Tensegrity and oscillations : exploring some constitutive and emergent features of virtual cell models

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1. Introduction

The key role of the coupling between mechanical forces and tissue growth and remodelling was suggested nearly twenty years ago (Trinkhaus, 1984), especially in the field of bone formation and remodelling. However, it is only recently that a large body of experiments highlighted the effects of physical forces such as tension, compression, gravity or shear stress at the cell level (Edwards et al., 1999; Huang and Ingber, 1999; Kaspar et al., 2000). Indeed, direct application of mechanical stresses to cultured cells can induce or modify cell differentiation, growth and migration as well as gene expression (Fujisawa et al., 1999; Wang et al., 2000; Meyer et al., 2000). However, we do not still fully understand how individual cells perceive mechanical signals and orchestrate them to produce a particular behaviour, both individually and collectively, through the control of cooperative phenomena at the cell population or tissue levels.

In some sense, the "cell challenge" is to sense all kind of signals but to generate a single, integrated response. Among these different cell signalling mechanisms, mechanotransduction, i.e. the conversion of a mechanical signal into a biological or biochemical response, has a kind of special status (Wang et al., 1993). Indeed, it already relies on the global structural characteristics of cells as opposed to biochemical events which can occur locally, for example near the cytoplasmic membrane or within an intracellular organelle. Mechanotransduction is why the development of theoretical cytomechanical model of the living cell is crucial : it provides the only way to understand how many simultaneous extracellular mechanical inputs (adhesion to extracellular matrix (ECM) proteins, junctions with other cells,…) combined with heterogeneous mechanical properties (local softening or hardening of the cytoskeleton) are integrated with other stimuli to provide a specific physiological or pathological cellular response (Lelièvre et al., 1996; Janmey, 1998).

We will briefly explore here some of these theoretical models, with special emphasis on the cell tensegrity paradigm proposed by D.E. Ingber (1993; 1997). Alternative cytomechanical models based on a simple elastic or viscoelastic continuum or stressed submembranous cortex will also be presented. Two issues will be presented. The first deals mainly with modelling the architectural properties of the cytoskeleton (CSK) as a physical basis for analysing both the mechanical properties of cells and the different way mechanical forces are transduced to yield an integrated cell response. The second is more concerned with cellular dynamics and related morphological changes since CSK remodelling is one of the intracellular factor controlling gene expression. Both presentations are based on the conviction that modelling how cells dynamically stabilise and self-organise their structure and shape is essential if we are to understand how cells sense their physical microenvironement and respond to mechanical signals through in/out bi-directional signalling pathways that connect the plasma membrane to the nucleus.

After reviewing the main aspects of both discrete and continuous mechanical models of cells, this paper ends with some propositions for testing a virtual, integrated cell model. These will be discussed in connection with the concepts, possibilities and limitations offered by

research programs on computational cells such as Virtual Cell and Electronic cell.

2. Discrete mechanical cell models

2.1. Cells as tensegrity structures

Since the eighties, D. E. Ingber and coworkers have promoted and architectural view of the living cell and of larger biological entities based on the concept of tensional integrity or tensegrity, according to which the structure of cells depends on tensile forces for its integrity (Ingber, 1993 ; 1997). As explain by Ingber, this proposition draws on a close analogy with the architectural principles developed for the construction of buildings by R. B. Fuller and K. Snelson, as exemplified by geodesic domes and later discovered as underlying the organization of viral capsids.

It seems worth emphazing in this introductory paragraph that the holistic approach proposed by Ingber has deep conceptual implications. First, it again drew attention to the need for unifying principles necessary to interpret the plethora of biological data successfully collected by a reductionism that cannot explain them on its own. Second, it strengthened the view of biological systems as non-linear systems with functions that are more than the sum of its parts.

How is a tensegrity system defined? According to Ingber and Jamieson (1985) " *A tensegrity system is defined as an architectural construction that is comprised of an array of compression-resistant struts that do not physically touch one another but are interconnected by a continuous series of tension elements*". At the cell level, the tensegrity framework proposed by D. E. Ingber is based on several initial assumptions.

The first assumption is that cell shape is stabilized by an internal mechanically active structure, the cytoskeleton. This assumption excludes both theoretical models treating the cell as a viscous fluid surrounded by a membrane, as well as mechanical models treating the CSK as a passive structure. Indeed, it is well-documented that living cells (and even non-muscle cells like endothelial or fibroblasts) can generate active tension through an actomyosin filament sliding mechanism similar to the one used in the contraction of smooth muscles. This is easily visualised, either at the cell level by the wrinkling of malleable substrata by cells (Dembo et al., 1996 ; Dembo and Wang, 1999), or at the population level via measurements of isometric tension or compaction rate of viscoelastic biogels (Kolodney and Wysolmersky, 1992; Eastwood et al., 1996). This internal contraction capability creates within the cell a prestress upon which external mechanical loads are superimposed. Tensegrity cell models have thus been developed by considering elastic and contractile actin microfilaments as tension elements and microtubules as compression-resistant elements (Wang et al., 2001).

The second assumption is that forces are not transmitted continuously across the cell but rather that transfer of mechanical loads and stresses take place at points where the cell is anchored to the extracellular matrix (ECM) and to neighbouring cells. Indeed, the cell anchors itself to the ECM by physically binding CSK elements to specific focal adhesion complexes (FACs) that cluster within localized adhesion sites. Such complexes include not only transmembrane proteins, mostly integrins, but also a scaffold of actin-binding proteins (talin, vinculin, -actinin, paxillin, …) which form a molecular bridge between the CSK and the intracellular part of the integrin. Similarly, specific cell-cell adhesions molecules (CAMs), like cadherins, selectins, cathenins, …) insure the transmembrane coupling of neighbouring cells CSK at localised junctional sites (adherens junctions, desmosomes).

