Running title: The cell and chloroplast cycle of *Seminavis robusta*

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Physiological and transcriptomic evidence for a close coupling between chloroplast ontogeny and cell cycle progression in the pennate diatom *Seminavis robusta* 


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ABSTRACT

Despite the growing interest in diatom genomics, detailed time series of gene expression in relation to key cellular processes, are still lacking. Here, we investigated the relationships between the cell cycle and chloroplast development in the pennate diatom *Seminavis robusta*. This diatom possesses two chloroplasts with a well-orchestrated developmental cycle, common to many pennate diatoms. By assessing the effects of induced cell cycle arrests with microscopy and flow cytometry, we found that division and reorganization of the chloroplasts is initiated only after S-phase progression. Next, we quantified the expression of the *S. robusta* *FtsZ* homolog to address the division status of chloroplasts during synchronized growth and monitored microscopically their dynamics in relation to nuclear division and silicon deposition. We show that chloroplasts divide and relocate during the S/G2 phase, after which a girdle band is deposited to accommodate cell growth. Synchronized cultures of two genotypes were subsequently used for a cDNA-AFLP-based genome-wide transcript profiling, in which 917 reproducibly modulated transcripts were identified. We observed that genes involved in pigment biosynthesis and coding for light-harvesting proteins were up-regulated during G2/M phase and cell separation. Light and cell cycle progression were both found to affect fucoxanthin-chlorophyll a/c-binding protein expression and accumulation of fucoxanthin cell content. Because chloroplasts elongate at the stage of cytokinesis, cell cycle-modulated photosynthetic gene expression and synthesis of pigments in concert with cell division, might balance chloroplast growth and confirms that chloroplast biogenesis in *S. robusta* is tightly regulated.
INTRODUCTION

Diatoms, an extraordinarily diverse group of heterokontophyte microalgae (Kooistra et al., 2003), dominate the primary production of many marine and freshwater ecosystems (Granum et al., 2005). Based on the shape and structure of their unique siliceous cell walls, two major architectural types are recognized: ‘centric’ diatoms, a paraphyletic group with radially patterned valves and ‘pennate’ diatoms, a monophyletic group characterized by a feather-like valve structure. Whole genome sequencing of *Thalassiosira pseudonana* (Armbrust et al., 2004) and *Phaeodactylum tricornutum* (Bowler et al., 2008), representatives of centric and pennate diatoms, respectively, has revealed that they combine plant- and animal-like characteristics and possess many genes that are completely unknown in other organisms. These genome studies enable the unraveling of the genetic basis of the unique properties underlying the ecological and evolutionary success of diatoms. Diatoms have long been known for their extremely high photosynthetic efficiency: compared to other photosynthetic eukaryotic unicells of comparable sizes, diatoms have the highest growth rates (Banse, 1982; Raven and Geider, 1988; Sarthou et al., 2005) and an unusually high photosynthetic flexibility that is essential for coping with large habitat-intrinsic fluctuations in irradiances (Lavaud et al., 2004). Their high growth rates might be attributed to their high rubisco carboxylase efficiency and the putative presence of an alternative C4-photosynthetic pathway, enabling temporary storage of carbon for use at times of low light irradiances (Riebesell, 2000; Wilhelm et al., 2006; Roberts et al., 2007). The photosynthetic flexibility of diatoms is related to their high capacity for energy dissipation through non-photochemical quenching, which can reach a 5-fold higher level than that in plants (Ruban et al., 2004).

Like in other groups of heterokontophyte algae, diatom chloroplasts originate from a secondary endosymbiosis, probably the engulfment of a red alga by a heterotrophic eukaryote (McFadden, 2001; Falkowski et al., 2004). As a result, diatom plastids are surrounded by four membranes instead of the usual two, typical for plants and green algae. Moreover, the outermost pair of membranes is connected with the nuclear envelope in a number of diatoms (Stoermer et al., 1965; Dawson, 1973; Hashimoto, 2005). These characteristics have implications for chloroplast protein targeting pathways (Apt et al., 2002; Kilian and Kroth, 2005) and possibly for its division mechanism and transmission to daughter cells (Hashimoto, 2005). While the chloroplast ultrastructure appears to be fairly uniform in diatoms, there is a wide variation in chloroplast morphology as well as in the number of chloroplasts and their
arrangement within the cells. The chloroplasts of polyplastidic diatoms, which comprise most centric diatoms but also several genera of pennate diatoms, have a very simple morphology (for a review, see Mann, 1996). In contrast, chloroplasts of mono-, di-, and tetraplastidic diatoms often have more elaborate shapes and can undergo complex changes in morphology and arrangement before and/or after cytokinesis. Chloroplast division involves constriction of the parent chloroplasts into two more or less equally sized daughter chloroplasts. Two types of chloroplast division are recognized among diatoms, referred to as autonomous and imposed division (Mann, 1996). In the autonomous type, believed to be the primitive condition, the chloroplast constricts into two without the obvious involvement of any other organelle. In imposed division, which appears to have evolved several times in different lineages of pennate diatoms, chloroplast division occurs synchronically with the formation of the cleavage furrow. In plants and some other eukaryotes, chloroplast division is orchestrated by a prokaryotic-derived division machinery, whose regulation is not fully understood (Osteryoung and Nunnari, 2003; Margolin, 2005; Adams et al., 2008) but which should somehow be coordinated with the regulation of the cell cycle machinery that enables to pace cell division rate on the surrounding environments and coordinates critical processes such as cell growth, karyokinesis, and cytokinesis (De Veylder et al., 2007).

Environmental factors mostly impinge on cell cycle control through cell cycle checkpoints at the G1/S or G2/M cell cycle phase transitions. Many universal core cell cycle genes that regulate these transitions have been found in the two sequenced diatom genomes (Armbrust et al., 2004; Bowler et al., 2008), but diatom-specific genes and gene regulations may contribute to the unique features that distinguish diatoms from other algae (Montsant et al., 2007; Huysman MJJ, Martens C, De Martino A, Rayko E, Allen EA, Gillard J, Heijde M, Mathieu B, Meichenin A, Montsant A, Siaut M, Vandepoele K, Van de Peer Y, De Veylder L, Inzé D, Bowler C and Vyverman W, unpublished data). Chloroplast development is difficult to address in polyplastidic cells, such as centric diatoms and cells of higher plants (Pyke, 1999). However, the observed coordination of chloroplast development with cell cycle progression in many pennate diatoms creates an opportunity to address more easily chloroplast development in relation to other cellular processes, because if chloroplast ontogeny is regulated by the cell cycle, the controlled progression of chloroplasts can be studied in synchronized cultures.

Here, we combined physiological experiments, cytological observations, and a cDNA-AFLP-based genome-wide transcriptome analysis to identify cell cycle-dependent checkpoints in chloroplast development and according gene expressions during synchronized growth. As a
model species, we used *Seminavis robusta* Danielidis & D. G. Mann (Danielidis and Mann, 2002), a representative of the diverse and ecologically important group of pennate *Naviculaceae*. As in most genera of this family, the two chloroplasts in *S. robusta* divide in an autonomous manner and move from the girdle to the valves and back, once during each cell cycle (Chepurnov et al., 2002) (Supplemental Fig. S1). *S. robusta* is particularly well-suited for cytological studies because of its large size (up to 100 µm) and, being a benthic species growing attached to surfaces (Thompson et al., 2008), the easy and non-intrusive observation of the behavior of cells. Moreover, sexual reproduction can be controlled easily, resulting in the first diatom pedigree that currently comprises approximately 110 clones (Chepurnov et al., 2008). Therefore, this species offers interesting perspectives for functional genomics studies as well as for forward genetics to investigate key life history traits, including the dynamics of their photosynthetic apparatus and its interaction with other cellular processes and the environment.

RESULTS

Cell and chloroplast division are both arrested in response to darkness and cell cycle inhibitor treatments

Using light deprivation and two cell cycle inhibitors, we investigated whether a relationship exists between the cell cycle checkpoints and the chloroplast division cycle. For light deprivation, exponentially growing cultures were transferred to complete darkness for 24 h and compared with light-grown control cultures by flow cytometry (Fig. 1 A). The tetraploid peak (G2+M-phase) present in the light-grown cultures was absent in the 24 h dark-treated cultures, indicating that in the latter the majority of cells were arrested in the G1-phase of the cell cycle. Microscopically, the light-grown cultures consisted of both dividing and non-dividing cells (Fig. 1 B). In contrast, the dark-arrested cultures only contained non-dividing cells (Fig. 1 C). These cells have their two chloroplasts undivided and located at the girdle sides of the cell (Fig. 1 D to G). In most cases, the ventral chloroplast had four sub-central lobes that, across the valves, reached the dorsal side of the cell (Fig. 1 F and G). Occasionally, some post-cytokinetic doublet cells could also be found in the dark-arrested cultures. These doublet cells are at a stage after cytokinesis, but before cell separation, during which the cell is devoted to frustule formation (Coombs et al., 1967a). Microscopically, the
doublet cells in dark-arrested cultures contained the G1-type of girdle-located undivided chloroplasts, previously described.

