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Neuronal Cytomechanics: The Actin-Based Motility of Growth Cones

STEPHEN J SMITH

The patterns of synaptic connection that underlie brain function depend on the elaborate forms characteristic of neurons. It is therefore a central goal of neuroscience to understand the molecular basis for neuronal shape. Neuronal pathfinding during development is one major determinant of neuronal shape: growing nerve axons and dendrites must navigate, branch, and locate targets in response to extracellular cue molecules within the embryo. The leading tips of growing nerve processes, structures known as growth cones, contain especially high concentrations of the ubiquitous mechanochemical protein actin. Force generation involving this cytoskeletal molecule appears to be essential to the ability of growing nerve fibers to respond structurally to extracellular cues. New results from electronically enhanced light microscopy of living growth cones are helping to show how actin-based forces guide neurite growth and synapse formation.

CELL MOTILITY MECHANISMS ARE FUNDAMENTAL TO DEVELopment of the nervous system: they are expressed during neuronal and glial proliferation and migration, neurite growth, and the selection of pathways and synaptic partners. Later, functional plasticity of the mature nervous system may also involve motility and structural change. This article will focus on the mechanisms used by growing neurites to select pathways and synaptic partners: mechanisms based primarily on the mechanochemical protein actin. While the motility mechanisms of neurons are probably similar to those in other metazoan cells, major questions remain about even the simplest of actin-based motions (*1–4*). This article will provide an overview of recent progress on the mechanisms of actin-based neuronal motility. In the process, I shall illustrate how electronically enhanced light microscopy can be used to study the dynamic aspects of cell motility and neuronal development.

The enlarged terminal ending of a growing axon or dendrite is known as the growth cone. This structure exhibits striking locomotory motility (*5–8*). The abilities of the growth cone to crawl, to explore, and to exert force enable developing neurites to reach their proper targets (*8–11*).The growth cone also may be the site at which neurite elongation occurs (*12*). It is probably helpful, however, to distinguish between the motility involved in neurite guidance and the process of neurite elongation itself (*10*). While the two processes must interact, guidance and elongation may be distinguishable on the molecular level: guidance at the growth cone may be mainly the realm of actin, while elongation is more fundamentally dependent on microtubules, another cytoskeletal constituent, and their tubulin subunits. In this article, I will focus on actin-based motility mechanisms rather than on the neurite elongation process.

The precise and specific nature of synaptic connection bespeaks strong regulation and guidance of neuronal motility. This guidance probably reflects the responsiveness of growth cones to temporal and spatial patterns of extracellular cue molecules (*13, 14*). These molecules may be parts of the extracellular matrix, they may be on the surface of other cells, or they may be diffusible, like hormones or neurotransmitters. These extrinsic cue molecules are presumed to act by binding to specific receptors on the surface of the motile cell, where they may generate physical adhesive forces or act as regulatory signals, either directly or via intracellular second messengers. The exuberant motility characteristic of the growth cone (Fig. 1) allows it to explore relatively large areas of its environment as it migrates, often contacting and "tasting" many surfaces before choosing one for further migration or synapse formation (*9, 15*).

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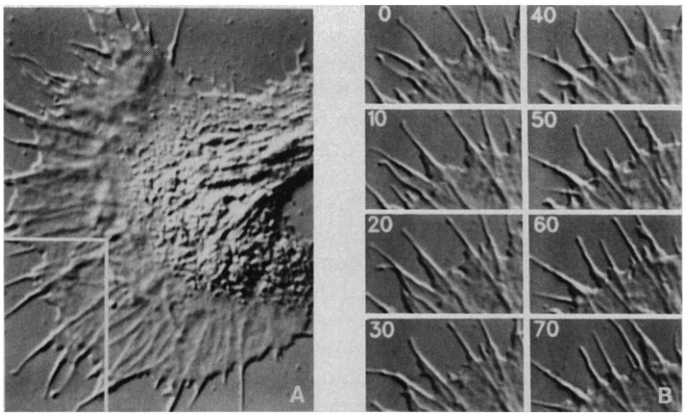

Fig. 1. Motility of the leading edge of an isolated growth cone in cell culture. (**A**) A digital video micrograph showing a single image of the entire growth cone. (**B**) A series of images samples at 10-s intervals from the area indicated by the box drawn in (A). Successive frames show different phases of the recurrent protrusion and retraction of the leading margins characteristic of active growth cones and many other motile cells. These images were provided by P. Forscher.

Mechanochemistry of Actin

Cellular structure, force generation, and motility depend upon a set of diverse cytoplasmic filaments called the cytoskeleton (*4, 16*). The cytoskeleton comprises three distinct sets of polymeric filaments: actin filaments, intermediate filaments, and microtubules. Actin filaments (approximately 7 nm in diameter, also known as microfilaments or F-actin) are helical polymers of actin (also known as G-actin), a 42-kD globular protein consisting of a single polypeptide chain of 375 amino acids (*17, 18*). Actin filaments of the cytoskeleton have essentially the same composition as the thin filaments of the muscle cell contractile apparatus. Intermediate filaments (8 to 14 nm in diameter) vary significantly among cell types. In neurons, they are called neurofilaments and are composed of a triplet of different subunits of approximately 68, 160, and 200 kD (*19*). Microtubules (22 to 25 nm in diameter) are hollow-cored tubular polymers of a heterodimeric subunit composed of α and β tubulin (*20*). Each type of filament is associated with an array of specific accessory proteins that regulate assembly and function of the cytoskeleton. The cellular functions and identities of most of these accessory proteins are not yet known.

