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Contractility and retrograde flow in lamellipodium motion

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Abstract

We present a phenomenological description of cell locomotion on a solid substrate. The material properties of the actin cytoskeleton in the lamellipodium are described by the constitutive equations of a viscous polar gel with intrinsic activity. The polymerization of the gel takes place in a localized region near the leading edge. Using a simple two-dimensional description, we calculate in the steady state the thickness profile of the lamellipodium which at the rear connects to the cell body; we also calculate the flow profiles and the forces exerted on the substrate. The cell velocity is estimated as a function of externally applied forces. Our description is consistent with experimentally observed properties of motile cells such as the existence of a retrograde flow in the lamellipodium and a dipolar force distribution exerted by the cell on the substrate.

Introduction

Locomotion allows cells to change their environment and plays an important role in many biological processes such as wound healing, remodeling of connective tissues, rebuilding of damaged structures or formation of metastases in cancer. Under all these circumstances, the cells crawl on a surface coated by an extracellular matrix to which it adheres by specific adhesion proteins [1]. The motility is based on the active behavior of the actin cytoskeleton of the cell. It involves several steps [2, 3]: protrusion of a thin layer of cytoskeletal structure (the lamellipodium) at the leading edge of the cell, adhesion on the substrate and contraction of the cytoskeleton; eventually motility also involves the disassembly of the actin network at the rear of the cell. The contraction pulls the rear part of the cell forward. The energy consumed by the cell motion is provided by the hydrolysis of adenosinetriphosphate (ATP), which drives actin polymerization and depolymerization and the motion of myosin motor proteins. The interaction of myosin with actin filaments creates the contractile stresses in the cytoskeleton that drive the retraction processes.

In the leading edge of the lamellipodium, new actin filaments are nucleated by branching off existing ones. Branching occurs when complexes of actin-related proteins (ARP) 2/3 bind to actin filaments close to the cell membrane [1]. This process together with filament growth by polymerization leads to the formation of a cross-linked and entangled filamental network or gel. The growth of the gel at the leading edge is controlled by the cell via signaling pathways and the activation of ARP 2/3 by proteins of the Wiskott–Aldrich syndrome family (WASP) which are localized in the cell membrane at the leading edge. Filament polymerization is also controlled by capping proteins which stabilize the ends of some filaments [4–6]. The localization of WASP proteins at the leading edge, in the cell membrane, is essential to ensure a controlled growth of the gel near the edge in the form of a thin layer. Below, we assume that WASP proteins are localized in a small region of size λ near the leading edge without describing the mechanisms that lead to this localization.

In the following, we propose a theoretical analysis in which we correlate the lamellipodium velocity and shape to biophysical parameters which can be measured independently in suitably designed experiments. The most obvious parameters are the polymerization and depolymerization rates of actin filaments. Another parameter describes how the lamellipodium exerts forces on its substrate via adhesion molecules [7–9]. As long as the lamellipodium velocity is small compared to a/τ_b where a is a molecular length and τ_b is the average time during which an adhesion molecule is

bound to the substrate, this force is proportional to the velocity of the actin gel relative to the substrate and thus involves simply a friction coefficient [10, 11]. This coefficient can be accessible in well-designed experiments [31]. More complex behaviors arise if the velocity is of the order a/τ_b but we postpone for further analysis the study of the corresponding regimes. The total integrated force which acts on the cell at any given time is zero if no external forces are applied. This force balance implies that if the front part of the cell pulls the cell forward, the rest of the cell pulls with exactly the same force in the backward direction. This balance of opposing forces can be characterized by a force dipole which can be determined from experimentally measured traction maps [7, 8]. Such experiments show that these forces correspond to contractile behavior of the cell characterized by a negative force dipole in a one-dimensional description.

The description of the actin network is more challenging. Actin filaments are cross-linked in a structure which under the electron microscope appears to be a gel [3]. This tells us that on time scales such that the cross-links do not have time to move, and on length scales large compared to the average distance between cross-links, the actin network should behave like an elastic body. However, the cross-linking proteins statistically unbind after a characteristic time τ, and on time scales longer than τ the actin network should behave like a fluid. These features are well captured in the Maxwell description of what is called a physical gel and have been measured experimentally in cells [12].

