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Cell Protrusion and Retraction Driven by Fluctuations in Actin Polymerization: A Two-Dimensional Model

Gillian L. Ryan^{1,2}, Danielle Holz², Sawako Yamashiro³, Daisuke Taniguchi³, Naoki Watanabe³, and Dimitrios Vavylonis^{2,*}

¹Department of Physics, Kettering University, 1700 University Avenue, Flint MI 48504, United States

²Department of Physics, Lehigh University, 16 Memorial Drive East, Bethlehem PA 18105, United States

³Department of Pharmacology, Kyoto University Faculty of Medicine, Yoshida-Konoe-cho, Sakyo-ku, Kyoto, 606-8501, Japan

Abstract

Animal cells that spread onto a surface often rely on actin-rich lamellipodial extensions to execute protrusion. Many cell types recently adhered on a two-dimensional substrate exhibit protrusion and retraction of their lamellipodia, even though the cell is not translating. Traveling waves of protrusion have also been observed, similar to those observed in crawling cells. These regular patterns of protrusion and retraction allow quantitative analysis for comparison to mathematical models. The periodic fluctuations in leading edge position of XTC cells have been linked to excitable actin dynamics using a one-dimensional model of actin dynamics, as a function of arc-length along the cell. In this work we extend this earlier model of actin dynamics into two dimensions (along the arc-length and radial directions of the cell) and include a model membrane that protrudes and retracts in response to the changing number of free barbed ends of actin filaments near the membrane. We show that if the polymerization rate at the barbed ends changes in response to changes in their local concentration at the leading edge and/or the opposing force from the cell membrane, the model can reproduce the patterns of membrane protrusion and retraction seen in experiment. We investigate both Brownian ratchet and switch-like force-velocity relationships between the membrane load forces and actin polymerization rate. The switch-like polymerization dynamics recover the observed patterns of protrusion and retraction as well as the fluctuations in F-actin concentration profiles. The model generates predictions for the behavior of cells after local membrane tension perturbations.

Key words/phrases

Actin Polymerization; Cell Motility; Lamellipodium; Excitable Dynamics; Mathematical Modeling

*Corresponding author: vavylonis@lehigh.edu.

Introduction

Directed motility is an essential aspect of cellular function in animal development, wound healing, and cancer metastasis. Cells crawl over two-dimensional surfaces by coordinating actin-based protrusion of the cell front, adhesion of the cell to the substrate, and myosin-based retraction of the cell rear (Parsons et al. 2010). Actin-based protrusion is an essential element of motility in many eukaryotic cells (Pollard and Cooper 2009). Lamellipodia are thin and flat protrusions that contain a dense actin filament network (Watanabe 2010). Addition of actin monomers to the free barbed ends of the lamellipodial actin network near the cell membrane results in pushing of the cell front forward. Polymerization at the leading edge combined with adhesion and myosin contraction at the rear of the lamellipodium leads to a retrograde flow of the actin network away from the leading edge of the cell (Pollard and Cooper 2009; Watanabe 2010; Parsons et al. 2010). Successful protrusion of the cell front requires coordination of actin polymerization along the leading edge of the cell that overcomes this retrograde flow.

In many cells, usually soon after they spread on a surface and before the onset of directed cell motion, the protrusion of the lamellipodium is followed by retraction (Ryan et al. 2012a). This leads to cycles of protrusion and retraction that are periodic and in some cells organize into traveling waves of protrusion along the cell front and sides (Giannone et al. 2004; Machacek and Danuser 2006; Barnhart et al. 2011; Ryan et al. 2012a; Ryan et al. 2012b; Allard and Mogilner 2013; Driscoll et al. 2012; Lee et al. 2015; Liu et al. 2015; Raynaud et al. 2016; Barnhart et al. 2017). This regular behavior involving fluctuations around a steady state can be used to study how the dynamics of actin polymerization are converted into cell motion (Ryan et al. 2012a).

In a previous study using XTC cells (Figure 1A), which are cells with flat lamellipodia, the waves of protrusion were accompanied by waves of LifeAct-labeled F-actin accumulation along the lamellipodium (Ryan et al. 2012b) (Figure 1B–D). This phenomenon belongs to the general class of cellular actin waves (Inagaki and Katsuno 2017). The XTC cells' leading edge had a period of 130–200 sec. In all three cells depicted in Figure 1B the cell extends 1–2 μm during a cycle of protrusion and retraction. We used active contours to track the position of the edge of the cells over time (Ryan et al. 2012b; Ryan et al. 2013), and calculated the distribution of the standard deviation of membrane position along the radial direction measured at 1-degree intervals along the arc-length of the cell, shown in Figure 1C. The average standard deviations of membrane position for the three cells were 1.4 μm , 1.1 μm , and 1.3 μm respectively. The protrusion velocity was anti-correlated with the total local F-actin concentration measured by integrating LifeAct intensity over a 5 μm distance into the cell (Figure 1E). The time-dependent cross-correlation function (Figure 1E) showed that, on average, the fastest protrusion (retraction) speeds occurred just 10 sec after a minimum (maximum) of integrated F-actin intensity at a given position along the arc-length of the cell.

The retrograde flow rate was approximately constant during protrusion and retraction, suggesting that the changing F-actin localization in these cells stems from variations in the assembly and disassembly of the actin network near the leading edge, as opposed to stemming from changing retrograde flow rates (Ryan et al. 2012b). The constant retrograde

flow and fluctuating actin polymerization near the membrane in XTC cell lamellipodia is evident in the kymographs of Figure 1B, in which LifeAct forms diagonal lines of high intensity starting at the cell edge. These indicate an F-actin network that forms near the edge of three different cells and processes into the cell as time increases to the right. Alternating high and low intensity over time in Figure 1B points to changing actin polymerization. Net actin polymerization near the membrane, as evidenced by increasing intensity, occurs during protrusion, although the total intensity within the lamellipodium is greatest during retraction, consistent with the anti-correlation of Figure 1E (Ryan et al. 2012b). The notion that fluctuations in actin polymerization can drive such dynamics is further supported by the experimental evidence that other cell types treated with blebbistatin continue to protrude and retract periodically, although the period of oscillations may change (Yamashiro et al. 2014; Burnette et al. 2011; Giannone et al. 2007).

