REGULATION OF CELL SHAPE IN EUGLENA
GRACILIS. IV. LOCALIZATION OF ACTIN, MYOSIN
AND CALMODULIN

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SUMMARY
The immunofluorescence patterns for actin, myosin, calmodulin and tubulin were observed in
Euglena gracilis Klebs strain Z during the biological clock-controlled shape changes observed with
division-synchronized cells, and during two shock responses that induce cell rounding. The
fluorescence patterns for actin, myosin, calmodulin and tubulin show a high degree of coincidence
and are visualized as lines running parallel to, and having the same spacing as, the pellicle strips
beneath the plasma membrane. The fluorescence patterns remain intact during the daily shape
changes, implying that the shape changes do not result from cycles of polymerization and
depolymerization of the microtubules and microfilaments. Resuspension of cells in Ca²⁺-free
medium induces cell rounding of many of the cells. The actin and calmodulin patterns are partially
disrupted by the Ca²⁺-free resuspension, while the myosin pattern is almost totally disrupted.
Microtubules are unaffected by this treatment. Prior exposure of cells to the calmodulin antagonist
trifluoperazine or to the microfilament-stabilizing peptide phalloidin stabilize the actin, myosin and
calmodulin patterns against disruption by the Ca²⁺-free resuspension and other shock responses.
The possibility of an actomyosin contractile system controlled by calmodulin is discussed.

INTRODUCTION
The unicellular flagellated alga Euglena gracilis can display numerous shape
changes. The swimming movements and rapid cell-shape changes termed metaboly
are the most widely known but least studied. Several investigators have reported cell
rounding in response to chemical or physical perturbations, including the lowering of
extracellular cation concentration (Lonergan, 1984, and references therein), exposure
to ethanol or chemical fixatives (Lonergan, 1983, 1984) and exposure to bright
illumination during microscopic examination (Murray, 1981). The most recently
reported Euglena cell-shape change is the slow transition of round cells to elongated
cells during a 12 h light/ 12 h dark growth cycle (Lonergan, 1983). This latter shape
change is coordinated by the biological clock, which controls the timing and speed of
this shape change.
All of the shape changes should theoretically involve a contractile mechanism,
presumably either an actomyosin contractile system or, perhaps, a spasmin-based
contraction. This paper presents the first evidence that actin and myosin are present
in Euglena; in addition, it presents the first immunofluorescence examination of how

Key words: Euglena, actin, myosin, calmodulin, cell shape.
T. A. Lonergan

actin, myosin and calmodulin are structurally organized in *Euglena*. Such an immunofluorescence examination was thought necessary before the contractile system was examined at the molecular level by electron microscopy. This investigation focuses on the immunofluorescence patterns for microfilaments, myosin and calmodulin during the biological clock-controlled shape changes and for a rapid cell rounding induced by resuspension of the cells in a Ca^{2+}-free growth medium, which represents a shock response. An immunofluorescence analysis of the pellicle-associated microtubules has previously been reported (Lachney & Lonergan, 1985) and is compared with the pattern reported here.

Materials and Methods

Cell culture

*Euglena gracilis* Klebs strain Z was cultured as previously reported (Lonergan, 1983, 1984). Samples of cells were removed from the culture by syringe and either fixed in suspension at room temperature by adding an equal volume of 6% paraformaldehyde (w/v), or added to a separate culture flask and exposed to various chemical agents followed by fixation.

Use of inhibitors

Phalloidin was dissolved in 95% ethanol (1 mg phalloidin/ml ethanol) and used at a final concentration of 50 μM. The appropriate volume of stock solution was added to an empty culture vessel and dried with a stream of air. Cells were added to the culture vessel and incubated in the appropriate growth conditions. In the experiments involving the calcium ionophore A23187, a 0.02M stock solution of A23187 dissolved in dimethyl sulfoxide (DMSO) was diluted by addition of the appropriate volume of cell culture. Trifluoperazine (TFP) was dissolved in growth medium, and was kindly supplied by Smith Kline and French Laboratories. Phalloidin and A23187 were purchased from Sigma. The cell density for all experiments was 8 × 10^6 cells/ml.

