Emergent features of cell structural dynamics: a review of models based on tensegrity and nonlinear oscillations

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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; Tabony et al., 2002). Indeed, direct application of mechanical stresses to cultured cells can induce or modify cell differentiation, growth and migration as well as gene expression (Schmidt et al., 1998; Fujisawa et al., 1999; Wang et al., 2000; Meyer et al., 2000; Chen et al., 2001). Despite this progress, we do not fully understand how individual cells perceive and orchestrate mechanical signals to respond either individually at the level of the cell itself or collectively, via cooperative processes, at the level of the tissue.

The challenge that confronts cells is how to integrate the information from many kinds of signals so as to permit a single appropriate response. Among these different cell signalling mechanisms, mechanotransduction, i.e. the conversion of a mechanical signal into a biological or biochemical response, enjoys a special status (Wang et al., 1993). This is because it relies on the structure of the cell, which is a global property, as opposed to its biochemistry, which is a local property (and which can be restricted for example, to the cytoplasmic membrane or an intracellular organelle). Mechanotransduction 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 allow a specific physiological or pathological cellular response (Lelièvre et al., 1996; Janmey, 1998). The importance of mechanotransduction makes it crucial to develop theoretical cytomechanical models of the living cell.

We explore here some of these cytomechanical models and pay special attention to models of cell tensegrity, as proposed by Don Ingber (1993; 1997), although we also examine alternative models considering either the cell are a mechanical (viscoelastic) continuum, or more simply the spatio-temporal organization of structural elements of the cell cytoskeleton (CSK) like filamentous actin. All models should help to understand how the cell architectural properties are dynamically regulated and provided a support to the transduction of intracellular signals and mechanical forces that lead to a wide range of integrated cellular responses. These modelling approaches are based on the conviction that it is essential to model how cells dynamically stabilise and self-organise their structure and shape if we are to

understand how they sense their physical microenvironment and respond to mechanical signals through bi-directional signalling pathways that connect the plasma membrane to the nucleus. In this framework, one expect a strong connection between the CSK remodelling and the control of gene expression.
With these considerations in mind, this paper reviews a representative range of discrete and continuous cytomechanical models of cells. We first explore the properties of purely structural models. We then present discrete and continuous models which investigate the self-organisation of the cell structure induced by nonlinear biochemical and biophysical processes, possibly coupled with changes of cell mechanical properties.

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 explained by Ingber, this proposition draws on the architectural principles developed for the construction of buildings by Buckminster Fuller and K. Snelson; these principles underlie the organisation of structures as diverse as geodesic domes and viral capsids.

It is worth emphasizing in this introductory section that the holistic approach proposed by Ingber has deep conceptual implications. First, it again draws attention to the need for unifying principles to interpret the plethora of biological data collected successfully 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.

What is the definition of a tensegrity system? 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 assumptions. The first assumption is that cell shape is stabilized by an internal, mechanically active, structure, the CSK. 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 cells 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. Recent works have shown a strong

correlation (a potential regulation) between the surface of such contacts and the amplitude of the transmitted forces (Balaban et al., 2001; Tan et al., 2003). The FACs include not only transmembrane proteins, mostly integrins, but also a scaffold of actin-binding proteins (talin, vinculin, α-actinin, paxillin, …) which form a molecular bridge between the CSK and the intracellular part of the integrin. Similarly, specific cell-cell adhesion molecules (CAMs), like cadherins, selectins, cathenins, …) ensure the transmembrane coupling to the CSK of neighbouring cells 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 mimics 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 whether 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 modulus

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:

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

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 modulus derived from the stress-strain relation ship. 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 leads 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 modulus, the integrated response is non-linear with apparent elastic modulus that is 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.

In a recent work, Canadas et al. (Canadas et al., 2002) used the same tensegrity model to analyse the viscoelastic response of the CSK. Their tensegrity structure still includes 6 bars, but now the 24 cables are viscoelastic elements modelled as Voigt bodies (elastic element in parallel with viscous dashpot). This time dependence of cables rheology introduces into the above constitutive equation an additional global-damping matrix [C] which, assuming small displacements, modelled the proportionality of external forces to the rate of nodal displacements {u'}.

$$\{\mathbf{F}\} = [\mathbf{K}]\{\mathbf{u}\} + [\mathbf{C}]\{\mathbf{u'}\}$$

Some of the results obtained with the viscoelastic tensegrity structure are qualitatively similar, with an increase of the normalized apparent elasticity modulus E* with increasing global strain ε_i (strain hardening). On the other hand, the normalized viscosity modulus varies slowly with ε_i and remains almost independent of changes in T*, the normalized basal internal tension of the tensegrity structure. This is not the case for E*, which increases approximately with the square root of T*. Let us also mentioned the recent work of Coughlin and

Stamenovic (Coughlin and Stamenovic, 2003) which explore the simulated mechanical response of a tensegrity cell model probed by current cytomechanical methods such as twisting magnetocytometry (Laurent et al., 2002).

The so simulated stiffening response of cells is in agreement with biological experiments (Wang et al., 2002; Stamenovic et al., 2002, 2003; Laurent et al., 2002, 2003). However, extended validation is very difficult since the CSK is not a fixed scaffold, but a dynamic network that can be modulated by internal and external clues. Some of these dynamical aspects will be addressed in section 3.

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., 2003) 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 cell0 is defined as a strict elastic discrete envelop, with no internal links. In cell1, we considered a reinforcement of the cell architecture with horizontal elastic cross-links modelling CSK fibres. Finally, cell2's architecture includes internal diagonal elastic links connecting the apical and basal physical cell surfaces.

