An emerging link between cytoskeletal dynamics and cell adhesion molecules in growth cone guidance

Daniel M Suter* and Paul Forscher†

It has become increasingly evident that growth cone guidance depends on the concerted actions of cytoskeletal proteins, molecular motors and cell adhesion molecules. Recent studies suggest that modulation of coupling between extracellular substrates and intracellular cytoskeletal networks via cell surface receptors is an important mechanism for regulating directed neuronal growth.

Addresses
Department of Molecular, Cellular and Developmental Biology, Yale University, PO Box 208103, New Haven, Connecticut 06520-8103, USA
*e-mail: daniel.suter@yale.edu
†e-mail: paul.forscher@yale.edu

Current Opinion in Neurobiology 1998, **8**:106–116

http://biomednet.com/elecref/0959438800800106

Abbreviations

ADF	actin depolymerizing factor
apCAM	*Aplysia* CAM
Arp	actin-related protein
BDM	2,3-butanedione-2-monoxime
CALI	chromophore-assisted laser inactivation
CAM	cell adhesion molecule
EGF	epidermal growth factor
F-actin	filamentous actin
FGF	fibroblast growth factor
Ig	immunoglobulin
Ig CAM	immunoglobulin superfamily CAM
IP_3	inositol trisphosphate
MAP	microtubule-associated protein
NCAM	neural CAM
NEM	*N*-ethyl maleimide
N-WASP	neural homologue of WASP
PIP_2	phosphatidyl inositol biphosphate
PLCγ	phospholipase Cγ
SH	Src homology domain
VASP	vasodilator-stimulated phosphoprotein
WASP	Wiskott–Aldrich syndrome protein

Introduction

The neuronal growth cone is a highly motile structure at the tip of growing axons. It may be viewed as a sophisticated signal transduction device, capable of recognizing extracellular guidance signals and translating them into directed neurite growth. Over the past fifteen years, an increasing number of guidance molecules have been identified that can be categorized as either short- or long-range cues that are either attractive or repulsive [1,2•]. The integration of these four fundamental guidance properties results in classic forms of growth cone behavior such as advance, turning, withdrawal, and target recognition.

Significant cytoskeletal rearrangements occur during growth cone guidance [3]. The two major cytoskeletal components of neuronal growth cones are filamentous actin (F-actin), which is located predominantly in the peripheral cytoplasmic domain, and microtubules, which are distributed in the central cytoplasmic domain (Figure 1a). Although growth cones vary considerably in their morphology depending on the growth substrate [4], neuronal cell type and age, this relatively distinct spatial segregation of actin filaments and microtubules has been observed in most growth cones studied to date. Our understanding of the cytoskeletal basis of growth cone motility has increased significantly in recent years and, in a similar vein, the morphogenic effects of neuronal guidance and signaling molecules have been described in great detail. However, how guidance cue information is transduced into the dynamic changes in cytoskeletal structure that underlie guidance responses has remained elusive. This review focuses on studies that begin to address this gap in our knowledge of growth cone motility and guidance.

Actomyosin-based growth cone motility

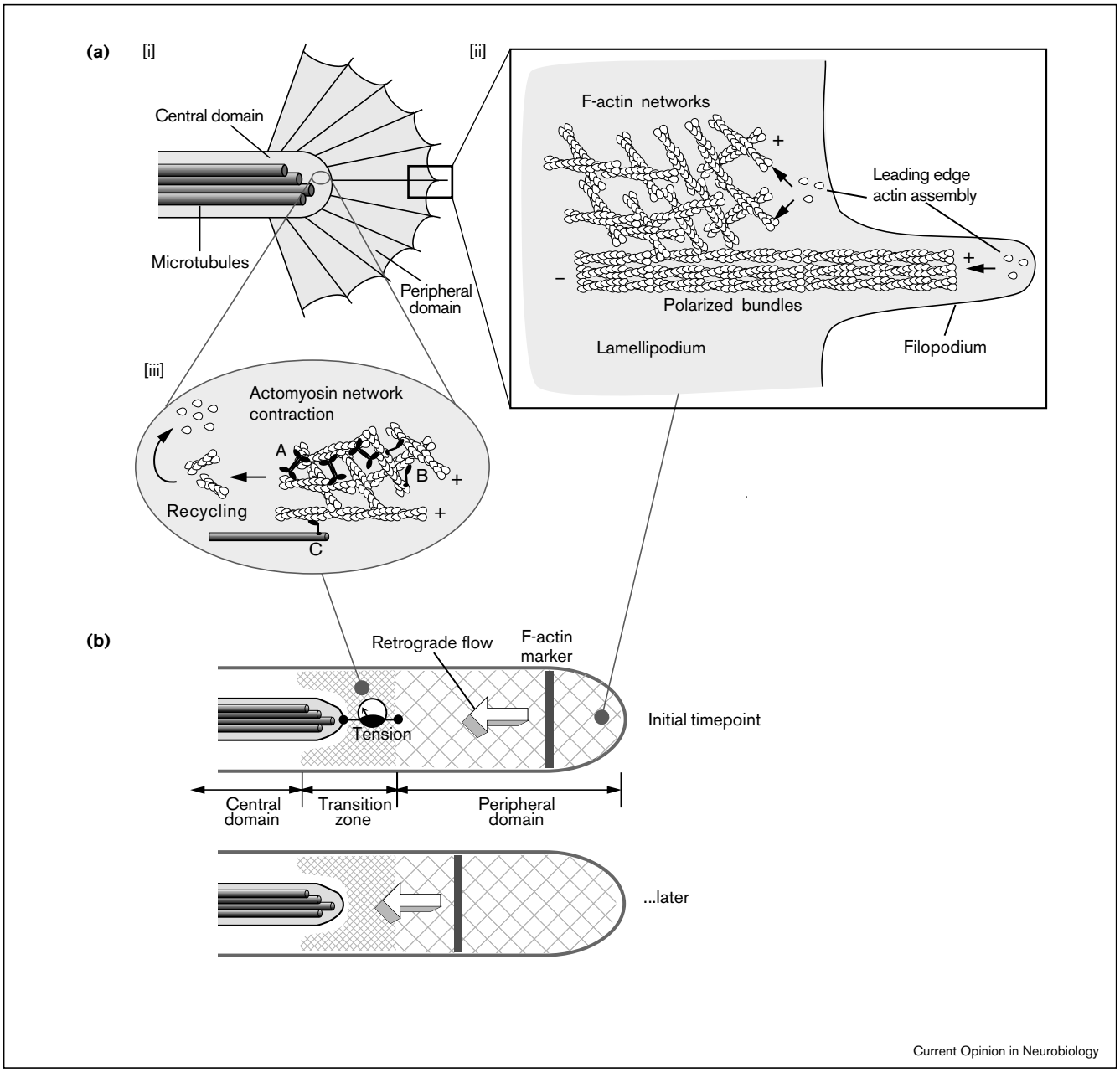
Ultrastructural studies reveal at least two distinct F-actin populations in the peripheral domain of growth cones: crosslinked networks in lamellipodial domains and bundles of parallel actin filament arrays that often span the width of the lamellipodia and extend into filopodia (Figure 1a) [5]. The latter population is highly oriented, with about 90% of the filaments displaying plus (barbed)-end distal polarity.

Motility in the growth cone peripheral domain is based on actomyosin and is characterized by three different processes: first, assembly of filaments at the leading edge; second, constant retrograde flow of F-actin networks; and third, proximal recycling of F-actin in a transition zone between the peripheral and central cytoplasmic domains in which microtubules and actin filaments overlap (Figure 1b) [6,7]. The above three kinetic processes have been observed in the lamellipodia of most motile cells, and regulation of each process could affect the rate of cell movement, as considered below [8•].

