

Dynamic Regulation of Cell Adhesion Molecules during Axon Outgrowth

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In the mature nervous system adhesion molecules appear to play a rather static role, maintaining synaptic connections, cell–cell contacts and stabilizing neuron–glial interactions. In contrast, during development and learning, adhesive mechanisms are dynamically regulated both spatially and temporally. It has been clear for many years that the expression of cell adhesion molecules (CAMs) can be regulated at the level of transcription. Now it is apparent that a CAM's functional activity at the cell surface can be regulated by restricting its location on the cell surface and also by its duration of expression on the membrane. Moreover, the expression can be influenced by very local events such as cell contact or neural activity. Perhaps this is not surprising, given the rapidly changing environments encountered by cells and their growing processes. Dynamic regulation of adhesion is critical for proper cell migration, axon guidance, and synapse formation during nervous system development. This review will focus especially on Ig superfamily (IgSF) CAMs such as apCAM, Fas II, L1, and axonin-1/Tag-1 and how their regulated expression participates in both the mechanics of axon outgrowth as well as proper axon guidance, synapse formation, and synaptic plasticity. A variety of model systems have been used to address dynamic regulation of adhesion. Important experiments have been done on integrins and cadherins as well as Ig superfamily CAMs. So we will draw conclusions from invertebrate and vertebrate systems, mixing electrophysiological and cell biolog-

ical studies with genetic and biochemical ones to describe a model of how CAM expression and function is regulated spatially and temporally during axon outgrowth.

THEORETICAL CONSIDERATIONS

Growth cone motility is a complex process involving adhesion to the substrate, membrane flow, and force generation via the cytoskeleton. How do cell adhesion molecules participate in this process? It is easy to imagine that adhesion between the growing axon and its substrate might be linked to the actin cytoskeleton so that the retrograde flow of actin can generate force to move the growth cone forward (Lin and Forscher, 1995). Indeed, the moving growth cone steers toward the region of the growth cone exerting the most stress (Bray, 1970). For growth cone movement to be directed by external cues, there must be a way to regulate coupling between cell surface adhesion molecules and the cytoskeleton. Both theoretical models and experimental studies of motile cells indicate that deadhesion is required for cell movement. Although this can be accomplished in many ways, the growth cone, moving rapidly at great distance from the synthetic machinery of the soma, would best be served by recycling both cell surface molecules and the cytoplasmic machinery involved in movement. Lauffenberger and associates have provided simulations describing fibroblast migration via integrins based on cellular experimental findings and mathematical models. Concepts from these models are worth considering for determining general principles of axon outgrowth. (Lauffenberger and Horwitz, 1996.)

Cell migration on a flat homogeneous substrate is

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considered to be a cyclical, continuous process (DiMilla et al., 1991; Lauffenburger and Horwitz, 1996). The first part of the cycle involves forward extension at the front of the cell of a thin piece of membrane and cytoplasm called a lamellipod. As membrane is extended, adhesion receptors must adhere to the substrate via specific ligand interactions in order to stabilize the lamellipod and prevent its retraction. The adhesion to the substrate occurs preferentially at the front of the cell, and this is substantiated experimentally by the increased concentration of adhesion receptors at the front. Extension of the forming lamellipod requires local polymerization of actin filaments and their appropriate organization in order to generate protrusion of the membrane. Exactly how the polymerization of the actin cytoskeleton occurs is unclear and is proposed to occur by either the Brownian ratchet model (Peskin et al., 1993; Mogilner and Oster, 1996) or the cortical expansion model (Condeelis et al., 1990). The lamellipod contains actin fibers organized into the lattice network that confers mechanical strength. After the lamellipod is formed and adheres to the substrate, the second part of the cycle begins, which involves myosin II-based contraction of the actin cytoskeleton. The association of the actin cytoskeleton with adhesion receptors on the membrane allows the force of contraction to move the membrane. Only if the adhesion receptors are disproportionately represented at the front will the cell displace forward. As the cell moves forward, some bonds between adhesion receptors and the substrate break because of the receptors' stronger attachment to the contracting cytoskeleton (Palecek et al., 1996). As the cell moves forward, adhesion receptors are removed from the surface, which requires that mechanisms exist to replace them at the leading edge; endocytic mechanisms may participate in this replenishment (Lawson and Maxfield, 1995). In the third part of the cycle, the cytoskeleton relaxes and adhesion receptors redistribute themselves. Some receptors remain clustered and move forward to readhere. Others are seen in intracellular vesicles, which move toward the perinuclear region. It is hypothesized that these receptors reinsert into the membrane and then move forward via cytoskeletal mechanisms (Bretscher, 1992; Forscher et al., 1992; Dai and Sheetz, 1995; Lauffenburger and Horwitz, 1996). The entire cycle then begins again.

Based on this model, migrating cells must assume a polarized morphology where the front differs from the back with respect to shape, chemosensory receptors, adhesion receptor distribution, and actin cytoskeletal arrangement. Additionally, the linkages between the adhesion receptors and the cytoskeleton

vary spatially (Lauffenburger and Horwitz, 1996). A gradient of adhesion receptor expression, high at the front of the cell and low at the rear, permits the rear to deadhere from the substrate while allowing the front to remain attached (DiMilla et al., 1991). This model offers answers as to how different biophysical and biochemical properties affect migration speed (Palecek et al., 1999). It is known that cell migration rates are affected by adhesive interactions between the cell and its environment, with highest speeds occurring at intermediate adhesiveness. Based on the model, the rear retraction rate of the uropod is determined by substrate adhesivity. When the substrate is too adhesive, the cell is slowed because the bonds between its CAMs and the substrate are stronger than the bonds between the CAMs and the actin cytoskeleton. In order to move forward, the CAM-substrate bonds are severed, and this dissociation is slower compared to the detachment rate between a CAM and its ligand on a less adhesive substrate. On substrates with a low concentration of CAM ligands, the cell is slowed by its inability to transmit cytoskeletal contraction into cell extension because it lacks adequate adhesion to the substrate (Palecek et al., 1999). These models of how CAM distribution and their ligand concentration on the substrate varies to affect cellular migration can be applied to growth cone mobility modeling, albeit on a smaller scale. Of course, differences exist between growth cone motility and cell migration such as actin organization (Forscher et al., 1992). But cellular models can be used to generate testable hypotheses to study growth cone motility.

CHANGES IN ADHESION DURING SYNAPTIC REMODELING AND DEVELOPMENT

ApCAM

The importance of dynamic CAM expression in biological systems is best illustrated by studies of apCAM and its role in synaptic plasticity of *Aplysia* neurons. *Aplysia californica* is a marine mollusk extensively studied as a model system for learning and memory. Its gill withdrawal reflex has been described systematically including its behavioral changes in response to stimuli and the extract neurons participating in the reflex as well as the molecular mechanisms that regulate both the reflex and its plasticity. In response to repeated sensory stimulation of the gill withdrawal reflex, the strength of the response increases (Carew et al., 1981). Studies have shown that the electrical responses elicited from the sensory-motoneuron syn-

apses are facilitated in a process referred to as long-term facilitation (LTF). The neuronal synaptic changes that occur in response to repeated activation of the reflex can be mimicked *in vitro* using a coculture system of L7 motor neurons and the sensory neurons that innervate them. *Aplysia* neurons express apCAM, a homologue of vertebrate NCAM, which is expressed in two forms resulting from alternative splicing. One form is attached to the membrane via a glycosylphosphatidylinositol (GPI) linkage, whereas the second has a transmembrane and cytoplasmic tail (Bailey et al., 1997). ApCAM expression on sensory neurons is downregulated in response to LTF. This downregulation of apCAM expression occurs both by reduction of newly synthesized apCAM molecules and by selective internalization of the transmembrane form of apCAM. Interestingly, internalization of apCAM is blocked by protein synthesis inhibitors, as is LTF, making apCAM's internalization the likely step in LTF induction that requires protein synthesis. This tight control of apCAM internalization might prevent inadvertent changes in synaptic architecture. The LTF-induced apCAM internalization is thought to enable defasciculation of axons, which might then be free to form new synapses or remodel old synapses. Indeed *in vitro* studies with anti-apCAM antibodies cause defasciculation of sensory axons (Peter et al., 1994). Because induction of LTF by serotonin causes specific reduction of apCAM on sensory neurites and does not reduce motoneuron apCAM, one hypothesis is that these selective changes in apCAM foster new sensory–motoneuron neurite interactions and weaken existing sensory–sensory interactions. This hypothesis is particularly supported by the observation that apCAM internalization appears prominently at sensory–sensory contacts during LTF [Fig. 1(a)] (Bailey et al., 1992; Peter et al., 1994).

Application of serotonin, 5-HT, both *in vivo* and *in vitro* elicits LTF of the gill withdrawal reflex. Bailey and colleagues have shown that 5-HT application to sensory neurons *in vitro* causes internalization of apCAM within 1 h (Bailey et al., 1992) and have demonstrated by epitope tagging that the transmembrane form but not the GPI form of apCAM is endocytosed. The remaining cell surface GPI-linked apCAM may participate in the synaptic stabilization accompanying the morphological changes that occur during LTF (Bailey et al., 1997). Application of 5-HT to motoneurons does not induce changes in cell surface apCAM; however, application of the neuropeptide FMRF induces internalization of motoneuron apCAM but not apCAM on sensory neurons. This motoneuron-specific reduction of cell surface apCAM causes motoneuron defasciculation *in vivo* and possibly partici-

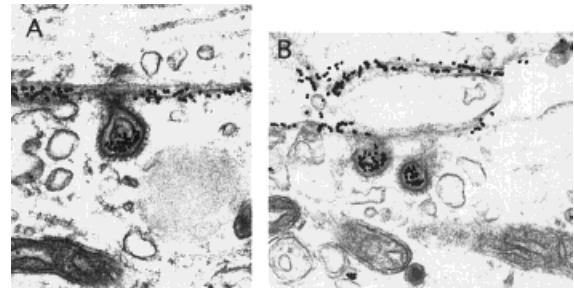


Figure 1 (A) *Internalization of apCAM by means of coated pits.* This figure shows the morphology of coated pits at apposed membranes. Note that only one membrane appears to be internalizing apCAM. Biochemical experiments have demonstrated a concomitant increase in expression of the light chain of *Aplysia* clathrin (ap-Clathrin) with apCAM internalization after 5-HT exposure. (Reprinted with permission from Bailey et al., 1992 © 1992 American Association for the Advancement of Science). (B) *5-HT Induced activation of Clathrin-Mediated Endocytic Pathway.* Note two coated pits in this electron micrograph which are heavily labeled with gold-conjugated Mab to apCAM. Removal of CAMs from these patches of apparent adhesion may lead to defasciculation of sensory neuron axons. This defasciculation is thought to be an initial step in the development of long-term facilitation. (This figure originally appeared in Hu et al., 1993 © Cell Press).

pates in synaptic depression *in vivo* (Peter et al., 1994; Zhu et al., 1995). The fact that two different neurotransmitters involved in synaptic plasticity both trigger internalization of apCAM but in different cell types suggests that cell-specific pathways regulate apCAM expression. In addition, apCAM internalization may participate in the structural changes that occur during synaptic plasticity. The endocytic pathways may redistribute membrane components to new sites of neurite contact or strengthen existing ones (Bailey et al., 1997).

