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2008 J. Phys. A: Math. Theor. 41 055101

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A simple model for dynamic phase transitions in cell spreading

A Bhattacharyay

Department of Chemistry, University of Warwick, UK

Received 25 October 2007, in final form 16 December 2007

Published 23 January 2008

Online at stacks.iop.org/JPhysA/41/055101

Abstract

There are some interesting experimental results on cell spreading at macroscopic scales (cell size) where people have observed various dynamic phases in terms of rate of spreading of area of adherence to the substrate. In the present paper, we develop a very simple phenomenological model to capture those apparent dynamic phases of a spreading cell without going into the microscopic details of actin polymerization which is the main driving force for such processes. Our conclusion is that the dynamic phases of cell spreading depend on some gross parameters of the cell rather than on complex signalling pathways.

PACS numbers: 87.15.La, 87.15.Rn, 87.17.Jj

Living cells move to perform various activities. While microbes often move in search for food following a chemical gradient, cells in multicellular organisms are required to move for immune-activity, healing, preferential positioning, etc. When cells move on a solid substrate, they move by periodically spreading and contracting themselves. The microscopic phenomenon acting as a driving force for such motion has been understood to be actin polymerization [1–4] at the leading edge of the lamellipodium. Lamellipodium is basically an active gel enclosed by cell membrane. Molecular motors like myosin and many other proteins play an important role by cross connecting the actin filaments to form a meshwork and keeping the filaments short and branched. A host of well-connected activities are actually performed at the microscopic level by several agents to enable the cell to stretch on the substrate. Particularly, the activity of myosin motors which cross connect actin filaments and produce localized strains on the meshwork by collectively moving on them are extremely important for a proper understanding of how such an active gel works [5–7].

Inside the cell, along the periphery of the spreading range, a network of actin filament grows in outward directions and pushes the cell membrane forward while the actin filaments predominantly depolymerize at the inner side of the network to supply actin monomers for the growth of filaments on the outer spreading edge. Basically, due to depolymerization at the inner and polymerization at the outer edge, a local gradient of actin monomers form which keeps the process going and the meshwork grows in the outward direction. It has been observed

that, for the cell to spread properly, the binding of the actin filaments to the substrate on which it moves is quite important [8]. This binding provides mechanical support to the actin network and thus helps the system avoid any breakup against mechanical restoring forces developed in the system as a result of spreading. It also helps the polymerization process by probably reducing positional fluctuations of the filaments. In this connection, the actin polymerization based motility of bacterium *Listeria monocytogene* (LM) and bio-mimetic systems like actA-coated polystyrene beads are worth mentioning [9, 10]. The LM, when it invades a cell, captures the actin machinery of the cell and polymerizes an actin tail on one side of it. The continuous polymerization of the actins against the cell membrane of LM keeps it moving on the opposite direction. The depolymerization of the actin tail on the far end creates the local actin monomer gradient which supplies required actin monomers to the polymerizing end.

With the advent of new powerful microscopy and imaging techniques, people are now looking at this world of small-scale biological activities at various levels. Particularly in cell spreading, experimental and theoretical attempts are being made to identify various universal features associated with the dynamics of spreading cells [11–13]. Although, the process of cell spreading is a complex and active phenomenon involving complicated bio-mechanical pathways, efforts are on to look at the problem on the basis of simple physical principles without involving all the microscopic details. In [12], it has been shown that the normalized contact area of a spreading cell to its substrate $\langle A(t) \rangle / \langle A(t)_{t \rightarrow \infty} \rangle$ is a universal function of time with a characteristic exponent which depends on the cell type. The exponent α , being a function of the cell type, reflects differences in the physiology of cell types or probably differences in the environmental conditions upon which depends the spreading process. Whereas, the same functional form of α reflects the basic underlying similarity of all the processes. In [12], a model has also been put forward to calculate the exponents from considerations of actin polymerization and depolymerization rates based on curvature of cell membrane at the leading edge, elastic properties of the substrate, etc. Where two distinct dynamic phases characterize the spreading of cells in [12], in [11] three dynamic phases have been identified with the spreading of mouse embryonic fibroblasts (MEF) on a fibronectin-coated substrate. In the present paper, we would propose a phenomenological model in order to account for the dynamic phases in a spreading cell being based on gross experimental findings without going into any microscopic details. In the following paragraphs, the experiments in [11] and the results are first presented in details and then the model has been constructed. The model is analysed and results presented followed by conclusion. We have concluded that the large-scale phases of cell spreading probably do not crucially depend on all the microscopic varieties of the intricate bio-mechanical pathways rather are a manifestation of some gross mean-field effects of all those molecular level intricacies.

