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GROWTH AND CELL CYCLE OF TWO CLOSELY RELATED RED TIDE-FORMING DINOFLAGELLATES: *GYMNODINIUM NAGASAKIENSE* AND *G.* CF. *NAGASAKIENSE*[1]

Frédéric Partensky,[2] *Daniel Vaulot*

Station Biologique (CNRS & Université Paris 6), Place Teissier, B.P. 74, F-29682 Roscoff cedex, France

and

Christiane Videau

Laboratoire de Physiologie végétale, Université de Bretagne Occidentale, F-29287 Brest cedex, France

ABSTRACT

Small-sized vegetative cells were found to co-occur with normal-sized cells in populations of the European bloom-forming dinoflagellate Gymnodinium *cf.* nagasakiense *Takayama et Adachi, currently known as* Gyrodinium aureolum *Hulburt, but not in populations of the closely related Japanese species* Gymnodinium nagasakiense. *We examined how cell size differentiation may influence growth and cell cycle progression under a 12:12-h light: dark cycle in the European taxon, as compared to the Japanese one. Cell number and volume and chlorophyll red fluorescence in both species varied widely during the photocycle. These variations generally appeared to be related to the division period, which occurred at night, as indicated by the variations of the fraction of binucleated cells (mitotic index) as well as the distribution of cellular DNA content. "Small" cells of* G. *cf.* nagasakiense *divided mainly during the first part of the dark period, although a second minor peak of dividing cells could occur shortly before light onset. In contrast, "large" cells displayed a sharp division peak that occurred 9 h after the beginning of the dark period. The lower degree of synchrony of "small" cells could be a consequence of their faster growth. Alternatively, these data may suggest that cell division is tightly controlled by an endogenous clock in "large" cells and much more loosely controlled in "small" cells. Cells of the Japanese species, which were morphologically similar to "large" cells of the European taxon, displayed an intermediate growth pattern between*

[1] Received 24 April 1991. Accepted 21 August 1991.
[2] Address for reprint requests.

the two cell types of G. *cf.* nagasakiense, *with a division period that extended to most of the dark period.*

Key index words: cell cycle; circadian clock; dinoflagellate; DNA; flow cytometry; growth patterns; Gymnodinium nagasakiense; Gyrodinium aureolum; *Pyrrophyta*

Occurrence of phased cell division in dinoflagellates in response to light : dark (LD) cycles is well documented both in culture and in the field (Sournia 1974, Chisholm 1981), partly because many authors have tried to estimate growth rates of natural populations from the observed variations of the fraction of dividing cells (e.g. Weiler and Eppley 1979, Elser and Smith 1985). However, whether the cell division cycle in this and other algal groups is directly entrained by the photocycle (Spudich and Sager 1980) or is coupled to an endogenous oscillator that is aligned with the photocycle (Edmunds and Adams 1981) is still debated. Interspecific variations in the temporal patterns of cell division and in the coupling between division and DNA replication have been identified among the different phytoplankton groups as well as among dinoflagellates (Chisholm 1981, Karentz 1983). Nevertheless, division patterns in response to particular entraining forces (e.g. the LD cycle) are commonly considered to be a conservative property of species, each species possessing its own "circadian chronotype," which is the temporal analogue of the phenotype (Chisholm 1981). The recent discovery of two different-sized vegetative subpopulations in culture and natural samples of the Northern European dinoflagellate *Gymnodinium* cf. *nagasakiense* (Partensky et al. 1988, Partensky and Vaulot 1989, Videau and Partensky 1990), a species misidentified in the literature as *Gyrodinium aureolum* Hulburt (as discussed in Partensky and Sournia 1986 and Partensky et al. 1988), led us to wonder whether cell size differentiation could affect the growth pattern and cell cycle of a single species. The closely related Japanese species *Gymnodinium nagasakiense* Takayama et Adachi, which can be distinguished from the European taxon by its much higher DNA content (Partensky et al. 1988, Boucher et al. 1991) and which does not show any cell size differences in culture (Partensky and Vaulot 1989), was also included in this study. Short-term variations in cell concentration and volume, chlorophyll (chl) red fluorescence, mitotic index, and cellular DNA content, and long-term variations of cell concentration and volume, were analyzed in batch cultures of both species, including cultures dominated by either "small" or "large" cells of *G.* cf. *nagasakiense* (i.e. with more than 90% of one cell type). These analyses revealed significant discrepancies in the growth physiology and its coupling to the LD cycle between the two cell types of *G.* cf. *nagasakiense* and between the "large" cells of this species and the typical cells of *G. nagasakiense.*

MATERIALS AND METHODS

One culture of the Japanese species *Gymnodinium nagasakiense* (GN1 from clone "Buzen-'85-2") and three cultures of the European species *G.* cf. *nagasakiense* (GCFN1 and GCFN2 from clone "Tinduff"; GCFN3 from strain "Iroise") were grown in 5-L Erlenmeyer flasks at 20° C in K medium (Keller et al. 1987) under a 12:12-h LD cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. These conditions were slightly different from the optimal culture conditions determined for the latter species by Nielsen and Tonseth (1991)—i.e. 17.5–22.5° C, K medium made up from seawater of 24.5–34‰ salinity, 18:6-h LD cycle, and 255 $\mu mol \cdot m^{-2} \cdot s^{-1}$—but led to similar generation times: 1.84 d (see Results), compared to the 1.64–1.92 d (0.52–0.61 $div \cdot d^{-1}$) reported by Nielsen and Tonseth (1991). For long-term analyses of cell number and volume changes, cultures were sampled at regular intervals (generally 1 d) shortly after light onset and until the poststationary phase. Several other cultures of both species grown in small volumes (500 mL) were also sampled daily to assess the variability of the observations made on large-volume cultures. Short-term variations in cell number and volume, chl red fluorescence, cellular DNA content, and the fraction of binucleated cells (mitotic index) were analyzed by sampling 5-L cultures during the exponential growth phase at 3-h intervals during two consecutive LD cycles. Cell concentration and volume measurements were performed on live samples with a Coulter counter model ZM (Coulter, Hialeah, Florida) interfaced to an IBM-PC-compatible computer, as described earlier (Partensky and Vaulot 1989). Cell volume distributions were analyzed with custom-designed software, CYTOPC (Vaulot 1989), using calculation windows. For bimodal populations of *G.* cf. *nagasakiense* (see Partensky and Vaulot 1989 for examples), window 1 ("small" cells) was set between the lower limit of the first peak and the trough (channel n) between the first and second peaks. Window 2 ("large" cells) was set between channel n + 1 and the upper limit of the second peak. Mean generation times (t_g in days) were calculated on each culture for 2-d periods of the exponential growth phase, as follows:

$$t_g = \frac{\ln 2 \times (t_1 - t_2)}{\ln N(t_1) - \ln N(t_2)}, \quad (1)$$

where N(t) = cell density at time t.

