Cytoskeletal control of fibroblast length: experiments with linear strips of substrate

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SUMMARY

In order to understand the factors determining the length of fibroblasts, three cell lines (mouse embryonic fibroblasts plus human fibroblast lines AGO 1523 and M19) were cultivated on the usual planar substrate (glass) and on specially prepared narrow linear strips of the same substrate, where the cells could spread only linearly. Morphometric measurements showed that the average length of cells of each type on the 'unidimensional' strips was no different from that on the usual 'bidimensional' substrate. The addition of colcemid significantly decreased cell length on both substrates, whereas cytochalasin D increased the length. We concluded that fibroblasts have an intracellular mechanism maintaining a relatively constant average cell length. This mechanism may involve the dynamic balance of centripetal and centrifugal forces developed by two cytoskeletal systems: the microtubules and the actin-myosin cortex. Three epitheliocyte cell lines (rat IAR2, canine MDCK and bovine FBT) were tested but, in contrast to fibroblasts, they did not maintain similar cell lengths on the usual substrate and on the linear strips, suggesting that control of length is cell-type-specific.

Key words: Fibroblast, Epitheliocyte, Microtubules, Microfilaments, Colcemid, Cytochalasin, Morphometry, Cell shape, Micropatterned adhesiveness.

INTRODUCTION

Control of cell dimension is an important but as yet poorly understood aspect of morphogenesis. One particular question related to this is how do elongated cells, such as cultured fibroblasts, control their length? These cells, when fully spread on a standard planar culture substrate, form several cytoplasmic processes of different length. Two alternative models explaining the control of cell length are possible. One of the models states that the sum of the lengths of all processes is controlled, so that when the number of processes is decreased, the length of the remaining extensions is increased. The other suggests that the maximum length of the cell is controlled regardless of the cell width and number of processes. These two possibilities are presented in (Fig. 1).

The ability of fibroblasts to orient themselves along the narrow linear strips of an adhesive surface is a manifestation of their 'contact guidance' ability (O'Neill et al., 1990; Matsuda and Sugawara, 1996; Ahmed and Brown, 1999). We found that the average length of the fibroblasts on linear strips was not increased when compared with those on the usual planar culture substrate. Further experiments suggested that this length-maintaining mechanism involved the interaction of two cytoskeletal systems: the actin-myosin cortex and the microtubules. Similar experiments with discoid epitheliocytes, cells with a different shape and cytoskeletal organization from fibroblasts, showed that they do not have cell-length-controlling mechanism.

MATERIALS AND METHODS

Cell cultures

The cells of two different morphological types were used in the experiments: fibroblasts (mouse embryo fibroblasts (MEF) and two human fibroblastic cell lines, AGO 1523 and M19); and epitheliocytes (rat liver epithelial cells IAR-2, canine MDCK line and bovine FBT). Cultures of mouse fibroblasts were prepared by trypsinization of 14-15 day embryos, secondary cultures were used for experiments after three to four days of growth. AGO 1523 fibroblasts derived from human foreskin (Heldin et al., 1981) were kindly provided by the National Institute of Aging Cell Culture Repository (Coriell Institute for Med. Research, Camden, NJ). Human fibroblast line M19 was derived from three-month-old embryos (Mironova et al., 1987). IAR-2 epithelial cells were derived from rat liver (Montesano et al., 1975). MDCK epitheliocytes were derived from canine kidney (Cereijido et al., 1978), and FBT epitheliocytes were derived from bovine trachea (Machatkova and Posposil, 1975).

The cells were grown in Dulbecco's modified Eagle's medium (Sigma), supplemented with 10% fetal calf serum (Gibco Biocult, Scotland), at 37°C in a humidified incubator supplied with 5% CO_2 in air. The cells were plated at an initial density of 100 cells/mm^2 on control substrates or 60 cells/mm^2 on the special substrates with narrow linear adhesive strips (see below), placed in 30-mm tissue culture dishes. Owing to low initial cell density, most cells (about 70%) on both substrates remained single after 24 hours, that is, they had no visible cell-cell contacts.

Colcemid (Demecolcine, Sigma; 0.2 µg/ml) or cytochalasin D (Sigma; 0.4 µg/ml) were added to the culture medium 19 hours after cell plating; the cells were incubated with the drugs for five hours before examination.

