Analysis of actin dynamics at the leading edge of crawling cells: implications for the shape of keratocyte lamellipodia

Summary of the model in the article by
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In the first article, the authors developed a model based on comparison of simple thermodynamic quantities. The present article turns a different approach; it includes the dynamic of the lamellipodium, with details such as dendritic nucleation, elongation, capping and disassembly of the actin filaments. This makes the model more prone to mistakes, but gives some explanation why the cells acquire exactly the shape they have, while the first article assumes very simplified shapes and does not provide any connection between geometric shape of the cell and the structure of lamellipodium. Here however, the authors analyze the feedback between the actin density and the edge shape and try to explain, why does the shape remain stable, when the cell moves forward. As in the first article the model is based on the fish epidermal keratocyte cells.

The authors implement a model using a computer simulation, which consist mainly of three steps:

1. update the actin density using the cell front

   Therefore they define their own "Mathematical model of actin dynamics"

2. compute local protrusion velocity from actin density

3. update the shape of the edge

   For the last two points, they use the formulas from the "Elastic polymerization ratchet model" by Mogilner and Oster (1996) and some from "Graded radial extension model" (Lee et al. 1993b).

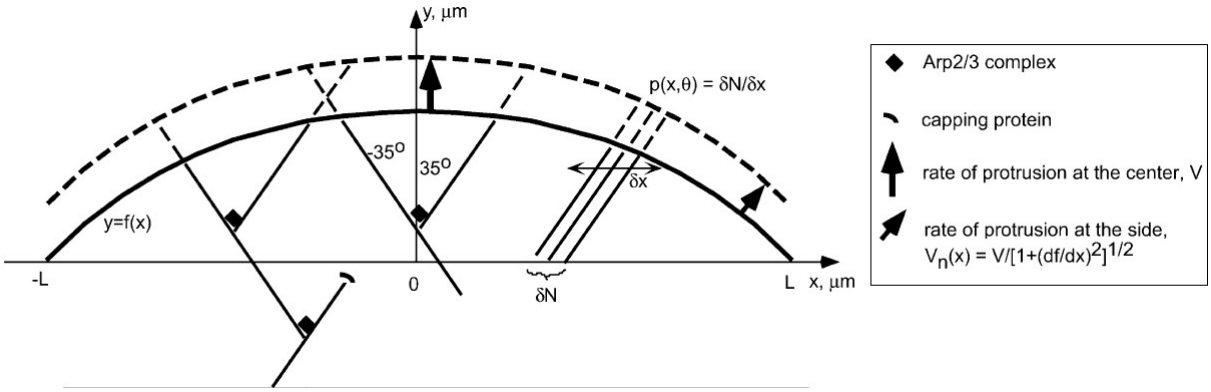
After describing the mathematical models mentioned above, the authors want to **adjust the parameters** in these models to match the biological facts. To do this they proceed in the following order:

1. They state the results of biological observations.
2. They investigate qualitatively the properties of actin density in few special cases in the proposed "Mathematical model of actin dynamics". More precisely, they:
   - investigate the case of flat leading edge. This allows them to prefere the "local" scenario over the "global" one. (see next page)
   - investigate how small indentations or bumps on the leading edge influence the actin density. They predict, that this leads to instability of the cell shape for smaller values of capping parameter and to stability for larger values. They conclude from this, that the capping parameter shouldn't be to small.
3. They present the average measured actin density and the average leading edge shape from experiment. Then they compare the actual average density with the densities predicted from the "Mathematical model of actin dynamics" applied on the given average shape. They do this for different capping parameters and choose the closest reasonable parameter. Important: On this stage they assume the leading edge shape as constant and do not use the "Elastic polymerization ratchet model".

4. Afterwards, they use the experimental data about the edge shape and calculate, which velocities are necessary for different sections of the edge, to retain the edge shape. (The measures show that the shape is stable). They put these velocities into a graph together with the experimental data for the actin density on the same section. Now they can adjust the parameters in the formula of the "Elastic polymerization ratchet model" to comply with the graph.

