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Relationship between photosynthetic metabolism and cell cycle in a synchronized culture of the marine alga *Cylindrotheca fusiformis* (Bacillariophyceae)

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The aim of this study was to characterize the variation and regulation of photosynthetic carbon metabolism in *Cylindrotheca fusiformis* during the cell cycle. The cells were synchronized using two cell cycle inhibitors: one for cells grown under light:dark cycles and one for growth in continuous light. We observed that the maximal photosynthetic capacity, P^B_m and ETR_m were lowest just before cell division, when the percentage of cells in G2 + M was maximal, and were highest after division, when the percentage of cells in G1 was maximal. These results clearly show that photosynthetic activity is related to the cell cycle. In addition, the role of different oxygen uptake processes was determined using $^{18}O_2$. We showed that light stimulated oxygen uptake, which increased with irradiance between 0 and 250 μmol photons m^{-2} s^{-1}. This variation was partly due to an increase of mitochondrial respiration, but mostly to a high Mehler activity. It seems that this increase was due to the Mehler reaction rather than photorespiration, because no increase of oxygen uptake was observed at low DIC (0.1 mM) concentration, which should have stimulated Rubisco oxygenase if there was diffusive CO_2 entry. The activity of the Mehler reaction was independent of the cell cycle and explained 50–60% of the light stimulated oxygen uptake at irradiances equal to or exceeding the growth irradiance.

Key words: cell-cycle, *Cylindrotheca fusiformis*, diatom, Mehler reaction, mitochondrial respiration, photosynthesis, photorespiration

Introduction

Cell division and growth proceeds according to different phases, and the succession of these phases is called the cell cycle. The cell cycle is classically divided into four phases, G1, S, G2 and M phase. DNA is replicated during the S phase, M corresponds to the period of mitosis and cell division, and G1 and G2 refer to 'gaps' in the cycle during which most of the cell growth occurs, and of which G1 is the major growth phase (Mitchison, 1971). Several studies of diatoms have shown a linkage between cell metabolism and the cell cycle; silicon metabolism is strictly linked to the cell cycle (Sullivan & Volcani, 1981; Sullivan, 1986; Brzezinski, 1992; Martin-Jézéquel *et al.*, 2000; Claquin *et al.*, 2002). Part of the nitrogen metabolism is also regulated during the course of the cell cycle (Olson *et al.*, 1986; Vaulot *et al.*, 1987; Hildebrand & Dahlin, 2000). For example, Hildebrand & Dahlin (2000) showed a relationship between the nitrate transporter (NAT) mRNA levels and the cell cycle in the diatom *Cylindrotheca fusiformis*. Some studies investigated the relationship between photosynthetic activity and the cell cycle. These studies were mainly conducted with green algal cells synchronized by a light/dark cycle. The rate of photosynthesis was maximal during the early phase of the light period, which corresponded to the beginning of cell division, remained high for a few hours and then decreased until the end of the light period (Sorokin, 1957; Kaftan *et al.*, 1999). Post *et al.* (1985) also observed a high photosynthetic activity for 'young cells' in a synchronized culture of *Scenedesmus protuberans* while the rate of photosynthesis was low for the 'old cells'.

The aim of the present study was to determine if variation and regulation of the photosynthetic metabolism occur in *Cylindrotheca fusiformis*

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during the cell cycle. We studied photosynthetic capacity, Mehler reaction activity, photorespiration and mitochondrial respiration in synchronized and unsynchronized cultures grown with a light/dark cycle or under continuous light. Diatoms do not naturally grow and divide synchronously. Therefore synchronization was achieved using cell cycle inhibitors. The advantage of these inhibitors is that they could be easily removed and, in this way, we were able to avoid the usual protocol of inducing synchronization in *C. fusiformis* by a long dark period and silicon starvation (Paul & Volcani, 1976; Hildebrand *et al.*, 1998), both of which could directly affect the photosynthetic metabolism of our cells.

Materials and methods

Cylindrotheca fusiformis (Bacillariophyceae, clone CCMP 343, Bigelow Laboratory, Booth Bay Harbor, Maine, USA) was grown axenically under a range of culture conditions as described below.

Synchronization with nocodazole under a light/dark cycle (6:6 h)

The cells were grown at 20°C under a light/dark cycle (6:6 h) in artificial seawater (Harrison *et al.*, 1980) modified by addition of selenium to a final concentration of 10 nM and enriched as f/2-medium (Guillard & Ryther, 1962; Guillard, 1975). Light was supplied by a 400 W Philips HPIT E40 daylight lamp. The culture conditions were the same before, during and after the treatment. The irradiance was about 250 μmol photons $m^{-2}\ s^{-1}$.

For synchronization, a stock solution of nocodazole (methyl (5-[2-thienylcarbonyl]-1H-benzimidazol-2-yl) carbamate, Sigma M1404) was made up in DMSO (33 μmol ml^{-1}). In order to obtain a final concentration of 1 nmol ml^{-1}, 30 μl of the stock solution was added to 1 l of culture in the exponential growth phase. After 18 h incubation, the inhibitor was removed by rinsing three times with fresh medium (centrifuged at 512 *g* for 10 min). The pellet was resuspended in 2 l of fresh medium. Measurements were first made 24 h after the removal of the cell cycle inhibitor in order to avoid interference from the treatment; before starting a new experiment, the samples were resuspended in fresh medium which contained 2.1 mM DIC at pH 8 (see below). Unsynchronized cultures were grown in the same conditions as synchronized cultures with DMSO addition, apart from the absence of nocodazole.

Synchronization with aphidicolin under continuous light

We were unable to synchronize cultures in continuous light with nocodazole, so that another cell cycle inhibitor, aphidicolin (Sigma A0781) was used. Cells were grown in continuous light for several generations under the conditions described above. A stock solution of aphidicolin (2.96 μmol ml^{-1}) was made up in DMSO, 1 ml of the stock solution was added to 300 ml of culture in the exponential phase to obtain a final concentration of 10 nmol ml^{-1}. After 24 h incubation, the inhibitor was removed as described above.

