*Journal of Experimental Botany*, Vol. 65, No. 10, pp. 2573–2584, 2014
doi:10.1093/jxb/ert387 Advance Access publication 25 November, 2013

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

# Molecular regulation of the diatom cell cycle

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Received 21 August 2013; Revised 21 October 2013; Accepted 24 October 2013

## Abstract

**Accounting for almost one-fifth of the primary production on Earth, the unicellular eukaryotic group of diatoms plays a key ecological and biogeochemical role in our contemporary oceans. Furthermore, as producers of various lipids and pigments, and characterized by their finely ornamented silica cell wall, diatoms hold great promise for different industrial fields, including biofuel production, nanotechnology, and pharmaceutics. However, in spite of their major ecological importance and their high commercial value, little is known about the mechanisms that control the diatom life and cell cycle. To date, both microscopic and genomic analyses have revealed that diatoms exhibit specific and unique mechanisms of cell division compared with those found in the classical model organisms. Here, we review the structural peculiarities of diatom cell proliferation, highlight the regulation of their major cell cycle checkpoints by environmental factors, and discuss recent progress in molecular cell division research.**

**Key words:** CDK, cell division, checkpoints, cyclin, diatom, mitosis.

## Introduction

In contrast to the terrestrial realm that is dominated by higher plants, animals, and fungi, aquatic life is characterized by a bewildering diversity of only distantly related groups of mainly unicellular organisms. How evolution led to the co-existence of such a high phylogenetic and species diversity remains a largely unsolved mystery (Smetacek, 2012). Many of these unicellular lineages include photosynthetic organisms commonly referred to as microalgae. Together, they account for about half the global biological carbon fixation, they fuel aquatic foodwebs, and they are key drivers of global nutrient cycles (Field *et al.*, 1998; Falkowski *et al.*, 2004; Raven, 2009). Among microalgae, diatoms stand out, as they are not only one of the most species-rich classes, but are also solely responsible for ~40% of all oceanic carbon fixation (Nelson *et al.*, 1995; Van den Hoek *et al.*, 1995; Armbrust, 2009). They thrive in well-mixed water columns of temperate and polar oceans and lakes, beneath sea ice, and in the plankton or on sediments of coastal seas and estuaries. These are among the most productive aquatic ecosystems, in which diatom productivity drives high production rates of higher trophic levels (Armbrust *et al.*, 2004; Armbrust, 2009). Diatoms typically have a 'bloom and bust' life cycle strategy characterized by short-term massive blooms alternated with cell death, sexual reproduction, and/or sinking of cells. The latter results in the export of carbon, silica, and other nutrients into the ocean interior, where it remains for centuries to millennia (Dugdale and Wilkerson, 1998; Falkowski *et al.*, 1998; Allen *et al.*, 2005).

As a group, diatoms belong to the Stramenopila, a completely separate evolutionary lineage from land plants (Katz, 2012). Diatoms possess a highly chimeric genome which arose through endosymbiotic and horizontal bacterial gene transfers, contributing to many unique features that are believed to

Abbreviations: AMPK, AMP-activated protein kinase; bZIP, basic leucine zipper; CDC, cell division cycle; CDK, cyclin-dependent kinase; CKI, CDK inhibitor; CKS, CDK subunit; CPF1, cryptochrome photolyase family 1; dsCYC, diatom-specific cyclin; $G_1$ phase, gap 1 phase; $G_2$ phase, gap 2 phase; LOV, light oxygen or voltage; MC, microtubule center; M phase, mitosis phase; MTOC, microtubule organizing centre; PC, polar complex; Rb, retinoblastoma; SDV, silica deposition vesicle; Snf1, sucrose non-fermenting-1; SnRK1, Snf1-related protein kinase 1; S-phase, synthesis phase; TF, transcription factor; TOR, target of rapamycin.

underlie their evolutionary and ecological success (Armbrust *et al.*, 2004; Bowler *et al.*, 2008; Moustafa *et al.*, 2009; Rayko *et al.*, 2010; Allen *et al.*, 2011; Fabris *et al.*, 2012; Gross, 2012; Prihoda *et al.*, 2012). The defining feature of diatom biology and key to understanding their evolutionary success is their haplo-diploid life cycle, controlled by a unique, endogenous cell size reduction–restitution strategy that is used as a clocking mechanism to switch from mitotic multiplication to cell differentiation and sexual reproduction (Lewis, 1984; Chepurnov *et al.*, 2004, 2008).

Our current understanding of the regulation of eukaryotic cell division relies mainly on studies in model systems, such as yeast, mammalian, and plant cells (Morgan, 1997; Inzé and De Veylder, 2006; Lloyd and Chan, 2006; Doonan and Kitsios, 2009; Harashima *et al.*, 2013), representing only two of the eight major eukaryotic groups (Baldauf, 2003). Although diatoms have long been the subject of cell division studies since Lauterborn's remarkable microscopic observations of diatom mitosis at the end of the 19th century (Lauterborn, 1896), only recently has significant progress been made to uncover the molecular secrets of diatom cell cycle regulation (Hogan *et al.*, 1992; Thamatrakoln and Hildebrand, 2007; Gillard *et al.*, 2008; De Martino *et al.*, 2009; Huysman *et al.*, 2010, 2013*a*). To date, only a few model species have been used for molecular studies of the cell cycle in diatoms. These include the pennate diatoms *Phaeodactylum tricornutum* (Fig. 1A, B) and *Seminavis robusta* (Fig. 1C, D), and the centric species *Thalassiosiora pseudonana*. Here, we summarize our present understanding of the diatom cell cycle and discuss the key regulatory mechanisms known to date.