Relative fluorescences of chl and DNA were determined on individual cells with an EPICS 541 flow cytometer (Coultronics) equipped with a 6-W argon laser (Coherent, Palo Alto, California) as the excitation light source (100 mW). These measurements were made on both subpopulations of *G.* cf. *nagasakiense.* Fluorospheres (9.85 μm diam; Fullbright, Coulter) were used as internal standards to normalize flow cytometric measurements of chl red fluorescence. Samples for red fluorescence analyses were distributed in 1.8-mL cryotubes, fixed with 1% glutaraldehyde (electron microscopy grade; Merck, Darmstadt, Germany), immediately frozen in liquid nitrogen, and stored frozen until analysis, as described in Vaulot et al. (1989). Flow cytometric red fluorescence was normalized by fluorometric determination of chl *a* (Yentsch and Menzel 1963). Four cultures of *G.* cf. *nagasakiense* grown at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and comprising different proportions of "small" and "large" cells were harvested onto GF/F filters (Whatman, Maidstone, England), which were extracted in 90% acetone at 4° C for 24 h and homogenized with a Teflon grinder (Thomas model C) prior to reading with a Turner Design fluorometer. This calibration (Fig. 1) showed that, under the light conditions we used, red fluorescence (RFL in relative units) was linearly correlated with chl content (pg $chl \cdot cell^{-1}$) for live cells of *G.* cf. *nagasakiense*:

$$RFL = 0.395\ chl + 0.089 \quad (n = 4,\ r = 0.996). \quad (2)$$

The fixation process slightly reduced the fluorescence of cell pigments (RFF) but did not affect the correlation between the

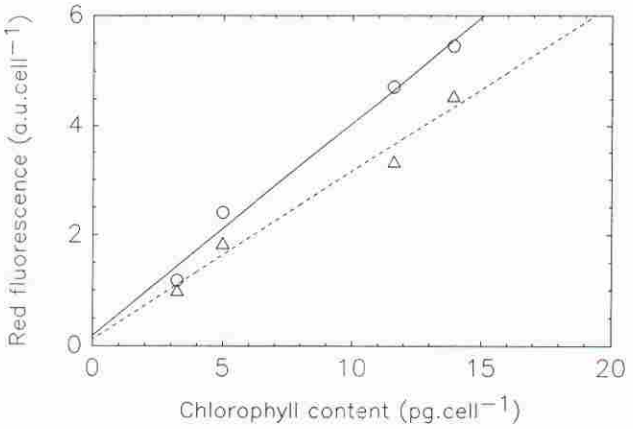

FIG. 1. Chlorophyll content determined by fluorometry vs. red fluorescence measured by flow cytometry for four different populations of *Gymnodinium* cf. *nagasakiense* grown under standard culture conditions before (○) and after (△) glutaraldehyde fixation.

two parameters:

$$\text{RFF} = 0.310\ \text{chl} + 0.058 \qquad (n = 4,\ r = 0.992). \tag{3}$$

For cell cycle studies, 15–25-mL samples were fixed with 1% glutaraldehyde and stored at 4° C in darkness until analysis. Prior to flow cytometric DNA measurements, cells were concentrated by centrifugation at 5000 rpm. All but 250 μL of the supernatant was discarded, and cells were resuspended by gentle shaking. Cells were then stained for 30 min with either 2.5 μg·mL^{-1} 4′-6′diamino-2-phenylindole (DAPI; Polysciences, Warrington, Pennsylvania) or 20 μg·mL^{-1} chromomycin A3 (CA3) and 0.1% Triton X-100 (both chemicals from Sigma, St. Louis, Missouri), final concentrations in K medium. Excitation light was either 351/364 nm (UV) for DAPI or 457 nm (blue) for CA3, and DNA fluorescence was collected (respectively) through a 480- or 580-nm band-pass filter (Glen Spectra, Stanmore, England). The fraction of cells within the G_1 phase of the cell cycle was determined on each sample by analyzing DNA distributions with a modification of Dean and Jett's (1974) method, as described in Vaulot et al. (1986). Only in culture GCFN3 were coefficients of variation of G_1 peaks sufficiently low to determine precisely the proportion of cells within the S (DNA-synthesizing cells) and G_2 + M (cells with a double concentration of DNA) phases of the cell cycle.