Photosynthetic capacity measurement

Chlorophyll *a* concentration *in vitro* was measured after extraction in 90% acetone (Jeffrey & Humphrey, 1975), and *in vivo* absorption was also measured according to Shibata *et al.* (1954). Chlorophyll-specific absorption cross section (a^*; m^2 $mgChl^{-1}$) was calculated from the chlorophyll concentration and the *in vivo* absorption (Dubinsky *et al.*, 1986). Absorption was measured throughout using a Uvikon 940 double-beam scanning spectrophotometer.

Photosynthetic activity was measured simultaneously by oxygen evolution and variable fluorescence (PAM) in a specially designed chamber (Dubinsky *et al.*, 1987) at growth temperature. The algae were dark-adapted for 10 min and then exposed for 4 min to 11 different irradiances (E) from 0 to 1200 μmol $m^{-2}\ s^{-1}$. The steady-state fluorescence (F_s) and the maximal fluorescence (F_m') were measured using a PAM 101-103 fluorometer (H. Walz, Effeltrich; Schreiber *et al.*, 1986). According to Genty *et al.* (1989), the effective quantum efficiency of PSII in actinic irradiance was calculated as:

$$\Delta F/F_m' = (F_m' - F_s)/F_m'. \quad (1)$$

$\Delta F/F_m'$ can be used to estimate the linear rate of photosynthetic electron transport (ETR) as of a single active PSII unit (Genty *et al.*, 1989; Hofstraat *et al.*, 1994):

$$ETR = \Delta F/F_m' \times E \times a^*_{PSII} \quad (2)$$

where a^*_{PSII} is the optical cross section of PSII. As we could not measure a^*_{PSII}, we calculated ETR per unit chlorophyll assuming that 50% of the absorbed photons are allocated to photoreactions in PSII (Gilbert *et al.*, 2000). ETR (μmol electron $mgChla^{-1}\ s^{-1}$) was calculated as follows:

$$ETR = \Delta F/F_m' \times E \times a^* \times 0.5 \quad (3)$$

where a* is the chlorophyll-specific absorption cross section (m^2 $mgChl^{-1}$). ETR_m (maximum electron transport rate) was then calculated as:

$$ETR(E) = ETRs\ (1 - e^{(-\alpha E/ETR_s)}\ e^{(-\beta E/ETR_s)} \quad (4)$$

$$ETR_m = ETR_s(\alpha/(\alpha+\beta))\ (\beta/(\alpha+\beta))^{\beta/\alpha} \quad (5)$$

where ETR_s is the maximum ETR without photoinhibition; α is the initial slope of a PE-curve, and β is the photoinhibition parameter (Platt & Gallegos, 1980).

Oxygen production was measured using a polarographic oxygen electrode (YSI-5331). We did not observe photoinhibition in the rate of oxygen evolution,

therefore P^B_m (maximal photosynthetic capacity, μmol O_2 mgChla^{-1} s^{-1}) was calculated as:

$$P^B = P^B_m(1 - e^{(-E\alpha/PB_m)}) - R \quad (6)$$

where α is the slope of the photosynthesis versus irradiance curve (Geider *et al.*, 1997).

Determination of different oxygen uptake processes

Discrimination between mitochondrial respiration, Mehler reaction and photorespiration was achieved as follows: After bubbling the algal suspension with nitrogen gas for 15 s, the oxygen incubation chamber (20 ml) was filled to approximately 90% of its volume and $^{18}O_2$ was injected to obtain an initial $^{18}O_2$ concentration around 15% of total O_2. Then, the chamber was filled and closed. As a result the final oxygen concentrations at the start of the measurements varied between 50 and 70% saturation. $^{18}O_2$ and $^{16}O_2$ were measured using a Membrane Inlet Mass Spectrometer (MIMS, a modified Omnistar Balzers instrument). The probe, a 1/16 inch RF-steel tube with 6 holes (0.25 mm diameter) drilled at the tip and fitted with silicon tubing, was inserted directly into the oxygen chamber, so that diffusion through the membrane inlet occurred at constant, growth temperature conditions.

After 10 min in darkness, the culture was illuminated for 20 min, and then 250 μl of DCMU was added to stop light-dependent oxygen uptake (Kana, 1992). After 20 min the recording was stopped. The difference between the slope of the $^{18}O_2$ evolution before and after the DCMU addition gave the light-dependent O_2 uptake. Indeed, DCMU addition should stop both the Mehler reaction and photorespiration by inhibiting non-cyclic electron flow. The O_2 consumption remaining after this treatment would be due to mitochondrial consumption (Peltier & Thibault, 1985). The percentage decrease in the slope indicates the sum of the Mehler reaction and photorespiration as a proportion of the total O_2 uptake. We call this percentage: the Mehler reaction-Photorespiration Percentage (MPP). However, MPP gives only an indication of the activity of these processes, as DCMU itself can also affect respiration, especially in blue light (Kowallik, 1982). O_2 uptake rate (U_0) and gross O_2 evolution rate (E_0) were calculated as:

$$U_0 = ((\Delta[^{18}O_2]/\Delta t) - k[^{18}O_2])(([^{18}O_2] + [^{16}O_2])/[^{18}O_2]) \quad (7)$$

$$E_0 = ((\Delta[^{16}O_2]/\Delta t) - k[^{16}O_2]) + U_0(([^{16}O_2]/([^{16}O_2]) + [^{18}O_2]) \quad (8)$$

where k is the rate constant of O_2 decrease due to the mass spectrometer consumption (Peltier & Thibault, 1985), $[^{16}O_2]$ and $[^{18}O_2]$ represent the amounts of each molecular species expressed in μmol O_2 mgChla^{-1}, and Δt is the time interval used in the calculation.

Because a rise in pH during photosynthesis might stimulate Rubisco oxygenase, some measurements were conducted at constant pH and at two dissolved inorganic carbon (DIC) concentrations. HEPES buffer solution (15 mmol l^{-1}, pH 8.0) in enriched artificial seawater was used to characterize the presence or absence of photorespiration. The buffer was enriched with a normal or a low concentration of bicarbonate (2.1 mM and 0.1 mM, respectively). Cells were harvested by centrifugation (512 *g* 10 min) and washed twice with HEPES buffer before measurements were started.