## Organellar and structural organization during the diatom cell cycle

Vegetative reproduction in diatoms is inevitably connected to their main characteristic feature, the diatom cell wall. This silicified frustule is composed of two unequal halves: a larger epivalve and a smaller hypovalve, which fit into each other like a lid on a box, and are held together by siliceous structures, called girdle bands (Round *et al.*, 1990). More than 100 years ago, the light microscopy studies of diatom mitosis by Robert Lauterborn already revealed that cell division in diatoms displays major differences compared with the classical paradigms of animal and plant cells (Lauterborn, 1896; translated and summarized in Pickett-Heaps *et al.*, 1984). Transmission

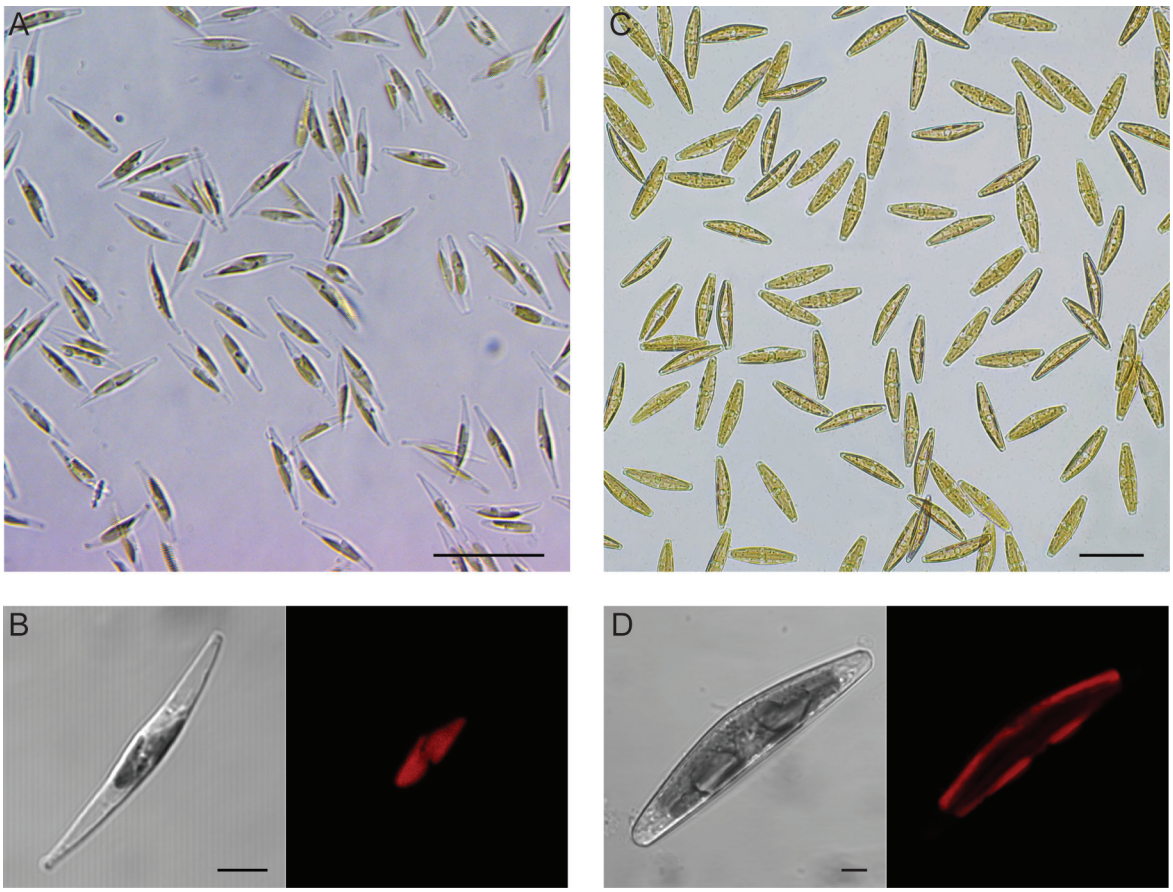


**Fig. 1.** Illustrations of two diatom species used for diatom cell cycle research in our lab. (A) Light microscopy image of a *P. tricornutum* culture. (B) Confocal laser scanning microscopy of a *P. tricornutum* cell. Left: transmission light. Right: autofluorescence of the chloroplast in red. (C) Light microscopy image of an *S. robusta* culture (with kind permission from O. Chepurnova). (D) Confocal laser scanning microscopy of an *S. robusta* cell. Left: transmission light. Right: autofluorescence of the chloroplasts in red (with kind permission from Dr J. Gillard). Scale bar in A and C = 50 μm. Scale bar in B and D = 5 μm.

electron microscopy studies of the internal structures of diatom cells have shown that diatoms contain unique ultrastructural and cytological characteristics (see later) suggesting the existence of diatom-specific features important for cell division (reviewed in De Martino *et al.*, 2009).

Among diatom species there exists considerable variation in the chloroplast number per cell, classifying diatoms into five different groups: monoplastidic, diplastidic, tetraplastidic, oligoplastidic, and polyplastidic species (Mann, 1996). In all except polyplastidic species, chloroplast movement, division, and development are tightly linked to cell cycle progression (Chepurnov *et al.*, 2002; Gillard *et al.*, 2008; Huysman *et al.*, 2010), probably contributing to an equal distribution of the daughter chloroplasts on each side of the future division plane. Cytological observations and transcriptional analysis of synchronized cultures demonstrated that chloroplast division precedes nuclear and cellular division in the pennate diatoms *S. robusta* (Gillard *et al.*, 2008) and *P. tricornutum* (Huysman *et al.*, 2010). However, the molecular mechanisms of chloroplast movement and division in diatoms remain unclear. In plants, the red alga *Cyanidioschyzon merolae*, and diatoms containing more than two chloroplasts, chloroplast movement has been shown to be actin dependent (De Francisco and Roth, 1977; Nishida *et al.*, 2005; Krzeszowiec *et al.*, 2007). The transcriptional induction of β-tubulin in *S. robusta* during chloroplast segregation and repositioning suggests the involvement of microtubuli, but does not exclude the possibility that actin may also be involved (Gillard *et al.*, 2008).

