Cell Division in Marine *Ceratium*

II. Mitotic behavior and phasing in cell division

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It is well known that the occurrence of cell division in photosynthetic dinoflagellates is confined to a certain time of the day. In regard to the genus *Ceratium*, a sub-class of dinoflagellates, it has been known that they divide at night or early morning under natural conditions (Gough 1905, Jørgensen 1911, Braarud and Pappas 1951 and Skoczylas 1958). In culture, the periodicity of cell divisions has been described in various dinoflagellates except the genus *Ceratium* (Sweeney and Hastings 1958 and Sweeney 1959).

In recent times, the study of nuclear division among primitive dinoflagellates under cultures has become the center of wide attention. But the study of this type for marine *Ceratium* has not been published to date as far as the author is aware.

The present paper deals with the periodicity of cell division and the mitotic feature of the marine dinoflagellate *Ceratium furca* (Ehrenberg) Claparede et Lachmann.

Materials and methods

The cell population of the genus *Ceratium* in the ocean is not large. However, some neritic species of *Ceratium* bloom as red-tide and so does *C. furca* in the coastal waters in Japan. But, since cultivation of the genus *Ceratium* in the laboratory is attended with difficulties, only a few instances have been reported.

Ceratium furca used in this experiments was originally collected from Sagami Bay and has been cultivated in laboratory since the spring of 1969.

Although *C. furca* and *C. tripos* were cultivated by Erdshreiber medium (Barker 1953 and Nordli 1957), when applied to *C. furca* the results are inconsistent. Von Stosch succeeded in keeping *C. horridum* for more than one month using a medium of his own formula (personal communication). However, the medium failed to be usable for *C. furca*. Thinking from the above facts *C. furca* may require more nutrients.

The author's culture medium "M" (Table 1) is natural sea water enriched with the nutrients tried by Provasoli, McLaughlin and Droop (1957) which can maintain *C. furca* without causing any aberrant forms during more than one month. The natural sea water was boiled and filtrated through absorbent cotton.

After inoculation, temperature was usually maintained at 20°C but it was occasionally shifted within the range of 15°C to 25°C at which *C. furca* grows well. The illumination was done by two 60-watt standard cool white fluorescent bulbs

Table 1. The compositions of A medium

Sea water	1000 ml	CaCl ₂	0.0003 g
KNO ₃	0.1 g	Na ₂ SiO ₃	0.0003 g
Na ₂ Glycerophosphate	0.002 g	P II metals*	5 ml
NTA	0.1 g	vitamin mix 8**	10 ml
Tris	0.5 g	pH=8.0	

* see Provasoli, McLaughlin and Droop (1957).

** see Pintner and Provasoli (1958).

The culture medium M was made by addition of 50 ml of Føyn's Erdschreiber medium into the 50 ml of A medium.

(about 2500 lux). Alternating light and dark period of 12 hours each was given and vessels used were Ehrenmeyer's flasks of 100 ml. Samples were taken for investigation every 1.5 hours through the day. Cells were precipitated by low speed centrifugation and were fixed in Schaudin liquid or Carnoy liquid.

For staining, Heidenhain's iron hematoxylin, Feulgen reagent and acetic carmin were used.

Results

Stage I (Interphase)

The nucleus of the stage I of *C. furca* is situated on the level of the epitheca (Fig. 1) or of the girdle (Fig. 2) as the case may be. The former is a daughter nucleus of the previous division which was allotted to the proter and the latter is the one located in the opisthe.

The nucleus of this stage is almost oval and is filled with small granular chromatin elements. There are a few nucleoli in the nucleus of stage I. However, the nucleoli do not usually stain but appear as defined unstained region of the nucleus as in other dinoflagellates (Dodge 1963). The nucleus of a relatively smooth contour is considered to be surrounded by the nuclear envelope.

Stage I is invariably found in all cultures (Table 2). An important point is

Table 2. Percentages of Stage I and Stage II over the total, taken under 12:12 light and dark regime. Thick line shows dark duration

hours	—10.5	—9	—7	—6	—4.5	—3	—1.5
Percentages							
Stage I (interphase)	20.7	21.3	22.9	44.7	17.6	18.9	18.6
Stage II (prophase)	74.7	73.5	75.3	54.6	75.1	70.6	68.7
hours	1.5	3	4.5	6	7.5	9	10.5
Percentages							
Stage I (interphase)	16.9	20.6	24.1	18.5	8.9	7.4	10.2
Stage II (prophase)	67.7	67.8	63.0	79.8	90.0	91.2	82.6

Figs. 1-4. Early stage of nuclear division in *Ceratium furca*. 1, stage I nucleus lying on the level of the epitheca, derived from a daughter nucleus of the anterior half in binary fission. 2, stage I nucleus lying on the level of the girdle, derived from a daughter nucleus of the posterior half of the previous division. 3, stage II nucleus. Chromatid pairs are appearing. 4, stage II nucleus. Pairs of chromatid are coiled together.

that the number of the stage I cells is rather few comparison with that of the stage II. In spite of it, the author considers this stage as interphase because of the presence of the nucleolus which vanishes as mitosis advances.