Studies of 5-HT-induced sensory neuron apCAM internalization illustrate that specific pathways regulate apCAM internalization. The LTF-specific downregulation of transmembrane apCAM is mediated via clathrin-dependent endocytosis [see Fig. 1(b)] (Hu et al., 1993; Bailey et al., 1997). This is supported by the selective increase in clathrin light-chain protein associated with apCAM internalization in sensory neurons in response to 5-HT application (Hu et al., 1993). The specific recognition sequence in the cytoplasmic domain of apCAM that binds to the clathrin adaptors is not known; however, the cytoplasmic domain is required for LTF-induced endocytosis. The cytoplasmic domain of transmembrane apCAM contains PEST sequences that target proteins for degradation. Additionally, two MAP kinase phosphorylation consensus

motifs are contained within the PEST sequence. When these sites are eliminated via single mutations or deletion of the cytoplasmic domain, apCAM internalization does not occur in response to 5-HT. LTF-induced internalization of apCAM also is eliminated by a MAP kinase-specific inhibitor, P098059. Thus, MAP kinase phosphorylation of apCAM appears to be a necessary step for both its internalization and the development of LTF-induced changes in the sensory neuron (Bailey et al., 1997; Martin et al., 1997).

Fasciclin II

A second example that highlights the role of CAM regulation in proper synaptic pattern formation comes from genetic analysis of another NCAM homologue, *Drosophila* Fasciclin II (Fas II) (Schuster et al., 1996; Davis et al., 1997). Motoneuron synapse formation in *Drosophila* is precise. Individual motoneurons stereotypically innervate particular muscle fibers and reproducibly create specific numbers of synapses. Fas II is expressed at high levels on certain motoneuron axons during initial outgrowth, whereas the muscle fibers to be innervated express relatively low levels across their surface. At the time of innervation when the axon first contacts the muscle fiber, the muscle concentrates its expression of Fas II at the forming synapse and downregulates expression elsewhere. Corey Goodman's group demonstrated that overexpression of Fas II on neighboring muscles during this period causes motoneurons to innervate ectopic muscles and to create more synapses than normal. Their work suggests that the normal temporal and spatial regulation of Fas II expression on specific muscle surfaces is necessary for proper innervation to occur. On the basis of studies of apCAM's internalization in response to induction of LTF (Bailey et al., 1992) and also on studies of proteolytic cleavage of NCAM at the synapse during LTP (Fazeli et al., 1994), it was speculated that Fas II expression is also controlled by endocytotic mechanisms responding to activity changes (Schuster et al., 1996).

Ng-CAM, Nr-CAM, and Axonin-1

A final example of dynamic expression of CAMs during a developmental process comes from a series of studies of the chick commissural neurons as they cross the midline to project to their targets contralaterally (Stoeckli and Landmesser, 1995; Stoeckli et al., 1997) reviewed in (Stoeckli and Landmesser, 1998). When commissural neurons extend processes in the spinal cord, several adhesion molecules are expressed on their axons. Axonin-1, the chick homologue of

mouse and rat Tag-1, is expressed as neurons begin to extend processes and is maintained as they grow ipsilaterally toward their midline crossing point. Expression of axonin-1 increases to maximal levels as axons reach the floorplate to cross the midline and then decreases substantially shortly after the axons reach the contralateral side. Ng-CAM is expressed throughout this period but its onset occurs shortly after the initial neurites begin outgrowth. Antibody perturbation studies have shown that both axonin-1 and Ng-CAM expression are necessary for proper bundling of axons but that only anti-axonin-1 antibodies disrupt proper axon pathfinding. Indeed, when anti-axonin-1 antibodies or soluble axonin-1 were injected in developing chick embryos, many axons failed to cross the midline and instead continued to grow ipsilaterally. Injection of either anti-axonin-1 and anti-Ng-CAM antibodies caused defasciculation of the axon bundles as they projected, but anti-Ng-CAM antibodies did not disrupt pathfinding.

Another Ig-CAM, Nr-CAM is expressed by the floorplate cells and commissural axons until embryonic day 6 (E6) in the chick. Nr-CAM also participates in pathfinding by the commissural axons. When either function blocking anti-Nr-CAM antibody (Stoeckli and Landmesser, 1995) or soluble chimeric Nr-CAM-FC fusion protein (Lustig et al., 1999) was injected *in ovo* into developing chick embryos, both caused axonal pathfinding errors. Because floorplate cells do not express axonin-1, Nr-CAM perturbation studies suggest that a heterophilic interaction between axonin-1 and Nr-CAM is necessary for proper decussation of commissural axons. Again, disruption of proper expression of either Nr-CAM or axonin-1 produces improper axon pathfinding. These results indicate that proper timing and proper location of expression of these CAMs is necessary for proper pathfinding by commissural neurons. The recent demonstrations that different cadherins are expressed at synapses in different anatomical regions supports the argument that regulated expression of CAMs is a critical aspect of the development of specific patterns of neuronal synaptic connections (Fannon and Colman, 1996; Shapiro and Colman, 1999; Wu and Maniatis, 1999).

L1

One Ig superfamily adhesion molecule that has been intensely studied using cell biological and biochemical approaches is L1. L1 is concentrated on axons and growth cones, so we will review important facts about L1 to provide a background for our discussion of CAM-mediated growth cone motility. L1

belongs to an Ig CAM subfamily along with Nr-CAM and neurofascin (Hortsch, 1996). L1 consists of six Ig like domains connected to five fibronectin type 3 repeats in the extracellular domain, followed by a single-pass transmembrane domain and a short cytoplasmic domain (Moos et al., 1988). L1 is a conserved molecule with homologues found in mammals, chick (Ng-CAM, mentioned above), and *Drosophila* (neuroglian). The cytoplasmic domain is the most conserved part of L1; it is identical in mammals and has two highly conserved regions that extend to invertebrates. L1 is coded for by a single gene, which is alternatively spliced, once in the extracellular region and once in the cytoplasmic region. The two exons code for "KGHHV" in the first Ig domain of L1 and for "RSLE" in the cytoplasmic domain (Miura et al., 1991). The RSLE cytoplasmic sequence of L1 regulates its cell surface expression (Kamiguchi et al., 1998b; Kamiguchi and Lemmon, 1998) and will be discussed in detail later. The two alternatively spliced exons are expressed in all neural cells that express L1. Nonneuronal L1-positive cells, including Schwann cells and melanoma cells, express L1 missing these two exons (Miura et al., 1991; Montgomery et al., 1996; Takeda et al., 1996).

The expression of L1 is restricted primarily to the nervous system and peaks during its development. It is expressed in both neurons and Schwann cells beginning at about E15 in the rat. A clue to L1's function as an adhesion molecule comes from its high expression on axons and growth cones. Functional studies have confirmed that it participates in neuronal axon outgrowth (Lagenaur and Lemmon, 1987) and fasciculation and *in vivo* studies show it is involved in axon guidance (Cohen et al., 1997). A clear understanding of how L1 directs axon guidance as opposed to axon growth is hampered by the fact that L1 can bind homophilically to itself on adjacent axons (Lemmon et al., 1989) and heterophilically to extracellular matrix (ECM) components as well as to other IgSF CAMs and integrins (Brümmendorf and Rathjen, 1994; Montgomery et al., 1996).

The importance of L1 function during development is clear because of the devastating effects caused by mutations in human L1. Mutations in either its extracellular or cytoplasmic domain cause X-linked hydrocephalus or MASA syndrome, which are characterized by varying degrees of mental retardation and hydrocephalus depending on the type of mutation in the L1 molecule (Yamasaki et al., 1997). The cytoplasmic mutations causing MASA syndrome were particularly interesting because they cause axon guidance problems, such as abnormal development of the corticospinal tract but rarely cause severe hydroceph-

alus or death. This supports the hypothesis that L1 participates in signaling pathways during neurite outgrowth. Studies of the L1 knockout mice (Dahme et al., 1997; Fransen et al., 1998; Kamiguchi et al., 1998a) have also confirmed the role of L1 in proper brain development. These animals have many similarities to humans with mutations in the L1 molecule, including hydrocephalus, mentation defects as determined by Morris water maze tests, and also malformation of certain axon pathways. Analysis of the knockout mice suggests that L1 not only participates in axon growth but also pathfinding. For example, axons in the corticospinal tract of L1 knockout mice fail to decussate properly in the hindbrain, suggesting that L1 is required for axon guidance in this system (Cohen et al., 1997).

POLARITY OF CAM EXPRESSION: DO BASOLATERAL SORTING SIGNALS MEDIATE AXONAL SORTING?

