

# Spot-like Area of F-actin Associated with the Migrating Nucleus in Post-mitotic Cells of *Closterium moniliferum* var. *submoniliferum* (Zygnematales, Chlorophyta)

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## ABSTRACT

Fluorescence microscopy using rhodamine-labeled phalloidin (RLP) staining revealed the association of a spot-like fluorescent area of RLP (F-actin spot) with the migrating nucleus in post-mitotic cells of *Closterium moniliferum* var. *submoniliferum*. The F-actin spot, which measured ca. 2  $\mu$ m in diameter, appeared at each polar region of telophase nucleus. After completion of septum formation, each daughter nucleus migrated from the middle of the cell to the annular constriction of dividing chloroplast along the convex side of the cell. During the course of this nuclear migration, the F-actin spot was always localized near the forward end of the migrating nucleus. When the nucleus reached the chloroplast constriction, the F-actin spot seemed to settle on the bundle of F-actin which girdled the chloroplast constriction. The F-actin spot became inconceivable accompanying the progress of chloroplast division. Electron microscopy revealed the presence of microtubule center (MC) near the forward end of each migrating nucleus. The MC was an electron-dense, ball-like structure, from which numerous microtubules emanated, some ensheathing the fore surface of the nucleus. The MC, which was ca. 1.5  $\mu$ m in diameter, moved to the distal end of the cell and finally entered the chloroplast constriction. The similarities between the F-actin spot and the MC in size and behavior during the nuclear migration suggest their correspondence.

## KEY WORDS

Cell division, *Closterium*, F-actin, microtubule, nuclear migration, Zygnematales

## 1. Introduction

In some members of the Zygnematales, prominent intracellular migration of daughter nuclei has been known to occur during post-mitotic restoration of cell symmetry (Pickett-Heaps and Wetherbee 1987, Meindl 1992). One of the representatives is *Micrasterias*, where the daughter nucleus migrates into the growing semicell at the beginning of cell development and then moves back to the center of the cell by the end of growth of new semicell (Meindl 1983, 1992). A fluorescence microscope investigation using fluorescently labeled phalloidin has demonstrated

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that actin filaments colocalize with the "post-telophase system of microtubules" (Meindl *et al.* 1994), confirming the earlier observations by electron microscopy (Kiermayer 1968, Meindl 1983). These actin filaments, in cooperation with the microtubules, are thought to be involved not only in the motive force generation for nuclear migration (Meindl 1983, Meindl *et al.* 1994, Lütz-Meindl and Menzel 2000) but also in the nuclear positioning (Meindl *et al.* 1994).

Another type of post-mitotic nuclear migration has been observed in *Closterium*, in which daughter nucleus moves back from the middle toward the distal end of the cell and finally resides in the annular constriction of dividing chloroplast (Lutman 1911, Pickett-Heaps and Fowke 1970). In *Closterium*, microtubules have been suggested to be responsible for the post-mitotic nuclear migration (Pickett-Heaps 1975, Pickett-Heaps and Wetherbee 1987), but the involvement of actin filaments in the migration of the nucleus and its subsequent positioning at the chloroplast constriction remained obscure.

The present study was carried out primarily to examine the changes of intracellular distribution of F-actin during the migration of daughter nuclei in *Closterium* by fluorescence microscopy, and unexpectedly revealed usual association of a spot-like fluorescent area of rhodamine-labeled phalloidin (F-actin spot) with the forward end of migrating nucleus. In this paper, the appearance and the behavior of the F-actin spot and its ultrastructural entity in *C. moniliferum* var. *submoniliferum* are described.

## 2. Materials and Methods

One strain of *Closterium moniliferum* Ehrenberg ex Ralfs var. *submoniliferum* (Woronichin) Krieger (NIES-182, supplied by National Institute for Environmental Studies, Ibaraki, Japan) was used in the present investigation. Cells were cultured in Ehrlenmeyer flasks, each containing C medium (Ichimura 1983), under a 16 h light ( $40 \mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ )-8h dark cycle at 20 °C.

Under these culture conditions, mitotic index was relatively high for several hours after the onset of dark, and, hence, the cells of this period were preferentially used for the present study.