Next, we studied chloroplast behavior upon treatment with chemical cell cycle inhibitors. First, hydroxyurea (HU) was applied to exponentially growing cultures, thereby inhibiting S-phase progression by depleting the cell of dNTPs (Young and Hodas, 1964). A complete S-phase arrest of cell division by HU was confirmed by flow cytometry after 72 h treatment (Supplemental Fig. S2), while the cultures remained healthy, because cell growth restarted after the inhibitor had been washed away (data not shown). The cells in these S-phase-arrested cultures all contained girdle-located undivided chloroplasts like in dark-arrested G1-phase cells, but the sub-central lobes were not observed (Fig. 2 A). From these observations, we conclude that chloroplast division and movements do not take place before the S-phase. However, these results should be interpreted with caution: because HU depletes the cell’s dNTP pool, chloroplast DNA replication might be affected by the drug and thus inhibition of plastokinesis might be a direct effect of the chloroplast’s inability to replicate its DNA. To validate the findings, aphidicolin, another cell cycle S-phase inhibitor that blocks the nuclear-specific DNA polymerase α (Ikegami et al., 1978) was applied on dark-arrested cultures that were re-illuminated immediately after addition of the drug. Microscopic analysis after 24 h revealed that treated cells did not divide and contained undivided chloroplasts located at the girdle sides (Fig. 2 B-D), while control cultures divided normally. In contrast with HU-arrested cells, ventral chloroplast lobes were observed during aphidicolin arrest (Fig. 2 D).

**Cytological changes during cell cycle progression in synchronized cultures**

Based on the uniform cell cycle arrest in dark-arrested cultures, we established a synchronization procedure to study cell cycle-modulated processes. To this end, exponentially growing cultures (12:12 L:D) were transferred into darkness for a period of 24 h and re-illuminated for 12 h. Two monoclonal strains, designated F1-8B and F1-9A, were synchronized in this manner and were used for the transcriptome analysis described below. Re-illumination resulted in the synchronous re-activation of cell cycle progression, starting from the G1-phase of the dark-arrested cells. Synchrony was evaluated by estimating the amount of dividing cells at hourly intervals, complemented by observations of chloroplast dynamics (Fig. 3). Dividing cells were operationally defined to include only doublet cells, characterized by the presence of a cleavage furrow, which is visible as an area of high contrast
separating the chloroplast pairs (see Supplemental Fig. S3 for an illustration of the counting procedure). Immediately upon re-illumination, all cells were in the G1-phase, containing undivided chloroplasts as previously shown. After 4 h, few cells with divided chloroplasts appeared in the cultures and after 6 h some cells were dividing. From 6 h onward the cultures contained S, G2 and M-phase cells, in which the proportion of dividing cells progressively increased until 50%, at the 9 h sampling point. At 9 h, the amount of dividing cells decreased as a consequence of daughter cell separation and initiation of the second cell division cycle.

The phenotypic cell cycle events during synchronization of the cultures were further characterized with confocal laser scanning microscopy and an appropriate set of stains for the nucleus (SYBRsafe™) and the cell wall (PDMPO, 2-(4-pyridyl)-5-((4-(2-dimethylaminoethylamino-carbamoyl)methoxy)phenyl) oxazole). Besides fluorescent cell wall labeling in diatoms (Shimizu et al., 2001; Leblanc and Hutchins, 2005), PDMPO visualizes acidic organelles (Diwu et al., 1999) and monitors the location of newly incorporated silica.

As shown previously by Chepurnov et al. (2002), chloroplast division occurs by central constriction (Fig. 4 A) and upon completion of constriction of the dorsal (Fig. 4 B) and ventral chloroplasts (Fig. 4 C), the daughter chloroplast pairs move bodily in opposite directions, describing a circular motion along the periphery of the cell, until they position against the valves (Fig. 4 D and E). By comparing these cells with the dark-arrested cells in valve and girdle view (compare Fig. 1 G with Fig. 4 D and Fig. 1 E with Fig. 4 E), the difference in location between undivided and divided chloroplasts could easily be seen. During this “reorganized chloroplast configuration” stage, the nucleus, positioned in the center of the cytoplasm, was still undivided (Fig. 4, E). Taking into consideration that the chloroplasts divide after S-phase initiation, this shows that plastokinesis and subsequent chloroplast relocation occur during S/G2-phase and is fully established before M-phase.

Before signs of mitosis appeared, but after chloroplast rearrangement, the first PDMPO signal was also detected as an elliptically-shaped thin band (Fig. 4 F). This signal probably represents a girdle band (see also Supplemental Fig. S4) that is added to the girdle as a result of cell growth. Chloroplast reorganization was followed by karyokinesis (Fig. 4 G) and cytokinesis (Fig. 4 H to K). Early on during cytokinesis, PDMPO fluorescence was initially detected inside two “vacuole-like” compartments located against the division plane running from pole to pole (Fig. 4 H), while a PDMPO stained cell wall was not yet visible. Since the fluorescence emission and intensity of PDMPO typically shifts when the intra-compartmental silica concentration increases above 3.2 mM (Shimizu et al., 2001; Hazelaar et
the observed fluorescence of PDMPO in these compartments could hypothetically be paralleled with silicon influx in preparation for silica deposition during the subsequent formation of the frustule. The deposition of cell wall material was observed as a straight line that ran between the two daughter nuclei and located at the same place of the previously observed cleavage furrow (Fig. 4 I). Only after this initial deposition of silica, while both cells were still attached to each other, the chloroplasts moved back from the valves to the ventral and dorsal girdle sides (Fig. 4 J). During this last relocation event, each chloroplast elongated until it completely covered the girdle area. Cell division was finalized when daughter cells separated (Fig. 4 K), creating two new cells containing one unstained valve, inherited from its mother, and one newly built valve stained with PDMPO (Fig. 4 L).

**Cell cycle-modulated expression of the *S. robusta* chloroplast division protein FtsZ**

The microscopically derived indications of cell cycle phase-dependent chloroplast division were validated by transcript quantification of the bacterial cell division homolog *FtsZ* in synchronized *S. robusta* cultures. Transcription of the *S. robusta FtsZ* gene was measured with a set of nested degenerate primers that, upon amplicon cloning and clone sequencing, enabled the design of a specific primer pair suited for real-time (RT) quantitative (Q) PCR (Supplemental Fig. S5). Three constitutively expressed genes were used for data normalization (see “Materials and Methods”) and RT-PCR of the *S. robusta FtsZ* ortholog was done on two replicated synchronizations of strain F1-8B (Fig. 5). Both showed that *FtsZ* expression is modulated during synchronization. Its expression 5 to 8 h after re-illumination increased 2.5- to 4-fold when compared to its average expression between 0 to 4 hours. In both replicates, *FtsZ* was maximally expressed at 7 h post-darkness, being 2 h before most cells were dividing.

**cDNA-AFLP expression profiling during cell cycle progression**

In parallel with the above-described sampling (Fig. 3) for cytological observations, samples were taken at 1-h intervals to conduct a cDNA-AFLP-based genome-wide transcript profiling to identify modulated gene expression profiles correlated with the cytological changes during the cell cycle. The complete data set, including annotations and expression data is available online (Supplemental Table S1).
The cDNA-AFLP analysis resulted in 2,908 transcript-derived fragments (TDFs) that were scored quantitatively. A considerable amount of expression variation between the two strains originated from genotype-dependent DNA polymorphisms in the transcripts, resulting in the absence/presence of cDNA-AFLP fragments (see Supplemental Fig. S6 for an example of a cDNA-AFLP electropherogram) (Vuylsteke et al., 2006). These expression profiles are of no value for expression analysis and were removed from the dataset; only expression profiles that were highly reproducible (Pearson’s correlation; $P<0.05$) across the two strains were kept for further analysis. In this manner, 955 TDFs were selected and used for adaptive quality-based clustering (De Smet et al., 2002). With high stringency settings (see “Materials and Methods”), 917 TDFs were grouped in nine expression clusters (designated C1 to C9) (Supplemental Fig. S7). Figure 6 shows the expression patterns of all TDFs during the cell cycle of both genotypes by hierarchical clustering (Eisen et al., 1998). TDFs in clusters C1 and C2 had their maximal expression during darkness (0 h) and the level of expression decreased drastically within the first few hours post-darkness. Expression of TDFs in clusters C3 and C4 was induced after illumination, peaking at respectively 2 and 3 h post-darkness. Average peak expression in cluster C5 occurred at 4-6 h post-darkness, corresponding with the initiation of chloroplast division and the appearance of the first dividing cells. This cluster contained all identified TDFs with nucleotide binding regions (Table I). Two of these were the universal S-phase-specific genes, histone H3 and MCM5. Histone H3 (Sr025) (Kapros et al., 1992; Menges et al., 2002) was maximally expressed at 7 h (Supplemental Fig. S8). The universal DNA replication licensing factor MCM5 (Sr027) had a discrete expression at 4 h. This latter gene is specifically involved in the initiation of DNA synthesis and unwinding of the DNA at the replication forks and has been shown to be temporally expressed at G1/S transition (Tsuruga et al., 1997; Takisawa et al., 2000). Therefore, the expression of the MCM5 ortholog represents a good marker for S-phase initiation and was used to demarcate G1 and S-phase during synchronization. While genes in cluster C6 were discretely expressed at 7 h, genes in cluster C7 had a broader expression range. They were induced at 6 h, in correspondence with the first occurrences of dividing cells, until the 12th h at the moment of cell separation. Genes in this cluster were expressed simultaneously with the enrichment of G2-phase cells and dividing cells. They consisted of an early and late expression subgroup, indicated as C7-Early (C7-E), containing genes induced at 6 h, and C7-Late (C7-L), containing genes induced at 8 h. Cluster C8 and C9 comprised a rather small number of expression profiles, peaking at three (3 h, 7 h and 10 - 12 h) and two (0 h and 12 h) non-consecutive time points, respectively.
In total, 378 (41%) of all TDFs with reproducible expression profiles across the two genotypes were selected for sequencing. Since we were interested primarily in the progression of the cell cycle, proportionally more TDFs were selected from cluster C5 and C7, where expression was up-regulated after re-illumination (Supplemental Table S2). Good quality sequences were found in 322 TDFs (85%) of which 100 with significant (E-value < 10⁻³) similarity (Table I) with genes in the in-house constructed database (see “Material and Methods”). Expression patterns of these annotated TDFs are presented in Supplemental Figure S9. Seventy-two TDFs showed homology with a gene with a putative allocated function, 18 were homologous with sequences without an allocated function (hypothetical proteins), and 10 TDFs matched with diatom orphan genes, for which no homologous counterparts exist (Table I). These latter genes were all confined to the late phases of the cell cycle, indicating that diatom-specific genes might be functionally associated with mitosis and cell separation. Based on the gene homology and the mapping of Gene Ontology (GO) labels and InterPro domains (see “Material and Methods”; Supplemental Tables S3 and S4), the TDFs with annotated functions were classified into 11 distinct functional groups (Table I).

