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MOLECULAR MECHANISM OF ACTIN-DEPENDENT RETROGRADE FLOW IN LAMELLIPODIA OF MOTILE CELLS.

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1. ABSTRACT

In motile, eukaryotic cells, a variety of cell-associated material (collectively termed here as 'particles') continuously flows, relative to the substratum, from the front to the back of the extreme margin of the cell (termed the 'lamellipodium'). This retrograde particle flow, occurs both over the surface of, and inside the lamellipodium. Force to drive retrograde particle flow in lamellipodia is dependent on actin filaments, but the precise mechanism of force generation, and function of the flow is generally not well understood. Actin filaments themselves, in lamellipodia of most motile cell types studied also flow retrograde relative to the substratum. This actin flow, in Aplysia bag cell neuronal growth cones, is known to be driven by activity of a myosin. In these growth cones, retrograde flow of cell surface-attached particles is coupled to retrograde actin flow. In Aplysia, force from retrograde actin flow may limit certain types of growth cone motility. In other motile cell types, such as keratocytes and fibroblasts, the mechanism of retrograde particle flow and function of retrograde actin flow in lamellipodia is poorly understood. For these cell types, recent data provide a basis for proposing alternative actin-based mechanisms to drive retrograde particle flow in lamellipodia. One mechanism is based on activity of a putative pointed end- directed actin motor, and the other on tension-driven surface lipid flow. Here I will review recent advances that have been made in determining the molecular mechanism of force generation to drive retrograde particle flow relative to the substratum in lamellipodia of motile cells. I will address the function of retrograde actin flow in lamellipodia, and apparent differences between Aplysia and other motile cell types.

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2. INTRODUCTION

A number of different types of motility occur in eukaryotic cells. In motile, eukaryotic cells adhering to solid substrata, one of the most dramatic is the continuous flow of cell-associated material, directed inwards from the cell periphery, both over the cell surface and inside the cell. In the literature, this flow is variously termed inward, centripetal, backward, or retrograde, flux or flow. Different types of flow of cell-associated material in eukaryotic cells have been observed for about two centuries, and is a fundamental property of all eukaryotic cells so far studied. In recent years, retrograde flow in adherent, motile, eukaryotic cells have been intensely investigated, although its function, remains for the most part, unknown. In these cells, observed retrograde flow may occur either relative to the cell (Fig 1A) or relative to the substratum (Fig 1B).

An adherent, eukaryotic, motile cell is composed of several distinct cell regions (Fig 2). At the front of the cell are leading edge structures. These comprise the lamellipodium (a thin cellular band, typically less than 0.5 μm thick, and 1-10 μm long from front to back), and long, thin, cylindrical extensions of the lamellipodium termed filopodia, or microspikes which are shorter. Behind the lamellipodium is a thicker cell region termed the lamella. Behind the lamella is the cell body, which is the bulkiest cell region comprising the nucleus and most of the organelles. Retrograde flow can occur in all of these cell regions (e.g. Fig 2 illustrates retrograde flow relative to the substratum in the lamellipodium and lamella). Also, retrograde flow is opposite to, and sometimes occurs simultaneously with, several types of forward cell motility (Fig 2). These are: protrusion which brings leading edge structures forward; cell body motility or traction which brings the bulk of the cell and nucleus forward; and tail retraction/deadhesion which brings the rear of the cell forward.

