



Substrate Deformation Determines Actin Cytoskeleton Reorganization: A Mathematical Modeling and Experimental Study

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A mathematical model has been developed to define the relationship between the actin cytoskeleton reorganization of a cell and substrate deformation acting on the cell. The model is based on the following major assumptions: (a) normal substrate strain, not the shear substrate strain, determines the actin cytoskeleton reorganization; (b) the normal substrate strain is transmitted to individual actin filaments; (c) each actin filament has a basal strain energy (BSE) when the cell adheres to the substrate without stretching; and (d) the actin filaments undergo disassembly when their strain energies are decreased to zero or increased to twice their BSEs. The resulting model predicts that the actin filaments are formed in the direction where their BSEs are minimally altered. This direction is therefore the one without normal substrate strain. The prediction was confirmed by experiments conducted on both fibroblasts and endothelial cells. The present model may be relevant for understanding better the effects of mechanical stimuli on the cells.

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Introduction

Actin cytoskeleton plays an important role in cell shape, motility and a variety of cellular functions (Shay, 1986; Bershadsky & Vasiliev, 1988; Janmey, 1991; Ingber, 1991). The actin cytoskeleton is composed of actin filaments, which constantly undergo dis-assembly and re-assembly. Furthermore, cell culture studies have shown that when cells were grown on a substrate subjected to mechanical stretching, the actin cytoskeletons of the cells were reorganized into bundles of actin filaments (i.e. stress fibers)

oriented towards a specific direction (Dartsch & Hammerle, 1986; Dartsch & Betz, 1989; Iba & Sumpio, 1991). These studies suggest that the actin cytoskeleton reorganization is tightly coupled with the substrate deformation. However, the precise relationship between the actin cytoskeleton reorganization and the substrate deformation has not been defined.

In cell culture studies, a planar artificial substrate (e.g. a silicone membrane), is usually used for growing cells. When cells are grown on the substrate, they adhere to it through focal adhesions along the basal cell surfaces (BurrIDGE *et al.*, 1988). The focal adhesions are discrete regions, which are composed of adhesive proteins such as fibronectin. Like short, discrete columns, the focal adhesions support the cell and link with the underlying substrate (Opas, 1987).

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Besides the connection with the substrate, focal adhesions are also associated, via integrins, with termini of actin filaments, which are prominent in many cultured cells (Heath & Dunn, 1978). The association provides a linkage between the substrate and the actin filaments. Through the linkage, on the one hand, inherent tensions in actin filaments can be exerted on the substrate (Harris *et al.*, 1980). On the other hand, substrate deformation can be transmitted to actin filaments (Ingber, 1991).

Substrate deformation can be characterized by normal and shear strains (Fung, 1994). Normal strain describes the length changes of a square element on the substrate surface, whereas shear strain defines change in the shape of the square. Therefore, normal substrate strain is probably the only strain that can be transmitted to the actin filaments, since it can change the length of an actin filament that anchors at its two ends to focal adhesions on the underlying substrate.

With the above background information, a mathematical model will be presented to define the actin cytoskeleton reorganization of a cell in response to substrate deformation. The experiments, which have been conducted to test the model, will also be described.

Mathematical Model

MODEL FORMULATION

We assume that (a) each actin filament of a cell on a substrate without deformation has a basal strain energy (BSE); (b) only normal substrate strain is transmitted to individual actin filaments of the cell; (c) the actin filaments cannot bear compression and (d) the actin filaments undergo disassembly if their strain energies are changed to zero or to a value greater than twice their BSEs. In addition, to simplify the model formulation, we assume that individual actin filaments are linearly elastic. Using this assumption and assumption (a) above, we obtain the BSE of an actin filament

$$E_b = k\delta^2/2, \quad (1)$$

where k is the spring constant of the filament, and δ is its initial elongation due to the inherent tension within the filament (Ingber, 1991).