Each set of filaments has a characteristic spatial distribution within cells. Actin filaments are more concentrated in cortical or peripheral cytoplasm (*4, 21*) and the intermediate filaments and microtubules are usually more centrally disposed. All three types of filament are present in axons and dendrites, but intermediate filaments appear to be relatively rare in dendrites, and both intermediate filaments and microtubules are rare in terminal endings such as growth cones and presynaptic terminals. Long microtubules are abundant within the central cytoplasm of axons and have been identified as the "tracks" along which fast axonal transport processes carry membranous organelles of various sorts (*22*). Although many microtubules extend part of the way into growth cones, they are found only rarely in the highly motile regions of the growth cone periphery, the lamellipodium and the filopodia (*23*). Figure 2 shows a schematic representation of the growth cone and its cytoskeleton, along with micrographs of the distribution of fluorescently stained actin filaments in a growth cone from the marine mollusk *Aplysia californica*. The staining for filamentous actin is most intense at the periphery of the growth cone, which focuses attention on the role of actin in peripheral motility. Electron microscopic information about the disposition of actin filaments in growth cones has been limited by the chemical fixation, extraction, and staining methods needed for high-resolution observations: actin filament organization appears to be highly susceptible to disruption during conventional preparation procedures. New methods of cryogenic specimen preparation (*21*) may provide important new information when applied to growth cones.

The ability of actin to polymerize and depolymerize in a dynamic fashion is fundamental to cell motility. Although purified actin monomers in vitro can reversibly self-assemble to form polymeric actin filaments, actin in cells probably does not behave as a simple equilibrium polymer. This is because the actin molecule possesses an adenosine triphosphate (ATP) binding site that governs polymerization behavior and can hydrolyse ATP. Under intracellular conditions, the actin polymerization-depolymerization cycle involves delayed hydrolysis of ATP (*17, 24, 25*) (Fig. 3). This ATPase activity makes it thermodynamically possible for cyclic polymerization and depolymerization of actin to proceed in a metabolically-driven steady state and to do physical work (*26*).

Though actin can polymerize and depolymerize in purified form, cells contain numerous actin-binding proteins (ABPs) that strongly influence these processes (*4, 17, 27*). These proteins can be divided into four classes; examples of each will probably be found in growth cones. Proteins that bind and sequester actin monomers can regulate polymerization by controlling the concentration of monomers available for polymerization. Perhaps the most important examples are the profilins, which are present in high concentration in many cells and may be regulated by products of ligand-dependent phospholipid metabolism (*28*). Other proteins bind to actin polymers and regulate their integrity by capping them against subunit loss or addition, severing them, or stabilizing them. Gelsolins are widely distributed members of this class that have a Ca^{2+}-dependent capping and severing action. Tropomyosin is another common protein that binds polymerized actin and appears to stabilize filaments. Still other ABPs mediate higher order assembly processes that cross-link actin filaments into bundles or networks or attach them to membranes. Fimbrin, spectrin, and vinculin are widely distributed members of this class. Synapsin I, a well-studied neuronal phosphoprotein, also binds both actin filaments and membranes (*29*). Certain microtubule-associated proteins (MAPs) (*30*) may bind to actin as well as microtubules. MAPs may thus be critical to the actin-microtubule interaction which occur in growth cones (*14, 31*).

Many other examples of ABPs in each of these three classes have been identified in different cell types and may yet be identified in growth cones. The localization and dynamics of actin in cells are probably regulated primarily by the localization and regulatory actions of ABPs in these first three classes. This notion has broad implications for the mechanics of neuronal motility and synapse formation, because many ABPs appear to be targets for extrinsic regulatory action and are modulated by Ca^{2+}, phospholipid metabolites, or protein kinases. One major caveat is that the functions of most ABPs are known only from in vitro studies, and a great deal more work is needed to establish their cellular roles.

The fourth class of ABPs are the myosins, which were originally discovered, with actin, as components of the force-generating apparatus of muscle cells. Actin filaments and myosin probably interact in nonmuscle cells to produce sliding forces analogous to those generated in muscle cell sarcomeres (*32*). This interaction is also driven by an ATPase activity, the actin-myosin ATPase, but this ATPase activity is distinct from the actin ATPase described above. The amount of myosin in nonmuscle cells is very small in comparison to muscle cells, and nonmuscle myosin is generally not found in the form of the conspicuous thick filaments characteristic of muscle.

Actin-myosin interactions generate polarized forces: myosin exerts a force on actin filaments that moves it from the pointed to the barbed end of an actin filament (Fig. 3D). Cellular effects of this sliding interaction depend entirely on the location and anchoring of actin filaments and myosin within the cell. Unfortunately, details of myosin organization and function in nonmuscle cells remain obscure, due in part to the lack of specific pharmacological agents to modify myosin function. New molecular genetic approaches to manipulation of cellular myosin content should help to elucidate the role of this molecule (*33*). It may also be anticipated that a recently discovered class of nonpolymerizing "small myosins," related to *Acanthamoeba* myosin I (*34*), will grow in importance as their significance in nonmuscle motility is elucidated.

Although purified actin and myosin can reconstitute ATPase activity, their interaction in cells is subject to many different regulatory influences. Actin-myosin regulation is best characterized in muscle cells, where regulatory schemes based on protein kinases or troponin and tropomyosin are well studied (*35*). Similar mechanisms probably operate in nonmuscle cells (*32*).

Protrusion and Retraction of the Growth Cone Periphery

The advance of migrating cells and growth cone lamellae has often been described as involving an interplay of protrusion and retraction at the leading edge (*1, 2*) (Fig. 1). The necessity of protrusive movement in order to advance a leading edge seems self-evident. The protrusions of the leading edge may take one of two seemingly distinct forms (see Figs. 1 and 2) (*36*). One is the lamellipodium (also called the lamella), where the cell margin spreads over the substrate in a flattened form, and the other is the filopodium, where a spike-like protrusion arises from the cell margin. Both lamellipodia and filopodia are rich in filamentous actin (Fig. 2). The bundled and radially aligned actin of filopodia can extend through a lamellipodial margin of less orderly actin filaments to the microtubule-rich central cytoplasmic domain (see also *23, 27, 38*). The basic mechanisms of lamellipodial and filopodial protrusion probably have a great deal in common. The two mechanisms will be discussed here without much effort to discriminate between them. Later in the discussion, an attempt will be made to justify such treatment.