Cell counts and cell cycle measurements

Cell number and cell size were determined by Coulter Counter Model ZM connected to Coulter Multisizer. For cell cycle measurements, 1 ml of culture was fixed with 10 μl of paraformaldehyde (10%). After 15 min at room temperature, the sample was frozen (−80°C) until analysis. The cellular DNA was stained with Pico Green (Molecular Probes) as described by Veldhuis *et al.* (1997). Cellular DNA was measured with a flow cytometer (FACS Calibure, Becton Dickinson). At least 10 000 events were collected. Distribution of G1, G2 + M and S-phases were analysed using Mcycle software (Phoenix Flow Systems, San Diego, CA, USA).

Results

For unsynchronized cultures under light/dark cycle (6:6 h) treatment (Fig. 1a,b), the percentages of the cell cycle phases remained almost constant during the experiment at around 56, 23 and 21% for the

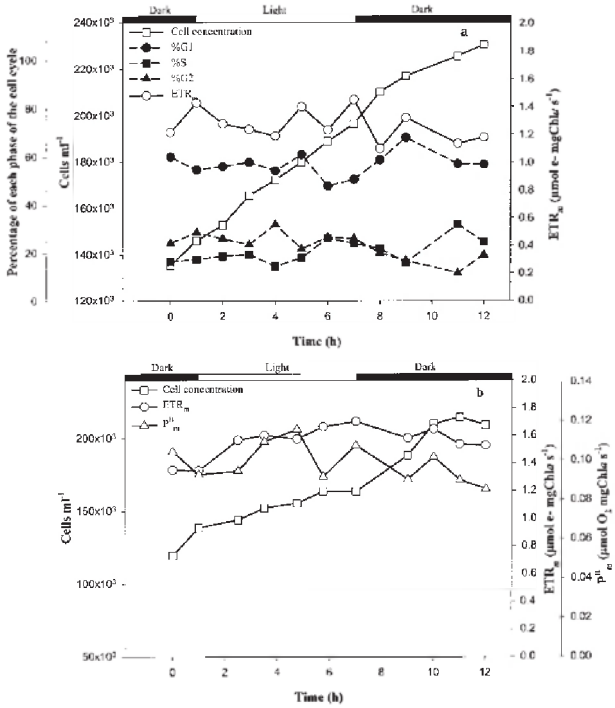

Fig. 1. Unsynchronized culture of *Cylindrotheca fusiformis* under light/dark cycles (6:6 h) at 2.1 mM DIC . (a) ETR_m (μmol e^- mgChla^{-1} s^{-1}), cell concentration (cells ml^{-1}) and percentage of cells in each phase of the cell cycle as a function of time. (b) ETR_m, P^B_m (μmol O_2 mgChla^{-1} s^{-1}) and cell concentration (cells ml^{-1}) as a function of time in a repeat experiment with a new culture; oxygen exchange was not measured.

G1, G2 + M and S phases, respectively. ETR_m also remained stable, and did not change between light and dark periods. P^B_m showed more variability than ETR_m, but was again independent of light and darkness. These results indicate that the maximum capacity of photosynthesis, measured by either PSII electron flow (a measure of gross photosynthesis) or net oxygen evolution, was not affected by the light/dark cycle.

Two similar experiments were performed with cultures synchronized by nocodazole under a light/dark cycle (6:6 h; Figs 2a,b). For both, cell division took place at the beginning of the light period. In the first 3 h of the light period, 70–100% of the cells divided and, consequently, the cultures could be considered as synchronized. For both experiments the percentage of cells in G2 + M was maximal (around 50%) before the cell division and decreased to zero or almost zero at the end of division. Consequently, the percentage of cells in G1 was minimal before cell division and reached 80–95% at the end of this phase. After cell division, the percentage of cells in G1 and G2 + M phases remained stable for several hours (Fig. 2a). However, for the second experiment (Fig. 2b) we observed a decrease in %G1 just after the division, which appeared to be due to an increase of the S phase, indicating preparation for a new division. P^B_m and ETR_m followed similar patterns in both experiments (Fig. 2a,b); they were constant and low before division, and increased during division to reach highest values at the end of division, broadly similar to %G1. Photosynthetic capacity of the population thus varied by a factor of 2 or 3 as a function of cell cycle stage.

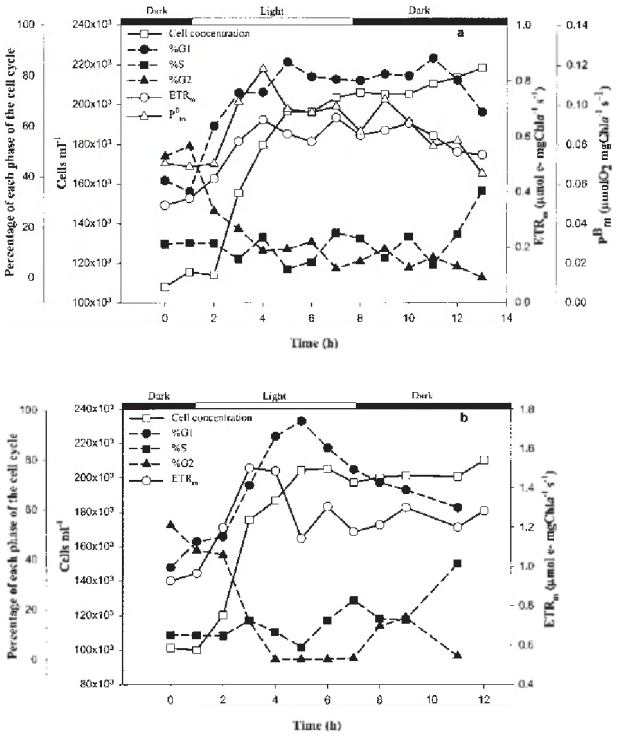

Fig. 2. Culture of *Cylindrotheca fusiformis* synchronized by nocodazole and light/dark cycles (6:6 h) at 2.1 mM DIC. (a) ETR_m (μmol e$^-$ mgChla^{-1} s^{-1}), P^B_m (μmol O_2 mgChla^{-1} s^{-1}), cell concentration (cells ml^{-1}) and percentage of cells in each phase of the cell cycle as a function of time. (b) ETR_m cell concentration and percentage of cells in each phase of the cell cycle as a function of time in a repeat experiment with a new culture; oxygen exchange was not measured.