Fluorescent antibody staining

The procedure for detergent extraction of cells and incubation with antibody has been reported (Lachney & Lonergan, 1985). Cell extractions were performed in the microtubule-stabilizing buffer previously reported. Sheep anti-bovine brain tubulin and sheep anti-rat-testis calmodulin were purchased from Caabco, Inc., Houston, Texas. Rabbit anti-chicken-muscle actin, rabbit anti-bovine-uterus myosin, goat fluorescein isothiocyanate-conjugated (FITC-conjugated) anti-rabbit immunoglobulin (IgG) and rabbit FITC-conjugated anti-sheep IgG were purchased from Miles-Yeda, Rehovot, Israel. Cells were stored refrigerated in 1 drop of microtubule-stabilizing buffer containing 1% NaN₃ (w/v) and analysed within 2 days. Approximately 1 μl of cell suspension and 1 μl of mounting medium consisting of 1% p-phenylenediamine (w/v) in glycerol at pH 8 were used to prepare wet mounts. Cells were viewed and photographed with an Olympus model BHT microscope with BH-RFL-W epifluorescence illumination, using an a 100 X oil-immersion objective lens. Cells were photographed on Kodak Plus-X pan film with 90 s manual film exposures using an Olympus OM-2 camera.

Detergent extraction method

For some experiments, the order of the fixation and extraction steps was reversed to test the sensitivity of the presumed contractile system to various treatments in the unfixed condition. This system was previously called a lysed cell system (Lachney & Lonergan, 1985). Cells were centrifuged and resuspended in microtubule-stabilizing buffer containing 2.5% Triton X-100 (v/v). Various agents were added to this buffer, including 180 μM-Ca^{2+}, 100 μM-phalloidin or 100 μM-trifluoperazine. Cells were agitated for 15 min in a gyratory shaker (1500 rev./min), centrifuged and fixed in 3% paraformaldehyde in microtubule buffer for 10 min followed by a
Cell shape changes in Euglena

15-min extraction in 80% acetone (v/v). Cells were processed for indirect immunofluorescence as previously described (Lachney & Lonergan, 1985).

Analysis of experimental results

The extent to which a population successfully cross-reacted with the antibodies, both for control populations and after experimental treatments of the cells, was analysed as follows. Cells from each control or experiment were examined and scored in one of three classes: cells having a fluorescent pattern over the entire cell (striated cells), cells having interrupted fluorescent striations over at least half of the cell (partially striated), and cells having no fluorescent pattern other than background autofluorescence (non-striated cells). Before the establishment of these criteria, it was determined

Fig. 1. Fluorescence patterns for actin, myosin, calmodulin and tubulin at the extremes
of cell shape. The round cells (A, C, E, G) represent typical cell shapes at the end of the dark period and the beginning of the light period. The elongated cells (B, D, F, H) represent typical cells from the middle of the light period of the light/dark cycle. A, B, actin; C, D, myosin; E, F, calmodulin; G, H, tubulin. ×1200; bar, 5 μm.
that the fluorescent patterns for microfilaments, myosin and calmodulin took the form of fluorescent striations parallel to the pellicle strips. The percentage of the population in each of these three classes was determined for consecutive experiments. The decision as to whether the percentage of the population in each class was significantly different from the percentage of the same class in the control population was based on the results of a $2 \times 2 \chi^2$ test for a significant difference at $\alpha = 0.05$ between the two classes (Lonergan, 1983).