5. Finally, they simulate the entire model with estimated parameters with the iterative procedure described above and get a stable shape of the leading edge, which approximately complies with the measurments.

1 The mathematical model of actin dynamics



The leading edge is described by the solid curve $y = f(x)$. We describe the actin network along the cell front between the lateral edges $-L < x < L$. When the cell movement is steady, the shape of the leading edge is constant (solid and dashed curves). Before a filament gets capped and disappears from the leading edge, its tip remains localized at the leading edge along which it glides laterally while growing (dashed segments). It is assumed that all filaments are oriented either $-35°$ or $35°$ relative to the x-axis, i.e. either negative or positive. With $p^-(x,\theta)$ and $p^+(x,\theta)$ respectively, we designate the number of filaments of the given orientation divided by the length of the segment parallel to the x-axis, which they intersect.

1.1 Main model equation

$$\underbrace{\frac{\delta p^{\pm}}{\delta t}}_{density\ change} = \underbrace{\pm\frac{\delta}{\delta x}(v^{\pm}p^{\pm})}_{lateral\ flow} + \underbrace{\beta b_{1,2}(p^-,p^+)}_{branching} - \underbrace{\gamma p^{\pm}}_{capping} \tag{1}$$

We assume initial conditions $p^-(L) = 0$ and $p^+(-L) = 0$ (i.e. no right- (left-) pointing filaments emerge from left (right) lateral edge).

The right side of the equation consists of three terms:

1. **Lateral drift** of the growing filaments; the corresponding rate of the flow, $v^{\pm}$, can be obtained from the geometric considerations:

$$v^{\pm}(x,t) = \frac{\mp\frac{\delta f}{\delta t}}{\frac{\delta f}{\delta x} - \cot(\pm 35°)}$$

Important: the term v contains the edge-shape function f. (the goal of the authors is to investige the feedback between the actin density and edge shape.) In the special case of the steady protrusion of the leading edge with constant shape and velocity V:

$$v^{\pm}(x,t) = \frac{V}{\pm\frac{\delta f}{\delta x} + 1.42}$$

The case of $\frac{\delta f}{\delta x} = 0$ (flat leading edge) is presented as an important example, as the equations can be solved analytically.

2. The second term describes **branching**, which is modeled by the constant rate β multiplied by some function dependent of the densities of the left- and right- oriented filaments. In the article, the authors consider two possible choices for such function:

   Local scenario assumes that the Arp2/3 complexes (which are distributed gradually along the edge) activate branching only at their present location. That means: there is a constant number of new branch nucleations for each section of the edge. New branches are always nucleated on the filaments of the opposite orientation. We assume that the filaments on the same location x have equal chance to nucleate new branch. A constant number β of nascent filaments are nucleated per second per micron of the cell boundary, therefore:
   $b_1(p^-, p^+) = \frac{p^{\mp}(x,t)}{p^+(x,t)+p^-(x,t)}$ (unclear notation; $\mp$ correspond the choice in equation 1.)

   Global scenario assumes that the Arp2/3 can cause nucleation of a new branch on any location along the leading edge (when e.g. the Arp2/3 is floating more freely along the cell edge). A constant number βL of new nascent filaments are nucleated per second over the whole leading edge:
   $b_2(p^-, p^+) = \frac{Lp^{\mp}}{\overline{P}}$, with $\overline{P} = \int_{-L}^{L}(p^+(x) + p^-(x))dx$ the total number of filaments.

3. The third term describes **capping** of the filaments. The capping proteins float freely, therefore each filament has equal probability to be capped.

2 The elastic polymerization ratchet model

by Mogilner and Oster is used to model the protrusion of the leading edge. It is

$$V_n \approx V_0 \exp\left(-\frac{f_r \delta}{k_B T}\right), \tag{2}$$

where V_0 is the free polymerization velocity, f_r is the membrane resistance force per filament, δ is the length increment of the fiber at one instance of monomer assembly and $k_B T$ is the thermal energy

The authors assume $f_r = \frac{F}{p(x)-p_0}$, where p_0 is some constant.