Substrates

For the usual planar substrate with isotropic adhesive surfaces, we used glass coverslips (Chance Propper Ltd., Smethwich, England). Substrates with narrow linear strips of adhesive surface were prepared as follows. The glass coverslips were coated with a thin non-adhesive layer of the biocompatible hydrogel, poly-2-hydroxyethyl

methacrylate (poly HEMA, Polysciences, USA) as described by Folkman and Moscona (Folkman and Moscona, 1978). Stock solution was made by dissolving of 6 g polyHEMA in 50 ml 95% ethanol. The mixture was turned slowly overnight at 37°C and then was centrifuged for 30 minutes at 2500 rpm to remove undissolved particles. This stock was diluted with 95% ethanol to a 10^{-1} solution, and was then pipetted onto the coverslip surface. The coverslips were dried on a level bench free of vibrations for 48 hours at 37°C. After the alcohol had evaporated, a thin film of optically clear polymer tightly bonded to the glass surface remained. Then the coverslips were heated at 120°C for two hours. Linear cuts through the coat to the coverslip surface were made with a razor blade. The width of the strips was 15±3 μm.

Differential interference contrast microscopy

The live 24-hour-old cultures were examined by video-enhanced microscopy using a Zeiss Axiophot microscope equipped with differential interference contrast (DIC) optic system with 40×0.7 Pl Fluotar objective and Hamamatsu Newvicon videocamera (Hamamatsu, Middlesex, NJ) For recording of images we used video tape recorder SVT-S3050P (Sony, Japan).

Morphometric analysis of cell shape

The outlines of DIC images of single cells, that is, of the cells without any cell-cell contacts, were used for morphometric analysis. The outlines of the 24-hour-old cells were entered into a PCAT computer by tracing on a digitizing tablet (Summasketch II, Summagraphics, UK). TRACER V1.0 software (Copyright Dr A. Brown) was used for entering and storing the cell outlines and for calculating their shape characteristics (Dunn and Brown, 1986). The four shape characteristics were: maximal cell length, cell area, dispersion and elongation indices.

Cell length was defined as the length of a direct line between two points at the maximal distance on a cell outline. Average values of length of groups of cells were calculated. They are designated in the text as the 'average length' of the cell population.

Dispersion and elongation indices were determined and calculated as described by Dunn and Brown (Dunn and Brown, 1986). The purpose of using elongation and dispersion to describe cell shape is that these have simple transformation properties and hence can detect fundamental transformations of cell shape. Elongation and dispersion describe two different aspects of how a shape differs from a circle. Both measure how much the total 'mass' of the shape extends away from its 'center of gravity' but elongation describes how much this extended mass can be reduced by compressing the shape along its long axis and dispersion describes how much extended mass remains.

Fluorescence microscopy

After 24 hours of culture, cells were washed with phosphate-buffered saline (PBS) containing 0.5 mM $CaCl_2$ and 3 mM $MgCl_2$, fixed in 3% paraformaldehyde in PBS for 10 minutes and permeabilized with 0.1% Triton X-100 for one minute at room temperature. For tubulin staining, the cells were fixed with methanol at –20°C for 10 minutes. F-actin was stained with TRITC-conjugated phalloidin (Sigma Chemical Co., St. Louis, MO). Tubulin was stained with an anti-α-tubulin mouse monoclonal IgG1 (clone DM1-A, Sigma). Paxillin was stained with an anti-paxillin mouse monoclonal antibody (Transduction Laboratories). For secondary antibodies, we used TRITC-conjugated goat anti-mouse IgG (Chemicon) and Oregon Green 488-conjugated goat anti-mouse IgG (Molecular Probes, USA). After several rinsings in PBS, preparations were mounted in buffered polyvinyl alcohol (Lennett, 1978). For fluorescence microscopy, an Aristoplan (Leitz, Germany) microscope equipped with epifluorescence illumination and a 50×1.0 Pl Fluotar water-immersion objective was used.
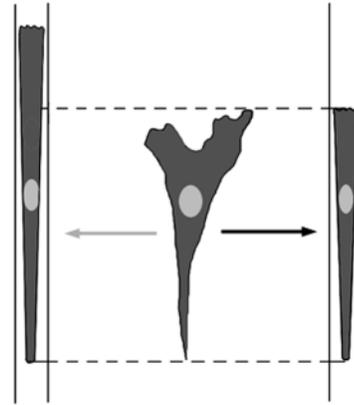

Fig. 1. Two alternative models of cell length control. Limitation of transverse cell spreading is compensated (gray arrow) or not compensated (black arrow) for by an increase in longitudinal spreading.