In [11], various dynamic phases of a spreading cell on a suitable substrate have been investigated and a sequence of transitions between successive dynamic phases been identified during the process of spreading. In this experiment, MEF cells were allowed to move on a glass surface coated with fibronectin. Fibronectin is an extra-cellular matrix protein which interacts with the cytoskeleton (an actin framework inside the cell) of the cell via the integrin receptors on the cell membrane. As has been mentioned above, this binding of the cytoskeleton to the extracellular matrix is very important for spreading of the cell by actin polymerization. A total internal reflection fluorescence microscopy and differential interference contrast microscopy of the spreading cells revealed three distinct dynamic phases. In the initial phase, the growth of the contact area of the cell is slow and is seen to be characterized by a small growth exponent $a_1 = 0.4 \pm 0.2$. It has been proposed that during this initial phase the cell basically tests the suitability of the surface to spread on and once this testing time is over the next phase of rapid growth follows. This second phase is characterized by a growth exponent $a_2 = 1.6 \pm 0.9$. In

the third phase, the cell boundary shows periodic local contractions and the area of adherence to the substrate starts to oscillate while the mean area of contact increases very slowly until it reaches the maximum limit. The growth exponent in this contractile expansion stage is $a_3 = 0.3 \pm 0.2$.

An important observation in [11] is that the cells taken in the experiment could be divided into two classes depending upon their growth rates in the fastest (middle) growing phase. Let the area of the cell at the point where the second dynamic phase starts from the first basal activity phase be A_1 and that at the point where the contractile phase takes it up from the rapid-growth middle phase be A_2 . All the cells in the experiment were found to belong to two classes depending upon the ratio $A_2/A_1 < 5$ or $A_2/A_1 > 5$. In the first class, the exponent in the middle phase was $a_2 = 0.9 \pm 0.2$ whereas for the second class ($A_2/A_1 > 5$) it was $a_2 = 1.6 \pm 0.2$. A large error bar appears in the measure of a_2 as mentioned in the previous paragraph due to the fact that its a mean of a_2 in these two distinct classes. So, the experiment suggests that the bigger the maximum area of spread the faster is cell's growth and this is an important observation to be noted to write a phenomenological model for such systems.

Let us consider the model in the form

$$\frac{\partial A}{\partial t} = \frac{1}{A} + pB - q \quad \frac{\partial B}{\partial t} = r - A, \quad (1)$$

where A is the area of the cell in contact with the substrate and B is the total polymerization rate of actin filaments, or the rate at which the actin meshwork grows. The growth rate of contact area A should depend proportionately on B since the actin polymerization is the principal driving force of cell spreading. A bigger area of contact with the substrate will definitely prevent a further increase in the polymerization rate simply because that will require even higher supply of actin monomers at the leading edge to maintain itself. This is modelled in the simplest possible way by making growth rate of B falling proportionately with A . There can always be other causes like development of stress on the cell membrane which should try to reduce the growth of B . Since at a larger A , the cell membrane will have a larger strain the polymerization process against the cell membrane should face greater mechanical resistance. So, as A increases, it should force B attain some dynamic equilibrium value where the growth rate of B is on average equal to the rate of degradation or breaking down of the actin meshwork due to restoring forces developed. Actually the constant r presents this limiting maximum value of A at which creation and destruction rates of the actin network inside the lamellipodium are on average the same. The growth rate of the area A should depend on how big the r is, because a larger r means larger initial growth for the polymerization rate B and that should make the contact area grow faster, presumably in accordance with the experimentally observed facts.