In order for adhesion molecules to participate in development, they must be properly expressed and localized in the neuron, often in a polarized distribution, either somatodendritically or axonally. This is critical not only for adhesion molecules but for many proteins including neurotransmitter receptors and ion channels that need a polarized distribution in neurons in order to function properly (Craig and Banker, 1994). Early studies of protein sorting in neurons suggested that it occurred by the same mechanisms identified for polarized epithelia (Dotti and Simons, 1990). Signals that sorted proteins basolaterally in epithelia would sort somatodendritically in neurons, and apical epithelial signals would sort axonally. For example, the low-density lipoprotein (LDL) receptor has been shown to use its basolateral sorting signal for dendritic localization. The LDL receptor was transfected into neurons using viral vectors, and its expression was localized to the dendritic surface. When critical tyrosines in the basolateral sorting signal were mutated, the LDL neuronal expression pattern changes from polarized in the dendrites (98% dendritic) to a uniform expression throughout the cell surface (Higgins et al., 1997). The transferrin receptor, a protein normally found in neuronal dendrites, can be mutated in its cytoplasmic domain so that its expression becomes both axonal and dendritic. Interestingly, its basolateral sorting signal used by MDCK cells is not required for dendritic sorting, but rather another cytoplasmic motif is used (West et al., 1997a,b). This finding calls into question the hypothesis that neurons

use the same epithelial basolateral sorting signals for somatodendritic sorting.

Studies of axonal sorting reveal the situation is even more complicated for neurons and that multiple mechanisms for sorting must exist. GPI-linked proteins have been shown to sort apically in epithelial cells (Simons and Wandinger-Ness, 1990; Rodriguez-Boulan and Powell, 1992). These proteins would be expected to sort axonally in neurons, based on early studies (Dotti and Simons, 1990; Dotti et al., 1991). There are many examples where GPI-linked proteins do sort axonally. For example, the acetylcholinesterase protein contains a GPI linkage and sorts axonally as expected (Incardona and Rosenberry, 1996). However, mutation of the GPI linkage to a transmembrane domain does not result in improper sorting, which calls into question the role of the GPI anchor in axonal sorting. Several Ig superfamily molecules from the axonin-1/TAG-1 subfamily including F11, F3, axonin-1/TAG-1, BIG-1/BIG-2 (Yoshihara et al., 1994; Yoshihara, 1995) and the new family member NB-3 (Kamei et al., 1998) contain GPI anchors. Some have been analyzed immunohistochemically and are shown to be expressed on axons and growth cones (Faivre-sarrailh and Rougon, 1993; Brummendorf and Rathjen, 1996; Dahlin-Huppe et al., 1997). However, in cerebellar type II interneurons, F3 and F11 are expressed somatodendritically and axonally (Faivre-sarrailh et al., 1992). Furthermore, the role of the GPI anchor is questioned by reports showing other GPI-linked proteins with diffuse axonal and somatodendritic cell surface expression (Kollins et al., 1999). For example, Thy-1, a GPI-linked IgSF CAM, is expressed on both cell bodies and axons in olfactory neurons and cerebellar Purkinje cells (Morris et al., 1985; Xue et al., 1990; Xue and Morris, 1990). Cepu-1, also a GPI IgSF CAM, is expressed diffusely on Purkinje cells (Spaltmann and Brummendorf, 1996). Recent evidence indicates that glycosylation of the region near the GPI linkage may be the recognition signal. So the role of the GPI anchor in axonal sorting is currently unclear.

Our laboratory has recently shown that the L1 cytoplasmic domain contains a short amino acid sequence that is required for axonal expression of L1 (Kamiguchi and Lemmon, 1998). The amino acid sequence "YRSL" is present only in L1-expressing neurons. Nonneuronal cells alternatively splice out the second cytoplasmic exon coding for RSLE, thereby eliminating the sequence. This tyrosine-based consensus motif conforms to the "YXXØ" pattern where Y is tyrosine, X is any amino acid, and Ø is a hydrophobic amino acid (Ohno et al., 1995). These sequences have been shown to participate in many

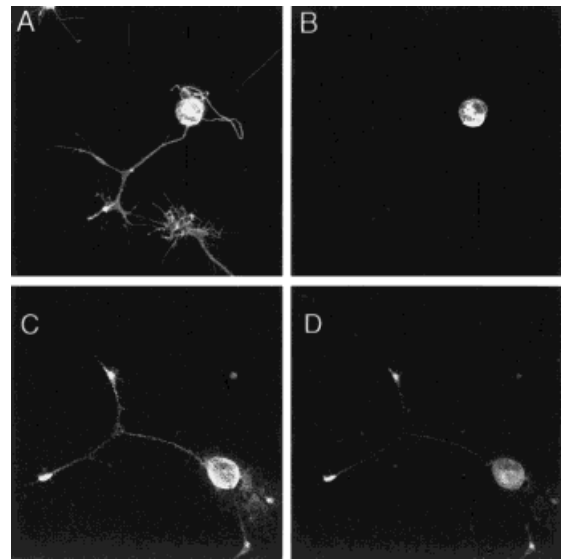


Figure 2 Sorting of L1 into the axon is regulated by a tyrosine based sorting signal in the L1 cytoplasmic domain. (C) and (D) Chick DRG neurons expressing full length L1 (B) is found at the tips of neurites visualized with anti-NCAM antibodies (C). When the tyrosine based sorting signal is disrupted by removing four amino acids (RSLE) to mimic the non-neural form of L1 it fails to exit the neuronal soma (B) into the neurite visualized with anti-NCAM antibodies (A). (These figures originally appeared in Kamiguchi & Lemmon, 1998.)

trans-Golgi mechanisms such as basolateral sorting, clathrin-dependent endocytosis, and lysosomal degradation (Mellman, 1996). Chick DRG neurons were transfected with human L1 cDNA constructs of either the full length L1 containing the YRSLE, a nonneuronal form that lacks the RSLE sequence, or a mutant L1 Y1176A where the tyrosine is changed to alanine (Fig. 2). When transfected into DRG neurons, the full-length L1 and a partial cytoplasmic truncation construct of L1 both containing the putative sorting sequence YRSL were expressed in the axonal growth cone and to a lesser degree the cell body. In contrast, expression of the two constructs containing mutations of the tyrosine-based sequence YRSL by either deletion of the RSLE-coding exon or mutation of the tyrosine to an alanine caused expression of L1 to be restricted to the cell body in most cases. Sometimes expression reached the proximal axon but never was found distally (Kamiguchi and Lemmon, 1998). These experiments demonstrate that the YRSL signal sequence is required for axonal sorting of L1.

In neurons, L1 is preferentially targeted to axons and is absent from dendrites. Based on the proposed hypothesis that epithelial apical and neuronal axonal membranes as well as epithelial basolateral and neu-

ronal somatodendritic membranes may be equivalent protein sorting domains, L1 is expected to show polarized distribution apically in epithelial cells. However, our preliminary results showed that L1 was also expressed on the basolateral surface of MDCK cells, further indicating that neurons and epithelial cells use distinct molecular mechanisms to mediate protein sorting (H. Kamiguchi and V. Lemmon, unpublished results).

The neural cell adhesion molecule, NCAM, was the first neural cell adhesion molecule identified and the founder of the IgSF CAMs (Acheson et al., 1991). A single gene encodes NCAM, and it generates three major membrane isoforms as well as a secreted form in the brain (Hemperly et al., 1986; Santoni et al., 1989). It is unclear what sorting signals are required for localization of the different NCAM isoforms to specific membrane compartments. NCAM-120 is a GPI-linked form that is located apically in MDCK-transfected cells (Powell et al., 1991). It is thought that a sequence in its ectodomain mediates apical sorting because a secreted form of NCAM sorts apically prior to secretion. NCAM-140 and NCAM-180 are sorted basolaterally in MDCK cells, but the recognition sequence is not tyrosine based. The recognition signal is diffusely located in a 40-amino acid cytoplasmic sequence. When the recognition sequence was added to a nonpolarized, mutated form a NCAM or to the p75 neurotrophin receptor, which sorts apically, the added signal redirected their expression to the basolateral surface in MDCK cells (Le Gall et al., 1997). Importantly, one must be careful in generalizing findings about sorting in epithelial cells to sorting in axons and dendrites. Studies of the transferrin receptor have shown that its basolateral signal for sorting in epithelial cells did not participate in its neuronal sorting (West, 1997a). It would be interesting to see similar mutation experiments of NCAM isoforms in neurons; these may identify new neuronal sorting mechanisms.

Some proteins expressed diffusely on the neuronal membrane are first directed axonally and then redirected somatodendritically. The β amyloid precursor protein (β APP), which is a transmembrane protein, sorts to the axon via two signal sequences in its ectodomain, but then it is endocytosed and transported for reexpression somatodendritically, a process termed transcytosis (Yamazaki et al., 1995; Tienari et al., 1996; Higgins et al., 1997). The signal that redirects the protein after endocytosis has yet to be identified. Transcytosis is thought to occur via two different sorting signals: the first signal acts during biosynthesis in the Golgi, and the second redirects the protein following endocytosis from the membrane.

Perhaps axonal targeting occurs through unique neuronal machinery. This idea was suggested by the recent identification of AP3, which is an adaptor complex that associates with non-clathrin-based coat proteins (Newman et al., 1995; Dell'Angelica et al., 1997; Simpson et al., 1997). AP3 consists of both a ubiquitously expressed AP3a and a neuronal-specific AP3b form. The AP3b contains distinct β and μ subunits which may be used in neuronal specific axonal sorting.

INTERNALIZATION AND RECYCLING OF CAMS

Clathrin-dependent endocytosis is a rapid form of internalization that has been studied extensively (Mellman, 1996). It is used to internalize membrane proteins whose cytoplasmic domains contain specific recognition sequences such as tyrosine-based signals YXXO (see above) or di leucine (LL) motifs, which are recognized by the endocytic machinery prior to internalization (Ohno et al., 1995, 1996; Marks et al., 1996; Rapoport et al., 1997). Recently, tyrosine-based motifs have been shown to interact directly with the μ 2 subunit of AP2 prior to clathrin dependent internalization. It is thought that LL motifs interact indirectly with the AP2 complex at a less well-determined site (Marks et al., 1996; Warren et al., 1998). L1 contains a tyrosine-based consensus motif YRSL in its cytoplasmic domain that is expressed only in neurons (Miura et al., 1991; Takeda et al., 1996). This YRSL sequence has been shown previously to mediate axonal sorting of L1 (see discussion above; Kamiguchi and Lemmon, 1998). Among mammals, the L1 cytoplasmic domain is completely conserved at the amino acid level and is highly conserved across all species. The tyrosine-based motif is conserved in all known L1 homologs, including chick, zebrafish, and mammals, and in the L1 subfamily members Nr-CAM and neurofascin (Hortsch, 1996). This strict conservation suggests that the sequence is important in L1 function.

