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Control of Cellular Morphology by Mechanical Factors

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Abstract. — This short review deals with the influence of mechanical factors on eucaryotic cell morphology and structure. We classify these factors into two types: i) external forces (*e.g.* gravitational forces or hemodynamic stresses), which when applied experimentally allow characterization of passive mechanical properties; and ii) internal forces, *e.g.* generated by molecular motors or polymerization processes. Perturbation of one or more of these forces induces significant changes in cell shape, cytoskeleton and pericellular matrix organization. We describe these phenomena in view of current models.

Résumé. — Cette brève revue traite de l'influence des facteurs mécaniques sur la morphologie et la structure des cellules eucaryotes. Nous classifions ces facteurs en deux catégories : i) les forces externes (par exemple les forces de gravitation et les contraintes hémodynamiques) qui, imposées *in vitro*, permettent de caractériser les propriétés mécaniques passives ; et ii) les forces internes, par exemple celles générées par les moteurs moléculaires ou les processus de polymérisation. La perturbation de l'une ou l'autre de ces forces provoque des changements significatifs de la morphologie cellulaire ainsi que de l'organisation du cytosquelette et de la matrice péricellulaire. Nous décrivons ces phénomènes en fonction de modèles existants.

Introduction

Mammalian cells are objects of microscopic dimensions (diameter on the order of 10 μm) which consist of interconnected protein polymers (the cytoskeleton), lipid membranes, and various organelles (among which the nucleus) [1]. They can move, *e.g.* spread, round up, elongate, extend processes, or locomote [2], such movements being essential to normal physiology (*e.g.* embryo shaping and neural development), repair situations (*e.g.* wound healing), and pathological processes (*e.g.* cancer metastasis). Cell movements are regulated by both chemical and physical factors. Shadowed for a while by enormous progresses made in molecular biology [3], the study of cell mechanics is taking a new impulse thanks to powerful techniques (*e.g.* routine cell culture, piezoelectric micromanipulation, videomicroscopy) which allow researchers to perform accurate mechanical tests on living cells [4]. In this brief review, we summarize data emphasizing the role of mechanical factors in the control of cellular morphology. We distinguish

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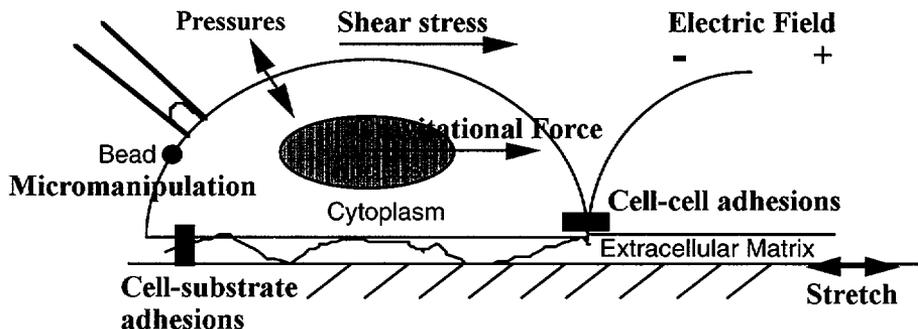


Fig. 1. — Summary diagram of the various external forces able to modulate cell morphology.

external forces, usually resulting in passive cell deformation, from internal, actively-generated forces, *i.e.* associated with chemical energy transduction.

1. External Forces

All cells in a living organism, not only those specialized in pressure wave or acceleration sensing (*e.g.* the hair cells of the ear), experience varying stress fields. As we shall see from various *in vitro* investigations (Fig. 1), these stresses are resisted by cohesive forces in the cell, carried in particular by the three major components of the cytoskeleton (actin microfilaments, microtubules and intermediate filaments), and result in dynamic cellular responses at the structural and functional levels.

