

## CATATAN PENELITIAN

# Vegetative Cell Division and Nuclear Translocation in Three Algae Species of *Netrium* (Zygnematales, Chlorophyta)

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Three species of *Netrium oblongum*, *N. digitus* v. *latum*, and *N. interruptum* were studied for their mode in the vegetative cell division and nuclear translocation during mitosis using light and fluorescence microscopy. The process of cell division in the three species began with the prominent constriction at the chloroplast in both semicells about half way from the apex. The constriction of chloroplast was mostly visible in *N. digitus* v. *latum*. Soon after nucleus divided, septum was formed across the cell and cytokinesis occurred. Observation with fluorescence microscope showed that the movement of nucleus moved back into the center of daughter cells was not always synchronous. Division of chloroplast in *N. oblongum* and *N. digitus* v. *latum* were different with that of *N. interruptum*. Chloroplast division in two former species occurred following the movement of the nucleus down semicell. However, in *N. interruptum*, chloroplast divided later after nucleus occupied the position at the center of the daughter cells. Cell restoration started after the completion of mitosis and cytokinesis.

Key words: Cell division, conjugating alga, mitosis, *Netrium*

The genus *Netrium* is one of the taxonomically problematic members of the conjugating green algae (Class Zygnematophyceae). The vegetative cell of *Netrium* is elongated and cylindrical, usually with rounded apices. Two large elaborately lobed and ridged chloroplasts, each with axial pyrenoids, occur per cell, separated by the centrally placed nucleus. The cell wall of *Netrium* is smooth without pores and unsegmented (Brook 1981; Graham & Wilcox 2000).

The last generic revision of *Netrium* is published by Ohtani (1990), with ten recognized species. He also proposed separation of the genus *Netrium* into two sections: *Netrium* Section and *Planotaenium* Section. *Netrium* Section included all members of *Netrium* having conspicuously notched chloroplast plates (*N. naegeli*, *N. digitus*, *N. nepalense*, *N. elongatum*, *N. minutum*, *N. lanceolatum*, *N. oblongum*), while other members with smooth chloroplast plates were put into *Planotaenium* Section (*N. interruptum*, *N. scottii*, *N. minus*). Recently, the study of phylogeny of conjugating green algae showed that genus *Netrium* was polyphyletic (Gontcharov *et al.* 2004). The three species of *N. digitus* v. *latum*, *N. oblongum* (strain SVCK 255 and strain M1367), and *N. interruptum* were diversified into three independent branches.

Vegetative cell division is one of the unique and intriguing aspects in the study of conjugating green algae. This is because the cell division produces two daughter cells which later reform the often, extremely complex (like those members of placoderm desmid), symmetrical shape of the parent semicell (Brook 1981; Harold 1990). In *Netrium* the onset of cell division can be recognized by the appearance of a constriction of

chloroplast and pyrenoid. Mitosis is followed by the formation of an ingrowing septum, which cuts the symmetrical cell in half. To restore its interphase symmetry, the chloroplast (and pyrenoid) in each daughter cell divides, and a new half-cell is formed by the control expansion of the cell wall originally derived from the septum (Biebel 1964; Pickett-Heaps 1975; Brook 1981; Jarman & Pickett-Heaps 1990; Gerrath 1993). Nuclear translocation occurred during cytokinesis follows the same pattern as other unconstricted genera, such as *Closterium* and *Hyalotheca* of placoderm desmids and *Cylindrocystis* of saccoderm desmids (Brook 1981; Meindl 1991).

While studying the phylogeny of conjugating green algae using the nuclear rDNA, Gontcharov *et al.* (2004) reported that among the three species of *Netrium* used, there were differences in the number of chloroplast per cell (1, 2, or 4), in the position of the nucleus in the cell, and nuclear behavior during cytokinesis. However, these observations lack of figure evidence and the authors did not discuss in detail. This brought a bias because the observations were different from the present knowledge about the course of vegetative cell division in *Netrium*. The aim of this study is to confirm the course of vegetative cell and chloroplast division in three species of *Netrium* in debate: *N. oblongum*, *N. digitus* v. *latum*, and *N. interruptum*, by light and fluorescence microscope as well as the translocation of nucleus during cytokinesis. Comparison of the morphology of chloroplast among *N. oblongum*, *N. digitus* v. *latum*, and *N. interruptum* will also be discussed.

Cultures of *N. oblongum* (strain SVCK 255) and *N. digitus v. latum* (strain SVCK 254) were obtained from Hamburg University Culture Collection (Sammlung von Conjugaten-Kulturen der Universität Hamburg). The culture of *N. interruptum* (strain Nint-781) was provided by Dr. Ohtani, Hiroshima University. Cultures were grown in screwcapped tubes containing 10 ml of CA (Ichimura & Watanabe 1974) or CAS Medium (Ohtani 1990), and maintained at 25 °C, under a 16:8 L:D cycle at about 35  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  provided by daylight-type fluorescence lamps. The cultures were subcultured once per month to maintain good growth condition. Examination of the life cycle of *Netrium* culture was proceed before the observation. This was important because usually cell division occurred before the beginning of dark period while elongation of cell (interphase) occurred during light period. Samples for observation were prepared as many as possible to get accurate data.

