CELLULAR SIGNALLING MECHANISMS OF NEURAL CELL ADHESION MOLECULES

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1. ABSTRACT

Neural cell adhesion molecules of the immunoglobulin superfamily are multidomain proteins involved in important cellular events pertinent to development and adult neurological function. This review attempts to give a concise overview of the complex intracellular signaling pathways enabling neural cell adhesion molecules NCAM and L1 to regulate axon growth, guidance, and synaptic plasticity. Recent research findings suggest that these molecules signal in part through integrins leading to cytoskeletal rearrangements locally in the growth cone or cell leading edge, and to MAP kinase, which has the potential to cause gene expression changes in the nucleus. Abnormal expression of NCAM on human chromosome 11q23 has been linked to schizophrenia in humans, a multigenic disease believed to be of neurodevelopmental origin. L1 at Xq28 is the target for mutation in a complex mental retardation disorder termed the L1 syndrome (also sometimes referred to as CRASH syndrome). Thus a full understanding of the mechanism of NCAM and L1 function will contribute to understanding both normal brain development and pathologies associated with cognitive dysfunction in schizophrenia and mental retardation.

2. INTRODUCTION

Current research has revealed that neural cell adhesion molecules are involved in multiple signal transduction processes important to cell migration, cell adhesion, neurite outgrowth, axon fasciculation and guidance, necessary for both the development of proper synaptic connectivity and synaptic plasticity associated with learning and memory. This review focuses on the ability of the neural cell adhesion molecules NCAM and L1 to signal via distinct pathways in response to multiple homophilic and heterophilic binding interactions. Abnormal expression or function of these adhesion molecules can contribute to pathologies in neurodevelopment and mental function.

3. NCAM SIGNALING

3.1. NCAM Structure and Isoforms

Neural cell adhesion molecule NCAM is an axonal growth-promoting cell recognition molecule within the immunoglobulin (Ig) superfamily. NCAM is expressed as three principal isoforms: two are transmembrane forms with either a short (NCAM140) or long (NCAM180) cytoplasmic domains, and another form lacking a cytoplasmic domain (NCAM120) is anchored to the plasma membrane by a glycophosphatidylinositol (GPI) linkage. All three NCAM isoforms have five Ig-like domains and two fibronectin type III (FN III) domains in the extracellular region. NCAM180 differs from NCAM140 in having a 261-amino acid insert in the cytoplasmic domain, which confers an ability to interact with spectrin (1). NCAM140 is localized to migratory growth cones and axon shafts of developing neurons, whereas NCAM180 is enriched at sites of cell-cell contact and postsynaptic densities of mature neurons (2). Unlike transmembrane isoforms, GPI-linked NCAM120 is preferentially expressed in glia. An alternative splice variant of NCAM containing the VASE exon in the Ig4 domain arises postnatally and serves to downmodulate axon growth (3). Among other
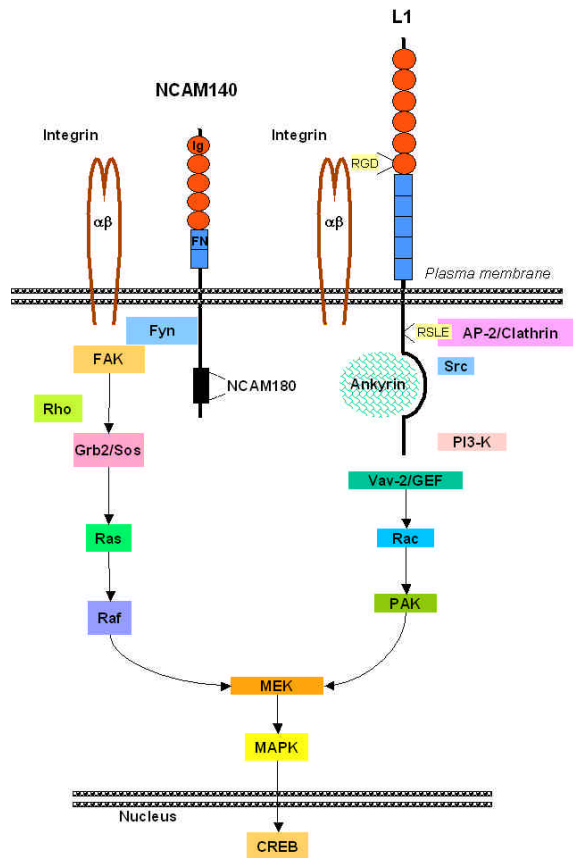
Figure 1. Downstream signaling pathways of NCAM, L1 and integrins culminating in MAPK signaling cascade leading to developmental events like neurite outgrowth, cytoskeletal organization and cellular migration.