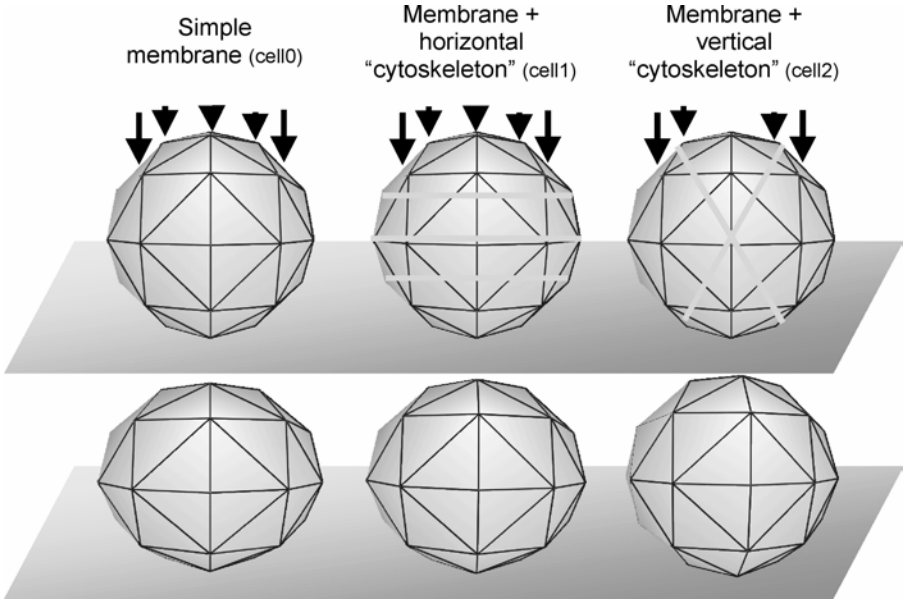


Figure 1: Influence of the "cytoskeleton" of the virtual on the mechanical response to vertical load. Three cell types are considered: no "cytoskeleton" (cell0), horizontal links (cell1), diagonal links (cell2). The first raw presents the initial 3D shape of each cell prototype. For a given fixed value 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 *Fs* 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_{elas} and the modulus of F_S. Figure 2 shows that k_{elas} could be chosen in order to model the linear part of the erythrocyte response, but also the nonlinear stiffening of the cell for forces up to 15pN.

The microplates 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: virtual plasma and nucleus membranes, both of them being represented by triangulated surfaces.

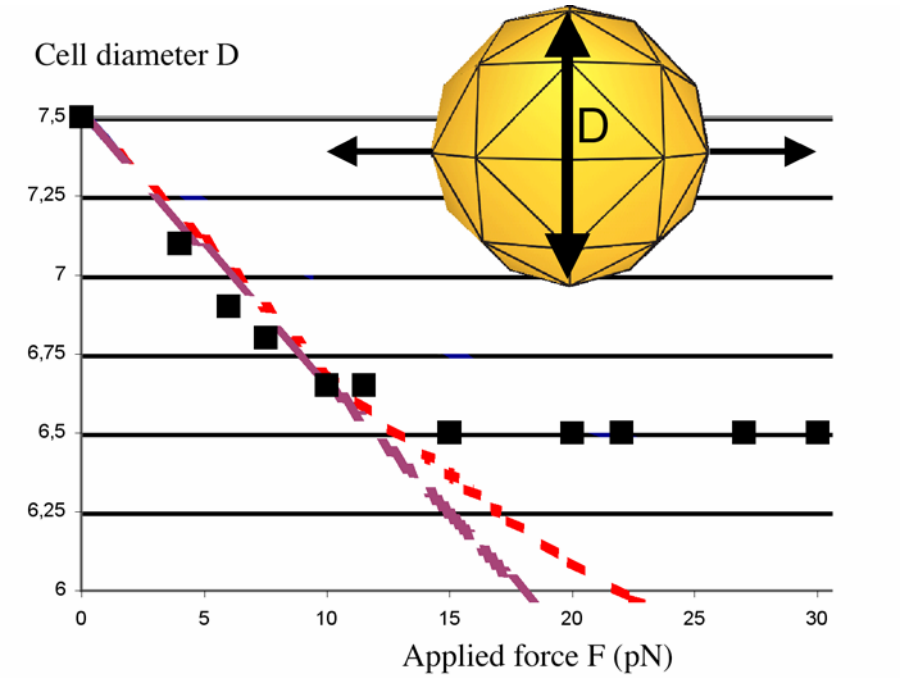
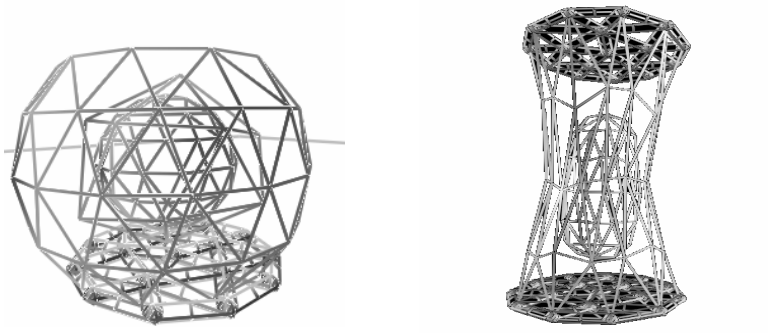


Figure 2: Simulated virtual spherical red blood cell (RBC) suspended in an hypotonic solution. Optical tweezers double trap is simulated by exerting locally a force F_S on two opposite nodes of the cell object contour (upper insert). The variation with load of the cell object diameter D(F_S) (in μm) 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.

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 on the right of figure 3.

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 indicates 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., 2003)

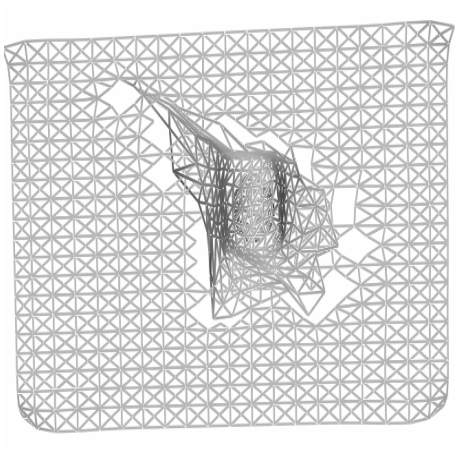


Figure 4: Simulated contraction of an elastic substratum by a virtual adherent fibroblast linked to this extracellular film by localised "focal contacts".