**Modulated expression of genes implicated in cell metabolism**

The majority of the TDFs for which GO labels were found, were involved in cellular and metabolic processes (Supplemental Fig. S10). TDFs involved in protein biosynthesis were dominant in S-phase cluster C5. Two genes were found involved in amino-acid synthesis, namely N-acetyl-γ-glutamyl-phosphate reductase (Sr029) and the bifunctional ATP sulfurylase-adenosine 5’-phosphosulfate kinase (Sr033). Various components of the translation machinery dominated this cluster: three TDFs encoded ribosomal proteins (Sr030, Sr031, and Sr032) and one aspartyl-tRNA-synthetase (Sr034). A translation initiation factor 3 (Sr050) was induced shortly after these genes, in cluster C7-E. In addition, a total of 9 post-translational modification proteins were identified (Sr001, Sr002, Sr009, Sr010, Sr028, Sr047, Sr048, Sr049 and Sr073). Four of these genes were expressed in cluster C7-E but two of them, a clpX-homolog (Sr001) (McGrath et al., 2006) and a ubiquitin-conjugating enzyme (Sr002), were highly expressed in the G1-phase cluster C2 when cells were released from the dark and were rapidly down-regulated after the first hour of light. A series of hypothetical proteins with undefined function displayed an identical regulation.

Several genes of the chloroplast-localized fatty acid biosynthetic pathways (Ohlrogge and Browse, 1995) were modulated during different phases of the cell cycle. β-hydroxyacyl-
ACP-dehydratase (Sr023) was identified in cluster C5, while two fatty-acid desaturases (Sr044; Sr045) were expressed some time later in cluster C7-E. Transcription of glycerol-3-phosphate dehydrogenase (Sr091), also referred to as dihydroxy-acetone phosphate (DHAP) reductase (Gee et al., 1988), was activated during cell separation in cluster C9, starting at 10 h after re-illumination.

For driving the anabolic pathways of protein and fatty-acid synthesis, the cell depends on an acetyl-CoA pool, which can be produced by the aerobic oxidation of carbohydrates (Fernie et al., 2004). In this respect, the glycolytic enzyme enolase (Sr017) was expressed in cluster C5 together with the mitochondrium-localized enzyme isocitrate dehydrogenase (Sr015) and a mitochondrial phosphate-carrier protein (Sr016), both needed during active citric acid cycling.

Two TDFs (Sr051 and Sr095), a subunit of the vacuolar-type H⁺-ATPase and a vacuolar type H⁺–pyrophosphatase, are known to play a role in vacuolar transport. More particularly, in being responsible for the acidification or maintenance of the acidity of organelles (Maeshima, 2001). Sr051 was activated during G2/M-phase in cluster C7-E, while Sr095 was initially expressed slightly during darkness and again highly abundant during cell separation (cluster C9).

Modulated expression of photosynthesis-related genes and genes involved in chloroplast movement

Fifteen TDFs were assigned to the functional category of photosynthesis (Table I). Using a X² correlation test (Table S5), this functional category was found to be dominantly expressed (p< 0.001) in cluster C7-L; one TDF was found in C7-E and two in C9. Nine copies of the chromophyte-specific fucoxanthin-chlorophyll a/c-binding proteins (FCPs) (Green and Durnford, 1996) were identified and they were complemented with four TDFs involved in the biosynthesis of their bound photosynthetic pigments: porphobilinogen synthase (Sr063), protoporphyrinogen IX oxidase (Sr068), and protoporphyrin IX magnesium chelatase subunit H (Sr046). They all encoded enzymes from the chlorophyll a/c-biosynthetic pathway (Vonwettstein et al., 1995). In addition, a ζ-carotene desaturase (Sr062) was identified, being an intermediate enzyme in the biosynthetic pathway of β-carotene and other carotenoids, such as fucoxanthin (Wilhelm et al., 2006). Several Calvin cycle enzymes were also expressed in C7-L and C9: phosphoribulokinase (Sr070), transketolase (Sr093), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Sr089). However, because GAPDH is also an enzyme
involved in glycolysis, it was assigned to the functional category of carbohydrate metabolism. Four genes operating in reactive oxygen metabolism were found in cluster C7-L: one TDF (Sr074) was highly similar to manganese superoxide dismutase (MnSOD) (Wolfe-Simon et al., 2006) and three TDFs (Sr075; Sr076; Sr077) to the peroxisomal membrane protein MPV17/PMP22 which is known to up-regulate activity of SODs in animal cells (Iida et al., 2003). These genes probably relate to the photosynthetic ROS generation, which is an inevitable part of the oxygenic photosynthesis (Horton et al., 1996; Apel and Hirt, 2004). β-tubulin (Sr019), which plays a fundamental role in cytoskeleton-based cellular movements, was highly induced from 4 h and maximally expressed at 6 h post-darkness. This induction (clustered within C5) slightly preceded chloroplast reorganization. Another TDF with similarity to a WD40-repeat protein (Sr018), putatively involved in cytoskeleton assembly, was analogously expressed; both genes were down-regulated after 6 h post-darkness but β-tubulin was again slightly induced at 8 h, in parallel with cell division (Supplemental Fig. S11). The involvement of microtubule-based cytoskeleton dynamics during chloroplast movement was expected and further validated by treatment of synchronized cultures with the microtubule inhibitor nocodazole. Nine hours after treatment, chloroplast division and reorganization were impaired when compared to untreated control cultures. Only after 26 h, chloroplast division and reorganization did proceed, arresting the cells at G2+M-phase (data not shown).

**Cell cycle versus light-regulation of photosynthesis and pigment biosynthesis**

The microscopic observations (Fig. 4) showed that chloroplasts elongated after cell division initiation when they relocate to the girdle sides of the cell. To investigate whether this elongation is paralleled with chloroplast growth, we determined fucoxanthin pigment turnover and validated the fcp expression with RT-(q)PCR in synchronized cultures. In addition, these data were compared with those from synchronized cultures to which the cell cycle inhibitor aphidicolin had been added shortly before re-illumination (Fig. 7). This was done to assess the dependency of pigment turnover and fcp expression on cell cycle progression versus light regulation.

Upon re-illumination, fucoxanthin content per cell increased under both conditions, but the trend was more pronounced in the dividing cultures than in the S-phase-arrested cultures. This fucoxanthin accumulation in the dividing cultures was obvious after 5 h of light and decreased after 10 h, corresponding with cell separation (Fig 3). In the S-phase-arrested
cultures, the accumulation curve was more gradual, without a clear peak and a smaller maximum. Although the difference between both profiles was obvious, a longitudinal ANOVA test was unable to prove its significance, which could be due to the small number of replicates.

In parallel, in response to re-illumination in both cultures, fcp expression was activated and subsequently down-regulated after 5 h in the arrested cultures, whereas, in cell cycle-progressing cultures, its expression was maintained throughout the 13-h time course (Fig. 7). These findings suggest that chloroplast growth occurs in advance of chloroplast elongation at cytokinesis, while chloroplast light-harvesting complexes are regulated by both the cell cycle and light.

**DISCUSSION**

**Regulation of chloroplast dynamics with respect to cell cycle progression and frustule silicification**

In diatoms, chloroplast division and development have not yet been considered in relation to cell cycle regulation. For example, darkness was shown to arrest the cell cycle in several diatom species (Vaulot et al., 1986; Brzezinski et al., 1990), but microscopic observations of the effect on chloroplasts are, to our knowledge, completely lacking. A putative reason is that mostly polyplastidic centric diatoms have been studied in which the small chloroplasts are stochastically partitioned upon cell division and, hence, have not such an obvious developmental cycle. In addition, the fact that flow cytometry is traditionally preferred to investigate cell cycle effects might have contributed to the general neglect of morphology and cytology.

As shown here for the pennate diatom *S. robusta*, chloroplast division and development are intimately linked with cell cycle progression. First, conditions that induce an arrest at the G1-to-S cell cycle checkpoint, i.e. light deprivation, hydroxyurea or aphidicolin arrest the development of chloroplasts at the plastokinesis stage, without inducing aberrant cell morphologies. Second, both divided chloroplasts rotate from the girdle to the valves before karyokinesis is initiated. And, at last, the chloroplast division homolog *FtsZ* is expressed in concert with chloroplast division in a cell cycle phase-dependent manner, during the S/G2 phases. We conclude that the G1-to-S-checkpoint controls chloroplast division and relocation and enables their synchronous division in *S. robusta* cultures. Previous
observations of chloroplast dynamics during the cell cycle in *S. robusta* had already shown that chloroplasts and nucleus divide in a coordinated manner (Chepurnov et al., 2002). Now, we observe that both also depend on each other and that common mechanisms to regulate their division should be involved. Similarly, in the red alga *Cyanidioschyzon merolae*, chloroplast and mitochondrium divisions are regulated at distinct cell cycle checkpoints (Nishida et al., 2005). However, in contrast with aphidicolin-arrested *S. robusta* cells, in *C. merolae* the mitochondrial division is restrained, while the chloroplast divides multiple times (Itoh et al., 1996; Nishida et al., 2005).