Cellular architecture seen in this way defines a mechanical network that provides a physical support to biochemical signal transduction pathways and that allows mechanical signals to be propagated from mechanoreceptors on the surface (in the form of cell adhesion molecules and transmembrane receptors) to targets deep within the cell.

2.2 Qualitative behaviour of cell tensegrity models

Some basic, qualitative properties of cell tensegrity models have been illustrated by Ingber and co-workers by constructing a physical structure made of multiple wood dowels interconnected with a series of elastic springs (Ingber, 1997). This "cellular toy" has interesting properties: if it is not subjected to an external force and if not attached to a rigid surface, it has a round shape because of the internal tension. However, it can spread out and flatten along a rigid surface if an external force is applied vertically. Moreover, if the toy is attached to a flexible surface while in its flattened configuration, and if the external force is then removed, the toy contracts spontaneously and returns to the round shape characteristic of its rest state, again because of the presence of internal tension. This relaxation is accompanied by a progressive wrinkling of the flexible substratum which closely mimicks the formation of wrinkles observed when living cells are cultured on malleable substrates like silicon rubber.

Simulations of tensegrity via the construction of virtual cells may eventually prove indispensable to understanding how the cell interprets its genome. In this context, an additional and particularly interesting feature of models of cellular tensegrity is their ability to fit naturally into a structural hierarchy. One can then include within such virtual cells smaller intracellular structures with their own mechanical properties. Of course, the cell nucleus is the first candidate. The nucleus has its own internal structure, the nuclear matrix, and a body of experimental findings are consistent with the nucleus being mechanically coupled to the rest of the cell. For example, when a cell spreads on a rigid substratum, its nucleus extends in parallel, even if some delay can be observed in migrating cells. The tensegrity model explains how the nuclei of living cells can respond directly to mechanical stimuli that are applied to specific surface receptors as those involved in cell adhesion. It thus provides a basis to understand how extracellular mechanical stimuli can modify gene expression through mechanical deformations of the nucleus (Maniotis et al., 1997).

2.3. Theoretical properties of cell tensegrity models.

Different theoretical studies have been undertaken to analyse the mechanical properties of cell tensegrity models. A minimal tensegrity structure composed of 6 compressive elements (bars) and 24 extensible elements (cables) with frictionless joints has been analysed by Stamenovic et al. (1996). This study was extended by Wendling et al. (1999) who investigated wether such a pre-stressed geometric structure could account for the stiffening response observed in living cells. They showed that, under large deformations, the tensegrity structure exhibits a non-constant stiffening response which depends on the loading conditions (extension, compression or shear). They further demonstrated that, although the Young's elastic moduli of each constitutive element stays constant, the apparent elastic modulus of the overall structure, the apparent elastic modulus of the overall structure does not stay constant.
They analysed the deformation of the 30-element cell tensegrity model governed by the constitutive equation :

$$\{ F \} = [K] \{ u \}$$

which relates the vector of external forces $\{F\}$ to the vector $\{u\}$ defined by the displacement (distance between the deformed and the reference state) of each of the 12 nodes through the rigidity matrix $[K]$ of the structure (cables and bars are assumed to be linearly elastic with Young's modulus E_c and E_b respectively, with $E_b >> E_c$). The external forces are applied at three upper nodes of the structure, while the contact of the structure with the inferior plane occurs through three fixed nodes : this latter condition is chosen to simulate a weak cell adhesion of a round cell onto a rigid substratum.

At the unloaded (reference) state, mechanical equilibrium results from the balance between pre-stretching stress in the cables and in the bars. For the different loading conditions, the tensegrity model response is analysed by considering the apparent elasticity moduli derived from the stress-strain relationship. With reference to continuous material, apparent elasticity modulus E_s and shear modulus G_s of the tensegrity structure have been defined as the derivative of the polynomial functions fitting the apparent normal and shear stresses to the apparent normal and shear strains respectively.

The numerical simulations of the tensegrity model response to extension and compression exhibit a non-linear mechanical response, characterised by linear relationship between the apparent elasticity modulus and the apparent strain. The modulus increases with strain during extension and is thus associated with a strain-hardening behaviour of the structure. On the contrary, a strain-softening occurs under compression, with decreasing values of the elasticity modulus as strain increases. This behaviour is qualitatively preserved when the length of the elastic elements or their pre-stress is changed by several orders of magnitude. Application of shear stresses lead to a more complex response, with initial stress-softening followed by stress-hardening.

This study illustrates how the response of the CSK of the living cell to applied stress might be a property of an integrated system and not a characteristic of individual components : even if each component of the tensegrity structure exhibits independently a linear elastic response with constant elastic moduli, the integrated response is non-linear with apparent elastic moduli that are strain-dependent. Such behaviour is related to the re-orientation of stresses which, even applied locally, are spatially distributed through the myriads of interconnected filaments composing the CSK. Evidence for such stiffening response of cells comes from several different, biological experiments. Moreover, these experiments provide additional information on the mechanical response of intracellular components such as the cell nucleus.

## 2.4. Simulation of virtual cell models : the example of physically based computational models

Despite the virtues of the tensegrity model, it should be noted that it cannot explains certain dynamic features of the CSK such as its remodelling. We briefly reported here a recently developed computational approach that provides an alternative way for simulating the mechanical response of virtual objects modelling living cells (Promayon et al., 2002) and that could be more extensible. This approach is inspired by the physically based computational framework proposed for simulating the respiration movements of the human trunk (Promayon et al., 1996 ; 1997). Based on algorithms operating within an object oriented programming language, this approach is able to take into consideration dynamic changes of objects properties and shapes. In this modelling framework, cells are considered as three-dimensional elastic bodies submitted to internal cohesive forces as in the tensegrity approach. In addition, external attractive forces (gravity, chemo-attraction, …) are also considered as possible control factors of the virtual cell dynamical features.

The virtual cell we have constructed within this framework is defined as a 3D incompressible object. From a computational point of view, this virtual cell is considered as an entity with its own properties (elasticity, contractility, …) and history (interactions with other cells or ECM, …). To simplify the calculations, a cell is defined by a 3D closed surface represented by a triangular mesh and its associated contour nodes.