RESULTS

Fibroblasts

Results of experiments with three types of fibroblasts (AGO 1523, M19 and MEF) were essentially similar and will be described together. In preliminary experiments, we examined the dynamics of morphometric parameters of fibroblasts during spreading. These experiments showed (Fig. 2) that fibroblasts on both substrates reached their fully spread shape, with nearly constant average length and areas, after 24-36 hours of cultivation. 24 hours was used as standard time of cultivation and measurement in further experiments.

The fibroblasts on standard glass substrates had the usual polygonal bodies often with two or three cytoplasmic processes; they had stable lateral edges and wide lamellae at the ends of these processes (Fig. 3a; Fig. 4a). AGO cells were somewhat more elongated than MEFs. The organization of the cytoskeleton was normal: these cells had well developed microtubule arrays filling the central bodies and radiating into peripheral lamellas. They also had several actin bundles and several paxillin-positive elongated focal contacts in each lamella.

The fibroblasts on the strips were elongated and oriented along its length (Fig. 3b; Fig. 4b). These fibroblasts had more
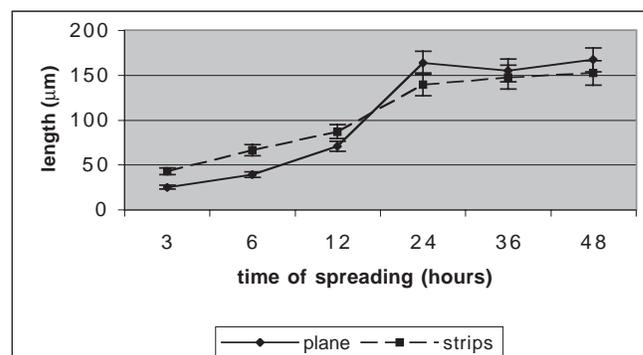

Fig. 2. Dynamics of average cell length changes in M19 fibroblasts on the usual planar substrates and on the narrow strips.
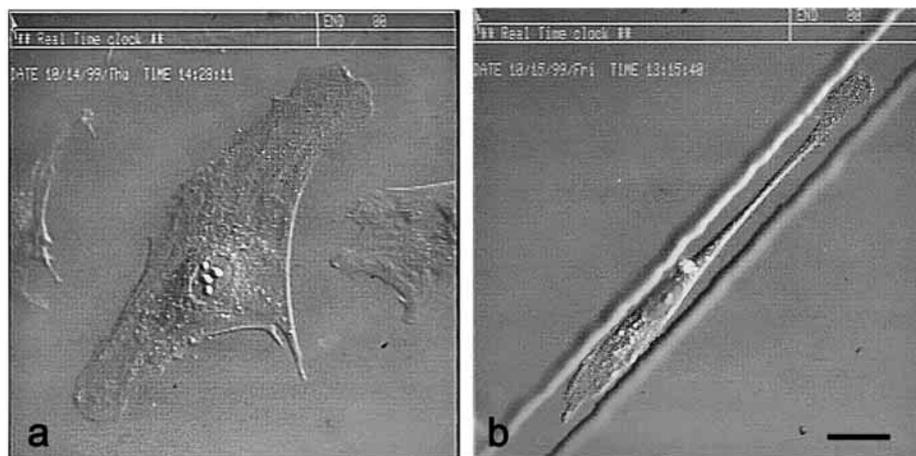
Fig. 3. Mouse embryo fibroblasts on the control substrate (a) or on the substrate with narrow adhesive strips (b). DIC microscopy. Bars, 20 μm.

refractive, thicker central parts of the body and two narrow peripheral processes with smooth lateral edges; often these processes had wider lamellae at the ends. These cells, and especially their processes, were not as wide as those on the planar substrate. Actin microfilament bundles were oriented along the strips; paxillin-positive focal adhesions were revealed at the actin bundle ends (Fig. 5).

