Cell Crawling: First the Motor, Now the Transmission

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Cell crawling is thought to be the result of three coordinated motility behaviors: (a) protrusion and adhesion of the front end, probably driven by actin assembly; (b) traction force that leads to the advance of the nucleus and bulk cytoplasm; and (c) release and retraction of the tail in most cell types. (In neurons, the last step is highly modified, and an axon elaborates from behind the advancing growth cone.) As Mitchison and Cramer (15) point out, it is the traction step that is least understood and seems most central to productive locomotion. Two papers in JCB now shed new light, both mechanical and molecular, on the traction step of cell crawling. A report in this issue of JCB on growth cone movements from the Forscher group (19) and a paper from the Borisy lab (20) on keratocyte motility provide new insights into the role of myosin in traction. Specifically, the two papers clarify the mechanical connections to the motor that produce the motile output of various animal cells. These papers also provide insight into how a conserved mechanism for animal cell crawling could underlie visually different crawling behaviors. Indeed, the cell types used in the new studies are at two extremes of cell crawling behavior. Neuronal growth cones change shape markedly during their constant, stop-and-go advance, which is accompanied by a variety of seemingly futile motile behaviors such as waving of, extending, and retracting filopodia, and a persistent retrograde flow of cortical actin. In contrast, fish keratocytes appear to glide smoothly over a culture surface, moving continuously with little or no change in aspect. Retrograde actin flow is not typically seen in these cells; indeed, most actin filaments remain stationary with respect to the substratum during movement (21).

Suter et al. (19) have extended their observations of the retrograde (toward the cell body) flow of cortical actin that occurs on the surface of growth cones of cultured Aplysia bag cell neurons. This behavior has been observed in other cultured cells as well (22, 23). Cortical actin, apparently as a newly assembled, cross-linked network, arises from the most distal margin of the growth cone and then moves smoothly backward across the thin cytoplasmic veil of the lamella only to disappear, presumably by disassembly, when it reaches the thicker, microtubule-rich, central region of cytoplasm. Suitably adherent beads on the cell surface can “go with the flow” on Aplysia neurons and be transported smoothly backward at the same speed as the actin (4, 10), as if the beads were riding an escalator. A key role for myosin contraction in powering this continuous retrograde movement is supported by pharmacological evidence (12). When Aplysia growth cones contact another cell, the actin flow slows at a rate that was inversely proportional to the rate of growth cone advance (10), suggesting a single motor underlying both the cortical flow and growth cone advance.

This older work has now been extended with two innovations. One is the use of beads coated with the cell-surface Aplysia cell adhesion molecule (ApCAM), or with antibodies to ApCAM that ride the flow, which revealed two states of coupling to the actin flow. Second, the use of glass needles to restrain these beads allowed for direct application and qualitative assessment of forces independent of the biological phenomena. This simple, reliable, external assay of motive forces in cell locomotion vastly increases the confidence in mechanical interpretations, revealing simple Newtonian behaviors and reducing the dependence on biological assumptions.

Suter et al. (19) find that ApCAM beads sprinkled on the top surface of the growth cone couple to the retrograde actin flow. For the first 10–20 min, these beads simply stop moving when restrained by the needle, but the retrograde flow continues uninterrupted as shown by the movement of smaller, non-ApCAM beads used as markers. Therefore, ApCAM beads initially couple weakly to the actin flow and can slip easily if restrained (Fig. 1a). After a variable latency period, however, restraint of the bead produces a set of integrated mechanical responses that the authors call a “restrained bead interaction” (Fig. 1b). First, the needle bends backward, indicating force generated from within the cell. Second, on-axis beads marking retrograde flow slow down and stop as the tension increases, suggesting that the same mechanism driving actin flow is responsible for needle bending. This result also indicates strong mechanical coupling between the ApCAM bead and the retrograde actin flow that is firm enough to support the force generated by the cell. At the same time, both the protrusion and traction phases of motility occur in the growth cone. Cytoplasm moves forward along the...
face receptors to the underlying cytoskeleton had previ-
usome long engagement of surface receptors with un-
pothesis that growth cone advance depends on engage-

Gravity's third law is that the same force is driving both.