splice variants, a secreted isoform consisting of most of the extracellular region of NCAM is expressed in brain and skeletal muscle (4).

All three isoforms can be post-translationally modified by the addition of polysialic acid (PSA) to the Ig5 domain (5-7). PSA is a homopolymer composed of alpha-2,8-linked sialic acid residues and is found almost exclusively on NCAM in vertebrate brain. Glycosylation of NCAM is regulated developmentally, decreasing during the embryonic to adult transition (8). In general, polysialylation of NCAM has the effect of enhancing axon growth, which occurs at the expense of axon fasciculation (9).

3.2. NCAM Mediated Cell Adhesion

NCAM plays a role in modulating adhesiveness of neurons and their processes through homophilic and heterophilic binding. The NCAM domains mediating homophilic binding are still debated but dimerization of the third Ig domain (10) and double reciprocal dimerization of the first and second Ig domains have been proposed (11-13). NCAM binds heterophilically to heparan sulfate proteoglycans (14) through heparin-binding sites in the first and second Ig domains, and to the extracellular matrix protein agrin (15) and several chondroitin sulfate proteoglycans, including neurocan (16) and phosphocan

(16, 17). Additionally, NCAM is capable of lateral binding to L1 via the NCAM Ig4 domain, which facilitates homophilic binding between L1 molecules apposed in trans (18, 19). Embryonic lethality of a targeted mutation of NCAM producing only a secreted form of NCAM consisting of its entire extracellular region illustrates the physiological importance of heterophilic interactions mediated by NCAM (20).

The presence of PSA on NCAM decreases NCAM-mediated cell adhesion (21). Fujimoto and co-workers (22) provide strong evidence for the effect of PSA on cell adhesion to be independent of binding or signaling properties of NCAM per se, but through steric inhibition of membrane-membrane apposition solely based on biophysical properties of PSA.

3.3. NCAM Signaling Mechanisms in Axon Growth

NCAM-mediated neurite outgrowth can result from activation of intracellular signal transduction pathways involving protein tyrosine kinases and phosphatases (23-27). NCAM crosslinking on the cell surface produces additional signaling responses including calcium rise, pH change, and phosphoinositide turnover that contribute to neurite growth (28, 29). An integrin-like signaling pathway has been elucidated for neurite outgrowth that is triggered by clustering of only the NCAM140 isoform (30, 31) (see Figure 1). NCAM140-mediated neurite outgrowth critically depends specifically on the Src family nonreceptor tyrosine kinase p59fyn and not pp60c-src as shown in cultures of cerebellar and sensory neurons from fyn-minus mice (26). p59fyn constitutively associates with NCAM140 in the plasma membrane, probably indirectly, but not with NCAM180 or NCAM120 (30). Crosslinking of NCAM140 with NCAM-Fc fusion protein or divalent NCAM antibodies induces the recruitment and transient activation of focal adhesion kinase (p125fak) (30), the nonreceptor tyrosine kinase pivotal to integrin signal transduction and formation of focal adhesive contacts with the substratum. Clustering of NCAM140 subsequently activates c-Ras1, the mitogen and extracellular signal regulated kinase MEK, and the MAP kinases, ERK 1 and ERK2. Importantly, inhibition of MAP kinase activation by MEK inhibitors impairs NCAM-dependent neurite outgrowth, as shown in primary neuronal cultures. The small G protein Rho also participates in NCAM140-mediated activation of MAPK but it is not clear where it acts in the pathway (31). The NCAM-Fak/Fyn-Ras-MAP kinase pathway was confirmed in a PC12 cell line where it was shown that NCAM-stimulated neurite outgrowth depends on activation of p125fak, p59fyn and Ras-MAP kinase (32). A central role of MAP kinase in neurite outgrowth on other substrates has also been demonstrated in primary cultures of chick retinal neurons growing on laminin or N-cadherin (33, 34).