Dynamical cell shape changes occur as a response to various forces (gravity, locally applied mechanical loads, …) applied to each node of the mesh. The dynamic of the local cell response is then determined by the mass initially assigned to each node. Since mass-spring networks are known to be rather unstable systems, cell elasticity properties have been modelled by defining a local shape memory (Promayon et al., 1996). This means that the elastic property

of a cell object is simply its ability to recover its original shape once deformed. This property is modelled by defining a local shape coordinate system in which each node of the structure is defined relatively to its neighbours by three parameters.

Some applications of this physically based computational approach are given below. First, as with tensegrity models, the mechanical response of specific cell architectures can be analysed. For example, one can simulate the effect of different intracellular organisation of the CSK which can mimic specific orientation of cell stress fibres. Figure 1 shows the simulated influence of transverse links within an elastic discrete envelope when the cell is submitted to uniaxial compression. The cell 0 is defined as a strict elastic discrete envelop, with no internal links. In cell 1, we considered a reinforcement of the cell architecture with horizontal elastic cross-links modelling CSK fibres. Finally, cell 2's architecture includes internal diagonal elastic links connecting the apical and basal physical cell surfaces.
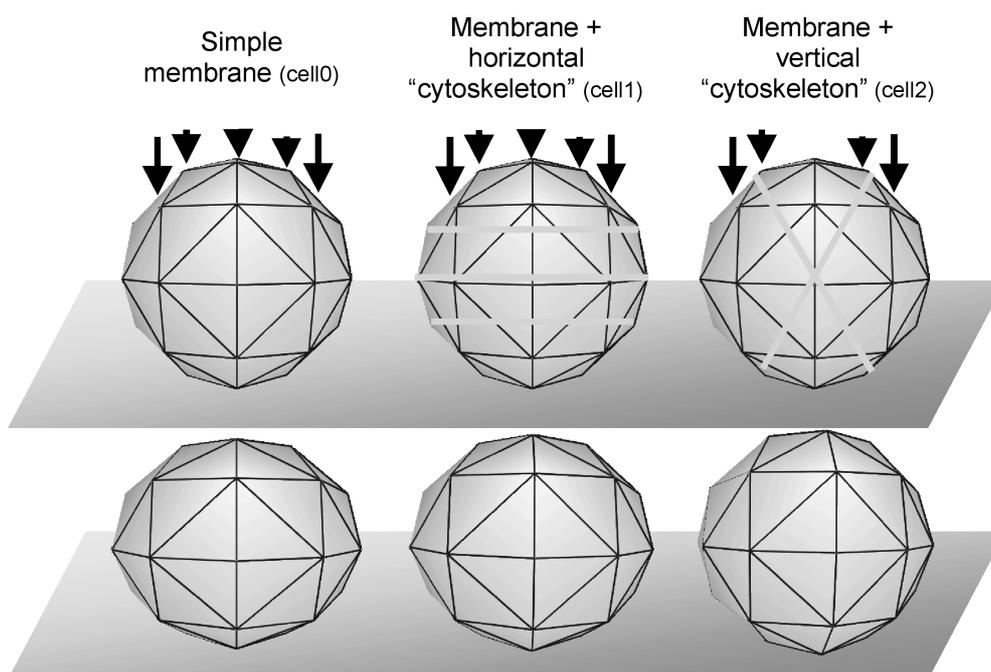
Figure 1 : Influence of the "cytoskeleton" of the virtual on the mechanical response to vertical load. Three cell types are considered: no "cytoskeleton" (cell0), horizontal links (cell1), diagonal links (cell2). The first raw presents the initial 3D shape of each cell prototype. For a given fixed value k_{elas} of the elasticity modulus of each cell object, the second raw in the figure indicates the equilibrium state which is reached when a vertical loading force F_c is applied on the 5 (cell0 and cell1) or 4 (cell2) nodes marked with arrows..

To make a closer comparison with real cells, we consider a virtual cell devoid of internal elastic links. This provides a model of living cells like human erythrocytes, where the cell membrane is entirely responsible for the elastic deformation of the cell, the inner cytoplasm being only viscous. The relevance of this modelling approach with regard to real experiments is illustrated in figure 2 where optical tweezers experiments of Henon et al. (1999) to deform nearly spherical erythrocytes have been simulated. In the experiments, a force F is exerted on two silica microbeads which are stuck to the erythrocyte membrane in diametrical position. By slowly incrementing the distance between the two trapped beads, an increasing stress is applied to the cell membrane. To simulate this experiment, a force F_s has been locally exerted on two opposite nodes of the physical cell membrane, pulling them apart. The simulation parameters

are the elasticity of the cell, i.e. k_{ela} and the modulus of F_s. Fig ZZZ shows that k_{ela} could be approximated to that the modelled cell: the real erythrocyte have the same behaviour.
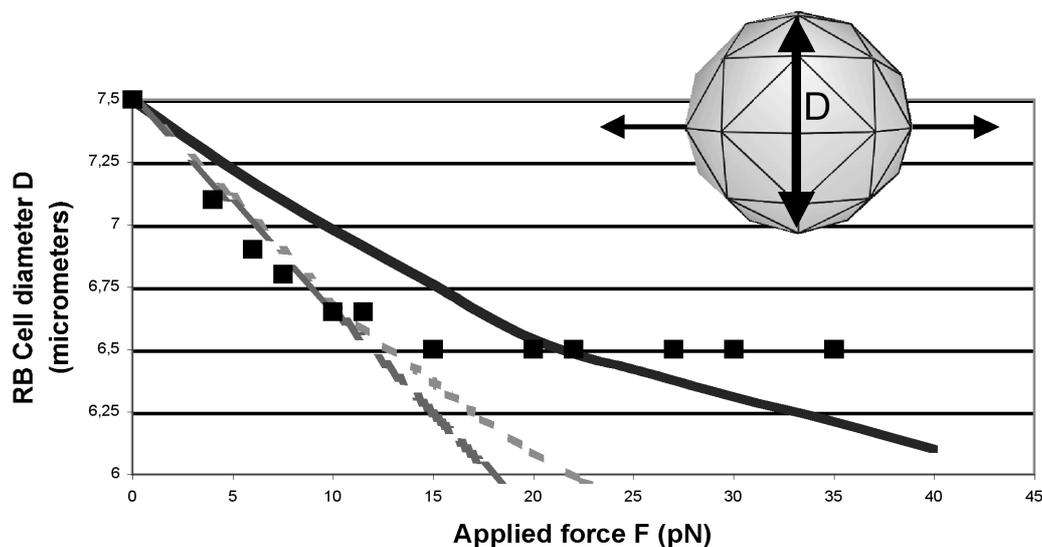