3. Oscillating cellular deformations and 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 changes in cell shape. 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 known 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 the filamentous actin (F-actin) network. It was once widely held 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 image 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 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 periods of 8-10s that are 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 in generating testable predictions.

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 activates 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 results in the gel swelling 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 (see also section 3.7). 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 into the cell 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 and 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) reconsidered this Brownian ratchet mechanism this model by assuming that the protrusion formation is more likely driven by the thermal bending undulations of semi-stiff F-actin filaments (Molginer and Oster, 2003a). This "elastic Brownian ratchet" mechanism has been recently reviewed (Molginer and Oster, 2003b) in order to account for the fact that actin filaments attach to the bacterium surface during actin-based propulsion of the bacterial pathogen *Listeria* (such bacterial cells being considered as simplified model systems for identifying essential factors of eukaryotic cell motility, see section 3.3 below). In an alternative work also considering the elastic polymerisation ratchet mechanism as the mechanical basis of cell protrusion, Molginer and Edelstein-Keshet (Molginer and Edelstein-Keshet, 2002) developped a reaction-diffusion-advection model which capture most of the biochemical (nucleation, growth, capping and depolymerisation of actin filaments) and geometrical aspects of actin dynamics in a cell protrusion. The prediction

of their model is that an optimal density of filaments barbed (fast-growing) ends is needed to insure a maximal protrusion velocity of the cell membrane. From available data, these authors derived an estimate protrusion velocity in the order of 0.4 μm/sec for a membrane resistance of 50 pN/μm, falling to 0.25 μm/sec when the membrane resistance is two times higher. They further speculate that the cell regulates its motility as well as its direction of motion by rapidly increasing the local density of uncapped barbed ends (through Arp2/3 activation, filaments uncapping, ...) and decreasing locally cell membrane resistance (disruption of cell cortex links to the membrane). However, a number of simplifications are likely to limit the range of validity of the results: one of them is the oscillatory feature of cell protrusions, which will be addressed in section 3.6.

3.3. Alternative to the ratchet model.

The pathogen bacterium *Listeria monocytogenes* uses actin polymerisation 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 ActA controls the activity of the complex Arp2/3 that initiates actin polymerisation (Welch et al., 1997). Actin dynamics is also controlled by an actin depolymerising 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 $\mu m.s^{-1}$) and was extended to explain the hopping motion observed in a *Listeria* mutant

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

3.4.1. 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 polymerisation/depolymerisation balance at the filament ends and the role of proteins in inducing actin polymerisation (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. $k_B^+(C_{eq} - C_{eq,B}) = -k_P^+(C_{eq} - C_{eq,P})$) 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 signalling (Machesky et al., 1999; Mullins, 2000) and movement generation (Borisy et al., 2000).

3.4.2. Models for actin network formation

In view of the complexity of the dynamics of actin networks, models were first formulated to analyse the polymerisation and fragmentation of isolated actin filaments *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 the formation of network. A further advance was made with the study of actin bundle formation using the kinetic approach (Edelstein-Keshet, 1998). This model was refined to explain 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 polymerisation or actin filament association in the absence of geometrical or mechanical constraints. A recent attempt to address the more complex question of the generation of actin networks was done by Maly and Borisy (2001) who developed a model for this generation as a process of self-organization. They were able to account for the preferential direction of the actin filament bundle observed *in vivo*. Finally, the analysis of actin gel formation on the surface of beads provided both experimental and theoretical insights into the regulation of actin networks in cells (Noireaux et al., 2000).

Models for spatio-temporal F-actin interactions *in vivo* were developed by LeGuyader 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 which takes into account free (Z(x,t) variable) and membrane-bound (Y(x,t) variable) F-actin as well as nucleation proteins (X(x,t) variable) along the antero-posterior axis which connects the two poles of the cell. The model equations are as follows:

$$\begin{cases} \frac{\partial X(x,t)}{\partial t} = K - \alpha XY + D_1 \frac{\partial^2 X}{\partial x^2} \\ \frac{\partial Y(x,t)}{\partial t} = \alpha XY - \beta YZ + D_2 \frac{\partial^2 Y}{\partial x^2} \\ \frac{\partial Z(x,t)}{\partial t} = \beta YZ - \gamma \frac{Z}{(\delta + Z)} + D_3 \frac{\partial^2 Z}{\partial x^2} \end{cases}$$

with zero-flux boundary conditions.

This model assumes the existence of non-linear reactions in which both the synthesis of F-actin and transformation of membrane-bound into free actin are autocatalytic. Furthermore, the diffusion coefficient of actin microfilaments bound to the membrane is very low, 4.10^3 times less than diffusion of nucleation proteins. Free actin microfilaments are partly integrated in the constriction ring, where they participate with myosin to the oscillatory

cell contraction. The above1D model generates oscillatory actin waves between the two poles of the cells (LeGuyader and Hyver, 1997). A still more representative aspect of this oscillatory dynamics is obtained by considering a 2D representation of the unfolded 3D cell cortex, projected onto a 2D surface. We simulated the behaviour the above model in such a 2D space, with additional scaling of the cortex deformation in the y direction by the F-actin concentration of the constriction ring (Figure 5). We thus obtained a back and forth motion of the constriction ring along the cell.