Such a description is not sufficient for two reasons. First, actin filaments which have 'barbed' and 'pointed' ends are structurally polar. Since they point on average with their growing end toward the leading edge of the cell, the network itself is polar. In the following, this polarity will mainly be important in the description of the lamellipodium surfaces (boundary conditions), but in general one has to keep in mind that we are dealing with a polar gel. Secondly, cross-links can be redistributed in response to myosin motor activity. Myosin oligomers can even act as moving cross-links. This confers a unique and original property to these biological systems as compared to conventional physical gels [13, 14]. We have recently derived a set of generic equations, based on symmetries and conservation laws, precisely designed for the description of actin gels in the presence of motor activity [15]. This description involves a small number of new parameters that account for all effects of motor activity. These parameters can be measured in separate experiments. In the following description, we will need only one such additional parameter. It describes the tendency for molecular motors to anisotropically contract the gel. The main limitation of this generic theory comes from the assumption of a system which is weakly out of equilibrium. In view of the fact that the force–velocity relation of motor proteins does not depart severely from linear behavior, we think that it is a valid starting point. Thus with suitable polymerization/depolymerization rules, an appropriate substrate friction coefficient and a description of the actin/myosin gel, one is in a position to calculate principles governing the shape and motion of cellular substructures such as lamellipodia.

In order to keep the algebra tractable and to obtain closed equations, we add into this first analysis a number of reasonable assumptions. First, the polymerization is assumed to take place in the immediate vicinity of the plasma membrane in a region of size λ, corresponding to the localization of the WASP proteins. Second, while depolymerization is not localized, it occurs mainly in the rear of the cell. We neglect depolymerization in the lamellipodium for simplicity, as this does not change the main features of our results. In order to describe the effects of the depolymerization in the rear part of the cell, we impose boundary conditions at a distance L from the leading edge, where L defines the lamellipodium length. These boundary conditions correspond to localized depolymerization in the rear and mimic the effects of depolymerization in the cell body. A similar assumption of localized polymerization and depolymerization described via boundary conditions on a gel has been used successfully and with predictive power in actin growth geometries inspired by the *Listeria* propulsion mechanism [16, 17]. A discussion of this assumption's validity has been given in [18]. In the case we discuss here it requires that the force f^L_{ext} exerted by the rest of the cell on the lamellipodium is large in a sense to be discussed below.

Finally, we discuss the limit of an incompressible actin gel and we neglect the effects of permeation of the solvent through the gel. Actin gels have a finite compressibility. The limit of an incompressible gel is a simplification which captures the features of deformations and force balances in a situation where shear deformations are large as compared to compression. This is, in particular, the case for an elastic body for which the ratio of shear and compression moduli is small. Perfectly incompressible gels have a Poisson ratio of exactly $1/2$. Experiments on actin gels provide us with values ranging between 0.4 and 0.5, which shows that such gels are only weakly compressible [19]. Our simple choice of an incompressible gel captures the relevant physics of such weakly compressible gels.

Within this framework, we can calculate in steady state the lamellipodium shape and velocity, the forces exerted on the substrate and the velocity field of the gel, as a function of a few independently measurable parameters. We show that there exists a new material parameter, namely the contractile stress, which is as important as the gel elasticity and viscous relaxation time. We can estimate the contractile stress and other key parameters by comparing our solutions to experimentally observed lamellipodium motion of keratocytes. The only unknown which is not easily accessible to experiments is the force exerted by the cell body on the lamellipodium. One of our key results is that the net lamellipodium velocity is controlled by the depolymerization rate and the contractile stress of the gel: the polymerization rate is controlled by the depolymerization process via the actin monomer conservation. Another remarkable feature is that a force which opposes the spontaneous motion exerted at the rear of the lamellipodium where the lamellipodium connects to the cell body can result either in a slowing down or a speeding up of the motion! This depends on a subtle comparison of the contractile stress and the force

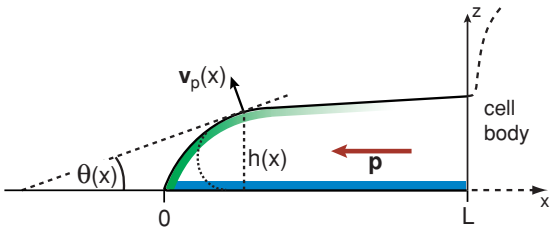

Figure 1. Schematic representation of the two-dimensional geometry of a thin gel layer corresponding to a lamellipodium of a moving cell. The height profile $h(x)$ of the gel is described as a function of the distance x from the leading end of the lamellipodium. The lamellipodium length is denoted as L. The gel in a lamellipodium is structurally polar with filaments pointing their plus ends toward the leading end of the lamellipodium. This polarization is described by the vector $\mathbf{p}$. Polymerization of new gel material occurs at the surface of the gel layer in a direction normal to the surface and with velocity $v_p(x)$. This velocity is proportional to the density of WASP molecules in the membrane which are assumed to be localized in the front with a density that decays exponentially (indicated in green). The angle of the height profile with respect to the horizontal is denoted by θ. The sharp angle at the tip $\theta(x = 0)$ is rounded as a result of membrane elasticity (dotted line).

dependence of depolymerization. This feature is reminiscent of the dependence predicted for nematode spermatocytes [20]. Third, we naturally obtain the retrograde flow of the gel at the lamellipodium edge [21, 22] and predict that if the force $f^L_{\rm ext}$ is small enough, an anterograde flow at the rear of the lamellipodium could be observed.