Diatom cells typically possess one microtubule centre (MC), which is a dense spherical body positioned next to the interphase nucleus, and from which microtubules radiate. The diatom MC resembles the microtubule organizing centre (MTOC) of animal cells, called the centrosome (Lloyd and Chan, 2006), but without centrioles (reviewed in Pickett-Heaps and Tippit, 1978; De Martino *et al.*, 2009). Besides its structural differences, the diatom MC behaves differently during the cell cycle. Whereas animal centrosomes duplicate before mitosis and give rise to the spindle poles (Azimzadeh and Bornens, 2007), the diatom MC is involved in initial spindle creation, after which it disintegrates (Fig. 2A, B). During

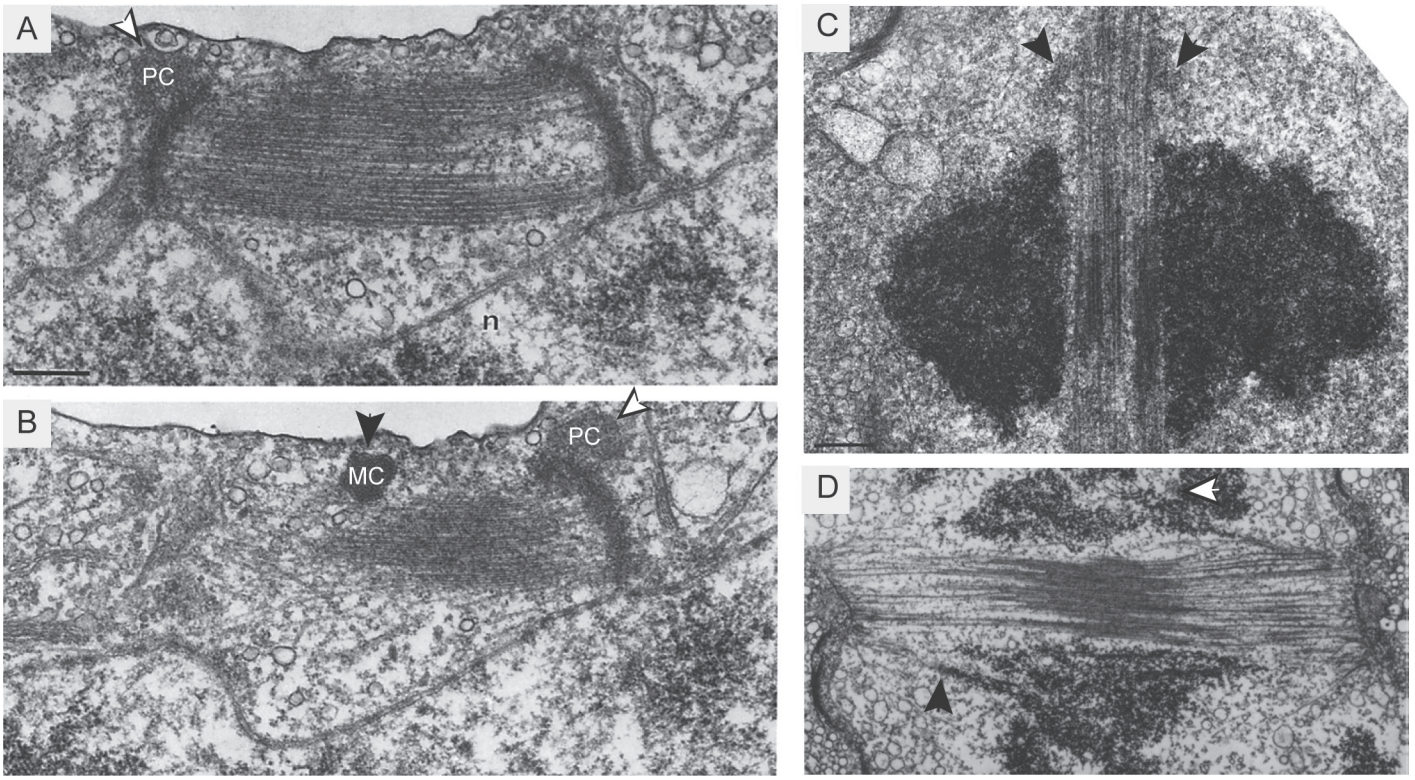


**Fig. 2.** Transmission electron micrographs illustrating the unique mitotic structures in diatoms. (A and B) Two different sections through the same late prophase spindle in the diatom *Surirella ovalis*. The polar complexes (PCs) each show a thickening at one end (white arrows). The microtubules forming the central spindle run from pole to pole, while other microtubules invaginate the nuclear envelope. The MC (black arrow) starts to disintegrate at this stage, and is not observed at metaphase and anaphase. n, nucleus. Scale bar=0.5 μm. Images reproduced with permission from Tippit DH, Pickett-Heaps JD. 1977. Mitosis in the pennate diatom *Surirella ovalis*. *Journal of Cell Biology* **73,** 705–727. (C) High voltage micrograph showing a thick section of a late metaphase *S. ovalis* cell. The arrows indicate a dense matric, called 'the collar', surrounding the central spindle between the chromatin and the poles. Bar=0.5 μm. Image reproduced with permission from Tippit DH, Pickett-Heaps JD. 1977. Mitosis in the pennate diatom *Surirella ovalis*. *Journal of Cell Biology* **73,** 705–727. (D) Attachment of chromosomes to the central spindle via 'presumptive kinetochores' (black arrow) during mid-prometaphase in *Hantzschia*. Some chromosomes still have no microtubules associated with their kinetochores (white arrow). x11000. Image reproduced with permission from Tippit DH, Pickett-Heaps JD, Leslie R. 1980. Cell division in two large pennate diatoms *Hantzschia* and *Nitzschia* III. A new proposal for kinetochore function during prometaphase. *Journal of Cell Biology* **86,** 402–416.

diatom mitosis, new functional MTOCs, designated the polar complexes (PCs), appear and cap the poles of the diatom spindle (Fig. 2A, B). The PCs disappear during cytokinesis, while a new MC arises (Pickett-Heaps and Tippit, 1978; Pickett-Heaps, 1991). In addition, diatom mitosis involves the action of an unusual, highly organized 'central spindle' that is initially formed outside the nucleus and consists of parallel microtubules arranged as two interdigitated half-spindles, creating a central overlap region of microtubules (Tippit and Pickett-Heaps, 1977; Pickett-Heaps and Tippit, 1978; Wordeman *et al.*, 1986) (Fig. 2A, B, D). The diatom spindle is encircled by a dense matrix, the 'collar' (Fig. 2C), which is supposed to help attach the spindle to an atypical type of 'presumptive kinetochores', the sites of chromosome attachment (Fig. 2D) (Tippit and Pickett-Heaps, 1977; Tippit *et al.*, 1980). These observations differ from what is known in animals and plants, and therefore diatoms appear to undergo cell division in a rather unique way (De Martino *et al.*, 2009).