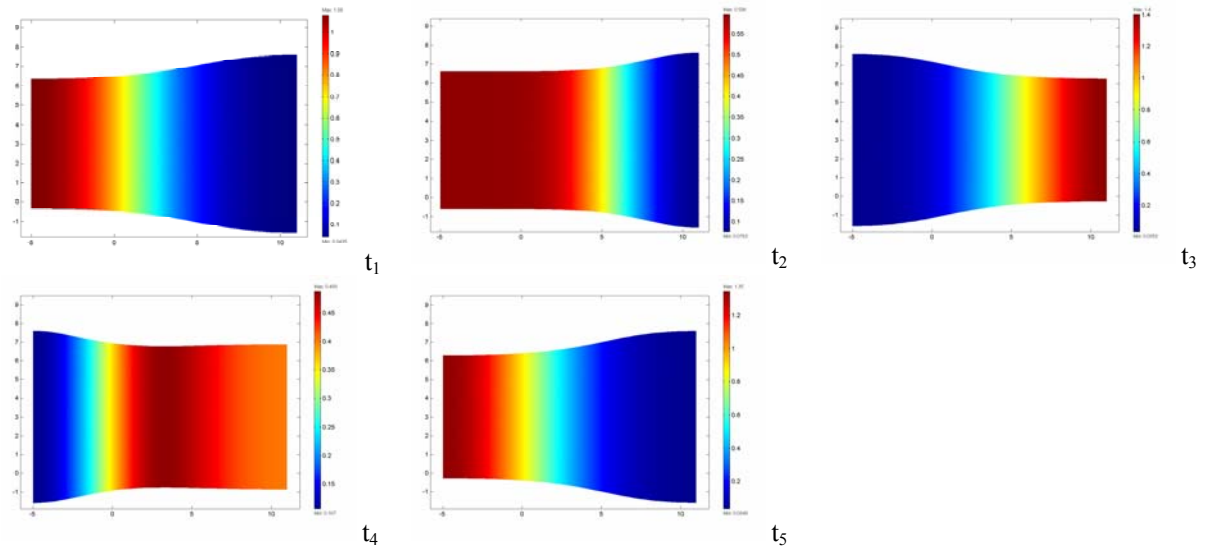


Figure 5: Simulated evolution of the back and forth propagation of the F-actin concentration Z(x,y,t) between the two cell poles. The period is about 10 time units and five consecutive times are presented. Constriction of the projected 2D cell cortex is obtained by rescaling the y dimension with the Z values along the x axis (see plate 25).

This theoretical behaviour is in agreement with the experimental work of Bornens et al. (1989) and with 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; Vicker, 2002).

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, 2003).

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., 2003, Fig. 6). 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 a three-dimensional free-boundary problem. However, for cells cultures on a rigid substrate, a simpler one-dimensional problem can be considered by considering the cell surface projection on the substrate and by further 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,θ), the remaining variables in the cytomechanical model are then: (i) the F-actin concentration in the cortex $a(\theta,t)$, (ii) the F-actin tangential velocity $v(\theta,t)$, (iii) the cell membrane position or the width of cell cortex annulus $L(\theta,t)$ measured from an virtual cell body delimited by an inner circle with radius R_0, with $L(\theta,t) << R_0$ (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 η of actin polymerisation/depolymerisation depends on the local value of F-actin concentration relatively to the chemical equilibrium value a*.

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

- 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 ϕ_1, the intracellular hydrostatic pressure β_1, the resistive elastic stress of the cell CSK controlled by the elasticity coefficient $\gamma_1$and a curvature-dependent stress due to the surface tension of the cell cortex modulated by the coefficient τ_1.

$$a.\phi_1 \frac{\partial L}{\partial t} = \beta_1 - \gamma_1.L.a + \frac{\partial}{\partial \theta}\left(\tau_1.a.\frac{\partial L}{\partial \theta}\right)$$

- 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

ϕ_0, a viscous stress with viscosity coefficient μ and the membrane curvature induced stress with coefficient τ_0.

$$a.\phi_0.v = \frac{\partial}{\partial\theta}\left[\mu_0.a.\frac{\partial v}{\partial\theta} + \sigma_0(a,a_{sat}) - \frac{\partial}{\partial\theta}\left(\tau_0.a.\frac{\partial L}{\partial\theta}\right)\right]$$

In addition, the contractile stress of the actomyosin network is modelled by the non-linear function σ_0 (a(θ,t), a_{sat}). Two mechanical states can be distinguished according to the value of the network F-actin concentration a(θ,t)). At low concentration values (a(θ,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(θ,t), a_{sat}) proposed by Alt and Tranquillo (1995) is the following:

$$\sigma_0(a,a_{sat}) = \psi_0.\, a^2.\, \exp(-a/a_{sat})$$

where the coefficient ψ_0 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. 6). 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. In an extension of this model (Stephanou and Tracqui, 2002), the influence of extra-cellular factors on protrusion dynamics and cell migration has been analysed.

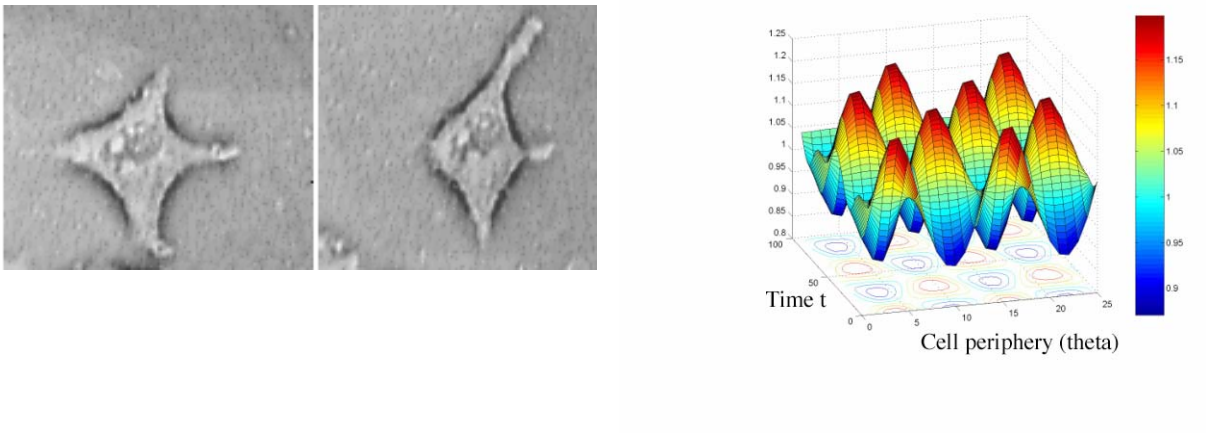


Figure 6: Spontaneous oscillatory protrusions of a L929 fibroblast observed at two consecutive times (left) and simulated (right) evolution with time t_s of the length L(θ, t_s) of the cell protrusion along the cell periphery (angular position θ) for an unstable spatial mode m=4 corresponding to oscillatory changes of cell shape with two protrusions occurring alternatively at perpendicular directions (see plate 26).

