Microfilament assembly during lens cell elongation in vitro

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ABSTRACT

Bovine epitheloid lens cells can be kept in culture for almost one year. During subculturing a gradual process of cell elongation is observed. Using several techniques, including immunofluorescence with antibodies against actin, electron microscopy, two-dimensional gel electrophoresis, drug treatment and measurement of actin polymerization by DNase I inhibition, it is shown that microfilament assembly parallels the process of cell elongation.

INTRODUCTION

Bovine epithelial cells in culture elongate spontaneously upon aging (1, 2) and change from a polygonal shape to long, fibrelive cells. This phenomenon can also be induced by dexamethasone (3) or by a soluble extract from the neural retina (4). Elongation of epithelial chick embryo lens cells in vitro can be induced by insulin or under certain growth medium conditions (5). It has been suggested that this process reflects, as far as morphology is concerned, in vivo differentiation (6).

It is now generally accepted that cell shape changes, as well as other forms of cellular motility processes, involve a redistribution of the intracellular cytoskeletal structures (7). For example, in elongating epithelial chicken lens cells Platigorsky and his colleagues (8) found a reorganization of microtubules. Furthermore, these workers were able to show that the process of elongation could be inhibited by colchicine. However, since the amount of microtubules in these cells is not impressive and no increase in tubulin synthesis was observed a secondary effect cannot be excluded. Beebe et al. (9) actually demonstrated that the presence of intact microtubules is no prerequisite for lens cell elongation. As Mousa and Trevithick (10) found that elongation of cultured rat epithelial cells is inhibited by cytochalasin B and D the question is reinforced as to whether microtubules or microfilaments play a major role in the morphological changes that occur in elongating epithelial lens cells.

In recent investigations (cf. ref. 11, 12) we have shown the presence of cytoskeletal structures (microfilaments, microtubules and intermediate-sized filaments) in bovine lens cells by means of immunofluorescence, demonstrating the main constituents of these structures to be actin, myosin, α-actinin, tubulin and vinilin. Further characterization was achieved by means of transmission electron microscopy and gel electrophoretic techniques.

In the present paper we provide evidence that in in vitro elongation of these cells.

MATERIALS AND METHODS

Bovine epithelial lens cells were cultured mainly as described earlier (1, 2). Calf eyes were obtained from the slaughterhouse and kept on ice. After washing the eyes in distilled water or 80% ethanol and removal of the lens either by an anterior or a posterior approach, cultures were set up in two different ways.

a) The capsule with adhering epithelial cells was isolated, spread on the bottom of a plastic tissue culture flask and allowed to stick to the surface for 30 min at 37°C. Then, tissue
culture medium was carefully added (1).

b) The capsules with the epithelium were treated with a 0.25% solution of trypsin in Ca$^{2+}$- and Mg$^{2+}$-free Tyrode's solution, and the isolated cells washed and resuspended in culture medium and seeded in plastic tissue culture flasks (2).

In both procedures a and b, the culture medium consisted of TC 199 and 0.5% lactalbumin hydrolysate in Hanks' solution (1:2 v/v). Newborn calf serum (Flow Labs, Scotland) was added to a final concentration of 10-15%. Cells were subcultured using 0.35% trypsin or 0.25% trypsin with 0.025% EDTA in Ca$^{2+}$- and Mg$^{2+}$-free Tyrode's solution. Routine transfers were done weekly at a split ratio of 1:4. Cell growth was monitored by phase-contrast microscopy. For the present study cells were grown on coverslips in Leighton tubes.

For electron microscopical observations the coverslips were coated with carbon. In some experiments the cells were treated either with cytochalasin B (5 µg/ml of growth medium) or colchicine (10 µg/ml of growth medium).