Our approach can be compared to other related approaches. An effective material description was introduced in [13] to describe cell locomotion. In this two-fluid description, contractile stresses were described as isotropic negative pressure. A two-dimensional continuum description based on an elastic sheet that is deformed by forces resulting from actin–myosin interactions was used to calculate cell shapes [23]. Such an approach requires numerical methods and is not based on generic physical arguments. More microscopic approaches are also complementary to our work [24, 25]. They essentially provide us with expressions describing the polymerization rate and with some of the coefficients describing the gel network behavior.

Thin active gels

We study a thin gel layer on a surface given by the x–y plane, as sketched in figure 1. Assuming that the system is homogeneous in the y-direction, and thus effectively two-dimensional, the thickness of the gel in the z-direction is given by a height profile $h(x)$. The length of the lamellipodium gel layer in x-direction is denoted by L. We denote the angle between the tangent to the height profile and the substrate by θ, so that $\tan\theta = {\rm d}h/{\rm d}x$.

The gel anisotropy found in the lamellipodium which results from the polymerization process suggests that the actin filaments are polarized in the direction x of motion. The polarization vector $\mathbf{p}$ is defined by averaging locally the orientation of unit vectors along the actin filaments. For simplicity, we ignore the gradients of the polarization vector and consider the polarization as a unit vector with coordinates $p_x = 1$ and $p_z = 0$. Our description captures the essential physics of the lamellipodium up to the cell body. The cell body itself and the rear of the cell, including the detailed depolymerization process of the gel, go beyond our simple theoretical approach. We include the relevant aspects of the physics of the cell body by imposing appropriate boundary conditions at $x = L$.

The polymerization of new gel material occurs at a velocity $\mathbf{v}^p$ at the gel surface $h(x)$. We suppose that the local polymerization velocity $\mathbf{v}^p$ is normal to the cell surface and is non-zero only if WASP proteins are locally present. The WASP proteins being confined in a region of size λ close to the edge, we write their surface density in the membrane as $\rho_{\rm wa}(x) = \rho^0_{\rm wa}\,{\rm e}^{-x/\lambda}$. The polymerization velocity is then expressed as $\mathbf{v}^p = \mathbf{n}k_p\rho_{\rm wa}(x)$ where $\mathbf{n} = (-\sin\theta, \cos\theta)$ is the normal vector to the cell surface. We choose to discuss a polymerization rate k_p which is independent of $\mathbf{n}\cdot\mathbf{p}$, the relative orientation of the polarization and the normal to the surface.

The actin cytoskeleton is a gel-like network. Such a gel exhibits elastic behaviors at short times but will behave like a viscous fluid when times are long compared to the lifetime of cross-links [26]. In the presence of active processes such as the force generation of myosin oligomers in the gel, active mechanical behaviors occur. In a condition of steady-state flow and in the linear regime, the gel has a liquid-like viscous behavior. The mechanical properties of such an active polar gel can be characterized by constitutive equations. The gel flow is characterized by the local velocity field v_α. The constitutive equations express the deviatory stress tensor $\sigma_{\alpha\beta}$ in terms of the strain-rate tensor $u_{\alpha\beta} = (\partial_\alpha v_\beta + \partial_\beta v_\alpha)/2$ and the actively generated stresses in the gel [15]. Here, we will need only the traceless part

$$2\eta u^a_{\alpha\beta} = \sigma^a_{\alpha\beta} + \zeta\,\Delta\mu\left(p_\alpha p_\beta - \tfrac{1}{3}p_\gamma p_\gamma \delta_{\alpha\beta}\right) \qquad (1)$$

of these constitutive equations because we will consider the case of an incompressible gel with $u_{\gamma\gamma} = 0$ for simplicity. The anisotropic parts of the stress and strain-rate tensors are $\sigma^a_{\alpha\beta} = \sigma_{\alpha\beta} - (1/3)\sigma_{\gamma\gamma}\delta_{\alpha\beta}$ and $u^a_{\alpha\beta} = u_{\alpha\beta} - (1/3)u_{\gamma\gamma}\delta_{\alpha\beta}$.

