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Hydrodynamics and physics of soft objects/Hydrodynamique et physique des objets mous

Propelling soft objects

Objets mous actifs

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Presented by Guy Laval

Abstract

Cells move by the action of specific proteins or small molecules that assemble and interact, constituting a dynamic network called the cytoskeleton. Actin, one of the major components of eucaryotic cells, exists as filaments (semi-flexible polymers) or as monomers. Local assembly of the actin network leads to cell movement as well as to the propulsion of objects like bacteria or liposomes within cells. In order to understand the force generated for this propulsion, we use biomimetic experimental systems that allow for the control of physical parameters. We show evidence for the role of elasticity in the propulsion mechanism. ***To cite this article: H. Boukellal et al., C. R. Physique 4 (2003).***

Résumé

Une cellule se déplace par l'action de protéines spécifiques ou de petites molécules qui s'assemblent et interagissent, formant un réseau dynamique, le cytosquelette. L'actine est un des constituants majeurs des cellules eucaryotes et existe sous forme de monomères ou de filaments (polymères semi-flexibles). L'assemblage local du réseau d'actine assure le mouvement de la cellule et également la propulsion d'objets comme des bactéries et des liposomes. Pour comprendre l'origine de la force nécessaire à cette propulsion nous concevons des systèmes expérimentaux biomimétiques permettant une variabilité des paramètres physiques. L'élasticité du réseau dynamique a une importance majeure dans le processus du mouvement. ***Pour citer cet article : H. Boukellal et al., C. R. Physique 4 (2003).***

Keywords: Actin; Polymerisation; Motility; Deformation; Elasticity; Droplet

Mots-clés : Actine ; Polymérisation ; Motilité ; Déformation ; Élasticité ; Goutte

1. Introduction

Actin assembly inside the cell is responsible for the deformation of the plasma membrane and cell movement. Actin filaments are asymmetric polymers with their growing (or barbed) ends oriented towards the cell membrane. Filaments depolymerise from the pointed ends which are oriented towards the cell body, and diffusion of released monomers back to the barbed ends permits the recycling of actin monomers for polymerisation. Other objects, like the bacterium *Listeria monocytogenes* [1] or

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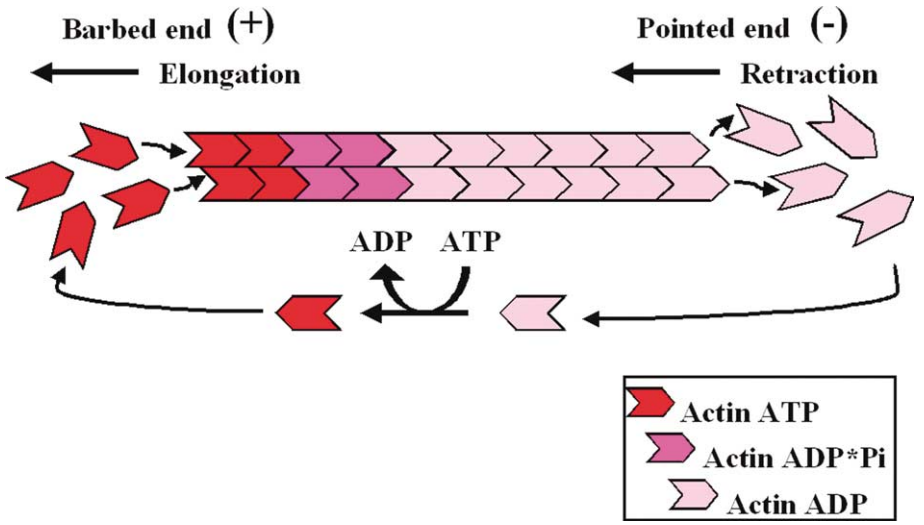

Fig. 1. Principle of growth and retraction of an actin filament in the presence of monomers and ATP (adenosine triphosphate).

liposomes [2], use the same mechanism for their propulsion inside cells and therefore constitute model systems for studying actin-based motility.

More precisely, there are several different species of pathogenic bacteria (*Listeria*, *Shigella flexneri*, *Rickettsia rickettsii*) and the Vaccinia virus that move within cells by actin assembly [3]. The size of these pathogens is on the order of one micrometer. Movement produced by actin polymerisation is on the order of a few micrometers per minute, and the velocity can vary depending on the cell type [4–6]. Vesicles called pinosomes [7] use the same mechanism of actin polymerisation for moving from the cell membrane to the cytosol. Endosomes and lysosomes [2] in *Xenopus* egg cells and extracts also undergo actin-based movement. All of these objects are propelled by generating 'comet tails' that are composed of actin filaments assembled at their surface. In order to study this phenomenon, extensive *in vitro* work has been done in cell extracts on the pathogen motility systems, especially *Listeria*. Recently, *Listeria* and *Shigella* motility was successfully reproduced in a mixture of purified proteins, greatly enhancing our understanding of the minimum biochemical requirements for motility [8].