Morphometric measurements revealed characteristic differences between the fibroblasts on the strips and the cells of the same origin on the standard substrate (Table 1). The cells on the strips had smaller areas, lower dispersion indices and higher elongation indices; all these differences were statistically significant. These differences, obviously, reflected decreased efficiency of spreading (decreased area) and absence of lateral processes (decreased dispersion indices) on the strips. Higher elongation indices (not to be confused with cell length) were also a result of a more regular cell shape and absence of lateral processes on the strips.

At the same time, the length of the fibroblasts on the strips was no higher than those on the standard substrate. These lengths were 240.4±10.29 μm and 265.6±10.65 μm in the experiments with AGO 1523 cells and 178.79±6.24 μm and 172.91±4.11 μm in those with MEF cells. The average length of M19 was even somewhat smaller on the strips than on the plane (124.2±5.9 μm and 149.4±5.0 μm). In other words, decreased transverse spreading across the strip was not compensated for by increased longitudinal spreading along the strip.

In order to find out more about the factors controlling fibroblast length, we tested the effects of two drugs affecting cytoskeletal systems: colcemid, which depolymerizes microtubules; and cytochalasin D, which destroys actin structures. Control tubulin staining showed that colcemid-treated fibroblasts had no cytoplasmic microtubules. These experiments showed that colcemid-treated fibroblasts had significantly decreased average cell length compared with untreated cells both on the standard substrates and on the strips (Table 1; Fig. 4b,c).

Cytochalasin-treated fibroblasts extended narrow cytoplasmic processes filled with microtubules and vimentin filaments; they had no lamellae at the ends of these processes. These cells had significantly increased average maximal lengths compared with control cells on both substrates (Table 1; Fig. 4b,d).

In a special experiment, M19 fibroblasts cultivated for 24 hours on the strips were re-seeded onto the standard substrate and examined after another 24 hours. These experiments showed that alterations caused by cultivation on the strips
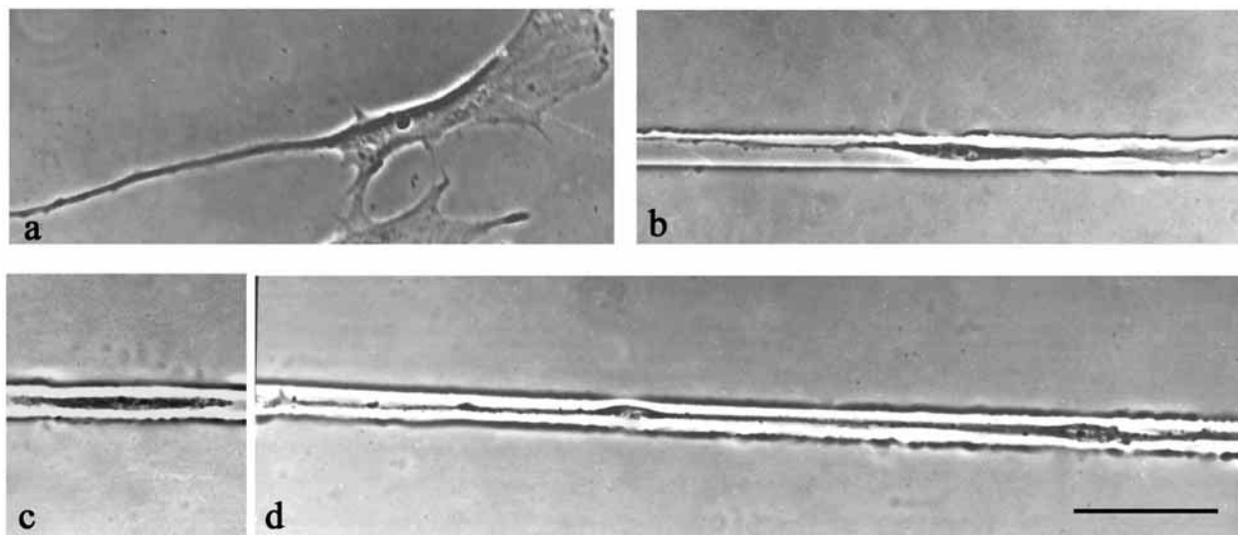