The periodicity of actin accumulation in XTC cells, coupled with traveling waves, is suggestive of an excitable system of actin assembly driving protrusion and retraction. This was the basis of the model of (Ryan et al. 2012b) where actin accumulation was modeled in one-dimension along the arc length of the cell. In this model, actin polymerization occurred near the cell membrane as the result of a diffusive membrane-bound activator. Accumulation of polymerized actin acted as a negative feedback, dampening activator accumulation. However the movement of the cell membrane was not addressed. In this paper we extend this prior one-dimensional model to incorporate the two-dimensional aspects of XTC lamellipodia and to examine the protrusion and retraction of the cell membrane over time. Related models (Wolgemuth 2005; Gov and Gopinathan 2006; Shlomovitz and Gov 2007; Carlsson 2010; Enculescu et al. 2010; Hecht et al. 2011; Xiong et al. 2010; Zimmermann et al. 2010) include pseudopodia formation in *Dictyostelium discoideum* (Xiong et al. 2010; Hecht et al. 2011) and models of cells where protrusion and retraction rely on changing myosin function and/or changing retrograde flow rates (Wolgemuth 2005), which is not necessary for XTC cells. A comparison of the one-dimensional excitable model to other models of related cell systems has been provided in (Ryan et al. 2012b; Ryan et al. 2012a). In the Discussion section of this paper we further compare the current work to more recent modeling works.

With our model we wish to capture the size and regularity of the XTC protrusions, while approximating the patterns of total F-actin generated by an excitable system. Our model can capture much of the membrane motion dynamics.

Model and Methods

The model describes the changing concentrations of free barbed ends, B , and F-actin, F , near the cell membrane as a function of position and time, as well as the position of the membrane (see Figure 2). It extends a previous one-dimensional model (Ryan et al. 2012b) that did not account for either membrane extension or actin concentration profiles. The model describes a part of the cell edge, for which the leading edge is along the x -axis, with the negative y -axis directed into the cell (we neglect the curvature associated with the shape of the whole cell). The displacement of the membrane along the y -axis is $h(s,t)$, where s is

length across the membrane. For simplicity, below we approximate $s \approx x$. Periodic boundary conditions are applied along the x -axis after a total width $20 \mu\text{m}$.

In this model we treat the F-actin network in the lamellipodium as a rigid network with sufficiently large modulus to be practically incompressible. This should be a good approximation for the XTC cells where the retrograde flow in the lamellipodium is approximately constant through leading edge protrusion and retraction cycles (Ryan et al. 2012b). Thus, we attribute protrusion and retraction to fluctuations of forces at the cell membrane, driven by fluctuations in barbed end concentration.

Assumptions on F-actin Assembly

F-actin assembly is distributed throughout the lamellipodium and does not occur solely where actin filaments touch the cell membrane. This is evident in single-molecule fluorescence microscopy where the association of a diffuse actin monomer or oligomer with the F-actin network appears as a single speckle ‘appearance’ event (Watanabe and Mitchison 2002; Smith et al. 2013; Yamashiro et al. 2014). In these studies, which addressed stationary lamellipodia (no protruding or retracting cycles), the dependence of the speckle appearance rate as a function of distance from the leading edge y has a peak within $1 \mu\text{m}$ of the leading edge. However polymerization events are observed as far as $8 \mu\text{m}$ into the cell. The appearance rate distribution is well-fit as a double exponential:

$$a(y) = A_1 e^{-y/\lambda_1} + A_2 e^{-y/\lambda_2}, \quad (1)$$

where $A_1 = 0.84$, $A_2 = 0.16$, $\lambda_1 = 0.5 \mu\text{m}$, $\lambda_2 = 4 \mu\text{m}$ (Smith et al. 2013). The first exponential term in Eq. (1) describes polymerization events occurring very close to the cell membrane while the second exponential describes basal polymerization further away. (Due to the microscope’s limiting resolution, some of the appearances attributed to the $\lambda_1 = 0.5 \mu\text{m}$ term may occur much closer to the cell membrane than $0.5 \mu\text{m}$.) The lifetime of the actin speckles can also be fitted by a double exponential with a half-life 24–30 sec (Watanabe and Mitchison 2002; Smith et al. 2013; Yamashiro et al. 2014).

To explain the observations in Figure 1, a model of fluctuations in actin polymerization would have to account for the time-variation of $a(y)$ as well as the variations in speckle lifetime. Since these time-dependencies have not been measured, we will make a few simplifying approximations. First, we assume that all the basal speckle appearance events are due to recycling oligomeric actin that break off and reassemble at the back of the lamellipodium (Smith et al. 2013). Second, we approximate the effective lifetime of F-actin in lamellipodia by a single exponential with a characteristic time $1/k_F^- = 100 \text{ sec}$. This lifetime is longer than the speckle lifetime due to several dissociation and local recycling events. We also assume it is independent of the protrusion velocity. Thus, in the model we examine here, fluctuations in actin polymerization arise from fluctuations in the polymerization rate very close to the membrane of the leading edge (in other words, they represent fluctuations of the amplitude of the λ_1 term in Eq. (1)). This would be consistent with our model’s other assumption of a slowly-diffusing membrane-bound activator. In summary, we assume that excitable dynamics of the total F-actin stems from changing

concentrations of free barbed ends adjacent to the membrane at the leading edge, and that these dynamics drive the periodic protrusion and retraction of the lamellipodium in XTC cells.