Assembly at the leading edge

F-actin assembly at the leading edge (Figure 1a[ii]) probably results from a combination of nucleation, polymerization, and annealing of short filaments. These processes are thought to be regulated by monomer-binding proteins (such as profilin and thymosin β4 [9]), barbed-end capping proteins, and proteins that regulate polymerization, such as the actin-related protein 2/3 (Arp2/3) complex [10••], vasodilator-stimulated phosphoprotein (VASP) [11], and Mena [12••]. It is interesting that many of these

Figure 1



Cytoskeletal organization and actin dynamics in growth cones. **(a)** Distribution of the two major cytoskeletal components in neurites and growth cones. [i] Microtubules are localized in the neurite and the central domain of the growth cone, whereas actin filaments are distributed in the peripheral domain. [ii] Blowup of [i] showing the organization of actin filaments in more detail [5]. Filopodia contain bundles of filaments with their plus (barbed) ends oriented towards the leading edge. These bundles can span the whole width of the lamellipodium. A second population of actin filaments form less polarized networks in lamellipodia. Plus (barbed)-end assembly occurs at the leading edge and at the tips of filopodia and is probably regulated by monomer-binding proteins (such as profilin) and by nucleation factors (such as the Arp2/3 complex). **(b)** Cross section of a growth cone demonstrating the dynamic processes involved in actin-based growth cone motility. Retrograde F-actin flow is indicated by a marker (e.g. flow-coupled bead). (a)[iii] Retrograde translocation of actin filaments is driven by myosin motors that may be in the transition zone. Labels A–C depict possible actomyosin combinations for tension generation: double-headed myosin II (A) and single-headed myosin I (B and C) subtypes are shown. The tension meter indicates low tension if actin networks are not stabilized by substrate interactions (compare with Figure 2). (a)[iii] Filament recycling occurs by action of putative severing proteins in the transition zone (such as gelsolin) and/or factors such as ADF/cofilin.

proteins have been implicated in the actin-based motility of intracellular pathogens such as *Listeria monocytogenes* [10••,11,12••] and *Shigella flexneri* [13]. These bacteria commandeer the host cell's actin assembly machinery as a means of intracellular propulsion and cell-to-cell spread, generating actin-tail-like structures behind them as they

move. It is important to note that similar actin structures, described as 'inductopodia', have been observed in *Aplysia* growth cones when either polycationic beads [14] or beads coated with antibodies to the *Aplysia* cell adhesion molecule apCAM [15•] were placed onto the lamellipodia. Such beads move on the growth cone surface without any preferential orientation, by a process driven by actin assembly and independent of retrograde F-actin flow [14].

Evidence suggests that formation of an F-actin tail and growth of a filopodium and/or lamellipodium appear to involve similar molecular scenarios. For example, the Arp2/3 complex was found to be sufficient for actin polymerization in *Listeria* motility [10••]; in addition, it was localized to lamellipodia of stationary and migrating fibroblasts, suggesting a potential role in coordinating F-actin assembly at the leading edge [16]. Finally, Gertler *et al.* [12••] implicated Mena, a murine relative of *Drosophila* Enabled and VASP, in the regulation of microfilament dynamics. Both Mena and VASP are believed to recruit profilin via proline-rich domains to the surface of *Listeria* [11,12••] and thereby promote actin assembly [9]. In support of a connection between bacterial F-actin tail and lamellipodia formation, expression of a neuronal isoform of Mena in fibroblasts resulted in the formation of actin-rich protrusions [12••]. Thus, studying the motility of invasive bacteria has given us important clues regarding the molecular basis of normal actin-based motility processes. It will be of considerable interest to investigate potential roles for these actin-regulatory proteins in growth cones and other neuronal processes that involve changes in actin structure.

Retrograde flow

In growth cones as well as other motile cells, F-actin filaments and networks can translocate from the leading edge towards the central cytoplasmic domain by a process called retrograde F-actin flow (Figure 1b) [3,7,17]. Lin *et al.* [18••] have recently shown that retrograde F-actin flow in *Aplysia* bag cell growth cones is driven by the action of myosin motors (Figure 1a[iii],b). Myosin inhibition either by injection of *N*-ethyl maleimide (NEM)-inactivated myosin S1 fragments or by cell treatment with 2,3-butanedione-2-monoxime (BDM), an inhibitor of myosin ATPase, results in a slowing of retrograde F-actin flow accompanied by protrusive growth of filopodia. This growth can be blocked by inhibition of actin polymerization using cytochalasin B, showing that it is attributable to continued actin assembly in the presence of the myosin inhibitors. These results suggest that leading edge growth could be achieved by either increasing the rate of actin assembly or decreasing the rate of retrograde F-actin translocation (flow), and that actin assembly and translocation are independent molecular processes.

The myosin subtype(s) driving retrograde flow remain to be elucidated; potential candidates are conventional myosin II and the unconventional myosins I and V, because these motors have been localized in growth cones (discussed in a recent review by Hasson and Mooseker [19]). The specific localization of myosins driving retrograde F-actin flow in growth cones has not yet been determined. In principle, a conventional or unconventional myosin motor attached to actin filaments, to other cytoskeletal structures such as microtubules or to the membrane cytoskeleton could be involved (Figure 1a[iii] labels A–C). Although it is currently not clear to what extent different myosins contribute to growth cone motility, it is generally accepted that actomyosin networks are involved in the generation of tension between the growth cone and neurite shaft, and that tension is related to rates of growth [20] (Figure 1b), as discussed further below.