Fig. 4. AGO 1523 cells on the control substrate (a) or on the substrate with narrow adhesive strips (b, c and d). Control cells (a, b), short colcemid-treated cells (c) and long cytochalain-treated cells (d). Phase contrast microscopy. Bar, 30 μm.
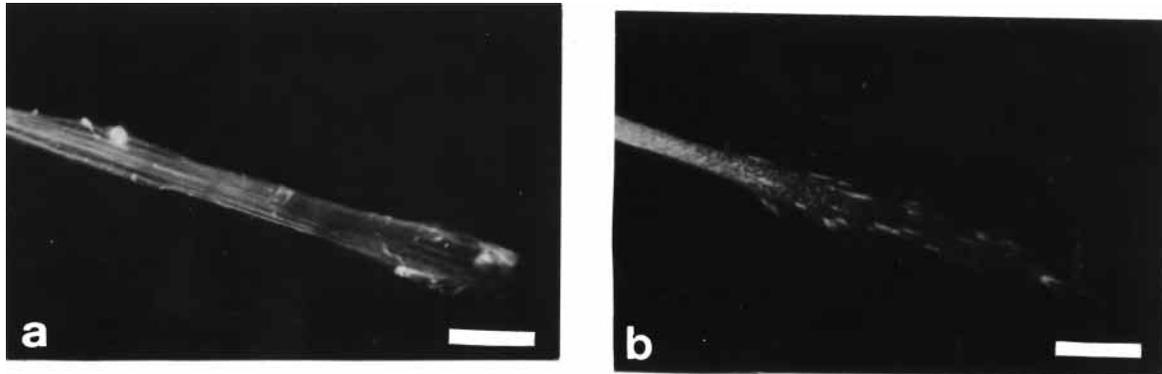
Fig. 5. AGO 1523 cells on the substrate with narrow adhesive strips. Fluorescent microscopy after staining for actin (a) and paxillin (b). Bar, 30 μm.

(area, elongation and dispersion) were reversible once transferred to the standard substrate (Table 3).

Epitheliocytes

Experiments with three types of epitheliocytes (IAR-2, FBT and MDCK) showed that all these cells had nearly discoid shape on the standard substrate at 24 hours (Fig. 6a). Accordingly, their dispersion and elongation indices were very low (Table 2). These cells had circular actin bundles at their periphery; microtubules radiated from central parts of the cell and acquired tangential orientation at the periphery proximal to the circular actin bundle. On the strips, all the epitheliocytes acquired ellipsoid shapes (Fig. 6b); the circular actin bundles were present along the whole cell periphery. Elongation and dispersion indices of the epitheliocytes on the strips were increased (Table 2). Average maximal length of the cells on the strips was considerably increased (Table 2); these increases were statistically significant.

The length of colcemid-treated epitheliocytes was shorter than those in untreated cells both on the standard substrate and on the strips (Table 2). The treatment with cytochalasin D did not cause any statistically significant changes in the lengths of epitheliocytes on both substrates (Table 2).

Additional experiments with MDCK epitheliocytes showed that morphometric changes observed on the strips were fully reversible: the cells re-seeded from the strips on the plane after

24 hours restored all the average parameters characteristic of epitheliocytes on this substrate (Table 3).

DISCUSSION

Fibroblasts have different mechanisms controlling longitudinal and transverse spreading

The data presented above show that fibroblasts on the strip, that is, on the substrate favouring 'unidimensional' spreading, do not exceed the average length characteristic of the cells spreading 'bidimensionally' on the standard substrate. This constancy of cell length was observed in the experiments with three types of fibroblastic cells independently obtained from different sources, having different histories and different lengths: in two different permanent lines of human fibroblasts and in secondary cultures of mouse embryo fibroblasts. Thus, constant length may be characteristic of cells with fibroblastic morphology.