The need for a retraction phase of leading edge advance may be less than obvious. Nonetheless, although the extent of retraction may vary with substrate type or other conditions, periodic retraction of leading edges is quite commonly observed, even in cases where the net advance of the migrating cell or growth cone is very brisk. Retraction may be intimately related to the process of advance, just as a leg is swung rearward in the power step of walking. Alternatively, retraction might be related to other leading edge functions such as the uptake of trophic substances or the testing of adhesive bonds. Another phenomenon that is probably closely related to leading edge retraction is ruffling, a wave-like elevation or thickening of the leading lamella. A striking feature of ruffles observed in the living state is their tendency to move across the lamella in a rearward or centripetal direction. The retrograde flow of ruffles on growth cones of cultured *Aplysia* bag cell neurons has an apparent velocity of about 0.1 μm/s (Fig. 4). Whatever the ultimate significance of retraction and ruffling, any complete model of locomotory motility must be able to explain these processes.

Observations on a wide range of cell types have led to two partially conflicting hypotheses about the leading edge motility of metazoan cells. These can be called the cortical actin flow hypothesis and the membrane flow hypothesis. Only the first hypothesis places actin in a central role, while the second, in its original and purest form, might explain motility with no reference whatever to actin or the cytoskeleton (*39*). According to the cortical actin flow hypothesis (*40, 41*), actin filaments are assembled preferentially at the leading edge of migrating cells, flow in the rearward or retrograde direction, and then depolymerize to allow diffusional recycling of actin monomers to the leading edge. Leading edge actin assembly would somehow generate protrusion, while the rearward flow gives rise to retraction and ruffling. The cortical actin flow hypothesis is strongly supported by the consistent presence of actin filaments where there is peripheral cytoplasmic motility and by experiments using actin-specific toxins called cytochalasins. Such experiments will be discussed at length below.

According to the membrane flow hypothesis, membrane lipid or protein components in cytoplasmic vesicles are inserted into the leading edges of motile cells by exocytosis, flow rearward over the lamellar surface and are then reinternalized by endocytosis for recycling through the cytoplasm to the leading edge (*39*). One observation suggestive of membrane flow is the rearward flow of

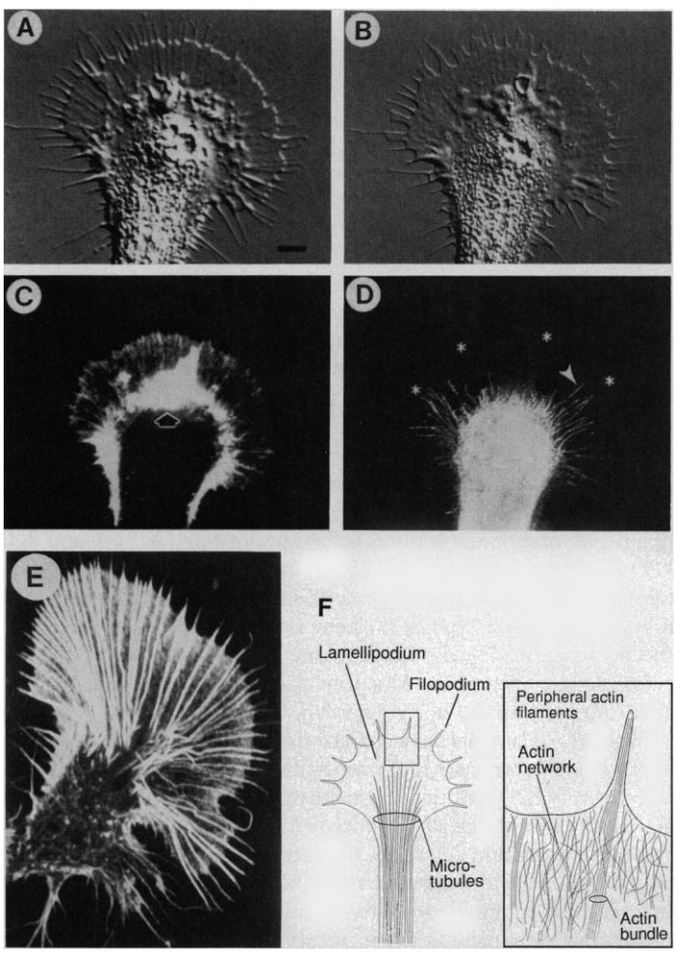

Fig. 2. Organization of the major cytoskeletal components of an *Aplysia* bag cell growth cone. (**A**) A living growth cone. (**B**) The same growth cone as in (A) after fixation with 4% formaldehyde. (**C**) Digitally enhanced conventional fluorescence micrograph of rhodamine-phalloidin bound to filamentous actin in the same growth cone. (**D**) Indirect fluorescein immunofluorescence of tubulin in the same growth cone, showing distribution of microtubules. (**E**) Higher magnification view of rhodamine-phalloidin stained filamentous actin in a different growth cone, obtained using a confocal scanning laser microscope (Bio-Rad MRC 540). (**F**) Idealized distributions of microtubules and actin filaments, and some basic terminology for the growth cone. Magnified view within box indicates that actin filaments may be organized in bundles, often aligned with filopodia, or in less orderly networks. [(A to D) reproduced from (*31*), by copyright permission of the Rockefeller University Press]

small particles dropped on or picked up by leading edges of migrating cells. Such transport is also consistent with an actin flow hypothesis, however, because actin filaments can attach to cytoplasmic domains of integral membrane proteins that are bound to particles on their extracellular domains (*40, 41*). Attempts to visualize the vesicles or other intracellular membranes necessary for exocytosis at growth cone leading edge have met with varied results (*42*). Cases where protrusion occurs without an intracellular membrane source may challenge the viability of the membrane flow hypothesis.