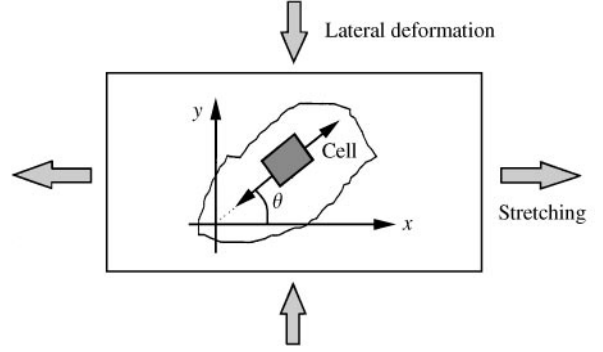


FIG. 1. Uniaxial stretching applied to the substrate to which the cell adheres. The applied stretch is ε_x , and the lateral deformation is ε_y , with $\varepsilon_y = -v\varepsilon_x$, where v is Poisson ratio of the substrate. The strain field produced by the stretch on the substrate surface can be described by normal strain (indicated by arrows) and shear strain (not shown), both describing length and shape changes of a small square on the substrate surface, respectively. See also Fig. 2, which illustrates how normal substrate strain changes with the orientation θ .

Without loss of generality, consider that uniaxial stretching is applied to a substrate (Fig. 1). From strain theory (Fung, 1994), the normal substrate strain in any direction θ with respect to the stretching direction is determined by

$$\varepsilon = \varepsilon_x \cos^2 \theta + \varepsilon_y \sin^2 \theta, \quad (2)$$

where ε_x is the applied stretch and ε_y , in the present case, is the lateral substrate deformation due to the Poisson effect (Fung, 1994).

Using the Poisson ratio definition, $v = -\varepsilon_y/\varepsilon_x$, and substituting it into eqn (2), we obtain

$$\varepsilon = -v\varepsilon_x + (1 + v)\varepsilon_x \cos^2 \theta. \quad (3)$$

The change of normal strain with respect to orientation θ is illustrated in Fig. 2.

According to assumption (b), the normal substrate strain is transmitted to the actin filament, which gives

$$\varepsilon_{af} = \alpha\varepsilon, \quad (4)$$

where ε_{af} denotes the actin filament strain, and α is the coefficient that represents the percentage of the normal substrate strain that is transmitted to the actin filament.

Two cases can be considered for the coefficient α . The first is that as $\varepsilon \geq 0$ (i.e. the tensile substrate strain), we let $\alpha = \alpha_t$. The second is that as $\varepsilon < 0$ (i.e. the compressive substrate strain), we denote $\alpha = \alpha_c$. Further, we assume that $\alpha_t < \alpha_c$, on the reasoning that under a tensile strain the slippage between two focal adhesions is more likely to occur than under a compressive strain,

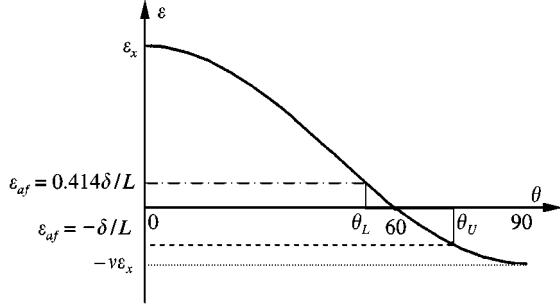


FIG. 2. Illustration of the change of the normal substrate strain with orientation θ . Note that the strain decreases monotonically with θ , and the orientation with zero normal strain is determined by $\cos^{-1}[v/(1+v)]^{1/2}$, where v is equal to 0.35 for the silicone substrate used in the present experiments. Two orientation limits, θ_L and θ_V , are also illustrated.

which shortens the actin filament to its less deformed state, and hence smaller forces would act on the focal adhesions. Previous studies also support the fact that tensile substrate strains are not completely transmitted to the cells (Winston *et al.*, 1989; Wirtz & Dobbs, 1990).

Now, consider the change in strain energy of the actin filament due to the actin filament strain, ε_{af} . It can be shown that

$$\Delta E = kL^2\varepsilon_{af}^2/2 + kL\delta\varepsilon_{af}, \quad (5)$$

where L is the length of the actin filament without substrate deformation (Fig. 3).