Since its initial discovery in *Arabidopsis thaliana* (Osteryoung and Vierling, 1995), homologs of the bacterial cell division protein FtsZ, are held responsible for organelle division in many other eukaryotes (Beech et al., 2000; Takahara et al., 2000; Kiefel et al., 2004) that occurs by FtsZ assembling into the bacterial cytokinetic apparatus, called the Z-ring (Osteryoung and Nunnari, 2003; Margolin et al., 2005). Accordingly, FtsZ expression is modulated during growth of *Nicotiana tabacum* BY2 (El-Shami et al., 2002) and *C. merolae* (Takahara et al., 2000; Nishida et al., 2005;) in synchronized cultures, in a circadian rhythm in *Chlamydomonas reinhardtii* (Hu et al., 2008) and in light/dark-synchronized cultures of *S. robusta* as well. Its expression is up-regulated after S-phase progression and prior to cell division, which agrees with microscopic observations. This shows that the chloroplast developmental cycle can be monitored unmistakably through synchronization of cell division in *S. robusta*.

Control of chloroplast division is governed by conserved cell cycle regulators of bacterial origin (Adams et al., 2008). One of these, the chaperone ClpX, identified here, was down-regulated upon re-illumination when cells re-enter the cell cycle. ClpX has been found to inhibit Z-ring formation in *Bacillus subtilis* (Weart et al., 2005) and ClpX-mediated proteolysis of the cell cycle master regulator CtrA initiates chromosome replication in *Caulobacter* (McGrath et al., 2006). It is therefore tempting to suggest that the activation of ClpX in a dark-arrested cell ensures the inhibition of plastokinesis in *S. robusta*. Another resemblance can be found with the regulation of plastokinesis at G1/S-transition in *Arabidopsis thaliana* (Raynaud et al., 2005), in which chloroplast division was regulated by the activity of the pre-replication factor, CDT1. In *S. robusta*, chloroplast division was paralleled by activation of the S-phase-specific gene MCM5, which is also a universal member of the pre-replication complex. Furthermore, our observations also suggest that ‘retrograde’ mechanisms (Koussevitzky et al., 2007) - involving signaling from the chloroplast to the nucleus – might be present as another way for coordinating the chloroplast
and nuclear division cycle. Retrograde signaling was, for example, reported in response to membrane tension as a possible way to regulate shape, size, and division of chloroplasts in *A. thaliana* (Haswell and Meyerowitz, 2006). Analogous feedback mechanisms might exist within diatoms, for example, to control G2/M-transition in response to the chloroplast developmental status, ensuring high-quality chloroplast segregation of daughter chloroplasts to the newly divided cells. In any case, control mechanisms may not be as strict in centric polyplastidic diatoms because - similarly to what is known for land plants - a stochastic partitioning of chloroplasts upon cell division will hardly result in cells without any chloroplast (Coleman and Nerozzi, 1999).

The molecular mechanism of chloroplast movement in diatoms is still unclear. In polyplastidic diatoms, the red alga *C. merolae*, and plants, chloroplast movements are known to be actin-dependent (Francisco and Roth, 1977; Nishida et al., 2005; Krzeszowiec et al., 2007). The transcriptional induction of β-tubulin in *S. robusta* suggests the involvement of microtubules during chloroplast rearrangement but does not exclude that actin may also be involved.

The intriguing question remains why pennate diatoms display this –sometimes complicated– cycle of repositioning the chloroplast during cell cycle progression. One possibility is that position of the chloroplasts at the valves might create a disadvantage for cell movement needed for adequate reaction upon environmental factors, such as light and nutrients (Cohn, 1992). Because cells glide across the surface by mucilage secretion from a valvar slit (called the raphe) (Hoagland et al. 1993; Wetherbee et al. 1998; Chiovitti et al. 2006), the underlying actin-myosin system that is held responsible for the raphe’s functionality could be impaired by a valvar location of the chloroplasts. In cultures of *S. robusta*, cells with a “reorganized chloroplast configuration” have indeed never been observed moving. This reasoning was first introduced in Mereschkowsky’s ‘law’ on diatom chloroplasts, which states that chloroplasts of diatoms have a tendency to leave the raphe as much as possible uncovered (Mereschkowsky, 1904). This is achieved in numerous diatoms (*Brebissonia* spp., *Cymbella* spp., *Gomphonema* spp., *Dickiea ulvacea*, *Didymosphenia* spp. and *Lyrella* spp.) by plastid invaginations beneath the raphe (Mann, 1996), whereas in other diatoms, such as in *S. robusta*, by positioning of the chloroplasts at the valves only during a short time of the cell cycle. As observed here, this positioning at the valves is necessary only to free the girdle area for addition of girdle bands, cytokinesis and frustule formation. The identified girdle bands were indeed observed late during the cell cycle, in cells with a “reorganized chloroplast configuration”. As such, the cyclic movement of chloroplasts could
create a large enough time frame for a cell to position itself optimally within the environment, maximally favoring cell growth, before cell division is committed. It is an interesting speculation that because centric diatoms are planktonic, lack a raphe, and are thus unable to move, they did not evolve such a well-orchestrated chloroplast development cycle. Also, variations in the chloroplast cycle in diatoms might explain the differences in timing of girdle band deposition among species (Coombs et al. 1967b; Kröger and Wetherbee 2000; Chiappino and Volcani 1977; Hildebrand et al., 2007). For example, Thalassiosira pseudonana which is polyplastidic has been illustrated recently to synthesize its girdle bands already during G1-phase (Hildebrand et al., 2007).

Girdle bands accommodate cell growth and, like frustules, are formed by polymerization of silica in a silica deposition vesicle (SDV) that is exocytosed (Pickett-Heaps et al. 1990; Kröger and Wetherbee 2000; Zurzolo and Bowler, 2001). Not much is known about the origin of the SDV, but it probably originates from other internal vesicle compartments, such as the endoplasmic reticulum, Golgi and lysosome (Lee and Li, 1992). In this regard, the TDF Sr051, identified as a subunit of the vacuolar-type H⁺-ATPase, is of particular interest because such energy-consuming proton transport proteins have a known functionality in the acidification of organelles (Maeshima, 2001). It was maximally expressed 1 h before initiation of cell separation (at 8 h post-darkness) when also the two “vacuole-like” compartments were visible by PDMPO accumulation. Because diatom silicification occurs inside an acidic SDV (Mayama and Kuriyama, 2002), the V-type H⁺-ATPase could be related to girdle or valve formation at the stage of SDV acidification. A P-type ATPase was recently also hypothesized to be involved in SDV formation, during expansion of its plasma membrane in T. pseudonana (Frigeri et al., 2006). Possibly both identified V-type and P-type ATPases are required in parallel during valve formation in diatoms.

**Cell cycle synchronization of S. robusta**

In many studies using phytoplankton cultures (reviewed by Pirson and Lorenzen, 1996; Tamiya, 1966; Krupinska and Humbeck, 1994), the endogenous cell cycle control mechanisms that respond to naturally phased light/dark conditions are exploited to study cell division in synchronous cultures (Otero and Goto, 2005). Diatoms are known to be capable of sustaining long periods of darkness and retain the ability to start growing rapidly upon re-illumination (Furusato et al., 2004). The synchronization protocol established here for S. robusta relies on this apparently fast release from the dark-arrested G1-phase. And as a result
of the identified common regulatory mechanisms in chloroplast division and cell cycle progression, this procedure was found successful for studying both processes simultaneously.

The uniformly dark-induced G1-phase arrest in *S. robusta* appears unusual when compared to other diatoms. The centric diatom *Thalassiosira weissflogii* accumulates, besides G1-phase cells, 60% G2+M-phase cells (Olson et al., 1986; Vaulot et al., 1986; Brzezinski et al., 1990) and other centrics (*Chaetoceros muellerii*, *Thalassiosira pseudonana*, *Chaetoceros simplex*, and *Minutocellus polymorhus*), plus one pennate (*Cylindrotheca fusiformis*), all accumulate G1-phase cells together with a smaller fraction (approximately 10%) G2+M-phase cells (Brzezinski et al., 1990). The pennate diatom *Phaeodactylum tricornutum* is the only diatom reported without dark-sensitive G2+M-phase and its facultative silicon requirement has been put forward as the main reason for this observation (Brzezinski et al., 1990). However, in diatoms the G2+M-phase fraction in flow cytometric signatures includes, besides G2- and M-phase cells, also post-cytokinetic doublet cells, which have completed cell wall formation but have not yet separated. Therefore, the “G2+M-phase” fraction in the dark-arrest experiment by Brzezinski et al. (1990) might have been post-cytokinetic doublet cells. At least, in *S. robusta* were the occasionally detected G2+M-phase cells at 24 h dark-arrest microscopically observed to be all post-cytokinetic doublet cells containing girdle-located chloroplasts, typical for G1-phase cells. Apparently, only the process of cell separation and not cytokinesis is hampered in these cells by the absence of light. Therefore, we suggest that it might be useful to address cell separation in diatoms analogously as in yeast cells, where it is defined as a post-mitotic phase and known to be molecularly distinct from the septum formation process during M-phase (Yeong, 2005; Sipiczki, 2007).