In our experiments we assessed only the projection of cell shape on the substrate plane in two dimensions; the third dimension (cell height) was not measured. Possibly, the height was increased when the cell was squeezed on the strip. Further experiments are needed to reveal these possible changes. In any case, it can be concluded from our experiments that the average length of fibroblasts remains relatively constant regardless of other shape parameters on the strip. Of course, only the average

Table 1. Morphometric parameters of fibroblasts

	Standard glass substrate				Substrate with narrow strips			
	Length (μm)	Area (μm^2)	Dispersion	Elongation	Length (μm)	Area (μm^2)	Dispersion	Elongation
	HUMAN FIBROBLASTS AGO 1523							
Control	265.6±10.65	5482.6±412.20	0.93±0.073	2.54±0.094	240.4±10.29	1903.6±114.67	0.36±0.041	4.38±0.099
Colcemid	135.75±6.564	6385.9±618.80	0.18±0.027	0.97±0.082	116.7±6.94	1188.3±69.09	0.12±0.013	3.14±0.113
Cytochalasin	332.9±16.65	7169.5±736.99	1.27±0.130	1.83±0.197	365.3±22.01	2831.9±248.41	0.33±0.044	4.65±0.113
	MOUSE EMBRYO FIBROBLASTS							
Control	172.91±4.11	5264.0±238.5	0.57±0.03	1.64±0.05	178.79±6.24	1522.0±83.82	0.30±0.02	3.98±0.05
Colcemid	97.14±2.81	4311.1±269.6	0.11±0.02	0.53±0.06	112.0±3.84	1279.4±96.05	0.16±0.02	2.95±0.09
Cytochalasin	242.64±7.48	4618.8±337.6	1.24±0.07	2.11±0.09	250.26±11.9	2094.6±109.04	0.45±0.03	4.20±0.09
	HUMAN FIBROBLASTS M19							
Control	149.4±5.0	1818.6±88.1	0.46±0.04	2.82±0.12	124.2±5.9	1174.7±62.9	0.11±0.009	3.35±0.07

Mean ± standard errors are given. No less than 50 cell outlines from three experiments were used for the calculations in each group.

length characteristic of the whole population is preserved – individual cells may oscillate around the average in the course of their life history. For instance, moving fibroblasts detach and contract their tail processes from time to time, but later their anterior lamella spreads forward restoring their length (Chen, 1979; Dunn and Zicha, 1995). Another example of length oscillation is cell rounding during mitosis. However, mitotic cells at any given time form a very small part of fibroblastic populations in our cultures, so that they could not significantly influence the shape.

Our data show that there are two distinct factors controlling the degree of spreading of elongating fibroblast: factors controlling spreading in the direction of cell length and factors controlling spreading in other directions. We will designate them as longitudinal and transverse spreading, respectively. A decreased degree of transverse spreading on the strips is not compensated for by an increase in longitudinal spreading. Jokingly, one can compare this situation with actors controlling the form of the human body: factors controlling the linear size are quite different from those controlling transverse dimensions.

Possible factors controlling the longitudinal spreading

Factors controlling longitudinal spreading determine the distance between the active edge and central part of fibroblast body. It is likely that the degree of longitudinal spreading is determined by the balance between the action of microtubules stimulating the extension of lamellipods in a longitudinal direction and contractile action of the actin-myosin system in the same direction. This suggestion is supported by the experiments with cytoskeleton-specific drugs, colcemid and cytochalasin D.

Experiments with colcemid show that the microtubular system is essential for maintenance of fibroblast length: this length decreased considerably after depolymerization of microtubules both on the planar substrate and on the strip. These cells were devoid of microtubules and had a collapsed system of intermediate filaments. Their only functioning component of cytoskeleton was the actin-myosin cortex. One may suggest that the mechanism maintaining the length in this situation is based upon the dynamic equilibrium between two activities of this system: the extension of lamellipods based on polymerization of actin microfilaments (Svitkina and Borisy, 1999; Borisy and Svitkina, 2000) and contractile tension of
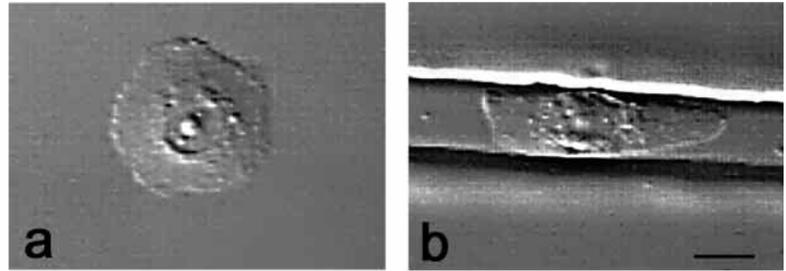