At the steady-state, actin filaments exist at a constant length where actin polymerisation at one end is balanced by depolymerisation from the other end. The source of energy for this process is adenosine triphosphate (ATP). Fig. 1 shows the principle of actin filament 'treadmilling'. Localised actin assembly is produced by the recruitment of actin polymerisation nucleating proteins to the sites of movement, creating filaments barbed ends at these sites. In the case of *Listeria*, the nucleator of actin polymerisation is the complex ARP2/3 [9], which is present in the cytosol and activated by the bacterial membrane protein called ActA [10]. The ARP2/3 complex is also an actin filament branching factor. In eucaryotic cells, the WASP (Wiskott Aldrich Syndrome Protein) family proteins are present at the plasma membrane and activate the ARP2/3 complex to lead to local actin polymerisation.

Due to the presence of actin filament cross-linking proteins in cells, actin networks act as gels whose elastic properties are of great importance in producing propulsion [11]. To further investigate this elastic effect and other physical properties of actin-based movement, we use biomimetic experimental systems that allow us to overcome the limitations imposed by living things like *Listeria*. With our system, it is possible to vary the size of the object, its deformability, and the surface concentration of actin polymerisation nucleators. In this paper, we will summarise the work that led to an understanding of the physical properties of actin gels growing from a surface. We will also show new direct evidence for the elastic properties of actin gels and the consequences of elasticity when propelling soft objects.

2. Model systems for propelled objects inside cells

Movement induced by polymerisation is an unknown phenomenon in physical chemistry, although the principle is simple: inserting material between two objects can lead to a displacement. From a physical point of view, there are several key questions. What is the mechanism that generates force and displacement? How can we predict the velocity of objects moving by actin polymerisation? What are the physical parameters that control the movement?

Many theoretical physical approaches have attempted to explain the movement induced by actin polymerisation. One is a rectified Brownian motion (or thermal ratchet) model [12] that supposes no attachment between the bacterium and the comet. This model has been contradicted by two experiments: in one of them [13], bacteria moving in *Xenopus* egg extracts are trapped in optical tweezers and cannot move independently from their comet, showing that bacteria are attached to their comet; in the other [14], bacteria move at the same speed in mouse fibroblast cells of different viscosity, showing that bacterial speed is independent of its Brownian motion. A model based on the Brownian motion of actin filaments [15] was derived later, and predicts no dependence of the bacterial velocity on its size, in contradiction with recent experiments [16] where beads of different radii (from 1 to 10 μm), placed in a mixture of purified proteins, move slower when the radius increases. A different theoretical approach is an elastic analysis [11] of the gel generated at the surface of the bacterium. The prediction is that the stresses on the bacterial surface and the friction exerted by the actin gel on the bacterium can lead to different regimes of motility, from 'saltatory' (or jerky) to continuous motion, explaining a phenomenon that has been observed in genetically modified *Listeria* moving in *Xenopus* egg extracts [17].

The need for experimental data to test these models encouraged the design of new systems where more parameters could be changed. Polystyrene beads coated with the recombinant protein ActA and placed in human cell extracts (HeLa cells) do not generate a comet, but instead develop a spherical actin gel whose thickness depends on the size of the beads [6]. Beads coated with ActA purified from *Listeria* and placed in *Xenopus* egg extracts mimic the movement of *Listeria* [18]. In these experiments, comets are formed when beads are small (less that 1 μm diameter) because symmetry breaks spontaneously, or when they are asymmetrically coated. Beads coated with VCA (a fragment of WASP) placed in HeLa cell extracts, can move by generating a comet tail made of a network of actin filaments [19]. Since this system contains no bacterial components, it provides a simplified experimental set-up for studying both the physical and the biochemical parameters of eucaryotic actin polymerisation and motility. This bead system can be used for structural characterisation of the actin gel, as shown in Fig. 2 where asymmetric beads of 500 nm diameter are observed by electron microscopy. The cross-linked structure of the gel is clear in the image of this sample, which was prepared by critical point drying and platinum shadowing. Other studies have shown a similar branched actin network in the lamellipodium of eucaryotic cells [20]. However, experiments in extracts are not appropriate for complete physical studies because only small beads (<1 μm) develop a comet, and the number of moving beads of 1 μm diameter is still low. The reasons for this are not clear but may be due to other proteins present in the extracts that prevent symmetry breaking and comet formation by strengthening the actin gel. In fact, experiments performed in a buffer containing just the proteins essential for motility show no limitation in comet formation. This motility medium [8] is an interesting alternative to cell extracts for actin-based motility studies since all the beads, ranging from 0.5 to 10 μm move in these reconstituted extracts. Using this system, we observe a wide range of behaviours depending on the size of the beads and the surface concentration of nucleators [16], allowing an investigation of the forces involved in actin-based movement. Friction at the gel/bead surface opposes the propulsion force as already analysed [11], therefore bead size and concentration of nucleators offer a direct way of changing the magnitude of each effect. Small beads move at a constant velocity of a few μm/minute. 10 μm diameter beads

