Physica D 318–319 (2016) 50–57

Contents lists available at ScienceDirect

Physica D

journal homepage: www.elsevier.com/locate/physd

Concentration profiles of actin-binding molecules in lamellipodia

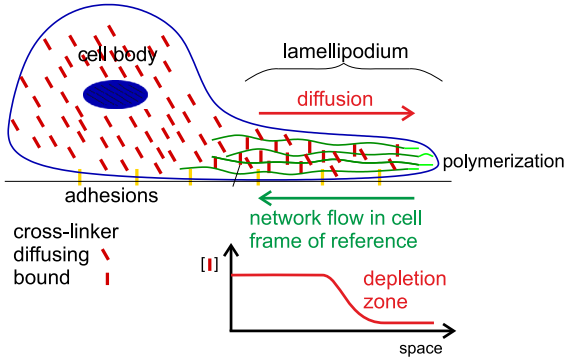
Martin Falcke [1]

Max Delbrück Center for Molecular Medicine, Robert Rössle Str. 10, 13125 Berlin, Germany

HIGHLIGHTS

- Actin network flow in lamellipodia causes gradients of actin-binding proteins.
- That depletes some proteins at the front in physiologically relevant cases.
- And entails a gradient in mechanical network properties.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 June 2015
Received in revised form
14 September 2015
Accepted 28 October 2015
Available online 4 November 2015

Keywords:
Cell migration
Cell mechanics
F-actin network
Actin cross-linkers
Mathematical modeling

ABSTRACT

Motile cells form lamellipodia in the direction of motion, which are flat membrane protrusions containing an actin filament network. The network flows rearward relative to the leading edge of the lamellipodium due to actin polymerization at the front. Thus, actin binding molecules are subject to transport towards the rear of the cell in the bound state and diffuse freely in the unbound state. We analyze this reaction–diffusion–advection process with respect to the concentration profiles of these species and provide an analytic approximation for them. Network flow may cause a depletion zone of actin binding molecules close to the leading edge. The existence of such zone depends on the free molecule concentration in the cell body, on the ratio of the diffusion length to the distance bound molecules travel rearward with the flow before dissociating, and the ratio of the diffusion length to the width of the region with network flow and actin binding. Our calculations suggest the existence of depletion zones for the F-actin cross-linkers filamin and α-actinin in fish keratocytes (and other cell types), which is in line with the small elastic moduli of the F-actin network close to the leading edge found in measurements of the force motile cells are able to exert.

1. Introduction

The crawling of many different cell types is essential for life. In the developing embryo, undifferentiated cells move towards the site, where they form a tissue or organ. Immune cells like neutrophils squeeze through the walls of blood vessels and crawl towards the site of an infection. Skin cells start crawling when they have to close a wound [1]. During metastasis, cancer cells dissociate from the primary tumor, crawl towards blood vessels and spread all over the body [2,3]. In vitro, cells are often plated on a two dimensional substrate. They form a flat membrane protrusion in the direction of motion, the lamellipodium, which is usually only about 120 nm thick but several μm broad and deep [4].

E-mail address: martin.falcke@mdc-berlin.de.
[1] Tel.: +49 (0) 30 94062753.

http://dx.doi.org/10.1016/j.physd.2015.10.019
0167-2789/

A dense network of branched actin filaments (F-actin) inside the lamellipodium pushes the leading edge membrane forward [5]. The mechanism driving the motion is treadmilling of the filaments [6]. It generates motion since the filament barbed (or plus) ends polymerize at the leading edge of the lamellipodium and the pointed (or minus) ends depolymerize at the rear [6]. The actin network moves relative to the lamellipodium leading edge due to polymerization with velocity v. That may even cause a rearward flow of the network in the lab frame observed as retrograde flow with some cells.

Cross-linker molecules establish links between filaments. Newly polymerized filament regions have not bound cross-linkers, yet. They bind them with rates determined by the rate constant k^+ and the free cross-linker concentration. Bound cross-linkers travel rearward with the network flow till they dissociate after the time $1/k^-$ (k^- dissociation rate). They travel on average the distance $d_t = v/k^-$ away from the leading edge with the retrograde flow between a binding event and the subsequent dissociation. Free cross-linkers move diffusively. Upon dissociation, molecules move on average the distance $d_d = \sqrt{D/k^+B_T}$ (B_T concentration of cross-linker binding sites on F-actin D diffusion coefficient) before they bind again to filaments. Due to this reaction–diffusion–advection (RDA) process, a gradient of the free cross-linker concentration and the degree of cross-linking arises. The existence of such a gradient has been also suggested earlier by measurements of the molecule ratio cross-linker to F-actin [7]. It is also suggested by the structure of the network in the lamellipodium, since often filaments are arranged in bundles further back in a part of the protrusion referred to as the lamella.

The gradient in mechanical properties also causes a variety of dynamic regimes of lamellipodium leading edge motion and shapes the initial phase of the force–velocity relation [8–11].