Staining of cells with rhodamine-labeled phalloidin (RLP: Molecular probes Inc., Eugene, Oregon) was carried out according to the method described by Sonobe and Shibaoka (1989) with slight modification. Some cells were exposed to 100  $\mu\text{M}$  *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester in phosphate-buffered saline (PBS, pH 6.9) for 30 min at room temperature. They were washed in PBS, fixed with 2% formaldehyde in PBS for 30 min, and stained with RLP (30 units/ml in PBS) for 30 min. They were then mounted in PBS containing *p*-phenylene diamine (1.0 mg/ml) and examined with a fluorescence microscope (Nikon XF-EFD). Some cells were treated with nonfluorescent phalloidin (120  $\mu\text{M}$  in PBS) for 20 min prior to the staining with RLP. The behavior of nuclei was observed either by differential interference contrast microscopy or by fluorescence microscopy employing a staining method with 4', 6-diamidino-2-phenylindole (DAPI).

For electron microscopy, some cells were first fixed with 2% glutaraldehyde in 0.1 M

phosphate buffer (pH 6.6) containing 6 mM  $\text{MgCl}_2$ , saponin (0.5 mg/ml), and tannic acid (2.0 mg/ml) for 1 h at room temperature. They were washed in several changes of phosphate buffer and postfixed with 1% phosphate-buffered osmium tetroxide for 30 min. They were then dehydrated in an ethanol series, replaced with propylene oxide, and embedded in Spurr's resin. Thin sections cut with a diamond knife were stained with lead citrate, and examined with a JEOL JEM-2000EX electron microscope, operating at 80 kV.

### 3. Results

The interphase cell of *C. moniliferum* var. *submoniliferum* is lunate, measuring ca. 225  $\mu\text{m}$  in length and 35  $\mu\text{m}$  in width. Each cell contains a nucleus situated in the middle of the cell and two chloroplasts localized on both sides of the nucleus (Fig. 1 A). The nucleus contains a single spherical nucleolus. When the cell commenced to divide, the nucleolus became obscure and many dot-like chromosomes appeared within the nucleus. They lay in the equatorial plane at metaphase (Fig. 1 B). Following the anaphase segregation of sister chromosomes (Fig. 1 C), centripetal growth of septum bisected the cell. The chloroplast within each daughter cell began to divide and slightly constricted about a third of the distance from the middle to the tip. Nucleolus reappeared within each daughter nucleus. The daughter nucleus moved toward the convex side of the cell along the septum (Fig. 1 D), and subsequently toward the annular constriction of the dividing chloroplast along the convex side of the cell (Figs. 1 E, 1 F). About 40 min were required for this nuclear migration from the middle of the cell to the chloroplast constriction. The split of septum and concomitant development of cell walls of new semicells lead to the separation of the two daughter cells. Within each daughter cell, the nucleus was lodged in the chloroplast constriction (Fig. 1 G).

The spatial and temporal changes in distribution of F-actin during the mitosis and subsequent restoration of cell symmetry were observed by fluorescence microscopy using RLP staining. In each interphase cell, many F-actin cables ran parallel to each other along the long axis of the cell within cortical cytoplasm (Figs. 2 A, 2 B). These cortical actin cables were observed throughout the cell cycle. In some post-mitotic cells examined, a brightly fluorescing area of RLP ca. 2  $\mu\text{m}$  in diameter was associated with the forward end of migrating nucleus. Treatment of these cells with nonfluorescent phalloidin prior to RLP staining abolished this fluorescent area as well as other intracellular RLP fluorescence. The spot-like fluorescent area is referred to as "F-actin spot" in this paper. At early telophase, the peripheries of daughter nuclei, either side of septum, and chloroplast ends facing the septum were lined by bright RLP fluorescence. RLP fluorescence also girdled the constriction of each dividing chloroplast. Most conspicuous of this stage was the appearance of the F-actin spot near the polar region of each daughter nucleus (Figs. 2 C, 2 D). During the course of post-mitotic nuclear migration, the F-actin spot always preceded the moving nucleus with a distance of 3–6  $\mu\text{m}$  from the forward end of the nucleus (Figs. 2 E, 2 F). The migrating nucleus was ensheathed by RLP fluorescence, which seemed to be continuous with the F-actin spot. The F-actin spot reached the chloroplast