The numbers of binucleated cells (BC) and total cells (TC) were counted for each sample with an Olympus BH2 epifluorescence microscope under UV light after staining cell nuclei for 10 min using 0.1% of a 1-g·L^{-1} DAPI solution in distilled water. Mitotic index (f in %) was calculated as follows (McDuff and Chisholm 1982):

$$f = \frac{BC \times 100}{TC}. \tag{4}$$

RESULTS

Long-Term Variations

Growth patterns. The growth pattern of the Japanese species *G. nagasakiense* (GN1; Fig. 2A, B) was simple, because cell volume distributions always were unimodal, even during periods of division (see Partensky and Vaulot 1989 for illustration). In contrast, the European species displayed a considerable variability in its growth pattern under standard culture

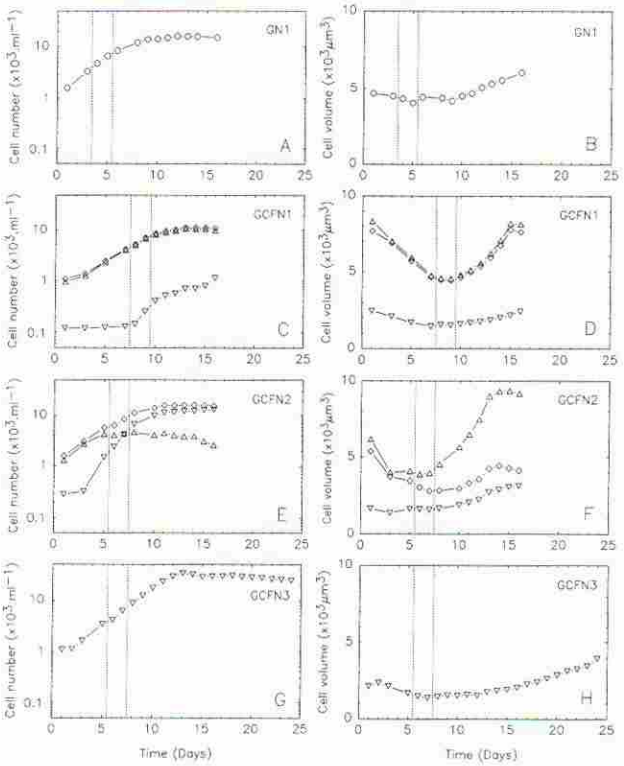

FIG. 2A–H. Long-term variations of cell number (A, C, E, G) and volume (B, D, F, H) for cultures grown under a 12:12-h LD cycle. A, B) Culture of *Gymnodinium nagasakiense* (GN1). C–H) Three cultures of *G.* cf. *nagasakiense*. C, D) Culture GCFN1 dominated by "large" cells. E, F) Culture GCFN2 with co-occurrence of "large" and "small" subpopulations. G, H) Culture GCFN3 dominated by "small" cells, the number of "large" cells being too low to determine cell volume and number. ○, Typical cells of *G. nagasakiense*; ◇, total cells of *G.* cf. *nagasakiense*; △, "large" cells of *G.* cf. *nagasakiense*; ▽, "small" cells of *G.* cf. *nagasakiense*. The 48-h periods during which cultures were sampled at 3-h intervals are indicated by the two vertical dashed lines (see Fig. 3).

conditions (see above), resulting from the occurrence of a subpopulation of vegetative "small" cells, which divided independently from normal-sized cells. This phenomenon was visualized by the bimodality of cell volume distributions, as shown earlier (Partensky and Vaulot 1989). Three different growth patterns of *G.* cf. *nagasakiense* were observed: dominance of "large" cells (GCFN1; Fig. 2C, D), dominance of "small" cells (GCFN3; Fig. 2G, H), and co-occurrence of both cell types (GCFN2; Fig. 2E, F). It is unlikely that these variations were strain-dependent, because GCFN1 and GCFN2, which displayed different growth patterns, were two cultures of the same clone of a "large" cell, this clone originating from a population of initially "large" cells isolated by cell sorting ("Tinduff" strain; Partensky and Vaulot 1989). However, patterns such as that observed in GCFN3 were noted only with the Iroise strain, which was nonclonal and also originated from a natural population of "large" cells.

Generation times. "Small" cells of *G.* cf. *nagasakiense*

TABLE 1. *Mean generation times (in days) of* Gymnodinium nagasakiense *and* G. *cf.* nagasakiense *cultures shown in Figure 2. Values were calculated both for a 2-d period at the beginning of exponential growth (Days 3–5) and for the periods during which cultures were sampled at 3-h intervals.*

Species	Strain	Culture code	Sampling period	Generation time (d)					
				Total cells		"Small" cells		"Large" cells	
				Days 3–5	SP[a]	Days 3–5	SP	Days 3–5	SP
Gymnodinium nagasakiense	Buzen-'85-2	GN1	Days 3–5	2.18	2.18	NA[b]	NA	NA	NA
Gymnodinium cf. *nagasakiense*	Tinduff	GCFN1	Days 7–9	2.37	2.46	ND[c]	ND	2.28	2.45
	Tinduff	GCFN2	Days 5–7	2.14	2.51	0.92	1.11	3.00	12.83
	Iroise	GCFN3	Days 5–7	1.84	1.88	1.84	1.88	ND	ND

[a] SP = periods during which cultures sampled at 3-h intervals.
[b] NA = not applicable.
[c] ND = not determined (cell concentration too low for accurate measurements).

displayed relatively short t_g, i.e. about 1.9 d when in pure population (GCFN3) and down to 0.92 d when in mixture with "large" cells (GCFN2; Table 1), although the latter value might be slightly overestimated because of the partial overlap of cell distributions of "large" and "small" cells (see Partensky and Vaulot 1989). In contrast, "large" cells of *G.* cf. *nagasakiense* had t_g longer than 2.2 d, both in GCFN1 and GCFN2. The generation time of the Japanese species *G. nagasakiense* (culture GN1; Fig. 2A) was intermediate between those of the "small" and "large" forms of the European taxon (2.18 d) and similar to the mean t_g of the mixed culture (GCFN2; Table 1). These values were consistent with those found in 500-mL flasks for populations of *G.* cf. *nagasakiense* dominated by "large" cells (t_g = 2.46 ± 0.12 d; n = 4) or "small" cells (t_g = 1.91 ± 0.07 d; n = 5), and for typical populations of *G. nagasakiense* (t_g = 2.04 ± 0.14 d; n = 5).