Model for barbed-end and F-actin concentrations

We assume that excitable biochemical kinetics govern the time-dependent variation of free barbed ends near (or very close to) the membrane. We assume free barbed ends of concentration $B(s,t)$ are generated by an autocatalytic membrane-bound activator with concentration $A(s,t)$. The accumulation of the activator is damped as a result of F-actin polymerization at the lamellipodium near the given arc-length position. The physical or biochemical origin of this inhibition has not been resolved experimentally and is not specified in our model. This combination of positive and delayed negative feedback provides the system with excitable dynamics. Random fluctuations in activator concentration can generate excitations by perturbing the system out of its stable state (Ryan et al. 2012b; Meinhardt and Gierer 2000; Xiong et al. 2010; Hecht et al. 2010). Denoting rate constants by symbols k and r and using the arc length $s \approx x$, the equations are:

$$\frac{\partial A(x,t)}{\partial t} = \left[r_0' + r_2' A(x,t)^2 \right] e^{-\int_{-\infty}^{h(x,t)} F(x,y,t)/F_{sat} dy} - k_A^- A(x,t) + D_A \frac{\partial^2 A(x,t)}{\partial x^2} + \sigma'(x,t), \quad (2)$$

$$\frac{\partial B(x,t)}{\partial t} = k_B^+ A(x,t) - k_B^- B(x,t), \quad (3)$$

$$\frac{\partial F(x,y,t)}{\partial t} = \gamma_{pol}(x,t) k_F^+ B(x,t) \delta[y - h(x,t)] - k_F^- F(x,y,t) + v_R \frac{\partial F(x,y,t)}{\partial y} \quad (4)$$

The first term on the right hand side (rhs) of Eq. (2) allows for spontaneous accumulation as well as non-linear self-recruitment of the activator. The exponential term that represents the negative feedback reduces the activator on-rate when the local F-actin concentration exceeds a saturation concentration, F_{sat} .

In Eq. (2) this concentration is measured by a line integral from the cell membrane towards the cell middle. The second term on the rhs in Eq. (2) represents deactivation. The third term on the rhs of Eq. (2) is diffusion of the activator and the last term is noise obeying Gaussian statistics with zero mean and variance $\langle \sigma'(x_1, t_1) \sigma'(x_2, t_2) \rangle = \sigma_0^2 \delta(t_1 - t_2) \delta(x_1 - x_2)$. Eq. (3) describes accumulation of free barbed ends as a result of the activation process. The rate constant k_B^+ describes the rate by which the activator generates new barbed ends. Rate constant $k_B^- \approx 0.4-8 \text{ s}^{-1}$ describes the rate of free barbed end loss through capping by

capping protein (Pollard et al. 2000). The first term on the rhs of Eq. (4) describes accumulation of F-actin as a result of polymerization at free barbed ends at the membrane. The polymerization rate is reduced compared to the polymerization rate in the absence of load by unitless reduction factor $\gamma_{pol}(x)$ with values between 0 and 1 that we describe in more detail below ($\gamma_{pol} = 1$ corresponds to polymerization under no load, while $\gamma_{pol} = 0$ corresponds to stalling of polymerization). The second term in the rhs of Eq. (4) is uniform F-actin disassembly rate, an approximation we discussed in section “Assumptions on F-actin Assembly”. The last term in Eq. (4) describes constant retrograde flow (v_R is a positive number), since the retrograde flow rate changed very little within 5 μm from the leading edge during protrusion and retraction XTC cells (Ryan et al. 2012b).

Since the capping rate, k_B , is much faster than the frequency of the protrusion and retraction events, generation of barbed ends must be fast enough such that B responds to changes in A quickly. This leads to $B \approx k_B^+ A / k_B^-$ that allows us to replace Eqs. (2) and (3) by:

$$\frac{\partial B(x, t)}{\partial t} = \left[r_0 + r_2 B(x, t)^2 \right] e^{-\int_{-\infty}^{h(x, t)} F(x, y, t) / F_{sat} dy} - k_A^- B(x, t) + D_A \frac{\partial^2 B(x, t)}{\partial x^2} + \sigma(x, t) \quad (5)$$

Here, unknown rate constant k_B^+ is absorbed into the new rate constants r_0 and r_2 and into the amplitude of the noise that is now σ_0 .

Model for membrane motion

We assume that the motion of the membrane at the leading edge results from the balance of forces due to actin polymerization, f_{pol} , membrane forces due to membrane tension and conserved cell surface area, f_{mem} (see Figure 2), and frictional forces, f_{fr} , that result from the breaking of links between the cell membrane and the F-actin network (Campas et al. 2012). Using $s \approx x$, applying force balance in the y-direction (here we assume variation of stresses in the x-direction can be neglected), and using units for f_{pol} , f_{mem} and f_{fr} of force per unit membrane length, one has for a given point on the cell membrane:

$$f_{pol} + f_{mem} + f_{fr} = 0. \quad (6)$$

The viscous resistance of the surrounding medium to the movement of the cell membrane is negligible compared to the forces of actin polymerization and membrane load (Enculescu et al. 2010) so we neglect it in Eq. (6).

Given a local concentration of free barbed ends, $B(x, t)$, that push against the membrane, the actin filament polymerization rate must adjust to satisfy Eq. (6). Assuming that a constant fraction of the actin filament elongation is converted into F-actin network expansion along the y direction, which occurs with rate $\partial h(x, t) / \partial t + v_R$, there exists a force-velocity curve

$f_{pol}[B(x, t), \partial h(x, t)/\partial t + v_R]$. The frictional force f_{fr} is also a function of the network expansion rate and the concentration of actin network links with the membrane (Campas et al. 2012). Here we will assume that the concentration of these links is approximately constant, which is consistent with approximately constant F-actin concentration near the membrane in certain cases (see Figure 6). Alternatively, f_{fr} could be negligible compared to the membrane force f_{mem} . In both cases, solving Eq. (6) for the membrane extension rate would give

$$\frac{\partial h(x, t)}{\partial t} = \gamma_{pol} [B(x, t), f_{mem}(x, t)] v_0 - v_R, \quad (7)$$

where v_0 is the network expansion rate at the leading edge in the absence of resisting force, and function $\gamma_{pol}(B, f_{mem})$, which can be evaluated at any point along the leading edge as a function of time, is the function that appeared in Eq. (4). Observed maximum protrusion speeds for XTC cells are similar in magnitude to retrograde flow speeds (Ryan et al. 2012b) so we estimate $v_0 \approx 2v_R$. Forward membrane motion requires a polymerization rate greater than the retrograde flow rate, $\gamma_{pol} > 1/2$. When $\gamma_{pol} = 1/2$, the system is balanced and the membrane does not move, while $\gamma_{pol} < 1/2$ leads to membrane retraction. In the remainder of this subsection we provide expressions for the restorative membrane force, $f_{mem}(x)$, which we assume depends on the shape and extension of the leading edge, and consider two possible functional forms of $\gamma_{pol}(B, f_{mem})$. Eqs. (4), (5) and (7), together with the following definitions of $f_{mem}(x)$ and $\gamma_{pol}(B, f_{mem})$, provide our complete model.