They introduce the notation $\omega = \frac{F\delta}{f_B T}$ and assume it constant. Altogether:

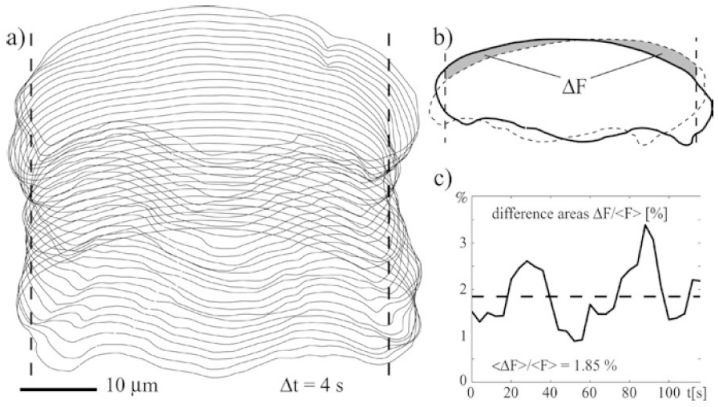
$$V_n(x) \approx V_0 \exp\left(-\frac{\omega}{p(x) - p_0}\right) \tag{3}$$

3 Experimental observations of the keratocyte cells

The theoretical considerations above allow us to choose different parameters describing branching and capping rate, the free polymerization velocity, etc. Moreover, we can choose between local and global branching model. The authors list some observations and compare them step by step with simulations of some special cases of the model, in each step adjusting some parameters.

3.1 Shape and motion of the cell are steady

The keratocytes have a comparably stable shape, especially on the cell front. The fluctuations can be measured by recording the difference in the area , ΔF_i, between the leading edge of the cell at time t_i and the leading edge of the time averaged cell shape (see picture). To record the changes of area at the leading edge only and to exclude the changes due to lateral displacement of the cell and the retraction at the rear, the measurment was restricted to a strip (dashed vertical lines) parallel to the direction of motion and between the rightmost position of the left boundary of the cell and the leftmost position of the right boundary of the cell. The measurements show, that ΔF_i averages around 1.85% and normally doesn't exceed 3%.

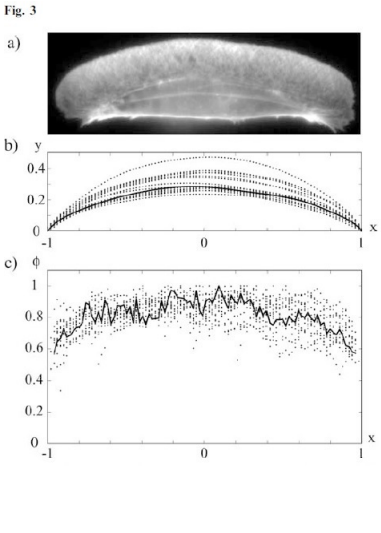


3.2 Actin filament density

Fluorescence microscopy reveals, that the filament density is at maximum in the center of the leading edge and decreases towards the edges.

Experiment: The authors treated the cell with a low concentration ($2\mu M$) of cytochalasin D, a drug that, similar to actin-capping proteins, blocks actin polymerization at the barbed filament end. This shall have the same effect as an increase of capping rate (and shall therefore decrease actin polymerization).

Result: After the treatment the cell edge became more flat-like.