Cell yield. Cell concentration at the end of the exponential phase was significantly lower in culture GCFN1, which was dominated by "large" cells of *G.* cf. *nagasakiense* (11,350 cells·mL^{-1}; Fig. 2C), than in GCFN2, where "small" and "large" cells co-occurred (16,220 cells·mL^{-1}; Fig. 2E). GCFN3, an almost pure population of "small" cells, had by far the highest final yield (37,860 cells·mL^{-1}; Fig. 2G). Surprisingly, the maximum cell concentration reached by the Japanese species *G. nagasakiense* (16,100 cells·mL^{-1}; Fig. 2A) was closer to that of GCFN2 than to that of GCFN1, although cells of this species had a volume similar to "large" cells of the European taxon (Fig. 2B, D). It is noteworthy that final yields in 5-L Erlenmeyer flasks generally were lower than yields observed in 500-mL flasks under the same culture conditions. In the latter flasks, populations dominated by "large" cells of *G.* cf. *nagasakiense* had an average yield of 18,190 ± 1,270 cells·mL^{-1} (n = 4), whereas populations dominated by "small" cells had a yield of 21,400 ± 1,880 cells·mL^{-1} (n = 6) (no statistical data on pure populations of "small" cells). Populations of *G. nagasakiense* grown in 500-mL flasks reached a final concentration of 20,510 ± 1,050 cells·mL^{-1} (n = 5). The discrepancies observed between flasks of different sizes may partly be due to the lower ratio of the surface in contact with air to the total volume in 5-L Erlenmeyer flasks, because cells of both species concentrated themselves near the surface by phototactism.

Cell volume. Long-term variations in cell volume followed a similar pattern in both species and in the two cell types of *G.* cf. *nagasakiense*: an increase during the lag phase (when any), a decrease to a minimum during exponential growth, and finally a new increase during the late exponential and stationary phases. These variations were more marked for "large" cells of the European species (Fig. 2D, F) than for typical cells of the Japanese species (Fig. 2B), with a cell volume increase of 78–133% for the former (4550–8120 μm^3 for GCFN1; 4010–9360 μm^3 for GCFN2) vs. 30% for the latter (4620–6020 μm^3 for GN1). The amplitude of cell volume changes noted in GN1 was, however, smaller than that observed in 500-mL flasks for *G. nagasakiense*, for which volume varied on average by 54.8 ± 14.6% (n = 5). "Small" cells of *G.* cf. *nagasakiense* displayed large cell volume variations (1410–3140 μm^3 for GCFN2, i.e. a variation of 123%; 1380–3960 μm^3 for GCFN3, i.e. a variation of 187%) during the experimental period and were still enlarging when sampling ended, although at that time they had attained a volume similar to the minimum volume observed for "large" cells.

In culture GCFN2, the ratio of "large" to "small" mean cell volumes decreased from 3.67 on Day 1 to 2.36 on Day 6, then increased and reached a maximum of 3.31 when "large" cells ceased to enlarge (Day 12). Because the volume of "small" cells went on increasing, peaks formed by "small" and "large" cells in volume distributions progressively drew nearer together. In some 500-mL cultures comprising both cell types, we sometimes observed that these peaks finally superimposed during the late stationary phase (not shown), such that the "large" to "small" mean cell volume ratio approached 1.

Short-Term Variations

Cell division. Cultures were sampled at 3-h intervals during two photocycles (Fig. 2, dashed lines). In culture GCFN1, the percentage of "small" cells of *G.* cf. *nagasakiense* never exceeded 5.5% during the 48-h sampling (Fig. 2C), such that average volume and biochemical changes (DNA, chl) reflected mostly those of "large" cells. In contrast, variations observed in culture GCFN3, where the "large" cell fraction was too low to form a definite peak in cell volume distributions, were characteristic of "small" cells (Fig. 2G). Observations made on GCFN2, a mixture of both cell types (Fig. 2E), also were useful to study the behavior of these subpopulations when co-occurring, although in this culture, only "small" cells were actively dividing during the 48-h sampling, whereas the number of "large" cells remained almost constant (Fig. 2E).

Diel variations of cell number and volume during the exponential growth phase appeared to be closely related to cell division, whatever the species or cell type (Figs. 3A–T, 4A–E). Changes in the percentage of binucleated cells (mitotic index) showed that division mainly occurred at night (Fig. 3E, J, O, T, Y). However, "large" cells of *G.* cf. *nagasakiense* displayed a very sharp division peak shortly before light onset (Fig. 3O), in contrast to cells of the Japanese species, which divided from the first hours of the dark period up to 3 h before light onset (Fig. 3E). For "small" cells of *G.* cf. *nagasakiense,* a first peak of dividing cells always occurred in the first hours of the dark period, sometimes followed by a second peak of division just before light onset (first LD cycle in GCFN2, Fig. 3T; second LD cycle in GCFN3, Fig. 3Y).

Cell cycle. The variations of the percentage of cells within the G_1 phase of the cell cycle (G_1%) confirmed these findings (Fig. 3D, I, N, S, X). The determination of the percentage of G_2 and S cells (G_2% and S%) was possible only in culture GCFN3, because the DNA staining method with CA3 used for this culture gave better results than that using DAPI (all other cultures). Moreover, the coefficients of variation of G_1 peaks always were higher for "large" cells than for "small" cells, even in a mixture of both cell types. This might be related to the differences in chromatin condensation previously observed between the two cell types (Partensky and Vaulot 1989). Variations of G_1%, although providing incomplete information about the cell cycle, may be interpreted if we suppose that each decrease of G_1% corresponds to an increase of S%, i.e. to the beginning of DNA synthesis, and each increase of G_1% corresponds to the release of the first daughter cells after completion of mitosis. The variations of cellular DNA in culture GCFN3 (Fig. 3X) confirmed these assumptions. Although the different cultures and cell types exhibited different cycling patterns, some generalizations can be made. First, G_1% began to decrease at the dark-to-light transition. The only exception was for the "large" cells in GCFN2, for which the G_1% decrease occurred 3 h later. This suggests that light may trigger entry into S phase, i.e. DNA synthesis. For "large" cells, the rate of entry into S phase appeared to be low during the light period and then increased during the first 3 h in the dark. The more erratic variations of the G_1 cell fraction observed during the second LD cycle in culture GCFN2 (Fig. 3N) might be related to the slow division rate of "large" cells during this period, as shown by the significant decrease in the maximum mitotic index between the first and second division bursts (Fig. 3O) and by the absence of cell number increase after the second division burst (Fig. 3K). For "small" cells, entry into S occurred at a more constant rate than for "large" cells and only took place during the light period. Second, for "large" cells of *G.* cf. *nagasakiense* and cells of *G. nagasakiense,* increase in G_1% began 3 h after the dark-to-light transition, whereas for "small" cells it occurred right at the light-to-dark boundary. This indicates that the cell division trigger has a fixed phase with respect to this boundary that differed by 3 h between "small" and "large" cells, in agreement with the mitotic index data.