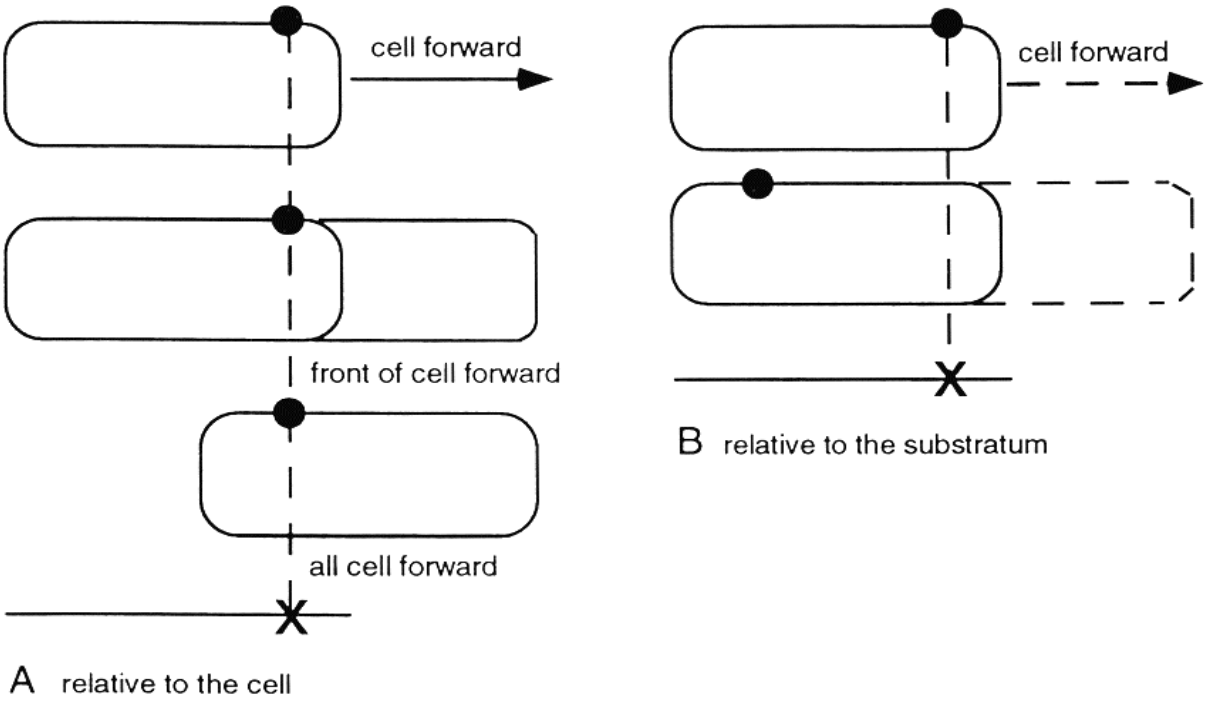

Fig 1. Types of retrograde flow in adherent, motile, eukaryotic cells. (A, relative to the cell) Top panel: cell-associated material (black sphere) is essentially stationary relative to the substratum (fixed point, X), as the cell (oblong) physically moves forward (arrow). Middle panel: if only the front of the cell moves forward, the material appears to flow retrograde from the cell front. Bottom panel, if all of the cell moves forward (cell locomotion) the material appears to flow retrograde to the back of the cell. This occurs for certain cell surface receptors in locomoting cells (termed capping). (B, relative to the substratum) (focus of this review) Cell-associated material (top panel, black sphere) physically flows retrograde (to new position, bottom panel), relative to a fixed point (X) on the substratum. This can occur (bottom panel) on both stationary (e.g., solid oblong) and moving (e.g., solid and dashed oblong), motile cells.

In the lamella and cell body the type of retrograde flow most understood typically occurs relative to the cell in locomoting cells. This is capping of cell surface receptors. Surface receptors flowing from the lamella and cell body cap over the nucleus or cell tail. From genetic studies, capping requires myosin II (1-3). Retrograde flow relative to the substratum in the lamella and cell body has in general been less studied. At least for certain types of cell-associated material, it is known that this is driven by a myosin (Waterman-Storer and Salmon, submitted), but not myosin II (3). In contrast in leading edge structures, mostly in lamellipodia, retrograde flow relative to the substratum, both over the lamellipodium surface and inside the lamellipodium, has been well studied. In both protruding and stationary lamellipodia, a variety of cell-associated material, including actin filaments, flows retrograde relative to the substratum (Fig 3). I will collectively refer to this material, except actin filaments, as particles. To distinguish between flow of particles and flow of actin filaments, I will use the terms 'retrograde particle flow', and 'retrograde actin flow', respectively. Many mechanisms have been proposed to drive retrograde particle flow in lamellipodia (4-7). It is now widely accepted that actin filaments are required to generate force to drive retrograde particle flow. Compelling evidence is that poisons of actin inhibit retrograde particle flow in lamellipodia (3, 8-10).

Early ideas on how actin filaments generated force to drive retrograde particle flow in lamellipodia were theoretical. One quite popular idea was that contraction of an actin filament network moved the lipid bilayer of a lamellipodium backward as a sheet, and structures on the moving sheet rode as passengers (4, 11). At the time this made sense; flow of particles on the surface of lamellipodia were thought to reflect a moving cell surface, and muscle proteins were just beginning to be identified in non-muscle motile cells (reviewed in (12)). This theory was not pursued once Singer and Nicholson (13) introduced the idea that the lipid bilayer was fluid. Of the several alternative explanations offered, the one that turned out to be the most pertinent came from a discussion between Wolpert and Harris in 1973 (11). Wolpert hypothesized that a ‘filamentous system’ directly moved particles retrograde. Precisely how has been debated since this time. Part of the problem is that over the last 10 years or so different types of particles have been studied in different motile cell types. For example, the tendency