To determine whether or not the tyrosine-based signal was functional for clathrin-dependent endocytosis, we tested for an interaction between the AP2 adaptor protein subunit μ 2 and the tyrosine-based sequence YRSL in the cytoplasmic domain of L1 (L1CD) using a yeast two-hybrid assay (Kamiguchi et al., 1998b). Yeast cotransfected with full-length L1CD containing the YRSL and μ 2 were able to grow on selection media, but cotransfected cells containing mutations in the YRSL sequence were not. Furthermore, L1 immunoprecipitates from rat brain probed

with either anti-AP2 antibodies or anti-AP180 antibodies detected interactions between the clathrin AP2 complex and L1. Cell culture experiments confirmed that L1 was endocytosed via clathrin. In L1-expressing 3T3 cells, endocytosed L1 colocalizes with the transferrin receptor (Kamiguchi et al., 1998b), a marker for clathrin-mediated endocytosis (Trowbridge et al., 1993). L1 internalization is clathrin dependent in these cells because it is specifically blocked by expression of dominant negative dynamin (Kamiguchi et al., 1998b). Previously, dynamin has been shown to be required for clathrin-dependent endocytosis (Damke et al., 1994; Hinshaw and Schmid, 1995). Endocytosed L1 could be detected in live growth cones within minutes of adding anti-L1 antibodies, which is consistent with a rapid internalization mechanism. Confocal studies of L1 endocytosis in the growth cone of DRGs revealed that L1 endocytosis was spatially dependent; staining for L1 colocalized with anti-Eps 14 staining (an early clathrin endocytic marker; Tebar et al., 1996; van Delft et al., 1997; Benmerah et al., 1998) only at the base of growth cones (Kamiguchi et al., 1998b) (Fig. 3). Because Eps 15 is a marker for the early stage of endocytosis, its colocalization with L1 implies that L1 endocytosis occurs preferentially in the rear of growth cones and not the leading edge. Local regulation of L1 endocytosis may be one mechanism regulating growth cone motility on L1 substrates. As the growth cone advances, the rear must deadhere to allow forward movement. Endocytosis of L1 at the back of growth cones would facilitate this detachment process. We have found that endocytosed L1 is then transported forward along microtubules for insertion at the leading edge of the growth cone (Kamiguchi and Lemmon, 2000), thereby facilitating attachment to the substrate, perhaps in response to guidance cues or force changes. Interestingly this recycling of L1 to the front of the growth cone occurs on L1 substrates but not on laminin, implying some form of substrate-dependent regulation of CAM recycling. As discussed earlier, endocytosis of integrins has been shown to participate in cell migration and growth cone motility (Lauffenburger and Horwitz, 1996). It seems plausible that the internalization and recycling of membrane CAMs from the rear to the front of growth cones creates a gradient of adhesivity similar to that seen in migrating cells, even though growth cones are smaller.

Little is known about which mechanisms trigger L1 endocytosis in the growth cone. However, one might imagine that extracellular cues stimulate changes in L1 cell surface expression via endocytosis. It has been shown that growth cones undergo rapid

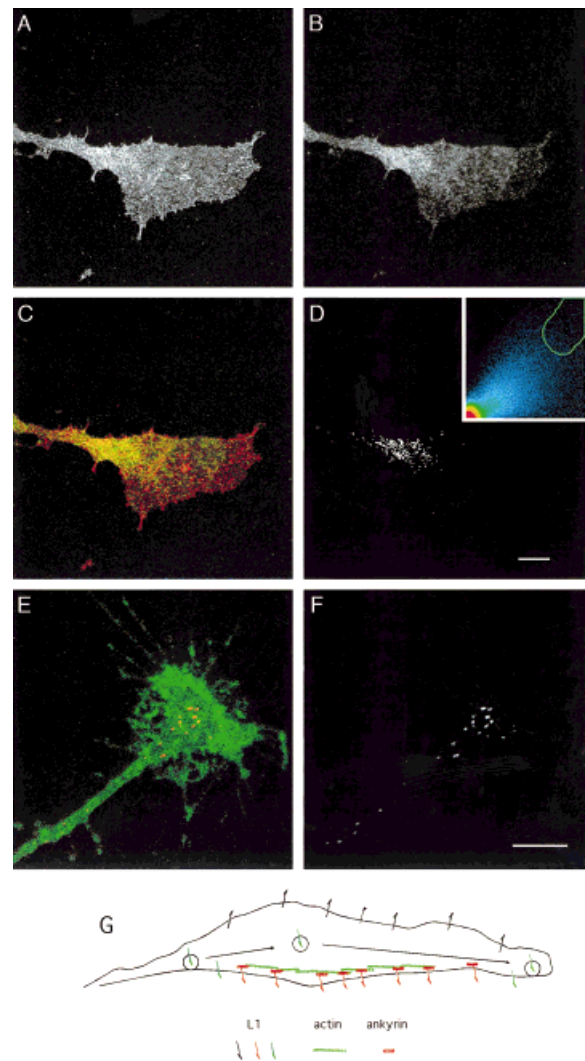


Figure 3 Internalization of L1 in growth cones. (A)–(D) Confocal section of a DRG growth cone growing on an L1 substrate. The growth cone was double labeled with anti L1-CD antibody (74-5H7) in A and with anti-Eps-15 in B. The superimposition image (C) of L1 in red and Eps-15 in green demonstrates colocalization (yellow) at the rear of the growth cone. A black and white mask shows the colocalized distribution of L1 and Eps15 (D). (E) and (F) Confocal sections of DRG growth cones growing on an L1 substrate. The living neurons were incubated with anti-L1 antibody for 15 minutes to label endocytosed L1. The fixed cells were double labeled with anti-NCAM antibody to visualize the outline of the growth cones. Endocytosed L1 is colored in red and NCAM in green. (F) shows just the internalized L1. (G) Simple model for L1 recycling in growth cones. See text for complete description. (Figures (A)–(F) originally appeared in Kamiguchi et al., 1998b.)

morphological changes in response to extracellular matrix cues, including new contact with L1 substrates (Burden-Gulley et al., 1995). L1–L1 homophilic bind-

ing may lead to L1 clustering and initiation of signaling cascades (Doherty et al., 1994; Williams et al., 1994). Some of these signaling pathways must regulate endocytosis and trafficking of L1 (see below) (Kamiguchi and Lemmon, 1997; Kamiguchi et al., 1998b) because L1-dependent adhesion does induce L1 internalization (Schaefer, Kamiguchi, Long, and Lemmon, unpublished results). Similarly, cell-cell contact appears to regulate E-cadherin endocytosis in epithelial cells (Le et al., 1999). E-cadherin is localized primarily to the cell surface in confluent epithelial cultures, whereas preconfluent cultures have an increased pool of internalized E-cadherin. When cell-cell contacts are disrupted by chelation of Ca^{2+} , an immediate internalization of E-cadherin from the cell surface is observed. Upon restoration of calcium levels, the cells quickly reexpress E-cadherin on the cell surface, suggesting that E-cadherin was recycled back to the membrane in response to renewed cell-cell contact. The ability of cells to traffic E-cadherin from the internalized pool to the cell surface responsively may be important for junction formation in development or tissue remodeling, processes that require dynamic regulation of adhesion over time (Le et al., 1999). A similar influence of cell-cell contact has been observed for apCAM endocytosis. During LTF induction in *Aplysia* sensory neurons, apCAM endocytosis occurs preferentially at sites of homophilic interaction (Bailey et al., 1992), which results in loss of neuritic contacts.

Multiple studies have demonstrated the importance of phosphorylation in dynamic regulation of endocytosis. Learning-induced apCAM endocytosis requires MAP kinase phosphorylation in sensory neurons (Bailey et al., 1997; Martin et al., 1997). Interestingly, elements of the MAP kinase cascade is also associated with L1 and the MAP kinase cascade is activated by clustering L1 on the cell surface (Schaefer et al., 1999). L1 immunoprecipitates contain raf, ERK, and p90rsk. Crosslinking L1 with antibodies induces a rapid activation of the MAP kinase cascade as evidenced by an increase in phosphorylated ERK. Curiously the phosphorylated ERK is associated with internalized L1 rather than cell surface L1, and if the internalization of L1 is prevented, then the activation of ERK does not occur. Activation of ERK by L1 internalization in theory could have several different consequences. One of the likely consequences is phosphorylation of L1 by ERK and p90rsk in regions of the L1CD important for interactions with the actin cytoskeleton. After L1 internalization, subsequent phosphorylation of L1CD might prevent an interaction with the actin cytoskeleton while it is being transported in vesicles along microtubules. Support-

ing this idea are peptide inhibition experiments where inhibition of the p90rsk interaction with L1 inhibited neurite growth on L1 but not laminin (Wong et al., 1996a). Another possible consequence of ERK activation of ERK activation might be to cause changes in L1-mediated adhesion (Schaefer et al., 1999). Activation of ERK during integrin-mediated cell motility is correlated with integrin deadhesion, which is required for efficient cell migration (Xie et al., 1998). As discussed above, ERK activation causes apCAM cell surface expression to be downregulated via endocytosis (Bailey et al., 1997). These experiments suggest that ERK activation may contribute to dynamic changes in L1 cell surface expression.