Cell volume. In both *G. nagasakiense* and *G.* cf. *nagasakiense,* cell volume underwent large circadian changes. In the Japanese species, mean cell volume regularly increased during the light period, when no division occurred, and decreased during the dark period (Fig. 3B), when cells divided. During the experiment, the decrease at night was greater (up to 29% of the volume at the end of the light period) than the increase during daytime (19%), accounting for the daily reduction in mean cell volume that occurred during the experimental period (Fig. 2B). Similarly, the cell volume decrease of "large" cells of *G.* cf. *nagasakiense* in GCFN1 (22–27%, Fig. 3G) and the parallel cell number increase (Fig. 3F) were clearly linked with cell division, which in this case was restricted to the latter part of the dark period (Fig. 3I, J). Interestingly, a decrease in mean cell volume was also visible to a lesser extent during the second dark period in culture GCFN2 (Fig. 3L) without significant increase in total "large" cell number (Fig. 3K), suggesting that the rates of cell division and mortality were balanced. In "small" forms of *G.* cf. *nagasakiense,* cell volume decreased regularly during the dark period and increased during the light period, but it is worth noting that cell number and volume could increase together during the light period (Fig. 3P, Q, second cycle; Fig. 3U, V). The slight decrease in "small" cell number following division bursts, which was particularly clear in culture GCFN3 (Fig. 3U), might correspond to a particular sensitivity of the newly divided "small" cells to shaking prior to cell counts. Absence of synchrony between cell volume variations of "small" and "large" during nighttime clearly appeared in culture GCFN2.

Consequently, the ratio of "large" to "small" mean cell volumes increased progressively during the first part of the dark period to a maximum of 2.8 and diminished sharply during the 3 h preceding the light period, i.e. after the division burst of "large" cells. In contrast, this ratio varied little during daytime (2.35–2.45).

Chlorophyll fluorescence. The variations of chl red fluorescence in cells of *Gymnodinium nagasakiense* (Fig. 3C) followed closely the changes in volume in these cells (Fig. 3B), although they were less pronounced (17–18% between the maximum and the minimum of fluorescence, corresponding to variation of 20–21% in chl *a* content, if we refer to Eq. 3), especially during the last hours of the dark period. Similarly, in "small" cells of *G.* cf. *nagasakiense,* chl content sharply decreased after the end of the light period but generally increased during the last hours of the dark period, in contrast to volume (Fig. 3R, W). Changes in chl red fluorescence in "large" cells of *G.* cf. *nagasakiense* were characterized by a diurnal increase, followed by a drop beginning at or shortly before the time of light offset, then by a peak occurring in the middle of the dark period (Fig. 3H, M). This peak of red fluorescence preceded a second, sharp drop occurring just before light onset.

DISCUSSION

The phenomenon of cell size differentiation was recently shown to occur repeatedly in cultures of *Gymnodinium* cf. *nagasakiense* (Partensky et al. 1988). Obvious discrepancies between "small" and "large" cell types of this species consist of different average cell sizes and degrees of nucleus condensation (Partensky and Vaulot 1989), but other, not yet characterized, biochemical discrepancies might exist between these two subpopulations, as suggested by their different growth physiologies. The ratio of "large" to "small" cell volume, which can reach up to 3.7 during the exponential phase of growth, however, approaches 1 when cells have ceased dividing. This illustrates the possible transformation of "small" cells into "large" cells. However, it is still unclear whether the "large" cells that issue from growth of "small" cells become absolutely identical to regular "large" cells from a biochemical point of view or if they possess some nonreversible biochemical alteration. Long-term variations in volume in both cell types of *G.* cf. *nagasakiense* suggest that the minimum volume is reached when the growth rate is maximum. In the short term, both subpopulations displayed large changes in cell volume and number and chl red fluorescence during the photocycle, but these changes did not occur at the same time in both types; this mainly reflected discrepancies in their division timing. "Large" cells had a single, sharp peak of division, occurring shortly before light onset, whereas "small" cells displayed either one or two division bursts, the main one at the beginning of the dark period. This discrepancy likely reflects a higher