For fluorescence microscope observations, samples were fixed with 1% formaldehyde in culture medium for 1 hr at room temperature. After fixation, samples were dropped onto a cover slip, dried at room temperature, and washed with PBS (136.9 mM NaCl, 2.7 mM KCl, 4.9 mM  $\text{Na}_2\text{HPO}_4$ , 1.5 mM  $\text{KH}_2\text{PO}_4$ , pH 7.4). Finally, samples were immersed in DAPI solution (4',6-diamidino-2-phenylindole, 0.5  $\mu\text{g ml}^{-1}$  in PBS) and observed with an Olympus epifluorescence microscope (BH2-RFK).

Morphology of *N. oblongum*, *N. digitus v. latum*, and *N. interruptum* were observed by light microscopy (Figure 1). The vegetative cell of *N. oblongum* was oblong-cylindric, gradually attenuated to rounded apices (Figure 1a). The length and width of cell was 150-240  $\mu\text{m}$  and 30-40  $\mu\text{m}$  respectively. Nucleus was located at the center of the cell, which (on the photograph) is obscured by chloroplast. The vegetative cell of *N. digitus v. latum* was oblong cylindrical with rounded apices (Figure 1b). The cell had 185-280  $\mu\text{m}$  in length and 60-80  $\mu\text{m}$  in width. The vegetative cell of *N. interruptum* was elongate to lanceolate while the apex was truncately rounded (Figure 1c). The length of cell was 180-250  $\mu\text{m}$  and the width was 35-45  $\mu\text{m}$ . Chloroplast was one per semicell with six longitudinal plates, which were deeply notched at their free margins (in *N. oblongum* and *N. digitus v. latum*) while chloroplast were two per semicell and smooth in *N. interruptum*. Nucleus was located at the center of the cell.

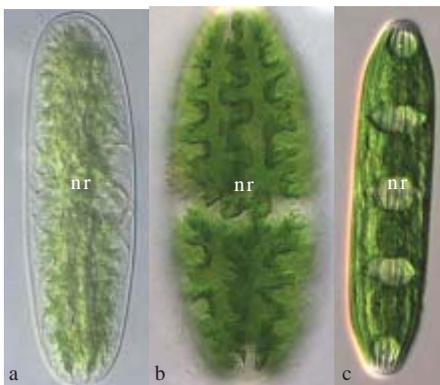


Figure 1. Vegetative cell of (a) *Netrium oblongum*, (b) *N. digitus v. latum*, and (c) *N. interruptum*. nr = nuclear region.

Prior to cell division, the chloroplast showed faint cleavages indicating imminent cell divisions. After nuclear division, a division septum was formed across the middle of cell (Figure 2a-c). Then, the daughter cells separated and the bulges of each daughter cell grew outward to form broadly

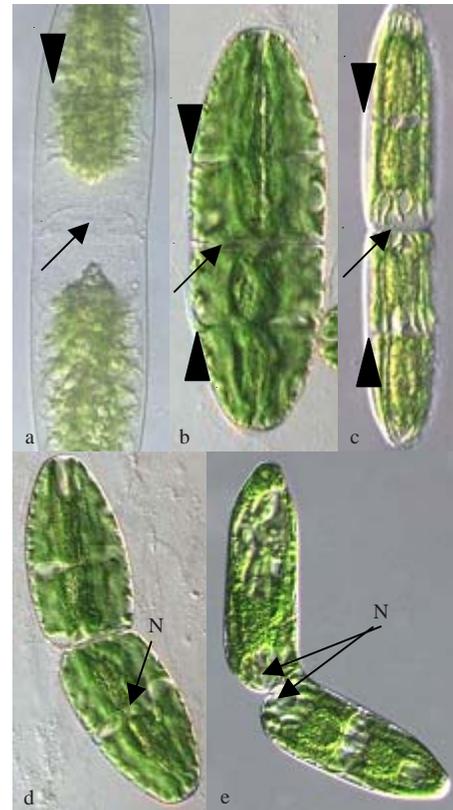


Figure 2. Vegetative cell division in (a) *N. oblongum*, (b, d) *N. digitus v. latum*, and (c, e) *N. interruptum*. N = nucleus a-c. Septum (arrow) was formed, divided the cell into two. Faint cleavages in the chloroplasts (arrowhead), indicating imminent cell division. d. The bulges around the septum grew, separating the daughter cells. e. Daughter nucleus was found near the new cell wall of separating daughter cells.