The best studied IgSF protein for its endocytic trafficking is the EGF receptor (EGFR). Recent work in fibroblasts has demonstrated that the overexpression of the kinase c-src causes increased EGFR internalization, resulting in a larger steady-state pool of internalized EGFR. The mechanisms by which c-src regulates EGFR internalization is unknown. However, it is hypothesized that c-src may participate in regulating the components involved in recruiting EGFR to the clathrin-coated pits. Another possibility is that c-src potentiates the function of existing dynamin. Dynamin is a GTPase required for clathrin-mediated endocytosis (van der Blik et al., 1993; Schmid et al., 1998) and has been shown to bind the SH3 domain of c-src *in vitro*, suggesting a possible *in vivo* interaction (Ware et al., 1997). This possibility is attractive because of studies of the β_2 adrenergic receptor, demonstrating that its internalization requires src-mediated phosphorylation of dynamin (Ahn et al., 1999). The role of src in regulating endocytosis events (Ware et al., 1997) may also apply to L1-mediated neurite outgrowth. In fact, neurite outgrowth from cerebellar neurons of src⁻ mice is substantially reduced on L1 substrates (Ignelzi et al., 1994), whereas there is no alteration of growth on NCAM substrates. However, src⁻ mice do not have any currently identified nervous system abnormalities analogous to the L1 knockout mice, suggesting src is not required for CAM-mediated neurite outgrowth (Soriano et al., 1991; Stein et al., 1992). Similarly, inhibitors of src kinases do not affect neurite outgrowth *in vitro*, so the role of src in L1-dependent neurite growth remains unclear (Bixby and Jhabvala, 1992; Doherty et al., 1994).

Other studies have shown that the tyrosine-based endocytic motifs can be masked by tyrosine phosphorylation, thus preventing recognition by adaptor proteins. This process is suggested to regulate endocytosis of certain proteins (Bradshaw et al., 1997; Shiratori et al., 1997). This appears to be true for the T-cell receptor CTLA-4 that binds receptors on anti-

gen presenting cells during T-cell activation (Shiratori et al., 1997). The cytoplasmic domain of CTLA-4 contains a tyrosine-based recognition sequence, "YVKM," which binds the $\mu 2$ chain of AP2 as detected by a yeast two-hybrid assay. The tyrosine is critical for $\mu 2$ recognition of the sequence. In addition, the sequence was necessary for internalization of the molecule, therefore suggesting a clathrin-mediated mechanism. When the tyrosine from this recognition sequence is phosphorylated, it also interacts with the tyrosine phosphatase Syp that is thought to initiate the CTLA-4 receptor's inhibitory signaling pathway. Although both $\mu 2$ and Syp bind at the YVKM sequence, phosphorylation of the tyrosine reduced the $\mu 2$ binding affinity (Shiratori et al., 1997). The reduction in $\mu 2$ binding suggests that its interaction with the YVKM sequence might be regulated by phosphorylation. Quite possibly, phosphorylation of this sequence modulates endocytosis of the CTLA-4 receptor.

CAMS AND CYTOSKELETON

Once adhesion molecules are properly polarized to the axonal membrane, they must maintain their distribution. Little is known about the mechanisms that maintain their distribution. Recently, experiments were done that indicate that cytoskeletal interactions with membrane proteins contribute a barrier to protein diffusion (Hollenbeck and Ruthel, 1999; Winckler et al., 1999). Winckler and others did laser tweezer experiments with silica beads coated with antibodies against specific membrane proteins or coated with lectins that bind nonspecifically to many cell surface proteins. They measured the distances that the beads could be dragged in the plane of the membrane before they escaped the laser tweezer trap and compared different regions of the neuron for resistance to movement. Beads in the distal axon could be dragged 10 times the distance compared to beads dragged in the proximal axon located in the initial segment just adjacent to the cell body. This impediment to diffusion in the initial segment was obliterated by disrupting the cytoskeleton with treatments such as latrunculin-B, an actin-disrupting drug. Surprisingly, the impediment was disrupted by 0.4% DMSO treatment alone, even though this did not affect other aspects such as neurite outgrowth rates or growth cone motility. It is supposed that this treatment alone may uncouple the tethers between the membrane proteins and the cytoskeleton, thus disrupting the barrier and permitting easier diffusion of membrane proteins. These studies demonstrate that there are membrane specializations

to separate axonal membrane components from somatodendritic membrane components.

Additional clues about adhesive dynamics of CAMs during axonal extension have come from laser tweezer studies of integrin molecules (Schmidt et al., 1995). These studies have shown that receptor localization in the growth cone influences interactions with the actin cytoskeleton. When polystyrene beads coated with anti- $\beta 1$ integrin antibodies were trapped by a laser on the surface of growth cones, the beads behaved differently, depending on the growth cone region. These results indicate regional differences in the interaction of membrane proteins with cytoskeleton. Beads were allowed to bind to integrins on the membrane for 10 s, and then the beads were towed laterally via the laser trap. Some beads moved easily, creating a tether between the bead and growth cone, and these beads were considered to be bound to integrins that were not associated with the actin cytoskeleton. Beads that could not be dragged were considered attached to integrins bound to the cytoskeleton; this cytoskeletal linkage stabilized the integrins' position in the membrane, thus preventing bead movement. In the leading edge region, a higher proportion of beads could not be dragged after binding to the integrins compared with beads bound at the base of the growth cone. This suggested that a higher percentage of integrins in the leading edge were bound to the actin cytoskeleton, thus stabilizing the integrins in the plane of the membrane and preventing bead movement. In the growth cone base, fewer beads were tightly attached, indicating that a higher percentage of rearward integrins were not attached to the cytoskeleton. These regional receptor cytoskeletal interactions in the growth cone fit predictions based on cell motility models (Lauffenburger and Horwitz, 1996) and experimental studies of integrin distribution during cell mobility (Lawson and Maxfield, 1995). Like migrating cells, the front region of growth cones requires greater stability and adhesion to the substrate during extension compared with the rear that must deadhere. During forward growth cone movement, more actin-integrin interactions would allow for increased transmission of force and thus locomotion via actin contractility. Because the leading edge of the growth cone is more strongly linked to the cytoskeleton, a larger transduction of force occurs at the leading edge that is thought to pull the weakly attached rear of the growth cone and the axon forward (Schmidt et al., 1995). Furthermore, it has been shown that retrograde actin flow is directly linked to growth cone advance (Lin and Forscher, 1995; Tanaka and Sabry, 1995). Therefore, as the actin cytoskeleton moves rearward, its

attachment to integrins creates force that pulls the growth cone and axon in a forward direction.

The function of CAMs can be altered in several different ways that depend on posttranslational modification of their cytoplasmic tails or of the molecules with which they interact. This can influence their locations and density and their interactions with the cytoskeleton or directly affect their affinity for extracellular ligands (Pomies et al., 1995; Oostendorp and Dormer, 1997). In response to increased extracellular matrix forces, the cytoskeleton-integrin linkages strengthen to increase rigidity of the region (Choquet et al., 1997). Possible ways to strengthen cytoskeletal linkages with cell surface adhesion molecules include increasing the number of integrin receptors in the region of attachment (Schmidt et al., 1995; Sheetz et al., 1998) or increasing the affinity of ligand binding (Felsenfeld et al., 1996). It has been shown that the number of integrins expressed in the growth cone is rapidly altered in response to varying concentrations of ECM ligands. This optimizes the relative concentrations of CAM and ligand to permit efficient axon growth (Condic and Letourneau, 1997). Alternatively, individual receptor affinity may be modulated directly. For example, phosphorylation of a tyrosine in the $\beta 3$ integrin cytoplasmic domain has been shown to affect $\alpha \beta 3$ ligand affinity (Blystone et al., 1997). The change in receptor affinity can occur simultaneously with receptor relocation, thus dramatically altering the number of functional receptors in the region. How do CAM receptors rapidly relocate in the growth cone? Because the response occurs within minutes, only posttranslational mechanisms can regulate their relocation. There is no evidence for the existence of significant intracellular pools of CAMs that could serve as reservoirs for rapid insertion into the membrane. Newly synthesized proteins in the soma are too far from the growth cone to function in the rapid growth cone responses to environmental signals.

Studies of gold particles coated with anti-integrin antibodies have demonstrated rapid (approximately 40 $\mu\text{m}/\text{min}$) directed movement of the particles toward the leading edge of the growth cone. This might occur via linkage to short actin filaments that are transported forward by motor proteins along the actin network (Sheetz et al., 1990; Schmidt et al., 1995; Suter et al., 1998). Recently, our laboratory has begun to study the role that endocytic mechanisms play in regulating L1 dynamics. We have demonstrated that L1 can be internalized via clathrin-dependent endocytosis. Experiments indicate that endocytosis of CAMs (Kamiguchi et al., 1998b; Kamiguchi, 2000)

may be an additional means for quickly relocating CAMs within the growth cone.

Extensive studies on Nr-CAM, neurofascin, and L1 by Bennett and his associates have revealed that these molecules can bind to ankyrin, a component of the subplasma membrane cytoskeleton (Garver et al., 1996). This is a critical interaction, permitting the cell surface CAMs to bind to the cytoskeleton. Perhaps, this interaction provides the critical linkage, permitting the actin cytoskeleton to transmit force through the plasma membrane to the substrate. This interaction is regulated by tyrosine phosphorylation in a conserved region at the C-terminal end of the cytoplasmic domain of these CAMs. CAM-dependent adhesion recruits ankyrin to the sites of cell contact, indicating that there is signal generated by the CAMs to regulate this interaction. Michael Horstch's group has done a fascinating experiment showing that Tag-1-mediated adhesion is sufficient to recruit ankyrin to L1 (Malhotra et al., 1998) (Fig. 4). Earlier work has indicated that Tag-1 and L1 binds in a *cis*-fashion in the plane of the membrane (Buchstaller et al., 1996). Perhaps the Tag-1-Tag-1 *trans*-binding recruits L1 to points of cell contact and in turn this recruits ankyrin.

The phosphorylation state of CAMs influences different aspects of neurite outgrowth as well as cell-cell contact. The cytoplasmic domain of L1 has been shown to be phosphorylated at several different sites (Schaefer and Lemmon, 1998). CKII phosphorylates L1 at serine 1181, which is immediately C-terminal to the YRSLE sequence (Wong et al., 1996b). The function of CKII in axon growth is not clear, but lack of CKII in neuroblastoma cells inhibits formation of neurites (Ulloa et al., 1993). CKII phosphorylation appears to be part of the response to L1 coclustering with axonin-1/Tag-1. It has been shown that L1 cytoplasmic domain phosphorylation is triggered in response to crosslinking L1 or axonin-1 and that the phosphorylation can be inhibited by the CKII inhibitor heparin (Kunz et al., 1996). Because axonin-1 and L1 have been shown to participate cooperatively during neurite outgrowth on some substrates (Buchstaller et al., 1996), it might be possible that CKII contributes to these processes. As mentioned above, L1 clustering activates the MAP kinase pathway, which results in L1 phosphorylation in areas of the cytoplasmic domain that are predicted to prevent interactions with ankyrin and indirectly the actin cytoskeleton (Schaefer et al., 1999). Therefore, second-messenger systems activated by CAMs, such as L1, can feedback to influence the adhesive functions of the CAMs in fairly direct ways.