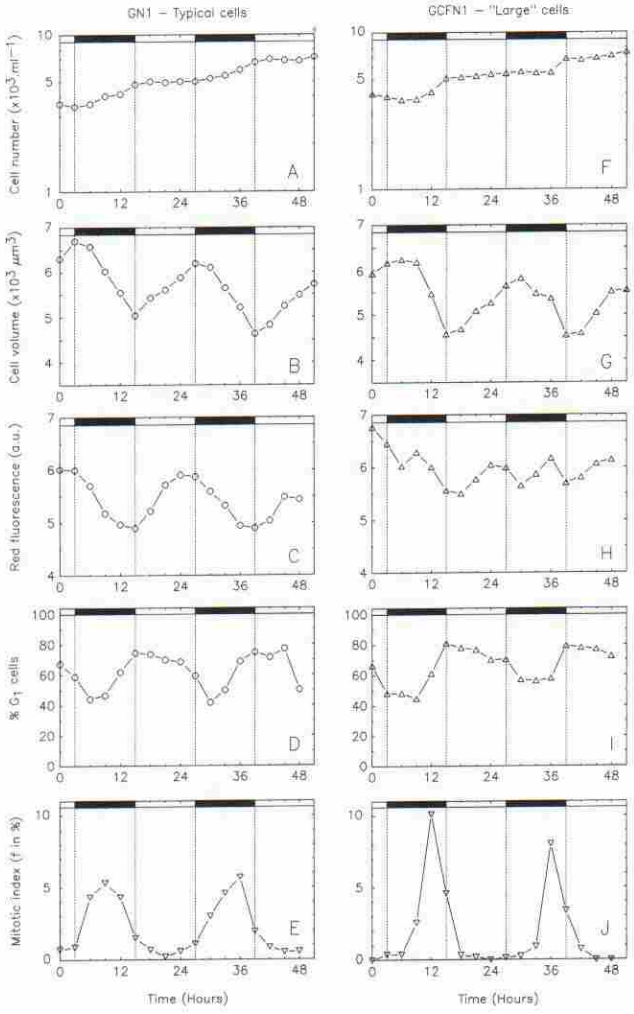

FIG. 3. Short-term variations of cell number, volume, chl red fluorescence, percentage of either (D, I, N, S) G_1 cells or (X) G_1, S, and G_2 cells, and percentage of binucleated cells for (A–E) typical cells of *Gymnodinium nagasakiense* (culture GN1), (F–J) "large" cells of *G.* cf. *nagasakiense* (culture GCFN1).

degree of phasing in "large" than in "small" cells, in response to the LD cycle. This assumption is supported by the staircase aspect of the growth curves of the "large" cells, dramatic increases in cell number being tightly correlated with division bursts. In contrast, "small" cell number appeared to increase at a roughly constant rate during the whole dark period and sometimes continued to increase at lower or similar rates during the light period. This diurnal increase might be explained by a cell-to-cell variability of the time of cell cycle completion. The occurrence in culture GCFN3 of a single peak of DNA-synthesizing cells (S phase) at the end of the light period, followed by either one or two peaks of G_2 + M and binucleated cells, confirms that a certain dispersion of the lengths of the G_2 phase and possibly mitosis may occur among individuals of a population of "small" cells.

Most slow-growing dinoflagellates studied so far

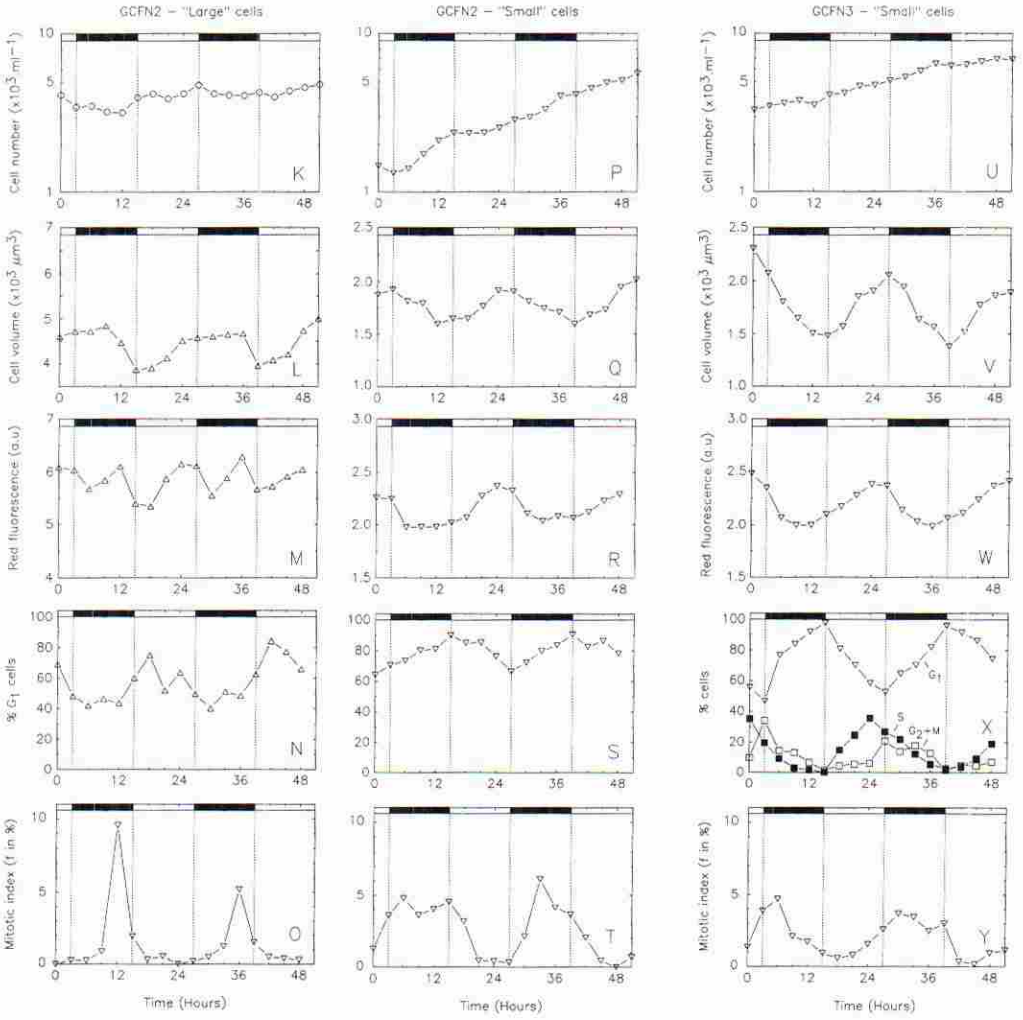

FIG. 3 (continued). (K–O) "large" and (P–T) "small" cells of *G.* cf. *nagasakiense* in culture GCFN2, and (U–Y) "small" cells of *G.* cf. *nagasakiense* in culture GCFN3. The densities of "small" cells in GCFN1 and of "large" cells in GCFN3 were too low during the sampling period to obtain reliable measurements. Black bars correspond to the dark period.