Based on the expression of the pre-replication factor MCM5 and the occurrence of dividing chloroplasts, the duration of the G1-phase in *S. robusta* can be estimated to last for 4 h (33% of the total division time) during synchronization under the applied culture conditions. Since the last 3 h were dominated by separating cells, 5 h (41% of the total division time) are left to fulfill the S, G2, and M-phases. Our results suggest that cell division during synchronization occurred faster (<12 h) than during normal exponential growth, even when compared to the highest recorded maximum division rate for *S. robusta*, being 16.6 h division\(^{-1}\) (approximately 1.5 division day\(^{-1}\) under continuous light at 200 μE). As suggested (Olson et al. 1986; Vaulot et al., 1986), part of the G1-phase might already be accomplished during dark arrest. Taking into account this comment, our microscopically estimated cell cycle stage durations of *S. robusta* correspond roughly with the flow-cytometric estimations on *P. tricornutum*, reported to spend half its cell division time in G1-phase (Brzezinski et al. 1990).
Gene expressions in relation to chloroplast functioning and photosynthesis

The overall results from the cDNA-AFLP experiment confirm the hypothesis that several biological processes are under temporal transcriptional control during the cell cycle of diatoms, as shown previously in similar studies on higher plants and animals (Cho et al., 2001; Menges et al., 2002; Breyne et al., 2002). One of the most distinct patterns in gene modulation concerns those genes encoding chloroplast proteins, associated with the functioning of chloroplasts during photosynthesis, including light-harvesting proteins, biosynthetic pigment enzymes, and genes coping with oxidative damage. Nearly all these genes were expressed during G2/M-phase progression, and clustered into the expression clusters C7-L. Most notably was the expression of fucoxanthin-chlorophyll a/c-binding proteins (FCPs), a chromophyte-specific type of light-harvesting proteins (Bhaya and Grossman, 1993; Eppard and Rhiel, 1998; Eppard and Rhiel, 2000; Guglielmi et al., 2005) in concert with cell division.

Regulation of fcp expression is known to modulate light-harvesting primarily during photo-acclimation independent of developmental processes (Falkowski and LaRoche, 1991; Durnford and Falkowski, 1997; Leblanc et al., 1999; Oeltjen et al., 2002; Oeltjen et al., 2004; Siaut et al., 2007). Nevertheless, cell and life cycle dependency of photosynthesis has been shown in several algae, such as Scenedesmus sp. (Post et al, 1985; Kaftan et al., 1999; Tukaj et al., 2003; Setlikova et al., 2005), Euglena gracilis (Winter and Brandt, 1986) Chlorella (Butko and Szalay, 1985) and the pennate diatom Cylindrotheca fusiformis (Claquin et al., 2004).

In S. robusta, FCP expression was found to be light activated in the first place, confirming previous results in centric (Leblanc et al., 1999; Oeltjen et al., 2002; Oeltjen et al., 2004) and pennate diatoms (Siaut et al., 2007). But, after 5 h, its expression became cell cycle dependent because it was maintained in dividing cultures while being repressed in S-phase arrested cultures. A possible molecular mechanism for explaining this difference relates to the retrograde inhibition of light-harvesting proteins known in C. reinhardtii and A. thaliana. In these organisms, light-induced expression of light-harvesting proteins is repressed by accumulation of the chlorophyll precursor Mg-Protoporphirine IX (Johanningmeier and Howell, 1984; Nott et al., 2006). Its accumulation, leakage from the chloroplast, and nuclear repression of photosynthetic genes are thought to occur during reduced chloroplast function to coordinate pigment biosynthesis with expression of light-harvesting proteins (Nott et al., 2006). In an analogous way, if chloroplast development is impaired in S. robusta a retrograde
signal might repress fcp. The fact that fucoxanthin does not decrease together with fcp, could be a result of the low turnover rates of diatom pigments, ranging from days to weeks (Riper et al., 1979; Goericke and Welschmeyer, 1992), compared to the low stability of messenger RNA.

A recent study of the circadian variations in pigment content in the pennate diatom P. tricornutum (Ragni and Ribera d’Alcà, 2007) showed that pigment synthesis follows the somatic growth preceding cell division. Furthermore, they observed that a pronounced circadian pattern of pigment synthesis was lost when cultures were continuously illuminated and became asynchronous. These studies support our findings that fucoxanthin content increases in preparation of cell division, until it is distributed into daughter cells.

Taking together our microscopic analysis, cDNA-AFLP results, and pigment accumulation patterns, we suggest that cell cycle-modulation of photosynthetic light-harvesting occurs in concert with chloroplast growth to prepare for chloroplast elongation at cytokinesis. Because chloroplasts in S. robusta elongate at the stage of cytokinesis, regulated synthesis of more functional light harvesting complexes would be expected to provide a balanced growth of chloroplasts at this stage, thereby optimizing the cell’s photosynthetic capacity during the subsequent G1-phase. Together with light-acclimation and circadian regulation, cell cycle-modulated biosynthesis of the large amount of pigments that are contained within diatoms (Chan, 1978, 1980; Falkowski et al., 1985; Tang, 1996) could as such coordinate cellular investments and improve cell fitness.

MATERIAL AND METHODS

Cell culture and image acquisition

The Seminavis robusta strains F1-8B and F1-9A were selected from a first generation of siblings obtained by crossing the wild-type clones 75 and 80 (Chepurnov et al., 2002). F1-8B and F1-9A belong to opposite mating types (Chepurnov et al., 2008) and had at the time of study an average apical cell length of 22.7 (± 1.6) µm and 18.9 (± 1.2) µm, respectively. Currently, they are maintained cryopreserved in the culture collection of the Laboratory of Protistology & Aquatic Ecology (http://www.pae.ugent.be/collection.htm). Cell cultures were grown in f/2 medium (Guillard, 1975) made with filtered (GF/C grade microfiber filter; Whatman) autoclaved sea water collected from the North Sea. F/2 nutrients were 0.2-µm-filter sterilized and added to the filtered autoclaved sea water. Na2SiO3 was added at a
concentration of 30 mg/L medium. Cultures were cultivated at 18°C with a 12:12-h light:dark period and ~85 \( \mu \text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) from cool-white fluorescent lights. Stock-cultures were reinoculated weekly by transferring small aliquots of cell suspension into fresh medium. Experimental cultures were prepared from stock cultures by inoculating an aliquot of cells from cell suspensions created by scraping the cells from the surface (cell scrapers; Sarstedt) or by detaching the cells by pipetting. Observations and cell culture photography were done with a Zeiss Axiovert 40 light microscope and a digital camera (Powershot G3; Canon). Fluorescence microscopy of cells was performed using Zeiss Axioscop 200, Axioacam MRm for image capturing and Zeiss filter set 14. Confocal fluorescence images were taken with a laser scanning confocal microscope (Zeiss Confocal LSM 510) equipped with software package LSM510 version 3.2 (Zeiss) and equipped with a 63X water-corrected objective (numerical aperture 1.2). Chloroplast fluorescence was visualized with Helium Neon laser illumination at 543 nm. Nuclear DNA was stained with SYBR Safe™ (Molecular Probes) that was visualized with Argon laser illumination at 488 nm and 500-530 nm band emission filter. Cell walls were visualized by incorporation of the Lysosensor™ Yellow/Blue DND-160 probe (PDMPO; Molecular Probes) and was applied during culture growth in concentration as described by Leblanc and Hutchins (2005). Emission fluorescence was captured in the line-scanning mode and for transmission light images, differential interference contrast optics were used.

**Cell cycle progression assays and flow cytometry**

The effect of darkness on cell morphology was tested on exponentially growing cultures of strain F1-8B (Chepurnov et al., 2008) that were inoculated at 1000 cells mL\(^{-1}\) in tissue culture flasks (CELLSTAR®, 175 cm\(^2\) growth surface with filter screw cap; Greiner Bio-One, Wemmel, Belgium) with 200 mL of growth medium. After 2 days of growth, the old medium was decanted from the flask-attached cells and the flask was refilled with 200 mL of fresh medium before transferring the cultures to complete darkness to assure that no nutrient limitation occurred during dark incubation. Hydroxyurea (Fluka) was tested at a final concentration of 6.5 mM, added to 100 mL of exponentially growing cultures of strain F1-31B. After addition, the cultures were incubated in continuous light during 72 h and compared with blank cultures to which an equal volume of DMSO was added. The effect of aphidicolin (Sigma-Aldrich; 0.5 \( \mu \text{g mL}^{-1}\)) was tested in triplicate on F1-8B cultures containing
approximately 5000 dark-arrested cells in 10 mL medium and compared after 24 h of light with DMSO-treated control cultures.

DNA content was measured on intact fixed cells. Therefore, at least 10 mL of a culture was centrifuged for 10 min at 1500 × g and the cells were fixed in 10 mL icecold methanol in the dark at 4°C (Vaulot et al. 1986). Fixed cells were rinsed 2-3 times with 4 mL TE (10 mM Tris, 1 mM EDTA, pH 8.0) by centrifugation for 10 min at 1500 × g and resuspended in 1 mL TE. Samples were incubated for 40 min at 37°C with 10 µl of 30.5 mg mL–1 RNase A (R4642; Sigma-Aldrich) and then placed on ice in the dark. To each sample, 1 µl of 4,6-diamidino-2phenylindole (DAPI) from a stock of 1 mg/mL was added, stained at least 10 min on ice and filtered over a 50-µm nylon mesh (CellTrics, Partec, Münster, Germany). The stained cell suspensions were analyzed with a CyFlow flow cytometer and the FloMax software (Partec, Münster, Germany).