According to assumption (d) above, we have $\Delta E = E_b$ and $\Delta E = -E_b$. Using eqns (1) and (5), we have

$$L^2\varepsilon_{af}^2 + 2L\delta\varepsilon_{af} - \delta^2 = 0, \quad (6a)$$

$$L^2\varepsilon_{af}^2 + 2L\delta\varepsilon_{af} + \delta^2 = 0. \quad (6b)$$

Equation (6a), a second-order equation with the unknown ε_{af} , has two roots, $\varepsilon_{af} = 0.414\delta/L$, and $\varepsilon_{af} = -2.414\delta/L$. The second root is discarded

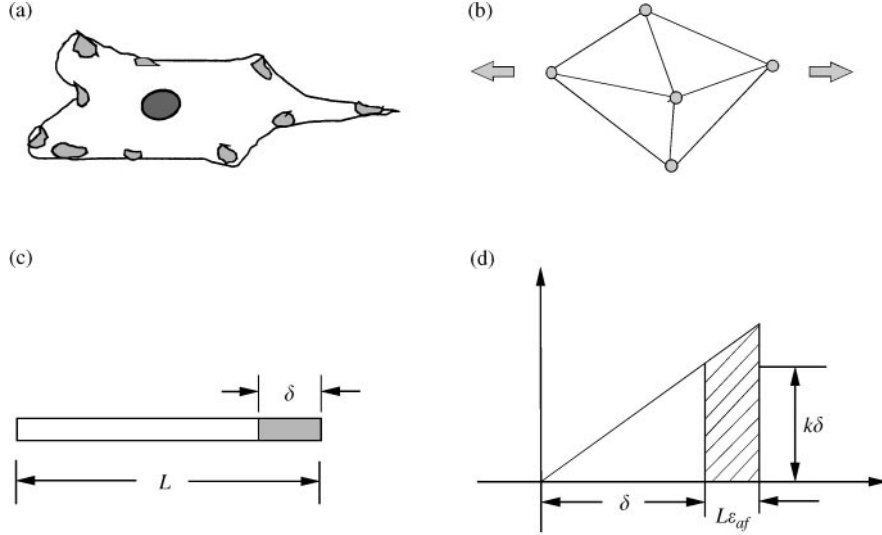


FIG. 3. (a) Illustration of a cell adherent to the substrate. The spots represent focal adhesions through which the actin filaments link with the substrate so that substrate deformation is transmitted to the filaments. (b) Network of actin filaments. The nodes represent the focal adhesions, and the arrows indicate that the filaments are subjected to stretching. (c) Each actin filament is represented by an elastic string, with spring constant k . When the cell resides in a substrate without stretching, the actin filament has a total length L , which includes its neutral length L_0 without internal force, and elongation δ . Note that $L = L_0 + \delta$. (d) Schematic of change in the basal strain energy (BSE) of an actin filament under stretching. The BSE of the actin filament is $k\delta^2/2$ (triangle without shadow), and the BSE increment is $kL^2\varepsilon_{af}^2/2 + kL\delta\varepsilon_{af}$ (shadowed area), where ε_{af} is the actin filament strain due to normal substrate strain transmitted to the filament.

because it indicates that the actin filament would be compressed, which contradicts assumption (c). Equation (6b) has one root, i.e. $\varepsilon_{af} = -\delta/L$.