The cortical actin flow hypothesis and the membrane flow hypothesis need not be considered mutually exclusive alternatives. There is ample evidence that both actin and membranes do flow in motile cells (*43*). One flow or the other may be both necessary and sufficient to produce motility, but this has certainly not been demonstrated. Even if such a demonstration had been accomplished, this would not rule out the possibilities that rearward flows of both actin and bulk membrane are important aspects of motility or its guidance.

Actions of Cytochalasins on Growth Cones

The most direct evidence for involvement of actin filaments in growth cone motility comes from experiments with cytochalasins, a class of fungal toxins that inhibit actin polymerization (*44*); these agents eliminate most of the filamentous actin from growth cones and severely derange and attenuate neurite outgrowth (*11, 45*). With electronically enhanced light microscopy applied to the large growth cones of *Aplysia* bag cell neurons, an especially striking temporal and spatial pattern of cytochalasin B (CB) action was observed by Forscher (*31, 46*) (Fig. 5). CB causes a rapid cessation of protrusive activity followed by recession of cytoplasmic actin networks away from the leading edge of the membrane envelope. Recession occurs at an initial rate of about 0.1 μm/s (about the same as ruffling waves) and continues until almost all actin filaments are eliminated. Upon removal of CB, actin filaments quickly reappear, beginning at the leading edge. Protrusion resumes as normal-appearing actin networks centripetally invade the membrane envelope at the same velocity (*47*).

The patterns of actin filament recession and regeneration in CB experiments are consistent with three predictions of the cortical actin flow hypothesis. First, elimination of actin filaments by CB within about 2 min confirms the high rate of monomer-polymer turnover presupposed by this hypothesis. It would appear that a given actin subunit remains part of a particular filament for only about 2 min (*48*). Second, the localization of the initial CB-induced loss of actin network at the leading edge, and the similar localization of the initial reappearance of actin network upon CB removal, suggests that polymerization of actin normally occurs most rapidly at the leading edge (see also *49*). Third, the similarities of the observed velocities for retrograde ruffling waves, actin network recession on CB onset, and actin reinvasion on CB removal (all about 0.1 μm/s) are as predicted by the actin flow hypothesis. The prompt arrest of protrusive activity as seen in Fig. 5, combined with the evidence for locally enhanced polymerization at the leading edge, suggest that leading edge protrusion may be a direct consequence of actin polymerization. Finally, although these experiments do not address the validity of the membrane flow hypothesis directly, they damage its appeal. All appearance of protrusion, retraction, or ruffling disappears after CB has eliminated actin filaments, so these phenomena cannot be used to argue for membrane flow without adding the complication that membrane flow depends on the presence of intact actin filaments.

Evidence for Myosin Participation

Although results like those of Fig. 5 strongly support the existence of an actin polymerization cycle at the leading edge, such a cycles is not by itself sufficient to explain those results. The retrograde flow of the lamellar actin matrix continues during the