The bending energy of elastic rods is length dependent. Filaments with long free length between supporting structures are floppy [12]. The persistence length characterizes the filament length at and above which the bending energy is of order k_BT, i.e., at which lengths filaments are semiflexible. The F-actin persistence length is between 2.2 and 17 μm [13–16]. Pure actin solutions show very small elastic moduli with weak concentration dependence (proportional to $[\text{actin}]^{1-1.2}$ [17,18]). Intuition suggests that branching could stabilize the network, but experiments in actin solutions suggest that it contributes very little to the elastic modulus of F-actin networks [19]. This is corroborated by a recent theoretical study showing that branching does not stiffen lamellipodial F-actin networks essentially [20]. For efficient transmission of force to the leading edge membrane and effective locomotion, the actin network has to be stabilized by cross-linking [21,22]. There is a critical molecule number ratio cross-linker:actin above which elastic moduli are affected by cross-linking. Studies with F-actin solutions showed rather coherently that elastic moduli start to change above a molecular ratio cross-linker:actin of 1:100 [23,18,24,19,25]. Filamin-A-induced cross-linking increases the viscosity of F-actin solutions at a molecular ratio of 1:1000, but only in the absence of Arp2/3 complex. If this is added, effects of filamin-A start at a molecule ratio of about 1:200 [19]. Remarkably, the effect of cross-linkers on mechanical properties has not saturated at a ratio of 1:1 yet [18,24,25].

The existence of a threshold in the molecule ratio for an effect of cross-linking on mechanical properties suggests the possibility that lamellipodia become very soft close to the leading edge, if the RDA process lowers the concentration of free cross-linkers towards that threshold value. Indeed, that is supported by the elastic modulus of that region found in force–velocity measurements, which is as soft as *weakly* cross-linked actin networks [25–28,11].

This study investigates concentration profiles of molecules subject to the reaction–diffusion–advection process. We will calculate the profile in a radial cut through the lamellipodium. The formulas derived here can be applied to any F-actin-binding protein in the lamellipodium.

2. Model

We consider a radial cut through the lamellipodium (spatial coordinate z). The small height of the protrusion (≈120 nm) and typical protein diffusion coefficients in the range of a few $\mu m^{-2}\,s^{-1}$ suggest to average concentrations across the height. The curvature radius of the leading edge is typically much larger than the depth of the protrusion such that we can use the onedimensional coordinate system instead of polar coordinates. We place the origin of the coordinate system $z = 0$ at the point where the network flow in the comoving frame vanishes because the flow velocity is 0. This point travels in the lab frame with the cell velocity, i.e., anterograde flow in the lab frame is equal to the leading edge velocity there. The distance of the leading edge to that point is the parameter L. Note, that L might be larger than the distance of the lamella–lamellipodium transition from the leading edge in some definitions of this transition line.

Free cross-linker molecules C diffuse with diffusion coefficient D in the lamellipodium. They might also experience advection, since Keren et al. report a convective flow of water towards the front, the velocity v_c of which has values in rapidly moving cells of about 40% of the cell speed [29]. Cross-linker molecules bind with the bimolecular rate constant k^+ to F-actin (total binding site concentration B_T). The bound cross-linkers B are transported with the flow velocity in the comoving frame of reference $V(z)$ towards the rear of the lamellipodium. The velocity $V(z)$ typically decreases with increasing distance from the leading edge. Its value at the leading edge is the sum v of the moduli of protrusion and retrograde flow velocity. The dynamics is described by the equations

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - v_c\frac{\partial C}{\partial z} - k^+(B_T - B)\,C + k^- B \tag{1}$$

$$\frac{\partial B}{\partial t} = \frac{\partial V(z)B}{\partial z} + k^+(B_T - B)\,C - k^- B. \tag{2}$$

The boundary conditions at the leading edge are $B = 0$, since newly polymerized filaments have not bound cross-linkers yet, and $\partial C/\partial z = 0$, since there is no cross-linker flux through the membrane. The boundary condition at $z = 0$ is $C = C_{bulk}$, the concentration in the cell body.

Depolymerization of F-actin and retrograde flow are necessarily coordinated in the stationary state of the lamellipodium. That can be expressed by the stationary continuity equation (we take the velocity in negative z-direction as positive).

$$\frac{\partial V(z)B_T}{\partial z} = r_d. \tag{3}$$

We assume the simplest case, i.e., a constant depolymerization rate r_d. That entails

$$V(z)B_T = r_d z. \tag{4}$$

We assume $B_T = constant$, i.e., the difference of the flux of F-actin into a given volume element and out of it is balanced by depolymerization. Measurements showed an exponential decay of B_T but with a decay length in steady motion very well justifying our approximation $B_T = \text{constant}$ [30]. In the range of distances from the leading edge considered here, B_T changes by about a quarter of its maximum value [30]. The estimate for the flux balance given below suggests that changes of B_T of that magnitude will not essentially affect our results. With $B_T = constant$, Eq. (4) reflects a velocity gradient in line with the observation that retrograde flow vanishes at some distance from the leading edge.

Depolymerization has consequences for the concentration dynamics of free and bound cross-linkers. Cross-linkers are set free when F-actin depolymerizes. The fraction of cross-linker binding