Figure 2 : Simulated virtual spherical red blood cell (RBC) suspended in an hypotonic solution. Optical tweezers double trap is simulated by exerting locally a force F_s on two opposite nodes of the cell object contour (upper insert). The variation with load of the cell object diameter D (F_s) in a plane perpendicular to the loading direction is simulated and compared to experimental data published by Henon et al. (1999) With appropriate scaling of the force, one can adjusted the parameter k_{elas} such that the experimental mechanical response of RBC can be nicely fitted in the linear elastic regime. Increasing the elasticity modulus k_{elas} induces a stiffer response which qualitatively reproduces the departure from the linear regime at larger traction forces.

The microplate experiments of Thoumine et al. (1997 ; 1999 ; 2000) can also be simulated in a similar fashion (Sauvaget, 2001) (Fig. 3). Experimentally, the adhesion of a fibroblast is realised between two glass microplates, one of them being slightly flexible. The mechanical of the cell response to stretching is measured. To qualitatively simulate these experiments, a cell object was defined as a two-region object: a virtual plama and nucleus membranes, both of them being represented by triangulated surfaces. Each node of the membrane is elastically linked to its neighbours as well as to the corresponding node in the other membrane. Microplates are modelled by two circular rigid objects attached to the apical and basal part of the external membrane, one of them being translated vertically. The corresponding shape of the virtual cell and nucleus at equilibrium is shown in figure 3 right.
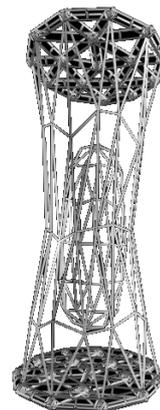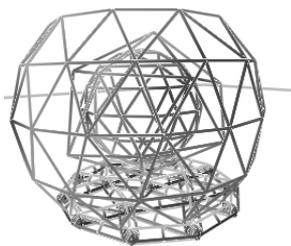
Figure 3: Simulated stretching of a virtual cell and its "nucleus" which mimics microplates experiments. Left : rest shape of the two embedded structures. Right : simulated cell and associated nucleus deformations.

For large amplitude deformations (hyperelastic behaviour), a non-linear mechanical response of the virtual cell could be observed. This property indictaes that, as observed with the cellular model of tensegrity of Ingber and col , the global mechanical behaviour of this virtual cell is not the sum of its individual component responses. Further developments are needed to analyse the theoretical properties of such a virtual cell, especially with regard to mechanical properties exhibited by continuous finite elements models. However, the simulation of the microplates experiments reported here illustrates the capability of this approach to deal with multi-scale dynamical phenomena. For example, it is also possible to simulate cell population behaviours such as tissues (figure 4) or cell interactions during cell migration (Promayon et al., 2002)
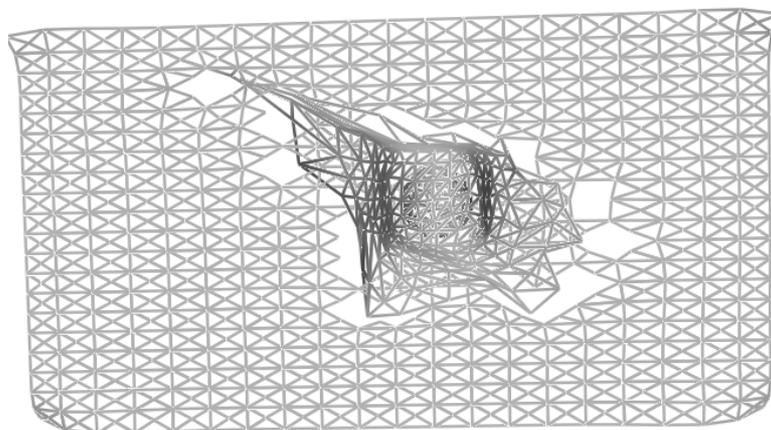
Figure 4 : Simulated contraction of an elastic substratum by a virtual adherent fibroblast linked to this extracellular film by localised "focal contacts"

3. Oscillating cellular deformations and continuous virtual cell models

The existence of a mechanical continuum within the cell means that oscillatory deformations could occur and affect gene expression. We therefore review some of the theoretical models proposed for analysing and modelling oscillations in cell shape. Each of them highlights a particular biophysical process as the central mechanism responsible for cell

shape changes. Nevertheless, the common theme is that all the interactions considered are integrated into a single response, namely an oscillation of cellular protrusions.

It has been shown for a long time that living cells change their shape by extruding and remodelling different types of membrane protrusions. Filopodia are finger-like protrusions of the plasma membrane while lamellipodia are sheet-like protrusions associated with Filamentous actin (F-actin) network. It was once generally believed that the cell membrane fluctuates without any particular direction in space and without any particular coherence in time. However, recent progress in cell imagery techniques and cell images analysis has revealed the organised dynamics of cell protrusions (Germain et al.,1999). For example, Killich et al. (1994) have reported the existence of different organize patterns of morphological changes in the amoebae *Dictyostelium discoideum*. Ehrengruber et al. (1995) show that neutrophils undergo periodic cytoskeletal rearrangements that lead to cycles of shape change with period of 8–10s that is associated with sinusoidal oscillations of F-actin. Different hypotheses have been proposed to explain the formation of cellular protrusions. These hypotheses have considerable implications for our understanding of intracellular signalling and theoretical models of the types reviewed here are essential tools in the testing and refining of such hypotheses.

