximal level of Rubisco (at 15.00) has been observed in *T. pseudonana* [111].

8. Conclusion

Regulation of the CBB cycle in diatoms is understudied relative to their ecological importance and seems to be very different from the Plantae. Given that there appears to be a large range of variability of CBB properties and regulation among diatoms, consistent with the large amount of genetic variation within the group [112], more species clearly need to be investigated.

Data accessibility. This article has no data.

Authors' contributions. All authors contribute to the writing and discussion of this review and E.J. also prepared both the figures.

Competing interests. We have no competing interests.

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