Fundamentals of cell polarity and motility

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Abstract

Cell polarity is a fundamental characteristic regarding cell motility and division. In this work, first we analyze polarization from a biological vantage point, namely which are the molecules taking part on it and how it is experimentally observed. Then, we evaluate this mechanism by implementing a simple mathematical model, so that the fundamental concepts can be understood, and afterwards, a more complex modelization is analyzed, involving the kinetics of two proteins, so that we show how a simplified two-variable model can reproduce the symmetry breaking needed for polarity. Finally, the validity of this model is considered.

Keywords: wave-pinning, bistable reaction-diffusion system, stationary front, cell polarization, Rho GTPases

1 Introduction

Cell polarity arises primarily through the localization of specific proteins to specific areas of the cell membrane, often reorganizing and defining a front and a back. It is the primary step in motility and cell differentiation, and this cellular process can be seen in various cell types, from amoeba to nerve cells. Mainly, the Rho GTPase family proteins are the ones involved in this process, presenting active and inactive forms. The active proteins are found cycled in the plasma membrane and the inactive ones are found in the cytosol. The front part has higher concentration of active forms than the back, making the cell polarize.

The best studied proteins of the Rho-GTPase family members are Cdc42, Rac, and Rho. In the active form Rho proteins are bound to guanosine triphosphate (Rho-GTP), and in the inactive form they are bound to guanosine diphosphate (Rho-GDP). This conversion is facilitated by three groups of proteins: GEF (guanine nucleotide exchange factors), which trigger the Rho-GTP-bound state, GAP (GTPase-activating proteins), which increase the Rho-GDP-bound state, and GDI (guanosine dissociation inhibitors), whose binding prevents anchorage of the Rho GTPases to the cell membrane.

The basic idea resides in an exchange between active and inactive forms of the chemicals with unequal rates of diffusion, which presents bistable kinetics. The local increase of the active form propagates through the cell membrane, leading to wave fronts. When this propagation eventually halts, it forms a stationary front, making the wave-pinning phenomenon arise. This pinned front represents a clear segregation of the cell into front and back, leading to cell polarity.

2 Biological background

Polarity is a fundamental property of most cells regarding motility: an apparently symmetric cell responds to directional cues provided by chemoattractants, creating a polarity axis. Chemoattractants are the spatial signals that initiate and maintain cell polarization during chemotaxis, which is the directed migration of a cell in response to a chemical stimulus. This directed cell movement allows, for example, leukocytes to crawl to sites of infection and inflammation, fibroblasts to enter a wound, and amoebae to form multicellular organisms.

Cell motility is caused because the active Rho GTPases activate a large flood of effectors that are responsible for remodeling the actin cytoskeletal elements, principally the actin filaments. These filaments are made up of identical actin proteins arranged in a long spiral chain with two structurally different ends, which are referred to as the 'barbed end' and the 'pointed end'. The growth of these actin filaments, which occurs predominantly at the barbed end, is initiated and regulated by the active proteins, and this way, the actin cytoskeleton is able to provide protrusive and contractile forces, which allows the cell to move.

Enhanced Green Fluorescent Proteins (EGFP) can be used to measure and observe cell polarity, when tagged onto active GTPases. It is used as a biosensor, since it exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. Recombinant DNA technology is used for inserting these markers, and this mechanism involves using enzymes and various laboratory techniques to manipulate the DNA. Scientists isolate the EGFP gene and combine it to the gene that produces the active GTPase proteins, and then they insert the complex into a cell. If the cell exhibits green fluorescence, scientists infer that the cell expresses the target gene as well, and can then track the movement of the proteins in question.

