Reanalysis of FLAP Data Showing Rapid Actin Transport in Motile Lamellae

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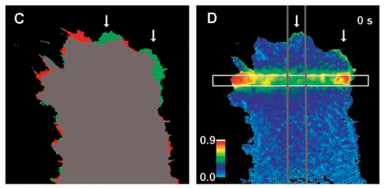
Abstract

A study by Zicha *et al.* [1] used the Fluorescence Localization After Photobleaching technique to analyse the transport of G-actin towards the front of the leading lamella in a highly motile malignant rat fibroblast line. Estimates by these authors indicated that diffusion alone is not fast enough to account for the observed speed and amplitude of the transport (up to 40 % FLAP signal at the leading edge only 2 seconds after bleaching of a stripe about 10 microns away). To explain these data, the authors proposed a model whereby actin monomer is carried by rapid advection through channels in the actin gel. Here we re-examine the basic experiments in terms of a more realistic two-dimensional model of a steadily protruding lamella based on the two-phase reacting flow approach [2]. Our results indicate that the channel advection hypothesis is not tenable because Taylor dispersion prevents significant advective transport of small solutes. Our analysis points to an alternative mechanism that explains the FLAP data without special transport. This mechanism requires the existence of a small region at the leading edge of the lamella where polymerization is fast and where retrograde flow of F-actin is equally fast. Our calculations show that this special front compartment condition allows for a very intense FLAP signal to develop on the observed timescale even if diffusion is the only transport mechanism responsible for G-actin delivery. If our hypothesis is correct, the observations of Zicha *et al.* constitute a novel and very sensitive indirect probe of critical reaction and retrograde flow rates in the leading edge compartment which are otherwise very difficult to study.

Experimental Data Shows Rapid Actin Transport

Fluorescence localization after photobleaching (FLAP):
•Actin molecules are labeled with two different fluorophores (YFP, GFP). At initiation, YFP channel is photobleached in a narrow strip across the lamella
•The FLAP ratio image is calculated as a normalized difference between the YFP and CFP intensity at each location (Ic-Iy)/Ic, thus revealing the local volume fraction of the bleached molecules



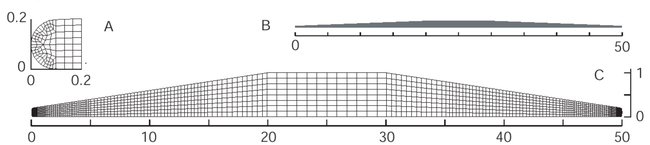
From Ziha et al. 2003
Images of a T15 cell taken immediately after a strip (white box) was bleached: **[C]** Protrusion, retraction, **[D]** FLAP ratio signal, **[E]** Integrated along grey line: GFP, YFP, FLAP ratio.

Bleached actin quickly appears at the leading edge:

• FLAP experiment in malignant rat fibroblasts shows rapid transport of actin towards the advancing leading edge (at a rate of ~10 µm in 2-3 sec)
• Generally, advancing portions of the leading edge showed high FLAP labeling 2 sec after bleaching. On retracting segments no such FLAP signal could be detected at such early times
• Zicha *et al.* deemed diffusion insufficient for the observed rate of transport and suggested a possibility of a pressure-driven advective transport through actin gel channels

Numerical Model Overview

A vertical cross-section of a cell [B] with the overall dimensions of 50 x 1 µm, tapering down to .2 µm at the leading edge cap [A], is represented by a finite element grid with 1,897 nodes (the vertical direction of plot [C] has been stretched to make the mesh more visible):



Two-phase flow paradigm [2] with simultaneous tracking of the cytosol and cytoskeleton volume fractions and velocities via implementation of a finite element method to solve the mass and momentum conservation equations in both phases.