Substituting $\varepsilon_{af} = 0.414\delta/L$ and $\varepsilon_{af} = -\delta/L$ into eqns (3) and (4), we obtain

$$\theta_L = \cos^{-1}\{v/(1+v) + 0.414/[(1+v)\alpha_t\varepsilon_x]\delta/L\}^{1/2}, \quad (7)$$

$$\theta_U = \cos^{-1}\{v/(1+v) - 1/[(1+v)\alpha_c\varepsilon_x]\delta/L\}^{1/2}, \quad (8)$$

and since $\alpha_t < \alpha_c$, as explained earlier, for simplification we assume $\alpha_t = 0.414\alpha_c$. Finally, letting $T = \delta/(\alpha_c L)$, we then have

$$\theta_L = \cos^{-1}\{v/(1+v) + 1/[(1+v)\varepsilon_x]T\}^{1/2}, \quad (9)$$

$$\theta_U = \cos^{-1}\{v/(1+v) - 1/[(1+v)\varepsilon_x]T\}^{1/2}, \quad (10)$$

where θ_L and θ_U denote the lower and upper orientation limits, respectively, of an actin filament under uniaxial stretching (see Fig. 2 for the illustration of the θ_L and θ_U). The lower and upper orientation limits mean that within their range, i.e. (θ_L, θ_U) , the strain energy (SE) of an actin filament is greater than zero but less than twice the BSE ($0 < SE < SE_b$). And if the orientation of the actin filament is equal to θ_L , SE is equal to twice the BSE; whereas if the orientation is equal to θ_U , SE is equal to zero.

PARAMETER AND MODEL PREDICTION

Three cases exist for the parameter T in eqns (9) and (10). (1) If $T > \varepsilon_x$, no solutions can be obtained from eqns (9) and (10). This means that the altered strain energy of an actin filament, due to the applied stretch, always lies between zero and twice its BSE, independent of the orientation angle θ . Therefore, the filament can form and orient in any direction, which means $\theta_L = 0$, and $\theta_U = 90^\circ$; (2) if $v\varepsilon_x < T < \varepsilon_x$, θ_L is determined by eqn (9), and $\theta_U = 90^\circ$; (3) if $0 < T < v\varepsilon_x$, both θ_L and θ_U are determined by eqns (9) and (10). The influence of the T value on θ_L and θ_U is illustrated in Fig. 4. As a special case, if T approaches zero, then according to the definition for T , δ must approximate zero. From eqn (6), ε_{af} must be zero. Substituting this into eqns (3) and (4) yields $\theta = \cos^{-1}[v/(1+v)]^{1/2}$. This means that when the initial elongation of an actin filament, δ , is sufficiently small, the filament will orient exactly in the direction with zero normal strain. The same conclusion can be reached by letting δ in eqns (7) and (8) approach zero.

When the parameter T is determined, eqns (9) and (10) indicate that the orientation limits, θ_L and θ_U , vary only with the applied stretch ε_x , because the first terms in the two equations are constant for a specific substrate with a fixed Poisson ratio v . Thus, for a large stretch ε_x , both orientation limits approximate a fixed angle, determined by $\cos^{-1}[v/(1+v)]^{1/2}$ (Fig. 5).

Note that in deriving eqns (9) and (10), no assumptions are made regarding the pattern of

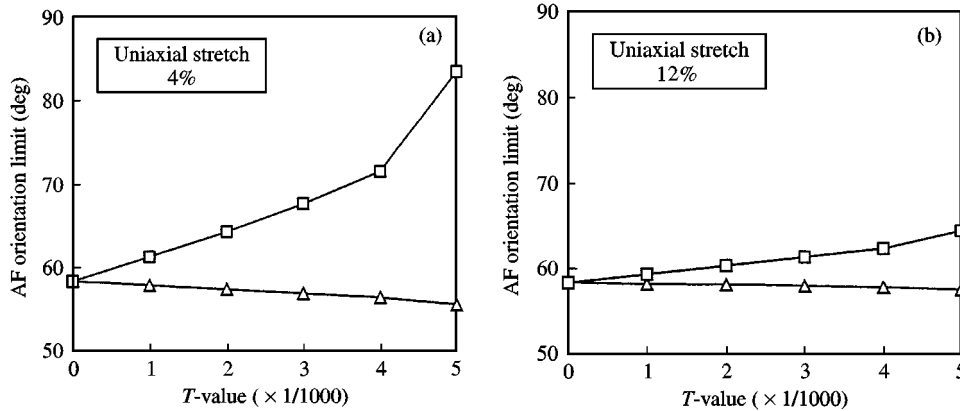


FIG. 4. The influence of the parameter T ($= \delta/(\alpha_c L)$) on the range between two orientation limits of the actin filament (AF). It is shown here that under a small stretch the range of the two orientation limits is wider than under a large stretch. This indicates that the parameter T is more influential under the small stretch.