**Synchronization of* S. robusta* cells and sampling of material**

For the synchronization, each *S. robusta* strain was grown in two tissue culture flasks. Because of the difference in cell size, F1-8B and F1-9A culture flasks were inoculated with 7.5×10⁵ and 1x10⁶ cells, respectively, into 200 mL of medium. Cell density estimates of stock cultures were obtained by microscopically counting cells in a 100 µL aliquot of a suspended culture put in a 96-well TC plate. Cultures inoculated for synchronization were grown for 2 days in a 12:12-h L:D regime. At the end of day 2, the dark period was extended for another 12 h, arresting the cell culture at the G1-phase. A first sample was taken just before the light was switched on, followed by 12 samples taken every hour after re-illumination. Just before sampling, of each culture, six pictures were taken with the Axiovert 40 microscope and a connected digital camera (Canon Powershot G3). Pictures were used to count different cell types using the open-source software ImageJ (http://rsb.info.nih.gov/ij/index.html) and the cell counter plug-in. M-phase cells were easily identified and distinguished from interphase cells by the presence of a newly built cell wall, situated between the two valve-located chloroplasts. As soon as daughter cells were separating, they were counted as non-dividing cells. In preparation for cell culture harvesting, the cells were concentrated by reducing the medium of the first flask to less than 50 mL by aspiration with a water pump and attached Pasteur pipette. After suspension through scraping (Sarstedt), the cells were then added to the second bottle from which the complete medium was aspirated. The collection of suspended cells from the two bottles was transferred to a 50 mL Falcon tube and centrifuged for 6 min. at
1500 × g. The supernatant was poured off and the tube containing the pellet was frozen in liquid nitrogen and stored at -80°C until RNA preparation.

**cDNA-AFLP-based transcript profiling**

Total RNA was extracted from each cell sample using the RNeasy® Plant Mini Kit (Qiagen). Cell lysis was achieved by mechanical disruption in 600 µL RNeasy Lysis Buffer (Qiagen) by highest-speed agitation with glass/zirconium beads (0.1 mm diameter; Biospec) on a bead mill (Retsch). All other steps for RNA extraction were done as according to manufacturer’s instructions. RNA concentration and purity were assessed by spectrophotometry (Nanodrop ND-1000 Spectrophotometer) and denaturing agarose gel electrophoresis. First and second-strand cDNA synthesis and cDNA-AFLP analysis, with BsuRI and MseI as restriction enzymes, were carried out according to Vuylsteke et al. (2007), starting from 2 µg total RNA. The final selection of the cDNA-AFLP amplification products was done with three selective nucleotides, resulting in 128 primer combinations. Expression profiles across the 13 time points for each genotype were quantified with AFLP-QuantarPro software (Keygene N.V., The Netherlands). The raw expression values within each genotype were normalized per gene by subtracting the average expression value of each gene from each data point in the time series and dividing it by the standard deviation. Reproducibility of expression profiles between the strains F1-8B and F1-9A was estimated for every gene by calculating a Pearson correlation between normalized expression data. Expression profiles showing a positive and significant ($p < 0.05$) correlation across the two strains were submitted to adaptive quality-based clustering (De Smet et al., 2002). The minimal number of genes in a cluster was set to 10 and the minimal probability of genes belonging to a cluster to 0.95. Hierarchical clustering (Eisen et al., 1998) was performed with the TMEV software (Saeed. et al., 2003).

**Identification of differentially expressed genes**

TDFs were purified from gel, followed by amplification, and subsequent sequencing as described by Vuylsteke et al. (2007). The sequence quality was checked through inspection of the electrophoretic peaks. With the good-quality sequences, a similarity search was done with BLASTN and BLASTX sequence alignments (Altschul et al., 1997) against nucleotide and protein sequences in the publicly available GenBank databases to which the latest versions of the more recently released genomes of *Phytophthora soja* (JGI, v1.1), *Phytophthora ramorum*...
(JGI, v1.1), *Phaeodactylum tricornutum* (JGI, v2.0), and *Thalassiosira pseudonana* (JGI, v3.0) were added. In case a BLASTN alignment of more than 30 nucleotides with 80% coverage between the query and the hit alignment was achieved, the hit sequence was used as a query for similarity searching with BLASTX. Based on the homology (E-value cut off: $1 \times 10^{-3}$; lowest % ID = 29) and the identity of functional Gene Ontology (GO) domains and InterPro domains, the TDFs were functionally annotated and classified in 11 functional groups. TDFs without functional annotation were classified as unknown. GO and Interpro annotation were performed with the online version of the Blast2GO v1.7.2 program (www.Blast2GO.de) (Conesa et al., 2005). The program extracts the GO terms associated with homologies identified with NCBI’s QBLAST and returns a list of GO annotations represented as hierarchical categories of increasing specificity. Because the diatom genome sequences are not included yet in the GenBank, we used the complete sequence of their corresponding diatom genes as input sequences. For construction of Supplemental Figure S9, the data were analyzed at level 2 and GO classes were set to represent at least five different TDFs.

**Cloning of putative FtsZ cDNA from *S. robusta***

Based on conserved amino acid regions of known *FtsZ* genes in *P. tricornutum* (JGI, v2.0 protein ID 14995 and 14426) and *T. pseudonana* (JGI, v3.0 protein ID 35728, 269655 and 15398), 12 oligonucleotide primers were designed with CODEHOP (Rose et al., 1998) and by manual design (Suppl. Fig. S4). These primers were tested in pairwise combinations and three (FP1, RP1b and CDH_FP4) were successfully used to amplify FtsZ cDNA. First, PCR with FP1 and RP1b primers corresponding to amino acid sequence W(A/S)(I/L/V)NTDAQA and (I/V)NVDFAD, respectively, was carried out using hot start PCR (AmpliTaq Gold® DNA polymerase, Applied Biosystems) in five steps: 9 min at 95°C; 4 cycles of 1 min at 94°C, 1 min at 35°C and 1 min at 72°C; 4 cycles of 1 min at 94°C, 1 min at 45°C and 1 min at 72°C; 30 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C; 1 cycle of 10 min at 72°C. A 20-fold diluted, pooled cDNA sample constituting all cell cycle phases during synchronization, was used as template and 20 pmole of each primers were used in the 25 µl reaction mixture. The PCR products were analyzed on a 1.5% agarose gel and a band of the correct size (~500bp) was isolated from the gel and extracted with Nucleospin Extract II (Macherey-Nagel). This extract served as a template in the subsequent PCR with nested primer CDH_FP4, corresponding to amino acid sequence VGIVTKPF, and primer RP1b under the following conditions: 9 min at 95°C, 35 cycles of 45 s at 94°C, 1 min at 52°C and 1
min at 72°C, followed by 1 cycle of 10 min at 72°C. The resulting DNA fragment of correct size (~300bp) was ligated into a pCR® 4 TOPO® Vector (Invitrogen) and sequenced. The DNA sequence of the insert was 92% identical to the *P. tricornutum* FtsZ protein 14995. Two specific primers (Sr FtsZ-F and Sr FtsZ-R) were designed with Roche ProbeFinder on the cloned sequence for RT-PCR.

**Real-time quantitative PCR assay**

RNA was isolated and quantified as before. Isolated RNA was treated with DNaseI (GE-Healthcare) to remove all contaminating genomic DNA. An aliquot of 0.5 µg of total RNA from each sample was used for cDNA synthesis. The reverse transcription was carried out in a total volume of 20 µL with oligo-dT primers and the Super-script II kit (Invitrogen) according to the manufacturer’s instructions. A 0.5-µL aliquot of a 5-fold dilution of the cDNA (2.5 ng) served as template for each Q-PCR reaction. Primers for FCP quantification were designed using the Beacon Designer 7.0 (PREMIER Biosoft International) (Suppl. Fig. S4) together with stringent set of primer design criteria, including predicted melting temperatures (*Tm*) of 58.0 °C +/- 2.0 °C, primer lengths of 17-22 nucleotides, and amplicon lengths of 75-150 bp. Primer pairs were tested by real-time PCR on a pooled cDNA sample under the same conditions as described below. Primer reliability was confirmed by the appearance of a single peak in the melting curve analysis performed by the PCR machine after completion of the amplification reaction.

Eight constitutively and moderately expressed cDNA-AFLP transcript-derived tags (TDFs) were selected as candidate genes to serve for internal reference during RT-PCR. These TDFs were excised from the cDNA-AFLP gels, reamplified, sequenced, and primer pairs were designed as before. The expression stability (*M*) of the putative normalization genes was analyzed with the geNORM™ program: genes with the lowest *M* value are the most stably expressed and were selected as normalization genes (Vandesompele et al. 2002). TDFs sr13af_M281.1 and sr07ae_M536.3 were identified by geNORM™ to be the two most stable expressed genes (*M* value = 0.426), followed by sr14ah12_M385.6 (*M* value = 0.540). The value for the pairwise variation between two sequential normalization factors (geNORM factor *V_{2/3}* ) was 0.146 during synchronization, which is smaller than the cut-off value of 0.15, proposed by Vandesompele et al. (2002) for reliable data normalization. Therefore, all three genes were used for data normalization in the subsequent Q-PCR analyses.
RT-PCR was performed on the Lightcycler 480 (Roche) platform. Each sample was assayed in triplicate under the following conditions: 2.5 ng template cDNA, 2.5 μL of the Lightcycler 480 SYBR Green I Master mix (Roche Applied Science) and 2 μl of primers a concentration of 0.5 μM. The cycling conditions comprised 10 min pre-incubation at 95°C and 45 cycles of 10 s at 95°C, 15 s at 58°C and 15 s at 72°C. Amplicon dissociation curves, i.e. melting curves, were recorded by heating at 95°C for 5 s and at 65°C for 1 min. Samples were cooled at 40°C for 10 s. The relative comparison ΔΔCt method (Pfaffl, 2001) was used to evaluate expression levels of the selected genes relative to the expression of the normalization genes in the same sample. Data were analyzed and normalized with qBase™ (Hellemans et al., 2007) for expression profile generation.