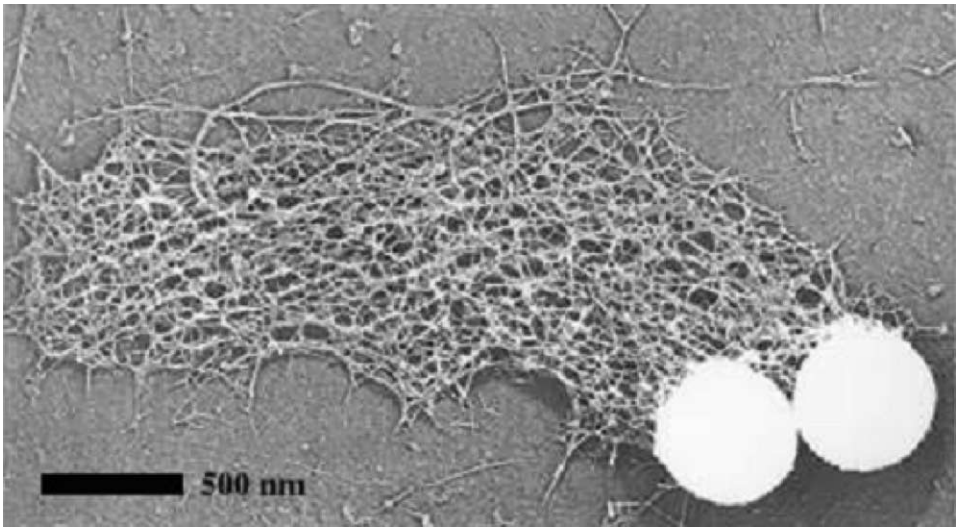

Fig. 2. Beads asymmetrically coated with a fragment of WASP that interacts with ARP2/3 and placed in HeLa cell extracts. Critical point drying and platinium replica observed by electron microscopy (collaboration with G. Resch and J.V. Small, Austrian Academy of Sciences).

move slower, but reproduce the saltatory motion previously observed with a genetically modified *Listeria* expressing a mutant ActA protein [17]. By simply changing the physical parameters of the system, we can mimic a behaviour produced by a mutant protein, showing for the first time the important effect of non-biochemistry components on actin-based motility.

3. Growing actin gels on hard spherical surfaces

Although the polymer networks of actin gels produced in solution have been extensively studied [21], surface grown actin gels are less well-characterised. One of the important physical parameters estimated for *Listeria* comet is the elastic modulus of the actin gel (10^3 to 10^4 Pa) measured by microsurgery on actin comets [13]. In spherical geometry, gels produced from surfaces are under a stress induced by the geometry because the insertion of a new monomer between the surface and the gel needs to deform and stretch the existing actin gel [6]. Because the stress is larger as the radius of curvature is smaller, the thickness of actin gels grown from polystyrene beads depends on the size of the bead. Two examples are shown in Fig. 3. Fig. 3(a) shows electron micrographs of thin sections of ActA coated beads. In Fig. 3(b), the same experiment was done with a fragment of ActA, called PRO, that does not interact with the ARP2/3 complex. We see in this figure that the thickness of the actin layer increases with the size of the bead, and a more careful analysis shows that the thickness of the actin gel is proportional to the radius of the bead for small beads (less than 5 μm diameter). For larger beads, the thickness of the actin gel is constant (1 μm for ActA coated beads) because actin monomer diffusion limits the gel growth, an effect predicted but not observed in [6]. Analysis of the data leads to an estimation of the diffusion coefficient of actin monomers which is 2×10^{-8} cm^2/s [22], 10 times smaller than the value measured in buffer.