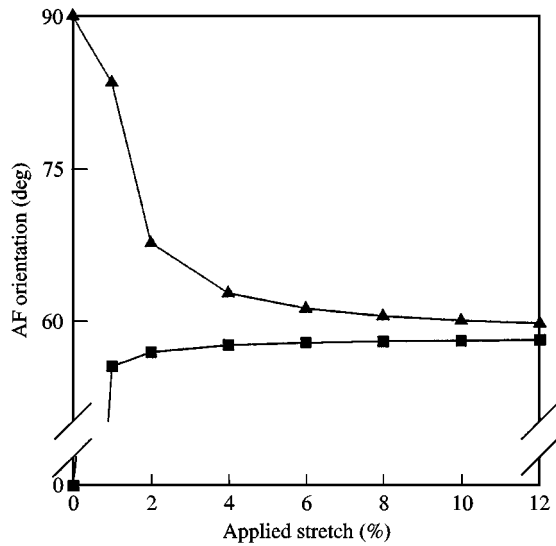


FIG. 5. The orientation limits of the actin filament (AF) for uniaxial stretching. Both lower (squares) and upper (triangles) orientation limits decrease with increased stretch. For a stretch greater than 10%, the orientation limits approximate about 60° , which was obtained from $\theta = \cos^{-1}[\nu/(1 + \nu)]^{1/2}$ for $\nu = 0.35$. In the figure, the parameter T (see text) is taken as 0.15%. Also, note that without stretching the two orientation limits are 0 and 90° , respectively. Zero degrees represents the stretching direction and 90° is its perpendicular. —■—, lower limit; —▲—, upper limit.

the actin cytoskeleton before stretching. Thus, the model predicts that under a large stretch, any pattern of actin cytoskeleton will be reorganized into stress fibers that orient towards a fixed direction. This direction has zero normal strain and is only dependent on the substrate material used. Previous studies have shown that a 10% stretch is large enough to induce actin cytoskeleton reorganization (Dartsch & Betz, 1989; Iba & Sumpio, 1991).

To test the present model, stretching experiments on human dermal fibroblasts and endothelial cells were conducted. The experiments are described in the following section.

Experimental Validation

In the first experiment, human dermal fibroblasts were grown on silicone substrates coated with $10 \mu\text{g ml}^{-1}$ ProNectin-F (ProNectin-Polymer Technologies, Inc., San Diego, CA). The cells were subjected to 12% uniaxial stretching for 24 hr. Immediately after the stretching, the cells

were fixed and stained with rhodamine phalloidin (Molecular Probes, Inc.). The stained cells were observed and photographed on a fluorescence microscope.

In the second experiment, human endothelial cells were grown in microgrooves made on silicone membranes coated with the same dose of ProNectin-F. The cells were subjected to 10% uniaxial stretching in the groove direction for 3 hr. After the stretching, again the cells were stained and photographed on a fluorescence microscope.

Figure 6(a) shows that after stretching the actin filaments of the fibroblasts uniformly oriented around 60° about the stretching direction. In contrast, without stretching, actin cytoskeletons of the cells had variable patterns (data not shown). Figure 6(c) shows that the endothelial cells in the grooves, which were parallel to the stretching direction, were devoid of actin filaments. The fact that the cells in the grooves not subjected to stretching had dense stress fibers parallel to the groove direction (data not shown), and the actin filaments were absent after stretching, suggesting that the actin filaments were disassembled but could not be re-formed in the stretching direction. Moreover, the cells outside the grooves had stress fibers oriented around 60° about the stretching direction. Since the silicone material had a Poisson ratio of 0.35, and because $\theta = \cos^{-1}[\nu/(1 + \nu)]^{1/2}$, the direction with zero normal strain is therefore about 60° with respect to stretching direction (see Figs 4 and 5). Thus, the experiments confirmed the model prediction that the actin cytoskeleton of a cell would be reorganized into stress fibers oriented around a specific direction with the least substrate deformation.