For $\Delta\mu = 0$, this expression is the standard relation between viscous stress and shear rate of a simple fluid with viscosity η. Active processes in the gel are driven by the chemical free energy per ATP molecule $\Delta\mu$. The coefficient ζ characterizes the generation of active anisotropic stresses in the gel. They are contractile if ζ is negative, which we consider in the following. The force balance in the gel is given by

$$\partial_\alpha\sigma_{\alpha\beta} = \partial_\beta P \qquad (2)$$

where P is the pressure. The interaction of the gel with the substrate is characterized by a friction coefficient ξ. The boundary condition at $z = 0$ is therefore $\sigma_{xz} = \xi v_x$. The cell surface $z = h(x)$ is a free surface and we impose as boundary conditions that the tangential and normal components of the total stress vanish: $\sigma_{\rm nt} = \sigma_{\rm nn} - P = 0$.

In the following, we ignore polymerization and depolymerization processes in the bulk and assume that they

occur only at the surfaces of the gel. We assume furthermore for simplicity that the gel is incompressible. With these assumptions, the flow field satisfies $\nabla \cdot \mathbf{v} = 0$, and the gel density ρ is constant. The pressure P is then a Lagrange multiplier that is introduced to satisfy the incompressibility constraint.

In the limit of a thin gel layer, $h \ll L$, and slowly varying thickness, $\mathrm{d}h/\mathrm{d}x \ll 1$, a thin-film approximation can be used to derive an effective one-dimensional description: we average the velocity, the stress and the pressure in the z-direction over the thickness of the gel $v = \langle v_x \rangle$, $\sigma = \langle \sigma_{xx} \rangle$ and $p = \langle P \rangle$. Note that here and in the following, the velocity v is always measured with respect to the substrate. The total force per unit length acting along the x-axis in a gel cross-section in the y–z plane is $F = \int_0^h \mathrm{d}z(\sigma_{xx} - P) = h(\sigma - p)$. The force balance on a slice of gel of size $\mathrm{d}x$ is obtained by balancing this force with the friction force on the substrate:

$$\frac{\mathrm{d}F}{\mathrm{d}x} = \xi v. \tag{3}$$

The boundary condition on the normal stress at the upper surface of the gel imposes $\sigma_{zz} = P$. The force F can therefore be written as $F \simeq h\langle \sigma_{xx} - \sigma_{zz} \rangle$. Using the incompressibility condition, $\partial_x v_x + \partial_z v_z = 0$, the constitutive equations lead to

$$\frac{\mathrm{d}v}{\mathrm{d}x} = \frac{1}{4\eta}\left(\frac{F}{h} + \zeta \Delta\mu\right). \tag{4}$$

We look for steady-state profiles of the lamellipodium which moves with constant velocity u in the negative x-direction, $h = h(x + ut)$. In a reference frame moving at a velocity $-u$ with respect to the substrate, the height profile is stationary. The gel flux in the x-direction in this reference frame is $j = \rho h(u + v)$. Mass conservation implies that the variation of this flux is compensated by the polymerization flux. To linear order in $\mathrm{d}h/\mathrm{d}x$ the steady-state height profile obeys

$$\frac{\mathrm{d}}{\mathrm{d}x}[(u + v)h] = k_p \rho_{\mathrm{wa}}. \tag{5}$$

The lamellipodium height can be expressed as a function of the velocity v by integrating this expression:

$$h(x) = \frac{k_p}{u + v(x)} \int_0^x \mathrm{d}x' \rho_{\mathrm{wa}}(x'). \tag{6}$$

Equations (3) and (4) represent two first-order equations for the force F and the gel flow v, the height profile being given by equation (6). They can be solved by imposing boundary conditions on the force at $x = 0$ (lamellipodium front) and $x = L$ (connection of the lamellipodium to the cell body). In order to take into account the combined effects of adhesion and external forces, we impose at the front $F(0) = -\left(W + f^0_{\mathrm{ext}}\right)$ and at the rear $F(L) = W' + f^L_{\mathrm{ext}}$. External forces f_{ext} act on the lamellipodium at its front and rear. The force at the rear includes that exerted by the cell body on the lamellipodium. We have ignored the effect of the membrane tension and bending rigidity at the free surface of the cell, but we include the effects of the adhesion energies per unit area of the lamellipodium (W) and the cell body (W') on the substrate.

