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Biological Systems at Sub-cellular Scale: Investigation of G-actin Transport in Filopodia

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Abstract. In our research of biological systems at the sub-cellular scale we focus on the cytoskeleton, particularly its components actin-filaments and microtubules, which are key mediators of axon growth and maintenance. Knowing how filaments and microtubules are regulated enhances our understanding of neural development, ageing, degeneration and regeneration. In the cytoskeletal machinery, finger-like, extremely narrow and long, membrane protrusions called filopodia act as sensors, facilitating proper cellular navigation and directed growth. Since explanations of dynamical and mechanical aspects of filopodia, centred in diffusion and transport processes, are being studied, we investigate the displacement of actins in the filopodia and discuss compensatory G-actin drift and diffusion towards the filopodia tip to supply the polymerisation of actins into filaments moving backwards. We conclude, through some simple calculations, that diffusion alone cannot provide the necessary actins to the polymerisation processes in all situations. Therefore, advection processes and Stokes equation need to be added to diffusion models to better simulate the colloidal fluid dynamics in filopodia cell-membrane protrusions.

Keywords. Dynamics model, diffusion, filopodia, polymerisation, cytoskeleton, F-actin flow

1 Introduction

Biological phenomena rely on physical and chemical interactions. As elements of a physical phenomenon, biological components interact freely with one another, exchanging mass and energy. While interacting chemically, they are subject to different chemical compatibilities of interacting elements. Biological interactions carry a rather complex

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aspect: entities often interact through the exchange of information (signals), without being necessarily in contact or even close together.

To understand biological systems at the sub-cellular scale, physico-chemical dynamics should be operationally documented as a working and consensual mathematical / computational model. Both types of observation, real experimental data and computer simulation, complement each other, by offering means to handle a wider range of parameters, or by suggesting the existence of particular interactions or structures that can then be experimentally tested.

In this work, we discuss if G-actin dynamics, considered solely as a diffusion process does supply enough molecules for the polymerisation processes that build filopodia; considering several configurations of parameters that are known to affect the maintainance of filopodial processes, like the number of filaments in the bundle, the polymerisation rate and the filopodial length. To satisfy mass balance within the protrusion tip, polymerisation must be continuously fed by G-actins coming from the base of the filopodia or other more central parts of the cell in sufficient quantity so that the retrograde flow of the filaments is fully supplied. That is, we investigate under which conditions the diffusion assumption is no longer sufficient.

2 Cytoskeletal Dynamics

2.1 Actin Dynamics

Actin is the most abundant protein in eukaryotic cells [13]. Actin exists as globular actin monomers called G-actin and polarised filaments called F-actin. Actin filaments are head-to-tail polymers of G-actin subunits. The minus, or pointed, end of actin filaments is relatively inert displaying slow growth *in vitro*. The opposite plus, or barbed, end grows much faster through exothermic polymerisation both *in vitro* and *in vivo* [4].

Processes of polymerisation and disassembly can be differentially and dynamically regulated through distinct classes of plus- and minus-end binding proteins, generating networks with different flow intensities, constantly changing filament length, used as scaffolds to pull and generate forces [3]. Of particular importance for our work is the plus-end polymerisation of actin and intermolecular associations of the intervening monomers [2].

2.2 Filopodia

Filopodia are relatively simple cellular compartments providing a realistic context in which to start modelling the complexity of actin network regulation in biological contexts. Filopodia are long, finger-like membrane protrusions with numerous roles in signalling and cell navigation [8].

Regulation of filopodial dynamics appears relatively simple, essentially governed by the proteins regulating polymerisation and disassembly processes, and the key challenge is to understand the high rate of polymerisation at the very tip. This requires constant delivery of actin monomers through these slender structures and where concentration of G-actin is expected to be very low. Therefore, how polymerisation can be sustained within

the highly challenging filopodial structure is a fascinating phenomenon which harbors key explanations for filopodial behavior.

3 Can diffusion alone explain G-actin delivery to the filopodial tip?

We first explore whether, and under which conditions, diffusion might be sufficient to supply barbed-end actin polymerisation processes in filopodia. For reasons of simplicity, our calculations do not consider filopodial elongation in a first moment, but analyse conditions where retrograde flow and polymerisation maintain filopodial shape, length, and F-actin retrograde flow in a steady state. For our calculations we used the parameter values in Table 1 [5]⁵.

Table 1: Parameters regarding filopodia and actin.