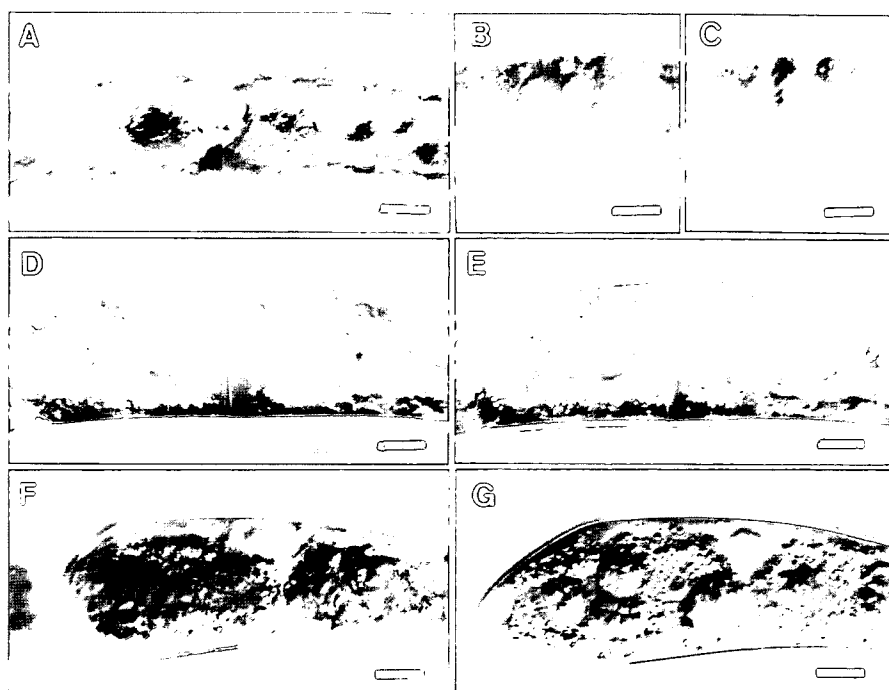


Fig. 1. Cell division and post-mitotic nuclear migration. A, Interphase cell. B, Metaphase cell. Chromosomes line up in equatorial plane. C, Anaphase cell. Two sets of sister chromosome are segregated from each other. D, Two daughter nuclei, each being localized at the convex side of the cell near the septum. E, Migrating nuclei. Each nucleus moves toward the constriction of dividing chloroplast along the convex side of the cell. F, Cell of a septum-splitting stage. Nucleus is lodged in the chloroplast constriction. G, Daughter cell developing new semicell. Nucleus enters the chloroplast constriction. Each bar = 10  $\mu$ m.

constriction, and seemed to settle on and unite with the F-actin bundle that encircled the chloroplast constriction (Figs. 2 G, 2 H). The F-actin spot became indiscernible as the progress of chloroplast division (Figs. 2 I, 2 J).

To demonstrate the ultrastructural features of the F-actin spot, post-mitotic cells were exclusively examined by electron microscopy. As already described by Pickett-Heaps and Fowke (1970), many microtubules extended from the forward end of the migrating nucleus toward the distal end of the cell, forming a long and broad bundle of microtubules. The microtubule bundle always had a spherical electron-dense area on its way (Fig. 3 A), from which many microtubules radiated. Following the naming by Pickett-Heaps and Fowke (1970), this area is referred to as "microtubule center" (MC) also in this paper. The MC measured ca. 1.5  $\mu$ m in diameter and usually appeared near the migrating nucleus with a distance of several  $\mu$ m from its forward end. Within the MC, together with the cross, longitudinal, and oblique sections of