RESULTS

*Immunofluorescence patterns for actin, myosin, calmodulin and microtubules during clock-controlled cell shape changes*

The possibility that *Euglena* cell shape changes are accomplished by an actomyosin system regulated by calmodulin depends on the presence of these proteins in *Euglena*. The presence of actin, myosin and calmodulin was demonstrated by indirect immunofluorescence. The immunofluorescence patterns for actin, myosin and calmodulin are compared in Fig. 1A–F with the pattern previously reported for microtubules (Lachney & Lonergan, 1985) (Fig. 1G, H). The patterns for both extremes of shape typical of *Euglena* (spherical and elongated) are shown for comparison. All four immunofluorescence patterns are coincident when compared in either of the extreme shapes. The frequency of the pellicle ridges (number of ridges/unit area) was determined from photographs using Nomarski differential interference microscopy to be $7.8 \pm 0.6$ (s.d.) per $25 \mu m^2$. The frequency of fluorescent striations (number of striations/unit area) was $7.8 \pm 0.8$ for the actin pattern, $7.4 \pm 0.4$ for the myosin pattern, $7.4 \pm 0.1$ for the calmodulin pattern and $7.8 \pm 0.6$ for the microtubule pattern. There was no significant difference between any of the fluorescent pattern frequencies when compared with the pellicle ridge frequency, implying that all of these fluorescent patterns could be associated with the pellicle ridges. All frequency measurements were made.

![Fig. 2. Immunofluorescence controls for actin. A. Immunofluorescent image of a cell incubated with rabbit anti-actin. B. Immunofluorescent image of a cell incubated with goat FITC-anti-rabbit antibodies. C. Immunofluorescent image of a cell incubated with anti-actin antibody preabsorbed with actin followed by goat FITC-anti-rabbit antibody. ×1200; bar, 5 μm.](image-url)
Cell shape changes in Euglena

from cells 45 μm in length and represent at least 25 separate measurements from different cells. A preliminary study of the spacing for actin fluorescence in round cells indicates that the spacing does not change. A more detailed study of this coincidence phenomenon is proposed in the Discussion. The immunofluorescence patterns for actin, myosin and calmodulin were present in virtually all cells at all times during the light/dark cycle, regardless of the cell shape. This implies that the round-to-long and the long-to-round shape transitions controlled by the biological clock are accomplished without the complete disassembly and reassembly of these protein patterns. The biological clock-controlled shape changes apparently result from a reorientation of the pellicle strips (Lachney & Lonergan, 1985) without depolymerization of the cytoskeleton and presumptive contractile systems. The coincidence of the actin and myosin fluorescence patterns with the pellicle ridges suggests that an actomyosin contractile system could operate to alter pellicle strip positioning. The pellicle-associated fluorescent striations can be seen on either cell surface by focusing through the cell (compare the pellicle orientation in Fig. 1B, D). No fluorescent patterns were identified in the cytoplasm. Unlike detergent-extracted fibroblasts, many of the Euglena cytoplasmic components and organelles remain intact after extensive extraction with Triton X-100 and acetone (Lachney & Lonergan, 1985). These cells have a pale yellow autofluorescence (Fig. 2A, B), which obscures fluorescent details in the cytoplasm.

The fact that the immunofluorescence patterns for microtubules, microfilaments, myosin and calmodulin showed coincidence necessitated appropriate immunofluorescent controls to exclude the possibility of non-specific staining of the pellicle material. No fluorescent striations were observed when cells were incubated with primary antibody alone (rabbit anti-actin, rabbit anti-myosin, goat anti-calmodulin, goat anti-tubulin) (Fig. 2A) or when incubated alone with the FITC-labelled antibody (FITC-anti-rabbit from goat or FITC-anti-goat from rabbit) (Fig. 2A). Autofluorescence comparable to that seen in Fig. 2A resulted when the primary antibody was replaced by rabbit or sheep IgG, followed by the appropriate fluorescent second antibody. It was predicted that if the antibodies specifically recognized their respective antigens in the detergent-extracted cells, then preabsorbing an antibody with purified antigen would bind the antigen recognition sites and would result in limited binding to the cellular antigen. Purified actin or myosin (500 μg, Sigma) were dissolved in 10 μl anti-actin or anti-myosin, respectively, and incubated at 2°C for 24 h. When preabsorbed anti-actin or anti-myosin were used in a typical experiment to localize microfilaments or myosin, no striations were observed before or after incubation with the FITC-labelled secondary antibody (Fig. 2C). The background fluorescence was somewhat greater than that of cells incubated with secondary antibody alone (compare with Fig. 2B), but no specific staining pattern was observed with either preabsorbed antibody.