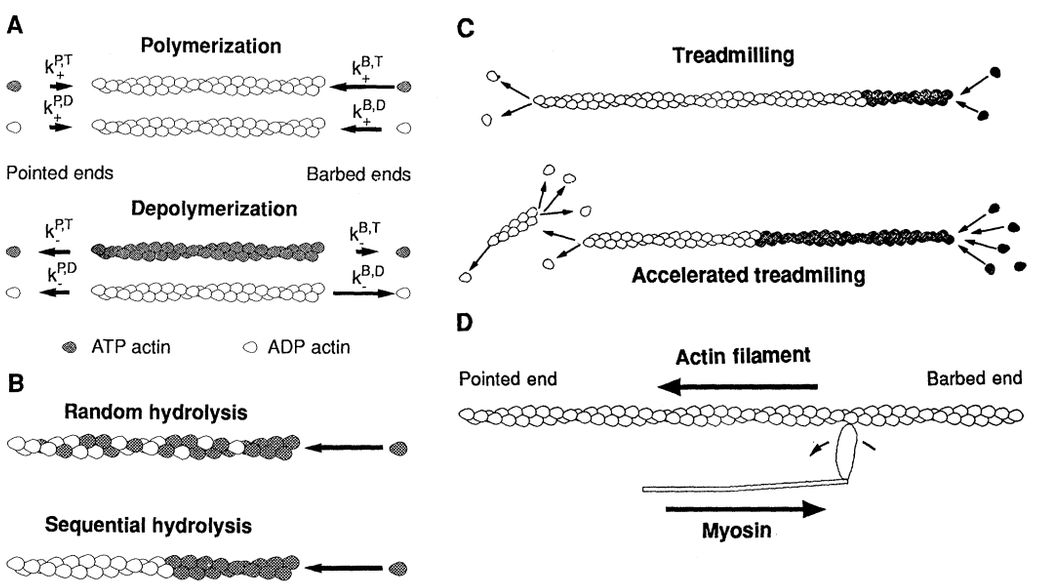

Fig. 3. Mechanochemical models for cytoskeletal actin dynamics. Pear-shaped symbols represent actin monomers; helical arrays of these symbols represent the polymeric actin filament. (**A**) Polymerization and depolymerization of actin filaments, and the influence of nucleotide species (ATP, filled actin symbol; ADP, open actin symbol) on polymerization and depolymerization reactions. The relative rates of the various possible reactions are indicated by the relative lengths of reaction arrows (*17, 25, 60*). Monomeric actin readily exchanges ADP for ATP under cytoplasmic conditions. The processes indicated are subject to modification by actin-binding proteins. (**B**) Release of inorganic phosphate (P_i) from ATP hydrolysis occurs in actin filaments with a delay on the order of a minute or two after ATP subunit addition (*17, 24, 25*). As indicated in (A), ATP hydrolysis may have significant control over polymer stability, and the associated free energy change may drive a steady-state polymerization cycle. Two possible schemes for the order of hydrolysis are indicated (*17, 25*). (**C**) Examples of "treadmilling": two possible types of actin polymerization cycles that might be driven in a steady state by hydrolysis of actin bound to ATP. Such processes may underlie the generation of protrusive force at leading edges and the ability of cells to sustain steady-state flows of cortical actin filaments. According to the relative rate constants indicated in (A), addition of ATP actin at the barbed ends is likely to occur rapidly. The "accelerated" treadmilling model involves severing of aged filaments and the rapid loss of ADP actin from barbed ends that may balance higher rates of barbed end filament growth. High rates of filament growth might result, for instance, from a high concentration of ATP actin near the leading edge membrane. (**D**) Diagram showing polarity of force generation in known actin-myosin interactions.

early stages of CB action (Fig. 5, B and C), even after a gap of filament-free cytoplasm appears and widens at the leading edge. If the flow were being "pushed" by polymerization at the leading edge, one would not expect flow to continue after blockage of polymerization, much less after the appearance of a filament-free gap is observed at the leading edge. In actuality, the velocity of apparent retrograde ruffling is little affected by CB until disruption of the lamellar actin matrix is nearly complete. These facts suggest that retrograde actin flow is normally driven by some force unrelated to polymerization.

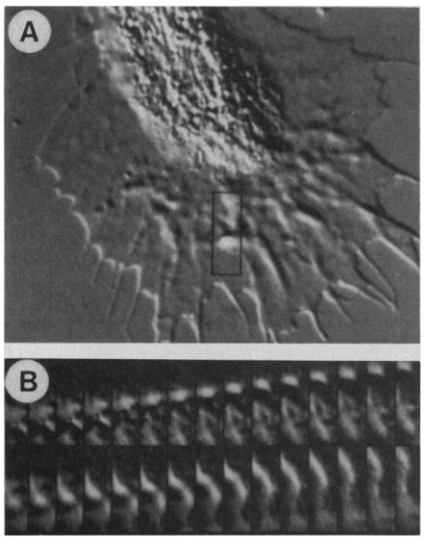

Fig. 4. Retrograde waves or rearward flow of ruffles on lamellipodium of an *Aplysia* bag cell neuron. (**A**) Digital video light micrograph of the entire growth cone. A rectangle is over the area where a time series was sampled. (**B**) Time series of images sampled at 2-s intervals over the rectangular area indicated in (A). Additional image processing was done to enhance contrast of ruffles. Rearward flow is evident from diagonal pattern across time series frames. These images were provided by P. Forscher.

Because myosin is present in growth cone lamellae (*23*), an actin-myosin interaction provides a plausible explanation for the observed retrograde actin translocation. Figure 6B, panel 1, schematizes a class of actin and myosin arrangements consistent with results like those illustrated in Fig. 5. This sketch suggests that an ATP-driven actin-myosin sliding force provides for a centripetal translation of actin filaments that can persist even after CB block of polymerization, as long as any filamentous actin remains to interact with myosin. Panels 2 through 5 in Fig. 6B show conditions corresponding to various stages in a CB experiment that would generate spatial patterns of actin filaments like those actually illustrated in Fig. 5. Unfortunately, there is little detailed information on the organization, attachment, or specific subtype of the myosin in growth cones, so the specific details of the actin-myosin interaction are not known. Figure 6C shows four possible arrangements of specific myosin subtypes that could correspond functionally to the situation diagramed in Fig. 6B. In two arrangements, myosin is distributed within a more or less random network of actin. The actin-myosin interaction here could lead to gel contraction and, if the gel were