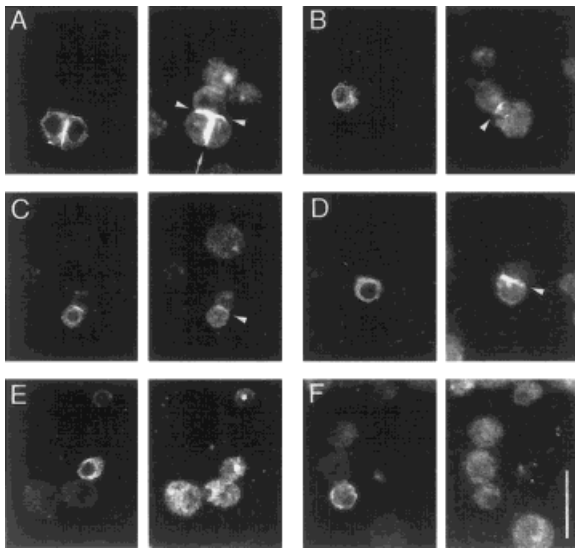


Figure 4 Ankyrin recruitment to human L1 is induced by homophilic TAG-1-mediated cell adhesion. S2 cells expressing human TAG-1 protein were mixed at a ratio of 20:1 with S2 cells coexpressing human TAG-1 and human L1 (A)–(D) or with S2 cells expressing only human L1 (E)–(F). This ratio of cells was selected to maximize the number of cell contacts between cells expressing both adhesion molecules with cells expressing only TAG-1, rather than contacts between cells expressing human L1 and TAG-1. After protein induction, cells were briefly allowed to aggregate and subsequently processed for double immunofluorescence using a rabbit anti-L1-CAM antiserum (left panels) and a mouse anti-*Drosophila* ankyrin antiserum (right panels). The *arrow* in A marks a cell contact between two cells expressing L1 as well as TAG-1. Cell contacts between cells expressing TAG-1 with cell expressing both adhesion molecules are indicated by *arrowheads*. Scale bar is 25 μm . (This figure appeared originally in Malhotra et al., 1998 © American Society for Biochemistry and Molecular Biology.) Note that L1 does not bind to Tag-1 on an opposed membrane (Buchstaller et al., 1996).

CONCLUSIONS

The models of the role of adhesion molecules in cell motility that have evolved over the past 10 years appear to apply nicely to growth cone movement as well. CAMs are targeted specifically to the growth cone where they are used over and over. The cycle begins with internalization at the rear of the growth cone via clathrin-coated pits. Movement of CAMs along microtubules permits reinsertion into the plasma membrane at the leading edge. When the CAMs bind to a ligand on an opposing membrane or are recruited to a contact site by a *cis*-interaction with a companion, they generate signals that attract molecules that provide linkage to the actin cytoskeleton.

When the adhesion complexes have completed their service and find themselves at the rear of the growth cone, the structures are disassembled, probably through the coordinated work of kinases and phosphatases. Then the CAMs are internalized to be shipped back to the front for another tour of duty. By using molecules with specific recognition sequences for sorting, internalization, and trafficking and by localizing the associated recognition machinery in very specific regions of the axon and growth cone, neurons are able to achieve incredibly precise control of when and where CAMs are expressed. Because CAMs are also expressed in the mature nervous system at sites involved in synaptic plasticity and sprouting, it is likely that the controlled internalization and expression of CAMs plays an important role in these processes, using similar to those molecular mechanisms required for axon growth and guidance.

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REFERENCES

- Acheson A, Sunshine JL, Rutishauser U. 1991. NCAM polysialic acid can regulate both cell-cell and cell-substrate interactions. *J Cell Biol* 114:143–153.
- Ahn S, Maudsley S, Luttrell LM, Lefkowitz RJ, Daaka Y. 1999. Src-mediated tyrosine phosphorylation of dynamin is required for beta2-adrenergic receptor internalization and mitogen-activated protein kinase signaling. *J Biol Chem* 274:1185–1188.
- Bailey CH, Chen M, Keller F, Kandel ER. 1992. Serotonin-mediated endocytosis of apCAM: an early step of learning-related synaptic growth in *Aplysia*. *Science* 256:645–649.
- Bailey CH, Kaang BK, Chen M, Martin KC, Lim CS, Casadio A, Kandel ER. 1997. Mutation in the phosphorylation sites of MAP kinase blocks learning-related internalization of apCAM in *Aplysia* sensory neurons [see comments]. *Neuron* 18:913–924.
- Benmerah A, Lamaze C, Begue B, Schmid SL, Dautry-Varsat A, Cerf-Bensussan N. 1998. AP-2/Eps15 interaction is required for receptor-mediated endocytosis. *J Cell Biol* 140:1055–1062.
- Bixby JL, Jhabvala P. 1992. Inhibition of tyrosine phosphorylation potentiates substrate-induced neurite growth. *J Neurobiol* 23:468–480.
- Blystone SD, Williams MP, Slater SP, Brown EJ. 1997.

- Requirement of Integrin B3 Tyrosine 747 for B3 Tyrosine Phosphorylation and Regulation of α v β 3 Avidity. *J Biol Chem* 272:28757–28761.
- Bradshaw JD, Lu P, Leytze G, Rodgers J, Schieven GL, Bennett KL, Linsley PS, Kurtz SE. 1997. Interaction of the cytoplasmic tail of CTLA-4 (CD152) with a clathrin-associated protein is negatively regulated by tyrosine phosphorylation. *Biochemistry* 36:15975–15982.
- Bray D. 1970. Surface movements during the growth of single explanted neurons. *Proc Natl Acad Sci USA* 65:905–910.
- Bretscher MS. 1992. Circulating integrins: α 5 β 1, α 6 β 4 and Mac-1, but not α 3 β 1, α 4 β 1 or LFA-1. *EMBO J* 11:405–410.
- Brümmendorf T, Rathjen FG. 1994. Cell adhesion molecules 1: Immunoglobulin superfamily. *Protein Profile* 1:963–1108.
- Brümmendorf T, Rathjen FG. 1996. Structure/function relationships of axon-associated adhesion receptors of the immunoglobulin superfamily. *Current Opinion in Neurobiology* 6:584–593.
- Buchstaller A, Kunz S, Berger P, Kunz B, Ziegler U, Rader C, Sonderegger P. 1996. Cell adhesion molecules Ng-CAM and axonin-1 form heterodimers in the neuronal membrane and cooperate in neurite outgrowth promotion. *J Cell Biol* 135:1593–1607.
- Burden-Gulley SM, Payne HR, Lemmon V. 1995. Growth cones are actively influenced by substrate-bound adhesion molecules. *J Neurosci* 15:4370–4381.
- Carew TJ, Walters ET, Kandel, ER. 1981. Classical conditioning in a simple withdrawal reflex in *Aplysia californica*. *J Neurosci* 1:1426–1437.
- Choquet D, Felsenfeld DP, Sheetz MP. 1997. Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. *Cell* 88:39–48.
- Cohen NR, Taylor JSH, Scott LB, Guillery RW, Soriano P, Furley AJW. 1997. Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. *Curr Biol* 8:26–33.
- Condeelis J, Bresnick A, Demma M, Dharmawardhane S, Eddy R, Hall AL, Sauterer R, Warren V. 1990. Mechanisms of amoeboid chemotaxis: an evaluation of the cortical expansion model. *Dev Genet* 11:333–340.
- Condic ML, Letourneau PC. 1997. Ligand-induced changes in integrin expression regulate neuronal adhesion and neurite outgrowth. *Nature* 389:852–856.
- Craig AM, Banker G. 1994. Neuronal polarity. *Annu Rev Neurosci* 17:267–310.
- Dahlin-Huppe K, Berglund EO, Ranscht B, Stallcup WB. 1997. Mutational analysis of the L1 neuronal cell adhesion molecule identifies membrane-proximal amino acids of the cytoplasmic domain that are required for cytoskeletal anchorage. *Mol Cell Neurosci* 9:144–156.
- Dahme M, Bartsch U, Martini R, Anliker B, Schachner M, Mantei N. 1997. Disruption of the mouse L1 gene leads to malformations of the nervous system. *Nat Genet* 17:346–349.
- Dai JW, Sheetz MP. 1995. Mechanical properties of neuronal growth cone membranes studied by tether formation with laser optical tweezers. *Biophys J* 68:988–996.
- Damke H, Baba T, Warnock DE, Schmid SL. 1994. Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. *J Cell Biol* 127:915–934.
- Davis GW, Schuster CM, Goodman CS. 1997. Genetic analysis of the mechanisms controlling target selection: target-derived Fasciclin II regulates the pattern of synapse formation. *Neuron* 19:561–573.
- Dell'Angelica EC, Ohno H, Ooi CE, Rabinovich E, Roche KW, Bonifacino JS. 1997. AP-3: an adaptor-like protein matical model for the effects of adhesion and mechanics on cell migration speed. *Biophys J* 60:15–37.
- Doherty P, Furness J, Williams EJ, Walsh FS. 1994. Neurite outgrowth stimulated by the tyrosine kinase inhibitor herbimycin a requires activation of tyrosine kinases and protein kinase c. *J Neurochem* 62:2124–2131.
- Dotti CG, Parton RG, Simons K. 1991. Polarized sorting of glypiated proteins in hippocampal neurons. *Nature* 349:158–161.
- Dotti CG, Simons, K. 1990. Polarized sorting of viral glycoproteins to the axon and dendrites of hippocampal neurons in culture. *Cell* 62:63–72.
- Faivresarrailh C, Gennarini G, Goridis C, Rougon G. 1992. F3/F11 Cell surface molecule expression in the developing mouse cerebellum is polarized at synaptic sites and within granule cells. *J Neurosci* 12:257–267.
- Faivresarrailh C, Rougon G. 1993. Are the glypiated adhesion molecules preferentially targeted to the axonal compartment. *Mol Neurobiol* 7:49–60.
- Fannon AM, Colman DR. 1996. A model for central synaptic junctional complex formation based on the differential adhesive specificities of the cadherins. *Neuron* 17:423–434.
- Fazeli MS, Breen K, Errington ML, Bliss TV. 1994. Increase in extracellular NCAM and amyloid precursor protein following induction of long-term potentiation in the dentate gyrus of anaesthetized rats. *Neurosci Lett* 169:77–80.
- Felsenfeld DP, Choquet D, Sheetz MP. 1996. Ligand binding regulates the directed movement of β 1 integrins on fibroblasts. *Nature* 383:438–440.
- Forscher P, Lin CH, Thompson C. 1992. Novel form of growth cone motility involving site-directed actin filament assembly. *Nature* 357:515–518.
- Fransen E, D'Hooge R, Van Camp G, Verhoye M, Sijbers J, Reyniers E, Soriano P, Kamiguchi H, Willemsen R, Koekkoek SKE, De Zeeuw CI, De Deyn PP, Van der Linden A, Lemmon V, Kooy RF, Willems PJ. 1998. L1 knockout mice show dilated ventricles, vermis hypoplasia and impaired exploration patterns. *Hum Mol Genet* 7:999–1009.
- Garver TD, Davis JQ, Ren Q, Bennett V. 1996. Association of ankyrin with the neuronal cell adhesion molecule neurofascin: regulation by both tyrosine phosphorylation and alternative exon usage. *Mol Biol Cell (Suppl)* 7:384a.
- Hemperly JJ, Edelman GM, Cunningham BA. 1986. cDNA clones of the neural cell adhesion molecule (N-CAM)