The first term on rhs of the equation for growth of the area A stands for the spreading of the cell by the pressure of the fluid inside exerted at the cell surface in contact with the substrate. Within the scope of our model, this is the passive spreading term which should always be there when a fluid like thing is enclosed inside a soft membrane and the system is acting under gravity if the outer membrane is not enough tight to overcome such a force. The exponent that has been found in the initial basal-phase of growth of the cells in [11] is roughly 0.5 and that also indicates the role of such a $1/A$ term in the initial spreading when active processes are not on. The pressure at the lower surface is proportional to the height H of the cell placed on the substrate. Considering the volume of the cell roughly constant, one has $H \sim 1/A$. The second term in the same equation is the one which represents the active process of cell spreading where p is a constant. As has been mentioned in [11], the cell initially takes some time to interact with the substrate in order to assess its suitability to be spreaded on; the constant p in our model has to be set very small to have the active spreading

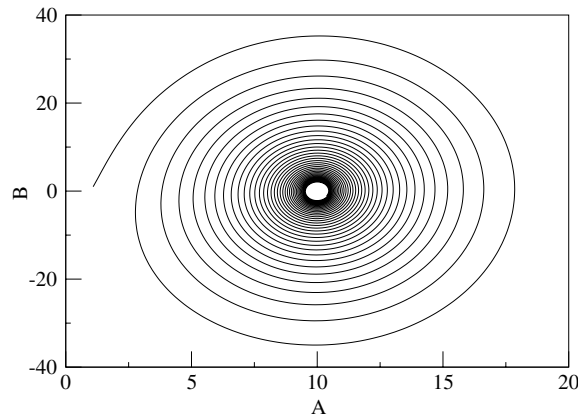


Figure 1. Phase portrait showing the spiralling down to the fixed point (A_0, B_0) .

coming into effect when B has grown by a good amount and up to that time the growth will be dominated by the other terms. The last term, a constant q , stands for the mean-field effect of all other things that constantly prevent spreading of the cell.

The fixed point of equation (1) is given by $A_0 = r$ and $B_0 = (q - 1/r)/p$. This fixed point actually corresponds to the final dynamic equilibrium state of the cell which has spreaded to the limit where rate of polymerization is equal to the rate of degradation of the actin filaments due to other forces. This can be easily understood if we do a linear stability analysis about this fixed point. Perturbing the system as $A = A_0 + a$ and $B = B_0 + b$, we have

$$\frac{\partial a}{\partial t} = -\frac{a}{r^2} + pb \quad \frac{\partial b}{\partial t} = -a. \tag{2}$$

The growth rate of the perturbation is given by $\lambda = -1/2r^2 \pm \sqrt{1/r^4 - 4p}/2$, which is always negative for not very large p . The phase trajectory should spiral down to the fixed point (A_0, B_0) so long as $p > 1/4r^4$ and initial values of A and B are far from the fixed point. Such a phase portrait is shown in figure 1 for $p = 0.05$, $r = 10$ and $q = 1.0$.

The ideal initial values for a numerical investigation of cell spreading on the basis of our model should be A very small and $B = 0$. The area of the cell in contact with the substrate is taken to be small when it is just placed on the substrate. One can also consider that there is a spreading A_0 at the start of the process beyond which the spreading of the cell is effectively considered in equation (1). We have actually taken A instead of $(A_0 + A)$ because the dynamics will remain the same in the region of our interest even if an extra A_0 is added to A . So, starting from such an initial condition, the trajectories spiral down to the fixed point when $p > 1/4r^4$ and we are interested in looking at the A versus t (t is time) plot on log-log scales. Initially, when B is very small and p is also very small, the growth rate of the area A will effectively be given as

$$\frac{\partial A}{\partial t} = \frac{1}{A} - q, \tag{3}$$

which can be solved to get

$$A + \frac{1}{q} \log(1 - qA) = -qt. \tag{4}$$

This equation clearly shows that A grows as $t^{1/2}$ for small enough A and q in the absence of contribution from active processes in spreading. This exponent $1/2$ corresponds quite well

