Dynamic Phase Transitions in Cell Spreading

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We monitored isotropic spreading of mouse embryonic fibroblasts on fibronectin-coated substrates. Cell adhesion area versus time was measured via total internal reflection fluorescence microscopy. Spreading proceeds in well-defined phases. We found a power-law area growth with distinct exponents $a_i$ in three sequential phases, which we denote basal ($a_1 = 0.4\pm0.2$), continuous ($a_2 = 1.6\pm0.9$) and contractile ($a_3 = 0.3\pm0.2$) spreading. High resolution differential interference contrast microscopy was used to characterize local membrane dynamics at the spreading front. Fourier power spectra of membrane velocity reveal the sudden development of periodic membrane retractions at the transition from continuous to contractile spreading. We propose that the classification of cell spreading into phases with distinct functional characteristics and protein activity patterns serves as a paradigm for a general program of a phase classification of cellular phenotype. Biological variability is drastically reduced when only the corresponding phases are used for comparison across species/different cell lines.

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Cells need to be mobile in order to perform many critical biological functions. The reorganization of extracellular matrix in wound healing, the positioning of nerve cells, or the engulfment of bacteria in the immune reaction of white blood cells are particular examples 
[1]. Accomplishing this variety of functions requires a diverse set of mechanisms and proteins. Most components of the molecular machinery of actin-based motility have been identified [2, 3, 4]. It has been possible to perform experiments with reconstituted systems of Listeria propulsion [5, 6] for which detailed elastic models have been developed [7]. In vivo, cell spreading on matrix-coated surfaces provides a simplified system of analyzing motile behavior. A substantial amount of experimental and theoretical work has been done along these lines [8, 9, 10, 11]. However, only quite recently, quantitative experiments of whole cell spreading and subsequent migration could be performed with high spatial and temporal resolution [12, 13]. We found that there are well defined and distinct states of spreading. It is the goal of this work to show that these states can indeed be considered phases of motility by demonstrating the existence of dynamic phase transitions between them.

Spreading cells extend a 200 nm thick sheet called the lamellipodium from the cell body onto the substrate, see Fig. 1. This process is driven by actin polymerization at the leading membrane edge, the precise mechanism of which is still under debate [14, 15]. The meshwork of actin fibers is crosslinked by various proteins. The molecular motor myosin II enables the meshwork to contract by moving along actin fibers and relative to other cytoskeletal elements. Thus, the lamellipodium is an active gel enclosed in a flat membrane bag adhering to the substrate. The physics of active gels has recently attracted a lot of attention. Rheological experiments of simple mixtures of purified actin and myosin solutions [16] and quite general theoretical modeling [17, 18] have been carried out. There are dynamic phase transitions involving extended and contracted actin density states as a function of myosin-actin coupling strength [17]. We will show that our cellular system exhibits similar transitions which express themselves prominently in the dynamics of the leading membrane edge.

FIG. 1: A: During cell spreading, a thin lamellipodial sheet extends from the cell body onto the substrate. B: Total internal reflection fluorescence micrograph of a spreading cell. The bright region corresponds to the area adhered to the substrate. C: Two overlayed snapshots of the leading membrane edge of a lamellipodium moving from right to left are shown in differential interference contrast. The edge position is marked with a white contour overlay.

Mouse embryonic fibroblasts (MEF) were allowed to settle onto fibronectin coated glass slides and observed with either total internal reflection fluorescence (TIRF) or differential interference contrast (DIC) microscopy. Fibronectin links the cell membrane to the extracellular matrix proteins mimicking natural cell adhesion. TIRF studies were performed at a moderate spatial and tempo-
eral resolution to capture overall spreading characteristics of the whole cell. Multiple cells could be studied simultaneously. High resolution DIC was used to characterize local membrane dynamics. Details of the methods may be found in our earlier work \[12, 13\].

In the following, we limit ourselves to the isotropic class. A suitable isotropically spreading cell was chosen and a basal phase where cells test the suitability of the substrate to adhere and area growth is minimal. We find \( a_1 = 0.4 \pm 0.2 \). Then follows a phase of fast continuous spreading, which is characterized by \( a_2 = 1.6 \pm 0.9 \). Finally, the cell slows down again exhibiting a sub-linear area growth with \( a_3 = 0.3 \pm 0.2 \). We will see below that the latter phase is characterized by periodic local contractions of the cell \[13\]. Nevertheless, the mean area growth leads to an effective power law behavior also in this phase. Histograms of exponents \( a_i \) for the three phases are shown in Fig. 3. There is a clear distinction of fast area growth in the continuous spreading phase with a rather broad distribution of the exponent \( a_2 \). However, we find two narrow clusters when discriminating with respect to the relative area growth, \( A_2/A_1 \), during that phase, where \( A_i \) denotes the adhesion area at the transition from phase \( i \) to \( i + 1 \). Small (\( A_2/A_1 < 5 \)) or large (\( A_2/A_1 > 5 \)) area increases correspond to small (\( m_2 = 0.9 \pm 0.2 \)) or large (\( m_2 = 1.6 \pm 0.2 \)) exponents, respectively. In addition, there were two single cells with even larger exponents \( m_2 \) which we excluded from the cluster average.

The transition from continuous to contractile spreading was further monitored using high resolution DIC. A suitable isotropically spreading cell was chosen and a well-resolvable and approximately straight membrane segment was selected for prolonged observation, see Fig. 1 B. Time-lapse sequences were obtained at video rate. Movies were digitized at \( 1/\Delta t = 3 \)Hz. Individual frames are counted using an index \( n \). The cell edge is determined with a custom C program by a local contour algorithm \[19\] allowing nanometer accuracy. We obtain a sub-pixel resolution of 15 nm for relative displacements, which translates into a minimal detectable velocity of 45 nm/s between frames. Further analysis proceeds using a cartesian coordinate system where the average membrane orientation is taken as the fixed y-axis. Points on the membrane are then labeled by their y-coordinates \( y_j \).
and the membrane velocity $v_j(n) = \Delta x_j(n)/\Delta t$ is measured along the x-axis which is normal to the average membrane orientation.