The patterns of the different cell cycle phases, and of P^B_m and ETR_m in cultures treated with continuous light and aphidicolin (Fig. 3) were quite similar to those observed in cultures grown in a light/dark cycle and synchronized with nocodazole (Fig. 2). Again, photosynthetic capacity was highest during the G1-phase of the cell cycle. Production was between 0.06 and 0.011 μmol O_2 mgChla^{-1} s^{-1} for the synchronized cultures and around 0.09 μmol O_2 mgChla^{-1} s^{-1} for the unsynchronized cultures, which is similar to values observed for other diatoms (Cullen & Lewis, 1988; Kromkamp & Limbeek, 1993).

We also investigated whether oxygen uptake varied through the cell cycle using $^{18}O_2$. Although oxygen uptake is often equated with respiration, this is not correct because not all oxygen uptake in the light is necessarily coupled to membrane bound respiratory electron transport. These measurements were performed under the growth irradiance (250 μmol m^{-2} s^{-1}) on cultures synchronized with nocodazole and grown in a light/dark (6:6 h) cycle (Fig. 4). Gross O_2 evolution rate (E_0) followed the same trend as P^B_m and ETR_m in synchronized cultures. The rate of total O_2 uptake (U_0) increased during cell division from 0.017 to 0.028 μmol O_2 mgChla^{-1} s^{-1} and then decreased to 0.014 μmol O_2 mgChla^{-1} s^{-1} by the middle of the next dark period (ANOVA, $p < 0.001$; Fig. 4).

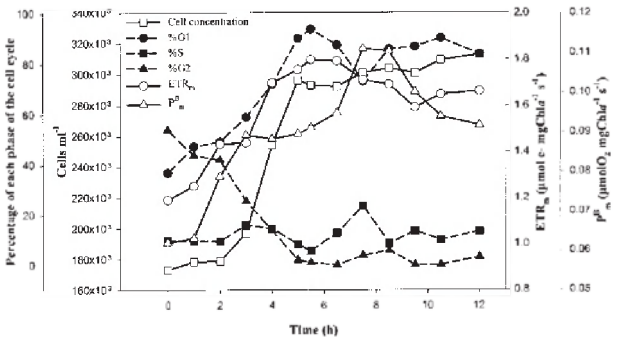

Fig. 3. Culture of *Cylindrotheca fusiformis* grown and synchronized by aphidicolin in continuous light at 2.1 mM DIC. ETR_m (μmol e$^-$ mgChla^{-1} s^{-1}), P^B_m (μmol O_2 mgChla^{-1} s^{-1}), cell concentration (cells ml^{-1}) and the percentage of cells in each phase of the cell cycle as a function of time.

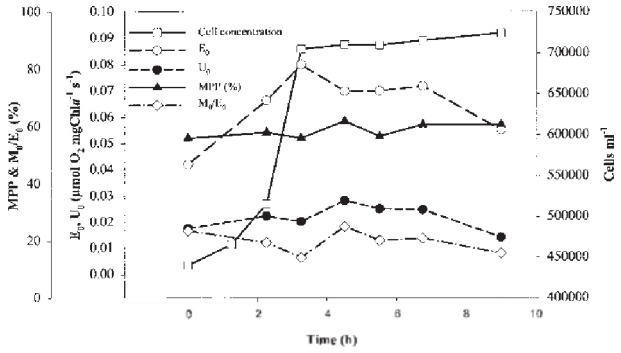

Fig. 4. Culture of *Cylindrotheca fusiformis* synchronized by nocodazole and light/dark cycles (6:6 h) at 2.1 mM DIC. Cell concentration (cells ml^{-1}), total O_2 uptake (U_0; μmol O_2 mgChla^{-1} s^{-1}), gross O_2 evolution (E_0; μmol O_2 mgChla^{-1} s^{-1}), MPP (%) and M_0/E_0 (%) at the growth irradiance as a function of time. $M_0/E_0 = (U_0 \times MPP)/E_0$.

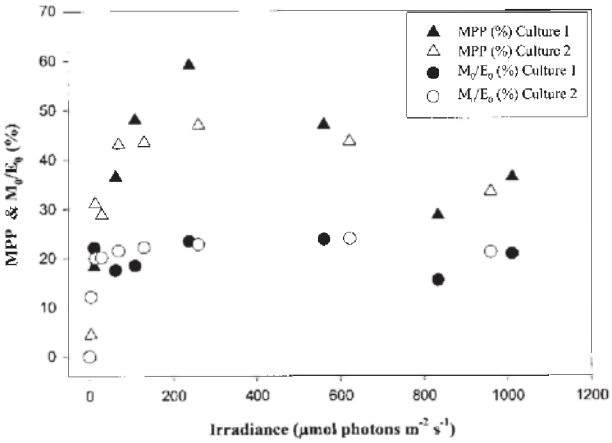

Fig. 5. Unsynchronized cultures of *Cylindrotheca fusiformis* at 2.1 mM DIC. MPP (%) and M_0/E_0 (%) as a function of irradiance. Two separate cultures were used for repeat experiments.

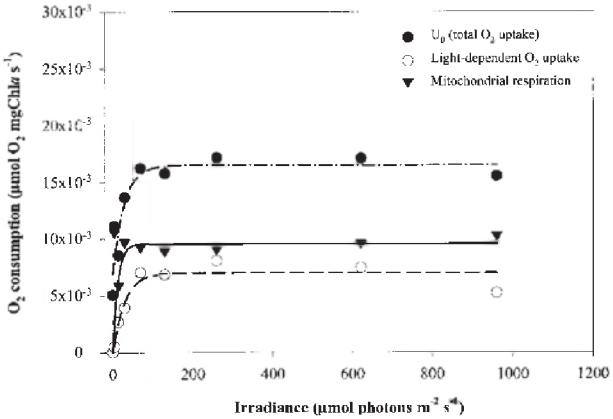

Fig. 6. Total O_2 uptake (U_0; μmol O_2 mgChla^{-1} s^{-1}), light-dependent O_2 uptake (μmol O_2 mgChla^{-1} s^{-1}) and mitochondrial respiration (μmol O_2 mgChla^{-1} s^{-1}) as a function of irradiance. Mass spectrometry was used to determine the MPP of 'Culture 2' (Fig. 5).