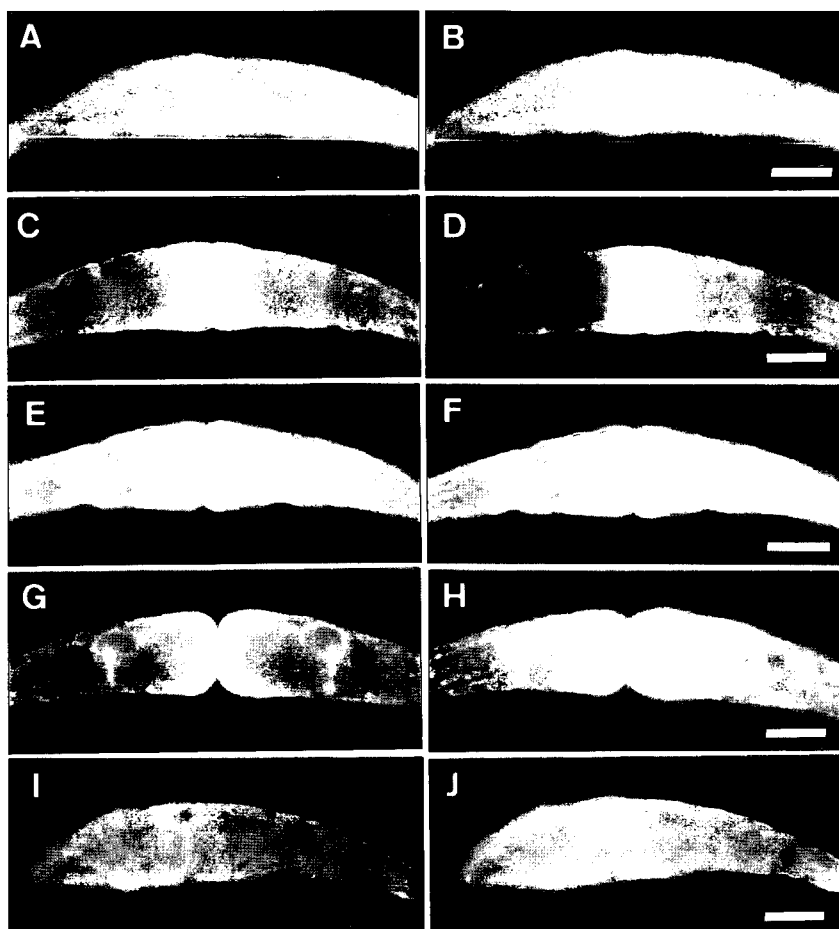


Fig. 2. Fluorescent images of cells stained with RLP and DAPI. RLP image (A, C, E, G, I), RLP-DAPI image (B, D, F, H, J). A and B, Interphase cell. F-actin cables run parallel to each other along the long axis of the cell. C and D, Telophase cell. A bright, spot-like fluorescence of F-actin (F-actin spot) is associated with each daughter nucleus near the pole. E and F, Cell, containing two daughter nuclei that begin to migrate. F-actin spot precedes each migrating nucleus. G and H, Cell of a septum-splitting stage. Each daughter nucleus is lodged in the chloroplast constriction. F-actin spot seems to settle on the F-actin bundle that encircles the chloroplast constriction. I and J, Cell expanding new semicell. F-actin spot disappears from the chloroplast constriction. Each bar = 20  $\mu$ m.

microtubules, electron-dense fibrils could be seen (Fig. 3 B). These fibrils seemed to be intertwined with one another. The MC finally resided in the constriction of dividing chloroplast.

#### 4. Discussion

The present fluorescence microscope study demonstrated several types of localization of F-actin in dividing and post-mitotic cells of *C. moniliferum* var. *submoniliferum*: F-actin cables in cortical cytoplasm running parallel to each other along the long axis of the cell; F-actin densely localized at the peripheries of septum and the chloroplast ends facing the septum; F-actin ensheathing daughter nucleus; F-actin encircling the isthmus of dividing chloroplast; and spot-like area of F-actin situated at the forward end of migrating nucleus (F-actin spot). These distribution patterns of F-actin are nearly in accordance with those observed in *C. ehrenbergii* (Hashimoto 1992), except for the F-actin spot. The F-actin spot appeared near each pole of telophase nucleus, and, then, it moved from the middle of the cell to the annular constriction of dividing chloroplast, being accompanied with the nucleus. The close association of similar fluorescent spot with the post-mitotic migrating nucleus could be observed also in *C. peracerosum-strigosum-littorale* complex (data not shown). Probably, this phenomenon commonly