1.1. GRAVITATIONAL FORCES. — Gravity plays a role in various cellular processes including cytoplasmic streaming in plant cells [5] and mammalian cell proliferation [6]. Experiments using low-speed centrifugation have allowed characterization of cytoplasm viscosity [7] and post-plating cell-substrate adhesion stresses [8]. In order to assess whether gravitational fields could directly influence cell morphology and dictate pattern formation in their cytoskeleton, we exposed epithelial cells to high centrifugal accelerations tangential to their substrate [9]. Three regimes describe the cellular response: i) cell morphology and density remain unaltered at forces below a threshold of about 10^{-7} N; ii) between this critical force and a second threshold of about 1.5×10^{-7} N, the number of adherent cells decreases exponentially with time and acceleration, with no alteration of cell morphology: this behavior can be modeled by a constant probability of detaching and by an exponential distribution of cell-substrate adhesive forces (see Paragr. 1.3.3.1 for additional details on these contact forces); iii) past the second threshold, cells that are still adherent exhibit elongated morphologies (with concomitant deformation of microfilaments and microtubules), the degree of elongation increasing linearly with the force. It is likely that the centrifugal force exerted on the cellular components of high mass density (*e.g.* the nucleus) is transmitted to cell-substrate adhesion sites (*e.g.* focal contacts) [10, 11] through the interconnected cell structure [12]: cell detachment corresponds to rupture at these contacts.

1.2. ELECTRIC FORCES. — Both fibroblasts and neutrophils exhibit directed migration (galvanotaxis) when exposed to continuous electric fields on the order of 1 V cm^{-1} , which can be interpreted whether chemically, in terms of a lateral redistribution of charged plasma membrane

glycoproteins involved in cell-substrate adhesion [13] or physically, in terms of two independent motors [14]. In addition, fibroblasts form protrusions in response to application of a local alternating current, due to different conductivities of the cytoplasm and the extracellular solution [15].

1.3. CONTACT FORCES

1.3.1. Hemodynamic Forces. — The pulsatile flow of blood in vessels imposes three types of mechanical constraints on endothelial cells: i) a hydrostatic pressure, acting normally to the vessel wall and comprised between 80 – 120 mm Hg ($1.0 - 1.6 \times 10^4 \text{ N m}^{-2}$) in human arteries; ii) a frictional shear stress, which acts tangentially and ranges from 1 – 100 dynes cm^{-2} ($0.1 - 10 \text{ N m}^{-2}$), depending on the location in the vasculature; and iii) a cyclic stretch acting circumferentially (its peak value is about 10% of the vessel diameter). Numerous *in vivo* and *in vitro* studies have shown that these hemodynamic stresses can affect endothelial cell morphology and function (for a review, see [16]). Laminar shear stress or stretch (either permanent or cyclic) are known to cause an elongation of endothelial cells [17, 18], whereas pressure induces retraction of cells from one another [19, 20]. These morphological changes are accompanied by a spatial reorganization of the cytoskeleton, cell-substrate focal contacts [21], and extracellular matrix [20, 22]. In particular, the pericellular fibronectin fibrillar network becomes more disperse in cultures exposed to hydrostatic pressure; by contrast, fibronectin fibrils aggregate in response to shear stress [20, 22].

The morphological response of endothelial cells to hydrostatic pressure and shear stress involves a coupling between: i) complex signal transduction events which might affect gene expression and protein synthesis, *e.g.* increases in intracellular calcium concentration [23]; and ii) direct force effects on the interconnected cell structure [24]. A mechanical analysis of the deformation of endothelial cells exposed to fluid shear stress shows that the stress-strain relationship follows a J-shaped curve [25], a behavior common to many biological samples [26], from intermediate filaments [27] to skin [28]. Cells exhibit a particular form of curve in J, where the incremental cell elastic modulus increases linearly with increasing stress [25, 29]. An explanation for this characteristics has been proposed recently, based on the tensegrity paradigm which states that cells are structures under continuous tension (*e.g.*, provided by the actin network) locally resisted by elements under compression (*e.g.*, microtubules) [24, 29].

1.3.2. Osmotic Stresses. — Osmotic forces also play an important role in cell mechanical integrity: cells swell in a hypotonic medium and shrink in a hypertonic medium, concomitant with major but mostly reversible structural alterations of the cytoplasm and cytoskeletal filaments [30]. It is also possible that local changes in osmotic strength be responsible for cell protrusion [31]. We found (unpublished data) that treatment of epithelial monolayers with a hypotonic buffer induced a thickening of the fibronectin fibrillar network (as did shear stress on endothelial monolayers), whereas treatment of cells with a hypertonic medium caused a dispersion of fibronectin into thinner fibrils (as did application of a hydrostatic pressure head on endothelial cell cultures). We hypothesize that stretching or wrinkling of plasma membranes in response to osmotic perturbation generate stresses in the cytoskeletal scaffold which support them [11]. These stresses may be conveyed through the interconnected cytoskeleton to cell-extracellular matrix transmembrane contacts at the basal surface [12, 24], where they induce phenomena such as association, dissociation, or rupture of pericellular fibrils which are initially stretched by cell tension [22]. However, these observations should be interpreted with caution since it is difficult to distinguish between mechanical and chemical (*i.e.* dramatic changes in intracellular water content) effects on structural constituents.