To investigate the occurrence of light-dependent O_2 uptake, we measured the contribution of the Mehler reaction and photorespiration by blocking these processes with DCMU. The MPP (Mehler reaction-Photorespiration Percentage) increased with irradiance up to the growth irradiance (about 250 μmol m^{-2} s^{-1}; Fig. 5) but then remained constant at around 50 %. The observed decreases in MPP at higher irradiances greater than the growth irradiance were not statistically significant. From the MPP (%) we calculated the absolute oxygen uptake due to these two processes (M_0) and divided this by the rate of gross oxygen evolution (E_0) in order to estimate the fraction of photosynthetic electron flow associated with the Mehler reaction and photorespiration. M_0/E_0 rose from zero in darkness to 23% at 250 μmol m^{-2} s^{-1}, but remained constant at higher irradiances ($p > 0.05$ for both cultures). MPP was also measured as a function of the cell cycle on a culture synchronized with nocodazole and a light/dark cycle (Fig. 4). MPP remained constant at around 58% throughout the cell cycle, and no variations were observed during cell division. Also, as can be seen in Fig. 4, 15–23% of the electron flow of E_0 was associated with the MPP reactions at the growth irradiance, which is in agreement with the data presented in Fig. 5.

The mass spectrometric measurements made for the MPP determination (Fig. 5) permitted an attempt at characterizing the contribution of individual oxygen-consuming processes to total O_2 uptake as function of irradiance (Fig. 6). Mitochondrial respiration was calculated by subtracting the light-dependent proportion of O_2 uptake attributed to the MPP reactions from the total O_2 uptake (U_0) for all irradiances, assuming that the DCMU treatment did not influence mitochondrial electron transport. Mitochondrial respiration was higher under light than under dark conditions. The rate of mitochondrial respiration depended on the irradiance: it rose steeply from 5.1×10^{-3} μmol O_2 mgChla^{-1} s^{-1} in the dark to approximately 9.2×10^{-3} μmol O_2 mgChla^{-1} s^{-1} at 40 μmol m^{-2} s^{-1} (Fig. 6). The rate of mitochondrial oxygen uptake reached its maximum at a lower irradiance than the total light-dependent uptake. The contribution of the Mehler reaction/photorespiration (i.e. light-dependent O_2 uptake) increased with irradiance and seemed to be responsible for most of the light stimulated O_2-uptake. It reached its maximum at the growth irradiance.

A high O_2/CO_2 concentration ratio might stimulate the oxygenase activity of Rubisco. In order to estimate the relative contribution of photorespiration to the total light-stimulated oxygen uptake measurements, we resuspended unsyn-

chronized *C. fusiformis* cultures in fresh medium containing either 2.1 mM DIC (the normal concentration in the growth medium), or in growth medium with low DIC concentration (0.1 mM DIC). The MPP values were very similar: 50.0 ± 3.7% ($n = 3$) and 49.0 ± 4.8% ($n = 3$) for the normal and low DIC concentration respectively, indicating that photorespiration played only a limited role in the light-enhanced oxygen uptake.

Discussion

The experiments using nocodazole or aphidicolin show comparable results (P^B_m, ETR_m, cell cycle patterns), which allow us to conclude that there was no effect of the inhibitors on the photosynthetic metabolism after they were removed. Nocodazole is an antimitotic agent that disrupts microtubules (Luduena & Roach, 1991; Vasquez *et al.*, 1997), arresting the cell cycle at G2/M phase. After washing, this cell cycle arrest is reversible. Aphidicolin inhibits eukaryotic and viral DNA replication by blocking DNA polymerases (Cheng & Kuchta, 1993; Spadari *et al.*, 1985) and blocks cells at the end of the G1 phase (Planchais *et al.*, 2000).

The photosynthetic parameters appeared to vary as function of the growth phase of cells in our synchronized cultures. The values of ETR_m and P^B_m were lowest just before the division when the percentage of cells in G2 + M phase was maximal, and highest after division when the percentage of cells in G1 phase was maximal (Fig. 2 and 3). This pattern does not seem to be affected by the light/dark cycle, as the same pattern was observed in light/dark cycles and under continuous light (Fig. 2 and 3). Consequently the photosynthetic capacity of a culture depends in part on the regulation of the cell cycle. Several studies describe cell-cycle dependent modification of the photosynthetic apparatus. Allen (1992) observed that phosphorylation of thylakoid proteins in a synchronized culture of *Scenedesmus obliquus* was correlated with an increase in photosynthetic capacity (Heil & Senger, 1986). Winter & Brandt (1986) described cell-cycle dependent changes in antenna size in *Euglena gracilis*. Strasser *et al.* (1999) and Szurkowski *et al.* (2001) also observed cell-cycle dependent changes in the photosynthetic apparatus for *Scenedesmus obliquus* and *S. armatus*, respectively.

Paul & Volcani (1976) synchronized *C. fusiformis* by using silicate starvation and a short photoperiod. After a long dark period, the cells were placed in continuous light, and photosynthetic activity decreased prior to cell division and increased after cell division, which is similar to our observations, despite the different treatments used to synchronize the cells. As mentioned before, this pattern was observed for the photosynthetic activity in other studies using synchronized green algae (Post *et al.*, 1985; Kaftan *et al.*, 1999). The high photosynthetic activity after cell division (i.e. in the G1 phase) may serve to increase the synthesis of carbon products and thus the cellular growth. Post *et al.* (1985) suggested that the high metabolic activity might be repressed by feed-back regulation which would take place when the amount of carbon produced by photosynthesis is sufficient to meet the cellular C-demands. This idea is supported by the results of Kaftan *et al.* (1999) using *Scenedesmus quadricauda,* who showed that, during high photosynthetic activity, cells used PSII at maximum capacity. After having accumulated enough reserves for the next division, photosynthetic activity declined followed by a reorganization of the thylakoid membranes, affecting photosynthetic activity.

When *C. fusiformis* grew asynchronously (Fig. 1), the photosynthetic capacities remained constant with time, because all phases of the life cycle were present at every moment; hence, we measured the average of different physiological states. Claquin *et al.* (2002) showed using *Thalassiosira pseudonana* that the cell cycle phases are controlled by the growth rate, therefore the variation of the photosynthetic capacities under different growth conditions can be partly due to cell cycle regulation.