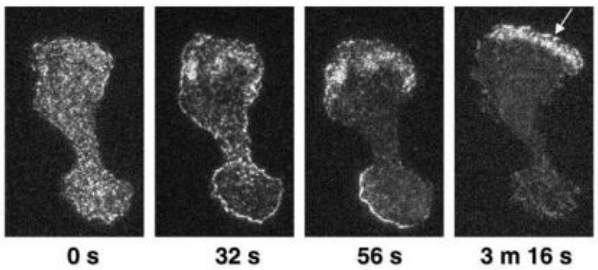

Figure 1: Polarization of a white blood cell being tracked by EGFP [2].

3 Mathematical modeling

We will first implement a simple mathematical model to observe the conditions needed for the arousal of cell polarity, and its basic characteristics. For simplicity, a one-dimensional mathematical model of the cell will be considered, divided in two, the cytosol and the membrane. Inactive Rho proteins are found in the former one, whereas active ones are found in the latter, and they diffuse along the axis of the cell in both cases; we will take the cell length to be $L = 10\mu m$. For a cell to be polarized, the concentration of active proteins in one end of the membrane should be higher than in the other one, dividing the cell in a front and a back region, as aforementioned.

It will be considered that the total number of proteins is held constant, since metabolic reactions are much faster than gene translation, so $\frac{da}{dt} = -\frac{db}{dt}$ must be satisfied, where a corresponds to the concentration of the active protein, and b to the one of the inactive protein.

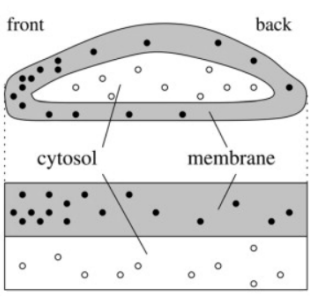

Figure 2: 1D diagram of the cell where active/inactive Rho proteins are represented by solid/open disks [5].

The temporal dependence of the concentration of the active form will be given by the equation:

$$\frac{da}{dt} = f(a,b) = b(k_0 + \frac{\gamma a^2}{K^2 + a^2}) - \delta a$$

Where k_0 is the inactive-active conversion rate, δ is the active-inactive one, γ is the maximal rate of the Hill function and K corresponds to the saturation parameter. The values used in the simulations are the following: $k_0 = 0.067s^{-1}$, $\gamma = \delta = 1s^{-1}$ and $K = 1\mu m^{-1}$.

This corresponds to a bistable system. For a fixed value of b, approximately in the range $b_{min} = 1.747 \leq b \leq 2.01 = b_{max}$, three equilibrium points can be found for a, the outer two being stable (a_- and a_+).

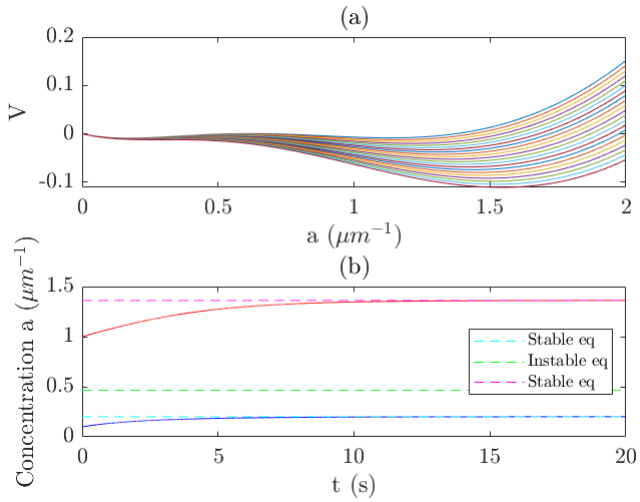

Figure 3: (a) Potential of the system for $1.8 \leq b \leq 2$. (b) Decay of the system to the stable equilibrium points for different initial conditions.