As well as the organization of the spindle, diatom cytokinesis also differs from that of animals and plants: whereas plant cells build their new cell membrane and cell wall centrifugally (inside-out), diatom cells first divide centripetally (outside-in) by invagination of their plasma membrane, like animal cells, and then create the new hypovalve centrifugally inside the silica deposition vesicle (SDV), an intracellular acidic tubular compartment unique to diatoms (Pickett-Heaps and Tippit, 1978; Pickett-Heaps, 1991; De Martino *et al.*, 2009). During this process, the new MC is positioned adjacent to the SDV, suggesting a role during valve synthesis (Boyle *et al.*, 1984; Edgar and Pickett-Heaps, 1984). When the new hypovalve is fully synthesized in the SDV, it is secreted from the cell by exocytosis, after which the daughter cells can separate.

## Diatom cell cycle checkpoints

The eukaryotic cell cycle comprises the coordinated succession of a phase of DNA replication or synthesis (S phase), a phase of physical separation of both copies of the genomes (mitosis or M phase), and cell division itself (cytokinesis). The S and M phases are separated by two gap phases, one preceding S phase [gap 1 ($G_1$) phase] and the other preceding M phase [gap 2 ($G_2$) phase]. A tight coordination of the cell division process is essential to the reproduction of every living organism. In eukaryotes, different cell cycle checkpoints (e.g. at the $G_1$–S and $G_2$–M transitions) ensure that the genetic information is inherited correctly by the daughter cells by inhibiting the replication and distribution of incompletely replicated or damaged chromosomes. The major eukaryotic cell cycle control points represent the onset of DNA replication (the $G_1$–S transition) and mitosis itself (the $G_2$–M transition) (Buchanan *et al.*, 2000). In addition, most organisms show during the mid-to-late $G_1$ phase a commitment point (known as START in yeast, restriction point in animals, or commitment point in *Chlamydomonas*), before which a number of conditions, depending on intra- and extracellular information, must be fulfilled (Oakenfull *et al.*, 2002). The seasonal dominance of diatoms in phytoplankton assemblages of marine and freshwater ecosystems suggests that they possess efficient sensing and signalling mechanisms that allow them to respond or adapt adequately to environmental fluctuations, such as light intensity and nutrient supply, through the activation of specific cell cycle checkpoints (Margalef, 1978; Falciatore *et al.*, 2000).

### *Nutrients*

Nutrient availability strongly affects diatom population dynamics. Nutrient limitation at the end of a diatom bloom period is often accompanied by switches in the diatom life cycle phase from vegetative division to spore formation or sexual reproduction (Smetacek, 2012). The major limiting nutrients of primary production in the oceans are nitrogen, phosphorus, iron, and silicon (Falkowski *et al.*, 1998). As a major constituent of amino acids and nucleic acids, nitrogen is indispensable for diatom growth (Valenzuela *et al.*, 2012; Yang *et al.*, 2013). In fact, nitrogen limitation and starvation in diatoms have been demonstrated to cause an extended duration of the $G_1$ phase or an arrest at multiple $G_1$ checkpoints (Olson *et al.*, 1986; Vaulot *et al.*, 1987).

Phosphate limitation has been reported in certain oceanic areas, such as the Eastern Mediterranean Sea and the Sargasso Sea (Wu *et al.*, 2000; Krom *et al.*, 2010; Howarth *et al.*, 2011), and has been hypothesized to have been more widespread during the glacial periods (Pichevin *et al.*, 2009). Van Mooy and colleagues showed that diatoms reduce their phosphorus demand upon phosphorus limitation, and maintain their growth by substituting phospholipids with non-phosphorus membrane lipids (Van Mooy *et al.*, 2009).

Dissolved silicon can be a major limiting factor for diatom reproduction. Silicon limitation in diatom cultures induces a cell cycle arrest at the $G_1$–S boundary and during the $G_2$–M transition (Vaulot *et al.*, 1987; Brzezinski *et al.*, 1990), and these arrest points have been linked to the requirement for silica during DNA replication and cell wall formation, respectively (Coombs *et al.*, 1967; Darley and Volcani, 1969; Okita and Volcani, 1980; Vaulot *et al.*, 1987). Furthermore, in some species such as *Chaetoceros* that deposit siliceous spine-like structures (called setae), silicon limitation experiments revealed an additional checkpoint early in the $G_1$ phase, related to setae formation (Brzezinski *et al.*, 1990).

In specific oceanic regions such as the Southern Ocean and the Eastern Equatorial Pacific Ocean, photosynthesis is limited by low concentrations of the trace metal iron (Behrenfeld *et al.*, 1996; Boyd *et al.*, 2000). Iron limitation has been shown to have a substantial impact on diatom cell division, both by laboratory experiments and by field studies (Marchetti *et al.*, 2009; Lommer *et al.*, 2012; Smetacek *et al.*, 2012). Several studies illustrate how diatoms have developed different strategies to survive upon and rapidly acclimate to iron limitation through transcriptional and biochemical reconfiguration of iron requirement and acquisition pathways and by the use of ferritin to maintain internal iron storage (Allen *et al.*, 2008; Marchetti *et al.*, 2009).

A general strategy in eukaryotes to couple nutrient sensing to cell division occurs through the TOR (target of rapamycin) signalling pathway or through the Snf1 (sucrose

non-fermenting-1)/AMPK (AMP-activated protein kinase)/SnRK1 (Snf1-related protein kinase 1) kinases (Thomas and Hall, 1997; Hardie *et al.*, 2012). Although several regulatory members of the these signalling pathways have been identified in the genomes of *P. tricornutum* and *T. pseudonana* (Serfontein *et al.*, 2010; van Dam *et al.*, 2011), it remains unclear if they play a role in nutrient perception in diatoms, since no functional evidence is available for these proteins to date.