With these conditions, for a given length L there exists one steady-state solution for any positive value of the velocity u. A unique value of this velocity is selected by the fact that in the rear the geometry of the cell body imposes a height $h(L) = h_0$. Depolymerization in the rear is essential for this solution to exist. The depolymerization velocity v_d in the steady state is related to the total velocity at the back by the conservation of the gel flux, $v_d = u + v(L)$. Monomers that result from depolymerization diffuse to the front where they control the polymerization rate k_p. The value of k_p depends on the local monomer concentration. If the length L of the lamellipodium is larger than the localization length λ of the WASP proteins, equation (6) imposes that

$$k_p \rho^0_{\mathrm{wa}} \lambda / h_0 = u + v(L) = v_d. \tag{7}$$

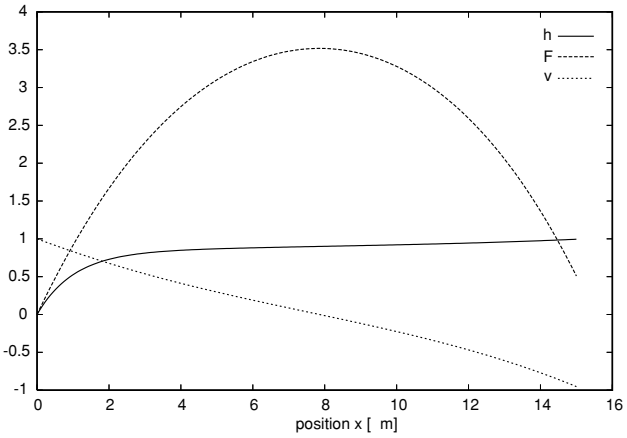

Figure 2. Calculated velocity, force and height profiles of a thin active gel layer corresponding to a lamellipodium moving on a substrate in the negative x-direction with velocity $u = 10\ \mu\mathrm{m\ min}^{-1}$. The position x is measured along the horizontal axis and given in μm, where the leading tip is at the origin of the coordinate system. The flow velocity v of the gel layer relative to the substrate is given in $\mu\mathrm{m\ min}^{-1}$ and the gel thickness h is given in μm. The integrated stress across the active gel layer F is given in units of 0.5 nN $\mu\mathrm{m}^{-1}$. The parameter values are $\zeta\Delta\mu/4\eta = -0.21\ \mathrm{min}^{-1}$, $\xi/4\eta = 1/(36\ \mu\mathrm{m})$ and $u = 10\ \mu\mathrm{m\ min}^{-1}$. Furthermore, we use $\xi = 3 \times 10^{10}$ Pa s m^{-1} and $k_p\rho^0_{\mathrm{wa}} = 9\ \mu\mathrm{m\ min}^{-1}$.

Height and flow profiles

Figure 2 presents numerically calculated steady-state height, force and velocity profiles for $f^0_{\mathrm{ext}} = f^L_{\mathrm{ext}} = W = W' = 0$. The gel velocity with respect to the substrate corresponds to a retrograde flow (toward the rear) in the front and to an anterograde flow (toward the front) in the rear. At the front the force ξv exerted per unit area on the substrate therefore also points to the rear, while in the rear it is oriented forward. The total force exerted by the lamellipodium on the substrate vanishes as imposed by macroscopic force balance but the force dipole $Q = \int_0^L \xi x v\, \mathrm{d}x$ is finite and negative in agreement with experiments that show that traction forces have opposite signs in the front and the back and their direction corresponds to contractile stresses in the cell. The cell body connected at $x = L$ to the lamellipodium can also exert a force which

modifies the velocity u. In this case, the total force exerted by the lamellipodium does not vanish but instead the total force exerted by the cell, including the cell body, vanishes. Again, the force dipole of the whole cell is finite. If external forces are applied in the front and the rear, both the height profile and the advancing velocity of the lamellipodium are modified.

If the length L of the lamellipodium is large, a simple solution of the steady-state profile can be found in the limit where the active stress $\zeta\Delta\mu$ is small. For simplicity, we consider only the case where there is no applied force at the front. The central region of size $L-2d$ of the lamellipodium has approximately constant height $h\simeq\bar{h}$ and vanishing gel velocity v. In the front of this central region, the height decreases to zero over a length d and the flow is retrograde. In the rear of the central region, the height increases to the value h_0 imposed by the cell body and the flow is anterograde. The size d of these regions is given by $d^2=4\eta\bar{h}/\xi$. This analysis is valid if $L\gg d\gg\lambda$.

At the front, there is a small region of size λ where the thickness h drops rapidly to zero while F and v are approximately constant. Outside this edge region of size λ, equation (6) for the height profile can be written as $h(x)=k_p\lambda\rho^0_{\rm wa}/[u+v(x)]$. This determines the lamellipodium thickness:

$$\bar{h}=k_p\rho^0_{\rm wa}\lambda/u=h_0v_d/u \tag{8}$$

which is estimated as the height at a position within the lamellipodium for which $v=0$ and thus $\mathrm{d}h/\mathrm{d}x=0$ in the absence of polymerization and where we have used equation (7). In the front region of size d, the velocity decreases exponentially as $v=-\mathrm{d}\zeta\Delta\mu/4\eta\,\mathrm{e}^{-x/d}$. Note that retrograde flow corresponds indeed to contractile active stress $\zeta\Delta\mu<0$. In the rear part of the lamellipodium, the velocity increases exponentially as $v(x)=v(L)\exp(x-L)/d$. The velocity at the rear is obtained by imposing that the force $F(L)$ be equal to the external force exerted by the cell body $f^L_{\rm ext}$: $v(L)=d/4\eta\big[f^L_{\rm ext}/\bar{h}+\zeta\Delta\mu\big]$. For a small external force the velocity is negative, corresponding to an anterograde flow.