We include two components in the membrane force: a global area-conserving force and a component due to membrane curvature, $f_{mem} = f_{area} + f_{curv}$. Component f_{area} is directed towards the cell center and is the same for all x at any given time; it depends only on the average extension of the lamellipodium $\langle h \rangle_x$ measured from a reference extension h_{ref} :

$$f_{area} = -k_{mem} (\langle h \rangle_x - h_{ref}). \quad (8)$$

The curvature component of the membrane force depends on the membrane curvature $\kappa(x) = \partial^2 h(x, t) / \partial x^2$ at position x :

$$f_{curv}(x) = S \kappa(x), \quad (9)$$

where membrane tension, S , is a constant (Enculescu et al. 2010). As both positive and negative membrane curvatures are possible, this force may point inwards or outwards towards the center of the cell. The dependence of $f_{curv}(x)$ on the second derivative of h represents the effects of membrane tension (Gov and Gopinathan 2006). For simplicity, we do not include more complex dependencies in Eq. (9), such as 4th order derivatives that represent elastic bending contributions.

For a large enough lamellipodium segment, fluctuations in $\langle h \rangle_x$ at steady state are very small. Thus f_{area} settles down to an approximately constant value independent of k_{mem} and h_{ref} such that there is no net lamellipodial extension, $\langle \gamma_{pol} \rangle_t \approx \langle \gamma_{pol} \rangle_x \approx 1/2$. The values of k_{mem} and S are related to one another since they both include the effects of membrane tension. However, since only changes in S will have a significant impact in our results, we treated k_{mem} and S as independent variables for convenience.

We examined two functional forms of γ_{pol} , corresponding to exponential (“Brownian-ratchet-like”) and switch-like force-velocity relationships, respectively. For both models we assume that the membrane force is distributed equally among the free barbed ends at the leading edge.

Ratchet Model. In this model we assume that each filament end grows with an exponential force-velocity curve as would be expected by a Brownian ratchet mechanism (Mogilner and Oster 1996; Peskin et al. 1993; Enculescu et al. 2010).

$$\gamma_{pol}^{ratchet}[f_{mem}(x, t), B(x, t)] = e^{-\alpha f_{mem}(x, t)/B(x, t)}. \quad (10)$$

In Eq. (10) the polymerization rate decreases exponentially with the force per filament, with a scaling factor $\alpha = \delta/k_B T = 0.66 \text{ pN}^{-1}$, where $\delta = 2.7 \text{ nm}$ is the filament elongation length per actin monomer. The ratchet model dictates a gradual change in the actin polymerization rate in response to changes in the ratio of the concentration of free barbed ends and the membrane load. When f_{mem} is approximately constant, a large change in B will be required to transition between maximum protrusion rates ($\gamma_{pol} = 1$) and maximum retraction rates ($\gamma_{pol} = 0$).

Switch-like Model—Interactions among filaments and attachment to the membrane (Enculescu et al. 2010) may give rise to non-linearities in the force-velocity curve leading to concave-down force-velocity curves as observed during gliding motility of keratocytes (Prass et al. 2006; Heinemann et al. 2011; Keren et al. 2008), as well as stick-slip behavior (multiple branches in force-velocity relationship) (Wolgemuth 2005; Dubrovinski and Kruse 2011; Cirit et al. 2010). Here we do not consider the more-complex possibility of stick-slip behavior, but investigate a concave dependence given by a Hill function. In our phenomenological description, filament polymerization shifts rapidly from being unencumbered to being stalled completely upon increasing load:

$$\gamma_{pol}^{switch}[f_{mem}(x, t), B(x, t)] = 1 - \frac{[\alpha f_{mem}(x, t)/B(x, t)]^n}{K_0^n + [\alpha f_{mem}(x, t)/B(x, t)]^n}. \quad (11)$$

We chose $n = 10$. The constant K_0 determines the location of the inflection point of the Hill function, and was chosen to preserve the fixed point of the model from the ratchet polymerization case ($\gamma_{pol} = 1/2$ at the same h_{ref} and f_{mem}/B). Similar dependencies were also assumed in models of actin network growth during protrusion of nerve growth cones

(Craig et al. 2012) and keratocytes (Adler and Givli 2013; Barnhart et al. 2017). As we will discuss below, this step-like change in leading edge speed is essential to produce protrusions and retractions with large amplitude and variation in curvatures.

Numerical Implementation

Computer simulations of the model were implemented on an $L_x = 40 \mu\text{m}$ wide and $L_y = 20$ to $40 \mu\text{m}$ tall system with $-L_y/2 < h < L_y/2$. The time step was $dt = 0.001\text{--}0.002$ s, with lattice spacing $dx = 5 \sqrt{2D_A dt} \mu\text{m}$, $dy = 0.025 \mu\text{m}$ and the system was evolved with the fourth-order Runge-Kutta method in time. The noise term was evaluated as in (Ryan et al. 2012b). Periodic boundary conditions were applied in the x-direction, along the arc-length of the leading edge. The F-actin concentration was set to 0 beyond the inner cell boundary along the y direction, a distance at which it decays to negligible values. Since the F-actin field moves into the cell by unidirectional transport, the rest of the simulation is not influenced by this boundary condition. Although the lattice points were discrete, membrane movement was allowed on a continuum. As a result, the lattice sites nearest the membrane had effective lattice spaces dy' , where $0 < dy' < dy$. Adjustments were made to calculations at lattice sites adjacent to the membrane such that retrograde flow and membrane movement conserve the total amount of F-actin in the system. Simulations were initialized with a flat membrane at $h = 0$. The system was allowed to relax for 1000 s without allowing any membrane movement to populate an initial distribution of barbed ends and F-actin throughout the system. The membrane was subsequently allowed to move for an additional relaxation time of 500 s before measurements were recorded for analysis.