3.7. Modelling neuron actin-driven deformation

Neuron plasticity presents different cases of cell deformation coupled to neuron development, such as axon guidance and spine formation during neuron potentiation (Engert and Bonheoffer, 1999, Da Silva and Dotti, 2002, Colicos et al., 2001, Matus, 2000). Basically, neuron actin-driven deformation is under the control of two intracellular signalling pathways mediated, respectively, by RhoA and Rac1/Cdc42 (see e.g. Ridley, 2001, Dickson, 2001). Although both can influence the actin polymerisation/depolymerisation process, they have opposite effects on cell modification. In addition, actin dynamics regulation by proteins such as ADF/Cofilin is also involved in the progression of diseases (Alzheimer); some evidences suggest that altered production and/or localization of these proteins leads to cognitive impairment (Bamburg and Wiggan, 2002).

In a recent study, N. Huc et al. (2002) considered a model for cell shape modification accompanying neuron potentiation or development (Huc et al., 2002). This model is based on a set of ODE for the signalling routes from the calcium influx to actin polymerisation activation (signalling dynamics). In a second step, we considered the mechanical properties of a dendrite.

Signalling dynamics. Basically, we use a simplified version of the model by Zhabotinsky (2000) for auto-phosphorylation of the CaMKII in response to calcium influx. In the original model, the CaMKII is a decameric enzyme and 11 states are possible, depending on the degree of subunit phosphorylation. In the present work, we simplify the equations so that the kinase is represented by a low, medium and highly phosphorylated state, denoted C_0, C_1 and C_2. The phosphatase PP1 and its inhibitor I1 are also considered. Calcium induces the dephosphorylation of the active form of the inhibitor through calcineurin activation. In contrast, the kinase A (PKA) phosphorylate the inactive form of the inhibitor.

$$
\begin{aligned}
\frac{dC_0}{dt} &= -K_1\left(\left[Ca^{2+}\right],\{C_i\}\right)C_0 + K_2\left(\left[Ca^{2+}\right],\{C_i\},P\right)C_1 \\
\frac{dC_1}{dt} &= K_1\left(\left[Ca^{2+}\right],\{C_i\}\right)C_0 - K_2\left(\left[Ca^{2+}\right],\{C_i\},P\right)C_1 \\
&\quad - K_3\left(\left[Ca^{2+}\right],\{C_i\}\right)C_1 + K_4\left(\left[Ca^{2+}\right],\{C_i\},P\right)C_2 \\
\frac{dC_2}{dt} &= K_3\left(\left[Ca^{2+}\right],\{C_i\}\right)C_1 - K_4\left(\left[Ca^{2+}\right],\{C_i\},P\right)C_2 \\
\frac{dP}{dt} &= -k_i PI + k_{-i}\overline{P} \\
\frac{dI}{dt} &= -k_i PI + k_{-i}\overline{P} + k_{PKA}\bar{I} - k_{CaN}\left(\left[Ca^{2+}\right]\right)I
\end{aligned}
$$

C_0, C_1 and C_2 refer to low, medium and high phosphorylation states of the multimeric CaMKII (Zhabotinsky, 2000); P is the phosphatase PP1 which is inhibited by I1 (ki and k-i are the association and dissociation rates.) CaMKII autophosphorylation is triggered by calcium (via the calmodulin.) Dephosphorylation depends on the phosphatase (PP1.) Kinetic rates *Ki* are non-linear functions of calcium, the different forms of the CaMKII and the phosphatase *P*; k_{PKA} and k_{caN} are the PKA-dependent phosphorylation and CaN-depend dephosphorylation rates of the inhibitor.

Mechanics. We assume that the material in the dendrite can be described in the framework of the elasticity equations. The force equilibrium inside the dendrite section reads:

$$\text{in the dendrite} \quad : \quad \rho \frac{d^2\mathbf{u}}{dt^2} = \nabla.\boldsymbol{\sigma}(\mathbf{u}, E(C_2))$$
$$\text{on the membrane} \quad : \quad \boldsymbol{\sigma}(u, E(C_2))\mathbf{n} = p(C_2, t)\mathbf{n}$$

$\boldsymbol{u}$ is the displacement of the material; $\boldsymbol{\sigma}$ is the stress tensor; $\mathbf{n}$ is the outward normal vector; E is the Young modulus of the material. We assume that the active form of the CaMKII (C_2) is responsible for a pressure term exerted on the material at the boundary. To connect the biochemical activation of CaMKII and the mechanics of spine protrusion, we assume that the kinase has a dual role. First, as suggested by experimental models, actin is directly involved in membane protrusion (Borisy and Svitkina, 2000.) Thus, we suppose that CaMKII activity controls a pressure p which is given by the first differential equation. In addition, there is a wealth of experimental evidence that cell filaments are severed or capped by proteins whose activity is Ca^{2+} or kinase dependent (Kwiatkowski, 1999.) Therefore, we suppose that CaMKII may control the material stiffness according to the second equation below.

$$\tau \frac{dp(C_2,t)}{dt} = P_0 f_2(C_2) - p(C_2,t)$$
$$E(C_2) = E_0 - (E_0 - E_1) f_1(C_2)$$