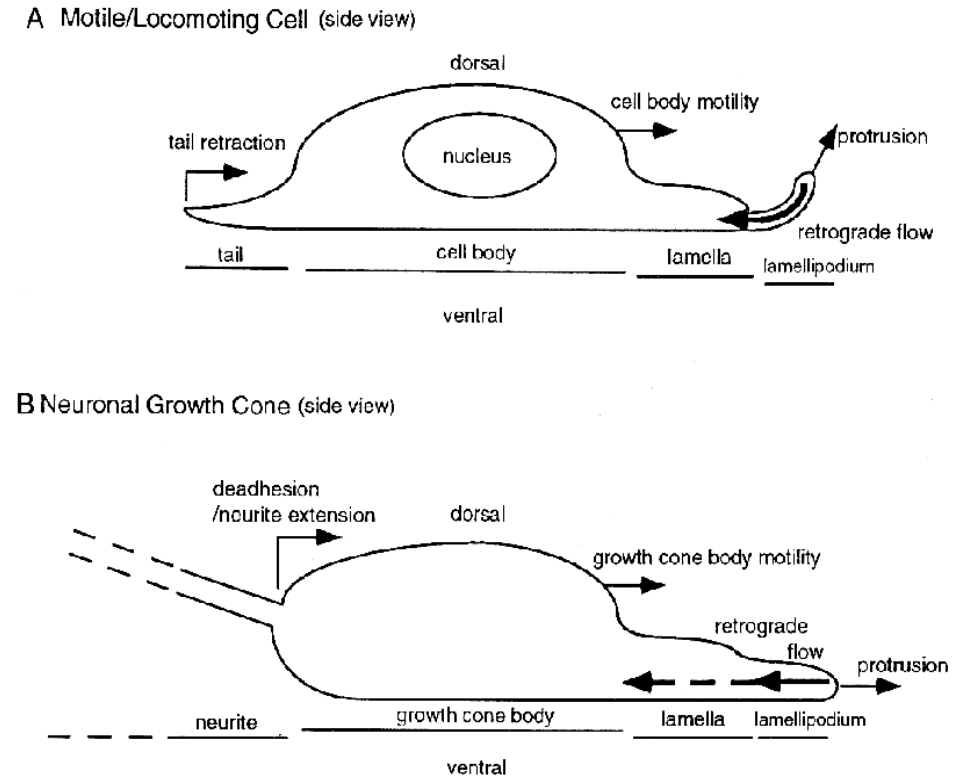

Fig 2. Cell regions and types of motility in motile cells. (A, motile/locomoting cell) In a fibroblast the lamellipodium is often raised up off the substratum. In certain other motile cell types such as keratocytes the lamellipodium is in constant contact with the substratum (as drawn in B). Retrograde flow relative to the substratum has mostly been studied for individual types of cell-associated material crossing the lamellipodium (thick arrow to left). Retrograde flow occurs opposite to the direction of certain types of forward cell motility that may occur in motile cells (protrusion, cell body motility and tail retraction). Strictly a motile cell is termed a locomoting cell, only if it undergoes net, protrusion, cell body motility and tail retraction, such that the entire cell boundary moves to a new position (e.g. Fig 1A, compare oblong, top and bottom panels). (B, neuronal growth cone) A growth cone is similarly organized to a motile cell except the nucleus is not located in the growth cone body, and the neurite replaces the tail. In the literature, the lamellipodium and lamella are sometimes collectively referred to as 'peripheral domain' and the growth cone body as 'central domain'. Growth cone body motility is sometimes referred to as central domain extension. Similarly, to locomote, a motile growth cone must undergo, net, protrusion, growth cone body motility and deadhesion/neurite extension. In contrast to motile cells, in growth cones, retrograde flow relative to the substratum has been mostly studied for individual types of cell-associated material crossing both the lamellipodium (thick arrow to left) and lamella (dashed thick arrow to left), and recently, mostly in Aplysia bag cell neurons.

has been to view particles flowing retrograde on the cell surface, as the same phenomenon as particles and actin filaments flowing retrograde inside the lamellipodium. It may turn out, however, that retrograde flow of particular types of particles associated with lamellipodia in some motile cell types, may be a separate phenomenon, driven by a distinct mechanism. Perhaps related to this, different results have been obtained in different motile cell types, particularly in Aplysia bag cell neuronal growth cones, fibroblasts and keratocytes. This has led to distinct views on both the mechanism of retrograde particle flow, and function of retrograde actin flow in lamellipodia.

In this review, I will briefly describe the organization of actin filaments in leading edge structures of adherent, motile cells, and in neuronal growth cones. Then, I will describe potential types of actin-dependent motile force to drive retrograde particle flow relative to the substratum in lamellipodia of these cells, and in growth cones of Aplysia neurons. I will present evidence in favor of each type of motile force, and discuss function of retrograde actin flow.

3. STRUCTURAL ORGANIZATION OF ACTIN FILAMENTS IN LAMELLIPODIA

Determining the structural organization of actin filaments in motile cells is crucial for solving the molecular mechanism of any type of actin-dependent cell motility (recently reviewed and discussed in detail (14). In lamellipodia and other