MAP kinase may affect growth cone motility through phosphorylation of local cytoplasmic or membrane-associated targets, such as myosin light chain kinase (35). Alternatively, MAP kinase activation is capable of inducing the expression of genes needed to extend the length of the axon through its ability to

phosphorylate and activate transcription factors such as Elk-1 directly. NCAM140 signaling also results in MAP kinase-dependent phosphorylation / activation of the transcription factor CREB (cAMP response element binding protein) on serine residue 133 (31). CREB is phosphorylated and activated by the serine/threonine kinase RSK, which is a direct target of MAP kinase. Signals from NCAM might be integrated with other cell stimuli at the level of CREB, since CREB can be phosphorylated on serine 133 by protein kinase C, calmodulin-dependent protein kinase and cAMP-dependent protein kinase, in addition to RSK. Such interactions may coordinately regulate neurite outgrowth, as NCAM-mediated neurite growth was shown to depend on both Ras-MAP kinase and cAMP-dependent protein kinase pathways in PC12 cells (36).

A different NCAM signaling pathway was described in which some isoforms of the FGF receptor mediate neurite outgrowth on all isoforms of NCAM through activation of phospholipase C-gamma (PLC-gamma) and diacylglycerol (DAG) lipase, culminating in production of arachidonic acid (24, 25, 37). An involvement of PLC-gamma in NCAM-dependent neurite outgrowth was confirmed in PC12 cells by the impairment of neurite growth on NCAM substrates in the presence of pharmacological inhibitors of PLC-gamma (32). Arachidonic acid can lead to neurite growth in some cells, and because arachidonic acid- induced neurite outgrowth can be inhibited by specific antagonists of N-and L-type calcium channels, calcium influx appears to be a participant in this cellular response (28, 32). It has been proposed that a short sequence of 10 amino acids, termed the "CAM homology domain", which can be found in the FGF receptors-1 and 2, might be able to bind directly with related sequences within the VASE exon-encoded region of NCAM, as well as short sequences in the extracellular regions of L1 and N-cadherin (38). In this model it remains to be established whether NCAM can physically associate with an FGF receptor, although NCAM140 and 180 have been shown to co-immunoprecipitate with a different FGF receptor (FGF receptor-4) and with N-cadherin in neuroendocrine tumor cell extracts (39).

Niethammer and coworkers (40) offer a unifying perspective for reconciling the divergent views of NCAM signaling leading to neurite outgrowth. Their findings show that distinct MAP kinase signaling pathways occur based on compartmentalization of different isoforms of NCAM in lipid rafts or non-rafts within the plasma membrane. NCAM140 in lipid rafts signals through p125fak and p59fyn leading to MAP kinase activation, while NCAM140 in the non-raft compartment requires an FGF receptor. A key feature of the mechanism is that NCAM140 must be activated in both compartments to signal neurite outgrowth effectively. In agreement with Beggs *et al.* (30), neither NCAM180 nor NCAM120 are neuritogenic. Kolkova and coworkers (32) also present evidence that the NCAM-p125fak/p59fyn-Ras-MAP kinase pathway and the NCAM-FGF receptor-PLC-gamma pathways are both required for neurite outgrowth on NCAM in PC12 cells. However, in contrast to Doherty *et*

al. (28), their studies indicate that protein kinase C (PKC) is critical for NCAM-dependent neurite growth, based on the ability of calphostin, a PKC selective inhibitor, to impair NCAM-dependent neurite outgrowth (32). They suggest that PKC may be activated by DAG thus serving as a link between the two pathways through PKC-mediated activation of the serine/threonine kinase Raf-1.The outcome of this pathway is to cause sustained rather than transient MAP kinase stimulation for neurite growth.