4. Deformation of soft objects by actin gel growth

In order to directly observe the pressure exerted by the actin comet tail, we use soft deformable beads instead of hard polystyrene spheres. Droplets of oil (ISIO4) are coated with the VCA fragment of WASP and placed in HeLa cell extracts. After 20 minutes incubation, a comet is formed on one side of the droplet which affects its curvature, transforming the round

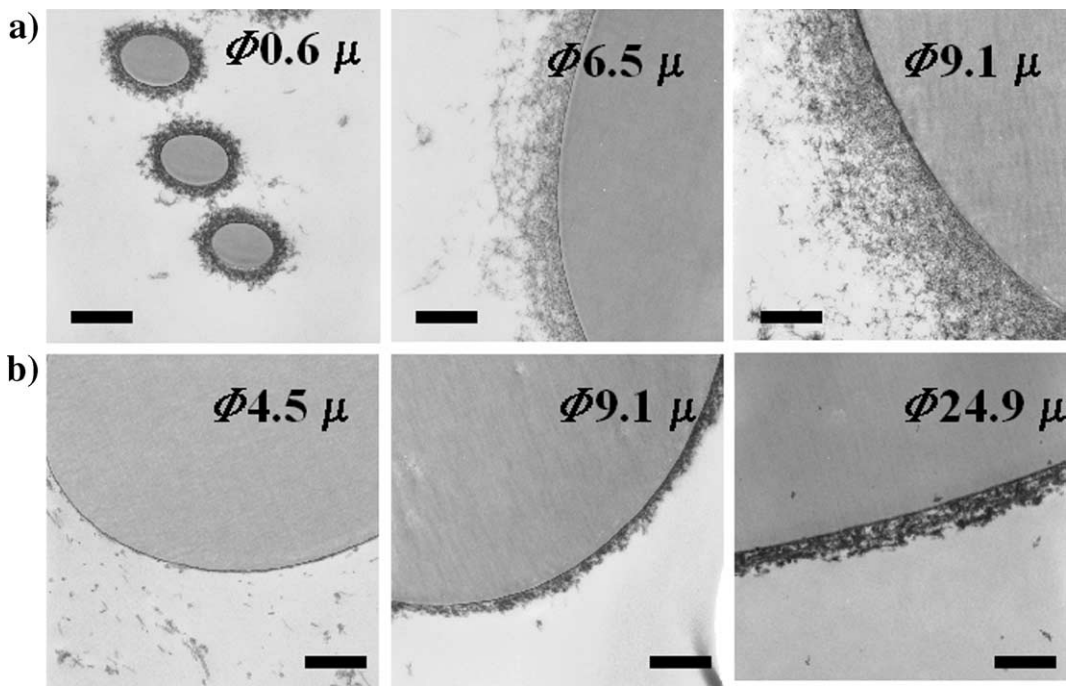

Fig. 3. Beads of various diameters coated with (a) ActA or (b) a fragment of ActA (PRO) and placed in HeLa cell extracts. Electron microscopy observation of thin sections. Bead diameter is indicated by the ϕ value on each image. Both protein-coated beads assemble actin at their surface when placed in cell extracts. The PRO-coated bead experiment is an example of an ARP2/3-independant polymerisation activity, as already shown in [24]. Bar, 500 nm.