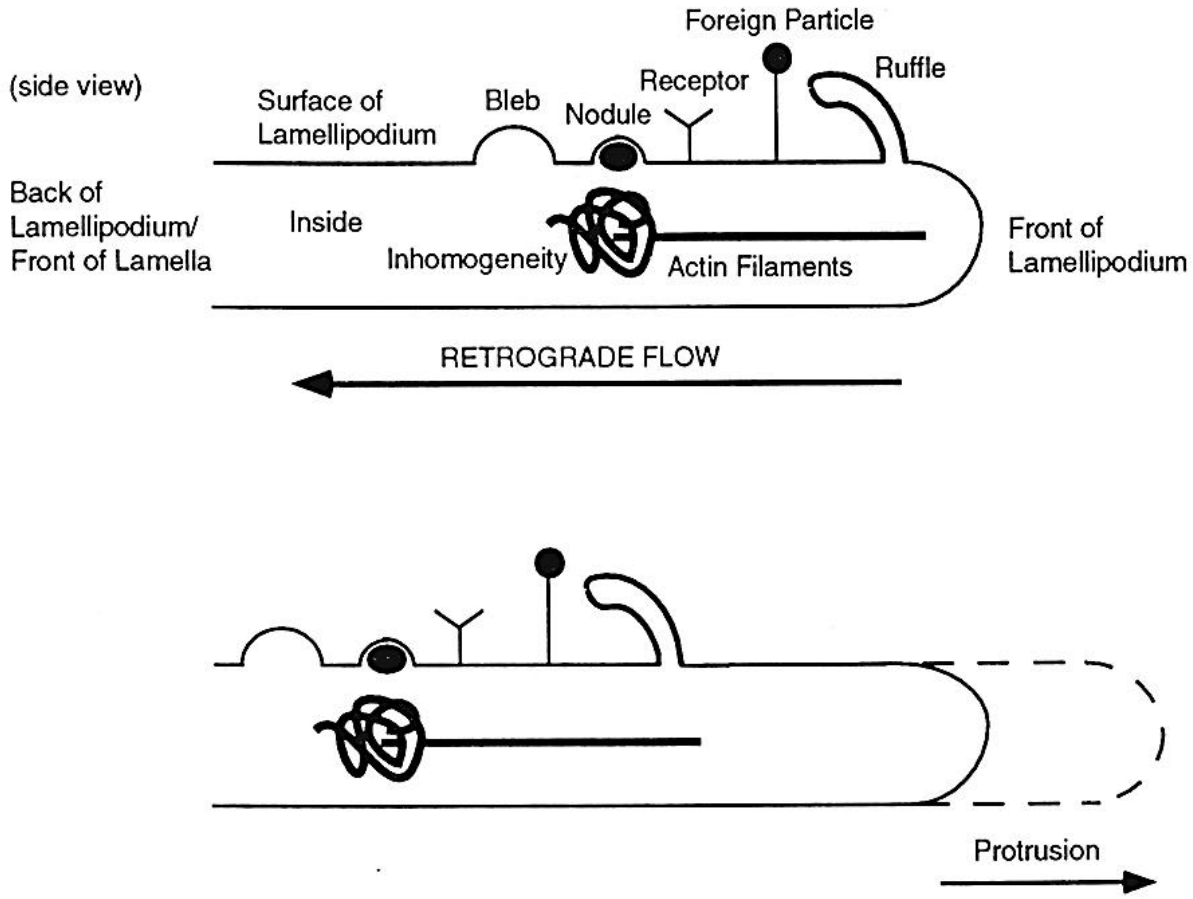

Fig 3. Different types of cell-associated material that flow retrograde relative to the substratum in lamellipodia. In motile and locomoting cells, retrograde flow (long arrow to left) is directed from the front to the back of both protruding (shorter arrow to right) and stationary lamellipodia, and other leading edge structures. Flowing retrograde over the surface of lamellipodia are: membrane ruffles, characteristic of fibroblasts due to lamellipodia that lift up off the substratum and flow retrograde; foreign-attached particles (e.g. beads, glass fragments); cell surface receptors; nodules; and blebs. These are shown flowing over the dorsal surface, but some, e.g. foreign-attached particles, have also been observed to flow over the ventral surface. Flowing retrograde inside the cell are: phase dense inhomogeneities; and fibrous material, including in most cell types studied, actin filaments.

leading edge structures the general organization of actin filaments is well known. Here, I will focus on certain details, that may turn out to be relevant for determining the precise source of actin filament organization that is responsible for driving retrograde flow of a particular type of particle.

Leading edge structures of motile cells are highly dynamic and are filled with dense arrays of actin filaments. Actin filaments, in general, where it has been possible to study, are organized with their barbed ends (fast growing, or plus ends) oriented preferentially in the direction of protrusion (15-19) (Fig 4A). One issue is whether there is a difference in the polarity of the actin network between the ventral and dorsal surfaces in these leading edge structures (Fig 4B). Such a difference has been reported for lamellipodia of growth cones of certain mammalian neurons (17). On the ventral surface of these growth cones, actin filaments are long and bundled and have expected uniform barbed end polarity facing the direction of protrusion. In contrast, actin filaments associated with the dorsal growth cone surface are shorter and apparently have more mixed polarity. Although it is unclear where these measurements were precisely made in the growth cone, information on the polarity of actin filaments associated with the dorsal surface of lamellipodia in other motile cell types is likely still missing. This is because experimental procedures in most studies of polarity involve extracting with detergent. Since the dorsal surface is more exposed than the ventral surface, detergent is more likely to disrupt an actin organization associated with the dorsal surface. Indeed in lamellipodia of keratocytes, detergent is thought to remove most of the dorsal-associated actin filaments (18). A distinct type of actin organization, similar to muscle sarcomeres (alternating polarity actin filament bundles; (19)) has been identified within 0.1-1 μm of the