3.1. Cortical F-actin solation/gelation models.

In the early eighties, Oster and Perelson (1985) proposed a model of lamellipodial motion based on the physical chemistry of actomyosin gels. The model consists of a sheet of cytogel attached to the substratum by elastic tethers. The rhythmic activity of extending and retracting lamellipods is assumed to be driven by alternating phases of solation and gelation of the cortical actomyosin gel respectively. This phase transition is controlled by intracellular levels of calcium which is stored in intracellular compartments and which is released into the cytoplasm; this release is under the control of mechanisms that operate in a complex, non-linear (autocatalytic) fashion known as the calcium-induced calcium release mechanism (CICR). In Oster and Perelson's model, raising levels of free intracellular calcium concentration activate solation factors which disrupt the F-actin gel network. Factors such as gelsolin or severin can either break the actin chains themselves or break the cross-links between the chains or induce a depolymerisation of the chains. Such breakdown induces the swelling of the gel up to a point where the swelling pressure is balanced by elastic resistance of the network.

One important aspect of this non-linear process is that calcium also triggers the actomyosin contractile machinery. There is thus a defined range of calcium concentration where contraction occurs. In addition to the CICR mechanism quoted above, it is clear, regarding non-linear systems theory, that the cell has all the physical and biochemical ingredients needed to induce spontaneous self-sustained oscillations above some critical threshold. The trigger is assumed here to be an initial leak of calcium at the leading edge of the cell membrane. Leaking can be induced by the bindings of extracellular factors to membrane receptors or by a mechanical stimulus exerted at adhesion site, with associated possibly accompanied by a modification of ion channels.

3.2. Intercalation of actin monomers : the Brownian ratchet mechanism

Peskin et al. (1993) formulated a theory to account for the force generated by the polymerisation process itself when the filaments are rigid. They proposed that the addition, below the cell membrane, of G-actin monomers at the end of F-actin growing filaments could exploit the Brownian motion of any diffusing object in the front of the filament. Thus, random fluctuations of the plasma membrane would create a free sub-membrane space where this intercalation could take place (Abraham et al.,1999 ; Borisy and Svitkina, 2000). This ratchet mechanism could explain the formation of thin cell protrusions when F-actin filaments are perpendicular to the membrane surface, but it cannot satisfactorily explain the formation of

lamellipodia.

Molginer and Oster (1996) extend this model by further assuming that the bending of the filament tips drives the protrusion formation. This bending mechanism would therefore provide a mechanical explanation for the appearance of lamellipodia type of protrusions. Moreover, Molginer and Oster inferred an optimal angle of 96 degrees between to two branching filaments.

3.3. Alternative to the ratchet model.

The pathogen bacterium *Listeria monocytogenes* uses actin polymerization to propel itself through the cytoplasm and the membrane of infected cells (Theriot et al, 1992; Frischknecht and Way, 2001). Experimental data show that the cell motility results from cooperation between the bacterium and the host cytoplasm proteins. The bacterium surface protein ActB controls the activity of the complex Arp2/3 that initiates actin polymerization (Welch et al., 1997). Actin dynamics is also controlled by an actin depolymerizing factor (ADF/cofilin) and capping proteins, which are in the cytoplasm host. The last two factors maintain a high level of actin monomers in the cytoplasm to achieve filament growth at the bacterium surface. In vitro studies proved that movement was possible with a limited number of proteins, including Arp2/3, ADF/cofilin and a capping protein (Loisel et al., 1999). Biophysical investigations demonstrate that the bacterium and its actin-tail are tightly bound, which rules out the ratchet model approach for this system (Gerbal et al., 2000). In addition, the same group measured the actin-tail Young modulus at a value of 103–104 Pa, a value 10 times larger than the cytoplasm rigidity. Using the framework of elasticity theory, Gerbal et al. (2000) proposed that the mechanical stresses generated at the *Listeria* cell surface are relieved at the back of the bacterium pushing the cell forwards. Their model accounted satisfactorily for the cell speed (about 0.1 m.s^{-1}) and was extended to explain the hopping motion observed in a *Listeria* mutant

3.3. Actin polymerisation, F-actin nucleation and reaction-diffusion models

3.3.1. Some experimental data

Actin dynamics plays a major role not only in cell movement (Condeelis, 1993) but also in cell adhesion or neuron plasticity (Colicos et al., 2001; Star et al., 2002). Characterisation of actin filaments growth proved the importance of the polymerization/depolymerization balance at the filament ends and the role of proteins in inducing actin polymerization (e.g. Arp2/3), severing actin filaments (e.g. gelsolin, ADF/cofilin) or protecting the filaments ends by capping proteins (Pollard et al., 2000). Actin monomers associate to form filaments with a polarity (barbed *vs.* pointed ends). At the barbed end, subunits associate rapidly, with a low equilibrium actin monomer concentration ($C_{eq,B} = 0.08\mu M$). In contrast, the dynamics is much more slower at the pointed end but with a larger equilibrium monomer concentration ($C_{eq,P} = 0.5\mu M$, Carlier et al., 1997). At steady-state, the actin monomer concentration is:

$$C_{eq} = \frac{k_B^+ C_{eq,B} + k_P^+ C_{eq,P}}{k_B^+ + k_P^+}$$

where k_B^+ and k_P^+ are the association rate of actin monomers to the barbed (B) or pointed (P) ends. At steady-state, the growth at the barbed end is exactly compensated by the disassembly at the pointed end, a dynamical state called *treadmilling*. However, the predicted treadmilling steady-state flux (i.e. $k_B^+ \left(C_{eq} - C_{eq,B} \right) = - k_P^+ \left(C_{eq} - C_{eq,P} \right)$) is too slow (0.2s^{-1}) to account for the rapid

turnover observed *in vivo*. This suggests that other cellular factors affect actin dynamics, including interactions with intracellular proteins (Carlier et al, 1997a, Pollard et al., 2000), intracellular signaling (Machesky et al., 1999; Mullins, 2000) and movement generation (Borisy et al., 2000).