Fig. 6. FBT cells on the control substrate (a) or on the substrate with narrow adhesive strips (b). DIC microscopy. Bar 15 μm.

cortex (Cramer, 1999). Possibly, in the course of cell stretching caused by extension and attachment of lamellipods during spreading, centripetal tension increases progressively, until it becomes high enough to stop further elongation.

In contrast to colcemid, cytochalasin D significantly increased the length of fibroblasts both on the planar substrate and on the strips. Cytochalasin D is known to profoundly disorganize the actin system. However, the effects of this drug at the cellular level are far from simple. Spreading of fibroblasts in cytochalasin-containing medium was not completely inhibited during the first 24 hours, but morphology of this spreading is profoundly changed: instead of wide lamellae with microfilament bundles, the cells in this medium formed narrow non-contractile processes packed with microtubules and intermediate filaments and needle-like accumulations of short actin microfilaments at their tips. After 24 hours the growth of these processes stops and they become completely immobile (Bliokh et al., 1980). The ability of cytochalasin-treated cells to extend narrow processes suggests that actin polymerization at their ends is not fully inhibited.

The growth of the processes in cytochalasin-containing medium is almost completely inhibited by the addition of colcemid (Bliokh et al., 1980). Thus, microtubules are essential for promoting their growth. One possible mechanism for this promotion is through the microtubules providing some factors, such as Rac1 protein, that enhance actin polymerization and lamellipod formation (Waterman-Storer et al., 1999).

In the control fibroblast with an intact microtubular and actin-myosin cytoskeleton, these two systems interact to establish the characteristic length of the cell; this length was found to be intermediate between that of cytochalasin-treated cells and colcemid-treated cells. On the basis of the data discussed above, we suggest that this interaction includes

Table 2. Morphometric parameters of epitheliocytes

	Standard glass substrate				Substrate with narrow strips			
	Length (μm)	Area (μm^2)	Dispersion	Elongation	Length (μm)	Area (μm^2)	Dispersion	Elongation
				IAR2 EPITHELIOCYTES				
Control	63.6±2.2	2677.8±201.2	0.02±0.002	0.18±0.02	194.8±6.9	95.8±4.4	882.1±69.1	0.15±0.02
Colcemid	52.6±1.4	1733.5±97.0	0.02±0.002	0.18±0.02	160.0±4.4	77.5±1.8	851.5±33.6	0.06±0.01
Cytochalasin	55.6±2.8	1484.4±68.6	0.11±0.03	0.44±0.08	162.3±7.2	83.0±3.2	762.3±77.0	0.14±0.02
				MDCK EPITHELIOCYTES				
Control	21.1±0.3	296.5±8.3	0.08±0.02	0.18±0.02	44.8±1.6	419.2±16.2	0.03±0.004	1.67±0.07
				FBT EPITHELIOCYTES				
Control	42.08±0.7	1120.7±31.6	0.01±0.007	0.21±0.02	63.8±1.9	808.5±19.9	0.06±0.007	1.82±0.07

Mean ± standard errors are given. No less than 50 cell outlines from three experiments were used for the calculations in each group.

Table 3. Reversibility of morphometric parameters of fibroblasts and epitheliocytes

	Substrate with narrow strips				Standard glass substrate			
	Length (µm)	Area (µm^2)	Dispersion	Elongation	Length (µm)	Area (µm^2)	Dispersion	Elongation
	HUMAN FIBROBLASTS M19							
	124.5±5.0	1338.3±65.3	0.10±0.01	3.26±0.06	143.7±5.2	2675.6±159.9	0.34±0.04	2.37±0.10
	MDCK EPITHELIOCYTES							
	43.0±2.2	393.6±19.2	0.05±0.009	1.43±0.11	20.4±0.3	243.8±7.8	0.008±0.0009	0.24±0.02

Mean ± standard errors are given. No less than 50 cell outlines from three experiments were used for the calculations in each group.