Stage II (Prophase)

Although the position, the size and the shapes of the nucleus of the stage II is the same as that of the nucleus of the stage I, in stage II the chromatin elements

Figs. 5-8. Middle stage of nuclear division in *Ceratium furca*. 5, stage III, the nucleus is flattened and pulled toward a point in the nucleus. 6, early stage IV nucleus. 7, stage IV nucleus. 8, mid stage V nucleus.

are thicker which later appear as pairs. As time advanced, the chromatin elements elongate twisting on each other (Figs. 3 and 4). This is obviously the prophase. The stage II was always found in cultures at the level of around 60% and the maximum was found to be 91.2% 9 hours after the onset of the light period (+9 hours). This is the highest frequency found in all stage inclusive.

Stage III

The nucleus of the stage III is a curved structure like a watch-glass. The chromatin threads of the stage III contrast in a striking way with those of other dividing nuclei, i.e., the chromatin threads become slenderer than those of the stage II and their twisting cannot clearly be discerned and they look converging toward a point in the nucleus (Fig. 5). The chromosomal arrangement of this stage rather resembles to that observed by Kubai and Ris (1969) in which chromosomes are converging toward the poles of the nucleus in the mid-dividing stage of *Gyrodinium cohnii* unlike the chromosomal arrangement of metaphase which was observed by Skoczylas in *Ceratium cornutum* (1958).

The maximum of this stage is found 3 hours before the beginning of the light period (-3 hours, Fig. 11. ●—●).

Figs. 9-10. Chromosome separation in *Ceratium furca*. 9, late stage V with the daughter chromosome moving toward the poles. Arrow shows beginning cleavage. 10, cell cleavage. Cleavage begins from the right anterior margin of the cell.

Stage IV

In the stage IV, the chromosomes become thicker than the stage III and appear as twisted. The chromosomes overlap densely on the equatorial zone (Figs. 6 and 7).

However, an attempt to visualize the spindle by various fixations including Wada's Cd-reagent combined with various stains failed to do so (Wada and Kusunoki 1964 and Wada 1970). In this case no distinct membrane appears about chromosomes like in that of blue-green algae.

The maximum of this stage was observed at +1.5 hours (Fig. 11 ■---■).

Stage V (Anaphase)

The stage V begins with the separation of the chromosomes toward the both poles. This stage will conveniently be called anaphase.

In the mid-anaphase, since the end of daughter chromatids form a line at the center of the nucleus (Fig. 8), the chromosomes must be roughly of equal lengths. The interval from the stage IV to the stage V is relatively short so that their maxima overlap. If the interval of fixation by 1.5 hours were made shorter, it may be possible to separate the two maxima (Fig. 11 ○---○).

In the middle of this stage, cytokinesis begins from the right anterior margin of the cell (Figs. 9 and 10).

Fig. 11. Mitosis in *Ceratium furca*. The occurrence rate of each stage was plotted as percentage of against every 1.5 hours. ●---● Stage III, ■---■ Stage IV, ○---○ Stage V, △---△ Stage VI. Thick line shows dark duration.

Stage VI (Telophase)

In this stage, the chromosomes move completely to the both poles and they return to the condition of the nucleus of the stage I. After cytokinesis, the one of the daughter nuclei is included in the proter and the other in the opisthe. After

that, the proter regenerates two posterior horns and opisthe acquires an anterior horn. As the horn regenerates, a daughter nucleus included in the opisthe moves a little toward the anterior part, reaching the level of the girdle which is also formed newly (Fig. 2).

Within the temperature range from 15°C to 25°C, the percentage of the frequency of each mitotic stage showed practically no variation in relation to the change of temperature.

Summary

In the marine dinoflagellate *Ceratium furca*, the duration and the percentage of mitosis were observed in culture. The periodic cell division with mitosis is strictly confined to the border from dark to light under the light regime of 12 hours each. The rate of mitosis scarcely varies in relation to the change of temperature between 15°C and 25°C. On the bases of long persistence of the prophase nuclei, the characteristic feature of the stages III and IV, lack of discernible spindle and the equatorial plate, the nucleus of *C. furca* may be considered as eukaryote which retains certain Cyanophyceae-like features.

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