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Consider a car stuck in the mud applying constant power to its
drive wheels (myosin acting to produce force). Because the
connection to the environment is fluid, the wheels spin, producing no forward motion. This slip interaction is
equivalent to the retrograde actin flow. If the mud now sol-
idifies, like the coupling of the ApCAM bead to the actin
after a latency period, and the ground is now immovable
(like the needle restraining the actin flow), then the stick
interaction occurs and the car moves forward, as does the
central cytoplasm in the growth cone. If there is some
combination of car movement (central cytoplasmic move-
ment) and wheel spinning (retrograde actin flow), then
these rates will be inversely proportional as required by
Newton's third law because the same force is driving both.

The above results provide strong support for the hy-
pothesis that growth cone advance depends on engage-
ment and disengagement of cell surface receptors with un-
derlying actin networks within the cytoplasm (11, 15).

An automobile analogy may help clarify how the above
conclusions were reached, as the traction observations
comprise a simple stick–slip mechanical interaction.

We have confirmed that the results provide no information about
the presence or absence of connection(s) between the central
cytoplasm and the myosin driving retrograde flow. (b) After a lat-
ency period, firm connections develop between the ApCAM
bead and the actin, associated with clustering of ApCAM side anal-
ysis of robust mechanical links from integrins through to the
nucleus (13). Connections from ApCAM to the central cy-
toplasm were confirmed when release of bead restraint
and its accompanying tension were immediately followed
by retreat of the central cytoplasm toward its original posi-
tion and revival of retrograde flow by the marker and Ap-
CAM beads. In our view, this is strong evidence that the
same motor that powers retrograde actin flow in static
cells does indeed drive the engorgement phase character-
istic of growth cone translocation, a somewhat controver-
sional idea. The rearward actin flow and forward flow of cen-
tral cytoplasm are both the result of the same motor,
confirmed by the observation that on-axis beads marking
retrograde flow slow down and stop at a rate that is in-
versely proportional to the rate of central cytoplasmic
movement, as previously observed for homophilic growth
cone interactions (10).

The above results provide strong support for the hy-
pothesis that growth cone advance depends on engage-
ment and disengagement of cell surface receptors with un-
derlying actin networks within the cytoplasm (11, 15).
Essentially, a two-state clutch (strong and weak coupling,
or stick and slip) exists between the membrane proteins
and the cytoskeleton (Fig. 1). Variable engagement of sur-
face receptors to the underlying cytoskeleton had previ-
ously been demonstrated (2). However, the forces applied
were small and there was no clear relationship between beha-
vors of cell crawling and the observed differences in
engagement. Suter et al. (19) provide persuasive evidence
that traction force requires different, firmer connections
between surface receptors and the cytoskeleton than the
connections required simply for retrograde flow of surface
markers, which are weak and slip easily. A “slipping clutch” may also explain the finding that, in some cells,
surface-marking beads move at a fraction of the rate of the
underlying actin flow (22). Suter et al. (19) also provide an
initial clue to the molecular basis of the two clutch states: a
clustering of ApCAM occurs along with the strongly cou-
ped state. Thus, the two states may simply reflect differ-
ces in the local concentration of coupling interactions.

Also confirmed is the notion that growth cone advance in-
volves central cytoplasm pulled forward by tension (6),
which requires the now demonstrated firm links between
the source of tension and the cytoplasm. Myosin appears
to be the source of this tension, based on the current evi-
dence linking cytoplasmic movement with retrograde flow.