Notation	Meaning	Value	Reference
L	Filopodial length	24 – 55 μm 1 – 10 μm	[1] [12]
N	Filaments in the filopodial bundle	10 – 30	References in [10]
C_0	G-actin concentration at filopodial base	10 μM	[10]
k_{on}	Polymerisation rate	10 $\mu M^{-1} s^{-1}$ 11.6 $\mu M^{-1} s^{-1}$	[10] [16]
N_0	Filaments to support protrusion	13	[10]
η	Conversion factor	20	[10]
D	G-actin diffusion coefficient	5 $\mu m^2/s$	[9]
V_{ret}	Retrograde flow	70 nm/s	[10]

For short filopodia (1-2 μm) the concentration of free actin at the filament base is sufficient to supply enough polymerisation through diffusion for an extension to occur [10]. This is in agreement with expression (1) for estimating the approximate time required for a particle to diffuse over a given distance x , in an environment where its diffusion coefficient is D , q_i is 2, 4 or 6 depending on the number of dimensions ($i = 1, 2$ or 3) [7]. Considering a linear displacement ($q_i = 2$) and a diffusion coefficient of 5 $\mu m^2/s$, a G-actin travels at 3.16 $\mu m/s$

$$t \approx \frac{x^2}{q_i D}. \tag{1}$$

⁵In the sequel quoted only as Table 1

For filaments longer than a few micrometers, we can analyze concentration of free actin according to expression (2) mentioned in [10]:

$$C(x) = C_0 - \frac{C_0 x}{L(t) + (D\eta e^{N_0/N})/(k_{on}N)}, \quad (2)$$

where $L(t)$ indicates filopodial length as a function of time (*i.e.* considering filopodial elongation). As mentioned above, we set $L(t) = L$ constant. Furthermore, we chose the filopodial length interval $L \in [1, 30]\mu m$ to cover for a wide range of physiologically occurring filopodia (Table 1).

Function C in (2) provides G-actin concentration at a given distance from the filopodial base ($x = 0$) towards the polymerisation zone at the filopodial tip ($x = L$). Under these conditions, the variation of free actin concentration at the filopodial tip can be described by (3). It is possible to note that expression (3) yields $C \rightarrow C_0$ when $L \rightarrow 0$, ensuring that concentration will be same as the one found closer to the base of filopodia

$$C = C(L, N, k_{on}) = C_0 - \frac{C_0 L}{L + (D\eta e^{N_0/N})/(k_{on}N)}. \quad (3)$$

With this information, one can calculate how many actins are polymerised in each scenario generated by variations of L , N and k_{on} , chosen values reported in Table 1. Let the quantity of polymerised actins be denoted by N_p . These data will be compared with the number of actins needed to maintain F-actin retrograde flow, here denoted by N_{ret} .

Our aim is to investigate in which conditions the polymerisation process and the retrograde flow are properly supplied by diffusive transport of G-actins alone, under the assumption that mass inside the filopodia is balanced and the rate of the retrograde flow of the actin bundle is $V_{ret} = 70nm/s$. To achieve this velocity, a polymerisation frequency of 30 actins/s per filament in the bundle is required when considering that every new actin elongates a filament by $\approx 2.7nm$ [10]. Therefore, in a bundle with N filaments, $N_{ret} = 30N$ actins would be polymerised per second.

At a G-actin concentration of $10\mu M$, the polymerisation rate per actin filament was reported to be $0.3\mu m/s$, which means that 110 actins are polymerised in a single filament per second [14]. As the number of polymerised actins is directly proportional to the concentration of monomers, on a concentration of $10\mu M$, 110 actins are polymerised per second on each filament, which provides us 11 actins/s on $1\mu M$. Then, for a bundle with N filaments, the number of polymerising actins N_p would be $11N/s$. When coupling this with expression (3) to estimate the behavior of the G-actin concentration along filopodia, we obtain $N_p = 11NC$, where N_p is the number of actin molecules polymerised for a given concentration $C = C(L, N, k_{on})$ at top of the filament bundle. Our calculations were performed with the algorithm described in [5].

Using that algorithm in combination with the values given in Table 1, we obtain that for 90.32% of the parameter tuples checked the polymerisation does not supply enough actin molecules for retrograde flow. In comparison, when setting the filopodial length interval to $L \in [0.5, 2]\mu m$, in just 15% of cases diffusion seems ineffective, suggesting that diffusion alone is not sufficient in many scenarios, particularly in long protrusions.

4 Discussion and Conclusion

The above calculations show that diffusion alone is, in general, not enough to supply the amount of G-actin needed to sustain the observed rates of polymerisation and backflow as one compensatory mechanism. It must be emphasized that, according to [10], diffusion is a key process of actin transport in filopodial protrusions, but this is restricted to filopodia at an initial stage of growth.

Other reports point to the same direction as the above calculations. For example, Monte Carlo simulations were used to investigate G-actin translocation during protrusion of the leading edge suggesting that diffusion alone was insufficient [15]. The use of compartmental and molecular stochastic models to study actin motion by diffusion leads to the conclusion that filopodia would reach a steady state length of as little as $\approx 1\mu m$ because the transport flux of G-actin monomers continuously diminishes as the protrusion becomes longer [6]. Work on filopodia-like acrosomal processes of sperm also found that the kinetics of diffusion-limited actin polymerisation were not sufficiently rapid to account for the observed acrosomal elongation dynamics [11].

Our next steps will be to generalise the above results to 2D and 3D configurations. Besides, we intend to analyse cytoplasmic flow effects on G-actin transport with Stokes equation, so as to understand the phenomena without considering just diffusion as a key transport process to drive essential features of filopodial dynamics.

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