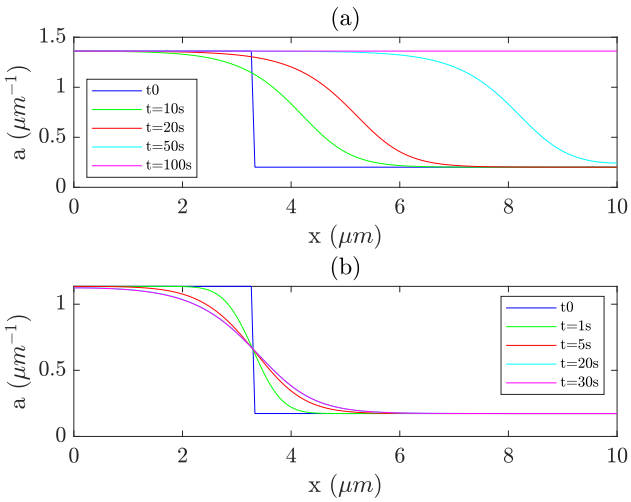

Figure 4: (a) Temporal evolution of the concentration of active proteins for concentration of inactive ones $b = 1.9$. (b) Temporal evolution of the concentration of active proteins for $b = 1.80161938462$, achieving wave-pinning.

3.1 Single variable case with diffusion (fixed b)

Now, we will analyze the formation of wavefronts and wave-pinning, so we will maintain the concentration of the inactive protein b constant, but a diffusion term will be added.

$$\frac{da}{dt} = b(k_0 + \frac{\gamma a^2}{K^2 + a^2}) - \delta a + D\frac{\partial^2 a}{\partial x^2}$$

Regardless of the initial conditions, the steady-state concentration of all points in space will be the one corresponding to the most stable equilibrium point. For a certain b, namely $b_T = 1.801619$, the fronts are halted, achieving wave-pinning, which happens because for this b, both equilibrium points are equally stable.

For $b_T < b < b_{max}$, we can see that, after a certain time, the concentration of the active protein throughout the whole cell will become homogeneous, and will have the value of the most stable equilibrium point a_+. Conversely, for $b_{min} < b < b_T$, at the end and for all points of space, a will correspond to the equilibrium point a_-. This switch is due to a change in the potential, and therefore a shift in the overall stability of both equilibrium points. For values out of this range, the potential has just an equilibrium point, losing bistability.

3.2 Full a, b system

After analyzing the basics of this modelization, we implement the full a, b system:

$$\frac{da}{dt} = b(k_0 + \frac{\gamma a^2}{K^2 + a^2}) - \delta a + D_a\frac{\partial^2 a}{\partial x^2}$$
$$\frac{db}{dt} = -b(k_0 + \frac{\gamma a^2}{K^2 + a^2}) + \delta a + D_b\frac{\partial^2 b}{\partial x^2}$$

Where D_a corresponds to the diffusion coefficient in the cell membrane, and D_b to the one in the cytosol. For computations, we will take $D_a = 0.1\mu m^2 s^{-1}$ and $D_b = 10\mu m^2 s^{-1}$, since the cytosol is an aqueous medium and the cell membrane is more dense. No flux at the ends of the cell is imposed, as well as mass conservation: $T = \int_0^L (a + b)dx$, where T is the total amount of proteins.

By inspection, it can be seen that if the initial concentration of the active protein is maintained the same (in our case the first fifth of the cell has a concentration a_+, and the rest a_-), the stability of the system depends on the parameter T. Qualitatively, for the concentration of the protein in the membrane, three different solutions can be obtained. Approximately for $T < 281.2$ and $T > 527.34$ we obtain an homogeneous response. For $304.65 < T < 312.39$, the whole system, after showing wave patterns, decays to the

most stable concentration of the membrane protein, and finally, for values $312.39 < T < 375.4$, wave-pinning arises. The same way as in previous modulations, the wave-front can travel backwards or forward; this shift happens at around $T = 320.16$.