**Electron microscopy**

For transmission electron microscopy the cultured cells were fixed in 2.0% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2; 300 mosm) for 1 h at 4°C, then transferred to 0.1 M phosphate buffer (pH 7.2; 300 mosm) at 4°C for at least 1 h and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) at 4°C for 1 h. The coverslips were subsequently rinsed in 0.1 M phosphate buffer (pH 7.2) at 4°C for at least 2 periods of half an hour. Dehydration was performed in an ascending series of ethanol. The coverslips were then transferred via a mixture of propylene oxide and Epon to pure Epon 812. After polymerization of the Epon on the coverslips, appropriate areas were selected under a phase-contrast microscope, the tissue layer stripped off on dry ice, the selected areas glued to Epon blanks, and sectioned parallel as well as perpendicularly to the cell layer with glass knives in a Reichert OMU3. Grey sections were picked up on copper grids, double contrasted with uranyl acetate and lead citrate, and examined in a Philips electron microscope EM 300.

For scanning electron microscopy the same fixation procedures were applied. After dehydration in an ascending series of acetone and processing by critical point drying using liquid CO$_2$, the dried mounted specimens were coated with gold and examined in a PSEM 500 (25 kV).

**Actin antibodies and indirect immunofluorescence microscopy**

Actin antibodies, kindly provided by Drs. M. Osborn and K. Weber, were raised in rabbits against denatured chicken gizzard actin (11). They were made monospecific by affinity chromatography on homogeneous monomeric pig brain actin covalently bound to Sepharose 4B. The antibody was used at 50 µg/ml in PBS.

Next to these, antibodies directed against tubulin, keratin and vimentin have been used in this study (cf. ref. 11). Cells grown on coverslips were treated with methanol and acetone at -20°C, air-dried, then the first antibody was added and the coverslips incubated for 40 min at 37°C. After several washings with PBS the fluorescein-labeled second antibody (goat antirabbit γ-globulins, Miles & Co., Kankakee, Ill) was added and the coverslips held for a further 40 min at 37°C. After a second series of washings with PBS the coverslips were mounted with Elvanol on microscope slides. The cells were viewed with a Zeiss microscope, equipped with epifluorescent illumination using the appropriate filters for fluorescein fluorescence. Pictures were taken with Planapo 40x and 63x oil immersion objectives on Kodak Tri-X film using an ASA-setting of 800.

**Labeling of lens cell proteins and preparation of cell lysates**

Confluent monolayers of bovine lens epithelial cells, growing in plastic tissue culture flasks of 200 cm$^2$ were incubated for 16-20 h with L-[35S]$^{-}$-methionine (obtained from the Radiochemical Center, Amersham, England, 5 µCi/ml medium) in the above mentioned tissue culture medium lacking methionine. After incubation the medium was decanted and the cells rinsed twice with Tyrode's solution. Then the cells were harvested from the surface with a
rubber policeman, centrifuged at low speed (5000 rpm in a Christ centrifuge) and washed with the Tyrode solution. The cells were lysed in a cytoskeleton-stabilizing buffer (50 mM Tris/Cl pH 7.4; 25 mM KCl; 5 mM MgCl₂, TMM buffer) with a Dounce homogenizer.

To obtain both TMM buffer soluble and insoluble proteins (the membrane cytoskeleton complex) the lysates were centrifuged at 10,000 x g and the resulting pellet washed three times in TMM buffer. For analysis of the proteins by gel electrophoresis either lyophilized or TCA-precipitated protein fractions were used.

*Gel electrophoretic analysis*

Protein analysis was performed by two-dimensional gel electrophoresis using both iso-electric focusing and non-equilibrium-pH-gradient isoelectric focusing in the first dimension, according to the method of O'Farrell et al. (13, 14). Radioactive spots were visualized by scintillation autoradiography (15), in combination with the drying procedure described by Berns and Bloemendal (16). Highly purified bovine brain β- and γ-actin and rat brain tubulin were used as protein markers. (Kindly provided by Drs. J. Vandekerckhove and I. Sandoval, respectively, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany).

Quantitative estimation of globular and filamentous actin

Monomeric and filamentous actin were quantitatively assayed in lens cell extracts using inhibition of deoxyribonuclease I essentially as described by Blikstad et al. (17).

*Drug incubation of the cells*

Cells grown on coverglasses in Leighton tubes were incubated with cytochalasin B (Calbiochem, Basel, stock solution 10 mg/ml in DMSO) or colchicine (Merck, Darmstadt) for 3-14 h. To reverse the effect of these drugs the medium was changed (neither cytochalasin B nor colchicine present) and the cells studied during several time intervals. Phase-contrast microscopy was performed with a Leitz Diavert.