Sensitivity of actin, myosin, calmodulin and microtubule fluorescence patterns to shock treatments

While actin, myosin and calmodulin patterns are associated with the biological clock-controlled cell shape changes, a rapid cell rounding (within minutes) can result
Table 1. Effect of resuspension in a Ca\(^{2+}\)-free growth medium on the actin, myosin, calmodulin and microtubule fluorescence patterns

<table>
<thead>
<tr>
<th></th>
<th>Actin</th>
<th>Myosin</th>
<th>Calmodulin</th>
<th>Microtubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Striated</td>
<td>82</td>
<td>80</td>
<td>86</td>
<td>92</td>
</tr>
<tr>
<td>Partially striated</td>
<td>13</td>
<td>12</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Non-striated</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Ca(^{2+})-free medium, 30 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Striated</td>
<td>33</td>
<td>12</td>
<td>42</td>
<td>96</td>
</tr>
<tr>
<td>Partially striated</td>
<td>37</td>
<td>16</td>
<td>29</td>
<td>3</td>
</tr>
<tr>
<td>Non-striated</td>
<td>30</td>
<td>72</td>
<td>29</td>
<td>1</td>
</tr>
</tbody>
</table>

Populations of elongated cells were fixed, extracted with detergent and incubated with the appropriate antibody as a control. A second batch of cells was resuspended for 30 min in a Ca\(^{2+}\)-free growth medium, then fixed, extracted with detergent and incubated with antibody. The cells were scored in three classes: those with fluorescent striations over the entire cell (striated), those with incomplete fluorescent striations over at least 50% of the cell (partially striated) and those with no visible fluorescent striations (non-striated). The values (%) represent measurements of 750 cells for each immunofluorescence assay pooled from three separate experiments. From certain chemical or physical treatments. The resuspension of cells in a Ca\(^{2+}\)-free growth medium has been reported to cause such a rapid cell rounding (Lonergan, 1984). Table 1 compares the numbers of cells retaining fluorescent striations representing actin, myosin, calmodulin and microtubules for control cells and cells resuspended in Ca\(^{2+}\)-free growth medium for 30 min. The resuspension in Ca\(^{2+}\)-free medium does not alter the microtubule fluorescence pattern, indicating the stability of the microtubules during the rounding. Both the actin and calmodulin fluorescence patterns are disrupted with decreases in the number of cells showing striations over the entire cell length (striated cells) and increases in the numbers of cells with partial striations (partial or interrupted striations over at least 50% of the cell) and non-striated cells. The rationale for using this scoring method has been previously discussed (Lachney & Lonergan, 1985). The percentage of striated cells is not significantly different for the actin and calmodulin patterns in the Ca\(^{2+}\)-free treatment (\(\alpha = 0.05\)). The cell response of the myosin pattern to the Ca\(^{2+}\)-free treatment, however, was significantly different when compared with either the actin or calmodulin patterns. Only 12% of the cells retained the myosin pattern, while 72% of the cells were non-striated, indicating that the myosin fluorescence pattern was the most sensitive to Ca\(^{2+}\)-free treatment. The similarities in the actin and calmodulin patterns in response to resuspension in Ca\(^{2+}\)-free medium imply that at least some of the calmodulin is associated with the actin.