The total O_2 uptake rate (U_0) measured in synchronized culture (Fig. 4) increased during the division and then decreased. There are three mechanisms that may be responsible for the O_2 consumption during photosynthesis: photorespiration, Mehler reaction and mitochondrial respiration (Badger, 1985; Peltier & Thibault, 1985; Weger *et al.*, 1989). In our study, the MPP (Mehler reaction–Photorespiration Percentage) was constant during the cell cycle (Fig. 4). The MPP was quite large, comprising 58% of the total O_2 uptake. In earlier studies, Peltier & Thibault (1985) and Xue *et al.* (1996) using the green alga *Chlamydomonas reinhardtii* and Weger *et al.* (1989) using the diatom *Thalassiosira weissflogii* did not measure any photorespiration or Mehler reaction as part of the total O_2 consumption in the light at growth irradiance. Their results suggested that O_2 uptake was exclusively due to mitochondrial respiration. However, Sültemeyer *et al.* (1986; 1987) concluded that, in *Chlamydomonas reinhardtii*, the O_2 consumption at high irradiance was mediated to a large extent by the Mehler reaction. Kana and coworkers (Kana, 1990, 1992; Lewitus & Kana, 1995, using two different cyanobacteria and six different estuarine phytoplankton species) and Kromkamp & Peene (2001; using several different cyanobacteria and eukaryotic marine unicellular algae) also found a significant, but variable, contribution of

the Mehler reaction to the total O_2 uptake at high irradiances, comparable to our values. At low bicarbonate concentrations, the oxygenase function of the Rubisco will be activated more and hence photorespiration can take place more easily. But in our study, the MPP was the same in both normal and low DIC concentration. We were not able to separate Mehler reaction and photorespiration with our method, but this result indicates that there was no increase of photorespiration at low DIC concentration. Although Paul & Volcani (1976) observed photorespiratory activity in *C. fusiformis* using enzymatic activity measurements, they did not measure any change of this activity throughout the cell cycle. The apparent absence of photorespiration has often been described in algae (Birmingham *et al.*, 1982) and particularly in diatoms, which have an efficient CO_2-concentration mechanism (Burns & Beardall, 1987; Raven *et al.*, 2000). Therefore the MPP in *C. fusiformis* is probably mainly due to the Mehler reaction. The MPP was a constant fraction during the cell life, which signifies that the variations of the total O_2 uptake during the cell life were due to proportional variations in mitochondrial respiration and Mehler reaction. The increase in respiration at the end of division (i.e. at the start of G1) could be linked to an increase in the production of low molecular weight compounds due to increased photosynthetic activity, which are used to synthesize new cell material and thus fuel respiration to recycle reducing equivalents, but it is also partly due to the light:dark cycle. The decrease in E_0 after cell division coincides with a decrease in U_0, which also indicates a strong link between respiratory and photosynthetic activity. In a previous study with *C. fusiformis*, dark respiration was constant throughout the cell cycle (Paul & Volcani, 1976).

Contrary to our expectations, the maximum activity of light-dependent O_2 uptake was observed at the growth irradiance (Fig. 5 and 6) and represented nearly 60% of the total oxygen uptake in the light (Fig. 5). We have suggested above that light-dependent O_2 uptake probably corresponds to the Mehler reaction. A strong Mehler reaction activity has been observed at high irradiance for several algal species (Kromkamp & Peene, 2001), although only a few studies (e.g. Kana *et al.*, 1990) actually measured this at the growth irradiance, and it is suggested that this reaction protects against photoinhibition by dissipation of excess photons (Osmond & Grace, 1995; Schreiber *et al.*, 1995). From the MPP data we could calculate the fraction of photosynthetic electron flow associated with the Mehler reaction (M_0/E_0; Fig. 4 and 5), and the electron flow associated with the Mehler reaction seldom exceeded 23% of the total photosynthetic electron flow. This also indicates that the Mehler reaction is not the missing alternative electron sink (Osmond & Grace, 1995) which is sometimes invoked to account for the discrepancy between estimated rates of P^B_m and ETR_m (Flameling & Kromkamp, 1998). The fact that non-photochemical quenching was still low (< 0.1, calculated as qN) also suggests that energy dissipation was still small at the growth irradiance and that the Mehler reaction did not play an important role with respect to energy dissipation. The decrease of the Mehler reaction activity at high irradiance may be due to damage of PSII, although we have no real evidence for this. The photoinhibition of photosynthesis that we observed frequently at high irradiance when using variable fluorescence measurements (data not shown) corroborates this assumption. Our results also demonstrate that the alga acclimated in such a way that it grew at E_k. At this special irradiance the alga has maximum flexibility with respect to light: it is no longer limited by light but the irradiance is not high enough to cause significant chronic photoinhibition. E_k thus seems the optimum irradiance for growth.

The higher mitochondrial O_2 consumption in the light than in the dark (Fig. 6) was also observed in several studies (Peltier & Thibault, 1985; Weger *et al.*, 1989; Xue *et al.*, 1996). One of the possible explanations for this phenomenon is that mitochondrial activity is substrate limited in the dark: the increase of the mitochondrial O_2 consumption may be due to the availability of recent photosynthate, which provides more substrate for the TCA cycle and thus entails more mitochondrial respiration (Falkowski *et al.*, 1985).

Conclusion

We have clearly shown that photosynthetic capacity is related to the cell cycle and that it is highest during the G1 phase, which is the main growth phase. Consequently this pattern in diatoms is similar to that in green algae. Increasing irradiance stimulates oxygen uptake, partly due to an increase in mitochondrial respiration but mostly to an increase in Mehler activity. The activity of the Mehler reaction accounted for 50–60% of the oxygen uptake at the growth irradiance, and the relative activity of Mehler reaction was constant throughout, and was independent of, the cell cycle. We could not demonstrate that the MPP is due more to the Mehler reaction than to photorespiration, but our results and the literature support this hypothesis. At present, there is no satisfactory hypothesis to explain such a high Mehler activity at the growth irradiance.

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References

Allen, J.F. (1992). Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta,* **1098**: 275–335.

Badger, M.R. (1985). Photosynthetic oxygen exchange. *Ann. Rev. Plant Physiol.,* **36**: 27–53.