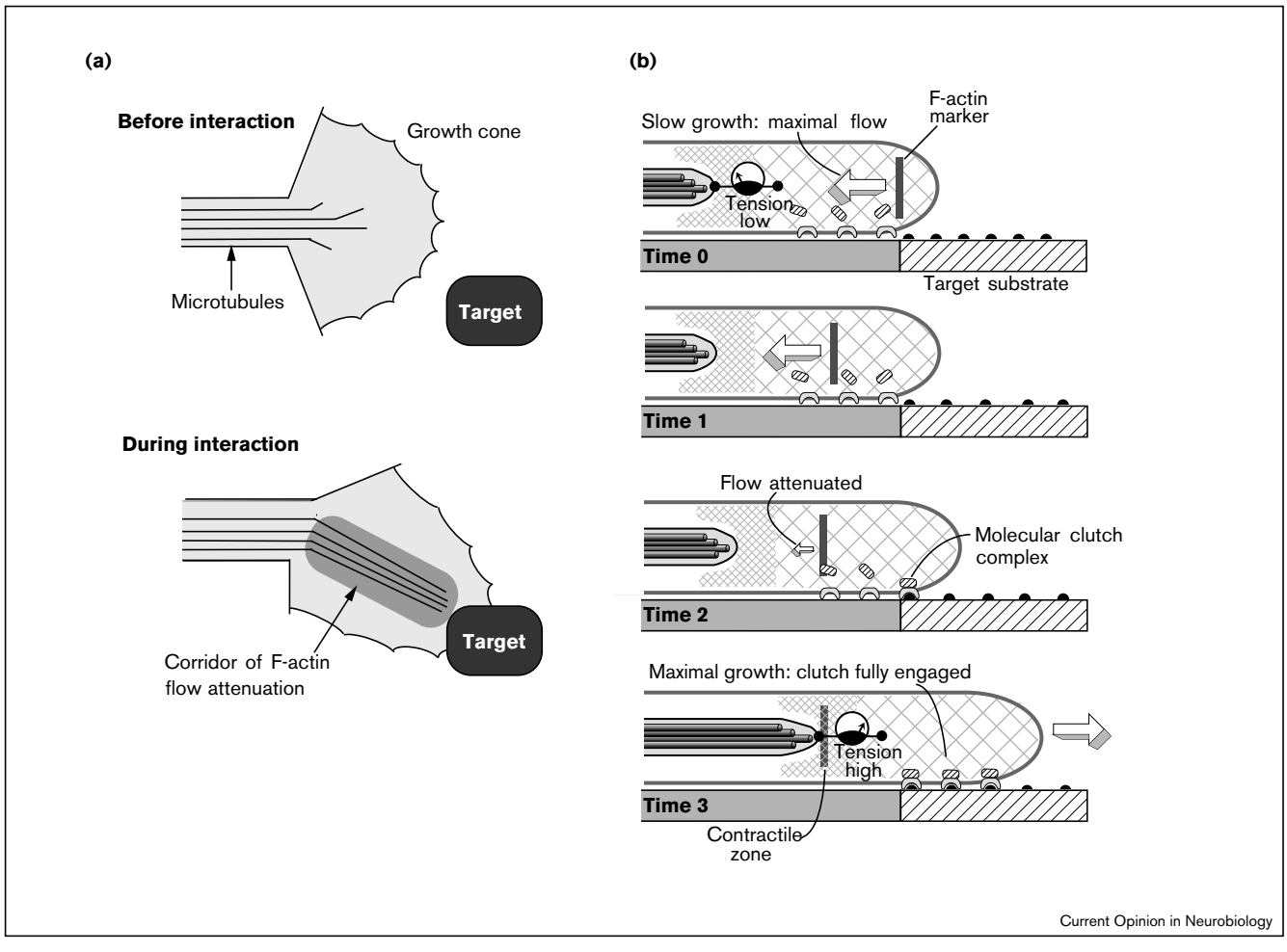
Proximal recycling

If a steady-state retrograde filament flux is to be maintained, actin filaments also have to be recycled in the transition zone (Figure 1a[iii]). This could be achieved by severing and/or depolymerization, mediated by filament-severing proteins such as gelsolin or by proteins of the ADF (actin depolymerizing factor)/cofilin family, respectively. It is of interest that growth cones obtained from *gelsolin* null mice are similar in size to cones from wild-type mice; in contrast, the number of filopodia per growth cone is higher in the *gelsolin* null mice, apparently as a result of delayed filopodial retraction [21]. Impaired F-actin severing at the base of filopodia could account for the delayed retraction rates observed. Cofilin is essential for actin filament turnover in yeast [22•], and ADF/cofilin (but not gelsolin) action is necessary for actin filament recycling in F-actin tail structures assembled by *Listeria* [23]. In addition to gelsolin and cofilin, N-WASP, a neural homologue of WASP (Wiskott–Aldrich syndrome protein), could contribute to actin recycling. This novel actin-depolymerizing and PIP_2-binding protein has recently been identified in brain and implicated in signal transduction between the epidermal growth factor (EGF) receptor and the actin cytoskeleton [24•]. Given the surprisingly normal phenotype of the *gelsolin* knockout mouse growth cone [21], it will be of interest to examine a potential role for ADF/cofilin and N-WASP in growth cone motility. Finally, as the rate of actin filament recycling may affect the location of the recycling (i.e. the transition zone) (Figure 1), recycling rates could also affect distal microtubule ends, as discussed below.

Growth cone guidance and the interactions between microtubules and actin filaments

Microtubules are the prominent cytoskeletal component in the neurite shaft and the central domain of the growth cone (Figure 1). Microtubules provide structural support and act as substrates for fast axonal transport of organelles. The mechanism by which microtubule arrays in the growing axon are established (i.e. the polymer versus the subunit transport model) is still controversial and has recently been discussed extensively elsewhere

Figure 2



Cytoskeletal rearrangements during interactions between the growth cone and its target. **(a)** The top view shows microtubule reorientation toward an attractive target after initial contact with filopodia and/or lamellipodia. Microtubule extension occurs specifically in a corridor of F-actin flow attenuation [35]. **(b)** Timecourse cross section demonstrates a mechanism for growth cone advance by substrate–cytoskeletal coupling [7,17]. Before target interaction there is maximal retrograde flow, no substrate coupling and little advance (Time 0 to Time 1). If the growth cone encounters a favorable target substrate (Time 2), molecular clutch complexes (including receptors and linkage proteins) form, linking the substrate to the actin cytoskeleton. Stabilization of peripheral actin leads to attenuation of retrograde flow and increased tension between central and peripheral domains (Time 3). Advance occurs by a combination of increased tension on the central domain and continued leading-edge actin assembly. See text for consideration of possible mechanisms of microtubule extension.

[25,26]. Microtubules entering the central growth cone domain splay out (Figure 2a) and continuously extend into and retract from the actin-rich peripheral domain [27]. This probably occurs by stochastic bouts of microtubule assembly and disassembly, a process referred to as dynamic instability, and/or by microtubule sliding, which is potentially mediated by the action of microtubule motors [7,20]. Pharmacological studies using low concentrations of vinblastine or nocodazole, which inhibit dynamic instability without causing marked microtubule loss, revealed that axonal advance depends on dynamic microtubule ends [28,29•]. The dynamic properties of microtubules may be regulated, at least in part, by microtubule-associated proteins (MAPs) [20].

A number of *in vivo* and *in vitro* studies in the past few years (reviewed in [3]) indicate that rapid rearrangement of the actin and microtubule cytoskeleton occurs when growth cones respond to attractive extracellular guidance cues. To summarize, during interactions between the growth-cone and its target, microtubules reorient and extend towards interaction sites, and F-actin tends to accumulate distal to microtubule ends [3] (Figure 2). More recently, similar cytoskeletal rearrangements have been observed in growth cones turning at substrate boundaries to avoid less favorable or inhibitory substrates [30–33].

Actin filaments appear to play a role in guiding microtubules during axonal steering [3,31,34]. Analysis of

retrograde F-actin flow during *Aplysia* growth cone–target interactions revealed an inverse relationship between rates of central domain extension and retrograde flow [35]. Microtubules were observed to preferentially extend in a corridor defining the target interaction axis in which retrograde flow was attenuated (Figure 2a). What could be the mechanism of such microtubule extension? We will discuss three possibilities; for two of them, development of tension in actomyosin networks, as a result of adhesive interactions with the underlying substrate, appears to be an essential ingredient [20].

One scenario involves myosin as a molecular linker between microtubules in the central domain and actin filaments in the transition zone (Figure 1a[iii] label C). Myosin interactions with polarized actin filaments would generate tension if distal regions of the peripheral domain were restrained by adhesions to the target substrate (Figure 2b). Such tension would tend to pull the central domain forward toward sites of target interactions. The microtubule-associated protein 2c (MAP2c) is a potential candidate for meditating actin–microtubule interactions [36]. A second possibility for generation of tension involves localized contraction of actomyosin filament networks in the transition zone (Figures 1a[iii] and 2b). A recent detailed analysis of the actin–myosin II system in fish keratocytes strongly supports such a dynamic network contraction model for cell body translocation [37••] — preliminary observations suggest a similar mechanism may be at work in growth cones as well (DM Suter, P Forscher, unpublished observations).

Microtubule extension might also be promoted by a mechanism not directly related to tension. Specifically, if actin filament recycling continues during target interactions that involve F-actin flow attenuation, forward displacement of the recycling zone should occur. This would result in progressive clearance of F-actin distal to microtubule ends. This effect in itself might promote microtubule advance, as it has been shown that microtubules tend to advance into growth cone regions in which F-actin has been depleted [6]. Furthermore, a recent elegant study using photoactivation of microtubule fluorescence in migrating newt lung cells has shown that microtubules protruding into F-actin-rich lamellipodia are actually in a steady state in which forward microtubule growth is matched by the rate of retrograde microtubule transport [38••]. Significantly, retrograde microtubule transport occurs as a result of association with actin filaments undergoing retrograde flow. It was also found that plus-end microtubule growth is inhibited by the presence of retrograde F-actin flow, suggesting that physiological processes that attenuate retrograde F-actin flow will tend to promote microtubule growth — exactly what was found during growth-cone–target interactions [35].

To summarize, when a growth cone turns towards an attractive substrate (Figure 2), cell surface receptors binding to the target substrate appear to make functional linkages with the underlying actin cytoskeleton, stabilizing it, and thereby attenuating retrograde flow. Stabilization (anchoring) of distal actin networks generates tension between central and peripheral domains, which promotes microtubule extension and also protrusive leading edge growth (Figure 2) [3,7,17]. Thus, growth cones may utilize cell adhesion molecules (CAMs) and associated cytoskeletal binding proteins, such as a 'molecular clutch', to mediate substrate–cytoskeletal coupling and thereby regulate rate and direction of growth (Figure 2b).