The conservation of the gel flux at the rear of the lamellipodium imposes $u=v_d-v(L)$. This allows us to obtain an explicit expression of the velocity of motion of the lamellipodium

$$u\simeq v_d-\left(\frac{h_0}{4\eta\xi}\right)^{1/2}\left(\zeta\Delta\mu+\frac{f^L_{\rm ext}}{h_0}\right). \tag{9}$$

If we ignore the force exerted by the cell body on the lamellipodium, the friction force exerted by the lamellipodium on the substrate is indeed a force dipole. In the limit where the length L is large, the force dipole is $Q\simeq L\bar{h}\zeta\Delta\mu<0$.

The lamellipodium length L in the steady state is determined by the transport of actin monomers to the front, where they are incorporated in the gel. Assuming that the transport of actin in the gel can be described by an effective diffusion coefficient D, and denoting the (two-dimensional) monomer concentration by ρ_m, the monomer flux toward the front is for $L\gg\bar{h}$ given by $j_m\simeq D(\rho_0-\rho_m(0))/L$ where $\rho_0=\rho_m(L)$ is the actin monomer density at the cell body that acts as a reservoir. We describe the gel polymerization by a rate proportional to the monomer concentration: $k_p=\alpha\rho_m$ where α is a kinetic coefficient. The steady-state condition becomes $v_dh_0\rho\simeq\alpha\rho_m(0)\rho^0_{\rm wa}\lambda\rho\simeq j_m\bar{h}$ where ρ denotes the monomer concentration in the gel. This determines both the length L and the actin monomer density $\rho_m(0)$ at the front of the lamellipodium:

$$\rho_m(0)\simeq\frac{v_dh_0}{\alpha\lambda\rho^0_{\rm wa}} \tag{10}$$

$$L\simeq\frac{D}{u\rho}(\rho_0-\rho_m(0)). \tag{11}$$

Here, we have assumed that the height h_0, the depolymerization velocity v_d and the monomer concentration ρ_0 are imposed by the cell body. Note that a stationary lamellipodium shape exists only if $v_d<\alpha\rho_0\rho^0_{\rm wa}\lambda/h_0$.

Conclusion and outlook

In summary, we have proposed a theoretical description which captures the main physical features of lamellipodium motion on a solid substrate. This approach allows us to introduce a new quantitative way to analyze experiments. A full comparison of this description with experiments would require measurements of the height, flow and force profiles of a moving cell. Existing data already provide an estimation of all the parameter values of our theory.

Taking the example of a crawling keratocyte, we estimate $L\simeq10\ \mu$m and $\bar{h}\simeq1\ \mu$m. The advancing velocity is $u\simeq10\ \mu\mathrm{m\ min^{-1}}$, and a retrograde flow exists at the front with $v(0)\simeq1\ \mu\mathrm{m\ min^{-1}}$ [27, 28]. The elastic modulus of an actin gel in a cell is of the order $E\simeq10^4$ Pa [11, 29, 30]; the typical visco-elastic relaxation time τ is of the order of 10 s [12]. From existing measurements of both surface stress $\sigma_{xz}\simeq4\times10^2\ \mathrm{N\ m^{-2}}$ [8] and the retrograde flow velocity $v(0)$, we can deduce the friction coefficient of a lamellipodium $\xi\simeq\sigma_{xz}/v(0)\simeq10^{10}\ \mathrm{Pa\ s\ m^{-1}}$. This value is very close to the friction coefficient of an actin gel on a latex bead $\xi\simeq3\times10^{10}\ \mathrm{Pa\ s\ m^{-1}}$ [31]. We find a length $d\simeq6\ \mu$m. These values used in figure 1 are consistent with the experimental results on keratocytes[28]; d is of the order L and the cell body exerts a finite force on the lamellipodium.