Incubation of the cells with extracts of the retinas (15 mg protein/ml extract; 0.5-1 mg protein/ml culture medium) for 48 h was essentially as described (4).

**RESULTS**

**Reorganization of the microfilament bundle distribution upon elongation**

Bovine epithelial lens cells grown in culture retain their polygonal shape at least during the first 15 passages. Occasionally, some elongated cells are observed. After about 20 transfers the cells start to change their appearance spontaneously resulting in elongated cell populations (Fig. 1). Elongation can also be induced by dexamethasone (3) or by an extract of the bovine retina (4; see Figs. 1c-e). In the phase-contrast microscope these elongated cells exhibit pronounced intracellular fibrillar organizations running parallel to the direction of elongation and traversing the whole length of the cell. These filaments can be visualized by the indirect immunofluorescence technique using highly specific antibodies directed against actin (see Fig. 2) and can thus be identified as microfilament bundles. When young, polygonal cells are incubated with the specific antibodies directed against actin a rather random distribution of the microfilaments (microfilament bundles) is observed (Fig. 2a). In elongated cells from cultures of higher passage number (30-40 transfers) heavy arrays of parallel stress fibers occur, often running through the whole length of the cell in the direction of elongation (Fig. 2b). Also young cells that have been incubated with retinal extracts elongate within 48 h. Again a drastic reorganization of stress fibers lining up in the direction of elongation is observed (compare Figs. 2c, d). In this case an optimal cellular density is needed to induce the morphological change, suggesting that cell-to-cell contact is necessary. To show that the observed immunofluorescence pattern is specifically due to actin-containing fibers, cells were treated with cytochalasin B. Fig. 2e shows that after incubation with this microfilament disrupting drug,
only spotty actin fluorescence is left. From these results it is concluded that the morphological differentiation of bovine lens cells in culture (resulting in elongated cells) is accompanied by a reorganization of microfilament bundles.

In cells of intermediate age (as reflected by the number of passages) which did not yet show pronounced elongation, a structure consisting of regular polygonal actin networks is observed (Fig. 2f). The highest concentration of these structures and greatest size is observed in cells that were subcultured 10-15 times. Further subculturing results in decreased amounts of polygonal networks and a decrease in their size (Table 1).

<table>
<thead>
<tr>
<th>Passage number</th>
<th>% Cells containing &quot;geodesic domes&quot;</th>
<th>Relative shape of &quot;geodesic domes&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16%</td>
<td>medium</td>
</tr>
<tr>
<td>10</td>
<td>23%</td>
<td>large</td>
</tr>
<tr>
<td>18</td>
<td>5%</td>
<td>small</td>
</tr>
<tr>
<td>34</td>
<td>9%</td>
<td>very small</td>
</tr>
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</table>

These results show that a transient organization of actin networks occurs in established flattened bovine lens cells in culture and that this
Fig. 2: Immunofluorescence micrographs of cultured bovine lens cells incubated with antibodies to actin
(e) Diffuse and spotty actin fluorescence after treatment of the cells with cytochalasin B;
(f) "Geodesic domes" in cells of intermediate passage number (11th passage). Bars indicate 20 μm.

(a and c) Young polygonal cells; c) confluent monolayer;
(b) Cell elongated spontaneously upon subculturing;
(d) Cells elongated after addition of a retinal extract to the growth medium;

organization represents an intermediate structure between the random distribution of actin fibers in polygonal cells and strongly organized parallel arrays of stress fibers in elongated cells.

Antibodies directed against tubulin and vimentin were also tested on both polygonal and elongated lens cells in culture (not shown). The results suggest that reorganization as shown for actin did occur neither for microtubules nor for intermediate-sized filaments during lens cell
elongation in vitro.