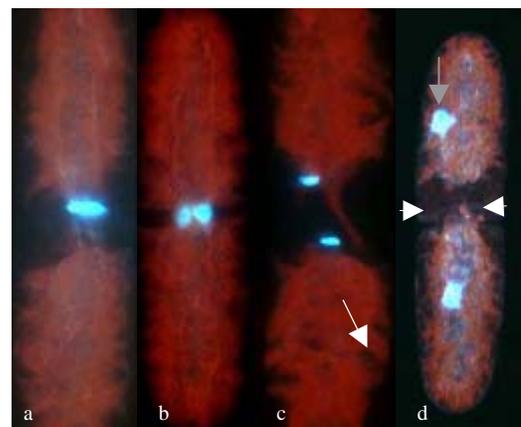


Figure 3. Nuclear translocation in cell division of *N. oblongum*. a. Nuclear division, b. Metaphase, c. Anaphase, d. One of the daughter nuclei was still in the opening cleavage of the two chloroplasts (green arrow), while the other one was already at the center of the cell. Long arrow showed the cleavage of the chloroplast. Short arrow showed the in-growing furrow that later initiated cytokinesis. Blue = nucleus; Red = chloroplast.

rounded end (Figure 2d, e). Sometimes daughter nucleus was found near the cross wall although the cell had already divided (Figure 2e). Finally, the daughter cells separated from each other and the cell began to elongate. Examination of nuclei during the cell division of *N. oblongum* was conducted by fluorescence microscopy (Figure 3a-d). Nuclear division occurred at the center of the two chloroplasts (Figure 3a, b). After mitosis and septum formation, each daughter nucleus moved along the semicell (Figure 3c) until it was insinuated at the chloroplast cleavage in each daughter cell (Figure 3d).

The manner of vegetative cell divisions in the genus *Netrium* observed by Biebel (1964), Jarman and Pickett-Heaps (1990), Ohtani (1990), and Pickett-Heaps (1975) are confirmed at the present study. Before mitosis, the chloroplast shows constriction about half way between the cell apices, which is obviously observed in *N. digitus v. latum*. This pattern is also found in other green algae where division site selection starts with the annular cleavage of chloroplast and pyrenoids (Fowke & Pickett-Heaps 1969; Pickett-Heaps *et al.* 1999). The septum quickly becomes more intense (thick) then the cross wall is stretching "cutting" the cell into two.

Cell division is followed by chloroplast division, which is slightly different between *N. oblongum*-*N. digitus v. latum* (cell having two chloroplasts) and *N. interruptum* (cell having four chloroplasts). In *N. oblongum* and *N. digitus v. latum*, chloroplast division occurred soon after nucleus divided. The process was rather difficult to be observed with light microscope. But, fluorescence microscope shows that at anaphase (at this time, septum has been formed), the chloroplast consists of two parts (Figure 3c), indicating that chloroplast division has been occurred, even before cytokinesis over. Biebel (1964) reported that chloroplast division in *N. digitus v. digitus* and *v. lamellosum* might occur before mitosis but in this study chloroplast division in *N. digitus v. latum* was always found after septum formation. On the contrary, chloroplast division in *N. interruptum* must occur not before the cytokinesis (Figure 2e; showing each daughter cell with only two chloroplasts).

In the conjugating cells, position of nucleus is at the central of the cell (between two chloroplasts). After cell division, daughter nucleus has to move back from the area near dividing septum into the center of new cell. Nuclear translocation can be observed by fluorescence microscope. In the present study, observation was focused on *N. oblongum* because there is no study has been conducted on this aspect using this species. As a result, nuclear translocation in the cell division of *N. oblongum* (and other two species studied) followed the same pattern as had been reported at the previous studies (Brook 1981; Meindl 1991). Nucleus divides at the center of the two chloroplasts then each daughter nucleus segregates into each daughter cells. The nucleus moves down along the semicell. As the new cell wall of semicell grows, the nucleus moves into the cleavage of the two chloroplasts. However, it is interesting to note that sometime at anaphase the chromosome is segregated into different directions in each daughter cell (Figure 3c). This phenomenon is not specific occurred in *N. oblongum* because it is also found in other species of *Netrium*

(unpublished observation). Another interesting observation is that the movement of the nucleus back into the center of the cell is not always synchronous between the daughter cells. Cell restoration seems depend on nuclear translocation. However, sometimes nucleus is found near the septum although the daughter cell has separated from each other. In this case, restoration of the daughter cells is delayed until nucleus moves back into the center of the two chloroplasts.

Chloroplast was always found one per semicell in *N. digitus v. latum* and *N. oblongum* and two per semicell in *N. interruptum*. Morphology of chloroplast in *N. oblongum* and *N. digitus v. latum* are different. The nature of radiating plates of chloroplasts is arranged like a thread in *N. digitus v. latum* and the notched in free margin of chloroplast are very deep. Meanwhile, the pattern of notched at the plate of chloroplast in *N. oblongum* is scattered and unclear. During this study, the growth culture of *N. oblongum* was the slowest among the others. The culture had been grown in several medium cultures but so far the appearance of cell morphology was not as healthy as the two others.

In conclusion, Gontcharov *et al.* (2004) reported that among the three species of *Netrium* used, there were differences in the number of chloroplast per cell (1,2, or 4), in the positions of the nucleus in the cell, and nuclear behavior during cytokinesis, to which those observations were not found in the present study. Fluorescence microscope definitely showed that each cell has two chloroplasts (in *N. oblongum* and *N. digitus v. latum*) or four chloroplasts (in *N. interruptum*). Sometimes the nucleus was obscured so that it may look that the cell has only one chloroplast.

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