The disruption of the actin pattern induced by the Ca\(^{2+}\)-free resuspension can be counteracted by incubating the cells with phalloidin for 24 h, indicating that the actin is organized as microfilaments. The actin pattern shows complete stabilization if the
Cell shape changes in Euglena

Table 2. Stabilization of the actin fluorescence pattern with phalloidin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control population</th>
<th>24 h phalloidin, no Ca(^{2+})-free resuspension</th>
<th>24 h phalloidin, Ca(^{2+})-free resuspension with phalloidin present</th>
<th>24 h phalloidin, Ca(^{2+})-free resuspension without phalloidin present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striated</td>
<td>86</td>
<td>82</td>
<td>75</td>
<td>55</td>
</tr>
<tr>
<td>Partially striated</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>Non-striated</td>
<td>2</td>
<td>6</td>
<td>12</td>
<td>17</td>
</tr>
</tbody>
</table>

Three samples of cells were removed from the control culture and incubated for 24 h with phalloidin (100 \(\mu\)M final concentration at a cell density of \(8 \times 10^5\) cells/ml). One sample of phalloidin-treated cells received no further treatment. Cells in the second sample were resuspended in a Ca\(^{2+}\)-free growth medium containing 100 \(\mu\)M-phalloidin (final concentration) for 30 additional minutes. Cells in the third sample were resuspended for 30 min in a Ca\(^{2+}\)-free growth medium without phalloidin. Cells from all samples were fixed, extracted with detergent and processed for actin immunofluorescence. Cells were scored in the three classes described for Table 1. The values (%) represent measurements of cells for each treatment pooled from two separate experiments.

Ca\(^{2+}\)-free medium also contains phalloidin (Table 2). The results for a 24 h exposure to phalloidin followed by a 30 min resuspension in Ca\(^{2+}\)-free medium containing phalloidin were not significantly different from the control. Omission of the phalloidin from the Ca\(^{2+}\)-free medium resulted in only partial stabilization of the actin pattern (55 % striated cells compared with the control value of 86 % striated cells), suggesting a dissociation of the phalloidin from the microfilaments under these conditions. When microfilaments were stabilized with phalloidin before and during the Ca\(^{2+}\)-free resuspension, the myosin and calmodulin patterns were also stabilized, giving cell profiles nearly identical (data not shown) to those reported as controls in Table 1.

Table 3. Effect of detergent extraction on fluorescence patterns

<table>
<thead>
<tr>
<th>Detergent extraction followed by fixation</th>
<th>Detergent extraction exposure to 180 (\mu)M-Ca(^{2+}), fixation</th>
<th>Detergent extraction exposure to 100 (\mu)M phalloidin, fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin</td>
<td>Myosin Microtubules</td>
<td>Actin</td>
</tr>
<tr>
<td>Striated</td>
<td>3 1 73</td>
<td>4 10</td>
</tr>
<tr>
<td>Partially striated</td>
<td>9 9 17</td>
<td>10 14</td>
</tr>
<tr>
<td>Non-striated</td>
<td>88 90 10</td>
<td>86 76</td>
</tr>
</tbody>
</table>

Cells were extracted with Triton X-100 before fixation to determine if a lysed cell system could be used to study the various fluorescence patterns. The effect of detergent extraction without Ca\(^{2+}\) was examined for the actin, myosin and microtubule patterns. Cells were also extracted in the presence of either 180 \(\mu\)M-Ca\(^{2+}\) or 100 \(\mu\)M-phalloidin and stained for actin. Cells were scored in the three classes described for Table 1. The values (%) represent measurements of 500 cells for each immunofluorescence assay pooled from two separate experiments.
The results reported so far used cells fixed, after the various chemical treatments, followed by detergent extraction. Another method commonly used to study cytoskeletal components is to extract the living cells with detergent and then follow this by treatment to perturb the remaining cytoskeleton or contractile complex. Fixation is the final step in this procedure. This system has been used successfully to study the stable microtubules in *Euglena* (Lachney & Lonergan, 1985). Detergent extraction before fixation completely disrupts the fluorescence patterns for actin, myosin and calmodulin, but does not significantly alter the microtubule pattern. The addition of Ca\(^{2+}\) or phalloidin at the time of detergent extraction has no effect on stabilizing any of the sensitive fluorescence patterns (Table 3).