1.3.3. Adhesive Forces. — On the ability of cells to form or break adhesive contacts with other cells and the extracellular matrix in a spatially and temporally regulated manner depend many cellular processes including spreading, migration, proliferation and differentiation, which are at the origin of tissue morphogenesis. For example, when cells are free of adhesive contacts (*e.g.* cultured cells treated with trypsin) they generally assume a spherical shape, due to cortical tension [32]. If they are given an appropriate adhesive substrate (*e.g.* rigid tissue culture plastic carrying surface charges), they will spread out and assume very flat shapes [33]. In this context, a physical description of adhesive processes at the cellular and molecular levels is essential [34,35]. We discuss here that cell-cell and cell-substrate mechanical interactions play an essential role in the maintenance of cell shape.

1.3.3.1. Cell-Substrate Adhesions. — The topology of the culture substrate can dramatically influence cell morphology. Using techniques of photolithography, some authors have fabricated adhesive islands of microscopic dimensions: when cells are plated onto these substrates, they adopt specific morphologies corresponding to the geometry of the islands (*e.g.* triangular shapes) [36,37]. We have sliced tissue culture plastic dishes many times in a parallel fashion using a scalpel to create intact adhesive strips of substrate, 20 – 100 μm wide, separated by non-adhesive grooves. Endothelial cells plated onto these strips spread and reach confluence, progressively adopting elongated morphologies with their major axes oriented in the direction of adjacent grooves (the smaller the width between grooves, the higher the degree of elongation); by contrast, cells cultured on unsliced plastic remain polygonal. Elongated morphology is associated with the orientation of cytoskeletal filaments (actin and vimentin), focal contacts and extracellular fibers parallel to the grooves [25]. We observed a similar elongation of cells plated on top of rectangular collagen gels, but not on circular gels except at the edges, confirming that anisotropic geometry of the substrate induces cell elongation and orientation [25].

1.3.3.2. Cell-Cell Adhesions. — In order to perturb intercellular adhesions, we used two different approaches: i) slicing of a confluent monolayer of endothelial cells with a razor blade, which causes rapid retraction of a layer of cells at the margin of the cut: these cells adopt ellipsoidal shapes, with their major axes oriented in the direction of the cut; and ii) culture of confluent endothelial cells in low external calcium concentrations, which induces cell elongation in random directions, the degree of elongation increasing linearly with decreasing calcium concentration [25]. This is probably due to a random rupture of some (but not all) cell-cell adhesions mediated by calcium-dependent molecules such as cadherins [38]. These results suggest that the polygonal shape of endothelial (or epithelial) cells in unperturbed monolayers is due to isotropic distribution of intercellular tension: introduction of anisotropy in cell-cell stress distribution causes dramatic morphologic reorganization, namely an elongation of cells in the direction of dominant fields of force.

1.3.4. Local Mechanical Perturbation by Micromanipulation. — In order to measure cell mechanical properties, investigators have used various experimental systems to apply contact forces to individual cells. These systems include i) aspiration into a micropipet, mainly performed on erythrocytes [39] and leucocytes [32]; ii) manipulation of cell surface extensions with microneedles [40–43] or poking with force-sensitive instruments [44,45]; iii) displacement of ingested [46,47] or surface-attached [29] microspheres using a magnetic field; iv) observation of cellular organelles under Brownian motion or manipulation of them with an optical trap (laser tweezers) [48]; and v) stretch of individual protoplasmic plasmodial strands [49,50] or muscle fibers [51]. Not only these studies show that physical forces induce cell deformation and specific orientation of cytoskeletal filaments [40], but they also provide quantitative