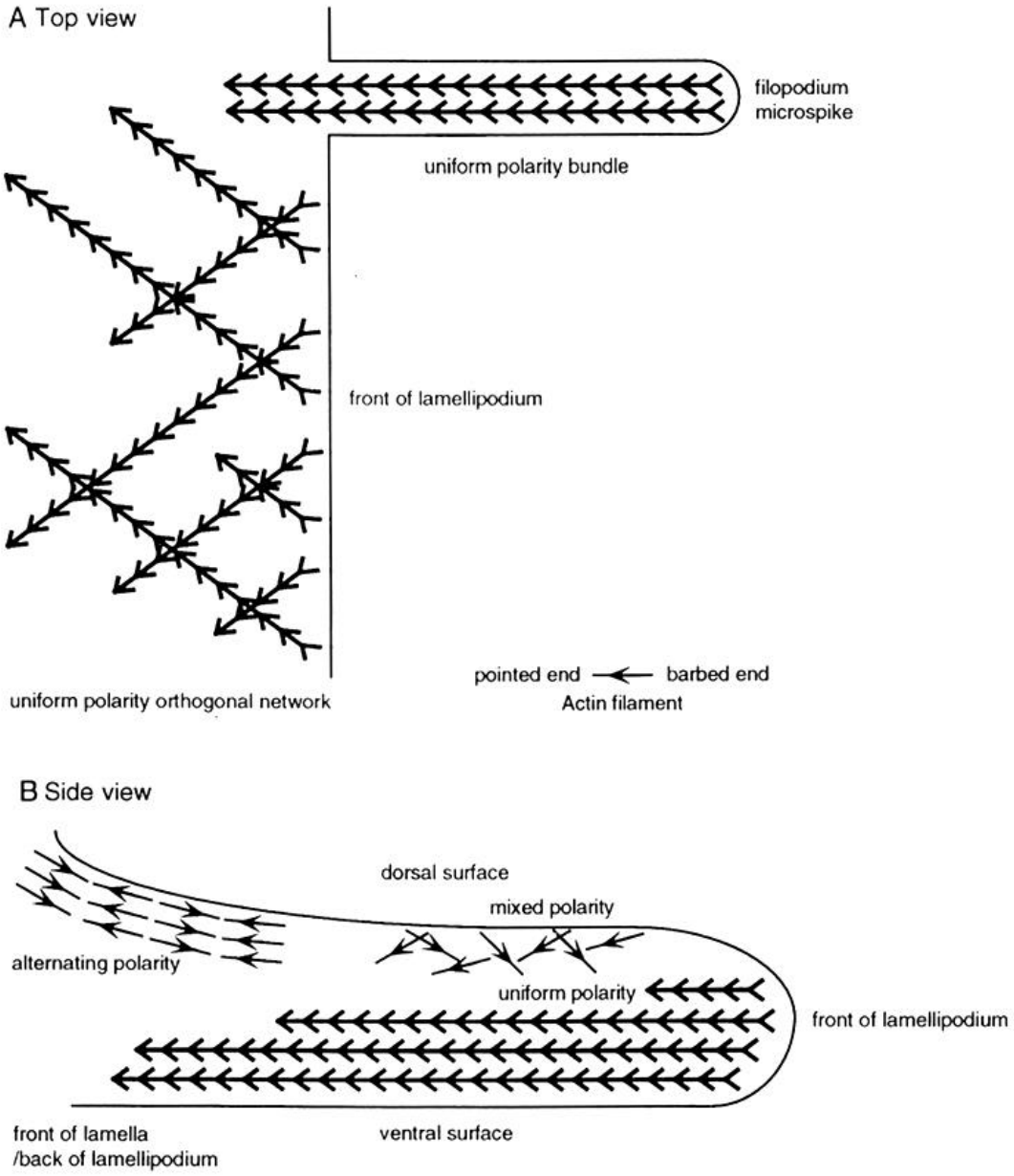

Fig 4. Actin filament organization in leading edge structures. (A, top view) In nearly every case, studies show that almost all the barbed ends of detected actin filaments face the front of the leading edge structure (facing the direction of protrusion). This is a type of uniform polarity. Filopodia and microspikes contain a tight bundle of long actin filaments. Lamellipodia contain an orthogonal, crosslinked, network, of actin filaments oriented at approximately 45^{o} to the direction of protrusion (18) The length of actin filaments in lamellipodia has been debated (14). (B, side view) Additional types of actin filament organization also seen in a few studies: short actin filaments of more mixed polarity under the dorsal surface of certain mammalian growth cones (17); alternating polarity bundles, comprised of short actin filaments, under the dorsal surface of the front of the lamella/back of the lamellipodium (19). It is not known if these structures are present in lamellipodia of other motile cell types. It is possible they are preferentially extracted during experimental procedures. Since the front of a lamellipodium, from the ventral to dorsal surface, is at most only about 0.5 μm thick (equivalent to roughly 50 actin filaments stacked on top of each other), these additional actin organizations are likely to be a minor component of lamellipodia.

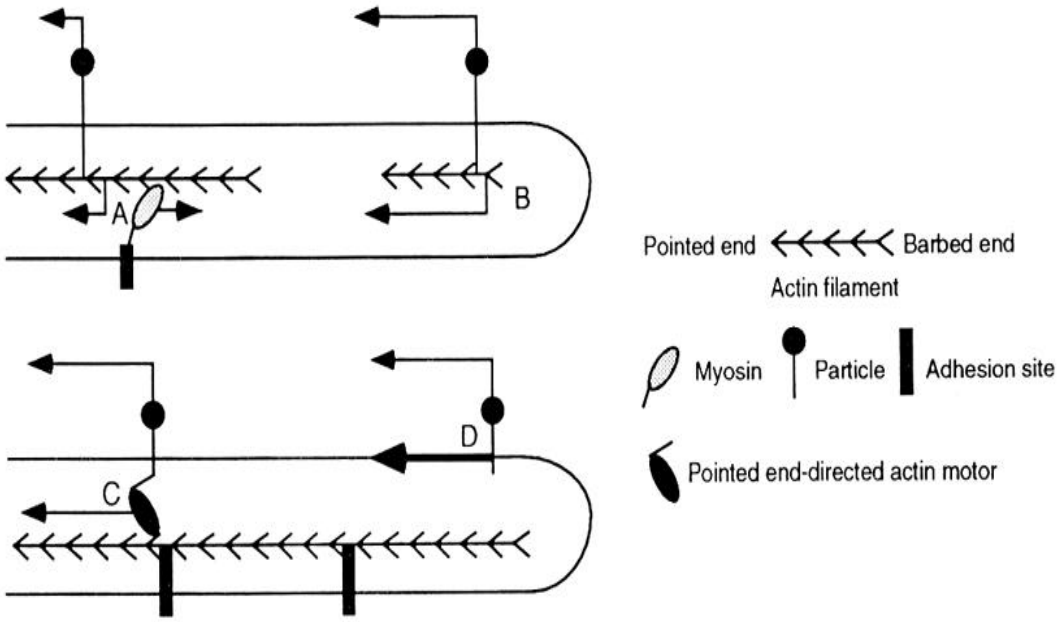

Fig 5. Types of actin-dependent motile force to drive retrograde particle flow relative to the substratum in lamellipodia. (A and B, actin flow-coupled mechanism) (A) Myosin (grey ball and stick), theoretically associated with an adhesion site (black bar) moves (short arrow to right) toward the barbed end of actin filaments (chevrons) and drives the filament retrograde (lower short arrow to left). Particles (lollipop) are coupled to this retograde actin flow. (B) In the same lamellipodium, some particles (lollipop) may be coupled to a population of actin filaments (chevrons) flowing at a different rate (lower long arrow to left) to the filaments in A. (C and D, alternative mechanisms to drive retrograde particle flow) (C) Particles attached to the cell surface (lollipop) or located inside the lamellipodium (not shown), are actively driven retrograde by a putative motor (black ball and stick) directed toward (lower long arrow to left) the pointed end of actin filaments (chevrons). In keratocyte lamellipodia, actin filaments are stationary relative to the substratum and are likely attached to adhesion sites (vertical black bars). In tissue culture fibroblasts actin filaments are not stationary, but flow retrograde slower than particles. If a pointed end-directed motor moves particles retrograde on these actin filaments in these cells, a mechanism must exist to prevent the filaments from undergoing net forward movement (which has not been reported to occur in lamellipodia). (D) Surface tension is higher at the back than the front of the lamellipodium and drives surface lipids (lower thick arrow to left) and surface-attached particles (lollipop) retrograde.