Electron microscopic observations

Polygonal cells of the early subcultures as well as elongated cells in later stages are shown to be linked by well-developed twisted junctions of the fascia adherens type. Bundles of microfilaments are attached to these structures. Intermediate-sized filaments and microtubules are present in abundant amounts. The former mostly surround the nucleus and criss-cros among the various organelles, the latter extend in various directions, often at different angles to the intermediate-sized filaments. Generally most organelles are well-distributed and localized in the central perinuclear areas.

The most striking changes of the cultured cells are without any doubt the differences in content and distribution of the already mentioned microfilaments. In the polygonal cells the arrangement of filaments delimits two distinct cytoplasmic areas. In the central perinuclear area fine bundles of microfilaments are running in the nuclear indentations and among the organelles (Fig. 3a). In the peripheral part of the cytoplasm larger bundles of straight microfilaments dominate. They are oriented in parallel or fan-shaped arrays (Fig. 3b). Dense plaques can be observed within the bundles of these stress fibers at more or less regular distances.

In polygonal cells of intermediate age transitory microfilamentous structures occur. After about 10–15 transfers the cells do not yet show pronounced elongation. As already mentioned in numerous cells a structure consisting of regular networks is observed, which appeared to be actin as confirmed by immunofluorescence microscopy (Fig. 2). These starlike structures are mainly localized in close proximity to the plasma membrane adjacent to the substrate. In several cells these stress fiber networks ("geodesic domes") also encompass the entire area around the nucleus. Compared to the cells of the monolayer in early subcultures the central region of elongated cells does not display any significant changes. The most striking feature of this stage is the presence of more extensive, longer microfilament bundles in the peripheral part (Fig. 4), as fan-shaped bundles, dominating other structures.

Microfilament assembly during in vitro lens cell elongation as quantitated by the DNase I inhibition assay

In the foregoing sections a correlation between the appearance of stress fibers, aligned with the long axis of the cultured cells, and cell elongation has been observed. In order to get an impression of the molecular basis of this process the ratio between globular-actin (G-actin) and filamentous actin (F-actin) was estimated both in young, polygonal and elongated cells in later subcultures. The method used for these estimations (17), is based upon the fact that G-actin specifically inhibits DNase I. The amount of F-actin is calculated by subtracting the amount of G-actin (estimated in the absence of guanidine hydrochloride) from the total amount of cellular actin (estimated in the presence of guanidine hydrochloride). The results summarized in Table 2 show that there is a shift from G-actin to F-actin upon lens cell elongation. The total amount of actin increases from 1.9% in young cells (6th passage) to 4.1% of total protein in elongating cells (22nd passage).

Decreased tubulin and crystallin synthesis in elongating bovine lens cells in culture

When polygonal and elongated bovine lens cells in monolayer cultures are compared with respect to their protein biosynthetic activity (Fig. 5) it is

<table>
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<tr>
<th>Passage number</th>
<th>G-actin</th>
<th>F-actin</th>
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<tr>
<td>6</td>
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Fig. 3: a) Transmission electron micrograph of the perinuclear area in a polygonal lens cell showing the organization of microfilaments (asterisks) in the nuclear indentations. Thin arrows: microtubules. (Bar = 1.7 μm).

b) Transmission electron micrograph of the peripheral part of a polygonal lens cell. A fan-shape organization of microfilament bundles is evident. Note numerous dense plaques (short thick arrows) within the bundles. Thin arrows: intermediate-sized filaments. (Bar = 4 μm).
obvious that the synthesis of neither crystallins nor tubulin is detectable in the elongated cells. This phenomenon is observed already after 2-3 passages, long before the cells start elongating. However, immunofluorescence shows the presence of some microtubules also in elongated cells, indicating that tubulin synthesis must occur at a low level. These low relative levels of tubulin and crystallin synthesis make it unlikely that these proteins are important for cell elongation in vitro. A role of microtubules in this process cannot be ruled out since the assembly of these structures may be independent of tubulin synthesis and may merely represent an equilibrium between a soluble and a cytoskeletal form of tubulin.