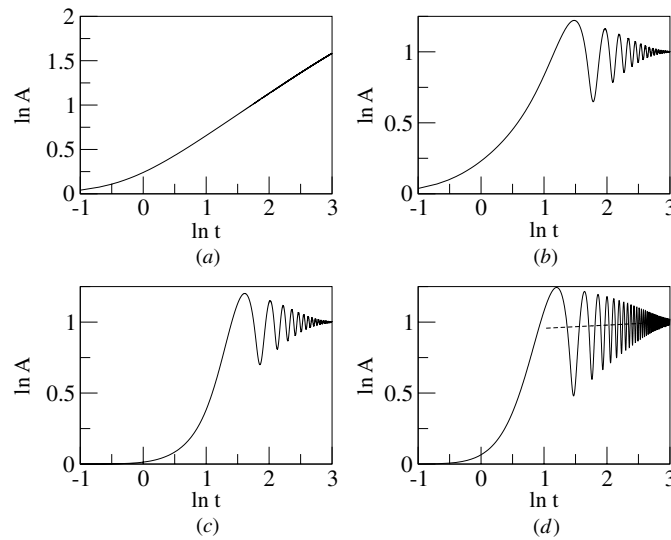


Figure 2. Apparent dynamic phases shown by our model on a plot of log of area of contact A against the logarithm of time t while spreading from an initial state given by $A_{\text{ini}} = 0.1$, $B_{\text{ini}} = 0$. The parameter values are (a) $p = 0$, $q = 0.01$; (b) $p = 0.01$, $q = 0.1$; (c) $p = 0.01$, $q = 1.0$ and (d) $p = 0.05$, $q = 1.0$ where $r = 10$ for all the graphs.

with the experimentally given one in [11] $a_1 = 0.4 \pm 0.2$. Such a spreading has been shown in figure 2(a) for $r = 10$, $p = 0$ and $q = 0.01$. Now, keeping $p = 0.01$, a small number, we set the parameter q at values 0.1 and 1.0 to plot the same in figure 2(b) and (c). There are three distinct states of spreading as appear in these figures. The initial phase of a very small growth rate. In this phase, the active part of our model, i.e. (pB), has hardly any influence on the spreading process. Next comes a rapid growth phase where the system grows quite rapidly to a larger surface of contact followed by an oscillatory spreading phase. In the oscillatory spreading phase, the contact area not only oscillates but there is a small increase in the mean area of contact with time. This oscillatory growth or contractile growth process is better reflected in figure 2(d) which has $p = 0.05$ and $q = 1.0$.

The better manifestation of the contractile growth with a larger p indicates that the contractile growth phenomenon is a characteristic of the competition between the active and other restoring passive processes in the system. In actual experiments, people have seen periodic breaking down of the actin mesh work at places along the circumference of the growing surface [4]. Such a local breakdown can happen as a result of development of mechanical stress as the cell spreads and supposedly due to some myosin density dependent generic contractile instability [14, 15].

To represent the three different with exponents comparable to those found in experiment, we have plotted figure 3 for $p = 0.001$, $q = 0.5$ and $r = 50$. We have done linear regression on the three distinct regions of the curve and have plotted straight lines (marked by crosses) to help guide eye. The exponents as obtained corresponding to these straight lines are $a_1 = 0.26$, $a_2 = 1.65$ and $a_3 = 0.07$. Where a_1 and a_2 are in good agreement with the experimentally obtained values a_3 is a little less. However, a positive a_3 actually captures the contractile expansion within the scope of the very simple mean-field model proposed and qualitatively represents the third phase of cell spreading.

To see if a bigger final area of contact corresponds to a larger growth exponent in the middle phase, we have plotted the same graph with $r = 50$ and $r = 10$ (by continuous and dashed

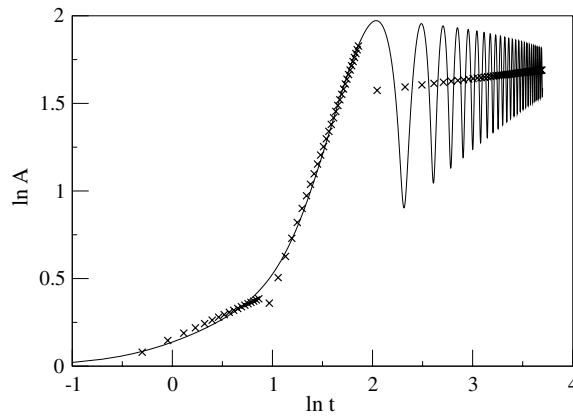


Figure 3. Three distinct dynamic phases are presented for parameter values $p = 0.001$, $q = 0.5$ and $r = 50$. The exponents in the three different phases are $a_1 = 0.26$, $a_2 = 1.65$ and $a_3 = 0.07$.