**The involvement of calmodulin in determining the stability of the actin and myosin fluorescence patterns**

The instability of the actin, myosin and calmodulin fluorescence patterns, caused either by resuspension of cells in Ca\(^{2+}\)-free medium (Table 1) or detergent extraction before fixation (Table 3), can be prevented by a 30-min prior exposure of the cells to the calmodulin antagonist trifluoperazine. When trifluoperazine-treated cells are resuspended in Ca\(^{2+}\)-free medium, more than 90% of the cells are entirely covered with fluorescent striations representing actin, myosin and calmodulin (Table 4). The same trend towards stabilization of the actin and myosin patterns was observed during detergent extraction (compare Table 4 with Table 3). The presence of trifluoperazine was not required for stabilization during either the actual Ca\(^{2+}\)-free resuspension or the detergent extraction. Only exposure of the cells to trifluoperazine before the subsequent treatment was necessary.

**The effect of elevated Ca\(^{2+}\) levels on the fluorescence patterns**

The effect of increasing intracellular Ca\(^{2+}\) levels, by using the calcium ionophore A23187, on the cell shape profile of a population has been reported (Lonergan, 1984). Incubation of cells with A23187, which theoretically should equilibrate the

<table>
<thead>
<tr>
<th>Striated</th>
<th>Actin</th>
<th>Myosin</th>
<th>Calmodulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partially striated</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Non-striated</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Elongated cells from the middle of the light cycle were incubated for 30 min with trifluoperazine (100 \(\mu\)M final concentration at a cell density of \(8 \times 10^6\) cells/ml) followed by either a 30 min re-suspension in Ca\(^{2+}\)-free medium or detergent extraction. Cells were fixed and stained for actin, myosin or calmodulin. Cells were scored in the three classes described for Table 1. The values (%) represent measurements of 750 cells for each immunofluorescent assay pooled from three separate experiments.
intracellular free Ca\(^{2+}\) with the 180 \(\mu\text{M}\)-Ca\(^{2+}\) in the growth medium resulted in an actin fluorescence pattern identical to that reported as the control in Table 1. Therefore, the actin pattern was insensitive to the increased Ca\(^{2+}\) level. The patterns for myosin and calmodulin were not examined, but previous studies have shown that the microtubules are not sensitive to similar treatment with A23187 (Lachney & Lonergan, 1985).

The detergent-extracted cell system discussed in a previous section was used to determine if the actin and/or myosin pattern were sensitive to increased Ca\(^{2+}\) levels, which have been shown to stimulate depolymerization of microtubules (Lachney & Lonergan, 1985). It was previously suggested that detergent extraction of cells before fixation results in a complete loss of fluorescent patterns for actin, myosin and calmodulin (Table 3). The fluorescent patterns can be stabilized by prior exposure of the cells to trifluoperazine (Table 4). Such a system, with no additional trifluoperazine in the detergent solution, was used with the addition of 180 \(\mu\text{M}\)-Ca\(^{2+}\) (growth medium concentration) to determine if increased Ca\(^{2+}\) levels affect microfilament stability after calmodulin inhibition. The increased Ca\(^{2+}\) concentration had no effect on either actin or myosin fluorescence patterns after a 30-min exposure to 180 \(\mu\text{M}\)-Ca\(^{2+}\). The fluorescence profiles for actin and myosin were not significantly different from the control values reported in Table 1.

Is there a correlation between cell rounding and microfilament integrity?