Effects of cytochalasin B and colchicine

The effect of cytochalasin B on calf lens epithelial cells in culture is shown in Fig. 6. Two stages of in vitro elongating lens cells are depicted which all exhibit the typical arborized shape when incubated with the drug (10 μg/ml growth medium). However, the effect of cytochalasin B seems to be more drastic in older cultures with elongated cells than in the younger ones containing the polygonal cells. Surprisingly the differential effect of cytochalasin B on calf lens cells becomes even more pronounced when the drug is withdrawn after several hours of incubation. The young cells regain their original polygonal shape within 3 hours, while the older, originally elongated cells do not restore their shape, even after maintenance for 24 h in normal serum. Cells of intermediate age show only a partial recovery after 24 h.

The effects of cytochalasin B and colchicine on the process of bovine lens cell elongation in
vitro under the direction of a retinal extract were also examined. Arruti and Courtois (4) showed an inhibitory effect of colchicine (in concentrations of $6 \times 10^{-5}$ to $6 \times 10^{-9}$ M) on the elongation of adult bovine epithelial lens cells. Van Vanzoëij et al. (3) demonstrated that cytochalasin B (5 μg/ml medium) did not prevent elongation of calf lens cells under the direction of dexamethasone. Therefore, one would expect a role of microtubules in the process of lens cell elongation in vitro as previously proposed for chick lens (6). Careful examination, however,
shows that concentrations of both cytochalasin B and colchicine which change the shape of normal non-elongating cells, also inhibit elongation (Table 3). On the other hand, as soon as the drug concentrations are so low that they do not affect the cell shape, they also do not prevent elongation.

A role of β-actin and myosin in lens cell elongation in vitro

Closer examination of cytoskeletal preparations from young polygonal calf lens epithelial cells and elongated cells revealed some interesting aspects concerning microfilament bundle assembly during elongation in vitro. Fig. 7 shows a one-dimensional gel containing samples of lens cell cytoskeletons. It can be seen that in the high molecular weight region a major band of the lens cells comigrates with rabbit muscle myosin. Since it has been described that myosin cannot be detected by the conventional 2D-gel electrophoretic technique (13, 16) we chose the modified procedure as described by O'Farrell (14). Newly synthesized proteins incorporated into the cytoskeletal fractions and isolated as described above, were analyzed in this way and autoradiographed. Cytoskeletal protein patterns from cells of increasing passage number are shown in Fig. 8.

From polygonal to elongated cells a distinct decrease in the intensity of vimentin relative to actin can be observed. This observation is parallel to the finding in vivo where a similar change was seen in electron micrographs of epithelium and fiber cells (11). Furthermore, the incorporation of myosin synthesized de novo gradually increases. At the same time the incorporated β-actin which is virtually absent in the cytoskeletal preparation from young cells (where only γ-actin is detected) becomes more pronounced. In total cell extracts of primary and elongated cells, however, both β-

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### Table 3

<table>
<thead>
<tr>
<th>R.E. (ml/5 ml medium)</th>
<th>Colchicine (M)</th>
<th>Elongation</th>
<th>Cell shape</th>
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<td></td>
<td>6.25 x 10^-7</td>
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</tr>
<tr>
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<td>elongated</td>
</tr>
<tr>
<td>200</td>
<td>6.25 x 10^-4</td>
<td>-</td>
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<table>
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<tr>
<td>300</td>
<td>2 x 10^-3</td>
<td>-</td>
<td>arborized</td>
</tr>
</tbody>
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**Fig. 7:** SDS-gel electrophoretic analysis of polypeptides that make up the cytoskeleton-membrane complex in cultured bovine epithelial lens cells

- Marker proteins (α-crystallin, 20,000 D; ovalbumin, 43,000 D; bovine serum albumin, 68,000 D and phosphohexase A, 93,000 D);
- A cytoskeletal preparation from cultured lens cells;
- Actin from bovine brain (N);
- Rabbit muscle myosin;
- Vimentin (V) in a Triton/KCl-cytoskeleton preparation from bovine lens cells in culture.
and γ-actin are present. In older preparations a hitherto unidentified 47 kD protein is found. Its intensity increases upon aging and elongation of the cells.

