

# The role of cell adhesion molecule L1 in axonal extension, growth cone motility, and signal transduction

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**Abstract.** Axonal pathfinding is a complex process dependent on cell-cell and cell-matrix interactions. L1 is a cell adhesion molecule that is abundant in the nervous system and that is concentrated on axons. As a culture substrate, L1 is a potent promoter of neurite outgrowth and elicits specific growth cone behavior. It interacts with the actin cytoskeleton via an ankyrin linkage and promotes specific distribution of F-actin within the growth cone. In addition, L1 has been implicated in signal transduction. For example, L1 is associated with kinases, L1-L1 binding regulates second messenger systems, and mutations in the L1 gene in humans result in abnormalities in the development of the corticospinal tract and corpus callosum. In this short review, recent advances in understanding the way in which L1 regulates growth cone behavior will be discussed.

**Key words:** Cytoskeleton – Cell adhesion molecule L1 – Axonal extension – Growth cones – Signal transduction

## Introduction

During the development of the nervous system, hundreds of millions of neurons must make appropriate connections with their respective target cells. Axons often traverse long distances through complex territories to reach their targets. A number of factors are known to be involved in axonal pathfinding, including growth promoting molecules, such as cell adhesion molecules (CAMs)

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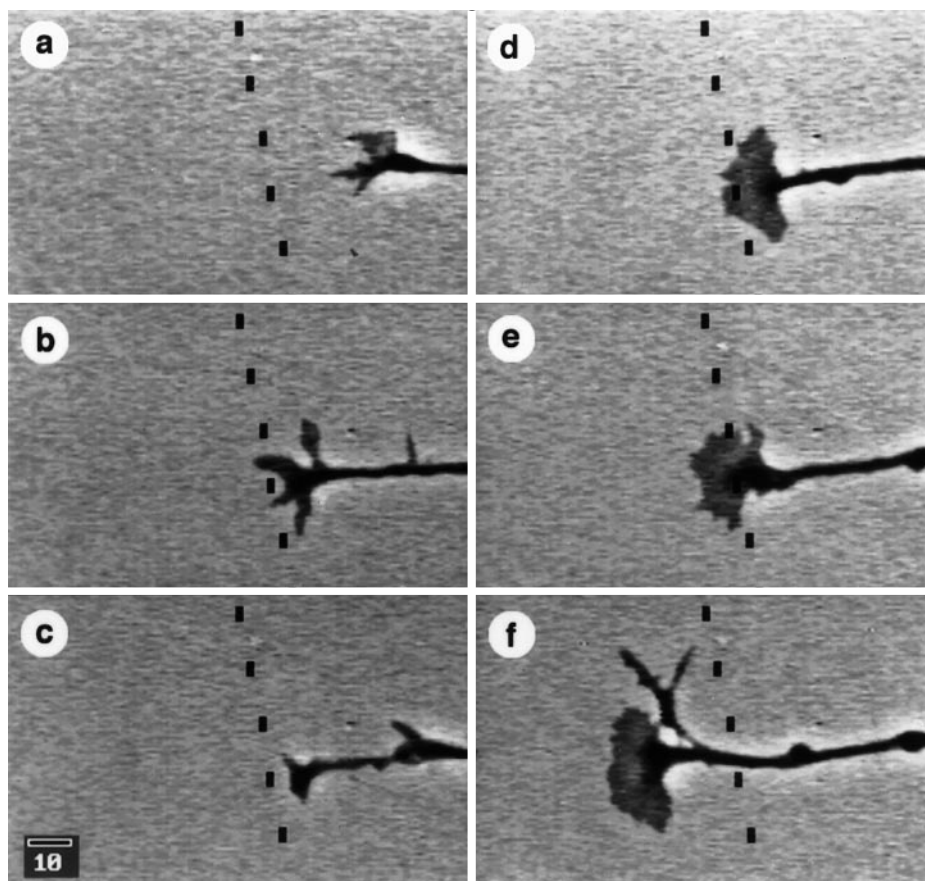
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and extracellular matrix molecules, physical channels, and diffusible molecules, such as chemoattractants and chemorepellants, which are thought to act in concert to guide axons. Reviews of many of these topics are found elsewhere in this issue. Our review will focus on L1, an adhesion molecule of the immunoglobulin superfamily, and its role in axonal outgrowth, growth cone motility, and signal transduction.

## L1-mediated adhesion

The L1 protein consists of six immunoglobulin domains, five fibronectin type 3 domains, a single transmembrane region, and an intracellular domain (Moos et al. 1988). Currently, two alternatively spliced forms of L1 are known. One form contains four amino acids, viz., RSLE (Arg-Ser-Leu-Glu), in the cytoplasmic domain plus a short stretch of extra amino acids near the N-terminus of L1; it is primarily expressed in neurons. The form lacking the RSLE domain and the N-terminal sequence is expressed in non-neuronal cells such as Schwann cells (Miura et al. 1991; Takeda et al. 1996). L1 binds in a homophilic fashion, i.e., one L1 molecule on the surface of one cell binds in a trans fashion with another L1 molecule on the surface of a second cell (McClay and Ettensohn 1987; Lemmon et al. 1989). In addition to promoting cell-cell adhesion, L1 is also a potent promoter of neurite outgrowth when the purified protein is used as a culture substrate or is expressed in cells (Lagenaur and Lemmon 1987; Williams et al. 1992). L1 homophilic binding occurs via the second Ig domain, a region that is required for neurite outgrowth from retinal cells (Zhao and Siu 1995). The homophilic adhesion function of L1 is relatively independent of its cytoplasmic domain (Hortsch et al. 1995; Wong et al. 1995a). L1 has also been shown to bind heterophilically with a number of other proteins, including TAG-1/axonin-1 (Kuhn et al. 1991), DM-GRASP (DeBernardo and Chang 1996), F3/F11/contactin (Brummendorf and Rathjen 1993), and the chondroitin sulfate proteoglycans phosphacan and neurocan (Milev