Constitutive equations and boundary conditions:
• Polymerization and depolymerization of actin monomers
• Cytoskeletal viscosity and solvent-network drag due to relative motion of the phases
• Network swelling, implemented as isotropic stress proportional to the network concentration
• Cytosol and cytoskeleton slip on free boundaries; no-slip condition for the network at ventral surface

[1] Zicha D., Dobbie I.M., Holt M.R., Monypenny J., Soong D.Y.H., Gray C., and G.A. Dunn. ***Rapid actin transport during cell protrusion.*** Science, 300:142-145, 2003.
[2] Dembo, M. and F. Harlow. ***Cell motion, contractile networks, and the physics of interpenetrating reactive flow.*** Biophysical Journal, 50: 109-122, 1986.

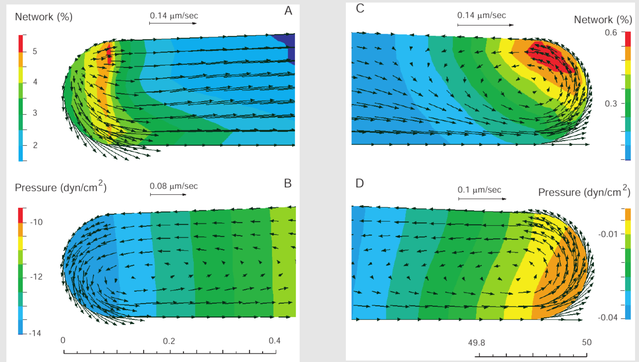
Steady State Flow in the Model Cell

The rapid polymerization in the cap compartment leads to a high network concentration which in turn causes local enhancement of the swelling stress so that the network phase expands and flows towards the cell center. The flow of polymerized material out of the cap leaves behind a partial vacuum that causes a zone of low pressure to develop. The suction of this pressure together with the drag exerted by the network combine to create a whirlpool of an eddy of cytosol. This eddy carries or circulates dissolved material from the center of the cell into the cap compartment along the lower boundary of the lamella and expels such material out of the cap along the upper surface. As network flows towards the center, it leaves the region where the polymerization rate is enhanced. Consequently, the tendency to depolymerize is no longer counterbalanced by polymerization, and the network concentration decreases. This basic treadmilling cycle of polymerization at the edges, inward expansion, and depolymerization at the center constitutes a steady flow of network and cytosol that is maintained indefinitely.

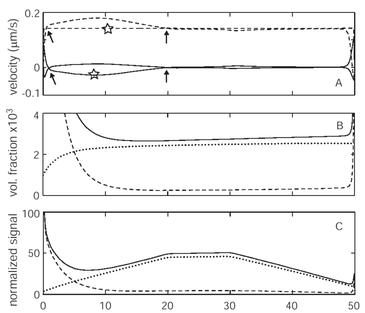
The traction forces [3] near each of the two caps tend to thrust the underlying substrate inward, towards the cell center. Due to symmetry, the edges of the cell are stretched with equal force in opposite directions.

Gliding Cell

To initiate cell motion, we ``deactivate" one of the cell edges so that the rate of actin assembly there is the same as in the bulk cytoplasm. The velocity fields and the associate viscous shear stresses on the substrate become asymmetric, and the cell is pulled by the remaining ``active" cap, whereas the deactivated cap drags against the substrate producing passive friction. The motion is a uniform translation like that of a keratocyte, and the velocity of this motion is implicitly defined by the requirement that the integral of the active and frictional forces on the substrate is zero at each instant of time (zero inertia limit).



Steady state solution of near leading (left panels) and trailing edges
All velocities (arrows) are shown in the frame of reference of the cell (cell velocity is -0.14 µm/s). (A,C) Network velocity and network volume fraction (color). (B,D) Cytosolic velocity and pressure (color, normalized so that the maximum pressure in the cell is zero).