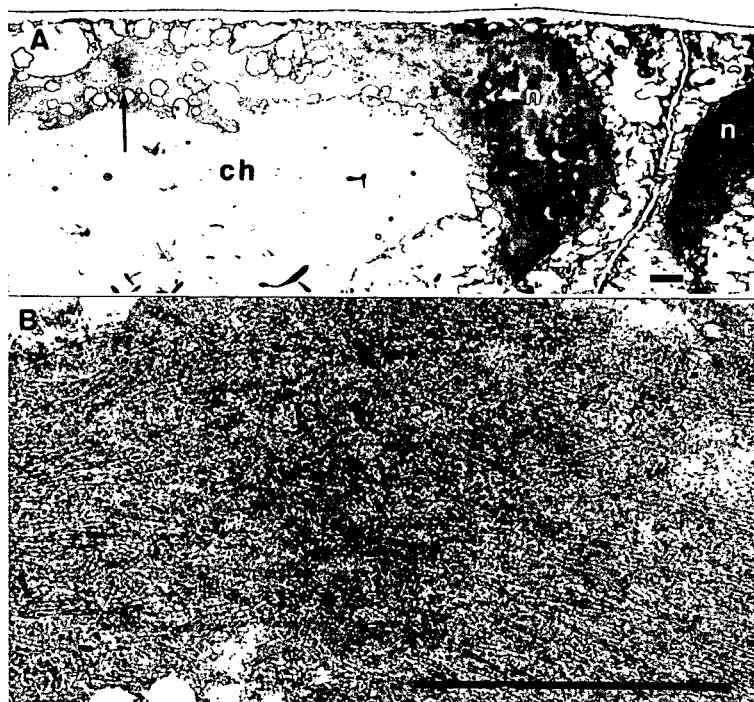


Fig. 3. Electron micrographs of part of a post-mitotic cell. A, Two daughter nuclei (n), moving toward the distal end of the cell. An electron-dense area (arrow), or microtubule center (MC), is observed at the forward end of one of the nuclei. Chloroplast (ch), septum (s). B, Close-up of part of A, showing the MC. Many microtubules radiate from the MC, where electron-dense fibrils are present. Each bar = 1  $\mu$ m.

occurs in *Closterium*. Hashimoto (1992) found a "marking pin-like" structure of F-actin appearing on the nucleus just prior to the chloroplast kinesis in *C. ehrenbergii*, but we have failed to observe such structures in *C. moniliferum* var. *submoniliferum*.

The occurrence of "microtubule center" (MC) near the polar region of telophase nucleus and its intimate association with the migrating nucleus have been shown in some members of the Zygnematales (*Closterium littorale*, Pickett-Heaps and Fowke 1970; *Micrasterias denticulata*, Meindl 1983; *Mougeotia* sp., Pickett-Heaps and Wetherbee 1987; *Netrium interruptus*, Jarman and Pickett-Heaps 1990; *Euastrum oblongum*, Url *et al.* 1992). The association of MC with post-mitotic migrating nucleus was also confirmed in *C. moniliferum* var. *submoniliferum*. The MC was an electron-dense spherical area of ca. 1.5  $\mu\text{m}$  in diameter, and many microtubules emanated from it, some ensheathing the forward end of the migrating nucleus. Like in *C. littorale* (Pickett-Heaps and Fowke 1970), the MC preceded the migrating nucleus and was finally lodged in the constriction of cleaving chloroplast. The F-actin spot was ca. 2  $\mu\text{m}$  in diameter and, as described above, it was always situated at the forward end of the migrating nucleus until its arrival at the chloroplast constriction. These observations indicate the similarity between the MCs and the F-actin spots in size and behavior, suggesting their correspondence. Within the MC, electron-dense fibrils were observed. Further investigations are needed to corroborate their entities.

The investment of migrating nucleus by actin filaments has been demonstrated in *Micrasterias denticulata* (Meindl *et al.* 1994, Pflügl-Haill *et al.* 2000), in which the perinuclear actin system originates from the MC as a brightly fluorescing, knob-like area of RLP that emerges at the beginning of nuclear immigration into developing semicell (Meindl *et al.* 1994). Likewise in *C. moniliferum* var. *submoniliferum*, each migrating nucleus was surrounded by F-actin, but the developmental process of this perinuclear system of F-actin could not be demonstrated by the present fluorescence microscope observation.

In *Closterium*, the mechanism involved in the precise positioning of daughter nucleus at the chloroplast cleavage remained unclear. The present fluorescence microscopy revealed the conspicuous behavior of the F-actin spot in conjunction with the nuclear positioning: The F-actin spot, which seemed to be continuous with the perinuclear sheath of F-actin, moved with the nucleus from the middle to the distal end of the cell and finally settled on the F-actin bundle that encircled the constriction of dividing chloroplast. As observed in *C. littorale* (Pickett-Heaps and Fowke 1970), the MC resided in the chloroplast cleavage also in *C. moniliferum* var. *submoniliferum*. Pickett-Heaps and Fowke (1970) have inferred the assistance of microtubules radiating out from the MC in holding the nucleus in the chloroplast constriction. If the F-actin spot corresponds to the MC, actin filaments and microtubules may be involved cooperatively in the nuclear positioning for establishing the restoration of cell symmetry in *Closterium*.

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