**Fig. 1a–f.** Time-lapse series of an RGC growth cone initiating growth on laminin and contacting L1 at a border region. Images of the growth cone are shown at 0:00 (a), 7:30 (b), 9:30 (c), 16:30 (d), 21:30 (e), and 37:30 (f) min after the first frame (a); time is denoted as min:sec. The border is indicated by the dashed line. The growth cone collapses (c) within minutes of initial contact with the L1 (b). Upon recovery and the second contact with L1 (d), the growth cone rapidly changes to the large fan-shaped morphology normally observed on L1, even though a large portion of the growth cone remains on the laminin. The growth cone crosses rapidly onto L1 after the second contact with the border (d–f). Bar: 10  $\mu$ m (reprinted from *J Neurosci* 15:4370–81, 1995, with permission)

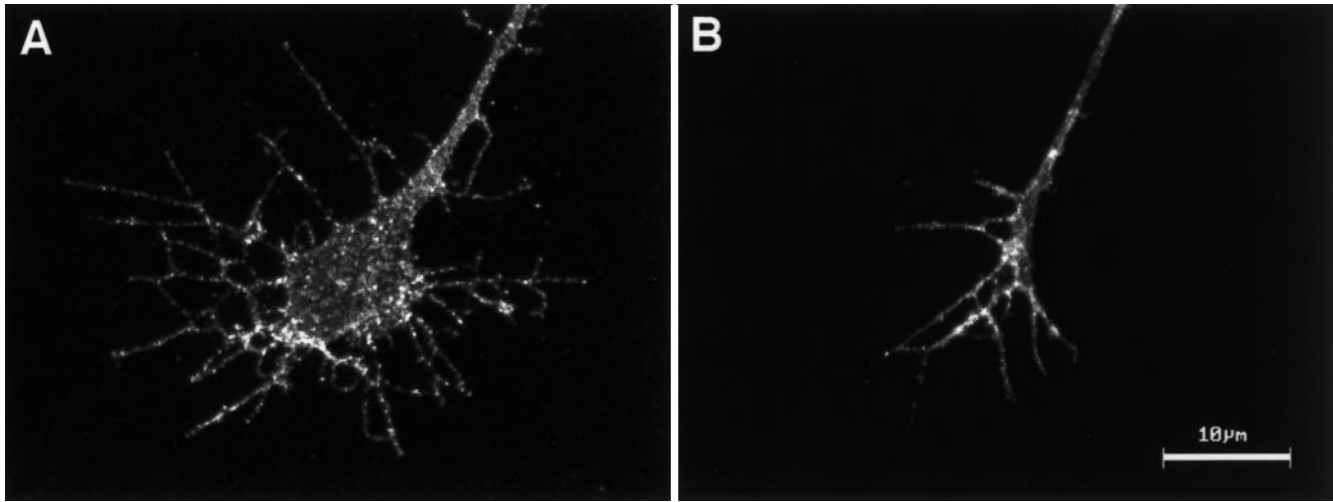
et al. 1994), laminin (Grumet et al. 1993), and a subset of integrins ( $\alpha_5\beta_1$ ,  $\alpha_v\beta_3$ ; Ruppert et al. 1995; Montgomery et al. 1996). Other than the promotion of cell-cell adhesion, the function of many of these heterophilic associations is still unclear, although interaction of TAG-1/axonin-1, F3/F11, or DM-GRASP with L1 can result in neurite outgrowth (Kuhn et al. 1991; Morales et al. 1993; DeBernardo and Chang 1996). Perhaps the most intriguing possibility is that cis rather than trans interactions between TAG-1/axonin-1 and L1 play a crucial role in regulating L1-mediated neurite growth (Buchstaller et al. 1996; Rader et al. 1996). These results illustrate the complexity of protein interactions that occur with L1 and suggest that L1 has different functions depending on the ligands to which it binds.

### Promotion of neurite growth by L1

Purified adhesion molecules can be used *in vitro* to examine neurite growth (Lagenaur and Lemmon 1987). For example, chick retinal ganglion cells (RGC) exhibit specific growth characteristics dependent upon the growth substrate (Lemmon et al. 1992; Payne et al. 1992). On L1, neurites form a dense but defasciculated monolayer. In contrast, neurites on laminin are highly fasciculated. The fasciculation of axons on laminin is the result in part of L1-L1 interactions between contacting axons, since it can be reduced by adding antibodies against L1 to the

culture medium (Stallcup and Beasley 1985; Drazba and Lemmon 1990).