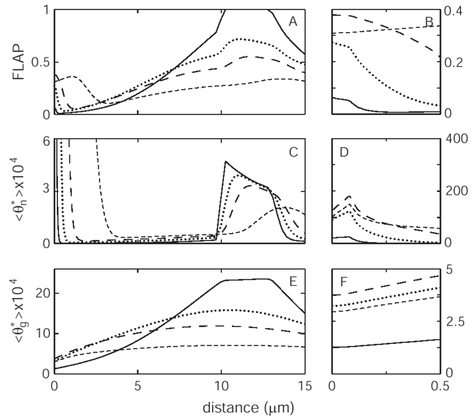


• (A) The network (*dash line*) and the solvent (*solid line*) velocity at the membrane. The retrograde flow rate in the lab frame can be found by subtracting the gliding velocity. The star indicates profiles at ventral membrane.
• (B) Volume fractions of G- and F-actin (*dot, dash lines*), and total actin (*solid line*).
• (C) The thickness average of the total actin vol. fraction over the cell height scaled to 50% at the cell midpoint (*solid line*). The breakdown of the total actin signal intensity into its The G- and F- components (*dot, dash lines*). The small local maximum of F-actin at the trailing edge is mostly due to 'sweeping' of the network by the advancing membrane.

[3] Dembo M. and Y.L. Wang. ***Stresses at the cell-to-substrate interface during locomotion of fibroblasts.*** Biophys. J., 76:2307-2316, 1999.
[4] Ponti A., M. Machacek, S.L. Gupton, C.M. Waterman-Storer, and G. Danuser. ***Two distinct actin networks drive the protrusion of migrating cells.*** Science, 305:1782-1786, 2004.

Numerical FLAP Experiment

The steady state described above serves as the initial condition for the numerical FLAP experiment. At initiation, a strip extending from 10 to 13 microns away from the leading edge of the lamella is instantly 'bleached' by introducing additional 'bleached' actin species. As the simulation time goes on, we track the transport of the bleached molecules directly, as well as by calculating the FLAP ratio.



A time series of the numerical FLAP experiment data recorded after the 2-sec bleaching period (*solid line*), as well as 2, 5, and 15 sec after the bleaching (*dot, long dash, short dash*). Panels to the right show the detail at the leading edge. (A,B) The FLAP signal. At 2 sec, the FLAP value at the leading edge is 27%, rising to 38% at 5 sec and declining towards the final value of 15% at 15 sec. (C,D) The thickness average of the bleached F-actin. The advection in this case is sufficiently fast so there is an observable transport of the F-actin out of the bleaching zone. (E,F) The thickness average of the bleached G-actin.

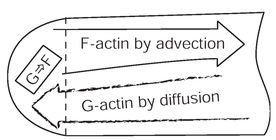
Observations:
• Distribution of the labeled G-actin or F-actin does not depend on the advection of the cytosol
• Within 2 seconds after the 2-second bleaching, enough bleached G-actin diffuses out of the bleaching zone to account for the leading edge FLAP ratio reported in [1]
• High network concentration in the lamella inhibits the magnitude of the FLAP ratio signal
• Protrusion stretches, or dilutes the network, mitigating the above effect

Summary

Simple diffusion of G-actin is sufficient to supply the monomer necessary for observed rates of protrusion and actin assembly. By the nature of diffusion this means that the supply of G-actin is quite unregulated and uniform at all segments of the leading edge be they protruding or retracting. Furthermore, this means that changes in the dynamics of the edge can be made quickly in response to local signalling events and that changes in protrusion activity are not contingent on a complex pre-existing logistical supply chain involving flows through networks of supply channels.

Advection of actin through narrow channels in the network is unlikely to contribute to the explanation of the FLAP data of [1]. The fundamental reason is that the walls of channels inside a porous gel are highly permeable and present a negligible barrier to diffusion. As a result, mixing of material inside and outside the channels is so fast as to negate the possibility of effective advective transport along the channel length. This conclusion is true even even for unrealistic assumptions that greatly favor advective transport, e.g. even if the channels are very wide and even if the flow in such wide channels is exceedingly fast.

The G-actin is delivered to the leading edge compartment by diffusion, where it is rapidly converted into F-actin. The filaments expand out of the leading edge forming a retrograde flow. Once outside the the leading edge cap, depolymerization becomes dominant, and the released actin monomers are once again free to diffuse back towards the edge.



FLAP data: The observed correlation between zones of edge protrusion and higher FLAP ratio [1] is due to the fact that protrusion leads to stretching and dilation of the F-actin near the front cap which favors rapid FLAP signal development. As a corollary, the inward motions of the leading edge inhibit such development due to an additional compaction of the F-actin by the retracting leading edge.

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