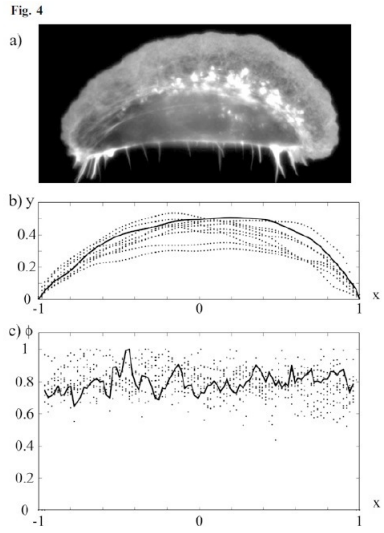


Fig. 3 Actin distribution in keratocyte lamellipodia. **(a)** Fish keratocyte stained for F-actin with rhodamine phalloidin. **(b)** Contours of the leading edges are plotted for 15 cells (*dashed curves*) between the lateral edges ($x = \pm 1$; the length scale is half the width of the cell, L; the y-coordinate is also scaled by L). The *solid curve* is the leading edge profile of the cell shown in **(a)**. **(c)** Along the leading edge, the intensity (proportional to the actin density, φ; normalized so that the maximal observed density is 1) is plotted for 15 cells (*dots*). Despite significant fluctuations, it is seen that the density is highest in the middle of the cell and decreases toward the lateral edges. The *solid line* is the measured intensity for the cell shown in **(a)**

Fig. 4 Actin distribution in the cytochalasin treated cells. **(a)** Fish keratocyte treated with cytochalasin and stained for F-actin with rhodamine phalloidin. The leading edge contours **(b**, *dashed*) and the actin density **(c**, *dots*) for eight cells treated with a low concentration (0.2 µM) of cytochalasin D. (x, y, φ are as in Fig. 3.) *Solid lines* show the data for one of the cells. Both cell boundary and actin density are less regular than those in Fig. 3. The average density profile is flat

4 Special case of the flat leading edge

I.e. when $f'(x) = 0$. This makes the rate on flow $v^{\mp}$ of the lateral drift constant and simplifies the equation 1 significantly.

The authors assume that the shape of the leading edge doesn't change, when the cell moves forward (i.e. f' stays equal 0). At least in the article they do not prove this with equation 3 from the elastic polymerization ratchet model, but only calculate the static distribution of actin density, without examining how this distribution would influence the shape of the leading edge. This hardly makes sense in the longer time scale, but in shorter time scale it allows us to observe some phenomena.

4.1 Analytic solutions for slow and fast capping

We can write the equation 1 in a nondimensional form and find approximate analytic solutions for special cases for very slow and very fast capping ($\gamma \ll 0.01\frac{1}{s}$ and $\gamma \gg 0.01\frac{1}{s}$ respectively).

	local	global
$\gamma \ll 0.01\frac{1}{s}$	$p \approx \frac{\beta L}{\sqrt{2V}}\sqrt{1-\left(\frac{x}{2L}\right)^2}$	$p \approx \frac{\beta L}{\sqrt{2V}}\cos\left(\frac{\pi x}{4L}\right)$
$\gamma \gg 0.01\frac{1}{s}$ at $-L+\epsilon < x < L-\epsilon$	$p \approx \frac{\beta}{\gamma}$	$p \approx \frac{\pi\beta}{4\gamma}\cos\left(\frac{\pi x}{2L}\right)$

4.2 Numerical solutions for the intermediate values of γ

For the intermediate values of γ, there is no analytic solution; therefore the authors calculated stationary solutions numerically for three different values; $\gamma = 0.001\frac{1}{s}$, $\gamma = 0.01\frac{1}{s}$ and $\gamma = 0.1\frac{1}{s}$. The obtained actin densities $p(x)$ have the following properties:

1. p(x)=p(-x)
2. The solutions are convex; they have maximum at the center and the density decreases to the sides; this is a result of the boundary conditions $p^-(1) = 0$ and $p^+(-1) = 0$ (All values on the graphs are scaled).

The shape of the density curves can be characterized especially by the following two properties:

1. The ratio between the center and the sides; $\frac{p(0)}{p(\pm 1)}$
2. The actin density can either grow gradually from the sides to the center or grow quickly at the sides and have a flat form in the center.