Birmingham, B.C., Coleman, J.R. & Colman, B. (1982). Measurement of photorespiration in algae. *Plant Physiol.,* **69**: 259–262.

Brzezinski, M.A. (1992). Cell-cycle effects on the kinetics of silicic acid uptake and resource competition among diatoms. *J. Plankton Res.,* **14**: 1511–1539.

Burns, B.D. & Beardall, J. (1987). Utilization of inorganic carbon by marine microalgae. *J. Exp. Mar. Biol. Ecol.,* **107**: 75–86.

Cheng, C.H. & Kuchta, R.D. (1993). DNA Polymerase-Epsilon - Aphidicolin Inhibition and the Relationship between Polymerase and Exonuclease Activity. *Biochemistry,* **32**: 8568–8574.

Claquin, P., Martin-Jézéquel, V., Kromkamp, J.C., Veldhuis, M.J.W. & Kraay, G.W. (2002). Uncoupling of silicon compared to carbon and nitrogen metabolism, and role of the cell cycle, in continuous cultures of *Thalassiosira pseudonana* (Bacillariophyceae) under light, nitrogen and phosphorus control. *J. Phycol.*, **38**: 922–930.

Cullen, J.J. & Lewis, M.R. (1988). The kinetics of algal photoadaptation in the context of vertical mixing. *J. Plankton Res.,* **10**: 1039–1063.

Dubinsky, Z., Falkowski, P.G. & Wyman, K. (1986). Light harvesting and utilization by phytoplankton. *Plant Cell Physiol.*, **27**: 1335–1349.

Dubinsky, Z., Falkowski, P.G., Post, A.F. & Van Hes, U.M. (1987). A system for measuring phytoplankton photosynthesis in a defined light field with an oxygen electrode. *J. Plankton Res.,* **9**: 607–612.

Falkowski, P.G., Dubinsky, Z. & Santostefano, G. (1985). Light-enhanced and dark respiration in phytoplankton. *Int. Ver. Theor. Angew. Limnol. Verh.,* **22**: 2830–2833.

Flameling, I.A. & Kromkamp, J. (1998). Light dependence of quantum yields for PSII charge separation and oxygen evolution in eucaryotic algae. *Limnol. Oceanogr.* **43**: 284–297.

Geider, R.J., MacIntyre, H.L. & Kana, T.M. (1997). Dynamic model of phytoplankton growth and acclimation: Responses of the balanced growth rate and the chlorophyll a:carbon ratio to light, nutrient-limitation and temperature. *Mar. Ecol. Prog. Ser.,* **148**: 187–200.

Genty, B., Briantais, J. & Baker, N.R. (1989). The relationship between the quantum yield of photosynthetic electron transport and of quenching chlorophyll fluorescence. *Biochim. Biophys. Acta,* **990**: 87–92.

Gilbert, M., Wilhelm, C. & Richter, M. (2000). Bio-optical modelling of oxygen evolution using in vivo fluorescence: comparison of measured and calculated photosynthesis/irradiance (P-I) curves in four representative phytoplankton species. *J. Plant Physiol.*, **157**: 307–314.

Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. In *Culture of Marine Invertebrate Animals* (Smith, W.L. & Chanley, M.H., eds), 26–60 Plenum Press. New York.

Guillard, R.R.L. & Ryther, J.H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.,* **8**: 229–239.

Harrison, P.J., Waters, R.E. & Taylor, F.J.R. (1980). A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *J. Phycol.,* **16**: 28–35.

Heil, W.G. & Senger, H. (1986). Thylakoid-protein phosphorylation during the life cell of *Scenedesmus obliquus* in synchronous culture. *Planta,* **167**: 233–239.

Hildebrand, M. & Dahlin, K. (2000). Nitrate transporter genes from the diatom *Cylindrotheca fusiformis* (Bacillariophyceae): mRNA levels controlled by nitrogen source and by the cell cycle. *J. Phycol.,* **36**: 702–713.

Hildebrand, M., Dahlin, K. & Volcani, B.E. (1998). Characterization of a silicon transporter gene family in *Cylindrotheca fusiformis*: sequences, expression analysis, and identification of homologs in other diatoms. *Mol. Gen. Genet.,* **260**: 480–486.

Hofstraat, J.W., Peeters, J.C.H, Snel, J.F.H. & Geel, C. (1994). Simple determination of photosynthetic efficiency and photoinhibition of *Dunaliella tertiolecta* by saturating pulse fluorescence measurements. *Mar. Ecol. Prog. Ser.*, **130**: 187–196.

Jeffrey, S.W. & Humphrey, G.F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen,* **167**: 191–194.

Kaftan, D., Meszaros, T., Whitmarsh, J. & Nedbal, L. (1999). Characterization of photosystem II activity and heterogeneity during the cell cycle of the green alga *Scenedesmus quadricauda*. *Plant Physiol.,* **120**: 433–441.

Kana, T.M. (1990). Light-dependent oxygen cycling measured by an oxygen-18 isotope dilution technique. *Mar. Ecol. Prog. Ser.,* **64**: 293–300.

Kana, T.M. (1992). Relationship between photosynthetic oxygen cycling and carbon assimilation in *Synechococcus* WH7803 (Cyanophyta). *J. Phycol,* **28**: 304–308.

Kowallik, W. (1982). Blue light effects on respiration. *Ann. Rev. Plant Physiol.* **33**: 51–72.

Kromkamp, J.C. & Limbeek, M. (1993). Effect of short-term variation in irradiance on light harvesting and photosynthesis of the marine diatom *Skeletonema costatum*: a laboratory study simulating vertical mixing. *J. Gen Microbiol.,* **139**: 2277–2284.

Kromkamp, J.C. & Peene, J. (2001) Oxygen consumption in the light by unicellular algae. Abstract S29-03. Proceedings 12th Int. *Congress on Photosynthesis*, Brisbane.

Lewitus, A.J. & Kana, T.M. (1995). Light respiration in six estuarine phytoplankton species: contrasts under photoautotrophic and mixotrophic growth conditions. *J. Phycol.*, **31**: 754–761.

Luduena, R.F. & Roach, M.C. (1991). Tubulin sulfhydryl-groups as probes and targets for antimitotic and antimicrotubule agents. *Pharmacol. Ther.*, **49**: 133–152.