Linear Stability Analysis

To interpret the numerical results, we performed linear stability analysis of the model without noise. This analysis is provided in Supplementary Text and Figures S1–S4. The fixed point of a homogeneous system without noise (at which $\gamma_{\text{pol}} = 1/2$) was calculated numerically, using $h_{\text{ref}} = 0 \mu\text{m}$ as the reference point. The switch-like model requires one more parameter than the ratchet model, K_0 . For any parameter set, K_0 was chosen to preserve the fixed point between both force-velocity models, so that $\gamma_{\text{pol}}^{\text{switch}} = \gamma_{\text{pol}}^{\text{ratchet}} = 1/2$ occurs at the same fixed point (B^*, F^*, h^*) in both models. The stability analysis shows that the system acquires oscillatory relaxation dynamics for wavenumbers q smaller than $1.5\text{--}5 \mu\text{m}^{-1}$ and these exist at the boundary of linear stability.

Results

Model reproduces features of protrusion and retraction observed in cells

Both the ratchet and switch-like model produce results that are in qualitative agreement with experiments. We start with the switch-like model that exhibits a sharper behavior. This model of force dependence of actin polymerization on membrane load, Eq. (11), coupled with our model of excitable actin dynamics leads to periodic protrusion and retraction of our model membrane, accompanied by periodic increases in the amount of F-actin in the modeled lamellipodium, using the parameter values in the Table (Figure 3A, Movie S1, which is qualitatively similar to experimental Movie S2 in (Ryan et al. 2012b)). Periodic

activity is driven by random concentration fluctuations generated by the noise term in Eq. (5). The noise generates local spikes of free barbed end concentration that spread across the leading edge, causing membrane protrusion. Actin polymerization rates peak during protrusion, leading to increases in F-actin at the membrane during those events. Accumulation of F-actin subsequently causes a reduction of the barbed end concentration (negative feedback), leading to retraction.

Retrograde flow of the actin network distributes the F-actin throughout the lamellipodium over time. Combined with the periodic nature of the polymerization rate, this flow causes striping in the kymographs of Figure 3B. The membrane shape and kymograph in Figure 3A, B are very similar to those observed experimentally (Figure 1A, B) with the αS value of 1 closest to experiment.

The standard deviation of membrane position, as well as the range of leading edge speed depend on the magnitude of the membrane curvature force, described by the dimensionless parameter αS (Figure 3C, D). Both quantities become smaller as αS is increased, indicating the increasing resistance of membrane tension to the formation of protrusions and retractions. As anticipated, the results are weakly dependent on the value of k_{mem} determining the area force (not shown).

To help illustrate how the model is able to reproduce experimental membrane protrusion and retraction, in Figure 3E the polymerization rate reduction factor γ_{pol} is plotted as a function of membrane force, f_{mem} , and barbed ends, B , using Eq. (11). Consider first small values of αS for which the curvature force is negligible and the membrane force is approximately constant over time and equal to the global area-conserving force $f_{\text{mem}} \approx f_{\text{area}} \approx 58 \text{ pN}/\mu\text{m}$ (orange color in Figure 3F). The concentration of free barbed ends, B , described by Eq. (5) varies by approximately a factor of two during an excitation at the leading edge (Ryan et al. 2012b). Indeed, Figure 3F shows the average value for B is of order $80 \mu\text{m}^{-1}$ and B varies mostly in the range between $40 \mu\text{m}^{-1}$ and $130 \mu\text{m}^{-1}$. Since the average value of B

corresponds to $\gamma_{\text{pol}} = \frac{1}{2}$, fluctuations in B cause the system to switch between 0 (stall, resulting in retraction at the speed of retrograde flow) and 1 (maximum polymerization rate, which is about twice the retrograde flow rate), see Figure 3E. The nonlinearity around

$\gamma_{\text{pol}} = \frac{1}{2}$ thus leads to a change from protrusion to retraction as B fluctuates.

Now consider large values of αS for which the curvature force becomes comparable to the area conserving force, f_{area} . The curvature force now works against the effect of polymerization on protrusion, acting inwards, along the direction of retraction, at a protrusion with positive curvature, and outwards, along the protruding direction, in a retracted membrane with negative curvature. Thus, the total membrane force increases during protrusion (higher B) and decreases during retraction (smaller B), see Figure 3F (blue

points). This trajectory of B and f_{mem} is along the diagonal $\gamma_{\text{pol}} = \frac{1}{2}$ of Figure 3E, resulting in a smaller variation of polymerization rate as compared to the case of lower membrane curvature force.

The results of the ratchet force-velocity model are shown in Figure 4. The ratchet model produces periodic increases in actin concentration at the membrane that populate the lamellipodium via retrograde flow similar to those observed in experiment (Figure 4A, B and Movie S2). These fluctuations are however accompanied by smaller membrane distortions and membrane speeds (Figure 4C, D) as compared to the switch-like model. It is thus rarer to have membrane retraction at a speed equal to the retrograde flow speed as observed in experiments (Figure 1B). The leading edge speed is also typically less than 20% of the maximum possible speed.

The reason for the smaller fluctuations in the ratchet model is the weaker dependence of γ_{pol} on the barbed end concentration B and membrane force f_{mem} near $\gamma_{pol} = \frac{1}{2}$ (Figure 4E): a change of barbed end concentration by a factor of two during an excitation changes γ_{pol} by a smaller fraction as compared to the switch-like model, for both small and large value of the membrane curvature force amplitude αS (see Figure 4E, F and compared to Figure 3E, F).

A comparison of the behavior between the two models is shown in Supplementary Figure S5, where we show the trajectories as function of protrusion rate and total actin concentration and compare to experimental measurements. Both models exhibit cyclical behavior as in experiment, however the switch model explores a larger range of values of membrane speed and position, with sharper features.

Protrusion waves and correlations between protrusion rate and F-actin concentration

Having established that the model is capable of generating protrusions of a size comparable to those observed in real cells, we now investigate whether or not our model generates short-range traveling waves of protrusion along the arc length of cells as in Figure 1D. Figures 5A, B show the F-actin concentration integrated over the lamellipodium width and leading edge velocity as functions of arc length and time resulting from a simulation with the same parameter set as Figure 3A, $\alpha S = 0.1$ (switch-like model). As in previous work (Ryan et al. 2012b), our model produces short-range traveling waves of F-actin along the arc length of the cell, which travel along both directions (Figure 5A). In addition, we find that the modeled cell also exhibits short-range traveling waves of protrusion and retraction (Figure 5B), similar to the experiments (Figure 1B).