DISCUSSION

Lenticular cells are characterized by the process of fibrogenesis. When the epithelial cells reach the equatorial zone of the lens they elongate into very long, tubelike fibers. In the embryo this differentiation process seems to be induced by factors from the tissue forming the neural retina [19]. In a previous paper [20] it was shown that in situ bovine lens cell elongation is accompanied by an increase in filamentous actin. This result suggests a controlled process of microfilament assembly during lens differentiation. Whether or not this process represents a driving force in lens fiber formation remains to be answered.

Since epithelial lens cells brought into culture elongate spontaneously [1, 7], as a result of hormone treatment [4] or after addition of an extract from the retina [4] we wondered whether this phenomenon had some aspects in common with the in vivo differentiation process. But some of the typical differentiation markers, the crystallins and the major intrinsic membrane protein, MP26, are undetectable in the elongated cells in culture. However, due to the complexity of tissue differentiation we made a search for other markers of lens cell elongation which might be found both in culture and in vivo.

The results presented show that assembly of microfilaments in lens cell elongation which is difficult to follow in situ, can be studied in bovine lens cell cultures. We are fully aware of the fact that cells in monolayers, growing under artificial conditions, have to adapt to their new situation, in which environmental and feedback mechanisms of regulation are completely different. However, especially those constituents involved in cellular motility processes and in maintaining cell shape appear to be retained in vitro.

In particular actin seems to play a role in the elongation of lens cells in culture. An increase in the assembly of microfilaments may be a factor that directs the onset of morphological changes. Furthermore a structure consisting of regular polygonal networks is observed especially in cells that do not yet show pronounced elongation. These structures have been described earlier by Lazarides [7] in fibroblasts and designated "geodesic domes". They encompass the entire area.
above and around the nucleus and have α-actinin localized at the vertices of the network. The vertices are thought to act as organization centers for actin-filament bundles resulting in or correlated with the elongation and flattening of fibroblasts. This temporary actin organization in fibroblasts is an intermediate structure between the diffuse meshwork state in rounded cells (for example seen after trypsinization) and the linear actin bundles in the fully flattened cells. However, as mentioned before, the correlation of the in vitro situation with events taking place in vivo may be too speculative, since stress fibers, for instance, seem not to occur in lens fibers in situ.

In cultured bovine lens cells the networks are also present after flattening of the cells in established cultures (Fig. 22) and they should thus not be considered as a temporary structure in cells of intermediate passage number. Thus during subculturing these "geodesic dome"-like structures occur mainly in young cells that have not elongated yet and do not yet exhibit pronounced parallel arrays of stress fibers.

Since tubulin synthesis diminishes, microtubules do not seem to play an important role in the elongation process.

Arutti and Courtois (4) reported that protein biosynthesis seems to be necessary for the elongation of cultured bovine lens cells under the direction of a retinal extract. These authors claimed also that protein biosynthesis is a requirement for the maintenance of the elongated shape. This was concluded from the reversion to the polygonal shape of previously elongated cells if treated with cycloheximide. The latter result is surprising as Piatigorsky (6) showed that chick lens epithelial explants are independent of protein biosynthesis for their elongation induced by insulin or under certain growth medium conditions. If the observation made by Arutti and Courtois, suggesting that elongation is dependent upon protein synthesis, holds also true for the calf lens system, one has to conclude that microfilaments are more likely to play a major role in lenticular cell elongation in vitro than microtubules. Assembly of microtubules, independent of tubulin biosynthesis can, however, not be excluded.

Mouss and Trevithick (10) have reported a differential effect of cytochalasins B and D on polygonal and elongated rat lens cells in culture. Young cells, which have not yet elongated are less sensitive to treatment with these drugs than are the elongated ones, which readily arborize. The cytochalasins inhibit elongation in cultures of rat lens epithelium explants (8). We made similar observations but also colchicine was found to inhibit elongation under the direction of a retinal extract and resulted in an arborized cell shape. This shows that both microtubules and microfilaments may be involved in elongation and/or maintenance of cell shape. Nevertheless taking into consideration all the available data we want to propose, in agreement with Dustin (21), a more static role for microtubules and a dynamic role for microfilaments in the elongation of epithelial lens cells.

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REFERENCES