Recent studies from our laboratory on an immunoglobulin superfamily cell adhesion molecule (Ig CAM) support this molecular clutch model. As mentioned above, apCAM, the *Aplysia* homologue of vertebrate NCAM (neural CAM), associates in different ways with the actin cytoskeleton as a function of crosslinking density [15•] and can trigger actin assembly when crosslinked by unrestrained antibody-coated beads.

In very recent studies, we found that physical restraint of beads coated with either apCAM antibody or purified protein resulted in growth cone steering events, tension development and cytoskeletal reorganization [39•]. These steering events elicited by restrained beads were similar to those observed during native growth cone target interactions [34]. Importantly, once again retrograde F-actin flow was attenuated exclusively in the bead interaction axis where microtubule advance occurred (Figure 2).

Interactions between cell adhesion molecules and the cytoskeleton

How well do the properties of known CAMs fit into this substrate–cytoskeletal coupling model of growth cone motility? Over the past years, an increasing number of ligands and receptors belonging to different structural families have been implicated as axon guidance molecules [1,2•]. For all of the major cell adhesion receptor families (Ig superfamily CAMs, integrins and cadherins), proteins that could interact with both the cytoplasmic domain of the receptors and the actin cytoskeleton have been identified [40]. However, our understanding of the function of such linkage proteins in growth cone guidance is limited.

Probably the best characterized cell surface receptors with respect to signaling and cytoskeletal linkage are the integrins. The molecular composition of integrin/cytoskeletal complexes has been analyzed mostly in focal adhesion complexes, which are formed by many cells in culture in response to extracellular matrix proteins [41]. Such focal adhesions consist of aggregated integrin receptors that link extracellular matrix components to actin stress fibers and contain a large number of structural proteins (such as talin and vinculin), as well as signal transduction proteins (such focal adhesion kinase [FAK], RhoA and src) [41]. It is reasonable to assume that analogous structures

exist in growth cones. In support of this assumption, it was demonstrated that sensory growth cones plated on fibronectin but not laminin substrates form adhesions resembling focal contacts [42]. Additional distinct effects of these two substrata on the rate of growth cone advance have been found when laminin- or fibronectin-coated beads were presented to growth cones [43].

To address the function of the focal adhesion proteins talin and vinculin in growth cones, Sydor *et al.* [44] recently used microscale chromophore-assisted laser inactivation (CALI). Local inactivation of talin resulted in cessation of both filopodial extension and retraction, whereas inactivation of vinculin caused bending and buckling of filopodia, suggesting that these actin-associated proteins play distinct roles in filopodial motility. As CALI results in only short-term loss of protein function, overall neurite outgrowth rates were unaffected; however, reduced long-term rates of neurite extension were observed in a preceding study in which vinculin-deficient PC12 cells were generated by antisense methods [45].

It is well established that catenins link cadherins to the actin cytoskeleton [40]. β-catenin and γ-catenin bind to the cytoplasmic domain of cadherin, as well as to α-catenin, which, in turn, is thought to link the cadherin/catenin complex to the actin cytoskeleton. Recently, *in vivo* expression of a dominant-negative *N-cadherin* mutant lacking a large portion of the extracellular domain revealed that N-cadherin function is necessary for axonal and dendritic outgrowth from retinal ganglion cells [46]. Interestingly, further experiments reported in this paper suggest that cadherin/catenin interactions, which are essential for cell–cell adhesion, are not involved in neurite outgrowth.

The Ig CAMs are far less well characterized with respect to cytoskeletal interactions than the integrins or cadherins. Biochemical studies have implicated spectrin and ankyrin as possible actin-linkage molecules for NCAM and CAMs of the L1 family, respectively [47,48]. Interestingly, cell–cell interactions mediated by homophilic binding of the *Drosophila* L1 homologue neuroglian promotes recruitment of ankyrin to cell contact sites, suggesting that ankyrin is only associated with clustered L1-like CAMs [49•]. Very recently, Garver *et al.* [50••] demonstrated that tyrosine phosphorylation of the cytoplasmic tail of neurofascin at a site highly conserved among members of the L1 family abolished the binding of neurofascin to ankyrin and resulted in an increased lateral mobility of neurofascin. These results suggest that the association of L1-like CAMs with the cytoskeleton via ankyrin may be regulated through tyrosine phosphorylation in response to extracellular stimuli.

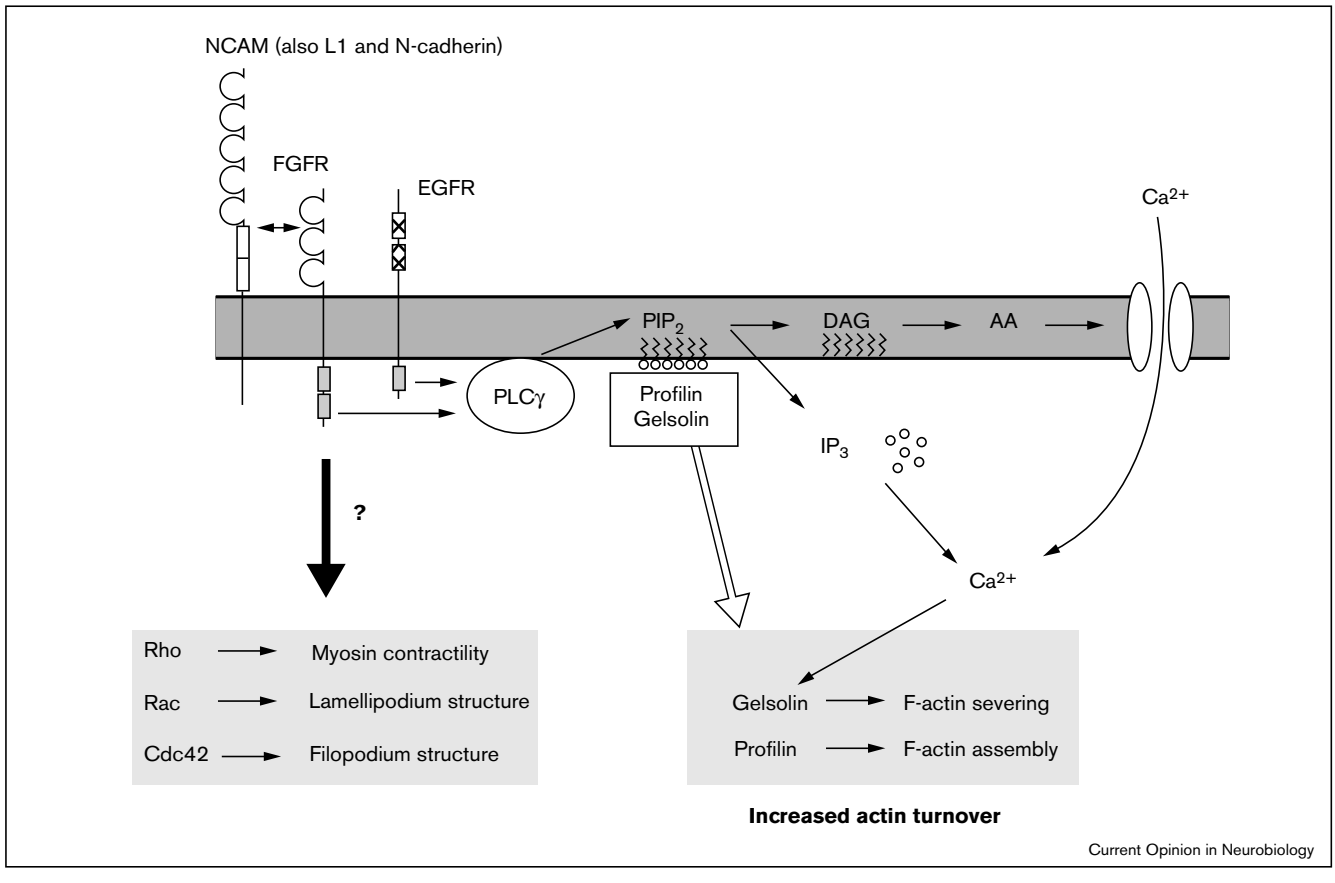
A key question with respect to growth cones relates to how linkages between CAMs and moving actin filament networks might be regulated. Is there a defined series of events that occurs in growth cones leading to substrate–cytoskeletal linkage? Can the strength or stiffness of these linkages be modulated? Some answers to these interesting questions are suggested by recent work, mostly in the integrin field. In an extensive study using fibroblasts, it was demonstrated that 32 cytoskeletal and signaling molecules can be classified into groups depending on their ability to form integrin transmembrane complexes in response to ligand occupancy, integrin aggregation, and tyrosine phosphorylation [51]. In addition, integrin receptor localization [52] and integrin ligand binding [53•] have been shown to influence the strength of receptor–cytoskeletal interactions.