A highly motile, membranous extension called the growth cone occurs at the distal tip of growing axons. Growth cones are specialized sensory structures that are thought to interact with localized cues in the environment to produce the directed growth of axons toward their appropriate targets (Bentley and Toroian-Raymond 1986; O'Connor et al. 1990; Lin and Forscher 1993). Growth cones of RGC on L1 predominantly take the form of lamellipodia with short filopodia. In contrast, growth cones on laminin are smaller, with a reduced lamellipodial area and several long filopodial processes. When cultured on purified protein substrates, RGC growth cones are more than twice as adhesive to L1 than to laminin but will grow equally well on either substrate when given a choice (Lemmon et al. 1992). Examination of RGC growth cones with time-lapse videomicroscopy has revealed distinct behavioral patterns during growth on L1 or laminin substrates. For example, growth cones on laminin tend to extend and retract several filopodial processes, and the lamellipodial veils are frequently reshaped during forward movement. Growth cones on L1 maintain a large lamellipodium with little alteration in size or shape, although some ruffling is observed as they progress forward at approximately half the rate of growth cones on laminin (Burden-Gulley et al. 1995); they actively respond to the new growth substrate encountered at sharp borders between laminin and L1. As growth



**Fig. 2A, B.** DRG growth cones have specific morphologies on L1-fc chimera or laminin substrates. DRG neurons were dissociated and plated on dishes coated with the L1-fc chimera (**A**) or laminin (**B**). The cells were labeled with rabbit polyclonal antibody against L1 and viewed by confocal microscopy. Note the large lamellipodi-

al expanse of the growth cone on L1-fc chimera and the numerous filopodial processes (**A**). In contrast, the growth cone on laminin possesses several long filopodial processes, but a much reduced lamellipodium (**B**). Single confocal optical section, 0.826  $\mu\text{m}$  thickness. Bar: 10  $\mu\text{m}$

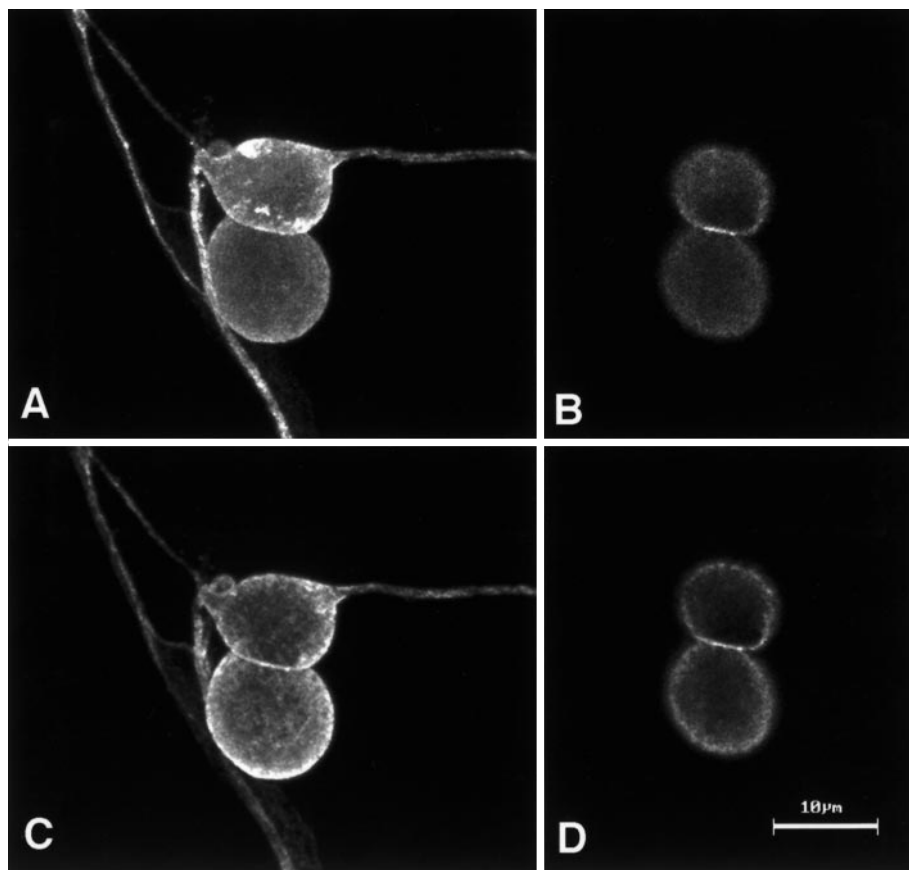
cones cross onto L1 from laminin, their morphology progressively changes from the small, predominantly filopodial type characteristically induced by laminin to a much larger fan-shaped type. This change in size occurs rapidly. Growth cones undergo an approximate 50% increase in size within the first minute of L1 contact (Burden-Gulley et al. 1995). In a subset of cases, initial contact with L1 from laminin resulted in transient growth cone collapse (Fig. 1c), which could have been a result of the reassembly of cytoskeletal elements stimulated by L1 contact (see below). In the example shown, the growth cone changed to the large fan-shaped morphology normally observed on L1, even though an extensive portion of the growth cone remained on the laminin (Fig. 1d). This result, in conjunction with the rapid rate of morphological change, suggests that L1 contact affects the intracellular signaling mechanisms that influence growth cone behavior.

The characteristic morphologies exhibited by growth cones on L1 and laminin are not limited to RGC neurons. When dorsal root ganglion (DRG) neurons are plated on laminin or L1-fc chimera substrates, they exhibit many characteristics similar to RGC neurons (Fig. 2; M. Pendergast and V. Lemmon, unpublished observations). DRG growth cones on laminin are dominated by long filopodial processes, with a small lamellipodial expanse. In contrast, a large lamellipodium is the predominant feature of DRG growth cones on L1-fc chimera, although they also possess many filopodial processes. Despite similar morphologies, some differences do exist between growth cones from RGC and DRG neurons. RGC growth cones are much more adhesive to L1 than to laminin, whereas DRG growth cones are less adhesive to L1 (Zheng et al. 1994). This could be a function of cell-specific signal transduction mechanisms that are initiated by L1 and that regulate the adhesiveness of L1 (see below; von Bohlen und Halbach et al. 1992).