- lacking a membrane-spanning region consistent with evidence for membrane attachment via a phosphatidylinositol intermediate Proc Natl Acad Sci USA 83:9822–9826.
- Higgins D, Burack M, Lein P, Banker G. 1997. Mechanisms of neuronal polarity. *Curr Opin Neurobiol* 7:599–604.
- Hinshaw, JE, Schmid SL. 1995. Dynamin self-assembles into rings suggesting a mechanism for coated vesicle budding. *Nature* 374:190–192.
- Hollenbeck PJ, Ruthel G. 1999. Sorting out the neuron. *Nature* 397:653.
- Hortsch M. 1996. The L1 family of neural cell adhesion molecules: old proteins performing new tricks. *Neuron* 17:587–593.
- Hu Y, Barzilai A, Chen M, Bailey CH, Kandel ER. 1993. 5-HT and cAMP induce the formation of coated pits and vesicles and increase the expression of clathrin light chain in sensory neurons of *aplysia*. *Neuron* 10:921–929.
- Ignelzi MA, Miller DR, Soriano P, Maness PF. 1994. Impaired neurite outgrowth of src-minus cerebellar neurons on the cell adhesion molecule L1. *Neuron* 12:873–884.
- Incardona JP, Rosenberry TL. 1996. Replacement of the glycoinositol phospholipid anchor of *Drosophila* acetylcholinesterase with a transmembrane domain does not alter sorting in neurons and epithelia but results in behavioral defects. *Molecular Biology of the Cell* 7:613–630.
- Kamei Y, Tsutsumi O, Taketani Y, Watanabe K. 1998. cDNA cloning and chromosomal localization of neural adhesion molecule NB-3 in human. *J Neurosci Res* 51:275–283.
- Kamiguchi H, Hlavin ML, Lemmon V. 1998a. Role of L1 in neural development: what the knockouts tell us. *Mol Cell Neurosci* 12:48–55.
- Kamiguchi H, Lemmon V. 1997. Neural cell adhesion molecule L1: signaling pathways and growth cone motility. *J Neurosci Res* 49:1–8.
- Kamiguchi H, Lemmon V. 1998. A neuronal form of the cell adhesion molecule L1 contains a tyrosine-based signal required for sorting to the axonal growth cone. *J Neurosci* 18:3749–3756.
- Kamiguchi H, Lemmon V. 2000. Recycling of the cell adhesion molecule L1 in axonal growth cones. *J Neurosci* 20:3676–3686.
- Kamiguchi H, Long KE, Pendergast M, Schaefer AW, Rapoport I, Kirchhausen T, Lemmon V. 1998b. The neural cell adhesion molecule L1 interacts with the AP-2 adaptor and is endocytosed via the clathrin-mediated pathway. *J Neurosci* 18:5311–5321.
- Kollins KM, Powell SK, Rivas RJ. 1999. GPI-anchored human placental alkaline phosphatase has a nonpolarized distribution on the cell surface of mouse cerebellar granule neurons in vitro. *J Neurobiol* 39:119–141.
- Kunz S, Ziegler U, Kunz B, Sonderegger P. 1996. Intracellular signaling is changed after clustering of the neural cell adhesion molecules axonin-1 and NgCAM during neurite fasciculation. *J Cell Biol* 135:253–267.
- Lagenaur C, Lemmon V. 1987. An L1-like molecule, the 8D9 antigen, is a potent substrate for neurite extension. *Proc Natl Acad Sci USA* 84:7753–7757.
- Lauffenburger DA, Horwitz AF. 1996. Cell migration: a physically integrated molecular process. *Cell* 84:359–369.
- Lawson MA, Maxfield FR. 1995. Ca²⁺- and calcineurin-dependent recycling of an integrin to the front of migrating neutrophils. *Nature* 377:75–79.
- Le Gall AH, Powell SK, Yeaman CA, Rodriguez-Boulan E. 1997. The neural cell adhesion molecule expresses a tyrosine-independent basolateral sorting signal. *J Biol Chem* 272:4559–4567.
- Le TL, Yap AS, Stow JL. 1999. Recycling of E-cadherin: a potential mechanism for regulating cadherin dynamics. *J Cell Biol* 146:219–232.
- Lemmon V, Farr K, Lagenaur C. 1989. L1-mediated axon outgrowth occurs via a homophilic binding mechanism. *Neuron* 2:1597–1603.
- Lin CH, Forscher P. 1995. Growth cone advance is inversely proportional to retrograde f-actin flow. *Neuron* 14:763–771.
- Lustig M, Sakurai T, Grumet M. 1999. Nr-CAM promotes neurite outgrowth from peripheral ganglia by a mechanism involving axonin-1 as a neuronal receptor. *Dev Biol* 209:340–351.
- Malhotra JD, Tsiotra P, Karagogeos D, Hortsch M. 1998. Cis-activation of L1-mediated ankyrin recruitment by TAG-1 homophilic cell adhesion. *J Biol Chem* 273:33354–33359.
- Marks MS, Woodruff L, Ohno H, Bonifacino JS. 1996. Protein targeting by tyrosine- and di-leucine-based signals: evidence for distinct saturable components. *J Cell Biol* 135:341–354.
- Martin KC, Michael D, Rose JC, Barad M, Casadio A, Zhu H, Kandel ER. 1997. MAP kinase translocates into the nucleus of the presynaptic cell and is required for long-term facilitation in *Aplysia*. *Neuron* 18:899–912.
- Mellman I. 1996. Endocytosis and molecular sorting. *Annu Rev Cell Dev Biol* 12:575–625.
- Miura M, Kobayashi M, Asou H, Uyemura K. 1991. Molecular cloning of cDNA encoding the rat neural cell adhesion molecule L1—two L1 isoforms in the cytoplasmic region are produced by differential splicing. *FEBS Lett* 289:91–95.
- Mogilner A, Oster G. 1996. Cell motility driven by actin polymerization. *Biophysical Journal* 71:3030–3045.
- Montgomery AMP, Becker JC, Siu CH, Lemmon VP, Cheresch DA, Pancook JD, Zhao X, Reisfeld RA. 1996. Human neural cell adhesion molecule L1 and rat homologue NILE are ligands for integrin $\alpha V\beta 3$. *J Cell Biol* 132:475–485.
- Moos M, Tacke R, Scherer H, Teplow D, Fruh K, Schachner M. 1988. Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. *Nature* 334:701–703.
- Morris RJ, Beech JN, Barber PC, Raisman G. 1985. Early stages of Purkinje cell maturation demonstrated by Thy-1