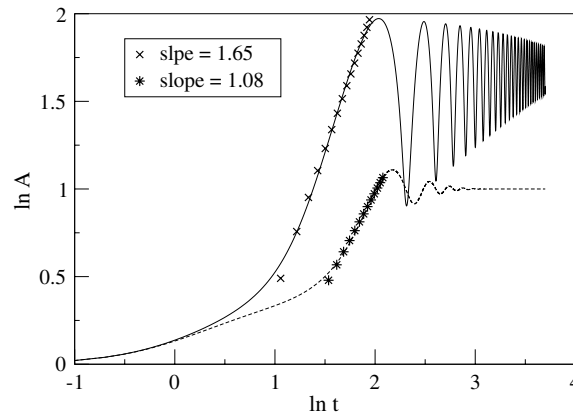


Figure 4. The fastest growing phase of a spreading cell is compared for the cases $A_2/A_1 < 5$ and $A_2/A_1 > 5$. In the case $A_2/A_1 < 5$ $a_2 = 1.08$ and in the other case $a_2 = 1.65$.

line, respectively) for $p = 0.001$ and $q = 0.5$ in figure 4. The exponents in the middle fastest growing phase are $a_2 = 1.65$ and $a_2 = 1.08$ for $r = 50$ and $r = 10$, respectively. Interestingly, if we the second fastest growing phase take up from the initial slow phase in both the cases at around $\ln A = 0.5$ we readily get the lower transition area $A_1 \simeq 3$. Now, quite logically, we can take $A_2 = r$ which results in $A_2/A_1 > 5$ for the steeply growing phase ($r = 50$) whereas $A_2/A_1 < 5$ for the slowly growing one ($r = 10$) in good correspondence with the experimental observations. The present analysis also indicates the fact that this increase in the exponent with the maximum area of contact should happen continuously rather than having classes of cells characterized by discrete exponents as is apparent from the experiment. This would be interesting to be further probed by experiments.

To conclude, we would like to mention that, the dynamic phases shown in cell spreading can easily be understood on the basis of dynamics of some macroscopic quantities and gross parameters. The detailed understanding of the microscopic-scale activities and their relations with the macroscopic parameters taken in the model are always important to realize the interplay between the small- and large-scale effects. However, the information we get by

qualitatively representing the experimental results with the simple phenomenological model is that the dynamics at the three different phases are really not that different. It is basically a spiralling journey to a stable fixed point starting from a far falling initial state. The initial basal activity phase is definitely very much different from the other two in the sense that the active part of the dynamics is not appreciably present in that phase, but the middle phase of steep growth is basically the first half of the first period of oscillatory expansion. In our interpretation of the model, the high growth rate of the polymerization rate at the beginning when the contact area of the cell with its substrate is small, is the cause of having this middle part as a separate phase on the log–log plot of the area against time. For the same dynamics, if the maximum area attainable by the spreading cell increases, it not only increases the growth rate of the system in this middle phase but also reveals this middle phase to be a part of the integral contractile phase as is evident from figure 4. This prediction can also be checked experimentally to understand the nature of these dynamic phases and assess the role of large-scale quantities on the controlling of cell spreading.

The phenomenon of cell spreading is definitely not isotropic as has been considered in the present model. The contractile phase actually shows periodic local contraction along the circumference of the cell and lateral waves of some universal nature have been observed to appear at the circumference of the cell at this phase [13]. Our simple isotropic model is really not in conflict with having local periodic contractions rather the universal spatio-temporal pattern of the lateral waves at the circumference of various cells indicates their common macroscopic origin. Thus, we have captured experimentally observed dynamic phases of cell spreading on the basis of a simple mean-field model. Our analysis leads to the conclusion that the classification of cells in phenotype depending upon their macroscopic spreading behaviour crucially depends on the gross difference in the cell types such as elastic nature of the membranes or binding to substrate for different cell types rather on having subtle difference in the complex signalling pathways in different cells.

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