In the above equations, τ is a relaxation time; P_0 is the maximal pressure exerted on the membrane; f_2 is a Hill function of degree 6; E_0 and E_1 are, respectively, the minimum and maximum Young modulus of the material; f_1 is a Hill function of degree 2. The stress tensor $\boldsymbol{\sigma}$ is related to the displacement $\boldsymbol{u}$ and to the Young modulus E by the classical constitutive relation of the elasticity theory (Landau and Lifchitz, 1967):

$$\boldsymbol{\sigma}(\mathbf{u}) = \lambda Tr(\boldsymbol{\varepsilon})\mathbf{I} + 2\mu\boldsymbol{\varepsilon}$$

where $\mathbf{I}$ is the identity matrix; $\mathrm{Tr}(\boldsymbol{\varepsilon})$ is the trace of the strain tensor $\boldsymbol{\varepsilon}$; λ and μ are the Lamé coefficients:

$$\lambda = \frac{E(C_2)\nu}{(1-2\nu)(1+\nu)} \quad ; \quad \mu = \frac{E(C_2)}{2(1+\nu)}$$

where ν is the Poisson coefficient of the material. The strain tensor $\boldsymbol{\varepsilon}$ is the symmetric part of the gradient of the displacement, $\boldsymbol{u}$, which reads:

$$\boldsymbol{\varepsilon} = \frac{1}{2}\left(\nabla\mathbf{u} + {}^T\nabla\mathbf{u}\right) = \frac{1}{2}\left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}\right)$$

Illustration. We consider a disk representing a dendrite section; the calcium influx is at the top of the disk. As shown in figure 7, the simulation of the above equations gives rise to a membrane protrusion (figure 7, left panel). Assuming that CaMKII controls the cytoskeleton mechanical properties (cf. the above section on mechanics), the dendrite protrusion is larger (figure 7, right panel).

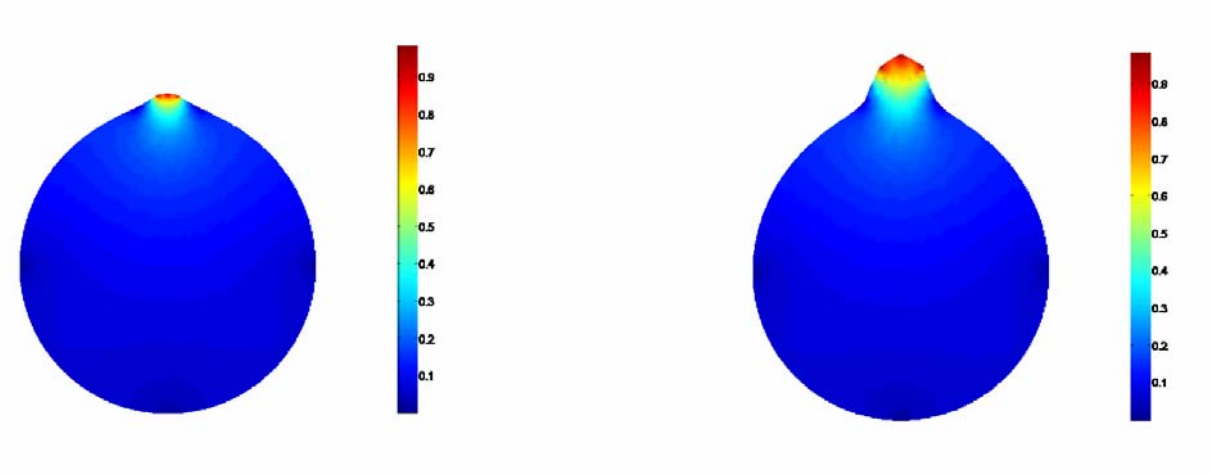


Figure 7: Force generation and membrane deformation. CaMKII-dependent induction of a stable spine protrusion in a dendrite. Active CaMKII generates a 5.5 pN force at the top of the dendrite section. The colormap code for the active CaMKII fraction. A noticeable protrusion is observed when the Young modulus of the cell decreases with CaMKII activity (compare right vs. left) deformation (see plate 27).

3.8. Simulated growth of branched actin filaments

To study the assembly, the movements and the dissociation of large amount of molecules in a virtual cell a simulation program has been developed. The simulator is driven by the description of the model written in a language we have also developed. With this language, the simulation program is not only dedicated to a particular model. We will present here a simulation of the growth of branched actin filaments in a prokaryote simulated cell.

The simulation program implements a three dimensional space bounded by a spherical membrane representing the virtual cell. This cell is initially filled with a population of molecules of various types. When the simulation begins, these molecules diffuse and interact according to the reaction rules described in the model. Periodically, the simulator shows the content of the cell with a 3D *OpenGL* user interface. During the simulation, the user can rotate the cell, focus to and zoom a particular area.

3.8.1. Simulator description

The simulator is a stochastic automaton driven by reaction rules between molecules. Each molecule is represented by a record including its type, its position, a list of links to some other molecules and other internal data. The simulator keeps track of each assembly in real time from the computer point of view.
A step of simulation is the done by examining each molecule and applying the following process: (the molecule *S* is chosen randomly in order to avoid artefacts)

- Check if close enough to *S* in a location randomly chosen *L* there is another molecule *T*.
- If so, and if a reaction rule is given between a molecule of the type of *S* and a molecule of the type of *T*, this rule is applied, according to a probability representing the reaction kinetics.
- Else, molecule *S* may move to the empty location *L*, according to a probability representing the diffusion speed.

When all the molecules involved in the cell have been processed, the current step is completed and new one can begin. The step simulated time slice is set to 100 micro-seconds, which correspond to the average time for a protein to move to a distance of 10 nanometres (approximately its diameter).