The rounding of cells can occur for numerous reasons. The cell rounding controlled by the biological clock has not been correlated with microfilament (Fig. 1) or microtubule (Lachney & Lonergan, 1985) depolymerization. While depolymerization of microfilaments is apparently not responsible for the slow cell rounding, the microfilaments do turn over during the 24 h period of growth in the light/dark cycle. A 5-h incubation of cells with cytochalasin B did not alter microfilament integrity (the fluorescence profile was not significantly different from the control values in Table 1), indicating that the previously reported short-term effects of cytochalasin on cell shape changes (Lonergan, 1983) cannot be attributed to a disruption of microfilaments. A 24-h incubation dramatically reduced the percentage of cells possessing striations to 25 %, with 25 % of the cells partially striated and 50 % non-striated, indicating that at some stage in the cell cycle, microfilaments are polymerized and the presence of the cytochalasin blocks the polymerization.

The treatment of cells with the calmodulin antagonist trifluoperazine causes a rounding of virtually 100 % of the cell population within 1 h (Lonergan, 1984). The fluorescence pattern for the actin in the rounded cells indicates that virtually all of the TFP-induced cell rounding represents cells with fluorescent striations over the entire cells (data not shown). This is a second example in which cell rounding is not correlated with the loss of microfilaments.

The cell rounding induced by shock treatments, such as resuspension in Ca\(^{2+}\)-free medium or detergent extraction before fixation, can be correlated with microfilament loss. When cells from the mid-light cycle are resuspended in Ca\(^{2+}\)-free medium, the percentage of round cells 15–24 \(\mu\text{m}\) in diameter increases from approximately 25 %
to approximately 50% (Lonergan, 1984). The round cells in the population before resuspension in Ca\textsuperscript{2+}-free medium are 97\% striated, whereas after resuspension in Ca\textsuperscript{2+}-free medium only 59\% of the cells are striated, with 41\% of the cells non-striated. The increase in non-striated cells from 3\% in the control to 41\% after resuspension in Ca\textsuperscript{2+}-free medium indicates that many of the cells become round and lose their microfilaments.

**DISCUSSION**

The nature of the mechanism(s) for altering cell shape in *Euglena* is unknown. It was originally speculated throughout these studies that *Euglena* might possess an actomyosin contractile system. Actin, presumably in the form of microfilaments, and myosin, presumably organized as bipolar filaments, were found by indirect immunofluorescence to be localized on the surface of the cells as discrete fluorescent striations (Fig. 1). The use of nitrobenzoxadiazole–phallacidin as a probe for filamentous actin was not attempted. The fluorescence patterns for actin and myosin were coincident with the spacing of the pellicle strips beneath the plasma membrane. Because of the identical spacing and the observation that the fluorescence patterns wind helically around the cells like the pellicle strips, it is speculated that the microfilaments and myosin are pellicle-associated. While there appears to be coincidence between the fluorescence patterns for microtubules, microfilaments, myosin and calmodulin, more detailed studies will be necessary before a precise model can be described to explain how these proteins are structurally arranged. There remains the possibility that the physical location of these proteins has been altered during the detergent extraction or subsequent procedures. Immunofluorescence techniques cannot be used to examine this problem, but the use of colloidal-gold-labelled antibodies in extracted and non-extracted cells could be used to localize microfilaments and myosin associated with the pellicle strips. The technique of Fey, Wan & Penman (1984) using non-embedded cells allows the cytoskeleton attachments to the *Euglena* pellicle to be examined. Present investigations are attempting to determine if any of these proteins is actin or myosin. The technique does not require detergent extraction of the cells, permitting this treatment to be used as a control for comparison.