HPLC pigment analysis

*S. robusta* pigments were sampled from light/dark synchronized cultures (see above) by filtering 25 mL of suspended culture over a pre-weighed 25 mm Whatman glass fibre filter (GF/F). Filters were wrapped in aluminium foil, frozen in liquid nitrogen and stored at -80°C. Before analysis the filters were lyophilized during 8 h and weighed (0.1 mg accuracy) to calculate the dry weight of each sample. Pigments were then extracted from the filters in 90% acetone by means of sonication (tip sonicator, 40 W for 30 s). Extracts were filtered over a 0.2 μm Alltech nylon syringe filter to remove particles and injected into a Agilent 1100 series HPLC system (ChemStation software) equipped with a Machery-Nagel reverse-phase C18 column (Nucleodur C18 pyramid; 5 μm particle sizes). Pigments were analysed according to the method of Wright and Jeffrey (1997), using a gradient of three solvents: methanol 80% - ammonium acetate 20%, acetonitrile 90% and ethyl acetate. Two detectors were connected to the HPLC system: an Agilent standard Fluorescence Detector (FLD) to measure fluorescence of chlorophylls and their derivates and an Agilent diode array detector (DAD) to measure absorbance of each peak at 436 nm and 665 nm and absorbance spectra over a 400 - 700 nm range. Fucoxanthin was identified by comparison of retention times and absorbance spectra and quantified by calculating response factors using pure pigment standards (supplied by DHI, Denmark).

Supplemental Data

The following material is available in the online version of this article.
Supplemental Figure S1. Schematic presentation of the chloroplastidic events during the cell cycle of *S. robusta*.

Supplemental Figure S2. Flow cytometric DNA-content histograms for assessing the S-phase cell cycle arrest by hydroxyurea.

Supplemental Figure S3. Estimation Method for percentage of cells in different cell cycle phases based on photographs of the synchronized culture

Supplemental Figure S4. Laser-scanning confocal photographs of a *S. robusta* cell to illustrate the addition of one girdle band.

Supplemental Figure S5. Identification of a *Seminavis robusta* FtsZ ortholog and design of specific RT-PCR primers.

Supplemental Figure S6. Section of an electropherogram of the cDNA-AFLP fingerprint of the two *S. robusta* strains

Supplemental Figure S7. The nine clusters identified by adaptive quality-based clustering (AQBC; De Smet et al., 2002) of 917 expression profiles which were highly reproducible.

Supplemental Figure S8. Expression of the *Seminavis robusta* MCM5 and histone H3 ortholog as a function of time after re-illumination.

Supplemental Figure S9. Clustering of the expression profiles of the cell cycle-modulated, annotated TDFs .

Supplemental Figure S10. Blast2GO pie-chart representing the distribution of functional GO labels for biological process.

Supplemental Figure S11. Expression of the *S. robusta* orthologs of β-tubulin and the WD40-repeat protein as a function of time after re-illumination.

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Supplemental Table S5. Calculation of $X^2$-square correlation between photosynthetic genes and expression cluster C7-L.

Sequence data from this article can be found in the GenBank data libraries.
ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1. Response to dark treatments of *S. robusta*. A, Flow cytometric DNA-content histograms of an exponentially growing culture before transfer to darkness (above), after 24 h of dark incubation (middle) and after 24 h of light (below). B to E, Light-microscopic photographs of the 24 h light-treated culture (strain F1-8B) (B), the 24 h dark-treated culture (strain F1-8B) (C), a 24 h dark-arrested cell in girdle view with focus on the ventral chloroplast (strain F2-31B), and a 24 h dark-arrested cell in valve view (strain F2-31B). The chloroplasts appear brownish and the cell wall is visible due to its high contrast. F and G, Confocal fluorescence microscopic photographs of cells from dark-arrested cultures (strain F2-31B) in which the chloroplastidic signal is red (autofluorescence), the nuclear signal green (SYBR Safe™) and the cell wall signal blue (PDMPO; Lysosensor™). Chloroplasts of dark-arrested cultures are undivided and pressed against the girdle. The ventral chloroplast has four subcentral lobes that extend across each valve towards the dorsal side of the cell. Bars: 10 µm.

Figure 2. *S. robusta* cells in cell cycle inhibitor-treated cultures. A and B, Light-microscopic photographs. C and D, Fluorescence microscopic photographs. A, HU-treated cell (strain F2-31B) arrested during S-phase. B, C, and D, Aphidicolin-treated cell (strain F1-8B) in girdle view with focus on the ventral chloroplast (C) and on the dorsal chloroplast (D). In each case both chloroplasts are undivided and located against the valves. The difference in cell morphologies between cells in A and B – D are due to the different average cell sizes of the used strains. Bars: 10 µm.

Figure 3. Evaluation of the synchrony of cell and chloroplast division in *S. robusta* synchronized strains F1-8B and F1-9A. Proportion of dividing cells is presented in function of time, during re-illumination of the 24 h dark-arrested cultures. Data are mean ± SE of six microscopic fields (0.09 mm²). The predominant morphological stages in each cell cycle phase are represented from light to dark grey in this order: undivided chloroplasts, dividing chloroplasts, dividing cells, and separating cells. G1 corresponds to the phase in which cells with undivided girdle located chloroplasts are present; the asterisk denotes the first observed cells with divided chloroplasts and also approximates the moment of S-phase initiation, according to the transcriptional induction of the MCM5 pre-replication factor (see Supplemental Fig. S6); G2/M corresponds to the phase in which dividing cells were...
increasingly represented with a maximum in the amount of dividing cells at 9 h. M/G1 corresponds to the stage of cell separation.

**Figure 4.** Cytological events during synchronized growth of *S. robusta*, observed with confocal fluorescence microscopy. The chloroplastidic signal is red (autofluorescence), the nuclear signal green (SYBR Safe™) and the silica tracer signal blue (PDMPO; Lysosensor™). In photographs A, C, D and H, the transmission light channel was permitted in order to visualize the outline of the cell. B to D, Cells shown in valve view; A, and E to J, and L, Cells shown in girdle view. A, Cell with focus on a dividing dorsal chloroplast, displaying the central constriction (arrow). B, Cell with the dorsal chloroplast divided. C, Cell with both chloroplasts divided. D and E, Cell with fully rotated, valve-located chloroplasts and containing an undivided nucleus (E). F, Cell with valve-located chloroplasts and showing a blue band in the middle of the cell, extending from pole to pole. This band probably represents a girdle band (see also supplemental Fig. S3). G, Cell just after karyokinesis. Each daughter nucleus is situated on a different side of the future division plane. H, Cell during cytokinesis. The division of the protoplast is apparent by the cleavage furrow, visible as a line of higher contrast running from pole to pole and in between both daughter nuclei. The PDMPO signal is confined to two “vacuole-like” compartments; as such, it appears that deposition of silica at the site of the cleavage furrow has not yet begun. I, Dividing cell during frustule formation. Deposition of silica into the frustule is visible as a line running from pole to pole and situated between the chloroplast pairs and daughter nuclei; J, Dividing cell with chloroplasts moving back from the valves toward the girdle. K, Separating daughter cells in slightly tilt valve view with focus on the dorsal chloroplasts, which are completely covering the girdle area. From the ventral chloroplasts only the subcentral lobes are visible. L, A newly divided cell with the newly formed valve stained with DPMO. The other valve is inherited from the mother cell.

**Figure 5.** Expression of the *S. robusta* FtsZ ortholog in function of time after re-illumination during two replicate synchronizations of strain F1-8B. The proportion of dividing cells is presented in function of time during re-illumination together with the relative expression values of FtsZ. RT-PCR data were normalized against three cDNA-AFLP-acquired reference genes.

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Figure 6. Hierarchical clustered expression profiles of 917 TDFs reproducibly modulated during the cell cycle of two synchronized cultures of strain F1-8B and F1-9A. Clustering was performed using the TMEV software (Saeed. et al., 2003) and the hierarchical clustering algorithm (Eisen et al., 1998). Each row represents a tag with the relative transcript accumulation patterns shown over 12 consecutive time points (columns) after re-illumination of dark-arrested cultures. Yellow and blue color intensities reflect, up- and down-regulation of gene expression relative to a range of +3 to –3, respectively; gray represents missing data. Cluster names (in accordance with adaptive quality-based clustering) and cell cycle phases are indicated at the right and the left, respectively. The arrow corresponds with the expression profile of the DNA replication licensing factor MCM5. C7-E and C7-L, C7-Early and C7-Late, respectively.

Figure 7. Comparison of fucoxanthin pigment turnover and fcp expression between light/dark-synchronized cultures and light/dark-synchronized cultures to which the cell cycle inhibitor aphidicolin was added shortly before re-illumination. A difference in fucoxanthin pigment (µg fuco · mg⁻¹ dry weight) accumulation is eminent after 5 h re-illumination. In the S-phase-arrested cultures, the build-up is less pronounced. In parallel, fcp expression is up-regulated between 1 and 5 h after re-illumination in both dividing and arrested cultures but its expression is down-regulated after the initial increase in arrested cultures, while it is maintained in the dividing cultures.

Table I. Identified cell cycle-modulated genes with significant E-value (<0.001)

Supplemental Figure S1. Schematic presentation of the chloroplastidic events during the cell cycle of S. robusta, illustrating chloroplast (red) movements with respect to the nucleus (green) and the valve and the girdle regions of the cell (VV, valve view; GV, girdle view). In A, B and C, two cells are shown: one in perspective view and one in girdle view. In the perspective view, a transverse section was created at one pole to facilitate the understanding of the S. robusta cell morphology. A, Newly divided cell in valve view. The ventral girdle side of the cell has straight edges and the dorsal side is curved. At this stage, both chloroplasts are undivided and located against the girdle sides of the cell. B, Chloroplasts divided by central constriction, creating two chloroplast pairs located against the girdles. C, Rotated chloroplast-pairs lying against the valves. D, Karyokinesis has taken place. The dotted line represents the future plane of division. E, Deposition of the daughter frustules at the division plane and
chloroplasts movement past each other, one to each girdle side in each daughter cell. F, Original location of the chloroplasts against the girdle sides of the cell. After this stage the daughter cells separate and the cycle restarts.