Lower capping rates; $\gamma = 0.001\frac{1}{s}$**,** $\gamma = 0.01\frac{1}{s}$

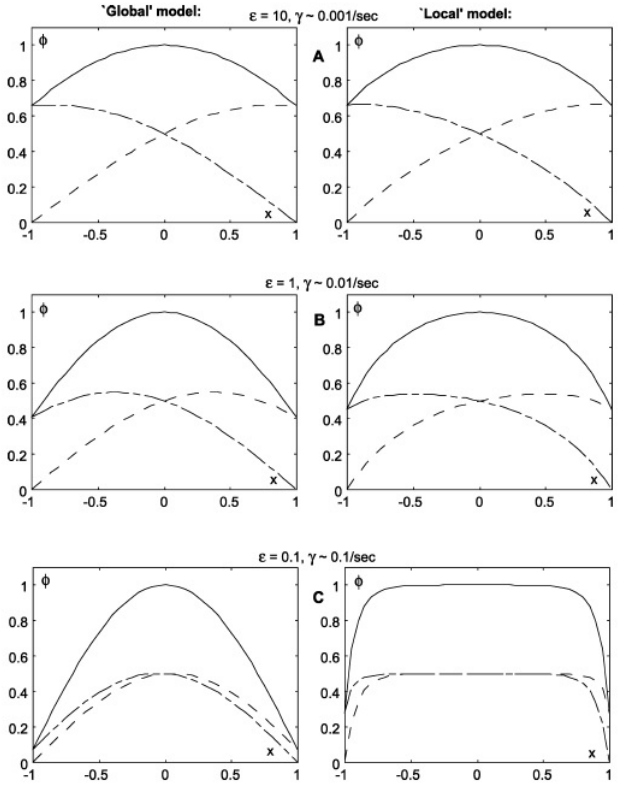
At the lower capping rates the actin filaments grow very long. This "globalize" the actin dynamics, as the actin is redistributed along the leading edge. This leads to very similar profiles for the global and local branching model. Lower capping rates are less biologically relevant!

Higher capping rates $\gamma = 0.1\frac{1}{s}$

In the local model, the actin density along the edge is determined by the balance between capping rate and the nucleation of new branches (which is here constant, as on every section of the leading edge, the same number of new branches will be nucleated. Only on the sides the number of new nucleations is lower). Therefore the density curve is flat.

On the other hand, in the global model, there are more new nucleations on the sections with higher actin density, therefore the differences between densities multiply. The curve gets a spike-like form.

Conclusion: We can predict that the local model is the right one, because in the experiment with increasing capping rate, the leading edge gets flatter.

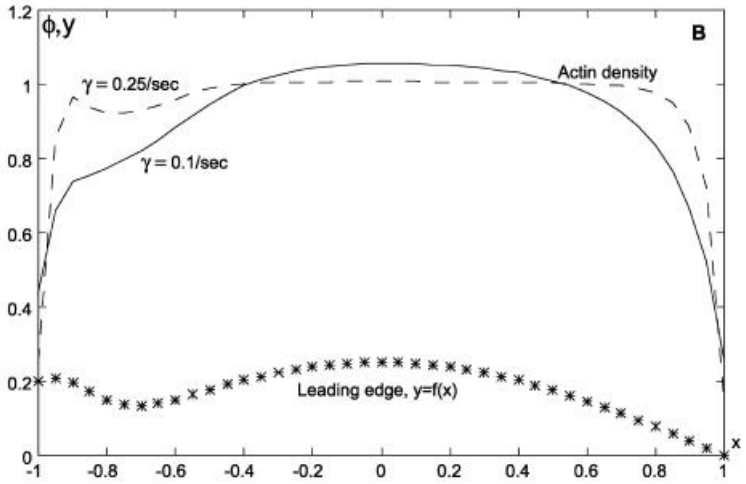


5 How the shape of the leading edge determines the actin density: theory

It is expected: If the leading edge is flattened or indented on some section, then the actin filaments are depleted here, which causes even lower polymerization and decreases the protrusion rate. This would cause an additional increase of the indentation, if not countered by some other impact.

On the other hand, if the leading edge has an outgrowth the filaments could accumulate there and the outgrowth would grow even more.

This is countered by the growth of very long actin filaments at lower capping rates which redistributes the actin along the edge.



On the figure we see stationary solutions of the filament density with the capping rate $\gamma = 0.25\frac{1}{s}$ (dashed) and $\gamma = 0.1\frac{1}{s}$ (solid) for given leading edge (stars). The first leads to instability, while the second suggests that the shape is more stable. This coincides with the experiment with the added cytochalasin D, which increases the capping rate and causes the cell shape to be more unstable

and undergo larger fluctuations.