Although we have found that F-actin polymerization is enhanced during the simulated protrusion (Figures 3A and 4A), we have not yet examined the correlation between the total F-actin concentration in the lamellipodium and the leading edge speed. As in Figure 1E, we measure the phase difference between the two signals by examining the average cross-correlation at fixed position as a function of time delay (see Figure 5C, D). We find that for both the switch and ratchet models of actin polymerization, the cross-correlation of the total actin concentration and the leading edge velocity is strikingly similar to that calculated for experimental measurements of LifeAct-mCherry intensity and leading edge velocity in Figure 1E, with a correlation coefficient of near-zero at zero time delay, and a large trough at 50 sec, indicating anticorrelation. The position of this minimum around 50 sec compares well with experiment in Figure 1E. The model also reproduces the same sequence of peaks in the cross-correlation, velocity autocorrelation, and LifeAct-mCherry intensity

autocorrelation as in Figure 1E at around 100 sec, which indicates the period of these signals (roughly consistent with the value of the period obtained from linear stability analysis, Figures S1C and S2C).

F-actin concentration profile during protrusion and retraction

We next checked to see if our model can capture the fluctuations of the F-actin concentration profile across the lamellipodium, despite our simplified treatment of basal polymerization, disassembly, and remodeling away from the leading edge. We analyzed experiments of XTC cells and measured the LifeAct-mCherry intensity as function of distance to the leading edge and membrane protrusion rate (Figure 6A, B). The measurements of intensity and leading edge velocity were performed by fitting an active contour to the cell boundary and averaging over time and position along the leading edge as described in (Ryan et al. 2012b; Ryan et al. 2013).

In Figure 6A we observe that the integrated F-actin concentration is maximum during retraction and minimum during protrusion, as expected from the cross-correlation function of Figure 1E. The profile has a sharper peak during protrusion at 1 μm into the cell that develops into a hump that peaks 2–3 μm into the cell during retraction. An example from another cell is shown in Figure 6B, also showing a similar development of a hump during retraction at 4 μm into the cell. However this trend is inverted at 1 μm into the cell where the intensity is maximum during retraction. The correlation curves for the cell in Figure 6B are very similar to those of the cell in Figure 6A shown in Figure 1E.

The switch-like model, using the parameters shown in the Table, reproduces a hump peaking 3–4 μm into the cell during retraction (Figure 6C). This trend is inverted close to the leading edge, where the concentration peaks during protrusion, as in the cell in Figure 6B.

Considering that analysis of experimental images involves uncertainty in measuring leading edge position of order 0.2 μm and velocity uncertainty of order 10 nm/s, the results of switch-like model in Figure 6C are consistent with the data in Figure 6A, B. Such errors are likely to blur a narrow sharp concentration peak close to the leading edge during protrusion, except in cases such as the cell in Figure 6B that exhibits a more uniform actin distribution. The ratchet model, using parameters from the Table, generates features similar to those of the switch-like model, however the magnitude of the concentration changes is enhanced (Figure 6D). The profiles in Figure 6D are less consistent with the experimental profiles in Figure 6A, B: the predicted concentration increase during protrusion and concentration dip during retraction within 1 μm from the leading edge would have been large enough to be detectable in Figure 6A or 6B.

We found the measured change of retrograde flow speed as a function of distance from the leading edge does not cause a significant modification of the concentration profiles. A moderate decrease in retrograde flow for distances of 5 μm or larger into the cell was measured in (Ryan et al. 2012b). Including this decrease in our model does not change the actin profiles significantly (Figure S6) but leads to movies that better resemble experimental movies (Movie S3).

In conclusion, our model that assumes that the excitable component of the actin network occurs close to the leading edge is also able to capture the density profile fluctuations, however we cannot exclude additional contributions of fluctuations in polymerization/depolymerization away from the leading edge.

Response of leading edge to membrane perturbation

Our model generates predictions for the response of lamellipodia to changes in membrane forces. Circularly-shaped XTC cells spread on a substrate transition from a state of stable non-oscillating lamellipodia to a transient state of large protrusions and retractions after treatment with blebbistatin (Yamashiro et al. 2014). During this response, the adhered cell surface area increases while no change in the retrograde flow rate within the lamellipodium can be detected. We suggest that the transient reduction in membrane tension after blebbistatin (Lieber et al. 2013) arising from the loss of contraction in the medial cell region, contributes to this behavior, in addition to other changes in biochemical reaction rates.

A simulation of a lamellipodium undergoing a perturbation in membrane forces is shown in Figure 7A. As initial conditions we use parameter values from the Table except for a higher value for the amplitude of the membrane curvature force αS that does not allow large protrusions and retractions (some fluctuations still remain). When the membrane curvature force and membrane area-conserving force are reduced simultaneously, the leading edge extends by a few μm and large protrusions and retractions develop, similar to the experiment in (Yamashiro et al. 2014) (Figure 7B).

The effect of membrane forces in lamellipodia undergoing excitable dynamics could be further tested by local mechanical perturbation of membrane forces, for example membrane pulling by optical tweezers. The case of a lamellipodium with the same initial state as in Figure 7A, perturbed by a local decrease in membrane force (simulating the effect of pulling by optical tweezers) is shown in Figure 7C, D. This results in a local extension of the leading edge, through local oscillatory behavior (Figure 7D).

Discussion

This work highlights how the mechanical and biochemical interactions combine non-linearly to generate fluctuations in actin polymerization that drive lamellipodial protrusions (Verkhovsky 2015). Our model extends the model of (Ryan et al. 2012b) by providing the F-actin profile as a function of distance away from the leading edge, the lamellipodium extension profile $h(x)$, and it also includes the effect of membrane tension. The resulting steady state of the system depends on a feedback between biochemical interactions and membrane tension. We find that increasing membrane tension (while keeping other parameters fixed) suppresses the amplitude of protrusions and retractions at a given x position and smooths out the $h(x)$ profile at any given time (Figures 3, 4). In the limit of very large membrane tension the profile becomes flat and the model reduces to that of (Ryan et al. 2012b), which allows excitable behavior with fluctuations of barbed end and F-actin concentrations without net membrane extension, with appropriate choice of rate constants.

Our model provides a framework for future whole-cell models that combine membrane deformation, actin network flow and distributed actin turnover.