### L1 and the cytoskeleton

Growth cone motility and axonal growth require coordinated interactions between cell surface molecules and intracellular elements. Receptors on the cell surface interact with ligands in the environment to mediate adhesion and direct growth. Insertion of new membrane at the leading edge of the growth cone is necessary for axonal extension, and a functional cytoskeleton is required to provide a structural scaffold throughout the axon and growth cone. Polymerization of globular actin into filaments allows rapid extension of filopodia from the growth cone and formation of transient lamellipodial veils (Smith 1988; Lewis and Bridgman 1992; Sheetz et al. 1992). Experiments with cytochalasins to disrupt actin filaments within growth cones have shown that the actin cytoskeleton is necessary for correct pathfinding in vivo (Bentley and Toroian-Raymond 1986; Chien et al. 1993) and for directed neurite outgrowth in vitro (Marsh and Letourneau 1984; Forscher and Smith 1988; S. M. Burden-Gulley, unpublished observations).

The L1 and cadherin families of adhesion molecules have been shown to bind indirectly to actin (Takeichi et al. 1992; Davis and Bennett 1994; reviewed in Gumbiner 1993), and the binding of these adhesion molecules on the surface of cells may mediate changes in the cytoskeletal scaffold, thereby resulting in stabilized adhesion or forward growth (reviewed in Lin et al. 1994). L1 has been shown to interact with the cytoplasmic protein ankyrin by means of a number of biochemical assays; ankyrin binds to the actin cytoskeleton by a spectrin bridge (Davis et al. 1993; Davis and Bennett 1994). Similar results have been acquired with the L1 homolog from *Drosophila*, viz., neuroglian, whose binding site for ankyrin is localized to the cytoplasmic domain (Dubreuil et al. 1996). The ankyrin-binding site in mammalian L1 has been localized to the C-terminus of the cy-



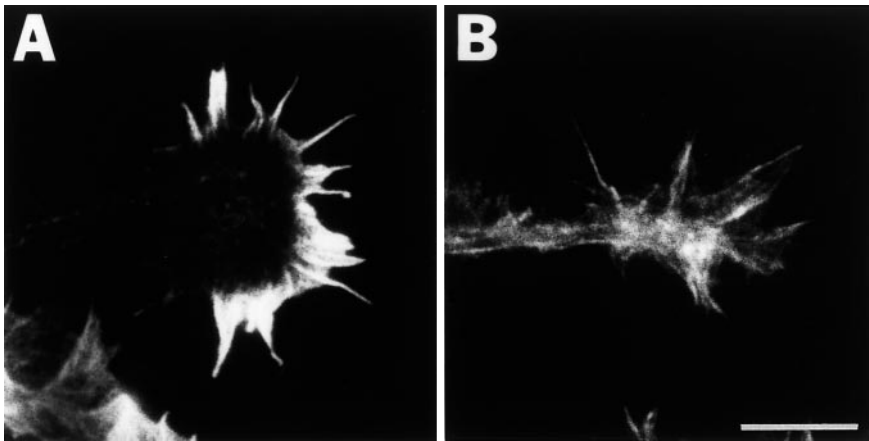
**Fig. 3A–D.** Co-localization of L1 and ankyrin at sites of cell-cell contact between DRG neurons. Chick DRG neurons were labeled with the 8D9 monoclonal antibody against L1 (**A, B**) and a rabbit antibody (Calbiochem) against chick erythrocyte ankyrin (**C, D**). The cells were viewed by confocal microscopy. Two contacting DRG cell bodies are presented as a compressed composite of 25 optical sections to show the entire cell body (**A, C**) and as a single 0.826- $\mu\text{m}$ -thick section to demonstrate the site of cell-cell contact (**B, D**). Both L1 and ankyrin are expressed along the surface of the cell bodies and axons, with a higher concentration and clear co-localization at the site of cell-cell contact (**B, D**). *Bar:* 10  $\mu\text{m}$

toplasmic domain (Davis and Bennett 1994), although the juxtamembrane region has also recently been shown to be involved in actin association (Dahlin-Huppe et al. 1997). Of interest, when neuroglian is expressed in *Drosophila* S2 cells that endogenously express ankyrin, it promotes the relocalization of ankyrin to the membrane and its concentration at sites of cell-cell contact (Dubreuil et al. 1996). L1 and ankyrin are also co-localized in chick neuronal cells at sites of cell-cell contact, providing further support for a functional interaction between L1 and ankyrin (Fig. 3; M. Pendergast and V. Lemmon, unpublished observations). These results suggest that adhesion of L1 on the surface of cells recruits cytoplasmic proteins such as ankyrin to points of cell-cell contact; this then stabilizes the adhesion through linkage with the membrane cytoskeleton. The finding that L1 involvement in outside-in signaling is conserved between *Drosophila* and higher vertebrates illustrates its importance (reviewed in Hortsch 1996).

When these results are extrapolated to the growing axon, it seems feasible that an L1-ankyrin interaction could be involved in stabilizing L1-mediated axonal fasciculation. In addition, this stabilization of adhesion may play a role in the generation of the force at the front of the growth cone necessary for forward movement (Lamoureaux et al. 1989; Lin et al. 1995). Retrograde flow of F-actin occurs in advancing growth cones, with the rate of flow being inversely correlated to the rate of forward growth (reviewed in Lin and Forscher

1995). When adhesion molecule receptors become activated by ligand binding, they are then thought to interact with molecular “clutch” proteins that mediate a linkage with the actin cytoskeleton (Lin et al. 1994; Schmidt et al. 1995). Ankyrin may be the clutch protein that links L1 to the cytoskeleton.