Recently, Choquet *et al.* [54••] have demonstrated that cells are able to strengthen integrin–cytoskeletal linkages in response to extracellularly applied force. The authors restrained fibronectin and integrin antibody-coated beads on fibroblasts with a given force using a laser tweezer. In order to re-trap beads associated with retrograde F-actin flow, they had to apply at least three times the original restraining force. The authors concluded that cells are able to respond to the rigidity of the extracellular matrix, thereby using the biophysical properties of the matrix, in addition to its biochemical characteristics, as a guidance cue. It is interesting to note in this context that the small GTP-binding protein Rho may play a role in force-dependent signal transduction events. The Rho family proteins are known to regulate actin-based structures, such as stress fibers, lamellipodia and filopodia [55]. In addition, it has been suggested that Rho regulates contractility by activating Rho kinase, which, in turn, downregulates myosin phosphatase [56]. The resulting increased levels of myosin light-chain phosphorylation promotes both contractility and focal adhesion formation [41,57•].

Ig CAM signaling

In marked contrast to the relatively sparse information available regarding proteins that link Ig CAMs to the actin cytoskeleton in neurons, tremendous progress has been made in the elucidation of signal transduction pathways associated with Ig-CAM-mediated neurite outgrowth. The laboratory of Walsh and Doherty (reviewed in [58•]) has shown in a series of publications that NCAM, L1, as well as N-cadherin signaling involves activation of a fibroblast growth factor (FGF) receptor tyrosine kinase-phospholipase Cγ (PLCγ) cascade that leads to Ca^{2+} influx (Figure 3). Saffell *et al.* [59••] have recently provided strong evidence that activation of the FGF receptor is necessary and sufficient to account for axonal growth mediated by NCAM, L1, and N-cadherin. Expression of a dominant-negative (kinase-deleted) FGF receptor in PC12 cells resulted in a complete loss of neurite outgrowth induced by above CAMs. In addition, immunoprecipitation experiments revealed that soluble NCAM and L1 are able to cause phosphorylation of the FGF receptor in the absence of FGF. Direct binding studies between the FGF receptor and CAMs would

Figure 3



Model for a potential crosstalk between CAM signaling and actin regulatory proteins. Evidence suggest that Ig CAMs (NCAM and L1), as well as N-cadherin, mediate neurite outgrowth by activation of the FGF receptor, which, in turn, activates a PLCγ signaling cascade (for details, see [58•]). PLCγ hydrolyzes PIP_2 to IP_3 (which causes release of Ca^{2+} from intracellular stores) and diacylglycerol (DAG), which is converted to arachidonic acid (AA). In turn, AA, stimulates Ca^{2+} influx through Ca^{2+} channels. PIP_2 hydrolysis may release actin regulatory proteins such as profilin and gelsolin, thereby making these proteins available for increased actin turnover/remodeling. Details of signal transduction between receptors and Rho proteins in growth cones remain elusive. The open arrow corresponds to protein translocation. EGFR, EGF receptor; FGFR, FGF receptor.

provide the ultimate support for this interesting new signal transduction pathway.

Aside from FGF receptor signaling, selective nonreceptor tyrosine and serine/threonine kinase signaling has been implicated for both NCAM- and L1-mediated neurite outgrowth [60–62,63•,64]. It remains to be established how these signaling molecules are related to the FGF receptor pathway. These nonreceptor kinases may enrich the repertoire of Ig CAM responses, as different CAM substrates induce distinct growth cone morphologies [4]. In addition, the nonreceptor kinases could intracellularly transduce signals between CAMs and the FGF receptor. Finally, it has been suggested that these kinases could mediate 'inside-out' signaling [58•], a phenomenon well established for integrin receptors.

How could CAM signaling pathways affect cytoskeletal remodeling? Polyphosphoinositide metabolism could provide a link (Figure 3). The phospholipid PIP_2 is a well established regulator of actin-binding proteins such as gelsolin and profilin [65], which are inactivated when in a PIP_2-bound state. CAM/FGF receptor interactions that activate PLCγ [58•] and lead to PIP_2 hydrolysis could promote release of gelsolin and profilin as well as generate IP_3 and diacylglycerol (DAG). Gelsolin is a Ca^{2+}-activated severing protein [65], whereas profilin promotes plus-end actin assembly by desequestering G-actin from the monomer binding protein, thymosin β4 [9]. Therefore, co-activation of gelsolin and profilin could promote actin filament turnover, assembly and remodeling of existing actin structures. In a similar vein, EGF receptor tyrosine kinase activation of PLCγ results in release of gelsolin [66•] and profilin [67] from PIP_2, thereby regulating cell motility and actin reorganization in fibroblasts [66•]. Finally, in addition to the PIP_2 pathway, there is genetic evidence for crosstalk between tyrosine kinase signaling and cytoskeletal regulatory proteins containing polyproline-rich domains. Specifically, a recent genetic study has identified an SH2/SH3 adapter protein essential

for photoreceptor axon guidance and target recognition in *Drosophila* [68•].

Rho family GTPases functions in growth cones

Increasing new evidence suggests that proteins of the Rho family of small GTP-binding proteins are candidates for signaling agents linking extracellular guidance cues and for regulation of the actin cytoskeleton in growth cones [69]. It was recently found that microinjection of Cdc42 and Rac1 into neuroblastoma cells resulted in formation of filopodia and lamellipodia, respectively [70]. Similar structures have been induced by injection of the C3 transferase [70], which abolishes RhoA-mediated functions such as neurite retraction [71]. Very recently, a yeast two-hybrid screen revealed two novel proteins that regulate RhoA-mediated control of contractility and neuronal morphology: a putative Rho-specific GDP/GTP exchange factor and a protein called p116Rip, which acts as a negative regulator of RhoA signaling [72•]. In summary, it appears that in growth cones, Cdc42 and Rac1 are involved in filopodia and lamellipodia formation, respectively, as initially demonstrated in fibroblasts [55], whereas RhoA may be involved in myosin contractility (Figure 3). In addition, Rac1 has been implicated in collapsin-1-mediated growth cone collapse [73].

At the systems level, analysis of Purkinje cells in transgenic mice expressing a constitutively active form of human Rac1 revealed a reduced number of presynaptic terminals and an increased number of dendritic spines, whereas dendritic tree morphology was not affected [74•]. These results suggest that different neuronal compartments are differentially affected by Rac1. Very recently, Threadgill *et al.* [75•] demonstrated that all three GTPases play a role in dendritic development of cortical neurons *in vitro*. Expression of dominant-negative mutants either of Rac or Cdc42 or of C3 transferase caused a reduction in the number of dendrites, whereas expression of constitutively active mutants led to an increased number of dendrites. Although several downstream effectors of Rho family proteins (such as Pak and WASP) have been identified in non-neuronal cells, their functions, as well as those of upstream regulators (Figure 3), in growth cones have not been established [69] — future work in this area should be illuminating.