The near-constancy of retrograde flow in XTC cells was an advantage since it allowed us to focus on the kinetics of actin polymerization and force balance at the membrane. Thus, we did not write equations for the stresses that develop within the lamellipodial network, the effect of myosin pulling at the back, and the force balance with traction forces at focal adhesions (Welf et al. 2013; He and Ji 2016). Such stresses should result in small changes of the retrograde flow rate in this system (Figure S6). Future experiments with modified XTC adhesion could allow addressing this next layer of complexity in a systematic way, using models of actin dynamics that include actin turnover and focal adhesion formation and maturation away from the leading edge.

The diffusing, self-amplifying activator of our model is most likely composed of more than one molecular species. The Arp2/3 complex, which accumulates in bursts along the leading edge preceding total F-actin maxima and which has autocatalytic activity (nucleating branches off branches) is likely involved (Ryan et al. 2012b). However, the effective diffusion calculated by random branching may be too slow to account for the activator's diffusion coefficient in our model (Ryan et al. 2012b). Other actin polymerization regulators such as VASP (Barnhart et al. 2017) or formins (Lee et al. 2015) may play a role too, however their diffusion coefficients when attached to the filament ends would be negligible. The value of the activator diffusion coefficient in our model suggests the involvement of regulators diffusing on the membrane. Several candidates, to be resolved in future studies, can play a role upstream of actin polymerization (Inagaki and Katsuno 2017), such as SCAR/WAVE (Millius et al. 2012), small GTPases (Machacek and Danuser 2006), and PIP3 (Taniguchi et al. 2013).

The mechanism driving protrusions and retractions in our model is fluctuations in actin polymerization rate (Ryan et al. 2012b). This mechanism was also the basis of a model of waves in keratocytes (Barnhart et al. 2017), however these authors did not model explicitly the process of local membrane protrusion. The changing density of free barbed ends in the Barnhart et al. model is due to VASP which localizes at the membrane and is depleted by adhesions with the free barbed ends. A cooperative force-velocity curve similar to the switch model was implemented, however, unlike our model, a global constraint on membrane tension allowed only one protrusion to occur in the membrane at a time. Another related model where protrusions are driven by nucleation of new filaments is the model by (Zimmermann and Falcke 2013; Zimmermann and Falcke 2014). In their model, capping and severing were assumed to decrease the actin filament density. The actin network in their model membrane consists of a semiflexible region close to the membrane and a gel like region consisting of cross-linked filaments further away (Zimmermann and Falcke 2013; Zimmermann and Falcke 2014). However, the Zimmerman and Falcke model was developed in one dimension.

Membrane protrusions coupled to an excitable biochemical network have also been studied in the context of *Dictyostelium* morphodynamics (Taniguchi et al. 2013; Huang et al. 2013; Moure and Gomez 2016). These studies used versions of the phase field method to track

movements of cell boundaries. In Taniguchi et al., the positive and negative feedbacks were due to PIP2 phosphorylation and PIP3 dephosphorylation while actin was not explicitly modeled. The model of Huang et al. included a slow excitable system, reflecting the behavior of the signal transduction excitable network and a fast oscillatory system, reflecting the behavior of the cytoskeletal oscillatory network. These two studies however did not study actin retrograde flow and actin density profiles as in our work. Moure and Gomez further used phase fields for actin that included cytosolic flow described by modified Stokes equations which included adhesion, membrane and wall forces. Future modeling work could investigate in more detail the similarities between *Dictyostelium* and animal cells, combining the models of signal networks with models of cytoskeletal flows, moving cell boundaries and models of biophysical regulation of actin polymerization (Shao et al. 2010; Danuser et al. 2013; Holmes and Edelstein-Keshet 2012).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Experimental observations of leading edge protrusion and retraction. (A) An XTC cell expressing LifeAct-mCherry. (B) Kymographs of the leading edge of XTC cells expressing LifeAct-mCherry from movies S2 (i) and panel A, S3 (ii), and S4 (iii) of (Ryan et al. 2012b). The cells protrude and retract periodically along the radial direction of the cell, with typical extensions during protrusion of 1–2 μm . F-actin polymerized near the leading edge during protrusion moves backwards into the cell via retrograde flow, resulting in the diagonal striping of intensity in the images. (C) Distribution of the standard deviation of the radial membrane position at a fixed angular position in the cells from movies S2 (blue, $N=360$ points), S3 (red, $N=70$ points), and S4 (green, $N=100$ points) of (Ryan et al. 2012b). The average standard deviation of the membrane position of the cells are 1.4 μm , 1.1 μm , and 1.3 μm respectively. (D) Normal leading-edge velocity (with respect to fixed substrate) versus angle and time, for cell in Figure 1G of (Ryan et al. 2012b). Positive (negative) velocities indicate protrusion (retraction). The retrograde flow speed for this cell (74 ± 3 nm/s, $n = 15$ measurements of bright features in kymographs) did not vary noticeably during observation. (E) Average correlation coefficients for leading-edge velocity autocorrelation, LifeAct-mCherry autocorrelation, and LifeAct-mCherry-velocity cross correlation, versus time and with no positional offset. Panels A, D and C reproduced from (Ryan et al. 2012b).

Figure 2.

Two-dimensional model of the leading edge of a cell. Our model follows the protrusion and retraction of the cell membrane in response to excitable dynamics of the actin network. Actin polymerization is stimulated by an autocatalytic, diffusive membrane-bound activator, A , which generates new free barbed actin polymer ends. We model the membrane height, h , the concentration of filamentous actin, F , and the concentration of free barbed ends of actin polymers, B , over time. Membrane movement is governed by protrusive forces stemming from actin polymerization, and forces that minimize both the global extension and local curvature of the membrane.

Figure 3.