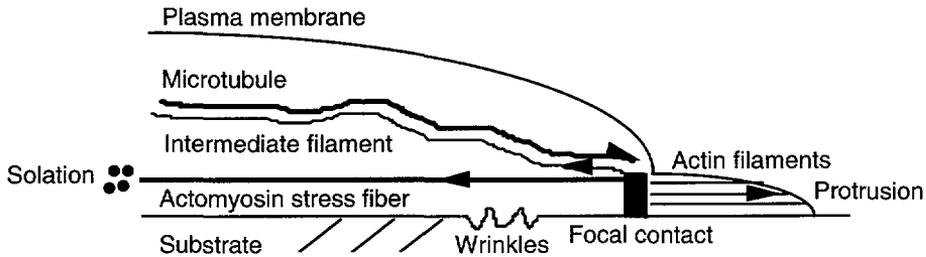


Fig. 2. — Side view of a cell edge, showing the mechanical actions of intracellular structural elements. Polymerization of actin filaments and microtubules can push the plasma membrane outwards. Cell traction, generated through sliding of actomyosin stress fibers or solation of the actin network, is resisted by the substrate at focal contacts; if the substrate is deformable enough, it will wrinkle or shrink. Intermediate filaments, guided by microtubules, probably behave as elements under tension [65, 76]. The question remains open of whether microtubules play a significant role in resisting cell traction.

information on the main mechanical characteristics of the cell structure, which are the following: i) the cell interior is under positive pressure [52, 53] and has viscoelastic properties, apparent viscosity values being highly scattered ($0.1 - 10^7$ Pa s) due to different methods of observation and to shear rate and temperature dependence [46, 54]; ii) there exists a permanent tension at the surface on the order of $0.03 - 0.1$ dyn cm^{-1} [32, 55], related to the actin cortex underlying the plasma membrane; and iii) cells are active materials in the sense that they can generate stresses (see Ref. [54] for a list of values) from the breakdown of energetic chemical bonds. We discuss below two examples of such forces.

2. Internal Forces

2.1. CELL TRACTION. — Actin and myosin are two proteins which can assemble into filaments. In muscle cells, these filaments organize as parallel bundles and can slide on one another so as to produce a contractile force: this involves a conformational change of myosin heads, which requires adenosine triphosphate (ATP) hydrolysis. In non-muscle cells such as fibroblasts, contractile actin-myosin bundles are also present, mostly at the basal surface of cells and are called stress fibers [56, 57]. These bundles usually terminate at focal contacts [10, 11], where traction forces are transmitted to the underlying substrate (Fig. 2). Cell contraction may involve not only motor proteins (*i.e.* myosin) but also local solation (*i.e.* disassembly) of the actin network [58]. In any case, fibroblast traction is likely to be responsible for the shrinking or wrinkling of malleable substrates onto which they are plated, for example gels of polydimethylsiloxane [59] or collagen matrices [60]. The extent of cell spreading positively correlates with the rigidity of the culture substrate [24, 61], indicating that the balance between tractional forces expressed at adhesive sites and substrate resistance plays a major role in controlling cell morphology.

In living cells, traction forces may be regulated through fluctuations of intracellular calcium and ATP levels. Experimentally, one can stimulate stress fiber contraction by treating cells with a critical concentration of detergent, then exposing these permeabilized cells to a buffer containing calcium and micromolar concentrations of ATP [62–64]. This induces rapid cell

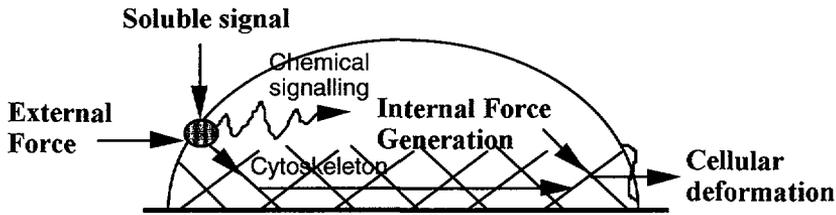


Fig. 3. — Schematic drawing of the different signals influencing cell morphology. Both externally-imposed forces and soluble substances can be transduced into cell shape changes, through two cooperating pathways: i) direct mechanical action on the cell structure; and ii) chemical signalling which then stimulates mechanisms of internal force generation.

rounding, when traction forces overcome cell-substrate adhesive forces. The velocity of cell retraction increases with ATP concentration as described by the equation of Michaelis-Menten for enzyme kinetics [65], in agreement with the fact that myosin functions as an actin-activated ATPase [66]. This type of approach allows the characterization of quantitative parameters describing cellular traction, which can be used to compare the contractile activity of different cell types. For example, it can be shown that normal fibroblasts exert greater contractile forces than do their transformed counterparts. Quantitative estimation of cell tractional forces give values on the order of $10^{-8} - 10^{-7}$ N [59, 67, 68].