6 Estimating γ by comparing theory and experiment

The authors measured the shapes of 15 fish keratocyte cells and compared the mean shape of the leading edge with the mean distribution of the actin filaments. Then they compare the actual average density with the densities predicted from the "Mathematical model of actin dynamics" applied on the given average shape. They do this for different capping parameters and choose the closest reasonable parameter. The visual impression is that $\gamma = 0.08\frac{1}{s}$ gives a better fit than $\gamma = 0.05\frac{1}{s}$ or $\gamma = 0.05\frac{1}{s}$.

7 How the actin density shapes the leading edge; adjusting the parameters of the ratchet model

The experimental observations show, that the leading edge retains steady shape. After the graded radial extension model (Lee et al. 1993), this means that the leading edge has to grow in the direction normal to the boundary with the velocity

$$v_n(x) = \frac{V}{\sqrt{1 + (f'(x))^2}}$$

where V is the cell protrusion velocity. As the velocity of each section of the leading edge is known, we can now compare the velocity with actin filament density on the same section and try to adjust the equation 3 from the elastic ratchet polymerization model, so that the equation would bring the wanted velocity by given actin density. The graphics show that this is indeed possible. The graph c) depictures results for a single cell, while d) depictures the scaled parameters for 15 different cells, obtained as in c). With exception of one (which we ignore), they are comparably close to each other. The author chose the mean values of these 15 examples as parameters for the model

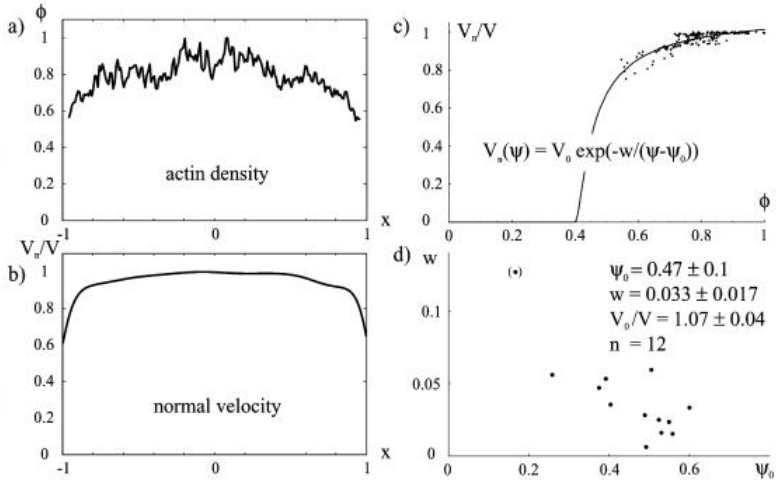


Fig. 8 Density–velocity relation. **(a)** Measured actin density normalized so that the maximal density is 1 (Fig. 3c; x, φ are as in Fig. 3). **(b)** Local normal protrusion velocity (V_n scaled by V) was computed from the leading edge profile, $y = f(x)$ (Fig. 3b) using Eq. (10) of the graded radial extension model. **(c)** Measured actin density **(a)** and the normal velocity **(b)** are compared at the grid points and plotted as *dots*. The theoretical result (*solid curve*) is fitted over the experimental data (*dots*). **(d)** Parameter values (for two of the three parameters; for 13 cells). V_0 is the free polymerization rate, $w = \omega/p(0)$ is the scaled membrane resistance, and $\psi_0 = p_0/p(0)$ is the scaled fraction of the "background" F-actin

8 Evolution and stability of the coupled (actin desity/cell shape system: theory:

Now we have all parameters and can combine both the equations for the impact of the edge shape to the actin density and the impact of the actin density to the edge shape to make an iterated simulation with a computer. The authors state that the obtained leading edge shape and the actin filament density are asymptotically stable and resistant to stochastic fluctuations of branching and capping rate if the capping rate is not set to high.