3.3.1. Models for actin network formation

In marked contrast with the actin dynamics complexity, models were first addressed to analyse the polymerization and fragmentation of actin filaments alone *in vitro* (Edelsetein-Keshet et al., 1998; Ermentrout et al., 1998). These models use the classical framework of kinetic differential equations without addressing the question of interactions between filaments and network formation. In the next step, actin bundle formation was considered including the kinetic approach (Edelstein-Keshet, 1998). This model was developed to account for the length distribution of actin filaments in a lamellipod (Edelstein-Keshet et al., 2001). Actin filament orientation was also studied using a Boltzmann-like equation (Geigant et al., 1998). However, these models are based on the kinetics of actin polymerization or actin filaments association without geometrical or mechanical constraints. A recent attempt to address the more complex question of actin network generation was done by Maly and Borisy (2001) who developed a model for the actin network formation as a self-organization process. They were able to account for the preferential direction of the actin filament bundle observed *in vivo*. Finally, one should mention the analysis of the actin gel formation on bead surface both experimentally and theoretically providing insight in the actin network regulation in cells (Noireaux et al., 2000).

Models for spatio-temporal F-actin interactions *in vivo* were developed by Le Guyader and Hyver (1997), who analysed the oscillatory dynamics of the cortical actomyosin ring of human lymphoblasts by interpreting it in terms of a reaction-diffusion process. They proposed a three-variable model that takes into account free and membrane-bound F-actin as well as nucleation proteins. By assuming the existence of a non-linear reaction in which the synthesis of F-actin is autocatalytic, their model generated oscillatory actin waves within a fixed area corresponding to the cell cortex. This theoretical behaviour is in agreement with the experimental work of Bornens et al. (1989) and more recent work showing that the disruption of the microtubule network by nocodazole induces cortical oscillations (Pletjushkina et al., 2001). Bornens et al. (1989) suggested that oscillating concentrations of nucleation proteins between the two poles of the cell would indeed create a polymerisation/depolymerisation wave of actin travelling through the cell. Such behaviour was also reported during the extension of pseudopods in *Dictyostelium discoïdeum* (Vicker et al., 2000).

3.5. Protrusive dynamics due to the modulation of stress-strain relationships within the actomyosin cytogel.

The cytomechanical model of Lewis and Murray (1992) extends the solation/gelation model by considering the stress-strain relationships within the actomyosin cytogel. The cytogel is modelled as a viscoelastic continuum submitted to active stress and osmotic pressure. In addition, the sol/gel transition is controlled by the resulting strain level within the cytogel. At high strain, the gelation rate is increased, while at low strain, the solation rate increases. The model dynamics is controlled by the non-linear stress-strain relationship defining qualitatively the contractile actomyosin stress. The inhomogeneous spatial solutions generated by this model have been specifically discussed with regard to the patterned formation of microvilli at the cell surface (Murray, 1993).

3.6. Coupling of actin dynamics with cell cortex curvature.

In a series of papers, Alt and col. (1995; 1999) proposed a modelling approach in which cell protrusions dynamics are due to the biophysical properties (viscoelasticity, contractility,...) of the cortical network of actin and myosin filaments underlying the cell membrane. This more or less dense network is able (i) to disassemble at locations where it becomes too condensed, (ii) to reassemble in cell protrusions like lamellipodia. Thus, cell protrusions mainly result from a mechanical balance between stresses acting on the cell cortex (mechanical forces generated by the actomyosin complex, tension forces due to the local membrane curvature (Raucher and Sheetz, 2000)) and associated F-actin polymerisation/ depolymerisation induced by intracellular network -free space variation in each moving protrusion.

A minimal three-variable model has therefore been developed to describe the spontaneous, self-organized, dynamics of cell deformations. This model has been used to study the spatio-temporal deformations of keratinocytes (Alt et al., 1995) as well the morphological changes in L929 fibroblasts (Stephanou et al., 2002a, Fig. 5). The model takes into account (i) the dynamics of F-actin polymerisation/depolymerisation in the cell cortex, (ii) the contractile activity generated by the actin/myosin interactions, (iii) the F-actin convection. The local amount of F-actin also determines the intensity of the resistive stress applied on the membrane as the result of CSK- cell cortex attachments. This resistive stress plus the stress induced by the cell cortex local curvature is assumed to balance the intracellular hydrostatic pressure.

The analysis of the morphological changes of adherent cells is at least a two-dimensional free-boundary problem. However, a simpler one-dimensional problem can be considered by assuming that the F-actin density as well as its convective tangential velocity is constant in the radial direction. In a cylindrical coordinate system (r,), the remaining variables in the cytomechanical model are thus: (i) the F-actin concentration in the cortex a(,t), (ii) the F-actin tangential velocity v(,t), (iii) the cell membrane position or the width of cell cortex annulus L(,t) measured from an virtual cell body delimited by an inner circle with radius R_0, with L(,t)<< R_0 (Alt and Tranquillo, 1995).