Certainly, growth cones on L1 display distinctive patterns of F-actin when compared with growth cones on other protein substrates (Fig. 4; Burden-Gulley and Lemmon 1996). RGC growth cones on laminin contain thick parallel actin filaments throughout the filopodia that extend deep into the growth cone central region (Fig. 4B). Other filaments of variable size fill both the peripheral and the central regions of the growth cone, resulting in a dense network of F-actin throughout the growth cone. This pattern is in sharp contrast with that observed in growth cones growing on L1 (Fig. 4A). In these growth cones, F-actin bundles originating in filopodia extend into the peripheral region but usually do not penetrate the central region of the growth cone. These bundles are then accompanied by a dense meshwork of fine microfilaments that fill the peripheral region but are not usually observed in the growth cone central body. This pattern of F-actin seems to occur under the direct influence of the growth substrate, since F-actin distribution has been observed to change in growth cones when they encounter a border between two substrates, and the change in pattern is to one appropriate for the new substrate encountered (Burden-Gulley and Lemmon 1996).



**Fig. 4A, B.** Distribution of F-actin in RGC growth cones. Growth cones growing on L1 (**A**) or laminin (**B**) were fixed, permeabilized, and incubated with rhodamine-phalloidin for the visualization of F-actin. Note the F-actin meshwork isolated in the peripheral portion of the growth cone on L1 (**A**) in comparison with the thick F-actin bundles throughout the growth cone on laminin (**B**). Bar: 10  $\mu$ m (reprinted from *Cell Motil Cytoskeleton* 35:1–23, 1996, with permission)

### L1 and second messenger systems

L1-induced neurite outgrowth is not only dependent on L1-L1 adhesion, but also requires activation of second messenger systems (reviewed in Doherty and Walsh 1996). The addition of purified CAMs or antibodies directed against CAMs results in changes in intracellular pH and in the levels of  $\text{Ca}^{2+}$ , inositol diphosphate, and inositol triphosphate (Schuch et al. 1989; von Bohlen und Halbach et al. 1992) and can stimulate neurite growth (Doherty et al. 1995). Doherty and colleagues have provided evidence suggesting that a G-protein-dependent activation of  $\text{Ca}^{2+}$  channels is involved in neurite outgrowth on N-cadherin, L1, or NCAM-expressing 3T3 cells, but not on laminin (Doherty et al. 1991; Saffell et al. 1992; Williams et al. 1992). An erbstatin-sensitive tyrosine kinase, PLCg, DAG lipase and, consequently, arachidonic acid also appear to be involved in the activation of the calcium channels for neurite outgrowth (reviewed in Doherty and Walsh 1996). The application of fibroblast growth factor (FGF) to cerebellar neurons also results in the activation of these intracellular messengers and enhanced neurite outgrowth (Williams et al. 1994). Of interest, a cell permeable PLCg inhibitor prevents neurite outgrowth stimulated by FGF and L1, NCAM, or N-cadherin (Doherty and Walsh 1996; Hall et al. 1996), suggesting that these molecules activate a common signaling pathway. A model has been proposed in which neurite outgrowth stimulated by these adhesion molecules requires a cis interaction with the FGF receptor (FGFR) in the plane of the membrane (reviewed in Doherty and Walsh 1996). Evidence to support this model includes the following: a CAM homology domain of about 20 amino acids is highly conserved between all FGFRs and shares homology with sequences in L1, NCAM, and N-cadherin (Williams et al. 1994). Neurons pretreated with antibodies against the FGFR or expressing a dominant negative form of the FGFR will not extend neurites following stimulation by the above CAMs, but integrin-stimulated neurite outgrowth is unaffected (Williams et al. 1994; Saffell et al. 1997). This model was originally described for trans homophilic interactions between CAMs, such as L1-L1 binding. However, it could also

be involved in neurite outgrowth stimulated by heterophilic interactions, although direct evidence for this is lacking.

Other signaling pathways may be activated following CAM binding. For example, L1 is a phosphoprotein that associates with and is phosphorylated by the serine/threonine kinases casein kinase II at serine<sup>1181</sup> (Wong et al. 1995c), and the S6 kinase, p90<sup>rsk</sup> at serine<sup>1152</sup> (Wong et al. 1996). Neurons loaded with peptides encompassing serine<sup>1152</sup> are prevented from extending neurites on L1 but not laminin substrates, suggesting that the p90<sup>rsk</sup> interaction with L1 is important for neurite outgrowth on L1 (Wong et al. 1996). The position of this serine residue is in close proximity to the juxtamembrane cytoplasmic domain of L1, a domain thought to mediate the interaction with the actin cytoskeleton (Dahlin-Huppe et al. 1997). Phosphorylation of this serine may therefore regulate an L1-actin interaction, although this has yet to be shown. Tyrosine kinases have also been suggested to regulate L1 function. For example, cerebellar neurons from *src*<sup>-</sup> mice display impaired neurite outgrowth on L1 but not on laminin, suggesting a partial role for pp60<sup>c-src</sup> in L1-mediated axonal growth (Ignelzi et al. 1994). Additionally, L1 has recently been shown to be phosphorylated on tyrosine (Heiland et al. 1996). L1-mediated adhesion and neurite outgrowth may be regulated by its interaction with the proteoglycan phosphacan/RPTP  $\zeta/\beta$ , a receptor type protein tyrosine phosphatase (Milev et al. 1994). The stimulation of protein phosphatase activity in growth cone membranes by an L1-dependent mechanism lends further support for a L1-phosphatase functional interaction (Klinz et al. 1995). An L1-phosphatase interaction is not surprising, since it provides a means for regulating L1-associated kinase activities; L1 is probably a substrate for several different phosphatases.