### *Light*

For any photosynthetic organism, including diatoms, light is an extremely important factor that influences growth. Because diatoms can grow over a wide range of light intensities and wavelengths, they are believed to have developed specific photoacclimation and photoadaptation mechanisms (Huisman *et al.*, 2004; Lavaud *et al.*, 2004, 2007; Schellenberger Costa *et al.*, 2013). As in most other phytoplankton species, timing of diatom cell division can be entrained by alternating periods of light and dark, implying that the cell cycle consists of light-dependent and light-independent segments (Vaulot *et al.*, 1986; Ashworth *et al.*, 2013). Accordingly, by both light limitation and deprivation experiments, light-controlled restriction points have been identified in several diatom species, either only during the $G_1$ phase, or during both the $G_1$ and $G_2$/M phases of the cell cycle (Olson *et al.*, 1986; Vaulot *et al.*, 1986; Brzezinski *et al.*, 1990; Gillard *et al.*, 2008; Huysman *et al.*, 2010). Interestingly, the photoperiod-determined cell division phasing in *Thalassiosira weissflogii* can be overruled by administration of periodic nitrogen pulses, suggesting that nutrient control of the cell cycle is downstream of the light checkpoint in this diatom or that the regulatory pathways controlling the nutrient and light checkpoint might be intricately connected (Olson and Chisholm, 1983).

Detailed investigation of the main transcription factor (TF) families in diatoms led to the identification of putative orthologues of TFs involved in light signalling (Rayko *et al.*, 2010). These include a group of Myb proteins containing a single Myb domain (Myb1R) that belong to the SHAQKYF-like family described in plants and green algae, which includes the clock genes *CCA1* (*circadian clock associated 1*) and *LHY* (*late elongated hypocotyl*) (Wang *et al.*, 1997). Furthermore, aureochrome-like sequences and bZIP-PAS proteins were found, which might represent classes of photoreceptors that contain putative light-sensitive and DNA-binding domains. Of particular interest, in addition to a bZIP (basic leucine zipper) domain, aureochromes also contain a LOV (light, oxygen, or voltage) domain, which is also present in the phototropin family of blue-light photoreceptors (Takahashi *et al.*, 2007; Ishikawa *et al.*, 2009). Recent studies of the *P. tricornutum* Aureochrome1a protein have indicated that this TF indeed functions as a blue-light sensor (Herman *et al.*, 2013; Huysman *et al.*, 2013*a*). Since blue light (350–500 nm) is the most prevailing band below the surface of oceanic waters (MacIntyre *et al.*, 2000), efficient blue light sensing and signalling mechanisms are expected to play a crucial role in the control of diatom growth. Related to this, a blue-light sensor cryptochrome photolyase family 1 (CPF1) has recently been identified and characterized in *P. tricornutum*. Overexpression of this protein affected blue light-induced expression of genes involved in cell cycle regulation and DNA repair, suggesting a role for CPF1 in perception and signalling of environmental light conditions and linking these to cell cycle progression (Coesel *et al.*, 2009).

## Diatom cell cycle synchronization

Because both nutrient and light deprivation cause a cell cycle arrest in diatoms (Olson *et al.*, 1986; Vaulot *et al.*, 1986, 1987; Brzezinski *et al.*, 1990), both strategies can be used to synchronize the diatom cell cycle naturally, without the need for chemical cell cycle inhibitors (Planchais *et al.*, 2000) that might cause stress to the cells. A synchronization method based on silicon starvation and repletion has been used to study cell cycle-related processes, such as cell wall formation, at the molecular level in the centric model species *T. pseudonana* (Thamatrakoln and Hildebrand, 2007; Shrestha *et al.*, 2012). However, silicon starvation cannot be used for synchronization of the pennate model diatom *P. tricornutum*, since it is one of the few species without a silicon requirement (Brzezinski *et al.*, 1990). On the other hand, *P. tricornutum* displays a light-dependent segment during its cell cycle occurring in the $G_1$ phase (Fig. 3) (Brzezinski *et al.*, 1990). Light limitation perturbs $G_1$ phase progression in *P. tricornutum* and illumination results in immediate and synchronous release of the cell cycle arrest (Huysman *et al.*, 2010). Applying this synchronization procedure allows the cell cycle checkpoints in $G_1$ and the commitment to division to be studied. A similar light-induced synchronization method has been proven useful to synchronize cell division in *S. robusta* (Gillard *et al.*, 2008). Therefore, this synchronization method has been used as a tool to study cell cycle-related processes in both diatom species (Gillard *et al.*, 2008; Huysman *et al.*, 2010, 2013*a*, *b*). As described above, cytological observations of the diatom chloroplast conformation during synchronized growth permitted the determination of the specific timing of chloroplast division and plastid movements during the cell cycle (Gillard *et al.*, 2008). As a result, chloroplast cytology might be used as a fast and easy tool to identify the cell cycle phase of a diatom cell possessing only one or two chloroplasts. This method can complement other more laborious techniques, such as flow cytometry or quantitative epifluorescence microscopy (von Dassow *et al.*, 2008), to determine the cell cycle stage in diatoms.

## Diatom cell cycle regulation: clues from the genome

Along with the sequencing of the genomes of both diatom model species, *T. pseudonana* and *P. tricornutum* (Armbrust *et al.*, 2004; Bowler *et al.*, 2008), genome-wide annotation studies were conducted to uncover the molecular mechanisms underlying the complex and tightly coordinated production and orientation of the unique mitotic and cytokinetic

structures in diatoms, as well as the decision to start the cell division cycle. Homology searches in the genomes of both sequenced diatoms for mitotic checkpoint and spindle-associated regulatory molecules resulted in the identification of genes important for chromosome segregation (including a kinetochore protein ZW10, several chromokinesins, and extra-spindle pole-like proteins) and genes involved in spindle assembly and elongation (diatom spindle kinesin 1 and several dyneins) (De Martino *et al.*, 2009). Furthermore, a centromere-specific histone H3 variant was detected in *P. tricornutum*, but not in *T. pseudonana*, suggesting a low level of similarity of centromeric proteins among diatoms (De Martino *et al.*, 2009).