The spatio-temporal evolution of these three variables is given by a system of three partial differential equations which define respectively:

variations of cortical F-actin concentrations, where the net rate of actin polymerisation/depolymerisation depends on the local value of F-actin concentration relatively to the chemical equilibrium value a*.

$$\frac{\partial(L.a)}{\partial t} + \frac{\partial(L.a.v)}{\partial q} = h.L.(a^* - a)$$

the balance of forces applied on the cell cortex in the radial direction. The model takes into account a viscous friction of the cell protruding over the rigid substratum, with coefficient $_1$, the intracellular hydrostatic pressure $_1$, the resistive elastic stress of the cell CSK controlled by the elasticity coefficient $_1$ and a curvature-dependent stress due to the surface tension of the cell cortex modulated by the coefficient $_1$.

$$a.f_1\frac{\partial L}{\partial t} = b_1 - g_1.L.a + \frac{\partial}{\partial q}(t_1.a.\frac{\partial L}{\partial q})$$

the balance of forces in the tangential direction. It includes the frictional drag of the actin cortex moving in the viscous cytosol, with magnitude controlled by the drag coefficient $_0$, a viscous stress with viscosity coefficient and the membrane curvature induced stress with coefficient $_0$.

$$a.f_0.\mathbf{v} = \frac{\partial}{\partial q}[m_0.a.\frac{\partial v}{\partial q} + s_0(a,a_{sat}) - \frac{\partial}{\partial q}(t_0.a.\frac{\partial L}{\partial q})]$$

In addition, the contractile stress of the actomyosin network is modelled by the non-linear function σ_0 (a(,t),a_{sat}). Two mechanical states can be distinguished according to the value of the network F-actin concentration a(,t)). At low concentration values (a(,t)< a_{sat}), the contractile stress increases, while above the saturation threshold a_{sat}, the contractile stress decreases exponentially as a consequence of the network swelling. The non-linear function σ_0 (a(,t),a_{sat}) proposed by Alt and Tranquillo (1995) is the following:

$$\sigma_0(a,a_{sat}) = y_0.\,a^2.\exp(-a/a_{sat})$$

where the coefficient ~ controls the magnitude of the contractile stress.

The existence and properties of protruding and retracting cell membrane protrusions are related to oscillatory solutions of the cytomechanical model. This theoretical analysis is performed in a standard way by looking for critical values of the model's parameters above which small random perturbations are amplified (Hopf bifurcation) until a coherent spatio-temporal pattern emerges with typical unstable modes or wave length (Fig. 5). As it may be expected intuitively, high values of the cell cortex surface tension reduce the number of cell protrusions whilst high values for the contractile efficiency of the actomyosin network contractility increase the number of oscillatory cell protrusions by favouring the destabilisation of higher unstable modes (Fig. 5 right). In an extension of this model by Stephanou et al. (2002b), the influence of extra-cellular factors on protrusivity dynamics and cell migration have been analysed.
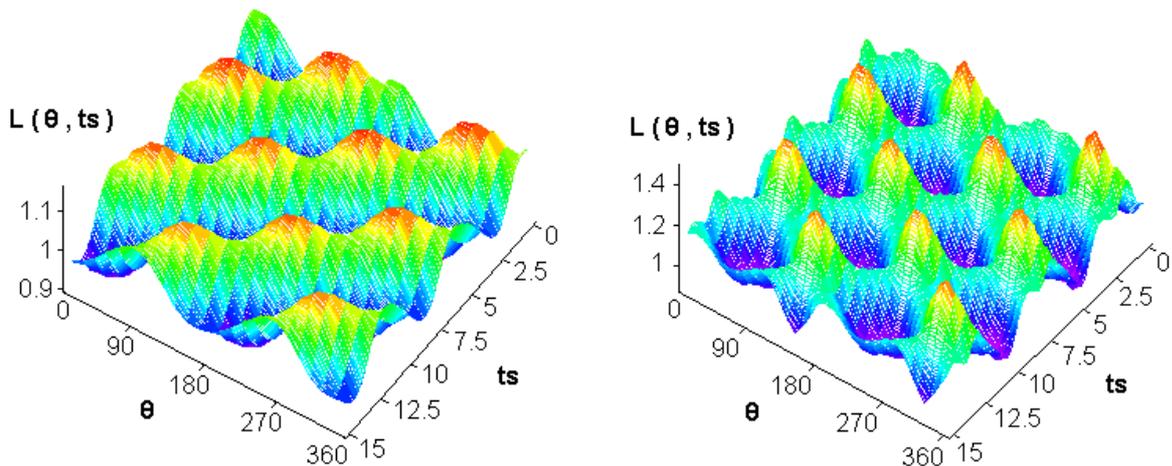
Figure 5 : Simulated evolution with time t_s of the length L(, t_s) of the cell protrusion along the cell periphery for different unstable spatial modes m. Left : mode m=2 corresponding to a single protrusion with apparent travelling motion around the cell body. Right : mode m=4 simulating oscillatory changes of cell shape with two protrusions alternatively occurring along to perpendicular directions.

4. Artificial tensegrity

Another approach to studying the coupling between mechanical forces and cytoskeletal dynamics is to construct an *in silico* system in which populations of artificial cells containing different proteins with cytoskeletal properties are subjected to selection for resistance to hydrostatic pressure. By allowing mutations to alter the properties of the proteins and by selecting the surviving cells, it might be expected that one or more types of CSK would evolve. In essence, the idea is to explore the parameters underlying the formation of tensegrity structures by feeding artificial cells and selecting for those that evolve the best structures. In our project, the initial cell consists of a lipid membrane in the form of a monolayer and several types of proteins, the membrane is under pressure and membrane units can diffuse.

Two cases are explored:
1.   The cell does not grow but there is either turnover of cellular constituents or migration of the entire cell.
2.   The cell grows. The cell is fed by the random insertion of proteins and lipids.
The following rules are applied:
1.   Turgor pressure results from the difference in concentration of molecules between the outside and the inside of the cell.
2.   A cell is maintained until it lyses where lysing is defined as having a breach in membrane integrity that cannot be repaired within a certain period.
3.   Two cells are compared and the one retained is either the one that lasts the longer or the one that maintains the higher turgor pressure or the one that can migrate.
4.   Mutations are made by introducing new types of proteins.
5.   Components that are used are less likely to be discarded than those that are not used (use is defined as forming part of a structure under tension or compression)

Components include:
   Membrane proteins that respond to curvature and to which other proteins can bind
   Proteins that cause filaments to branch
   Proteins that cross-link filaments
   Lipids of two types (cone and inverted cone) that form a monolayer.
   Calcium (in the form of a gradient that is higher outside the cell)
Variables include:
   Binding affinities that may depend on the tension in the system or on activation by another protein (equivalent of post-translational modification)
   Proteases
Mechanical aspects:
   Turgor is calculated from the density of molecules within the cell. Individual molecules produce more turgor than those that are in the form of polymers or aggregates (molecules with no free spaces around them generate no turgor). This turgor then acts at the membrane. The membrane can deform by movement of lipids normal to its plane.