- immunohistochemistry on postnatal rat cerebellum. *Journal of Neurocytology* 14:427–452.
- Newman LS, McKeever MO, Okano HJ, Darnell RB. 1995. β -NAP, a cerebellar degeneration antigen, is a neuron-specific vesicle coat protein. *Cell* 82:773–783.
- Ohno H, Fournier MC, Poy G, Bonifacino JS. 1996. Structural determinants of interaction of tyrosine-based sorting signals with the adaptor medium chains. *J Biol Chem* 271:29009–29015.
- Ohno H, Stewart J, Fournier MC, Bosshart H, Rhee I, Miyatake S, Saito T, Gallusser A, Kirchhausen T, Bonifacino JS. 1995. Interaction of tyrosine-based sorting signals with clathrin-associated proteins. *Science* 269:1872–1875.
- Oostendorp RA, Dormer P. 1997. VLA-4-mediated interactions between normal human hematopoietic progenitors and stromal cells. *Leukemia Lymphoma* 24:423–435.
- Palecek SP, Horwitz AF, Lauffenburger DA. 1999. Kinetic model for integrin-mediated adhesion release during cell migration. *Ann Biomed Eng* 27:219–235.
- Palecek SP, Schmidt CE, Lauffenburger DA, Horwitz AF. 1996. Integrin dynamics on the tail region of migrating fibroblasts. *J Cell Sci* 109:941–952.
- Peskin CS, Odell GM, Oster GF. 1993. Cellular motions and thermal fluctuations: the Brownian ratchet. *Biophys J* 65:316–324.
- Peter N, Aronoff B, Wu F, Schacher S. 1994. Decrease in growth cone neurite fasciculation by sensory or motor cells in vitro accompanies downregulation of aplysia cell adhesion molecules by neurotransmitters. *J Neurosci*.
- Pomies P, Frachet P, Block MR. 1995. Control of the alpha 5 beta 1 integrin/fibronectin interaction in vitro by the serine/threonine protein phosphatase calcineurin. *Biochemistry* 34:5104–5112.
- Powell SK, Cunningham BA, Edelman GM, Rodriguez-Boulan E. 1991. Targeting of transmembrane and GPI-anchored forms of N-CAM to opposite domains of a polarized epithelial cell. *Nature* 353:76–77.
- Rapoport I, Miyazaki M, Boll W, Duckworth B, Cantley LC, Shoelson S, Kirchhausen T. 1997. Regulatory interactions in the recognition of endocytic sorting signals by AP-2 complexes. *EMBO J* 16:2240–2250.
- Rodriguez-Boulan E, Powell SK. 1992. Polarity of epithelial and neuronal cells. *Annu Rev Cell Biol* 8:395–427.
- Santoni MJ, Barthels D, Vopper G, Boned A, Goridis C, Wille W. 1989. Differential exon usage involving an unusual splicing mechanism generates at least eight types of NCAM cDNA in mouse brain. *EMBO J* 8:385–392.
- Schaefer A, Lemmon V. 1998. Signal transduction, neurite growth and Ig superfamily adhesion molecules in the vertebrate nervous system. In: Sonderegger P, editor. *Ig superfamily molecules in the nervous system*. Amsterdam, Netherlands: Harwood Academic Publishers. p. 313.
- Schaefer AW, Kamiguchi H, Wong EV, Beach CM, Landreth G, Lemmon V. 2000. Activation of the MAPK Signal Cascade by the Neural Cell Adhesion Molecule L1 Requires L1 Internalization. *J Biol Chem* (274:37965–37973).
- Schmid SL, McNiven MA, De Camilli P. 1998. Dynamin and its partners: a progress report. *Curr Opin Cell Biol* 10:504–512.
- Schmidt CE, Dai J, Lauffenburger DA, Sheetz MP, Horwitz AF. 1995. Integrin-cytoskeletal interactions in neuronal growth cones. *J Neurosci* 15:3400–3407.
- Schuster CM, Davis GW, Fetter RD, Goodman CS. 1996. Genetic dissection of structural and functional components of synaptic plasticity. II. Fasciclin II controls presynaptic structural plasticity. *Neuron* 17:655–667.
- Shapiro L, Colman DR. 1999. The diversity of cadherins and implications for a synaptic adhesive code in the CNS. *Neuron* 23:427–430.
- Sheetz MP, Baumrind NL, Wayne DB, Pearlman AL. 1990. Concentration of membrane antigens by forward transport and trapping in neuronal growth cones. *Cell* 61:231–241.
- Sheetz MP, Felsenfeld DP, Galbraith CG. 1998. Cell migration: regulation of force on extracellular-matrix-integrin complexes. *Trends Cell Biol* 8:51–54.
- Shiratori T, Miyatake S, Ohno H, Nakaseko C, Isono K, Bonifacino JS, Saito T. 1997. Tyrosine phosphorylation controls internalization of CTLA-4 by regulating its interaction with clathrin-associated adaptor complex AP-2. *Immunity* 6:583–589.
- Simons K, Wandinger-Ness A. 1990. Polarized sorting in epithelia. *Cell* 62:207–210.
- Simpson F, Peden AA, Christopoulou L, Robinson MS. 1997. Characterization of the adaptor-related protein complex, AP-3. *J Cell Biol* 137:835–845.
- Soriano P, Montgomery C, Geske R, Bradley A. 1991. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell* 64:693–702.
- Spaltmann F, Brummendorf T. 1996. CEPU-1, a novel immunoglobulin superfamily molecule, is expressed by developing cerebellar Purkinje cells. *J Neurosci* 16:1770–1779.
- Stein PL, M LH, Rich S, Soriano P. 1992. p59fyn mutant mice display differential signaling in thymocytes and peripheral T cells. *Cell* 70:741–750.
- Stoeckli ET, Landmesser LT. 1995. Axonin-1, Nr-CAM, and Ng-CAM play different roles in the in vivo guidance of chick commissural neurons. *Neuron* 14:1165–1179.
- Stoeckli ET, Landmesser LT. 1998. Axon guidance at choice points. *Curr Opin Neurobiol* 8:73–79.
- Stoeckli ET, Sonderegger P, Pollerberg GE, Landmesser LT. 1997. Interference with axonin-1 and NrCAM interactions unmasks a floor-plate activity inhibitory for commissural axons. *Neuron* 18:209–221.
- Suter DM, Errante LD, Belotserkovsky V, Forscher P. 1998. The Ig superfamily cell adhesion molecule, apCAM, mediates growth cone steering by substrate-cytoskeletal coupling. *J Cell Biol* 141:227–240.
- Takeda Y, Asou H, Murakami Y, Miura M, Kobayashi M, Uyemura K. 1996. A nonneuronal isoform of cell adhe-

- sion molecule L1: Tissue-specific expression and functional analysis. *J Neurochem* 66:2338–2349.
- Tanaka E, Sabry J. 1995. Making the connection: Cytoskeletal rearrangements during growth cone guidance. *Cell* 83:171–176.
- Tebar F, Sorkina T, Sorkin A, Ericsson M, Kirchhausen T. 1996. Eps15 is a component of clathrin-coated pits and vesicles and is located at the rim of coated pits. *J Biol Chem* 271:28727–28730.
- Tienari PJ, De Strooper B, Ikonen E, Simons M, Weidemann A, Czech C, Hartmann T, Ida N, Multhaup G, Masters CL, Van Leuven F, Beyreuther K, Dotti CG. 1996. The beta-amyloid domain is essential for axonal sorting of amyloid precursor protein. *EMBO J* 15:5218–5229.
- Trowbridge IS, Collawn JF, Hopkins CR. 1993. Signal-dependent membrane protein trafficking in the endocytic pathway. *Annu Rev Cell Biol* 9:129–161.
- Ulloa L, Diaznido J, Avila J. 1993. Depletion of casein kinase-II by antisense oligonucleotide prevents neuritogenesis in neuroblastoma cells. *EMBO J* 12:1633–1640.
- van Delft S, Schumacher C, Hage W, Verkleij AJ, van Bergen en Henegouwen PMP. 1997. Association and colocalization of Eps15 with adaptor protein-2 and clathrin. *J Cell Biol* 136:811–821.
- van der Blik AM, Redelmeier TE, Damke H, Tisdale EJ, Meyerowitz EM, Schmid SL. 1993. Mutations in human dynamin block an intermediate stage in coated vesicle formation. *J Cell Biol* 122:553–563.
- Ware MF, Tice DA, Parsons SJ, Lauffenburger DA. 1997. Overexpression of cellular Src in fibroblasts enhances endocytic internalization of epidermal growth factor receptor. *J Biol Chem* 272:30185–30190.
- Warren RA, Green FA, Stenberg PE, Enns CA. 1998. Distinct saturable pathways for the endocytosis of different tyrosine motifs. *J Biol Chem* 273:17056–17063.
- West AE, Neve RL, Buckley KM. 1997a. Identification of a somatodendritic targeting signal in the cytoplasmic domain of the transferrin receptor. *J Neurosci* 17:6038–6047.
- West AE, Neve RL, Buckley KM. 1997b. Targeting of the synaptic vesicle protein synaptobrevin in the axon of cultured hippocampal neurons: evidence for two distinct sorting steps. *J Cell Biol* 139:917–927.
- Williams EJ, Furness J, Walsh FS, Doherty P. 1994. Characterisation of the second messenger pathway underlying neurite outgrowth stimulated by FGF. *Development* 120:1685–1693.
- Winckler B, Forscher P, Mellman I. 1999. A diffusion barrier maintains distribution of membrane proteins in polarized neurons. *Nature* 397:698–701.
- Wong EV, Schaefer A, Landreth G, Lemmon V. 1996a. Involvement of p90rsk in neurite outgrowth mediated by the cell adhesion molecule L1. *J Biol Chem* 271:18217–18223.
- Wong EV, Schaefer AW, Landreth G, Lemmon V. 1996b. Casein kinase II phosphorylates the neural cell adhesion molecule L1. *J Neurochem* 66:779–786.
- Wu Q, Maniatis T. 1999. A striking organization of a large family of human neural cadherin-like cell adhesion genes. *Cell* 97:779–790.
- Xie H, Pallero MA, Gupta K, Chang P, Ware MF, Witke W, Kwiatkowski DJ, Lauffenburger DA, Murphy-Ullrich JE, Wells A. 1998. EGF receptor regulation of cell motility: EGF induces disassembly of focal adhesions independently of the motility-associated PLCgamma signaling pathway. *J Cell Sci* 111:615–624.
- Xue GP, Calvert RA, Morris RJ. 1990. Expression of the neuronal surface glycoprotein Thy-1 is under post-transcriptional control, and is spatially regulated, in the developing olfactory system. *Development* 109:851–864.
- Xue GP, Morris RJ. 1990. Evidence for cell-type differences in the regulation of neuronal expression of Thy-1. *Biochem Soc Trans* 18:441–442.
- Yamasaki M, Thompson P, Lemmon V. 1997. CRASH syndrome: mutations in L1CAM correlate with severity of the disease. *Neuropediatrics* 28:175–178.
- Yamazaki T, Selkoe DJ, Koo EH. 1995. Trafficking of cell surface beta-amyloid precursor protein: retrograde and transcytotic transport in cultured neurons. *Journal of Cell Biology* 129:431–442.
- Yoshihara Y, Kawasaki M, Tani A, Tamada A, Nagata S, Kagamiyama H, Mori K. 1994. BIG-1: a new TAG-1/F3-related member of the immunoglobulin superfamily with neurite outgrowth-promoting activity. *Neuron* 13:415–426.
- Zhu H, Wu F, Schacher S. 1995. Changes in expression and distribution of aplysia cell adhesion molecules can influence synapse formation and elimination in vitro. *J Neurosci* 15:4173–4183.