Generally, cell cycle regulation in eukaryotes is controlled at multiple points by an evolutionarily conserved set of proteins, the cyclin-dependent kinases (CDKs) and cyclins, that can form functional kinase complexes (reviewed in Morgan, 1997; Inzé and De Veylder, 2006). In these complexes, the CDKs and cyclins act as catalytic and regulatory subunits, respectively. CDKs were first discovered in *Xenopus* eggs as the active component of maturation-promoting factor (MPF), a complex that expresses kinase activity and enables entry into mitosis (Gautier *et al.*, 1988). Since then, multiple CDKs have been identified in different eukaryotic organisms and, based on the cyclin-binding motif present, the CDK family can be subdivided into different groups (Doonan and Kitsios, 2009). Cyclins were initially identified in fertilized sea urchin eggs as proteins with particular oscillatory patterns of gene expression and protein destruction during the cell cycle (Evans *et al.*, 1983). Because cyclins determine the substrate specificity of the CDK complex, the fluctuation of cyclin abundance creates different kinase specificities and activities of the CDK–cyclin complex at different stages of the cell cycle. In general, eukaryotes express two main groups of cyclins: the G1 cyclins that regulate $G_1$–S transition and the mitotic cyclins that control $G_2$–M transition (Oakenfull *et al.*, 2002). Interestingly, comparative genomics revealed that the cyclin gene family represents one of the expanded gene families in diatoms, together with histidine kinases and heat-shock TFs (Bowler *et al.*, 2008). In addition to members of each of the canonical families of cyclins, a substantial number of diatom-specific cyclin (dsCYC) genes were found (Bowler *et al.*, 2008; Huysman *et al.*, 2010). Several of these *dsCYC* genes have been found to be specifically up-regulated under certain conditions (Fig. 3), such as light (*dsCYC2*), phosphate repletion (*dsCYC5*, *dsCYC7*, and *dsCYC10*), and the presence of silica (*dsCYC9*) (Sapriel *et al.*, 2009; Huysman *et al.*, 2010, 2013*a*; Valenzuela *et al.*, 2012). This large number of cyclins contradicts previous suggestions that expansion of cell cycle protein families in both metazoans and plants would be linked to a multicellular lifestyle (Harashima *et al.*, 2013). Rather, the dramatic expansion of this gene family may reflect on the one hand the unique characteristics of diatom life cycles associated with constraints imposed by the rigid nature of their cell walls, such as the control of cell size reduction or the activation of sexual reproduction at a critical size threshold, and on the other hand the need to integrate a multitude of environmental cues into the cell cycle machinery. It will be interesting to see if other groups of unicellular eukaryotes have undergone similar expansions in key families of cell cycle regulators.

Monitoring the transcription of the annotated diatom cell cycle genes during synchronized growth in *P. tricornutum* allowed the assignment of particular CDKs and cyclins to a specific cell cycle phase (Huysman *et al.*, 2010). This resulted in a set of cell cycle marker genes that can be used in the laboratory and might constitute good biomarker candidates for field studies. The development of molecular cell cycle probes might be valuable to assess and monitor diatom growth events, either species or group specific, in nature, and might, for example, help to detect early bloom stages of toxin-producing species (Diercks *et al.*, 2008). The type of probes that can be designed, either universal or specific, and their applicability will largely depend on the degree of sequence conservation of the genes of interest. In the case of the diatom-specific cyclins, development of universal probes might be difficult given their high sequence variation and possible functional divergence amongst different species (Huysman *et al.*, 2010; Shrestha *et al.*, 2012), but in turn, they might be interesting candidates for the evaluation of species-specific responses to environmental stresses.

CDK–cyclin complex activity is controlled not only by cyclin association, but also by phosphoregulation of the CDK subunits by regulatory proteins and interaction with CDK inhibitors (CKIs) or scaffolding proteins such as CKS1/Suc1 (CDK subunit) (Pines, 1996; Harper, 2001; De Clercq and Inzé, 2006). A CKS1 protein could be identified in the *P. tricornutum* genome, but no CKIs have been found yet based on sequence homology (Huysman *et al.*, 2010), which might not be that surprising, given the observation that different species use distinct types of only poorly related CKIs to control their cell cycle (De Clercq and Inzé, 2006).

Except for yeasts, all eukaryotes share the retinoblastoma (Rb)-mediated pathway for $G_1$–S regulation, involving the Rb protein and E2F/DP TFs (Weinberg, 1995; Claudio *et al.*, 2002). Upon mitogenic stimulation (by external and/or internal factors) during $G_1$ phase, active CDK–cyclin complexes phosphorylate the Rb protein. In hypophosphorylated form, the Rb protein binds to the E2F and DP TFs, rendering them inactive. Phosphorylation and hence dissociation of the Rb protein results in the activation of the E2F–DP complex and the subsequent transcriptional activation of genes involved in DNA replication and S-phase onset (Weinberg, 1995; de Jager and Murray, 1999; Claudio *et al.*, 2002). In *P. tricornutum*, all members of the Rb-mediated pathway are present, suggesting a similar regulation of the $G_1$–S transition to that in plants and animals (M.J.J. Huysman *et al.*, unpublished data) (Fig. 3). However, *de novo* motif prediction led to the identification and characterization of a diatom-specific E2F motif that deviates from the conserved animal/plant motif (M.J.J. Huysman *et al.*, unpublished data).