A typical velocity map along the contour over time is shown in Fig. 4. We find that a region of continuous, uninterrupted spreading (red shadows) precedes a sequence of periodic membrane retraction events (blue stripes). These two different states of membrane dynamics correspond to the continuous spreading and contractile phase of the lamellipodium, found above. The two phases can be clearly distinguished using the discrete Fourier transformation $v_j(s)$ of the velocity map $v_j(n)$ defined as

$$v_j(s) = \frac{1}{N} \sum_{n=1}^{N} v_j(n) \exp\left(2\pi i \frac{(n-1)(s-1)}{N}\right),$$

(2)

where $N$ is the number of frames. Averages are taken over spatial regions of interest. The continuous spreading phase is characterized by a strong boundary maximum of the spectrum $|v_j(s)|$ at $s = 1$, see Fig. 5 A. In contrast, in the contractile phase the spectrum develops a pronounced peak at $s = s_{\text{max}}$, see Fig. 5 B, which signals oscillatory behavior with a period

$$T = \Delta t \frac{N}{s_{\text{max}} - 1}.$$  

(3)

Thus, the peak position of the power spectrum serves as an excellent phase indicator. We calculate the spectrum inside a small time window - with a width on the order of the repeat time - and sweep across the phase boundary. Indeed, there is a well defined transition between the two phases as seen in Fig. 5 C. However, the periodic contractions do not take place simultaneously along the leading edge, see Fig. 4. In fact, there are lateral waves of maximum contraction velocity running in both directions. These waves have a speed on the order of 200 nm/s. Moreover, there are sudden phase shifts of the periodic contractions up to half a period, see encircled region in Fig. 4.

To summarize our experimental findings, we have seen clear signatures of dynamic phase transitions in the spreading behavior of MEF cells. Since actin polymerization does not stop during membrane retraction events 13, one concludes that the actin network contracts and/or is actively pulled back by myosin motor activity. Kruse et al. 17 have modeled oriented fibers connected into a network by molecular motors. They find an instability of homogeneous fiber density towards a contracted state as a function of fiber-motor coupling strength. Moreover, their generic theoretical model allows for oscillatory solutions. Our cellular system ex-
hibits similar behavior. Indeed, the periodic contrac-
tions are absent when myosin light chain kinase (MLCK),
which activates myosin, is inhibited [13].

In the following, we give a systems biology oriented
view of cell motility. Several questions arise: What is the
functional role of the phases described above? How are
these dynamic phases of the structural motility network
regulated and connected to the signaling network? Can
we disentangle the complex set of motility related pro-
teins and simplify description by considering functional
modules and hierarchical levels of control?

During the initial spreading phase, a MEF cell assem-
bles the cytoskeletal structure necessary to probe the
mechanical suitability of the substrate in the following
contractile phase where it periodically pulls on the sub-
strate. Indeed, cells require stiff substrates for growth
and move toward stiffer regions [11]. The machinery for
this stiffness sensing is organized into i) the basic struc-
tural elements consisting of the actin cytoskeleton, the
myosin II motors, as well as the plasma membrane, ii)
factors directly regulating protein coupling strength and
activity, and, finally, iii) these regulatory proteins are
controlled by a signaling network coordinating spatially
distant and/or logically separate functional events in
the cell. In order to link these cellular components to the
physics of dynamic phase transitions, we note that it is
the basic structural elements which exhibit the various
phases we have identified in this work. The phase param-
eters are given by the regulating proteins, e.g., MLCK ac-
tivity and concentration. The different dynamic phases
correspond to functional regions in the regulating para-
ter space. The trajectories in this parameter space are
determined by the cellular signaling network. This hier-
archical identification provides an immediate conceptual
advantage: The topology of the motility phase diagram is
independent of the complex signaling network, i.e., the
relative positions of all the motility phases do not de-
pend on the trajectories in parameter space followed by
the cell. Indeed, the basic phase characteristics can be
probed and modeled separately. This was demonstrated
using the observed linear relationship between the period
of contractions and the lamellipodia width [13]. We find
that the contraction period is the same for equal lamel-
lipodia width, independent from the variations in the bio-
chemical pathway(s) induced in order to achieve a certain
width. On the other hand, cell motility is a unique case
where the interconnections of the structural and signal-
ning networks, which depend on gene expression, can be
probed in order to establish a quantitative link between
phenotype and genotype. We are currently identifying
functional modules in a large scale screening of spread-
ning phenotypes across various fibroblast cell lines with
mutant genotypes.

The idea of phases in cell behavior can be applied
quite generally. Phases of motility should be consid-
ered analogous to the phases of the cell cycle, phases of
varying metabolic activity or different protein expression.
We propose to classify cellular behavior in well defined
phases. Their number will be considerable less than an
enumeration of concentration and activity levels of all
molecular components of the cell. Thus, one can hope
to accomplish a simplified description. Currently, phase
classification is not generally done and cellular phenotype
cannot be sensibly compared across different genotypes.
We expect that some fraction of the variability encoun-
tered in biological experiments and the often conflicting
results between laboratories stem from the fact that find-
ings corresponding to different cellular phases and bound-
ary conditions are spuriously compared to each other. In
conclusion, we feel that the classification of motility in
phases can serve as a paradigmatic example for a power-
ful general ordering principle in quantitative biology.

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