cell surface in locomoting heart fibroblasts. Since some of these bundles are associated with the dorsal surface (but not ventral surface) of the front of the lamella, the possibility remains that they are also associated with the dorsal surface at the back of the lamellipodium (Fig 4B). If alternating polarity actin bundles are associated with the dorsal surface of other motile cell types, under experimental conditions, less-than-optimal for preserving the dorsal surface, they might appear to have more random polarity.

4. TYPES OF ACTIN-DEPENDENT MOTILE FORCE TO DRIVE RETROGRADE PARTICLE FLOW RELATIVE TO THE SUBSTRATUM IN LAMELLIPODIA

4.1. Actin flow-coupled mechanism

The classic photobleaching work of Wang (20) together with more recent work has lead to the view that actin filaments are formed at the front of lamellipodia (21-23), and then the filaments continuously flow retrograde relative to the substratum (19, 24-26), before disassembling further back in the lamellipodium. This, in conjunction with results showing that in some cell types cell surface-attached particles flow retrograde at the same rate as internal filamentous structures (8, 27), has led to the prevalent idea that particles flow retrograde in lamellipodia because they are coupled to the retrograde flow of actin filaments. In Aplysia bag cell neuronal growth cones, this is supported by a direct test; actin filaments marked by photobleaching of phalloidin move at the same rate as surface-attached foreign beads (26). The natural question then is 'how does the actin flow?' While it was initially thought that actin assembly itself might drive retrograde actin flow (20), flow occurs in the absence of actin polymerization (28). This result switched investigator's attention to alternative candidates for driving retrograde actin flow in lamellipodia. In Aplysia growth cones, one candidate is that the motor activity of a myosin drives actin flow (e.g. Fig 5A). In these cells actin flow is inhibited (29) by a low affinity inhibitor of myosin ATPase (BDM, (30, 31)) and microinjection of cells with NEM inactived-myosin heads. It is not known which myosin drives retrograde actin flow or where

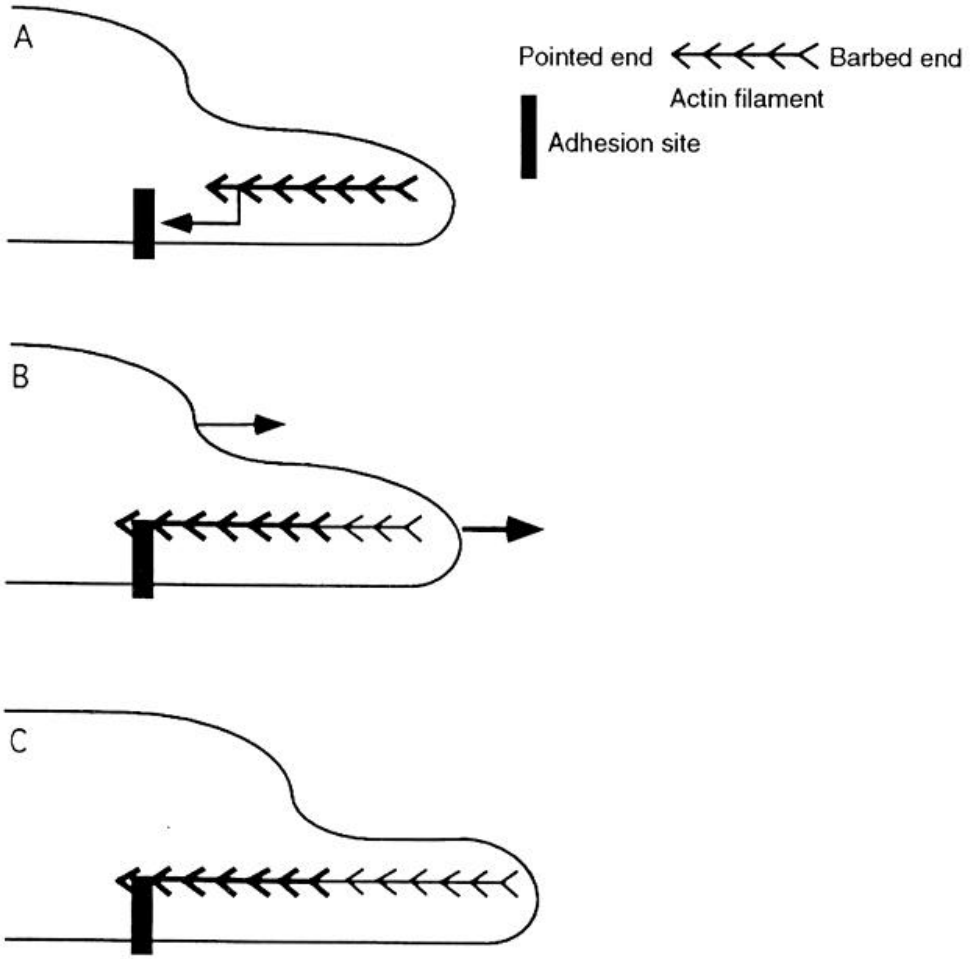

Fig 6. In Aplysia growth cones, retrograde actin flow is inversely correlated with growth cone body motility and protrusion (26). (A) Actin filaments (thick chevrons) flow retrograde (arrow). (B) Actin filaments attach to adhesion sites (vertical black bar) and retrograde actin flow stops. The gap at the front of the lamellipodium created by retrograde actin flow in A, is filled by actin filament assembly (thin chevrons). (B to C) Growth cone body motility occurs (upper arrow in B, to new position in C). Current data can not determine if force from attenuation of retrograde flow is directly harnessed to drive growth cone body motility (see text). The absence of retrograde flow no longer limits protrusion; there is no gap to fill at the front of the lamellipodium, and on going actin assembly (C, thin chevrons) is instead coupled to net protrusion (lower arrow in B, to new position in C).

the myosin is spatially located.