Discussion

In this study, a mathematical model has been established to define the actin cytoskeleton reorganization of a cell in response to substrate deformation. The model predicts that the actin cytoskeleton of a cell subjected to substrate deformation will be reorganized in such a way that the actin filaments orient in a direction with minimal alteration of their basal strain energies, and thus the direction most probably with the

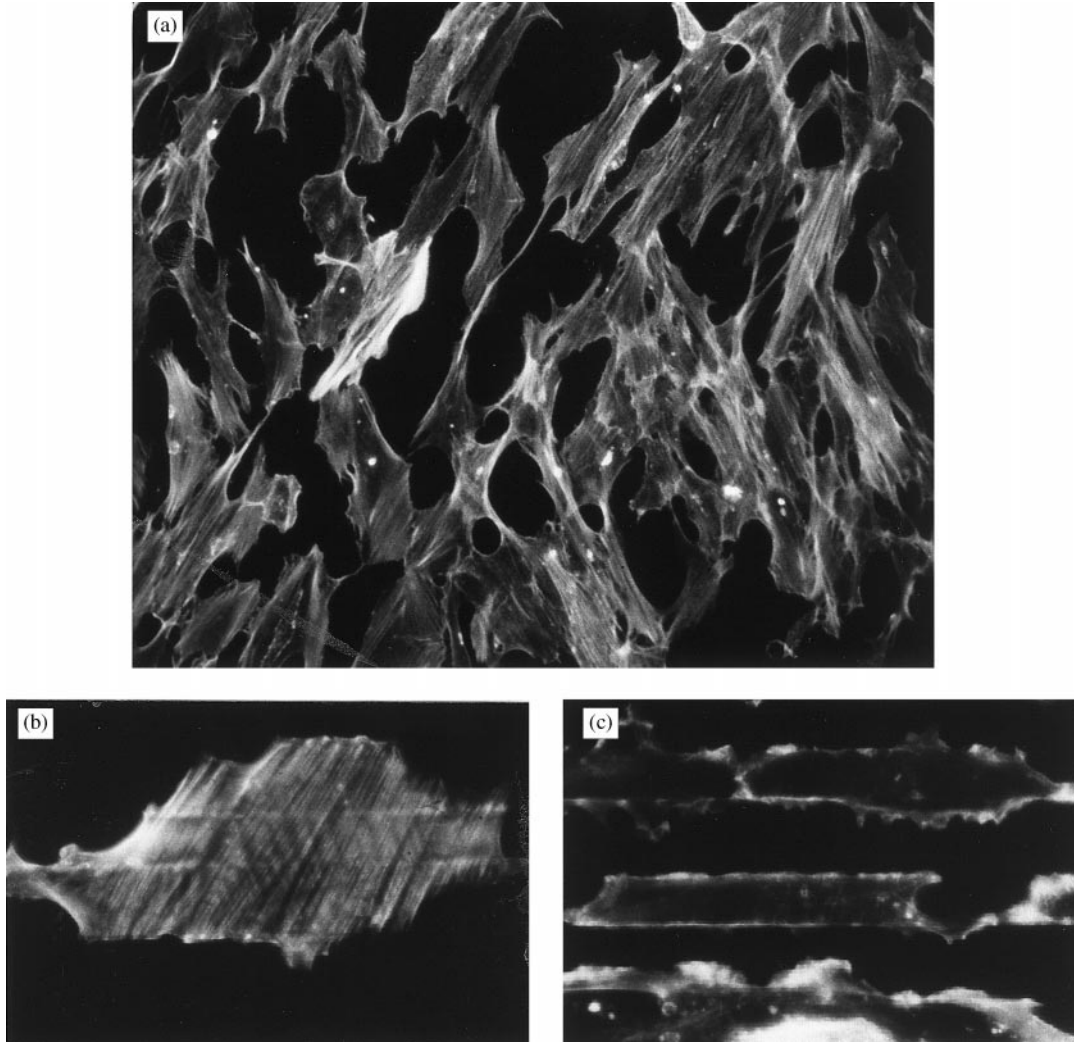


FIG. 6. The reorganized actin cytoskeleton of the human skin fibroblasts (a) and endothelial cells (b, c) after cyclic stretching. The fibroblasts and endothelial cells were subjected to uniaxial stretching, 12 and 10%, respectively. The cells were stained with rhodamine phalloidin. It is shown that the actin filaments uniformly oriented around 60° with respect to the stretching direction (horizontal). Note that the endothelial cells in the microgrooves (c) had no detectable actin filaments, but the cells outside the microgrooves had dense stress fibers oriented around 60° with respect to the groove direction, i.e. the stretching direction (b).

least substrate deformation. The prediction was confirmed by stretching experiments on human dermal fibroblasts and endothelial cells.

The present model can explain the notion of strain threshold introduced in the cell orientation response to mechanical stretching (Wang *et al.*, 1995). It can be shown that the strain threshold is equivalent to the parameter T , which is equal to $\delta/(\alpha_c L)$. Therefore, it becomes clear that the strain threshold defines the combined factors of the initial strain state of the actin filament (i.e. δ/L), and the extent of the transmission of the normal

substrate strain to the actin filament. Thus, the present model clarifies that the cell strain threshold is related to the responsiveness of the actin cytoskeleton to mechanical stretching. In addition, the strain states of actin filaments, and therefore the parameter T , may differ within a cell and also between cells. An effective approach to handling the complicated situation is to consider the parameter T as a random variable that follows a normal distribution—this is exactly how the cell strain threshold was defined previously (Wang *et al.*, 1995).

The present model considers only uniaxial stretching. But it can be readily extended to strip biaxial stretching, which is also one-directional stretching but without lateral deformation. Under this type of stretching, only the second terms remain in eqns (9) and (10). Thus, with a large stretch, the actin cytoskeleton will be reorganized to align around perpendicular to the stretching direction. The prediction was supported by the fact that the actin filaments of the endothelial cells at the periphery of a cyclically deformed circular membrane align along the periphery, along which the substrate strain is zero (Iba & Sumpio, 1991).