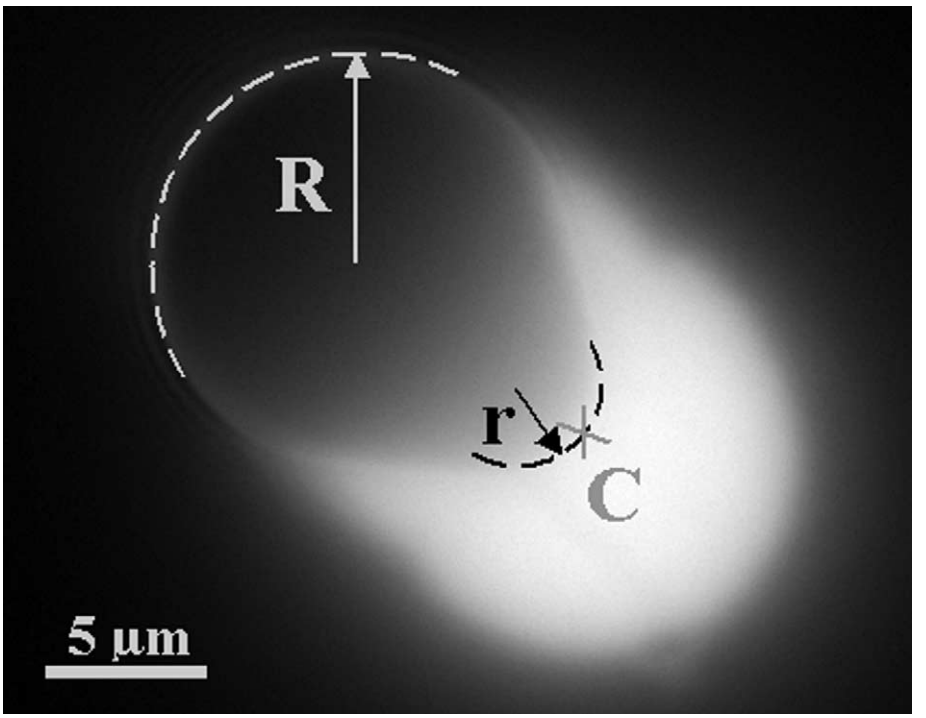

Fig. 4. Droplet of oil coated with VCA (fragment of WASP) and placed in HeLa cell extracts. Actin is marked with rhodamine.

bead into a pear shape (Fig. 4). This deformation is also observed by electron microscopy in the case of endosomes and lysosomes propelled by actin comets [2]. This effect can be explained by the stress exerted by the gel on the soft substrate. We take $\sigma_{\perp\perp}(M)$ as the normal stress exerted on the surface at point M. With $\rho(M)$ and $\rho'(M)$ as the radii of curvature at any point M on the droplet surface, the Laplace equation gives:

$$\gamma_M\left(\frac{1}{\rho(M)}+\frac{1}{\rho'(M)}\right)+\sigma_{\perp\perp}(M)=\big(P_{\text{int}}(M)-P_{\text{ext}}(M)\big), \tag{1}$$

where γ_M is the local surface tension at M, and $P_{\text{int}}(M)$ and $P_{\text{ext}}(M)$ are the hydrostatic pressures on the inside and the outside the droplet, respectively. We assume a constant surface tension $\gamma_M=\gamma$ for any point M situated at the oil/VCA/extracts interface. P_{int} is constant inside the droplet and P_{ext} is constant outside the droplet. If R is the radius of curvature at the face of the droplet free of gel (see Fig. 4), Eq. (1) applied to the extreme point of that side leads to an expression for $P_{\text{int}}-P_{\text{ext}}$ as a function of the geometry of the droplet:

$$\frac{2\gamma}{R}=P_{\text{int}}-P_{\text{ext}}. \tag{2}$$

Taking r as the radius of curvature at the extreme point C of the droplet-comet side (see Fig. 4), and writing Eq. (1) at point C with the use of Eq. (2), we find Eq. (3) where $\sigma^C_{\perp\perp}$ is the normal stress applied at C by the actin comet:

$$2\gamma\left(\frac{1}{r}-\frac{1}{R}\right)=-\sigma^C_{\perp\perp}. \tag{3}$$

Since the left-hand side of Eq. (3) is positive ($r<R$), $\sigma^C_{\perp\perp}$ is negative. This means that the droplet of oil is aspirated by the actin comet. The value of this negative pressure increases to reach a positive value at a lateral point of the droplet.

An estimate of the force generated by the actin comet tail can be made by considering that the pressure exerted on the deformed part of the droplet is on the order of $(-\sigma_{\perp\perp})$ on the hemisphere of radius r. Measurements of the droplet radii of curvature gives $r=2.5\pm0.2$ µm and $R=6.5\pm0.2$ µm. γ can be measured using the pendant drop method [23], and is estimated at 5 ± 1 mN/m at the oil/water interface with the protein adsorbed. From Eq. (3) we deduce a value of 2550 ± 30 Nm^{-2} for the normal stress $(-\sigma_{\perp\perp})$. This leads to a total force of 100 ± 30 nN exerted by the actin comet on the bead. This estimate could explain why *Listeria* escape from optical tweezers [13] that only exert a maximal force of about 100 pN. Unlike *Listeria*, the surface is fluid in these experiments, and the actin polymerisation activator can migrate on the surface. Fluorescent labeling

of VCA shows a higher concentration at the side of the droplet where the actin comet is building. Since the surface tension decreases as the number of adsorbed molecules increases, this effect should lower the estimation made above, although other proteins from the cell extracts may counterbalance this effect by also adsorbing.

5. Conclusions

Experimental model systems that mimic biological behaviours are of great interest for understanding the physical mechanism of movement induced by actin polymerisation. In our work, we have shown by different approaches the effect of elasticity, monomer diffusion, friction and depolymerisation on the propulsion mechanism. Since depolymerisation replenishes the pool of actin monomers to be used for polymerisation, it can be a limiting factor for movement in biological systems. However, if a flow of monomers can be injected into the system, there might be no need for depolymerisation during the movement, if the system is already asymmetric. A chemical system designed with macromolecules could be of interest in testing this assumption.

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