2.2. POLYMERIZATION FORCES. — Actin polymerization (which depends on ATP hydrolysis) is used in diverse types of biological movements such as the formation of the acrosomal process in sperms [69], the displacement of the bacterium *Listeria Monocytogenes* during mammalian cell infection [70], and the projection of filipodia and lamellipodia during fibroblast locomotion [71]. Microtubule polymerization (which relies on guanosine triphosphate hydrolysis) is essential for guiding chromosome movement in mitotic cells [72] and for axonal extension [42, 73]. It is clear that polymerization forces alone can push plasma membranes outwards, through either a motor-driven process or thermal fluctuations [74], thereby causing morphological changes at the cell surface (Fig. 2). In addition, polymerization of actin or tubulin can be promoted by mechanical tension, *e.g.* within tethers pulled from a neutrophil [75] or in processes initiated from neurons [73], respectively.

These specific roles set apart, the question arises of the overall contribution of microtubules and actin microfilaments to the mechanical integrity of the cell structure (Fig. 2). Some authors [24, 76] have postulated that microtubules resist actin tension, and thereby bear compressive forces. This hypothesis is potentiated by several observations: i) having a large geometric cross-sectional moment of inertia, the tubular structure of microtubules is well suited for resisting compressive loads [26]; ii) in a viscometer, microtubule suspensions rupture at low shear stresses [27], suggesting that microtubules are not made to resist large tensile loads; iii) the tensile force generated by one actomyosin sarcomere is quantitatively similar to the compressive force that one microtubule is able to sustain (see appendix); and iv) an increase in the expression of cell traction has been documented upon disruption of microtubules by specific drugs (*i.e.* colchicine or nocodazole) in a variety of cell types [42, 77-79]. Additional experiments will have to be performed to solve this interesting issue.

Conclusion

We have seen that cell morphology, architecture, and movement are under the tight control of mechanical factors acting in concert. Although we put the accent on physical determinants of cell shape, chemical factors are equally important, as illustrated by leucocytes locomoting towards a source of chemoattractant. Intracellularly, an interplay occurs between biochemical and mechanical regulations (Fig. 3). Thus there is a need to better understand how physical constraints are transduced into appropriate molecular signals [29], and in turn, how molecular mechanisms (*e.g.* motors, polymerization) are responsible for generation of force [80].

A general physical model of cell morphology has to take into account the complex molecular and structural reality unraveled by cell biologists. Quick progress is being made in characterizing the thermodynamic and mechanical properties of individual components [81–83]. The difficulty now consists in putting such information into a consistent model describing the structurally interconnected cell structure, in agreement with quantitative data of cell mechanics and locomotion. A few recent models have been proposed on the basis of different views of the role of cytoskeletal networks, *e.g.* continuous tension [76] or assembly-disassembly [58, 84, 85].

Further cooperation between physicists and biologists should eventually lead to a precise quantitative description of the mechanical interactions between cells and their environment and thereby to a better understanding of tissue and organ physiology [86, 87].

Appendix

The isometric stress produced by a glycerinated muscle fiber stimulated with physiologic values of ATP (0.1 – 1 mM) is around 0.1 N mm^{-2} [51]. Therefore, the contractile force generated by one myosin filament encircled by 6 actin filaments (cross sectional area on the order of 10^{-9} mm^2), as in a sarcomere, is around 10^{-10} N . This value is consistent with measurements of tension at the surface of fibroblasts (around $15\text{--}30 \text{ m dyn } \mu\text{m}^{-1}$) [43]. On the other hand, the critical compressive load to induce microtubule buckling can be derived from Euler's formula for an elastic beam: $F = k\pi^2 EI/L^2$ [26], where E is the Young's modulus, I is the geometric moment of inertia of the cross section, L is the length of the beam, and we suppose a buckling mode $k = 1$. Experimental evaluations of the flexural rigidity of microtubules (E times I), considering curvature variations in response to hydrodynamic flow, thermal fluctuations, or compression forces applied with optical tweezers, give values around 10^{-23} Nm^2 [83, 88, 89]. The flexural rigidity is actually higher (by a factor of 2 to 4, depending on the study) *in vivo*, due to the presence of microtubule-associated proteins [88, 90], or intermediate filaments [91] which stabilize microtubules, *e.g.* by bundling. If we take a length L of $2.5 \mu\text{m}$ (the length of a sarcomere), we obtain a value F on the order of 10^{-10} N , comparable to actomyosin traction. (The elastic beam theory may not be fully valid for analyzing non-homogeneous, nanometer-scale objects such as microtubules; nevertheless, the theoretical value agrees rather well with one experimental measurement of microtubules buckling force [89].)

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