(i.e. with a $t_g > 1$ day), including *Gonyaulax polyedra*, *Ceratium furca*, and *Pyrocystis fusiformis*, are known to display a tight cell cycle phasing when grown on 24-h photocycles, with mitosis typically restricted to a 4–6-h gate near the dark-to-light transition (Chisholm 1981). Some other division patterns have been observed in dinoflagellate species, such as a division period extending to the entire dark period (*Gymnodinium splendens* and *Scrippsiella trochoidea*) or beginning in the middle of the light phase and completed by the onset of darkness (*Scrippsiella sweeneyi*, *Prorocentrum micans*, and *Gyrodinium uncatenum*; Hastings and Sweeney 1964, Chisholm 1981, Cetta and Anderson 1990). In species with high intrinsic growth rates, such as *Amphidinium carterae*, division patterns are usually characterized by a fairly broad division burst (Nelson and Brand 1979, Chisholm and Brand 1981). These discrepancies in division pattern have led to the concept of species-specific circadian "chronotype" in response to particular environmental conditions (Chisholm 1981). A given chronotype can be explained by two types of hypotheses. In the case of slow-growing populations, tight synchrony has been attributed to the coupling of cell division to an endogenous oscillator (circadian clock) with a period close to 24 h, which is reset by the environmental periodicity but can free-run in the absence of external cues (Edmunds and Adams 1981). Alternatively, the cell cycle may be controlled directly by the photocycle at specific "transition" points (Spudich and Sager 1980) that are crossed when a certain number of biochemical requirements are met (critical size or protein or DNA levels; Alberghina and Sturani 1981). The latter hypothesis accounts for a wide range of division patterns under photoperiodic conditions (Vaulot and Chisholm 1987). Our work suggests that the circadian chronotype is not species-specific, as suggested by Chisholm (1981), but also depends on the phenotype, because phenotypic discrepancies between the two cell types

of *G.* cf. *nagasakiense* are accompanied by "chronotypic" discrepancies. The division patterns observed in "large" cells of *G.* cf. *nagasakiense* match the most common trend in dinoflagellates to divide before the dark-to-light transition (Chisholm 1981). However, a division phase that extends to the whole dark period with the main division burst occurring during the early dark period, as observed in "small" cells of *G.* cf. *nagasakiense,* seems to be rare in this algal group. In cultures comprising both "small" and "large" specimens, the observed division pattern is the result of the superimposition of two different patterns. In culture GCFN2, for instance, the period of division of the total population (i.e. "small" + "large" cells) is extended to the entire dark period, although the division peak of solely the "large" cells is very tight. Weiler and Eppley (1979) provided a similar example with *Ceratium furca,* a dinoflagellate species that also forms "small" cells (called "microswarmers") at high temperature (25° C) or high cell density (i.e. more than 200 cells·mL^{-1} at 20° C). Interestingly, cultures of typical cells of *C. furca* had a sharp peak of division at the time of light onset, whereas cultures composed of a large number of microswarmers peaked shortly after light offset, which also resulted in a decrease in synchrony (Weiler and Eppley 1979).

Division patterns of *G.* cf. *nagasakiense* can be interpreted in the framework of the clock hypothesis (Edmunds and Adams 1981): division could be regulated by a circadian clock in "large" cells, whereas in "small" cells, coupling between the clock and division could be looser. Cell size differentiation would therefore be accompanied by a significant disturbance in the control of the circadian clock over division. Alternatively, the observed patterns do not conflict with the "block-point" hypothesis (Spudich and Sager 1980). The tight division of the "large" cells of *G.* cf. *nagasakiense* is typical of cells with a light-controlled transition point and a small variability in the length of the light-dependent phase (Vaulot and Chisholm 1987). The loss of synchrony of the "small" cells can then be interpreted as a mere consequence of their shorter generation time, which results in an increased heterogeneity in individual cell history with respect to the photocycle (Vaulot and Chisholm 1987). This assumption could also account for the division pattern observed for the Japanese species *G. nagasakiense,* namely a single division burst extended to the whole dark period, a pattern that appears to be intermediate between those of "large" and "small" cells of *G.* cf. *nagasakiense,* as was the growth rate of *G. nagasakiense* cells. This hypothesis is also supported by the fact that for all populations but the "large" cells of culture GCFN2, which were growing very slowly, entry into S phase appears to be triggered by light onset. This suggests the existence of a light-dependent transition point at the G_1–S boundary as observed in other phytoplankton species (Olson and Chisholm 1986, Vaulot et al. 1987, Brzezinski et al. 1990). In some dinoflagellates, such as *Gyrodinium uncatenum* and *Gonyaulax polyedra,* entry into S seems, however, to be independent of light (Homma and Hastings 1989, Cetta and Anderson 1990). The size and biochemical (RNA, proteins) requirements to be met to exit from the environmentally controlled segments and for division initiation (Alberghina and Sturani 1981) are obviously different for the two cell types, because "small" cells divide at a smaller size than "large" cells. However, the interdependence of cell size and cell division likely holds for each of the two subpopulations taken independently. The biochemical requirements of the smaller population prior to division are probably determined as soon as, or shortly after, a "small" cell, which is potentially at the origin of a clonal population of "small" cells, is formed by budding of a typical cell of *G.* cf. *nagasakiense* (Partensky and Vaulot 1989).

Temporal change of any cellular parameter averaged over the population is the result of competition between growth processes, which will increase its average value, and division processes, which will decrease its average value. The general tendency of average volume to increase during the light period and stagnate or decrease during the dark period, even in the absence of strong division, suggests that cell volume growth of both *G. nagasakiense* and *G.* cf. *nagasakiense* can occur only in the presence of light and that cell volume remains constant or even decreases during the dark. Changes in red fluorescence were more complex. The good correlation existing between the chl content and the fluorescence measured by flow cytometry, even after glutaraldehyde fixation, led us to interpret the variations of fluorescence as variations of pigment content. However, such correlation might only hold for the particular light conditions we used (100 μmol·m^{-2}·s^{-1}), because fluorescence per unit chl *a* in *Amphidinium carterae* decreased at high concentrations of chl due to photoadaptation (Sosik et al. 1989). Red fluorescence of the "small" cells of *G.* cf. *nagasakiense* decreased during the first part of the dark period, i.e. at the time of the main division burst, and increased thereafter. This suggests that chl is continuously synthesized during the photocycle and that chl per cell only decreases as a result of the division process. The same is true for cells of *G. nagasakiense.* In contrast, "large" cells of *G.* cf. *nagasakiense* displayed a particular chl fluorescence pattern, with a first maximum 3 h before the end of the light period, a decrease, and then a second maximum in the dark, just before the division burst. These data suggest that "large" cells synthesize chl in the light. They cease synthesizing chl shortly before the beginning of the dark period and even lose chl in the first hours of the dark period. Then they have a second period of chl synthesis during the second part of the dark