Conclusions

It has become clear over the past years that growth cones are efficient devices for relaying information between extracellular substrates and intracellular motility machinery. Studies on the actin-based motility of intracellular pathogens suggest a number of proteins potentially related to regulation of actin dynamics in growth cones. Recent work in the integrin field and emerging Ig CAM studies provide strong support for a mechanism of substrate–cytoskeletal coupling. Cell surface receptors from different structural families may regulate directed growth cone movement by modulation of cytoskeletal coupling efficiency. In addition, an increasing number of receptor- and cytoskeleton-associated signaling pathways in growth cones and non-neuronal cells have been identified. In order to formulate molecular mechanisms for growth cone guidance, a future challenge will be to determine how these signaling pathways interact with each other and regulate proteins involved in cytoskeletal remodeling and motility.

Acknowledgements

We apologize for omissions of references due to space limitations, especially as this review is integrating work from different fields. The authors thank Tama Hasson for valuable comments on the manuscript. DM Suter is supported by a postdoctoral fellowship from the Roche Research Foundation and P Forscher is supported by a National Institutes of Health RO1 grant.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Goodman CS: **Mechanisms and molecules that control growth cone guidance.** *Annu Rev Neurosci* 1996, **19**:341-377.

2. • Tessier-Lavigne M, Goodman CS: **The molecular biology of axon guidance.** *Science* 1996, **274**:1123-1133.
The authors of this excellent review categorize four different mechanisms of growth cone guidance: contact attraction, chemoattraction, contact repulsion, and chemorepulsion. After highlighting recently discovered guidance molecules and receptors, the authors discuss the respective roles of these different mechanisms in guidance decisions.

3. Tanaka E, Sabry J: **Making the connection: cytoskeletal rearrangements during growth cone guidance.** *Cell* 1995, **83**:171-176.

4. Burden-Gulley SM, Payne HR, Lemmon V: **Growth cones are actively influenced by substrate-bound adhesion molecules.** *J Neurosci* 1995, **15**:4370-4381.

5. Lewis AK, Bridgman PC: **Nerve growth cone lamellipodia contain two populations of actin filaments that differ in organization and polarity.** *J Cell Biol* 1992, **119**:1219-1243.

6. Forscher P, Smith SJ: **Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone.** *J Cell Biol* 1988, **107**:1505-1516.

7. Mitchison T, Kirschner M: **Cytoskeletal dynamics and nerve growth.** *Neuron* 1988, **1**:761-772.

8. • Welch MD, Mallavarapu A, Rosenblatt J, Mitchison TJ: **Actin dynamics *in vivo*.** *Curr Opin Cell Biol* 1997, **9**:54-61.
This well written review from the Mitchison laboratory summarizes the recent findings on actin dynamics in lamellipodia. The authors discuss the role of several proteins regulating actin polymerization, depolymerization, and retrograde flow.

9. Pantaloni D, Carlier MF: **How profilin promotes actin filament assembly in the presence of thymosin beta 4.** *Cell* 1993, **75**:1007-1014.

10. •• Welch MD, Iwamatsu A, Mitchison TJ: **Actin polymerization is induced by Arp2/3 protein complex at the surface of *Listeria monocytogenes*.** *Nature* 1997, **385**:265-269.
A seven-subunit complex was purified from cytoskeletal extracts from human platelets and demonstrated to be sufficient to induce actin polymerization at the surface of *Listeria monocytogenes*. Two of the seven subunits have been identified as Arp2 and Arp3 proteins by peptide sequencing.

11. Kang F, Laine RO, Bubb MR, Southwick FS, Purich DL: **Profilin interacts with the Gly-Pro-Pro-Pro-Pro-Pro sequences of**

vasodilator-stimulated phosphoprotein (VASP): implications for actin-based Listeria motility. *Biochemistry* 1997, **36**:8384-8392.

12. •• Gertler FB, Niebuhr K, Reinhard M, Wehland J, Soriano P: **Mena, a relative of VASP and Drosophila Enabled, is implicated in the control of microfilament dynamics.** *Cell* 1996, **87**:227-239.
Mena and Evl have been identified as two murine homologues of *Drosophila* Enabled, which is required for the formation of proper axonal connections. Focusing on Mena, the authors show that this VASP-related protein localizes to focal contacts, binds profilin, and is recruited to the surface of *Listeria monocytogenes* in infected cells. Most importantly, expression of neuronal-enriched Mena isoforms in fibroblasts results in the formation of actin-rich protrusions.

13. Laine RO, Zeile W, Kang F, Purich DL, Southwick FS: **Vinculin proteolysis unmasks an ActA homolog for actin-based Shigella motility.** *J Cell Biol* 1997, **138**:1255-1264.

14. Forscher P, Lin C-H, Thompson C: **Novel form of growth cone motility involving site-directed actin filament assembly.** *Nature* 1992, **357**:515-518.

15. • Thompson C, Lin C-H, Forscher P: **An *Aplysia* cell adhesion molecule associated with site-directed actin filament assembly in neuronal growth cones.** *J Cell Sci* 1996, **109**:2843-2854.
For the first time, a member of the Ig superfamily, apCAM, is shown to trigger actin assembly. Interestingly, this *Aplysia* NCAM (neural CAM) homologue associates in different ways with the underlying actin cytoskeleton depending on the crosslinking density.

16. Welch MD, DePace AH, Verma S, Iwamatsu A, Mitchison TJ: **The human Arp2/3 complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly.** *J Cell Biol* 1997, **138**:375-384.

17. Lin C-H, Thompson CA, Forscher P: **Cytoskeletal reorganization underlying growth cone motility.** *Curr Opin Neurobiol* 1994, **4**:640-647.

18. •• Lin C-H, Espreafico EM, Mooseker MS, Forscher P: **Myosin drives retrograde F-actin flow in neuronal growth cones.** *Neuron* 1996, **16**:769-782.
The first evidence that myosin motor action drives retrograde F-actin flow in neuronal growth cones. Two different approaches have been used to inhibit myosin function: injection of NEM-inactivated myosin S1 fragments into cultured neurons and pharmacological treatment with BDM. In combination with cytochalasin experiments, the results demonstrate that growth is regulated by both actomyosin motility and actin polymerization.

19. Hasson T, Mooseker MS: **The growing family of myosin motors and their role in neurons and sensory cells.** *Curr Opin Neurobiol* 1997, **7**:615-623.

20. Heidemann SR: **Cytoplasmic mechanisms of axonal and dendritic growth in neurons.** *Int Rev Cytol* 1996, **165**:235-296.

21. Lu M, Witke W, Kwiatkowski DJ, Kosik KS: **Delayed retraction of filopodia in gelsolin null mice.** *J Cell Biol* 1997, **138**:1279-1287.

22. • Lappalainen P, Drubin DG: **Cofilin promotes rapid actin filament turnover in vivo.** *Nature* 1997, **388**:78-82.
The function of cofilin in actin depolymerization was analyzed in yeast strains expressing *cofilin* mutants generated by site-directed mutagenesis. Interestingly, endocytosis, but not cortical actin patch motility, was reported to be affected in these *cofilin* mutants, suggesting that the former but not the latter process is dependent on actin filament turnover.

23. Rosenblatt J, Agnew BJ, Abe H, Bamburg JR, Mitchison TJ: **Xenopus actin depolymerizing factor/cofilin (XAC) is responsible for the turnover of actin filaments in Listeria monocytogenes tails.** *J Cell Biol* 1997, **136**:1323-1332.

24. • Miki H, Miura K, Takenawa T: **N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP_2-dependent manner downstream of tyrosine kinases.** *EMBO J* 1996, **15**:5326-5335.
In an attempt to identify binding proteins of Ash/Grb2, the authors characterize a novel protein, N-WASP, that is highly expressed in the brain and exhibits PIP_2-binding and actin-depolymerization activity. In addition, the data suggest that N-WASP mediates EGF-induced rearrangement of the actin cytoskeleton.