The cells were grown on the strips for 24 hours, then some cells were measured (left half of the table); other cells were removed from the strips, re-seeded on the planar glass and measured after another 24 hours (right half of the table).

competition between two groups of cytoskeletal mechanisms – the centrifugal growth of microtubules promoting actin polymerization and extension of lamellipodia and the centripetally directed contractile tension of the actin-myosin system, which may counteract and eventually balance the centrifugal growth. Manifestations of the competition between microtubular growth and actin-myosin contractility are well known (Waterman-Storer and Salmon, 1999). One particularly well known manifestation of this competition was demonstrated in the experiments showing that actin-myosin contractility is increased by drug-induced depolymerization of microtubules (Danowski, 1989; Pletjushkina et al., 1998; Liu et al., 1998; Elbaum et al., 1999).

Possible factors controlling transverse spreading of fibroblasts

In contrast to longitudinal spreading (which determines cell length), transverse spreading determines the width of the active edge. The following hypothetical model of transverse spreading can be proposed. Attachment of each lamellipod at the active edge is followed by the formation of new lamellipods. These lamellipods are extended not only radially, that is, along the direction of longitudinal spreading, but also in perpendicular tangential directions. Attachment of these tangential lamellipods leads to gradual widening of the active cell edge. At least two factors are essential for this component of spreading. One obvious factor is the availability of sufficient area of adhesive substrate surface for attachment of transverse lamellipods. Another factor is the transversely directed contractility of the actin-myosin cortex. This contractility, caused by tangentially oriented actin microfilaments in the lamella, may limit the width of this lamella and of the active edge. Lower the transverse contractility, wider the edge. This contractility may be much higher in the central parts of the fibroblast body than in the leading lamella owing to the higher concentrations of myosin II (Verhovsky et al., 1999). Microtubules may increase transverse contractility, especially, in the central parts of the cell's body; for instance, as suggested by Waterman-Storer and Salmon microtubules in this part may release some Rho-activating factor activating myosin contractility (Waterman-Storer and Salmon, 1999). These differences in transverse contractility may be responsible for the fan-like shape of fibroblasts.

Epitheliocytes have no mechanism for the control of cell length

Experiments with three lines of epitheliocytes show that mechanisms controlling the shape of single discoid cells of this

tissue type are significantly different from those of fibroblasts. When squeezed on the linear strip epitheliocytes acquired an ellipsoid shape with a maximal length considerably higher than the diameter of discoid cells on the planar substrate. In the experiments with MDCK cells, these changes to the length proved to be reversible: these cells were restored to their original epithelial shape 24 hours after return from the strips to the planar substrate. Colcemid had no significant effects on the cell length on the strip. The shape of epitheliocytes on the strips remained smoothly elliptical but they did not form stable lateral edges or narrow 'waists' proximal to lamellae.

Thus, as expected, epitheliocytes did not undergo microtubule-dependent polarization even in the conditions where substrate shape maximally favoured elongation.

Obviously epitheliocytes have no control over their maximal length. During their spreading, longitudinal and transverse directions are not distinguished. Most probably, these cells form and attach lamellipods in all possible directions eventually acquiring a discoid or ellipsoid shape. It is well known that spatial patterns of microfilament bundles and of the microtubular system are quite different in epitheliocytes and in fibroblasts. These differences extend to microtubular dynamics in cytoplasts prepared from the cells of these two types (Rodionov et al., 1999). Probably, differences in control of cell length between epitheliocytes and fibroblasts are due to these differences in cytoskeletal organization. However, the exact factors responsible for these differences are not known.

One cannot also exclude that, beside balanced dynamic interaction of cytoskeletal structures, there are other factors controlling cell length in fibroblasts and these may be absent in epitheliocytes. For instance, anchorage modulations of protein synthesis on the planar substrate and on the strip may be involved in this control.

To summarize, the experiments presented in this paper show the existence of cell-length control and cell-specific differences in this control. Cultivation of cells on narrow linear strips of adhesive substrate may provide a convenient experimental system for furthering the analysis of these phenomena.

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