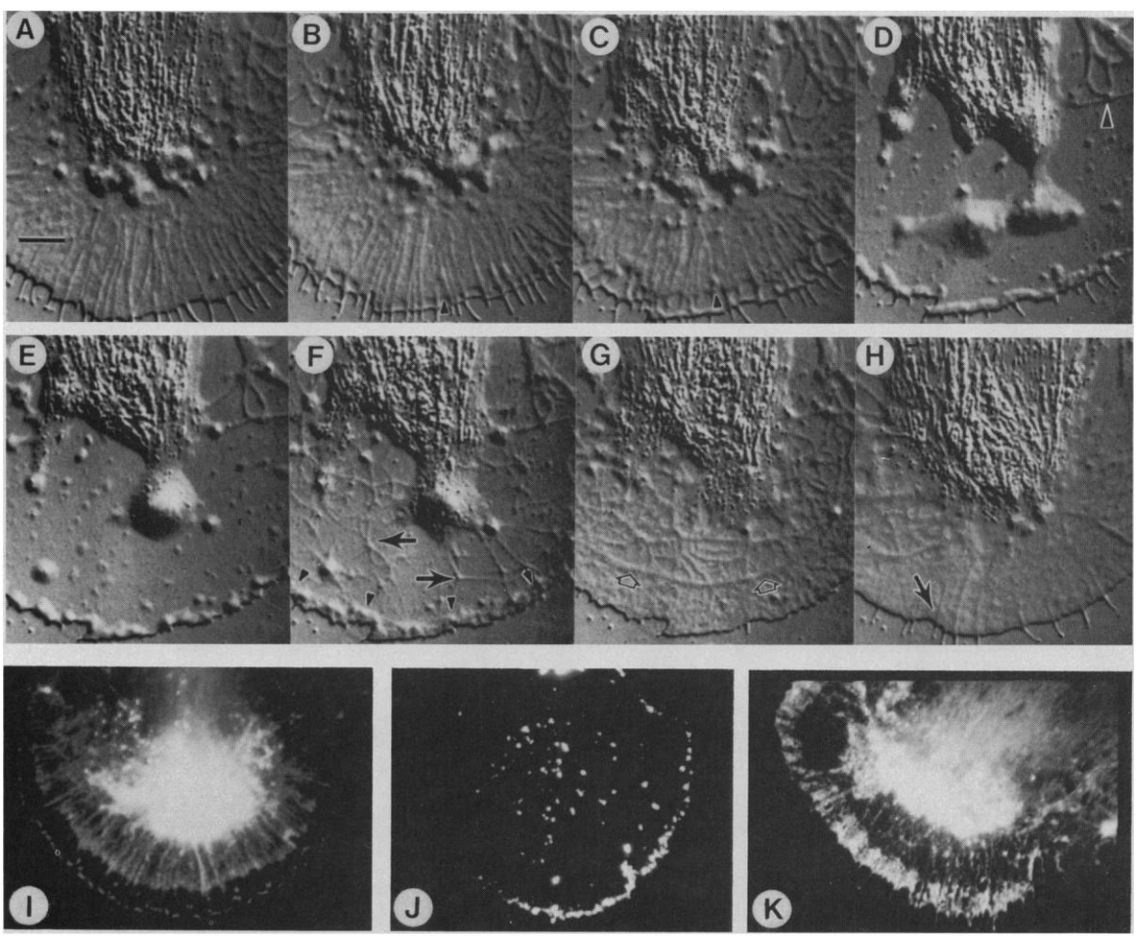

Fig. 5. Disappearance and reappearance of lamellar ruffling and actin filament network of an *Aplysia* bag cell growth cone in response to a brief application of 10 μM cytochalasin B (CB) [reproduced from (*31*), by copyright permission of the Rockefeller University Press]. (**A**) Control condition, Nomarski differential interference contrast (DIC) image of a living growth cone; (**B**) 30 s and (**C**) 60 s after CB application. Note gap at leading edge and recession of boundary indicated by arrow. The region central to this boundary exhibits relatively normal ruffling waves, while the more distal band is devoid of motility. (**D**) Three minutes and (**E**) 9 min after CB application. Lamellar motility has disappeared entirely. (**F**) Sixty seconds after removal of CB from perfusing solution, motility commences again within the lamella. The cytoplasmic matrix reappears as sparse cables throughout the lamella and as normal-appearing ruffling cytoplasmic matrix (arrowheads) spreading in from the leading edge. (**G**) Three minutes after removal of CB, normal-appearing ruffling matrix has spread centripetally throughout lamellar region. (**H**) Seventeen minutes after CB removal, the growth cone has a nearly normal appearance and ruffling motility. Rhodamine-phalloidin stains of growth cones fixed at various time point after CB treatment show that the matrix evident in the DIC images is equivalent to the actin filament network. (**I**) Early phase of CB action: fixation after 80 s in CB [timepoint corresponds to (C)]. (**J**) Late phase of CB action: fixation after 30 min in CB [timepoint corresponds to (E)]. (**K**) Early phase of recovery from CB action: fixation 65 s after removal of CB [timepoint corresponds to (F)].

anchored in the central region of the growth cone, retrograde flow. In the other two arrangements, myosin is postulated to be fixed more or less directly to the substrate over which the growth cone is crawling. Retrograde flow would then result from the interaction of myosin with actin filaments oriented with their barbed ends toward the periphery (see *14*).

Actin Polymerization and Protrusion

The scheme of Fig. 6B, panel 1, may explain protrusive force generation as well as rearward flow. The addition of subunits to the forward end of an actin filament butting against the cell surface could somehow push forward on the membrane (*26, 50*). There are arguments against this simple possibility, however (*51*). Nonetheless, a more general view of a spatially structured polymerization-depolymerization cycle may lead to a more acceptable (if less easily stated) model for protrusion.

Whenever the processes of polymerization and depolymerization are spatially displaced from one another, an entropic potential is created that could give rise to a protrusive force. This potential would reflect the tendency of newly formed actin filaments to escape to regions of lower concentration, away from the polymerization site. This potential would be expressed most readily as an expansive force acting in all directions, including the direction necessary to push forward the leading edge membrane. Though various processes might restrain or counterbalance such a force, in the steady state the corresponding stored energy must finally be released. For instance, if filaments are cross-linked as they are formed, the entropic force may be restrained as a gel pressure (*51*), but in a steady state cross-links must be broken as fast as they are formed. This would then lead to expression of a gel swelling force, and possibly leading edge protrusion, at the time and place of cross-link breakage (*52*). According to such a model, the energy input needed for protrusive work would be provided by the ATP hydrolysis associated with actin polymerization. Hill and Kirschner (*26*) have shown that the free energy associated with that ATP hydrolysis is adequate for such a mechanical role.