25. Baas PW, Brown A: **Slow axonal transport—the polymer transport model.** *Trends Cell Biol* 1997, **7**:380-384.

26. Hirokawa N, Terada S, Funakoshi T, Takeda S: **Slow axonal transport—the subunit transport model.** *Trends Cell Biol* 1997, **7**:384-388.

27. Tanaka EM, Kirschner MW: **Microtubule behavior in the growth cones of living neurons during axon elongation.** *J Cell Biol* 1991, **115**:345-363.

28. Tanaka E, Ho T, Kirschner MW: **The role of microtubule dynamics in growth cone motility and axonal growth.** *J Cell Biol* 1995, **128**:139-155.

29. • Rochlin MW, Wickline KM, Bridgman PC: **Microtubule stability decreases axon elongation but not axoplasm production.** *J Neurosci* 1996, **16**:3236-3246.
This detailed study from the Bridgman laboratory demonstrates that low doses of nocodazole decrease both dynamic properties of microtubule ends and axon extension rates, but not microtubule mass. The analysis of the light and electron microscopy data suggests that dynamic microtubules are required for axonal extension and support the findings of a previous report [28].

30. Tanaka E, Kirschner MW: **The role of microtubules in growth cone turning at substrate boundaries.** *J Cell Biol* 1995, **128**:127-137.

31. Challacombe JF, Snow DM, Letourneau PC: **Actin filament bundles are required for microtubule reorientation during growth cone turning to avoid an inhibitory guidance cue.** *J Cell Sci* 1996, **109**:2031-2040.

32. Williamson T, Gordon-Weeks PR, Schachner M, Taylor J: **Microtubule reorganization is obligatory for growth cone turning.** *Proc Natl Acad Sci USA* 1996, **93**:15221-15226.

33. Challacombe JF, Snow DM, Letourneau PC: **Dynamic microtubule ends are required for growth cone turning to avoid an inhibitory guidance cue.** *J Neurosci* 1997, **17**:3085-3095.

34. Lin C-H, Forscher P: **Cytoskeletal remodeling during growth cone-target interactions.** *J Cell Biol* 1993, **121**:1369-1383.

35. Lin C-H, Forscher P: **Growth cone advance is inversely proportional to retrograde F-actin flow.** *Neuron* 1995, **14**:763-771.

36. Cunningham CC, Leclerc N, Flanagan LA, Lu M, Janmey PA, Kosik KS: **Microtubule-associated protein 2c reorganizes both microtubules and microfilaments into distinct cytological structures in an actin-binding protein-280-deficient melanoma cell line.** *J Cell Biol* 1997, **136**:845-857.

37. •• Svitkina TM, Verkhovsky AB, McQuade KM, Borisy GG: **Analysis of the actin-myosin II system in fish epidermal keratocytes—mechanism of cell body translocation.** *J Cell Biol* 1997, **139**:397-415.
Another excellent study from the Borisy laboratory on the localization of cytoskeletal elements in motile cells. In addition to ultrastructural data on the actin–myosin II system of keratocytes, analysis of myosin dynamics support a hypothesis of dynamic network contraction as a mechanism for cell body translocation.

38. •• Waterman-Storer CM, Salmon ED: **Actomyosin-based retrograde flow of microtubules in the lamella of migrating epithelial cells influences microtubule dynamic instability and turnover and is associated with microtubule breakage and treadmilling.** *J Cell Biol* 1997, **139**:417-434.
Using elegant photoactivation of fluorescent microtubule-marking techniques and time lapse imaging, Waterman-Storer and Salmon provide several important new insights into microtubule dynamics in migrating cells. Marking experiments revealed that microtubules in the lamella move towards the cell body at the speed of retrograde flow by an actomyosin-dependent mechanism. In addition, retrograde F-actin flow was found to attenuate microtubule growth, quench dynamic instability and enhance microtubule breakage. The latter effect often generated short microtubules fragments that could translocate by subunit treadmilling.

39. • Suter DM, Errante LD, Belotserkovsky V, Forscher P: **The Ig superfamily cell adhesion molecule apCAM mediates growth cone steering by substrate–cytoskeletal coupling.** *J Cell Biol* 1998, **141**:in press.
The authors provide strong evidence that the *Aplysia* cell adhesion molecule apCAM can mediate growth cone advance and steering by a mechanism involving substrate–cytoskeletal coupling. A novel assay was used in which beads derivatized with specific molecuar substrates were restrained from retrograde translocation, resulting in a build up of tension between the restrained bead and the moving actin cytoskeleton. Build up of tension was correlated with directed axonal microtubule advance.

40. Gumbiner BM: **Proteins associated with the cytoplasmic surface of adhesion molecules.** *Neuron* 1993, **11**:551-564.

41. Burridge K, Chrzanowska-Wodnicka M: **Focal adhesions, contractility and signaling.** *Annu Rev Cell Dev Biol* 1996, **12**:463-519.

42. Gomez TM, Roche FK, Letourneau PC: **Chick sensory neuronal growth cones distinguish fibronectin from laminin by making substratum contacts that resemble focal contacts.** *J Neurobiol* 1996, **29**:18-34.

43. Kuhn TB, Schmidt MF, Kater SB: **Laminin and fibronectin guideposts signal sustained but opposite effects to passing growth cones.** *Neuron* 1995, **14**:275-285.

44. Sydor AM, Su AL, Wang FS, Xu A, Jay DG: **Talin and vinculin play distinct roles in filopodial motility in the neuronal growth cone.** *J Cell Biol* 1996, **134**:1197-1207.

45. Varnum-Finney B, Reichardt LF: **Vinculin-deficient PC12 cell lines extend unstable lamellipodia and filopodia and have a reduced rate of neurite outgrowth.** *J Cell Biol* 1994, **127**:1071-1084.

46. Riehl R, Johnson K, Bradley R, Grunwald GB, Cornel E, Lilienbaum A, Holt CE: **Cadherin function is required for axon outgrowth in retinal ganglion cells in vivo.** *Neuron* 1996, **17**:837-848.

47. Pollerberg GE, Burridge K, Krebs KE, Goodman SR, Schachner M: **The 180-kD component of the neural cell adhesion molecule N-CAM is involved in a cell-cell contacts and cytoskeleton-membrane interactions.** *Cell Tissue Res* 1987, **250**:227-236.

48. Davis JQ, Bennett V: **Ankyrin binding activity shared by the neurofascin/L1/NrCAM family of nervous system cell adhesion molecules.** *J Biol Chem* 1994, **269**:27163-27166.

49. • Dubreuil RR, MacVicar G, Dissanayake S, Liu C, Homer D, Hortsch M: **Neuroglian-mediated cell adhesion induces assembly of the membrane skeleton at cell contact sites.** *J Cell Biol* 1996, **133**:647-655.

Expression of neuroglian in *Drosophila* S2 cells leads to cell aggregation by homophilic neuroglian interactions and to recruitment of ankyrin to sites of cell–cell contact, whereas non-interacting plasma membranes are devoid of ankyrin. These results suggest that homophilic cell–cell adhesion is necessary to target ankyrin to L1-like CAMs.

50. •• Garver TD, Ren Q, Tuvia S, Bennett V: **Tyrosine phosphorylation at a site highly conserved in the L1 family of cell adhesion molecules abolishes ankyrin binding and increases lateral mobility of neurofascin.** *J Cell Biol* 1997, **137**:703-714.

This excellent work presents evidence that tyrosine phosphorylation of neurofascin at a site conserved among L1 family members affects the interaction between neurofascin and ankyrin, providing a mechanism for the regulation of CAM–cytoskeleton interactions.

51. Miyamoto S, Teramoto H, Coso OA, Gutkind JS, Burbelo PD, Akiyama SK, Yamada KM: **Integrin function: molecular hierarchies of cytoskeletal and signaling molecules.** *J Cell Biol* 1995, **131**:791-805.