At this stage, artificial tensegrity is simply a gedanken or thought experiment Nevertheless, several testable predictions can be made. Firstly, large cells should have less problem with turgor pressure than small cells. Secondly, calcium should have an important role in strengthening the CSK to resist lysis (hence a small leak is self-repairing). Thirdly, cells that do not grow or move have a CSK parallel to the membrane whilst those that can grow or move have one that is perpendicular. Fourthly, there should be a reserve pool of lipids and cytoskeletal components near the membrane in readiness for incorporation in the membrane in time of need.

5. Discussion

This brief presentation underlines the advantage and limitations of continuous versus discrete modelling approaches to cell behaviour. Continuous models can account for a large variety of cellular dynamics including the protrusive activity which is coupled to modifications of continuous mechanical properties such as membrane tension, cell cortex viscoelasticity or mechanical stresses developed by the F-actin network.. However, a more refined description of CSK organisation, including the orientation of filaments or the formation of stress fibres, is hardly compatible with a continuous formulation, although recent models bridge the gap between mesoscopic mechanical properties of the actin cortex and a description at the molecular level (Maly et al, 2001). Models for cell dynamics, including lamelipod formation, modification of the cell architecture or cell adhesion to a substrate should incorporate actin dynamics to allow cell reshaping and motility in response to extracellular signals. Conversely, discrete tensegrity models seem to provide a more adequate description of the cell as a physical object, as evidenced by the larger number of criticisms encountered by these models compared to others.

Indeed, the tensegrity paradigm is still a matter of active controversy, as illustrated by recent papers (Ingber et al., 2000; Wang et al., 2001). For D. E. Ingber, the intransigence of the remaining critics seem to " … largely result from an overly strict definition of what tensegrity is and how it can be applied" (Ingber et al., 2000). As quoted above, the tensegrity model states that: (i) cells and tissues exhibit integrated mechanical behaviour through use of specific structural principles, namely the discontinuous-compression / continuous-tension construction submitted to a pre-existing tension or prestress (Pourati et al., 1998), and (ii) the cell has an elastic submembranous skeleton with its associated lipid bilayer which can be linked to the internal CSK depending on type of cell adhesion.

Can we propose experiments to discriminate between the models? It seems worth reporting here the different interpretations of similar experiments such as the induction of cell deformation through a direct manipulation of transmembrane receptors. According to Ingber and col., application of mechanical stresses to integrins using surface-bound micropipettes pre-coated with fibronectin induces CSK reorganisation, nucleus elongation along the tension lines as well as reorganisation within nucleoli, i.e. deep inside the nucleus (Maniotis et al., 1997). On the other hand, and as expected, such a reorganisation was not observed when similar mechanical stresses were applied to membrane receptors which are only linked to the submembranous F-actin CSK.

Opposite conclusions were drawn by Heideman and col. (1999; in Ingber et al., 2000) based on the application of similar mechanical stresses to integrin membrane receptors with glass needles treated with laminin, an ECM adhesion protein. Formation of an actin spot was observed on the cytoplasmic side of the membrane, inducing a locally high deformation of the membrane rather than a global change in cell shape. Heideman and colleagues thus conclude that the elastic cortical CSK is not connected to the internal microtubule cytoskelton, which is in complete disagreement with the fundamental tenet of tensegrity.

Analysing the argument in detail is beyond the scope of this paper. Maybe we should simply mention that Ingber's reply is that experiments showing a lack of action at a distance when pulling on the cell via integrins *before* focal adhesion formation are not valid as proofs of the failure of the tensegrity model. It is certainly clear that the tensegrity paradigm highly stimulates both theoretical and experimental work, including the development of new physical methods of quantification. For example, this has led to experiments to determine the significance of the compression of microtubules compression for cell mechanics. Ingber (Ingber et al., 2000) reported that microtubules counterbalanced approximately one-third of the total cellular prestress within a cell whose activation has been stimulated by histamine, a chemical constrictor, whilst noting that cell attachment to a rigid substratum would decrease this prestress level. Prestress within the cell can be estimated from microbeads displacement when deformable polyacrylamide gels containing small fluorescent microbeads are used as cell

culture substratum, but looking for other quantification methods is the subject of current investigations.

From a theoretical point of view, a clear advantage of the tensegrity model paradigm is to provide an alternative view to cell engineering models that would only describe cell mechanical behaviour by ad hoc "data fitting" models, based for example on combination of rheological elements. Trough the cell tensegrity model, more specific questions can be addressed regarding specific cell behaviours such as strain-hardening or CSK stiffness (Volokh et al., 2000; Wendling et al., 2000), or the process of mechanotransduction. In the latter case, tensegrity-based predictions can be compared to theoretical predictions inferred from other cell models like the percolation model of Shafrir et al. (2000). This possibility of exploring various mechanistic hypotheses is a real advantage of the tensegrity model when compared to other cell simulation models such as Electronic Cell (http://eeell.org/) or Virtual cell () where cytomechanical parameters are absent from the theoretical framework. That said, the dynamic remodelling of the cell is not yet taken into account in the current tensegrity model. This is one of the requirements for future modelling work in which the integration of mechanical and biochemical properties may provide an appropriate framework to simulate cells in a way that may ultimately enable a better interpretation of genomic data..

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