The existence of two seemingly distinct forms of growth cone protrusion, lamellipodia and filopodia, raises the question of how similar or different the underlying dynamics and mechanisms may be. Most of the considerations discussed above could conceivably apply to both. Filopodia and lamellipodia exhibit similar periodicities and velocities of protrusion and retraction, and similar rates of retraction upon CB treatment (see Fig. 3), suggesting that they are driven by a common mechanism. At one extreme, it seems possible

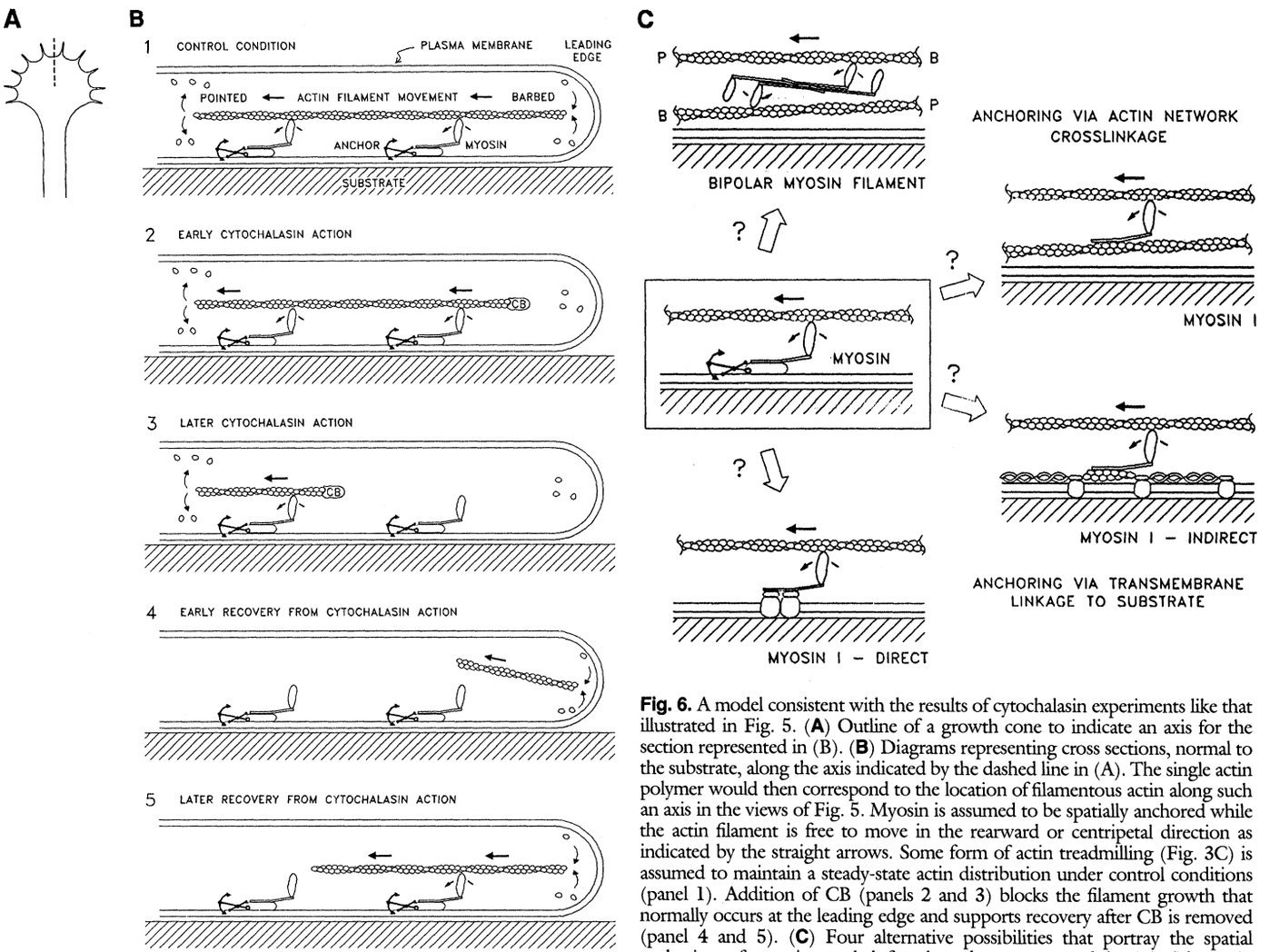

Fig. 6. A model consistent with the results of cytochalasin experiments like that illustrated in Fig. 5. (**A**) Outline of a growth cone to indicate an axis for the section represented in (B). (**B**) Diagrams representing cross sections, normal to the substrate, along the axis indicated by the dashed line in (A). The single actin polymer would then correspond to the location of filamentous actin along such an axis in the views of Fig. 5. Myosin is assumed to be spatially anchored while the actin filament is free to move in the rearward or centripetal direction as indicated by the straight arrows. Some form of actin treadmilling (Fig. 3C) is assumed to maintain a steady-state actin distribution under control conditions (panel 1). Addition of CB (panels 2 and 3) blocks the filament growth that normally occurs at the leading edge and supports recovery after CB is removed (panel 4 and 5). (**C**) Four alternative possibilities that portray the spatial anchoring of myosin needed for the scheme proposed in (B). The actin filaments indicated in the network cross-linkage models need not be rigidly aligned (*4*). Polarity of the additional actin filament (B, barbed end; P, pointed end) is indicated as necessary for the bipolar myosin diagram. Polarity of the movable actin filament in each case is as indicated in (B).

that filopodial actin dynamics may differ from those of lamellae only by the greater linear order and bundling in filopodia. A better understanding of this issue will require new information about the true order of actin filaments within the apparently random matrix of the lamella and also about the detailed structure and dynamics of the relevant actin-myosin interactions.

Regulation of Growth Cone Motility

Extrinsic cue molecules that bind to specific cell surface receptors are probably the major regulatory input to the actin-based mechanisms of growth cone and dendrite motility. At least three kinds of receptor binding effects are probably important. First, receptor binding may generate second messengers with global regulator influences on actin dynamics (*53*). Second, receptor binding may have a local organizing influence on the cytoskeleton (*54*) that involves receptor activation, aggregation, or localized second messenger action. Third, receptor binding to molecules on other cell surfaces or extracellular matrix may generate adhesive forces that constrain actin mechanically and steer or otherwise regulate growth cone motility (*14*). All of these actions must involve ABPs, but our specific information about such mechanisms is at present rudimentary.