The new observation is that during a restrained bead inter-
action the central microtubule-rich cytoplasm also surges
forward, which is the key traction movement of growth
cone crawling. That is, tension forces are seen to naturally
produce the “engorgement” phase of growth cone ad-

Figure 1. Highly schematic diagram of the forces and mechanical
connections inferred from the results of Suter et al. (19). (a) Dur-
ing the latency period after attachment of ApCAM-coated beads,
restraint of the bead causes it to stop, with no evidence of tension
exerted in the needle. Retrograde actin flow continues uninterr-
upted, suggesting a weak, slipping interaction between the bead
and the underlying actin network. The question marks are in-
tended to illustrate that the results provide no information about
the presence or absence of connection(s) between the central
cytoplasm and the myosin driving retrograde flow. (b) After a lat-
ency period, firm connections develop between the ApCAM
bead and the actin, associated with clustering of ApCAM be-
neath the bead. Restraint of the bead now causes development of
tension in the needle and retrograde flow to stop. Because the act-
in is no longer able to slip relative to the surface, the tension gen-
erated by the myosin motors is now accommodated by forward
flow of cytoplasm at the leading edge and in the central microtu-
bule-containing cytoplasm. This indicates mechanically resistant
connections among the surface, actin, myosin motors, and sur-
rounding cytoplasm. See text for further explanation.
and the earlier results that retrograde flow depends on myosin ATPase activity (12). A major question is what cytoplasmic architecture might underlie the connections between the cell surface, the motor, and the deep cytoplasm. The diagram in Fig. 1 illustrates the mechanical aspects suggested by the new results but is clearly unrealistic in terms of cytoskeletal arrangements. For example, although some actin filaments in lamellar regions of growth cones are oriented parallel to the axis of movement, most filaments form a crisscross network (9). Further insight into the nature and functioning of some of the connections is provided by the paper on fish keratocytes (20).

Fish keratocytes are wing-shaped cells whose long axis is perpendicular to the direction of advance. The forward arc of the cell consists of a broad, thin lamella and the rear part of the cell contains the nucleus and cell body. Keratocytes’ peculiar ghostly locomotion, in which the cell moves smoothly and continuously at high speed (10 µm/min) with essentially no change in shape, is thought to be due to nearly perfect coordination among the protrusion, traction, and retraction phases (15). It is known from watching keratocytes move on and deform rubber sheets that the cell exerts most of its traction force near the two lamellar sides (8), rather than along the front-back axis as one might expect. Curiously, the cytoplasm and organelles of the cell body rotate like the drum of a steamroller as the cell moves forward (1). Svitkina et al. (20) address these unusual phenomena in a study that confirms previous observations of a marked gradient in the actin filament network (18): dense at the lamellar margin, less dense toward the cell body, but then very dense in the form of actin bundles in the transition region between the lamella and cell body. The new data indicate that myosin is also arrayed in a gradient. Fluorescent myosin II, as bipolar filaments, formed small clusters near the lamellar margin that increased in size toward the cell body, aggregating into a network in the transition region. In moving cells, the myosin clusters in the lamella are initially stationary with respect to the substratum, so that as the cell moves, the cell body gets closer to the clusters and the lamellar margin moves further away. In static cells, myosin clusters move slowly backward toward the cell body.

Fig. 2 illustrates a model “dynamic network contraction” proposed by Svitkina et al. (20) to explain their results. Actin assembly is nucleated at the lamellar margin to form a network that is disassembled nearer the nucleus, as observed for several cell types (2). Myosin filaments and clusters also arise spontaneously within the network, as previously observed in fibroblasts (14). The actomyosin-based contraction of this network pulls the cell body forward and also causes the network to collapse into bundles at the transition zone (Fig. 2 b). This concentration of cytoskeletal filaments near the bottom of the cell body creates a drag force that, combined with the forward translocation force, causes the cell body to rotate (just as the nondrive wheels of a car spin from being dragged along the ground) (Fig. 2 d). The authors postulate a stick-slip clutch connecting the cell body to the cytoskeletal network to explain how the cell body can be pulled forward by the contracting network and also rotate. Typically, contractile mechanisms have been assumed to involve contraction parallel to the direction of advance (15, 16), but a network postulated to contract uniformly throughout would exert a significant fraction of the force perpendicular to the direction of advance. This new model could then explain the substantial traction forces exerted laterally (8). The uniform contraction produces leftward and rightward forces at the sides of the lamella that cannot be accommodated by net leftward or rightward locomotion of the cell because of cell integrity, but would stretch the cell out perpendicular to the direction of advance. Nevertheless, the forces on a rubber substratum would be seen to be greatest at these lamellar sides, and not along the front-to-back axis where the forces are accommodated by net locomotion.