In yeast and metazoans, CDC25 phosphatases are known to activate CDKs by opposing the activity of the WEE1/MYT1/MIK1 family of inhibitory kinases (Perry and Kornbluth, 2007). Despite the presence of a *MYT1* homologue, the *P. tricornutum* genome does not contain a *CDC25* gene (Huysman

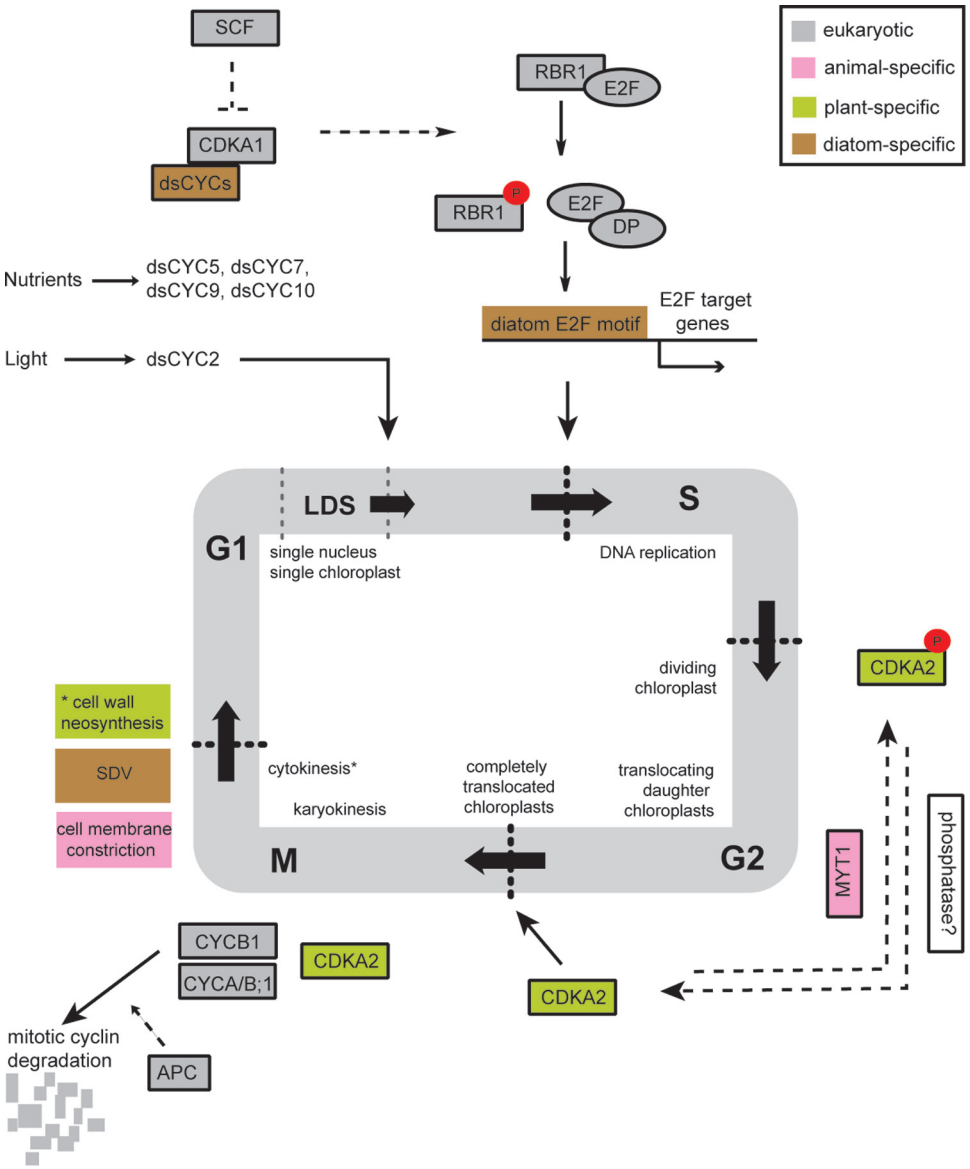


**Fig. 3.** Overview of the cell cycle regulation and conservation in *P. tricornutum*. The start of the cell cycle at the $G_1$ phase is initiated by the presence of non-limiting amounts of external stimulatory factors, including light and nutrients. Perception of these growth stimuli induces an intracellular signalling cascade that leads to the transcription of several diatom-specific cyclins (dsCYCs). These dsCYCs subsequently interact and activate $G_1$-expressed CDKs. The active CDK–cyclin complexes most probably phosphorylate the Rb-related protein 1 (RBR1), resulting in the release and activation of the E2F transcription factor. E2F together with DP binds and transactivates a diatom-specific *cis*-regulatory E2F motif in the promoter of E2F target genes, leading to S phase progression. After DNA synthesis has been completed, a mitotic CDK, *CDKA2*, is expressed. In analogy with the DNA damage checkpoint known in other eukaryotes, this complex is probably kept inactive during S–$G_2$ transition through inhibitory phosphorylation by the MYT1 kinase. Possibly, this inhibitory phosphorylation is later removed by an as yet unspecified phosphatase. Activation of CDKA2 leads to the progression of $G_2$ to M and finally results in nuclear (karyokinesis) and cellular (cytokinesis) division. Cytokinesis involves the centripetal invagination of the plasma membrane, and the centrifugal neosynthesis of a new valve within the silica deposition vesicle (SDV). Chloroplast division and translocation both precede karyokinesis and cytokinesis. Conserved regulators or regulatory pathways in eukaryotes are indicated in grey, characteristics common with animals in pink, features common with plants in green, and diatom-specific properties in brown. Arrows and dashed lines indicate activation and hypothetical regulations, respectively. LDS, light-dependent segment.

*et al.*, 2010). DNA damage or mitotic defects trigger the activation of cell cycle checkpoints, resulting in CDK inactivation by phosphorylation of Thr14 and/or Tyr15 residues. Dephosphorylation of these residues in human CDK1 by CDC25 renders the CDK–cyclin complex active and hence stimulates cell cycle progression (Lew and Kornbluth, 1996). Although the phosphorylation status of Tyr15 in plants is important to arrest the cell cycle in the presence of replication defects (Cools and De Veylder, 2009), it does not seem to be crucial for $G_2$–M progression (Dissmeyer *et al.*, 2009). The

detection of an intriguing number of parallels in transcriptional, biochemical, and functional properties of mammalian CDC25 and plant B-type CDKs led to the suggestion that the CDC25-mediated regulatory mechanisms might have been replaced in plants by a mechanism governed by the plant-specific B-type CDKs (Boudolf *et al.*, 2006). In diatoms, the function of the CDC25 phosphatase might be taken over by CDKA2, given its expression timing and sequence similarity to B-type CDKs (Boudolf *et al.*, 2006; Huysman *et al.*, 2010) (Fig. 3). In addition, recent analysis using reverse genetics indicates a role for CDKA2 during mitosis (M.J.J. Huysman *et al.*, unpublished data).