3.4. NCAM Isoforms in Synaptic Function

In addition to stimulating axon growth NCAM plays an important role in synaptic plasticity and function. NCAM is preferentially expressed in regions of the adult brain associated with synaptic plasticity (e.g., the hippocampus and cerebellum; (41). Mutant mice lacking all isoforms of NCAM (5) are deficient in hippocampal long-term potentiation (LTP), spatial learning, and long-term depression LTD (42, 43). Function-blocking NCAM antibodies have been shown to diminish LTP in hippocampal slice assays (44). It is noteworthy that LTP deficiency in hippocampal slices from NCAM knockout mice can be rescued with exogenous application of brain-derived neurotrophic factor (BDNF), implicating crosstalk between NCAM and BDNF signal transduction pathways, both of which activate Ras-MAP kinase (45).

Postsynaptic localization of NCAM is strongly implicated in modulating synaptic function. The expression of NCAM in postsynaptic hippocampal neurons increases synaptic strength in an activity-dependent manner by regulating the number of synapses (2). Specifically, it is the NCAM 180 isoform that is confined mostly to postsynaptic membranes, whereas NCAM 140 is expressed both pre-and postsynaptically (46, 47). The importance of NCAM180 in synaptic function is further revealed by its ability to reverse aberrant serotonin receptor sensitization and emotional behavior in NCAM null mutant mice (48).

A role for NCAM in the organization and modulation of pre-synaptic machinery has been identified recently at the neuromuscular junction. As shown in total NCAM knockout mice, NCAM is needed to make the developmental switch from an immature, brefeldin-sensitive type of synaptic vesicle cycling, which uses L-type calcium channels, to the mature form of cycling, which uses P/Q calcium channels (49, 50). Vesicle release in NCAM null mutant mice occurs abnormally in the preterminal region of the axon and upper part of the nerve terminal rather than being restricted to the nerve terminal. As a result, neuromuscular junctions of NCAM knockout mice lack paired pulse facilitation and show strikingly depressed responses to repetitive stimuli. In the absence of NCAM it appears that synaptic vesicles are continuously released from the preterminal sites and that release cannot be further induced. These results suggest that NCAM participates in organizing the presynaptic machinery or in regulating vesicle cycling or release.

Seemingly contrary to the situation in vertebrates, where there is a positive correlation between NCAM and synaptic plasticity, synaptic strengthening in invertebrates

occurs by a reduction of cell surface NCAM (51-53), but see (54). For the formation of new synapses during long-term facilitation (similar to LTP) in Aplysia, downregulation of the Aplysia NCAM homolog (apCAM) must occur. This process involves internalization of NCAM from the pre-synaptic axonal membrane in sensory neurons, and requires MAP kinase activity (55, 56). Similarly in Drosophila, the NCAM homolog Fasciclin II, which is present pre- and post-synaptically, has to be downregulated for activity-dependent synaptic growth in nerve-muscle synapses (57). The presynaptic release machinery in flies expressing 50% of wild type levels of Fasciclin II is upregulated by CREB- and cAMP-dependent protein kinase A-mediated transcription (57, 58). A resolution to the apparent difference with regard to mammalian NCAM may be related to the ability of NCAM isoforms to undergo receptor-mediated endocytosis (59). Clustering and endocytosis of NCAM might be an early step needed for intracellular signaling to MAP kinase, which subsequently regulates synaptic vesicle cycling or neurotransmitter release. Receptor-mediated endocytosis of NCAM140 and 180 has shown to occur by a clathrin-dependent pathway in astrocyte cultures (59).