Results of switch model show a broad range of possible leading edge speeds, leading to protrusions similar to those observed in experiment. (A) Snapshots of the simulation for αS values of 0.01, 1, and 15. (B) Kymographs of the leading edge for the same αS values as in panel A. Intensity indicates local F-actin concentration. (C) Average standard deviation of the membrane position (average over all positions in the simulation at steady state, over 500 s) versus membrane curvature force amplitude αS . (D) Histogram of leading edge velocities for the same membrane curvature force amplitude αS as in panel A, calculated over several

rounds of protrusion and retraction. (E) The polymerization factor γ_{pol} for the switch model is insensitive to small changes in the number of free barbed ends, B , and membrane bending force f_{mem} , except near a transition diagonal region where small changes lead to a sharp transition between $\gamma_{poly} = 0$ and $\gamma_{poly} = 1$. (F) Trace of values of B and f_{mem} at a given membrane position over the course of a simulation, for magnitude of membrane curvature force amplitude equal to $\alpha S = 0.01$ (orange) and $\alpha S = 10$ (blue). For high values of αS , B and f_{mem} are located along the diagonal, causing less variation of γ_{pol} over one period (compare to panel E). This leads to smaller variation of velocities and extension as shown in panels C and D. Unless otherwise specified, all data shown are for the values shown in Table I.

Figure 4.

Ratchet model exhibits protrusion and retraction but does not sample the full range of possible leading edge speeds. Panels show same quantities as Figure 3, using values shown in Table I. Unlike the switch model, in panel E the polymerization factor γ_{pol} varies gradually as a function of the local density of free barbed ends and the membrane force. Thus the changes in either value during protrusion and retraction (panel F) lead to little change in the polymerization rate (panel D) and smaller membrane extension compared to the switch model (panel C).

Figure 5.

Protrusion waves and correlations between protrusion rate and F-actin concentration. (A) Total F-actin concentration versus arc length and time for switch-like model. (B) Same as A, but for leading edge velocity. (C) Correlation coefficient versus time delay and with no positional offset for leading edge velocity autocorrelation, total F-actin autocorrelation, and the cross correlation between panel A and B. (D) Same as panel C, for the ratchet model. Model parameter values from Table I, using $\alpha S = 0.1$ (similar results are found for $\alpha S = 1$).

Figure 6.

Concentration profile across the lamellipodium as function of protrusion rates. (A) Experimental measurement of LifeAct-mCherry intensity as a function of distance from the leading edge, sorted by leading edge speeds. Data is for the same cell as Figure 1B. We find that the intensity of LifeAct, and thus F-actin, increases during retraction (denoted by negative speeds). (B) Same as panel A, for a different cell. (C) Simulated F-actin concentration as a function of distance from the leading edge, sorted by leading edge velocity in the model with switch-like polymerization. (D) Same as panel C, for the ratchet model. All simulation data shown are using values from Table I using $\alpha S = 0.1$ (similar results are found for $\alpha S = 1$).

Figure 7.

Simulations of the response of the lamellipodium to a global and local perturbation of membrane force. (A) Simulation with initial conditions from Table I ($\alpha S = 1$). At $t = 0$ the membrane area force is reduced by $200 \text{ pN}/\mu\text{m}$ globally along the length of the cell and the membrane curvature force is reduced to $\alpha S = 0.01$. Simulation shows the cell is initially quiescent and starts to expand upwards, generating large protrusions and retractions after the perturbation. (B) Graph showing the average position of the leading edge over a $5\text{-}\mu\text{m}$ -wide region in the cell of panel A. (C) An upward $750 \text{ pN}/\mu\text{m}$ force is applied to the membrane of a cell initialized as in panel A over an area of $0.5 \mu\text{m}$. This force mimics the pulling of the membrane by optical tweezers. The section of the membrane undergoing an external force oscillates and is stretched by approximately beyond the previous membrane position. (D) Graph showing the position of the leading edge in the simulation of panel C.

Table I**Model Parameters**

Parameter	Physical Meaning	Value
F_{sat}	F-actin saturation concentration	1000 $\mu\text{M } \mu\text{m}^a$
k_{F}^+	Effective actin assembly rate	0.224 $\mu\text{M s}^{-1} b$
k_{F}^-	Effective actin disassembly rate	0.01 $\text{s}^{-1} c$
k_{A}^-	Activator deactivation rate	0.03 $\text{s}^{-1} c$
D_{A}	Activator diffusion coefficient	0.1 $\mu\text{m}^2 \text{s}^{-1} c$
r_0	Activation rate constant	240 $\mu\text{m}^{-1} \text{s}^{-1} d$
r_2	Nonlinear activation rate constant	0.1 $\mu\text{m s}^{-1} e$
v_r	Retrograde flow rate	0.05 $\mu\text{m s}^{-1} f$
L	Membrane length	40 μm
n	Hill Coefficient	10 g
σ_0	Noise coefficient	9.5 $\mu\text{m}^{-1/2} \text{s}^{-1/2} c$
α	Scaling for polymerization rate	0.66 $\text{pN}^{-1} h$
αk_{mem}	Coefficient of area conserving force	12 $\mu\text{m}^{-2} i$
αS	Coefficient of membrane tension	1 j

^aValue corresponding to 200 μM in (Ryan et al. 2012b) that considered the local concentration of F-actin over a distance of 5 μm .

^bThis assembly rate is two times as large as actin assembly rate of (Ryan et al. 2012b), due to our steady state of $\gamma_{\text{pol}} = 1/2$ that corresponds to a polymerization rate of 66 subunits per second per filament (Watanabe 2010).

^cSame as (Ryan et al. 2012b).

^dChosen to reproduce a period comparable to experiment for switch-like model. This value is equivalent to 0.4 $\mu\text{M s}^{-1}$ which is 12.5 times smaller than in (Ryan et al. 2012b).

^eValue equivalent to 60 $\mu\text{M}^{-1} \text{s}^{-1}$ of (Ryan et al. 2012b).

^fTypical retrograde flow rate in XTC cells (Ryan et al. 2012b).

^gChosen for a concave-down curve similar to gliding motility of keratocytes (Prass et al. 2006; Heinemann et al. 2011; Keren et al. 2008).

^hScaling factor for the polymerization rate, $\alpha = \delta/k_{\text{BT}}$ with δ the elongation distance per monomer.

ⁱChosen for computational efficiency, results are not sensitive to this value.

^jCorresponds to membrane tension $S = 1.5$ pN. Taking into account the lamellipodium height h , the value of S/h is of order 10 pN/ μm (Enculescu et al. 2010), close to 10 pN/ μm for Dictyostelium (Simson et al. 1998) and 275 pN/ μm for keratocytes (Lieber et al. 2013).