The anisotropic active stress is obtained from the retrograde flow velocity as $-\zeta\Delta\mu\simeq4\eta v(0)/d\simeq10^3$ Pa. Note that in this case the force transmitted by the lamellipodium does not vanish since it balances the force $f^L_{\rm ext}$ exerted by the cell body on the lamellipodium, where $f^L_{\rm ext}\simeq\xi v(0)d\simeq-\zeta\Delta\mu\bar{h}\simeq1\ \mathrm{nN\ \mu m^{-1}}$. For a lamellipodium with effective lateral size $L_y\simeq30\ \mu$m, the total force is therefore 30 nN; this order of magnitude is consistent with the force of 10 nN obtained in [8] by the integration of the stress. The force dipole of the cell is $QL_y\simeq f^L_{\rm ext}L_cLy\simeq6\times10^{-13}$ J, where $L_c\simeq20\ \mu$m is the total length of the cell. The lamellipodium length is determined by $L\simeq D\rho_0/u\rho$. Using $D\simeq3\times10^{-12}\ \mathrm{m^2\ s^{-1}}$, $\rho_0\simeq200\ \mu$M and $\rho\simeq300\ \mu$M [32, 17], we obtain $L\simeq12\ \mu$m.

We have used for simplicity a one-dimensional description within a thin-film approximation. A more realistic

three-dimensional description is possible within the same framework; however, it requires a precise analysis in a more complex geometry. The thin-film approximation requires that the localization length of the WASP proteins λ is small enough and that the decay length of the retrograde flow d is large enough, $\lambda, \bar{h} < d$. The second condition is realized if $4\eta/(\xi\bar{h}) > 1$ which is the case for a keratocyte.

All the above considerations depend neither on the particular distribution of WASP proteins nor on the details of the polymerization kinetics, provided they are localized. The localization of the WASP proteins in a small region of size λ has been observed but the physical mechanisms which are responsible for this localization are still under investigation. It has been suggested that the protein might favor the large membrane curvatures at the leading edge. A second possibility is that WASP in the membrane is transported actively toward the leading edge by motor proteins which advance with velocity v_m along actin filaments adjacent to the membrane. Since the actin gel is polarized with the plus ends pointing forward, such a transport would localize WASP proteins at the leading edge. Assuming that WASP diffuses in the membrane with diffusion coefficient D_m as it detaches from the transporting motors, the localization length is given by $\lambda \simeq D_m/v_m$. A third possibility is that WASP interacts strongly with the plus ends of the filaments and is transported in the gel by diffusion or by motor proteins. We cannot, however, rule out other mechanisms as long as they lead to a sufficiently low WASP concentration along the lamellipodium such that the effects of their presence is negligible as compared to controlled gel growth at the leading edge.

The active stress of the cytoskeleton mostly controls the retrograde flow at the leading edge. The retrograde flow is localized over a length d and its amplitude increases with $|\zeta\,\Delta\mu|$. In the limit $|\zeta\,\Delta\mu| = 0$, there is no retrograde flow and the advancing velocity in a steady state is equal to the depolymerization velocity $u = v_d$. At a finite value of $|\zeta\,\Delta\mu|$, the advancing velocity increases as a function of the contractile stress (equation (9)). The cytoskeleton is visco-elastic and behaves at short times as an elastic solid and at long times as a viscous fluid. As a consequence, at the front the gel behaves as a solid over a length $\tau v_d \simeq 1\ \mu$m. The fully viscous description is valid beyond this length and thus our theory is consistent, if this length is smaller than d. In the same vein, we have not described in detail the interaction of the growing gel with the plasma membrane. We postpone a detailed discussion to future work. Yet one can foresee that the sharp angle of the gel shape at the leading edge which our analysis predicts will be rounded up on a length scale $(\kappa/W)^{1/2}$ as suggested by the dotted line in figure 1. Here, W denotes the total energy per unit surface of the membrane and κ is the bending rigidity of the membrane.

We have assumed that the active stress is constant throughout the lamellipodium. Experimentally, the surface density of myosins increases toward the rear of the lamellipodium. Part of this effect is captured in our model by the fact that the lamellipodium is thicker at the rear. A more elaborate description would require keeping track of both the local myosin density and the gel elasticity at short times. The fact that the myosin density becomes small at the leading tip can be captured in our description by assuming that the contractile stress $\zeta\,\Delta\mu$ is position dependent and vanishes at the front.

The depolymerization velocity v_d influences the advancing velocity u and the length of the lamellipodium L. The advancing velocity increases with v_d (equation (9)) and the length of lamellipodium decreases, as expected, with the depolymerization velocity (equation (10)). For small external forces, the thickness of the lamellipodium $\bar{h}$ is inversely proportional to the advancing velocity; it increases with increasing depolymerization velocity. Lamellipodium formation requires the depolymerization velocity to be smaller than a critical value that increases with the actin monomer density ρ_0 and the polymerization rate α.