3.8.1.1. The rules

The simulator implements four kinds of interaction rules between two molecules: the source *S* and the target *T*:

- **Reaction**: *S* reacts with *T* to produce two other types of molecules *S′* and *T′*.
- **Association**: *S* get bound to *T* to produce the complex *S′*-*T′*. Of course *S′* (resp. *T′*) can keep the type of *S* (resp. *T*).
- **Dissociation**: the complex *S*-*T* can break and leave individual molecules *S′* and *T′*
- **Catalyse**: the complex *S*-*T* can be transformed to *S′*-*T′*.

Each rule is given a probability of execution, which, on the long run correspond to a reaction kinetics. For the association rule, a maximum number of links can be specified.

3.8.1.2. Configuration

The simulator use a configuration file to describe the model the user wants to simulate. This file contains four sections. The first one describes the molecules involved in the model. The second specifies the diffusion rate of each molecule. The third section describes all the reaction rules which will be applied during the simulation. The last section describes the initial population and location for each kind of molecules. Here is an example of the types of molecules involved in the simulation of the growth of actin filaments:

```
molecule
      BR (0, 200, 200),      // branching protein.
      P (200, 0, 0),         // filamentous actin 'plus' end.
      M (0, 200, 0),         // filamentous actin 'minus' end.
      AF (200, 200, 0),      // actin inside the filament.
      AG (200, 100, 0);      // phosphorylated globular actin.
```

With these definitions, the *plus* end (**P**) will be displayed in red, the *minus* end (**M**) in green, the filament itself (**AF**) in yellow and the free globular actin (**AG**) in orange. The branching protein (**BR)** will be displayed in cyan.
In this example, only the free G-actin molecules and the branching protein can diffuse. The filaments themselves are frozen:

```
      speed (AF) = 0.0;      // diffusion speed is zero
      speed (P) = 0.0;       // for the filaments.
      speed (M) = 0.0;
      speed (AG) = 1.0;      // high diffusion speed.
      speed (BR) = 1.0;
```

The following rules show the formation of the polarised dimers from two free phosphorylated free G-actin molecules:

```
      AG  +  AG  ->   M(1) * P(1)      [0.05];
```

```
M   *  P   ->   AG   + AG        [0.5];
```

The right part of the first specify that a minus end **M** can be bound to only one plus end **P**, and conversely, a plus end **P** can be bound to only one minus end **M**. The second rule is the reverse reaction, the depolymerisation of the complex, giving back two free G-actin molecules.
The next rules show the growth of a filament from the plus end:

```
AG  +  P  ->   P(1)  =  AF(1)    [0.8];
P   *  AF ->   AG    +  P        [0.001];
```

The first rule shows how a free G-actin molecule can be bound to the plus end of an already existing filament. The equal sign in the right part of the rule specify that the link must be aligned with the filament. The second rule shows the reverse reaction, the depolymerisation from the plus end giving back one free G-actin molecule.

The next two rules show the growth of a filament from the minus end. One can notice that the polymerisation kinetic is lower than the previous one.

```
AG  +  M   ->   M(1)  =  AF(1)   [0.3];
M   *  AF  ->   AG    +  M       [0.005];
```

This last rule shows how the branching protein makes grow a filament from another one: the branching protein is bound to a filamentous F-actin molecule with an angle of 70° (this is the meaning of the slash in the right part of the rule) and become the plus end of the new filament:

```
B  +  AF  ->   P(1) / AF(1)      [0.5];
```

3.8.1.3. Initialisation

The simulation is initialised by the statement:

```
cube (0, 0, 0, 12, AG);
cube (10, 0, 0, 5, BR);
```

A cube of length *12* located at the centre of the cell *(0, 0, 0)* is filled with 12^3 = 1728 molecules of free globular actin. Another cube of length *5* located at 10 along the X axis is filled with 125 branching proteins. When the simulator is started, these molecules will diffuse and react according to the rules. Polarised dimers first assemble and then the filaments grow until one end touches the membrane or an equilibrium state is reached (see Fig. 8).

3.8.2. Simulation results

First, we have observed that the number of primary filaments depends only on the average number of dimers which in turn depends on the concentration of G-actin and on the dimerisation kinetics. Second, the lengths of the filaments are distributed in two groups: a small number of very long filaments and a gaussian distribution of the length of the other ones. Quite all the filaments are stopped by the membrane of the cell. As the polymerisation speed is greater for the plus end, this end is statistically the one which touches the membrane.

The other end is growing and shrinking and reaches a stationary state (depending again on the concentration of G-actin and on the polymerisation kinetic).