52. Schmidt CE, Dai J, Lauffenburger DA, Sheetz MP, Horwitz AF: **Integrin-cytoskeletal interactions in neuronal growth cones.** *J Neurosci* 1995, **15**:3400-3407.

53. • Felsenfeld DP, Choquet D, Sheetz MP: **Ligand binding regulates the directed movement of beta1 integrins on fibroblasts.** *Nature* 1996, **383**:438-440.

The movement of fibronectin or β1 integrin antibody-coated gold particles on fibroblasts was analyzed using nanometer-resolution tracking techniques. The addition of RGD-containing peptides caused directed movements of antibody-coated beads, suggesting that ligand binding is an important step in the process of integrin–cytoskeleton association.

54. •• Choquet D, Felsenfeld DP, Sheetz MP: **Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages.** *Cell* 1997, **88**:39-48.

Using similar tools to those used in [53•], the authors clearly demonstrate that application of external forces influences the strength of integrin–cytoskeleton linkages. This work provides important biophysical evidence that cells are able to sense the rigidity of the extracellular matrix.

55. Nobes CD, Hall A: **Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia.** *Cell* 1995, **81**:53-62.

56. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K *et al.*: **Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase).** *Science* 1996, **273**:245-248.

57. • Chrzanowska-Wodnicka M, Burridge K: **Rho-stimulated contractility drives the formation of stress fibers and focal adhesions.** *J Cell Biol* 1996, **133**:1403-1415.

The authors provide evidence for an interesting pathway whereby Rho-mediated contractility induces the formation of stress fibers and focal adhesions. They propose that Rho affects contractility by regulation of myosin light-chain phosphorylation.

58. • Doherty P, Walsh FS: **CAM-FGF receptor interactions: a model for axonal growth.** *Mol Cell Neurosci* 1996, **8**:99-111.

This well-written review discusses evidence for a model suggesting that CAMs, such as NCAM, L1 and cadherin, mediate neurite outgrowth by activation of the FGF receptor, which, in turn, activates a PLCγ-dependent signaling cascade. Considerations of binding motifs based on structural analysis provide further support for this model.

59. •• Saffell JL, Williams EJ, Mason IJ, Walsh FS, Doherty P: **Expression of a dominant negative FGF receptor inhibits axonal growth and FGF receptor phosphorylation stimulated by CAMs.** *Neuron* 1997, **18**:231-242.

The strongest evidence to date for the signaling pathway discussed in [58•]. Neurite outgrowth mediated by NCAM, L1 and N-cadherin was abolished in PC12 cells or primary neurons from transgenic mice expressing a kinase-deleted dominant-negative form of the FGF receptor. Addition of soluble NCAM– and L1–Fc chimera resulted in phosphorylation of the FGF receptor – a key step in receptor activation.

60. Maness PF, Beggs HE, Klinz SG, Morse WR: **Selective neural cell adhesion molecule signaling by Src family tyrosine kinases and tyrosine phosphatases.** *Perspect Dev Neurobiol* 1996, **4**:169-181.

61. Kunz S, Ziegler U, Kunz B, Sonderegger P: **Intracellular signaling is changed after clustering of the neural cell adhesion molecules axonin-1 and NgCAM during neurite fasciculation.** *J Cell Biol* 1996, **135**:253-267.

62. Wong EV, Schaefer AW, Landreth G, Lemmon V: **Involvement of p90rsk in neurite outgrowth mediated by the cell adhesion molecule L1.** *J Biol Chem* 1996, **271**:18217-18223.

63. • Beggs HE, Baragona SC, Hemperly JJ, Maness PF: **NCAM140 interacts with the focal adhesion kinase p125(fak) and the SRC-related tyrosine kinase p59(fyn).** *J Biol Chem* 1997, **272**:8310-8319.

Immunoprecipitation studies revealed that the NCAM140 isoform is associated with the nonreceptor tyrosine kinases p59fyn and p125fak, which are known to be involved in integrin-dependent signaling. Although the role of p125fak in growth cone guidance remains to be established, this kinase could provide a means of integrating signal transduction pathways associated with Ig CAM and integrin receptors.

64. Kamiguchi H, Lemmon V: **Neural cell adhesion molecule L1: signaling pathways and growth cone motility.** *J Neurosci Res* 1997, **49**:1-8.

65. Janmey PA: **Phosphoinositides and calcium as regulators of cellular actin assembly and disassembly.** *Annu Rev Physiol* 1994, **56**:169-191.

66. • Chen P, Murphy-Ullrich JE, Wells A: **A role for gelsolin in actuating epidermal growth factor receptor-mediated cell motility.** *J Cell Biol* 1996, **134**:689-698.

This paper provides compelling evidence that gelsolin is an important effector of EGF-induced and PLCγ-mediated cell motility and reorganization of the actin cytoskeleton in protruding lamellipodia. The data suggest that activation of PLCγ via the EGF receptor causes mobilization of gelsolin from PIP_2, making it available for actin turnover.

67. Goldschmidt-Clermont PJ, Kim JW, Machesky LM, Rhee SG, Pollard TD: **Regulation of phospholipase C-gamma 1 by profilin and tyrosine phosphorylation.** *Science* 1991, **251**:1231-1233.

68. • Garrity PA, Rao Y, Salecker I, McGlade J, Pawson T, Zipursky SL: **Drosophila photoreceptor axon guidance and targeting requires the dreadlocks SH2/SH3 adapter protein.** *Cell* 1996, **85**:639-650.

A genetic screen for defects in photoreceptor axon guidance led to the identification of the Dreadlocks SH2/SH3 adapter protein, which could provide a link between tyrosine phosphorylation and cytoskeletal regulatory proteins.

69. Luo L, Jan LY, Jan YN: **Rho family small GTP-binding proteins in growth cone signalling.** *Curr Opin Neurobiol* 1997, **7**:81-86.

70. Kozma R, Sarner S, Ahmed S, Lim L: **Rho family GTPases and neuronal growth cone remodelling: relationship**

between increased complexity induced by Cdc42Hs, Rac1, and acetylcholine and collapse induced by RhoA and lysophosphatidic acid. *Mol Cell Biol* 1997, **17**:1201-1211.

71. Jalink K, van Corven EJ, Hengeveld T, Morii N, Narumiya S, Moolenaar WH: **Inhibition of lysophosphatidate- and thrombin-induced neurite retraction and neuronal cell rounding by ADP ribosylation of the small GTP-binding protein Rho.** *J Cell Biol* 1994, **126**:801-810.

72. • Gebbink MF, Kranenburg O, Poland M, van Horck FP, Houssa B, Moolenaar WH: **Identification of a novel, putative Rho-specific GDP/GTP exchange factor and a RhoA-binding protein: control of neuronal morphology.** *J Cell Biol* 1997, **137**:1603-1613.
Two novel regulators of RhoA involved in neurite contractility and outgrowth have been identified: a putative RhoA-activating nucleotide exchange factor inducing neurite retraction and a negative regulator of RhoA-promoting neurite outgrowth.

73. Jin Z, Strittmatter SM: **Rac1 mediates collapsin-1-induced growth cone collapse.** *J Neurosci* 1997, **17**:6256-6263.

74. • Luo L, Hensch TK, Ackerman L, Barbel S, Jan LY, Jan YN: **Differential effects of the Rac GTPase on Purkinje cell axons and dendritic trunks and spines.** *Nature* 1996, **379**:837-840.
Expression of a constitutively active form of Rac1 in transgenic mice causes different effects on Purkinje cell axon terminals, dendritic trunks and spines, suggesting that establishment of different neuronal compartments may involve distinct mechanisms.

75. • Threadgill R, Bobb K, Ghosh A: **Regulation of dendritic growth and remodeling by Rho, Rac, and Cdc42.** *Neuron* 1997, **19**:625-634.
This *in vitro* study shows that all three Rho-related GTPases (Rho, Rac and Cdc42) are involved in dendritic growth and remodeling of cortical neurons, suggesting an important role of these regulatory proteins for the development of the cortex.