An alternative candidate for driving retrograde actin flow has come from a mathematical model (32). In this model, flow is driven by loss of actin filaments from a crosslinked actin network at the back of the lamellipodium. This is predicted to induce greater stress in the remaining network at the back of the lamellipodium, creating a tension gradient, sufficient to drive retrograde flow of the actin network. Also, since in this model the crosslinks in the actin network allow the stress to develop, a gradient in actin crosslinks, higher at the back of the lamellipodium, is also predicted to generate a tension gradient. For some motile cell types, this is a very attractive model. For example in Ascaris sperm cells, where actin filaments are replaced by major sperm protein filaments (that flow retrograde relative to the substratum (33)), no cytoskeletal motors have been identified. Also this model may not be at odds with a role for a myosin in driving retrograde flow of actin filaments, as above in Aplysia growth cones. Instead of using the motor activity of a myosin, as drawn in Fig 5A, a myosin may instead act to crosslink the filament network. Certainly, myosin II crosslinks actin filaments into a non-sarcomeric, 'zig-zag' array at the back of lamellipodia of certain tissue culture fibroblasts (34, 35).

4.2. Alternative mechanisms to drive retrograde particle flow in lamellipodia

Outside of the Aplysia system it is unclear if particles couple to the retrograde flow of actin filaments. In lamellipodia of keratocytes, and MC7 and IMR 90 tissue culture fibroblasts,

surface-attached particles, and phase-dense inhomogeneities flow retrograde relative to the substratum faster than actin filaments (25), compare (36) and (37). Retrograde particle flow is dependent on an intact actin cytoskeleton. Particles flowing at different rates may simply be driven by different populations of actin which are flowing at different rates in the same lamellipodium, but are not equally detected by methods used in different motile cell types (Fig 5B). Consistent with this possibility, particles have been observed to flow retrograde relative to the substratum at different rates over dorsal and ventral surfaces respectively in the same fibroblast lamellipodium (38). Alternatively, these data also fit a model in which particles, either on the surface, or inside the lamellipodium, are actively driven retrograde by the action of an actin-based motor. Genetic studies in amoeba do not support a role for myosin II (3), nor for myosin IA/1B, IB/IC or IB/ID (39) in driving retrograde particle flow in lamellipodia. Chromophore assisted laser inactivation studies in chick dorsal root ganglia do not report such a role for myosin IB or V (40). BDM does not inhibit retrograde particle flow in lamellipodia in either newt lung cells (Waterman- Storer, and Salmon, submitted) or heart or MC7 fibroblasts (Cramer and Mitchison, unpublished). While it is too early to exclude a role for myosin in driving retrograde particle flow in lamellipodia, one possibility is that the motor is a yet to be identified pointed end-directed actin motor protein (Fig 5C). In mitotic cells, the theoretical existence of such a motor to drive a type of retrograde particle flow is the simplest explanation of experimental data (41). It is consistent with the polarity of most actin filaments detected in lamellipodia (15-19). Supporters of this idea need to find alternative roles for the myosins enriched in lamellipodia. One obvious role, but so far not reported in the literature outside of the Aplysia system, is to drive retrograde flow of actin filaments. Alternatively, video tracking has shown that certain cell surface proteins can move rapidly forward in lamellipodia (42-44). This movement requires actin filaments and may be driven by a myosin, allowing receptors to rapidly promote substrate sensing and adhesion. This is consistent with the localization of a myosin I isoform to forward moving particles in lamellipodia of coelomocytes (45). Other work implicates roles for unconventional myosins in protrusion and retraction of leading edge structures (40), also see (46), vesicle transport/secretion (reviewed in (47), and stabilization of actin containing structures (48).

A distinct alternative mechanism for driving retrograde flow of surface-attached particles is tension-driven surface lipid flow (Fig 5D). Recent studies show that surface lipid in chick dorsal root ganglia neurites flows retrograde relative to the substratum at 4-7 μm/min along a shallow surface tension gradient (49). The observed rate of lipid flow is certainly sufficient to drive observed retrograde flow relative to the substratum of particles attached to the surface of lamellipodia in keratocytes (5 μm/min, calculated from (36)) and fibroblasts (1-2 μm/min, (25)). The neurite data differ significantly from previous views of lipid flow; where, in locomoting cells, surface lipid has instead been invoked to flow retrograde relative to the cell, but remain essentially stationary relative to the substratum (50, 51). Also the data are in contrast with previous convincing reports which do not reveal retrograde surface lipid flow relative to the substratum over the cell body, lamella, or portions of lamellipodia (9, 36, 52). In these studies, however, measurements were not reported from 0 to 1-4 μm from the front of lamellipodia. Since this is typically where retrograde particle flow is fastest, it remains a formal possibility that there is local retrograde surface lipid flow relative to the substratum in lamellipodia. Supporters of this idea need to find a source of lipid to move retrograde from the front of the lamellipodium, and for removing excess lipid that would otherwise pile up at the back of the lamellipodium. In the neurite study one source of lipid is likely to come from the secretory pathway, and in motile cells polarized insertion of lipid vesicles has been observed at the front of lamellipodia (reviewed in (51)). Polarized removal has been observed at the back of protrusive structures in several motile cells types (see (53)). Also the exact source of tension in the cell surface needs to be found. In the neurons studied above, tension is at least partly generated by activity of the actin cytoskeleton (54). In lamellipodia, could the known organization of actin filaments (Fig 4) generate a tension gradient? The bulk, uniform polarity actin filament network, may generate a tension gradient as predicted by mathematical modeling (32) (as described above). Presumably this tension gradient could be transmitted to the cell surface through integral membrane proteins. Alternatively, alternating polarity actin bundles may generate contractile force (discussed in (19)). These bundles are in a prime position to contract under the dorsal surface at the front of lamella/back of lamellipodium.