Soluble factors and neurotransmitters or their second messengers affect neurite growth in both invertebrate and vertebrate neurons (*55*). Effects of neurotransmitters on motility and structure are especially interesting because neural activity (and thus neurotransmitter liberation) can have profound but specific effects on neuronal structure and synaptic connection (*56*). Neurotransmitter sensitivity of growth cone behavior could provide one link between neuronal activity and synapse formation processes. In many cases, these effects probably involve second messengers acting via protein kinases on ABPs. Which ABPs, if any, are the critical targets in neurotransmitter effects on growth cones or dendrites remains to be determined.

Summary

A rudimentary model of the neuronal motility fundamental to synapse formation is beginning to emerge from molecular and physiological studies of actin dynamics. The locomotory motility of growth cones and many metazoan cells appears to result from two cyclic and metabolically driven cytomechanical processes: the actin polymerization cycle and actin-myosin interactions. An ATP-energized actin polymerization cycle may drive the protrusive phase of locomotory motility, while an ATP-energized actin-myosin interaction drives the retraction phase. The interplay of these two processes permits axonal growth cones to crawl over substrates, to explore embryonic spaces, to form and mechanically test adhesive contacts, to exert tensile forces on the growing axon and prospective target cells, and perhaps to initiate uptake of trophic substances.

Similar processes may underlie an ability of dendrites to participate actively in establishing the specific cellular contacts necessary for the initiation of synapse formation. Dendrites are now recognized as more active and motile partners in synapse formation than might have been supposed (*57*). In some cases at least, dendrites exhibit abundant transient protrusions at the times during development or regeneration when they are approached by the "right" growth cones, and these filopodial protrusions may help to initiate the productive synaptic contact. Protrusion of dendritic filopodia may also be triggered by neurotransmitters (*58*).

Dendritic filopodia are rich in actin and their protrusion during synapse formation may reflect actin-based processes similar to those operating in growth cones. Changes in dendritic structure are also implicated in synaptic plasticity within mature nervous systems, and again actin may be fundamental to these changes (*56*).

Our understanding of locomotory motility must still be considered tentative at best. Outstanding unresolved questions concern the regulation of actin polymerization and the involvement of myosin. First, even if one assumes that the cortical actin flow hypothesis provides an accurate and relevant outline for locomotory motility, one must still ask why actin should polymerize mainly at the leading edge and what governs and subsequent the subsequent depolymerization process. Closely related questions can be asked about the effects of extrinsic cue molecules on polymerization and other actin functions. To all of these questions, it seems most likely that the answers will involve actin-binding proteins, their subcellular localization, and their mechanical or signal transduction linkages to receptors that are integral to the plasmalemma (*59*). Second, while the availability of specific pharmacological probes has placed the involvement of actin in motility on fairly solid ground, the evidence for myosin involvement is still circumstantial. Much more definitive tests of myosin's participation are needed and could still lead to major revisions of our ideas about motility. Further understanding of the classification and distribution of myosin subtypes will perhaps be the major key to a better understanding of cellular locomotion. Finally, there are also numerous questions about the flow of lipid and protein membrane components that may accompany the flow of actin. Given the present high level of interest in these problems and the power of modern molecular and physiological methods, I anticipate that answers to many of these questions will be available soon.

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47. In these experiments, elimination of lamellipodial actin by CB was associated with a fairly rapid distal extension of microtubule ends. Upon removal of CB and the reappearance of actin filaments, these microtubule endings retreated to their former central locations. This phenomenon may reflect regulation of microtubule elongation by an interaction with the peripheral actin matrix, as suggested from a variety of different observations [H. C. Joshi, D. Chu, R. E. Buxbaum, S. R. Heidemann, *J. Cell Biol.* **101**, 697 (1985); P. C. Letourneau, T. A. Shattuck, A. H. Ressler, *Cell Motility* **8**, 193 (1987)].
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61. I am grateful to P. Forscher for many helpful discussions and for providing much of the published and unpublished data reviewed here and to J. Buchanan, M. Cooper and C. F. Stevens for additional helpful discussions. I also thank G. Oster and M. Sheetz for extensive comments on an earlier version of the manuscript.

Cellular and Molecular Mechanisms of Drug Dependence

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The molecular and cellular actions of three classes of abused drugs—opiates, psychostimulants, and ethanol—are reviewed in the context of behavioral studies of drug dependence. The immediate effects of drugs are compared to those observed after long-term exposure. A neurobiological basis for drug dependence is proposed from the linkage between the cellular and behavioral effects of these drugs.

SUBSTANTIAL PROGRESS HAS BEEN MADE IN ANALYZING THE molecular and cellular actions of three major types of abused drugs: opiates, such as heroin and morphine; psychostimulants, such as cocaine and amphetamine; and alcoholic beverages (ethanol). The growing effects of drug addiction on society include increased criminal behavior as well as the direct consequences of drugs on health and their associated costs (*1*). Although our understanding of the biology of drug addiction is improving, no effective preventative strategies have been attained. Attention and resources have been focused instead on treatment after addiction. To generate obsessive drug-seeking and drug-taking behavior, an addictive drug must act on the cells and molecules of the nervous system. However, the sites and mechanisms that participate in these effects have not been well resolved, and the basis for individual variation in addictive liability is unknown.

In this article we describe our attempts to determine whether the molecular, cellular, and behavioral data on acute and chronic effects of addictive drugs form an internally consistent sequence of events in which molecular events generate cellular effects that in turn link to behavioral phenomena to explain the common features of drug dependence (*2*). We discuss the basic phenomenon of drug dependence and some theories of addiction and survey recent progress in studies of the pharmacological characterization of the three proto-

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