A likely candidate for mediating the L1-induced actin distribution and lamellipodial morphology in growth cones is rac, a member of the Ras superfamily. Rac is a small GTPase that has been shown to promote the formation of lamellipodial veils and membrane ruffling in Swiss 3T3 cells upon activation by growth factors (Ridley et al. 1992; Mackay et al. 1995). F-actin distribution in the veils of rac-activated 3T3 cells is remarkably similar

to that of growth cones on L1. It is concentrated in a meshwork of fine bundles near the periphery of the veils and throughout filopodial processes (Ridley et al. 1992). Rac is an essential element in the epidermal growth factor (EGF)-induced production of arachidonic acid and calcium influx that is required for cytoskeletal rearrangements in 3T3 cells (Peppelenbosch et al. 1995, 1996). The binding of EGF to its receptor is thought to activate rac through phosphatidylinositol-3 kinase (Nobes et al. 1995), with the subsequent production of phosphatidylinositol diphosphate, which is required for increased actin polymerization (reviewed in Ridley 1996). In addition, rac has been shown to activate pp70<sup>S6</sup> kinase (Ridley 1996). The parallels between the signaling pathways activated by rac and those activated by L1-binding for neurite outgrowth are striking. It has yet to be shown whether a similar rac signaling pathway is activated by growth factor binding to the FGFR, although evidence for a direct role of rac in axonal pathfinding has been obtained from *rac* gene mutants in *Drosophila* (Luo et al. 1994). It will be interesting to explore whether L1-induced effects on growth cone behavior involve rac activation.

### L1 and human brain development

Support for the idea that the L1 cytoplasmic domain is essential for regulating axon growth comes from an analysis of humans with mutations in the L1CAM gene. In 1992, Kenrick and associates reported that L1 mutations cause severe abnormalities of brain development (Rosenthal et al. 1992), including agenesis of the corpus callosum, corticospinal tract (CST) hypoplasia, hydrocephalus, and spasticity of the lower limbs (Wong et al. 1995b). The syndromes associated with L1CAM mutations include X-linked hydrocephalus, MASA syndrome, X-linked spastic paraplegia, and X-linked agenesis of the corpus callosum. These syndromes have been combined under the acronym CRASH syndrome for corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraplegia, and hydrocephalus (Fransen et al. 1995). At present, 80 families with 75 different mutations have been identified and are described on the L1 home page (<http://hgins.uia.ac.be/dna-lab/l1/>) maintained by P. Willems and associates. An analysis of individuals who have L1 mutations and whose clinical signs have been published reveals a striking correlation between the type of mutation and the severity of the disease (Yamasaki et al. 1997). Mutations that result in a truncation of the protein in the extracellular domain produce a severe phenotype with high risk of death before 12 months of age, very low intelligent quotients, and a high incidence of severe hydrocephalus. These phenotypes are probably a result of the loss of L1-mediated adhesion that, in turn, disrupts neuronal migration. In contrast, mutations that only affect the cytoplasmic domain give a phenotype with excellent survival beyond 12 months, mild hydrocephalus, and mild rather than severe retardation. However, mutations of the cytoplasmic domain cause spastic paraplegia, indicating hypoplasia of the CST. This shows that structural alterations of the L1 cytoplasmic domain alter axon growth or guidance and result in abnormal

development of the CST. Since truncation of the L1 cytoplasmic domain does not lead to a loss of L1 adhesiveness (Wong et al. 1995a), the failure of correct CST development is probably caused by L1 signal defects or loss of L1 interactions with the cytoskeleton.

### Conclusions

L1 is an essential adhesion molecule for the normal development of the human central nervous system. It has an extremely complex biology, having multiple extracellular ligands and a variety of intracellular associations with kinases and the cytoskeleton. Revealing the way in which these various interactions coordinate growth cone behavior and axon growth remains an exciting challenge.

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### References

- Bentley D, Toroian-Raymond A (1986) Disoriented pathfinding by pioneer neurone growth cones deprived of filopodia by cytochalasin treatment. *Nature* 323:712–715
- Brummendorf T, Rathjen FG (1993) Axonal glycoproteins with immunoglobulin and fibronectin type-III-related domains in vertebrates – structural features, binding activities, and signal transduction. *J Neurochem* 61:1207–1219
- Buchstaller A, Kunz S, Berger P, Kunz B, Ziegler U, Rader C, Sonderegger P (1996) Cell adhesion molecules NgCAM and axonin-1 form heterodimers in the neuronal membrane and cooperate in neurite outgrowth promotion. *J Cell Biol* 135:1593–1607
- Burden-Gulley SM, Lemmon V (1996) L1/8D9, N-cadherin and laminin induce distinct distribution patterns of cytoskeletal elements in growth cones. *Cell Motil Cytoskeleton* 35:1–23
- Burden-Gulley SM, Payne HR, Lemmon V (1995) Growth cones are actively influenced by substrate-bound adhesion molecules. *J Neurosci* 15:4370–4381
- Chien CB, Rosenthal DE, Harris WA, Holt CE (1993) Navigational errors made by growth cones without filopodia in the embryonic *Xenopus* brain. *Neuron* 11:237–251
- 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* (in press)
- Davis JQ, Bennett V (1994) Ankyrin binding activity shared by the neurofascin/L1/NrCAM family of nervous system cell adhesion molecules. *J Biol Chem* 269:27163–27166
- Davis JQ, McLaughlin T, Bennett V (1993) Ankyrin-binding proteins related to nervous system cell adhesion molecules – candidates to provide transmembrane and intercellular connections in adult brain. *J Cell Biol* 121:121–133
- DeBernardo AP, Chang S (1996) Heterophilic interactions of DM-GRASP: GRASP-NgCAM interactions involved in neurite extension. *J Cell Biol* 133:657–666
- Doherty P, Walsh F (1996) CAM-FGF receptor interactions: a model for axonal growth. *Mol Cell Neurosci* 8:99–111
- Doherty P, Ashton SV, Moore SE, Walsh FS (1991) Morphoregulatory activities of NCAM and N-cadherin can be accounted for by G protein-dependent activation of L-type and N-type neuronal Ca<sup>2+</sup> channels. *Cell* 67:21–33
- Doherty P, Williams E, Walsh FS (1995) A soluble chimeric form of the L1 glycoprotein stimulates neurite outgrowth. *Neuron* 14:57–66