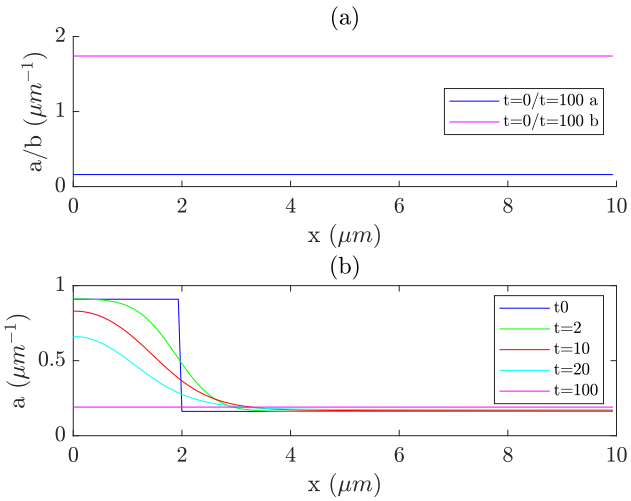

Figure 5: (a) Evolution of the concentration of the active protein for $T = 281.2$, with homogeneous solution. (b) Evolution of the concentration of the active protein for $T = 304.65$; the solution decays to a steady-state value.

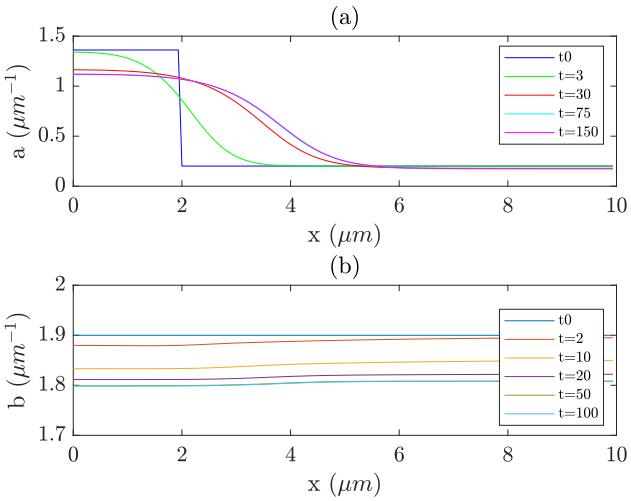

Figure 6: (a) Evolution of the concentration of the active protein for $T = 344.63$, where wave-pinning appears. (b) Evolution of the concentration of the inactive protein for $T = 344.63$.

When it comes to the variable b, since $D_b >> D_a$, basically its concentration through the whole cell will be the same, though it will have a slight temporal dependence. If the wave-front of a is travelling backward (a decreases in the membrane), the value of b increases, whereas if it is going forward (concentration a increases), its value decreases.

4 Discussion

Studying simplified systems is of the utmost importance since they help understand complex biological systems. We have seen how a single pair of proteins in active and inactive forms, cycling between plasma membrane and cytosol are enough to provide a basic cellular polarization mechanism.

Several types of experimental findings give support to the application of this mathematical model for polarization by Rho GTPases. Some experiments strongly suggested the existence of at least two stable values of the activated form of the protein a, which correspond to the values of a at the front and at the back of polarized cells. What is more, biochemical evidence of a positive feedback loop in Cdc42 activation supports the Hill function term in the kinetics employed, and in yeast, the cycling of Cdc42 between its GTP and GDP bound states is known to be crucial for polarization, among much other evidence.

The parameters used for the computational models correspond to the ones observed in biological systems. For instance, when it comes to the protein diffusion coefficient, it has been measured that for *E.coli* in the cytoplasma it is of around $D = 8\mu m^2 s^{-1}$ and in the cell membrane $D = 0.2\mu m^2 s^{-1}$, with similar values for other types of cells or organisms. For computations, we have used that the value of this coefficient in the cytosol is two orders of magnitude bigger than in the membrane, in agreement with nature.

To conclude with, this modelization could be more accurate provided some input stimuli or randomness had been added, but just this simple implementation provides a deep insight into cell polarization. Creating and enhancing this type of mathematical models is paramount for research and technological development, as they give a clear idea of the physics that lays behind the systems or organisms that surround us.

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