Both the amplitude of the retrograde flow and the advancing velocity decrease with increasing friction ξ. The length L and the thickness h of the lamellipodium increase for increasing friction ξ. We have considered here that the adhesion force is proportional to the local velocity on the substrate. Finally, an externally applied force on the lamellipodium modifies both the polymerization/depolymerization rates and the stress distribution. The effects of a force on the advancing velocity therefore depends on its position of application [20]. In the case of a pulling force $f^L_{\rm ext} > 0$ applied at the rear the two effects are competing. Such a force is naturally exerted by the cell body but could also be modified by external manipulation. If we ignore the force dependence of the depolymerization rate, then equation (9) predicts a decrease of u with increasing force. However, the depolymerization velocity can increase under the action of this force, which tends to increase the velocity u (and could lead to a negative mobility as suggested in [20]). These two contributions could be separated experimentally by their time dependence. The effect on the stress profile is almost instantaneous, while the velocity change due to a variation of the depolymerization rate is effective only after a time L^2/D corresponding to the diffusion of actin monomers to the leading edge.

At small forces, the thickness $\bar{h}$ and the length L of the lamellipodium increase with increasing force. The force exerted by the cell body on the lamellipodium results from the adhesion of the cell body to the substrate. On the time scales considered here, this force can be described by a viscous friction force $f^L_{\rm ext}$. If the friction is linear, $f^L_{\rm ext} = \xi_{\rm cb} L_{\rm cb} u$ is proportional to the advancing velocity, where $\xi_{\rm cb}$ is a surface friction coefficient and $L_{\rm cb}$ is the size of the cell body. The velocity u can be calculated self-consistently from equation (9):

$$u \simeq \left[v_d + |\zeta\,\Delta\mu| \left(\frac{h_0}{4\eta\xi} \right)^{1/2} \right] \Bigg/ \left[1 + \frac{\xi_{\rm cb} L_{\rm cb}}{(4\eta\xi h_0)^{1/2}} \right]. \quad (12)$$

Here, we have expressed u in terms of the depolymerization velocity v_d because it does not depend on the actin monomer concentration. At steady state, the polymerization velocity adjusts to balance this depolymerization velocity by selecting the appropriate length of the lamellipodium. The gel contractility provides an additional contribution which

increases the advancing velocity. For the parameter values used for keratocytes, we estimate this contribution to about 10% of the total velocity. This implies that a myosin inhibition should reduce the advancing velocity while suppressing the retrograde flow. Note that such a retrograde flow could still be driven by the membrane tension exerting a force $f^0_{\rm ext}$ at the leading edge.

If the friction force $f^L_{\rm ext}(u)$ varies in a non-monotonic way as a function of velocity such that a range of negative slope exists, the resulting motion may exhibit a stick-slip or saltatory behavior. This is the case if the friction force exerted by the cell body on the substrate is elastically coupled to the lamellipodium [18]. Such a mechanism could explain the inchworm motion observed for fibroblasts.

Glossary

Lamellipodium. The lamellipodium is the thin sheet-like front part of a cell that is moving on a solid substrate. The lamellipodium contains a network of actin filaments assembled near the moving front. It is enclosed by the plasma membrane and linked to the substrate by specific adhesion molecules.

Force dipole. A cell moving on a solid substrate to which it adheres generates a distribution of traction forces with density $\vec{f}(\vec{r})$ acting on the substrate at position $\vec{r}$ measured from the cell center. Such traction maps show that forces exerted at opposite sides of the cell largely cancel, such that the net total force $\int {\rm d}^2 r\, \vec{f} \simeq 0$. In such a situation the dipolar structure of the force distribution can be characterized by the matrix $Q_{\alpha\beta} = \int {\rm d}^2 r\, r_\alpha f_\beta$, where α and $\beta = x, y$. If the y-dependence is unimportant, $Q = Q_{xx}$ describes the dipole of forces projected on the x-axis. Q is negative if balancing forces are contractile.

Retrograde/anterograde flow. When a cell moves on a substrate, the actin gel in the cell flows at a speed that is different from the cell velocity of motion. If this actin flow moves with respect to the substrate in a direction opposite to the direction of motion of the cell, this flow is called retrograde; movement in the same direction is anterograde flow.

Visco-elastic gel. A gel is a network of polymers or filaments which are cross-linked. A cross-linked gel with permanent links is a solid elastic body. If the links between polymers have a finite lifetime, the gel is still elastic if deformed only briefly, but exhibits liquid-like behavior if stresses are applied for a sufficiently long time. This combined behavior is called visco-elastic.

Active processes. Processes such as the force generation of motor proteins in the cytoskeleton are driven by the chemical energy of ATP hydrolysis. If such active processes take place in a system, thermodynamic equilibrium is not reached. In such a nonequilibrium situation, force and motion generation at the molecular scale modifies material properties of a cytoskeletal gel as compared to the passive case.

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