While the fluorescence patterns for microtubules (Lachney & Lonergan, 1985), microfilaments, myosin and calmodulin all show a high degree of coincidence, the microfilament, myosin and calmodulin patterns can be dissociated from the microtubule pattern. When cells are briefly resuspended in Ca\textsuperscript{2+}-free medium or extracted with detergent before fixation, the microfilament, myosin and calmodulin patterns are disrupted, while the microtubule pattern is unaffected (Table 1). The myosin pattern is the most sensitive to disruption by resuspension in Ca\textsuperscript{2+}-free medium, while the extent of disruption of the microfilament and calmodulin patterns is nearly identical. This implies that the calmodulin is associated, at least in part, with the microfilaments. Dual fluorescent labelling of cells exposed to decreased levels of Ca\textsuperscript{2+} should reveal whether the calmodulin is coincident with the microfilaments, the microtubules or both.
Cell shape changes in *Euglena* 207

The sensitivity of the microfilament, myosin and calmodulin patterns to Ca\(^{2+}\)-free resuspension can be counteracted by prior exposure of the cells to the peptide phalloidin, which is thought to stabilize microfilaments. The *Euglena* were cultured autotrophically, and the phalloidin must have been taken into the cells by phagocytosis, a process not previously attributed to *Euglena*. Phalloidin stabilization of the microfilaments also stabilizes the fluorescent patterns of myosin and calmodulin. While not conclusive, this result implies that one effect of Ca\(^{2+}\)-free resuspension is to disrupt the integrity of the microfilaments. This investigation represents the first report of microfilaments in *Euglena*, and it is not known whether microfilament-fragmenting proteins exist in *Euglena* or whether such proteins would be activated by resuspension in Ca\(^{2+}\)-free medium.

The microfilament, myosin and calmodulin patterns can also be stabilized by brief prior treatment of the cells with the calmodulin antagonist trifluoperazine (Table 4). *Euglena* cells become round as a result of exposure to trifluoperazine (Lonergan, 1984), but the round cells maintain their immunofluorescence patterns for microfilaments, myosin and calmodulin. These results collectively imply that the resuspension in Ca\(^{2+}\)-free medium or detergent extraction before fixation activates calmodulin, possibly resulting in the depolymerization of the microfilaments. Inhibition of calmodulin with trifluoperazine would thus prevent the calmodulin-stimulated depolymerization. Such an interpretation should be considered with caution, however, in that the specificity of the TFP interaction with calmodulin can be questioned. At the cell densities typically used for the immunofluorescence studies, TFP inhibited the rate of whole-cell photosynthesis (measured as the rate of oxygen evolution) by 95%, while respiratory oxygen consumption was inhibited by 30%. It is possible that the TFP-induced rounding results from a disruption of cellular energy charge instead of, or in addition to, the inhibition of calmodulin. Changes in the ATP level might affect membrane transport properties, thus resulting in an osmotically induced shape change. In addition, it has been reported that phenothiazine drugs alter membrane fluidity (Salesse et al. 1982).

Biochemical evidence for the presence of actin and myosin in *Euglena* is desirable to support the hypothesis that an actomyosin contractile system is operative, even though immunofluorescence controls indicated that anti-actin and anti-myosin are specific in their recognition of actin and myosin in *Euglena*. Immunoblotting is being used at present to confirm the cross reactivity of the antibody preparations with *Euglena* actin and myosin extracted from the flagella. Actin and myosin have been localized by immunofluorescence in human sperm flagella (Virtanen, Badley, Paasivuo & Lehto, 1984) as well as in *Euglena* flagella (data not shown).

There have been several reports on immunofluorescence patterns for actin and myosin in other cells, including sperm cells (Virtanen et al. 1984), mouse blastomeres (Lehtonen & Badley, 1980; Sobel, 1983) *Dictyostelium* (Yumura, Mori & Fukui, 1984) and *Saccharomyces* (Adams & Pringle, 1984; Kilmartin & Adams, 1984). While the immunofluorescence in these systems generally reveals diffuse staining patterns, the patterns reported for *Euglena* are specific, with a high degree of coincidence. There is no direct evidence for the involvement of an actomyosin contractile...
system during the various *Euglena* shape changes, although the presence of actin and myosin in the pellicle region makes such a mechanism feasible.

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REFERENCES


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