Martin-Jézéquel, V., Hildebrand, M. & Brzezinski, M.A. (2000). Silicon metabolism in diatoms: implications for growth. *J. Phycol.,* **36**: 821–840.

Mitchison, J.M. (1971). *The biology of the cell.* Cambridge University Press., Cambridge.

Olson, R.J., Vaulot, D. & Chisholm, S.W. (1986). Effects of environmental stresses on the cell cycle of two marine phytoplankton species. *Plant Physiol.*, **80**: 918–925.

Osmond, C.B. & Grace, S.C. (1995). Perspectives on photoinhibition and photorespiration in the field - Quintessential inefficiencies of the light and dark reactions of photosynthesis. *J. Exp. Bot.,* **46**: 1351–1362.

Paul, J.S. & Volcani, B.E. (1976). Photorespiration in diatoms IV: Two pathways of glycolate metabolism in synchronized cultures of *Cylindrotheca fusiformis*. *Arch. Microbiol.*, **110**: 247–252.

Peltier, G. & Thibault, P. (1985). O_2 uptake in the light in *Chlamydomonas*. *Plant Physiol.*, **79**: 225–230.

Planchais, S., Glab, N., Inzé, D. & Bergounioux, C. (2000). Chemical inhibitors: a tool for plant cell cycle studies. *FEBS Lett.*, **476**: 78–83.

Platt, T. & Gallegos, C. L. (1980). Modelling primary production. In *Primary productivity in the sea* (Falkowski, P. G., editor), 339–351 Plenum Press. New York.

Post, A.F., Eijgenraam, F. & Mur, L.R. (1985). Influence of light period length on photosynthesis and synchronous growth of the green alga *Scenedesmus protuberans*. *Br. Phycol. J.*, **20**: 391–397.

Raven, J.A., Kübler, J.E. & Beardall, J. (2000). Put out the light, then put out the light. *J. Mar. Biol. Ass. U.K.*, **80**: 1–25.

Schreiber, U., Schliwa, U. & Bilger, W. (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynt. Res.*, **10**: 51–62.

Schreiber, U., Hormann, H., Asada K. & Neubauer, C. (1995) O_2-dependent electron flow in intact spinach chloroplasts: properties and possible regulation of the Mehler-ascorbate peroxidase cycle. In *Photosynthesis: from light to Biosphere* (Mathis, P. editor), 813–818 Kluwer Academic Publishers. Dordrecht, The Netherlands.

Shibata, K., Benson, A.A. & Calvin, M. (1954) The absorption spectra of suspensions of living micro-organisms. *Biochim. Biophys. Acta*, **15**: 461–470.

Sorokin, C. (1957). Changes in photosynthetic activity in the course of cell development in *Chlorella*. *Physiol. Plant*, **10**: 659–666.

Spadari, S., Focher, F., Sala, F., Ciarrocchi, G., Koch, G., Falaschi, A. & Pedrali-Noy, G. (1985). Control of cell division by aphidicolin without adverse effects upon resting cells. *Arzneimittelforschung*, **35**: 1108–1116.

Strasser, B.J., Dau, H., Heinze, I. & Senger, H. (1999). Comparaison of light induced and cell cycle dependent changes in the photosynthetic apparatus: A fluorescence induction study on the green alga *Scenedesmus obliquus*. *Photosynth. Res.*, **60**: 217–227.

Sullivan, C.W. (1986). Silicification by diatoms. In *Silicon biochemistry*. (Everes, D. & O'Connor, M., editors), 59–89 Wiley Interscience. Chichester.

Sullivan, C.W. & Volcani, B.E. (1981). Silicon in the cellular metabolism of diatoms. In *Silicon and siliceous structures in biological systems* (Simpson, T. L. & Volcani, B. E., editors), 15–42 Springer Verlag. New York.

Sültemeyer, D., Klug, K. & Fock, H.P. (1986). Effect of photon fluence rate on oxygen evolution and uptake by *Chlamydomonas reinhardii* suspensions grown in ambient and CO_2-enrichied air. *Plant Physiol.*, **81**: 372–375.

Sültemeyer, D., Klug, K. & Fock, H.P. (1987). Effect of dissolved inorganic carbon on oxygen evolution and uptake by *Chlamydomonas reinhardii* suspensions adapted to ambient and CO_2-enrichied air. *Photosynth. Res.*, **12**: 25–33.

Szurkowski, J., Bascik-Remisiewicz, A., Matusiak, K. & Tukaj, Z. (2001). Oxygen evolution and photosynthetic energy storage during the cell cycle of green alga *Scenedesmus armatus* characterized by photocoustic spectroscopy. *J. Plant Physiol.*, **158**: 1061–1067.

Vasquez, R.J., Howell, B., Yvon, A.M.C., Wadsworth, P. & Cassimeris, L. (1997). Nanomolar concentrations of nocodazole alter microtubule dynamic instability in vivo and in vitro. *Mol. Biol. Cell*, **8**: 973–985.

Vaulot, D., Olson, R.J., Merkel, S. & Chisholm, S.W. (1987). Cell-cycle response to nutrient starvation in two phytoplankton species *Thalassiosira weissflogii* and *Hymenomonas carterae*. *Mar. Biol.*, **95**: 625–630.

Veldhuis, M.J.W., Cucci, T.L. & Sieracki, M.E. (1997). Cellular DNA content of marine phytoplankton using two new fluorochromes: Taxonomic and ecological implications. *J. Phycol.*, **33**: 527–541.

Weger, H.G., Herzig, R., Falkowski, P.G. & Turpin, D.H. (1989). Respiratory losses in the light in a marine diatom: measurements by short-term mass spectrometry. *Limnol. Oceanogr.*, **34**: 1153–1161.

Winter, J. & Brandt, P. (1986). Stage-specific state I-state transitions during the cell cycle of *Euglena gracilis*. *Plant Physiol.*, **81**: 548–552.

Xue, X., Gauthier, D.A., Turpin, D.H. & Weger, H.G. (1996). Interaction between photosynthesis and respiration in the green alga *Chlamydomonas reinhardtii*. Characterization of light-enhanced dark respiration. *Plant Physiol.*, **112**: 1005–1014.