period. The sharp decrease appearing after the fluorescence peak is linked to cell division, which might mask the exact extension of the dark period of chl synthesis in these cells. Similarly, in *Amphidinium carterae*, the volume-normalized chl begins to increase in the middle of the light phase to reach a maximum 2 h after the beginning of the dark period, then drops sharply before the onset of cell division; this also suggests a loss of chl intercalated between two periods of chl synthesis (Gerath and Chisholm 1989). The maximum amplitude of variation of chl per cell (about 100%) in this species was significantly larger than in *G.* cf. *nagasakiense* (27–30% in both cell types), which may partly be due to differences in generation times.

Cell size differentiation may have important consequences for the *in situ* growth dynamics of *G.* cf. *nagasakiense*, as previously discussed (Partensky and Vaulot 1989). Whether the lower degree of synchrony in "small" than in "large" cells also has ecological implications is more difficult to assess. Cell cycle asynchrony might allow "small" cell populations of *G.* cf. *nagasakiense* to better use those nutrient resources that are needed only at a given point of the cell cycle (Vaulot et al. 1987) by spreading cell uptake over a longer part of the photocycle. Moreover, if division is spread over a long period rather than limited to a narrow time interval, some of the recently divided cells might have a better chance to escape predators performing vertical migrations.

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ULTRASTRUCTURE OF VEGETATIVE AND DIVIDING CELLS OF THE UNICELLULAR RED ALGAE *RHODELLA VIOLACEA* AND *RHODELLA MACULATA*[1]

Laura M. Patrone

Department of Biological Sciences, State University of New York at Binghamton, Binghamton, New York 13901

Sharon T. Broadwater and Joseph L. Scott[2]

Department of Biology, College of William and Mary, Williamsburg, Virginia 23185

ABSTRACT

Rhodella violacea *(Kornmann) Wehrmeyer and* Rhodella maculata *Evans were investigated for ultrastructural details of vegetative and dividing cells.* Rhodella violacea *has a nuclear projection into the pyrenoid similar to that found in* R. maculata, *although the nuclear projection in* R. maculata *traverses a starch-lined area before contacting the pyrenoid. Unlike most red algae, the two* Rhodella *species lack a peripheral encircling thylakoid in the chloroplast and have dictyosomes associated solely with endoplasmic reticulum (ER) instead of with both mitochondria and ER. Both species also have a well-developed peripheral system of ER connected to the plasmalemma by tubules, a situation found only in red algal unicells. Cell division was studied primarily in* R. violacea; *a less thorough examination of* R. maculata *showed no essential differences. Both have small, double-ringed, nucleus-associated organelles (NAOs) surrounded by moderately electron-dense material, metaphase–anaphase polar gaps in the nuclear envelope, absence of perinuclear ER, and short interzonal spindles. This pattern of mitosis is similar in most respects to that reported in the unicell* Flintiella. *Following mitosis, microtubules extend from the region of each NAO to its associated nucleus and to the undivided pyrenoid. The NAOs appear to apply tension to the nuclei and the pyrenoid and may be the mechanism for ensuring equal partitioning of both organelles. Two different forms of pyrenoid–nucleus association occur during mitosis. Nuclear projections into the pyrenoid, prevalent during interphase and early stages of mitosis, recede at metaphase. Then, the pyrenoid extends protrusions into the nuclear polar areas, forming a cup that partially surrounds the nucleus. Cell division and vegetative characters confirm the close taxonomic affinity of these two species of* Rhodella *and support their separation from the genus* Porphyridium.

Key index words: cell division; cytokinesis; mitosis; nucleus; polar ring; pyrenoid; Rhodella maculata; Rhodella violacea; *Rhodophyta*

The genus *Rhodella* was described by Evans (1970) to accommodate a unicellular marine red alga isolated from sand at Essex, England. This alga, *R. maculata*, exhibited chloroplast and nuclear features that were significantly different from all other red algal unicells. Wehrmeyer (1971) transferred *Porphyridium violaceum* to *Rhodella* (renaming it *R. violacea*) due to several striking ultrastructural features that suggested a much closer affinity to the genus *Rhodella* than to *Porphyridium* (Gantt and Conti 1965, Gantt et al. 1968).

A thorough investigation of cell division in *R. violacea* (Kornmann) Wehrmeyer and a partial study in *R. maculata* Evans were undertaken in our laboratory as part of a continuing effort to use cell division characters to better understand the phylogeny of the Rhodophyta. Prior to this study, only two genera of unicellular red algae had been thoroughly studied for ultrastructural aspects of mitosis (Schornstein and Scott 1982, Scott 1986). In addition to extending our knowledge of rhodophytan mitosis, this study supports the distinction between *Rhodella* and *Porphyridium* and, by the elucidation of further ultrastructural characters, confirms the close affinity between these two species of *Rhodella*. We believe that the other two members of the genus *Rhodella*, *R. cyanea* (Billard and Fresnel 1986, Fresnel and Billard 1987) and *R. grisea* (Deason et al. 1983, Pekárková et al. 1988, Fresnel et al. 1989), are unlikely members of this genus, but their taxonomic affinities will be discussed in a future paper.

MATERIAL AND METHODS

Rhodella violacea was obtained from the Northeast Pacific Culture Collection, Vancouver, British Columbia (NEPCC #14), and was subsequently deposited at the University of Texas Culture

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[2] Address for reprint requests.