**Supplemental Figure S2.** Flow cytometric DNA-content histograms for assessing the S-phase cell cycle arrest by hydroxyurea (HU). A, culture after 72 h of light incubation in the presence of DMSO. B, culture after 72 h of light incubation in the presence of HU.

**Supplemental Figure S3.** Estimation Method for percentage of cells in different cell cycle phases based on photographs of the synchronized culture F1-8B, made by inverted microscopy (Zeiss, Axiovert 40). When a cell is not indicated by a sign, the cell was counted as non-dividing cell, whereas an asterisk denotes a cell that was counted as dividing, and a triangle a separating cell counted as non-dividing because daughter cells were partially detached. A, 4 h after re-illumination. B, 10 h after re-illumination. C, 12 h after re-illumination.

**Supplemental Figure S4.** Light-microscopic and laser-scanning confocal photographs of a *S. robusta* cell in slightly tilt girdle view to illustrate the girdle band that was deposited during cell growth. A, Chloroplast auto-fluorescence signal (red). B, Bright field. C, cell wall-associated PDMPO (Lysosensor™) fluorescence signal (blue). D, Nuclear SYBR Safe™ signal (green). E, all signals combined. The cell has the “reorganized chloroplast configuration”, containing two pairs of valve-located chloroplasts and one undivided nucleus. Upon laser-illumination, the cell slightly opened up on one side, thereby facilitating the interpretation of the picture that the blue PDMPO signal is confined to the area in-between both valves (indicated by arrows). The PDMPO fluorescence runs elliptically around the cell’s longitudinal periphery, thereby indicating that it represents a girdle band.

**Supplemental Figure S5.** Identification of a *Seminavis robusta* FtsZ ortholog and design of specific RT-PCR primers. A, Degenerate primers tested for amplification of FtsZ, together with two specific primers (Sr FtsZ-F and Sr FtsZ-R) used during RT-PCR. B, Gel electrophoresis of PCR products. Lanes 1 and 5, SmartLadder SF molecular weight marker (Eurogentec); lane 2 and 3, PCR products using primers FP1 and RP1b on 5-fold (lane 2), 10-fold (lane 3), and 20-fold diluted cDNA-pool. 500-bp band (arrow) was isolated and used for nested PCR with primers RP1b and CDH FP4, resulting in a single 300-bp band (lane 6) that
was used for cloning into the pCR-4 TOPO vector. C, Sequence of the cloned FtsZ fragment. Magenta and green marked sequences correspond to nested primer CDH FP4 and primer RP1b, respectively D, Multiple alignment of FtsZ sequences in Arabidopsis thaliana, Phaeodactylum tricornutum, Thalassiosira pseudonana, and Seminavis robusta. Primer sequences corresponding to the CDH FP4 and RP1b were omitted because they contain multiple bases at degenerate positions due to mismatching during PCR.

Supplemental Figure S6. Section of an electropherogram of the cDNA-AFLP fingerprint of the two S. robusta strains F1-8B and F1-9A with primer combination BstYI+CC/MseI+CG (PC1) and BstYI+CC/MseI+CT (PC2). First and middle lane are size markers. The other lanes represent the 13 time point taken from each genotype during synchronization. Representative examples of cDNA-AFLP sequence (SP) and expression polymorphisms (EP) can be observed.

Supplemental Figure S7. The nine clusters identified by adaptive quality-based clustering (AQBC; De Smet et al., 2002) of 917 expression profiles which were highly reproducible (Pearson correlation; P<0.05) in both strains F1-8B and F1-9A across the 13 time points. The minimal number of genes in a cluster were set at 10 and the minimal probability of genes belonging to cluster at 0.95.

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**Figure 1.** Response to dark treatments of *S. robusta.* A, Flow cytometric DNA-content histograms of an exponentially growing culture before transfer to darkness (above), after 24 h of dark incubation (middle) and after 24 h of light (below). B to E, Light-microscopic photographs of the 24 h light-treated culture (strain F1-8B) (B), the 24 h dark-treated culture (strain F1-8B) (C), a 24 h dark-arrested cell in girdle view with focus on the ventral chloroplast (strain F2-31B), and a 24 h dark-arrested cell in valve view (strain F2-31B). The chloroplasts appear brownish and the cell wall is visible due to its high contrast. F and G, Confocal fluorescence microscopic photographs of cells from dark-arrested cultures (strain F2-31B) in which the chloroplastidic signal is red (autofluorescence), the nuclear signal green (SYBR Safe™) and the
cell wall signal blue (PDMPO; Lysosensor\textsuperscript{TM}).
Chloroplasts of dark-treated cultures are undivided and pressed against the girdle. The ventral chloroplast has four subcentral lobes that extend across each valve towards the dorsal side of the cell. Bars: 10 µm
Figure 2. *S. robusta* cells in cell cycle inhibitor-treated cultures. A and B, Light-microscopic photographs. C and D, Fluorescence microscopic photographs. A, HU-treated cell (strain F2-31B) arrested during S-phase. B, C, and D, Aphidicolin-treated cell (strain F1-8B) in girdle view with focus on the ventral chloroplast (C) and on the dorsal chloroplast (D). In each case both chloroplasts are undivided and located against the valves. The difference in cell morphologies between cells in A and B – D are due to the different average cell sizes of the used strains. Bars: 10 µm.
Figure 3. Evaluation of the synchrony of cell and chloroplast division in S. robusta synchronized strains F1-8B and F1-9A. Proportion of dividing cells is presented in function of time, during re-illumination of the 24 h dark-arrested cultures. Data are mean ± SE of six microscopic fields (0.09 mm²). The predominant morphological stages in each cell cycle phase are represented from light to dark grey in this order: undivided chloroplasts, dividing chloroplasts, dividing cells, and separating cells. G1 corresponds to the phase in which cells with undivided girdle located chloroplasts are present; the asterisk denotes the first observed cells with divided chloroplasts and also approximates the moment of S-phase initiation, according to the transcriptional induction of the MCM5 pre-replication factor (see Supplemental Fig. S6); G2/M corresponds to the phase in which dividing cells were increasingly represented with a maximum in the amount of dividing cells at 9 h. M/G1 corresponds to the stage of cell separation.
Figure 4. Cytological events during synchronized growth of S. robusta, observed with confocal fluorescence microscopy. The chloroplastic signal (autofluorescence) is red, the nuclear signal (SYBR SafeTM) green and the silica tracer signal (PDMPO; LysosensorTM) blue. In photographs A, C, D and H, the transmission light channel was permitted in order to visualize the outline of the cell. B to D, Cells shown in valve view; A, and E to J, and L, Cells shown in girdle view. A, Cell with focus on a dividing dorsal chloroplast, displaying the central constriction (arrow). B, Cell with the dorsal chloroplast divided. C, Cell with both chloroplasts divided. D and E, Cell with fully rotated, valve-located chloroplasts and containing an undivided nucleus (E). F, Cell with valve-located chloroplasts and showing a blue band in the middle of the cell, extending from pole to pole. This band probably represents a girdle band (see also supplemental Fig. S3). G, Cell just after karyokinesis. Each daughter nucleus is situated on a different side of the future division plane. H, Cell during cytokinesis. The division of the protoplast is apparent by the cleavage furrow, visible as a line of higher contrast running from pole to pole and in between both daughter nuclei. The PDMPO signal is confined to two “vacuole-like” compartments; as such, it appears that deposition of silica at the site of the cleavage furrow has not yet begun. I, Dividing cell during frustule formation. Deposition of silica into the frustule is visible as a line running from pole to pole and situated between the chloroplast pairs and daughter nuclei; J, Dividing cell with chloroplasts relocating from the valves toward the girdle. K, Separating daughter cells in slightly tilt valve view with focus on the dorsal chloroplasts, which are completely covering the girdle area. From the ventral chloroplasts only the subcentral lobes are visible. L, A divided cell with the newly formed valve, stained with DPMO. The other valve is inherited from the mother cell and is not stained.
Figure 5. Expression of the *S. robusta* *FtsZ* ortholog in function of time after re-illumination during two replicate synchronizations of strain F1-8B. The proportion of dividing cells is presented in function of time during re-illumination together with the relative expression values of *FtsZ*. RT-PCR data were normalized against three cDNA-AFLP-acquired reference genes.
Figure 6. Hierarchical clustered expression profiles of 917 TDFs, reproducibly modulated
during the cell cycle of two synchronized cultures of strain F1-8B and F1-9A. Clustering was performed using the TMEV software (Saeed. et al., 2003) and the hierarchical clustering algorithm (Eisen et al., 1998). Each row represents a tag with the relative transcript accumulation patterns shown over 12 consecutive time points (columns) after re-illumination of dark-arrested cultures. Yellow and blue color intensities reflect, up- and down-regulation of gene expression relative to a range of +3 to –3, respectively; gray represents missing data. Cluster names (in accordance with adaptive quality-based clustering) and cell cycle phases are indicated at the right and the left, respectively. The arrow corresponds with the expression profile of the DNA replication licensing factor MCM5. C7-E and C7-L, C7-Early and C7-Late, respectively.
Figure 7. Comparison of fucoxanthin pigment turnover and fcp expression between light/dark-synchronized cultures and light/dark-synchronized cultures to which the cell cycle inhibitor aphidicolin was added shortly before re-illumination. A difference in fucoxanthin pigment (µg fuco · mg\(^{-1}\) dry weight) accumulation is eminent after 5 h re-illumination. In the S-phase-arrested cultures, the build-up is less pronounced. In parallel, fcp expression is up-regulated between 1 and 5 h after re-illumination in both dividing and arrested cultures but its expression is down-regulated after the initial increase in arrested cultures, while it is maintained in the dividing cultures.