5. FUNCTION OF RETROGRADE ACTIN FLOW IN LAMELLIPODIA

Retrograde flow of actin filaments relative to the substratum in lamellipodia has often been proposed to play some role in cell motility, although retrograde movement is not immediately reconcilable with net forward displacement of either leading edge structures (protrusion), or the cell body (cell body motility). As with studies of mechanism, those for function of retrograde flow have yielded seemingly ambiguous results. In Aplysia growth cones, as the rate of retrograde actin flow relative to the substratum decreases, the rate of growth cone locomotion increases, both in terms of protrusion and growth cone body motility (26) (Fig 6). This is consistent with a model in which retrograde actin flow is attenuated by coupling to substrate, and in Aplysia the myosin which drives retrograde actin flow instead generates 'forward thrust' (as in a 'molecular clutch' proposed in (55)). There is tentative support for this idea for growth cone body motility, but not protrusion in Aplysia. When myosin force in Aplysia is killed with BDM, retrograde flow and growth cone body motility are inhibited, but protrusion is promoted (from (29)). Further studies are required to determine if myosin force from retrograde flow is directly harnessed for growth cone body motility in Aplysia. This is because BDM inhibits more than one myosin (30, 31) and so theoretically different myosins may independently generate force for growth cone body motility and retrograde actin flow respectively. For protrusion in Aplysia the implication is that force from a myosin is not directly harnessed for protrusion, but that the rate of actin flow limits net protrusion (Fig 6).

In contrast to Aplysia, in stationary tissue culture fibroblasts (25) and locomoting heart fibroblasts (19) there is no correlation between retrograde actin flow relative to the substratum in lamellipodia and either protrusion or cell body motility. Also, in locomoting Ascaris sperm cells the rate of retrograde flow of major sperm protein filaments in lamellipodia is unrelated to cell speed, although flow is faster in stationary cells (33). For protrusion, the difference between Aplysia and fibroblasts may simply reflect a different geometry. Aplysia growth cones are in contact with the substratum, whereas fibroblast lamellipodia are often raised up off the substratum, thus preventing efficient coupling with the substratum. For cell body motility, the difference between Aplysia and fibroblasts may reflect the exact nature of an Aplysia growth cone. Perhaps the front of an Aplysia growth cone is simply one structure, rather than a distinct lamellipodium and lamella. In a single structure, a single type of force has the potential to drive retrograde actin flow across the entire region from the front margin of the growth cone to the front of the growth cone body. In this case, it is easy to imagine why in locomoting Aplysia growth cones, there is a relationship between retrograde actin flow, and both protrusion and growth cone body motility. In contrast, in locomoting fibroblasts the force that drives retrograde actin flow in lamellipodia is spatially separated from the cell body by stationary actin filaments in the lamella (19). Instead, in fibroblasts, retrograde actin flow may regulate the formation of certain actin bundles (56).

A different role for retrograde flow has been proposed in locomoting newt lung epithelial cells (Waterman- Storer, and Salmon, submitted). In these cells, retrograde flow alters the spatial orientation of microtubules, which, in turn influences microtubule plus-end assembly dynamics. This might be important for communication between actin and tubulin cytoskeletal systems in locomoting cells and neuronal growth cones.

6. PERSPECTIVE

I have described a number of actin-dependent motile forces to drive retrograde particle flow and actin flow relative to the substratum in lamellipodia. The prevalent mechanism in which all retrograde flow of particles reflect coupling to moving actin filaments needs to be tested more rigorously in different motile cell types. To solve this issue, and those of function, taking advantage of known motile systems that are defective in specific myosins, and developing better myosin inhibitors is needed. Also required is the ability to detect markers of actin filaments, a variety of different particles, and lipids at better resolution. This may require development of new markers. For example, are there actin filaments in lamellipodia whose dynamic behavior is yet undetected by current methods? Also, it is a distinct possibility that more than one mechanism operates in the same lamellipodium. For example, some particles (either internal or cell surface-attached) could be coupled to actin flow, independent of distinct cell surface-attached particles coupled to lipid flow. Similarly, if all actin filaments are stationary in lamellipodia, retrograde flow of internal particles may be driven indepedently to retrograde flow of distinct particles on the cell surface.

Another direction for the future is to determine how retrograde flow relative to the substratum in lamellipodia is coordinated with that in the lamella and cell body of the same motile cell. For example it is clear that in Aplysia growth cones and amoeba cells certain individual particles can traverse, relative to the substratum, both the lamellipodium and lamella (3, 8, 26). In amoeba, this can continue, relative to the substratum, across the cell body (3). How does this occur? In Aplysia growth cones, the data are more consistent with a single type of force continuously transporting the same particle. In amoeba, the data are less clear, and instead distinct forces may differentially contribute to transporting the same particle in different regions of the same cell (e.g. as can occur for motor-driven intracellular vesicle transport).

7. ACKNOWLEDGMENTS

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