In addition to contraction, cell crawling requires making and breaking attachments to the substratum (7). Combining ideas about surface-to-cytosol connections from Suter et al. (19) with the contractile network ideas from Svitkina et al. (20) produces a speculative model that can explain a variety of the motile phenomena associated with cell crawling. We assume, as in other models, that the motile phenomena observed on the top, free surface of cells reflects mechanisms also functioning on the bottom, attached cell surface. During traction, the actin network is stably and tightly coupled to surface receptors, as shown by Suter et al. (19) that, in turn, are coupled to the substratum. Myosin-generated tension causes network contraction, and the inner cytoplasmic mass or cell body moves forward (Fig. 1 b), but the lamellar actin remains attached to and stationary with the substratum (21), as does myosin.

Figure 2. Diagram illustrating the dynamic network model of Svitkina et al. (20) in a locomoting fish keratocyte. (a and b) A network of actin (light grey lines) and myosin (dark bipolar filaments) contracts causing reorganization of the network. Myosin is clustered and actin is brought into alignment as parallel bundles. (c) Seen at the level of the entire cell, this network contraction causes forward translocation of the cell body. (d) In this cross-section, the forward rolling of the cell body is seen as a combination of the forward-directed force and a drag force at the bottom of the cell body/nucleus created by the accumulation of contracted network. Similar to nondrive wheels of a car, the combination of forward force and dragging along the bottom surface causes rotation of a rounded object.
suspended within the network (20). Persistent movement is possible because the network is essentially regenerated continuously for each change of location by network assembly at the forward edge and disassembly at the rear. Retrograde movement of actin and/or myosin in stationary cells is the result of network contraction in the presence of weak interactions between the membrane and the network (Fig. 1a). These slip, allowing the entire network to slip backward relative to the membrane, as shown by Suter et al. (19) for growth cones. In stationary keratocytes, we imagine that tension rips loose some of the moorings and, combined with network assembly/disassembly, creates a slow retrograde movement of the network.) Presumably, the stop-and-go advance of growth cones then reflects oscillating periods of slip or stick between the cytoskeleton and substratum. And the differences between myosin playing a contractile or transport function in cell traction (15) would seem to narrow considerably, reduced (arguably) to different clutch engagements. Localized contraction of the network combined with protrusive events could underlie membrane ruffling and filopodial waving often seen on the dorsal surface of cells. Both keratocytes and Aplysia growth cones are highly lamellipodial with few of the filopodia that garnish the front ends of many crawling cells. These finger-like projections contain a bundle of uniformly oriented actin (9). Thus, forces and cytoskeletal arrangements would correspond even more closely to that shown in Fig. 1, suggesting little fundamental difference between filopodial and lamellipodial crawling.

Speculations aside, the current work raises many important questions for future study. Some in the field remain skeptical of the simple role for myosin in retrograde flow and cell crawling shown in Fig. 1. In any case, how does the myosin function in motile cells? For example, at least one observation of Suter et al. (19) argues against myosin in Aplysia growth cones being organized as in the lamella of keratocytes. Suter et al. (19) show that traction force can be exerted in a local region of the growth cone while retrograde flow continues elsewhere. That is difficult to explain with a uniformly contracting network. Does Aplysia myosin also move retrogradely during actin flow as predicted by dynamic network contraction? Or put another way, just how general are the findings from fish keratocytes? The evidence for variable coupling between surface receptors and the underlying cytoskeleton also raises a host of questions about the molecular and mechanical bases for this complex-regulated connection. And what underlies the connections between the motor and deep cytoplasm? Is a clutch at this position, postulated by Svitkina et al. (20), a special feature of keratocytes? In cells without rolling motion, there may always be a connection. Whether the motor produces traction could then depend on whether there was a simultaneous connection to a system that permitted the force to be dissipated with-out moving the cell (“sling mud”). Because of these and many other issues, the pages of JCB are likely to be graced by important advances in our understanding of cell crawling for some time to come.

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