- Drazba J, Lemmon V (1990) The role of cell adhesion molecules in neurite outgrowth on Muller cells. *Dev Biol* 138:82–93
- Dubreuil RR, MacVicar G, Dissanayake S, Liu C, Homer D, Hortsch M (1996) Neuroglial-mediated cell adhesion induces assembly of the membrane skeleton at cell contact sites. *J Cell Biol* 133:647–655
- Forscher P, Smith SJ (1988) Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. *J Cell Biol* 107:1505–1516
- Fransen E, Lemmon V, Van Camp G, Vits L, Coucke P, Willems PJ (1995) CRASH syndrome: a clinical spectrum of corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraparesis and hydrocephalus due to mutations in one single gene, L1. *Eur J Hum Genet* 3:273–284
- Grumet M, Friedlander DR, Edelman GM (1993) Evidence for the binding of Ng-CAM to laminin. *Cell Adh Commun* 1:177–190
- Gumbiner BM (1993) Proteins associated with the cytoplasmic surface of adhesion molecules. *Neuron* 1:551–564
- Hall H, Williams EJ, More SE, Walsh FS, Prochiantz A, Doherty P (1996) Inhibition of fgf-stimulated phosphatidylinositol hydrolysis and neurite outgrowth by a cell-membrane permeable phosphopeptide. *Curr Biol* 6:580–587
- Heiland PC, Hertlein B, Traub O, Griffith LS, Schmitz B (1996) The neural adhesion molecule L1 is phosphorylated on tyrosine and serine residues. *Neuroreport* 7:2675–2678
- Hortsch M (1996) The L1 family of neural cell adhesion molecules: old proteins performing new tricks. *Neuron* 17:587–593
- Hortsch M, Wang Y, Marikar Y, Bieber AJ (1995) The cytoplasmic domain of the *Drosophila* cell adhesion molecule neuroglian is not essential for its homophilic adhesive properties in S2 cells. *J Biol Chem* 270:18809–18817
- 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
- Klinz S, Schachner M, Maness PF (1995) L1 and N-CAM antibodies trigger protein phosphatase activity in growth cone-enriched membranes. *J Neurochem* 65:84–95
- Kuhn TB, Stoeckli ET, Condrau MA, Rathjen FG, Sonderegger P (1991) Neurite outgrowth on immobilized axonin-1 is mediated by a heterophilic interaction with L1(G4). *J Cell Biol* 115:1113–1126
- 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
- Lamoureux P, Buxbaum RE, Heidemann SR (1989) Direct evidence that growth cones pull. *Nature* 340:159–162
- Lemmon V, Farr K, Lagenaur C (1989) L1-mediated axon outgrowth occurs via a homophilic binding mechanism. *Neuron* 2:1597–1603
- Lemmon V, Burden SM, Payne HR, Elmslie GJ, Hlavin ML (1992) Neurite growth on different substrates: permissive versus instructive influences and the role of adhesive strength. *J Neurosci* 12:818–826
- Lewis AK, Bridgman PC (1992) Nerve growth cone lamellipodia contain two populations of actin filaments that differ in organization and polarity. *J Cell Biol* 119:1219–1243
- Lin CH, Forscher P (1993) Cytoskeletal remodeling during growth cone-target interactions. *J Cell Biol* 121:1369–1383
- Lin CH, Forscher P (1995) Growth cone advance is inversely proportional to retrograde F-actin flow. *Neuron* 14:763–771
- Lin CH, Thompson CA, Forscher P (1994) Cytoskeletal reorganization underlying growth cone motility. *Curr Opin Neurobiol* 4:640–647
- Lin CJ, Lamoureux P, Buxbaum RE, Heidemann SR (1995) Osmotic dilution stimulates axonal outgrowth by making axons more sensitive to tension. *J Biomech* 28:1429–1438
- Luo L, Liao YJ, Jan LY, Jan YN (1994) Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes Dev* 8:1787–1802
- Mackay DJG, Nobes CD, Hall A (1995) The Rho's progress: a potential role during neuritogenesis for the Rho family of GTPases. *Trends Neurosci* 18:496–501
- Marsh L, Letourneau PC (1984) Growth of neurites without filopodia or lamellipodial activity in the presence of cytochalasin b. *J Cell Biol* 99:2041–2047
- McClay DR, Etensohn CA (1987). Cell adhesion in morphogenesis. *Annu Rev Cell Biol* 3:319–345
- Milev P, Friedlander DR, Sakuri T, Karthikeyan L, Flad M, Margolis RK, Grumet M, Margolis RU (1994) Interactions of the chondroitin sulfate proteoglycan phosphacan, the extracellular domain of a receptor-type protein tyrosine phosphatase, with neurons, glia and neural cell adhesion molecules. *J Cell Biol* 127:1703–1715
- 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
- 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
- Morales G, Hubert M, Brummendorf T, Treubert U, Tarnok A, Schwarz U, Rathjen FG (1993) Induction of axonal growth by heterophilic interactions between the cell surface recognition protein-F11 and protein-Nr-CAM/Bravo. *Neuron* 11:1113–1122
- Nobes CD, Hawkins P, Stephens L, Hall A (1995) Activation of the small GTP-binding proteins rho and rac by growth factor receptors. *J Cell Sci* 108:225–33
- O'Connor TP, Duerr JS, Bentley D (1990) Pioneer growth cone steering decisions mediated by single filipodial contacts in situ. *J Neurosci* 10:3935–3946
- Payne HR, Burden SM, Lemmon V (1992) Modulation of growth cone morphology by substrate-bound adhesion molecules. *Cell Motil Cytoskeleton* 21:65–73
- Peppelenbosch MP, Qiu RG, deVries-Smits AMM, Tertoolen LGJ, deLaat SW, McCormick F, Hall A, Symons MH, Bos JL (1995) Rac mediates growth factor-induced arachidonic acid release. *Cell* 81:849–856
- Peppelenbosch MP, Tertoolen LGJ, deVries-Smits AMM, Qui RG, M'Rabet L, Symons MH, deLaat SW, Bos JL (1996) Rac-dependent and independent pathways mediate growth factor-induced  $Ca^{2+}$  influx. *J. Biol. Chem.* 271:7883–7886
- Rader C, Kunz B, Lierheimer R, Giger RJ, Berger P, Tittmann P, Gross H, Sonderegger P (1996) Implications for the domain arrangement of axonin-1 derived from the mapping of its NgCAM binding site. *EMBO J* 15:2056–2068
- Ridley AJ (1996) Rho: theme and variations. *Curr Biol* 6:1256–1264
- Ridley AJ, Paterson HF, Johnston CL, Diekmann D, Hall A (1992) The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* 70:401–410
- Rosenthal A, Jouet M, Kenwick S (1992) Aberrant splicing of neural cell adhesion molecule L1 messenger RNA in a family with X-linked hydrocephalus. *Nat Genet* 2:107–112
- Ruppert M, Aigner S, Hubbe M, Yagita H, Altevogt P (1995) The L1 adhesion molecule is a cellular ligand for VLA-5. *J Cell Biol* 131:1881–1891
- Saffell JL, Walsh FS, Doherty P (1992) Direct activation of 2nd messenger pathways mimics cell adhesion molecule-dependent neurite outgrowth. *J Cell Biol* 118:663–670
- Saffell JL, Williams EJ, Mason IJ, Walsh FS, Doherty P (1997) Expression of a dominant negative FGF receptor inhibits axonal growth and FGF receptor phosphorylation stimulated by CAMs. *Neuron* 18:231–242
- Schmidt CE, Dai JW, Lauffenburger DA, Sheetz MP, Horwitz AF (1995) Integrin-cytoskeletal interactions in neuronal growth cones. *J Neurosci* 15:3400–3407