The major cell cycle regulatory protein degradation complexes, the Skp1/Cullin/F-box protein (SCF) complex and the anaphase-promoting complex (APC) (Harper *et al.*, 2002; Vodermaier, 2004), were also recently identified in *P. tricornutum* and have been found to be transcribed at the $G_1$/S and M phases (Huysman *et al.*, 2013*b*), respectively, suggesting that they might control CDK–cylin activity during these phases in diatoms (Fig. 3).

## Functional analysis of cell cycle regulators

Although much effort has been devoted to the identification of cell cycle regulators in diatoms, until now only one cell cycle gene has been functionally characterized: *dsCYC2* of *P. tricornutum*. Initially, *dsCYC2* was discovered as a cyclin that is rapidly up-regulated upon light exposure of dark-arrested cells (Huysman *et al.*, 2010). Interaction with CDKA1 and complementation of a G1-cyclin-deficient yeast strain demonstrated its function as a G1-cyclin. Silencing of *dsCYC2* resulted in the perturbation of the $G_1$–S transition in light/dark-grown cells, but not in continuous light-grown cells, suggesting that it functions as a rate-limiting factor controlling the light-dependent cell cycle onset in *P. tricornutum*. In addition, *dsCYC2* transcription is regulated directly by the blue-light receptor Aureochrome1a, reinforcing its role during the light-dependent cell cycle onset upon blue light exposure (Huysman *et al.*, 2013*a*).

## Future directions

Although the findings discussed in this review, along with the results of the cytological and physiological studies from the past, allow us to fill in some major gaps in the understanding of diatom cell cycle regulation (Fig. 3), many questions remain unresolved. The functional characterization of *dsCYC2* as a blue light-regulated cell cycle regulator hints at a role for the *dsCYC* genes as signal integrators, but their exact role during the cell cycle and the reason for their expansion remain enigmatic. It is also unclear how the transition from the vegetative phase to other life cycle stages, such as sexual reproduction, resting spore formation, and cell death, is regulated at the molecular level. In addition, it was suggested recently that epigenetic mechanisms might be involved in the regulation of cell division in diatoms (Ashworth *et al.*, 2013). The recent mapping of the methylation patterns of the *P. tricornutum* genome will definitely help to investigate this hypothesis (Veluchamy *et al.*, 2013).

The works discussed above all resulted from so-called reverse genetics experiments. A major drawback of this approach lies in the dependence on what is already known, resulting in a lack of knowledge of novel genes that show no homology to genes in other species. As diatoms possess several unique mitotic structures (De Martino *et al.*, 2009) and have evolved strategies to live in the most extremely varying conditions, it is to be expected that at least part of the unknown sequences, which account for almost half of the gene repertoire (Bowler *et al.*, 2008), could play a role in the regulation of the diatom life cycle. In contrast to reverse genetic techniques that are now well established for diatoms, forward genetic screening in diatoms appears to be more difficult (reviewed in Saade and Bowler, 2009). Forward genetics involves the mutagenization of the cells, followed by screening for altered phenotypes of the mutants. However, due to the lack of a good experimental diatom model in which sexual reproduction can be controlled, and because of the time-consuming and laborious process of mutant mapping, reports on forward genetic screens in diatoms are scarce (Alonso *et al.*, 1996; Huesemann *et al.*, 2009; Saade and Bowler, 2009). Nevertheless, ongoing work may contribute to make this possible in the near future by investigating the potential of *S. robusta*, a pennate diatom in which the sexual cycle can be easily manipulated, as a model system (Chepurnov *et al.*, 2008). *Seminavis robusta* has been the subject of several cell cycle and life cycle studies in our lab during the last years. Gillard *et al.* (2008) described the first genome-wide transcript profiling, using a cDNA amplified fragment length polymorphism approach, of *S. robusta* cells progressing synchronously through the mitotic cell cycle, and ongoing work in this species using the same approach, as well as deep transcriptomic sequencing, aims to uncover the molecular mechanisms associated with the cell size reduction process, the switch from mitosis to meiosis, and sexual reproduction itself. Without doubt, these results and the current genome sequencing of *S. robusta* (A. Bones, personal communication) will shed new light on a set of genes for which no expression or functional data exist. A crucial step towards the exploration of *S. robusta* as a model organism will be the establishment of genetic transformation tools to enable reverse genetic functional analysis of the unknown genes identified in the expression studies. Transformation protocols for *S. robusta* by biolistic particle bombardment, the method routinely used to transform *P. tricornutum* (Falciatore *et al.*, 1999) and *T. pseudonana* (Poulsen *et al.*, 2006), are currently being optimized.

An improved understanding of the diatom life and cell cycle will also require the confirmation of the function assigned to a gene by lab-based studies in the natural environment, e.g. by the development of molecular probes that can selectively detect the gene from environmental samples. In addition, metatranscriptomic approaches (consisting of the large-scale sequencing of transcripts retrieved from natural communities), combined with in-depth analysis of the prevailing physicochemical parameters, hold great promise for understanding the regulation of mixed eukaryotic populations, including diatoms, in response to environmental

variations, and can facilitate functional assignment of specific encoded sequences (Rusch *et al.*, 2007; Yooseph *et al.*, 2007). In particular, this strategy would be extremely valuable for the functional characterization of the large set of unknown and diatom-specific genes (Armbrust *et al.*, 2004; Bowler *et al.*, 2008; Gillard *et al.*, 2008). However, the major bottleneck to apply metatranscriptomics approaches in diatoms comes from the availability of only a few diatom reference genomes to which to anchor the environmental sequences. Nevertheless, the advent of modern, next-generation sequencing techniques that allow high-throughput parallel sequencing at a lower cost might tremendously expand our current diatom genome sequence resources in the coming years.

## Acknowledgements

The authors wish to thank Annick Bleys for help in preparing the manuscript. We thank Olga Chepurnova and Dr Jeroen Gillard for providing pictures of *S. robusta*. This work was supported by a grant from the Research Foundation Flanders (G.0288.13).

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