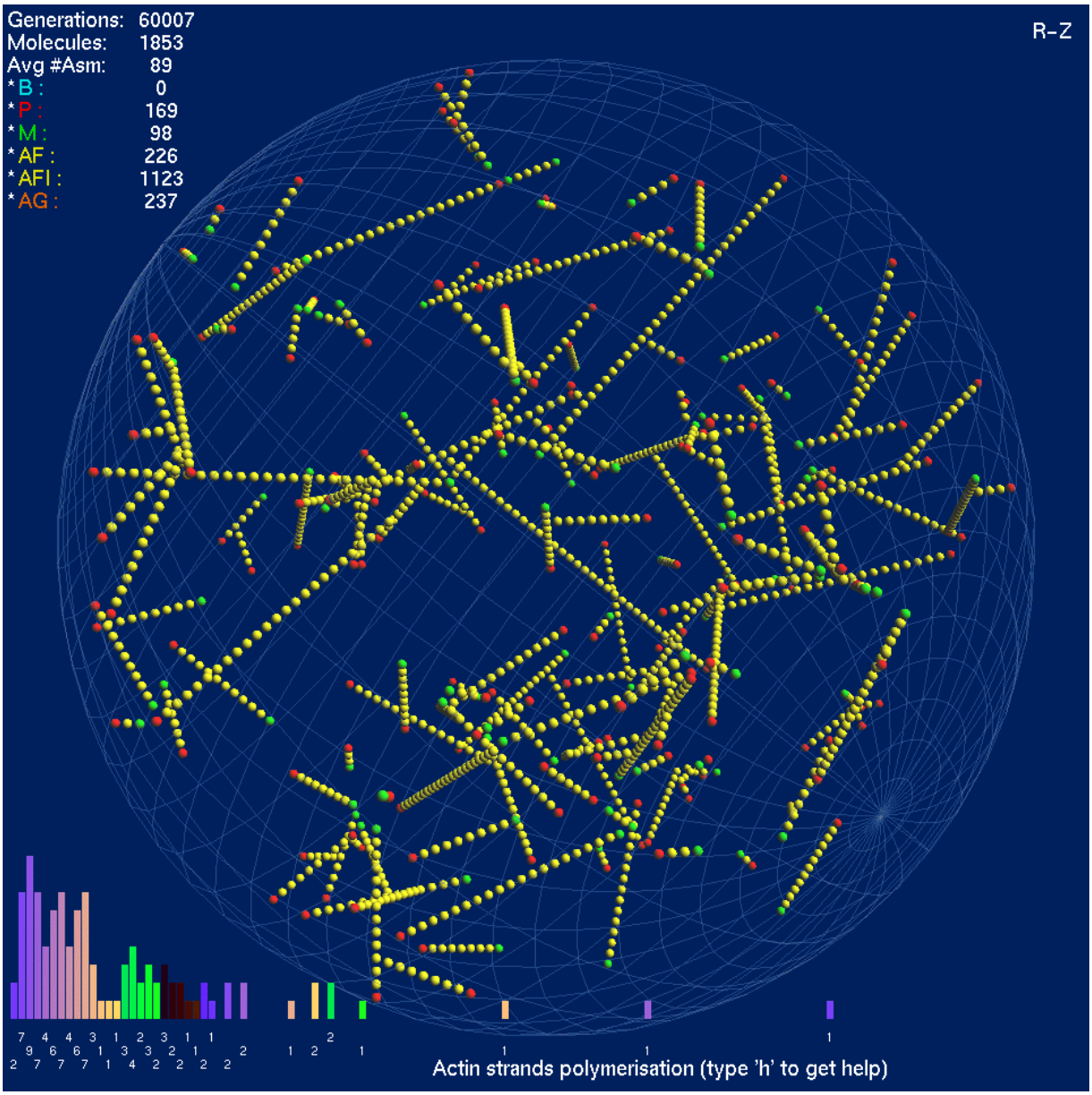


Figure 8: A view of the virtual cell filled with growing actin filaments. On the bottom left of the screenshot there is a histogram of the lengths of the filaments (see plate 28).

The long filaments are those which pass near the centre of the cell, some of them extends to the membrane by their two ends. The small ones are close to the membrane and nearly parallel to it. In this particular simulation, the branching protein is removed just after initiating a new branch of actin. As a consequence, the number of branches is at most equal to the number of branching proteins. It may have fewer branches because the depolymerisation process can crunch a whole branch. Because of the presence of the membrane, the concentration of branching protein is a bit higher near the membrane than in the centre of the cell. This is the reason why the filaments have branches more often near the membrane than in the centre of the cell.

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. 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.
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)
6. Tension in polymers can rupture links or increase lateral interactions (by 'deforming' constituent proteins so as to strengthen the polymer)

Components may include:

- Membrane proteins that respond to curvature and to which other proteins can bind
- Proteins that cause filaments to branch at different angles
- Proteins that nucleate radial structures
- Proteins that can act as anchors in the membrane
- Proteins that cross-link filaments
- Proteases
- Proteins that change affinities for other proteins under stress
- 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). These affinities should allow for the possibility of dynamic assembly and disassembly of polymers.
- Growth by insertion of new proteins and lipids into 'neutral spaces' in different ratios and at different relative rates.

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 predictions can be made that might be tested by selection over many generations. Firstly, large cells should have fewer problems 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) should have a CSK parallel to the membrane whilst those that can grow should 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. Finally, division should occur spontaneously between two segregated cytoskeletal assemblies.

5. Discussion

As recently pointed out by T. D. Pollard (Pollard, 2003) in the context of cellular motility, the complexity of the mechanisms driving cell dynamics will force cell biologists to depend increasingly on mathematical models to test their hypotheses. This brief review emphasizes the advantages 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). Thus, discrete tensegrity models seem to provide a more adequate description of the cell as a physical object. However, the tensegrity paradigm is still a matter of active controversy, as illustrated by recent papers (Ingber et al., 2000; Wang et al., 2001). For 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 experiments be designed to discriminate between the models? It is worth reporting here the different interpretations of apparently similar experiments such as those based on the induction of cell deformation through a direct manipulation of transmembrane receptors. According to Ingber and col., on the one hand, 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, application of similar stresses to membrane receptors that are not linked to the F-actin CSK did not result in such reorganisation. Opposite conclusions, however, were drawn by Heideman and col. (1999; cited 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 concluded 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 review but we should mention that Ingber's reply is that experiments showing a lack of action at a distance when pulling on the cell via integrins *before* the formation of focal adhesion complexes are not valid as proofs of the failure of the tensegrity model. It is clear that the tensegrity paradigm is stimulating both theoretical and experimental work greatly and is leading to the development of new physical methods of quantification. For example, it 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 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 the displacement of small fluorescent microbeads within a deformable polyacrylamide gel which can be used as a cell culture substratum, the search for other quantification methods is now on.

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 can only describe the mechanical behaviour of cells by ad hoc "data fitting" models based for example on a combination of rheological elements. Through 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, 2002 ; Volokh, 2003 ; Wendling et al., 2000 ; Canadas et al., 2002) 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://e-cell.org/) or Virtual cell (http:// www.nrcam.uchc.edu/ vcell_development/ vcell_dev.html) 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 models. This is one of the requirements for future work on modelling in which the integration of mechanical and biochemical properties into a single model may genomic data to be interpreted.

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