As a special case, the present model can also apply to biaxial stretching, a type of stretching often used in cell stretching studies (Winston *et al.*, 1989; Hung & Williams, 1994). Accordingly, the model predicts that if the substrate deformation is large enough, new actin filaments will not form in the substrate plane, because no direction in the substrate plane is without deformation. The only direction in which the actin filaments can be formed is the one perpendicular to the substrate plane. This prediction has been confirmed by the observation that after 10% equal-biaxial stretching, the actin cytoskeleton of the endothelial cell formed a three-dimensional structure protruding from the substrate (unpublished observations).

Not only can the model apply to the conditions of uniaxial, strip biaxial, and biaxial stretching, but it can also apply to the condition of compressive strain. Under this condition, similarly, the model predicts that actin cytoskeleton will be reorganized so that the resulting actin filaments are oriented in the zero-normal-strain direction. In other words, both tensile and compressive strains are unfavorable signals that cause reorganization of the actin cytoskeleton. This prediction was supported by the result from uniaxial stretching. Under this type of stretching, the cells were subjected to tensile strain in the stretching direction but to compressive strain in its perpendicular. The experimental results showed that the newly formed actin filaments avoided the perpendicular direction, in addition to the stretching direction (Fig. 6). A more strict test will be an experiment, where equal-biaxial compression is applied to the cells. Under this

condition, the model predicts that the actin filaments will not be formed in the substrate plane if the compression is large enough. This prediction is experimentally testable.

The central assumption in the present model is that individual actin filaments of a cell are considered to be pre-stretched, extensible and linearly elastic. The notion of pre-stretch is similar to that of inherent tension in the tensegrity model (Ingber, 1993). In addition, the introduction of extensibility and linear elasticity is based on the previous finding that single actin filaments show extensibility and elasticity (Kojima *et al.*, 1994). However, the assumption of linear elasticity of the actin filaments in the present model should be considered to be a first order of approximation, since actin filaments also show viscoelastic behavior (Janmey, 1991).