- Schuch U, Lohse MJ, Schachner M (1989) Neural cell adhesion molecules influence second messenger systems. *Neuron* 3:13–20
- Sheetz MP, Wayne DB, Pearlman AL (1992) Extension of filopodia by motor-dependent actin assembly. *Cell Motil Cytoskeleton* 22:160–169
- Smith SJ (1988) Neuronal cytom mechanics: the actin-based motility of growth cones. *Science* 242:708–715
- Stallcup WB, Beasley L (1985) Involvement of the nerve growth factor-inducible large external glycoprotein (NILE) in neurite fasciculation in primary cultures of rat brain. *Proc Natl Acad Sci USA* 82:1276–1280
- Takeda Y, Asou H, Murakami Y, Miura M, Kobayashi M, Uyemura K (1996) A noneuronal isoform of cell adhesion molecule L1: tissue-specific expression and functional analysis. *J Neurochem* 66:2338–2349
- Takeichi M, Hirano S, Matsuyoshi N, Fujimori T (1992) Cytoplasmic control of cadherin-mediated cell-cell adhesion. *Cold Spring Harb Symp Quant Biol* 57:327–334
- Williams EJ, Doherty P, Turner G, Reid RA, Hemperly JJ, Walsh FS (1992) Calcium influx into neurons can solely account for cell contact-dependent neurite outgrowth stimulated by transfected L1. *J Cell Biol* 119:883–892
- Williams EJ, Furness J, Walsh FS, Doherty P (1994) Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, N-CAM, and N-cadherin. *Neuron* 13:583–594
- Wong EV, Cheng G, Payne HR, Lemmon V (1995a) The cytoplasmic domain of the cell adhesion molecule L1 is not required for homophilic adhesion. *Neurosci Lett* 200:155–158
- Wong EV, Kenwrick S, Willems P, Lemmon V (1995b) Mutations in the cell adhesion molecule L1 cause mental retardation. *Trends Neurosci* 18:168–172
- Wong EV, Schaefer AW, Landreth G, Lemmon V (1995c) Casein kinase II phosphorylates the neural cell adhesion molecule L1. *J Neurochem* 66:779–786
- Wong EV, Schaefer A, Landreth G, Lemmon V (1996) Involvement of p90<sup>rsk</sup> in neurite outgrowth mediated by the cell adhesion molecule L1. *J Biol Chem* 271:18217–18223
- Yamasaki M, Thompson P, Lemmon V (1997) CRASH syndrome: mutations in L1CAM correlate with severity of the disease. *Neuropediatrics* (in press)
- Zhao X, Siu CH (1995) Colocalization of the homophilic binding site and the neuritogenic activity of the cell adhesion molecule L1 to its second Ig-like domain. *J Biol Chem* 270:29413–29421
- Zheng J, Buxbaum R, Heidemann SR (1994) Measurements of growth cone adhesion to culture surfaces by micromanipulation. *J Cell Biol* 127:2049–60