The present model indicates that if actin filaments of a cell are subjected to a large strain, the actin filaments will be under disassembly. However, the mechanism of the actin filament disassembly by substrate strain is not clear. One possibility is that the focal adhesions, due to the tension or compression acting on them, disassociate themselves from anchored actin filaments. But this seems unlikely, because if so, the actin filaments will not be reformed in the least normal strain direction, since the focal adhesions that are connected to newly formed actin filaments are still subjected to tension or compression. Another possibility is that the actin filaments are *directly* broken by the substrate deformation transmitted to the filaments. But there is little evidence for this. A more likely mechanism is that the over- or less-deformed actin filaments are more susceptible to action of molecules which are activated and/or produced by substrate strain and which can break the filaments. Candidates may include reactive oxygen species such as hydrogen peroxide. It has been shown that exogenous hydrogen peroxide remodels the actin cytoskeleton (Huot *et al.*, 1996) and mechanical stretching can induce the production of hydrogen peroxide (Howard *et al.*, 1997). Also, gelsolin fragments cleaved by caspases have been shown to have the ability to disrupt the actin filaments (Kothakota *et al.*, 1997). Therefore, if the caspases can be shown to be activated by substrate strain, they could play a role in "breaking" the actin

filaments. Other candidates may include the small GTP-binding protein Rho, which plays a crucial role in regulation of stress fiber assembly (Ridley & Hall, 1992; Chrzanowska-Wodnicka & Burridge, 1996; see also reviews by Craig & Johnson, 1996; Zigmond, 1996). Under the condition of cyclic mechanical stretching, a previous study has shown that ADP-ribosylation of Rho with C3 transferase decreased the formation of actin filaments (Yano *et al.*, 1996), suggesting that Rho is involved in the disassembly of actin filaments under mechanical stretching. Regardless of what the mechanisms are, however, the present study supports the notion that the actin filaments must be stretched or compressed to a certain degree before they can break down.

To define the relationship between the cellular structure and functions, the tensegrity model has been developed (Ingber, 1991). The model can explain many cellular behaviors and functions, such as cell spreading, motility, and mechanical signal transduction (Wang *et al.*, 1993; Ingber, 1993; Stamenovic *et al.*, 1996). The key assumption of this model is that continuous tension exists in the tensile structure (i.e. actin filaments and intermediate filaments). As an alternative viewpoint, the present model considers that discrete basal strain energies exist in *individual* actin filaments. In other words, the basal strain energy is not necessarily the same for all actin filaments, partially because no continuous tension in the actin filaments is assumed in the present model. Furthermore, the tensegrity model considers that overall change in tension results in cytoskeletal change, whereas the present model specifies that the change in the basal strain energies in individual actin filaments leads to the reorganization of actin cytoskeleton.

It has been well recognized that actin cytoskeleton plays a variety of roles in cellular functions such as proliferation and differentiation, and that mechanical stretching can alter the cellular functions. But how stretching alters the cellular functions is not completely understood. One possibility is that the pattern of the actin cytoskeleton itself represents a metabolic state of a cell. Evidence that supports this notion is that the enzyme, procollagenase, is induced only after reorganization of the actin cytoskeleton

(Unemori & Werb, 1986). Thus, it seems likely that the altered pattern of the actin cytoskeleton by substrate deformation leads to another metabolic state of the cell. This means the cellular functions are now explicitly related to the substrate deformation, since the present model shows the resulting pattern of the actin cytoskeleton is dictated by applied substrate deformation.

In summary, a mathematical model which relates the actin cytoskeleton reorganization to applied substrate deformation has been established. The model predicts that substrate deformation determines the resulting pattern of actin cytoskeleton, and this prediction was verified experimentally. The present model may help us understand better how mechanical stimuli alter cellular functions.

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