3.5. NCAM in Cortical Circuitry Relevant to Schizophrenia

Schizophrenia is a neuropsychiatric disorder that is currently believed to originate from disturbances in neurodevelopment (60, 61). NCAM knockout mice show several hallmarks of schizophrenia: increased size of lateral brain ventricles, impaired sensory motor gating manifested by reduced prepulse inhibition of acoustic startle (62), and deficits in hippocampal / amygdala-dependent learning and LTP (5). They are also more aggressive and anxious than their wild-type littermates (63). NCAM knockout mice display defects in the structure of the hippocampus (6), one of several brain regions implicated in schizophrenia. In particular hippocampal mossy fibers of NCAM-minus mice produce ectopic synapses due to a failure in remodeling (64). Furthermore, there is a reduction in polysialylated NCAM, which is needed for proper axon guidance, in the brains of both NCAM knockout mice and human schizophrenics (65). Neuronal process formation in cerebral cortical structures implicated in schizophrenia, such as the prefrontal cortex, has not yet been analyzed in NCAM knockout mice.

Abnormal levels of an NCAM fragment that includes most of its extracellular domain are secreted in affected brain regions and cerebrospinal fluid of schizophrenic patients, correlating with severity of the disease (66-71). Soluble NCAM could arise by proteolytic shedding of transmembrane isoforms (72, 73) or enzymatic cleavage of GPI-linked NCAM (74, 75), but apparently not from overproduction of the alternatively spliced secreted form of NCAM (69). Abnormal release of the NCAM extracellular fragment could interfere with normal homophilic or heterophilic interactions of NCAM, either by constitutively activating NCAM signaling or by serving as a competitive inhibitor of homophilic or heterophilic NCAM binding interactions.

A transgenic mouse strain has been generated in which developing neurons in the brain secrete the entire extracellular region of NCAM from the neuron-specific enolase promoter at the onset of terminal neuronal differentiation and extending into postnatal life (76). This approach was designed to circumvent the embryonic lethality that occurred when the NCAM extracellular region was expressed in mice through deletion of the transmembrane-coding region in the NCAM gene in mouse embryonic stem cells (20). The NCAM extracellular region was expressed in transgenic brain at 2-3 fold higher levels than endogenous NCAM 180/140, and over 50% of the transgenic NCAM protein in the brain is in the soluble fraction. The extracellular NCAM fragment was preferentially localized within process-rich layers of the cerebral cortex, hippocampus, and cerebellum. Behavioral studies revealed that these mice have reduced prepulse inhibition of acoustic startle, display hyperactivity in the open field, and show increased stereotypic activities, consistent with aspects of the schizophrenia phenotype (76). An evaluation of learning and memory showed that the transgenic NCAM animals are also deficient in contextual and cued fear conditioning. Abnormal secretion of NCAM during development may thus perturb brain circuitry leading to pathological behaviors relevant to schizophrenia.

4. L1 SIGNALING

4.1. L1 Structure and Function

L1-related neural cell adhesion molecules (L1-CAMs) comprise a class of the immunoglobulin superfamily of proteins expressed across the animal regnum from birds and mammals (L1/NgCAM, NrCAM, Neurofascin, and the Close Homolog of L1 or CHL1) to C. elegans (LAD-1) and Drosophila (Neuroglian). These cell recognition molecules are characterized by the presence of an extracellular domain with six Ig-like domains (77) and four to five FN III domains, followed by a transmembrane region and a short (~110 residue), highly conserved cytoplasmic domain. L1-CAMs are widely expressed in the nervous system and have been implicated in a variety of developmental processes including neuronal migration, axon growth and guidance, axon fasciculation, myelination and synaptic plasticity. L1 is of timely importance, as the human L1 gene on the X chromosome (Xq28) has been identified as the target for mutation in a pleiotypic form of mental retardation, termed the L1 syndrome (78). A site on the World Wide Web is available for current information on human L1 mutations (79). Some pathological L1 mutations are known to truncate the L1 protein, giving rise to a null mutation. Missense mutations occuring throughout the L1 extracellular and intracellular domains can generate a protein with altered function or cause failure to be transported to the plasma membrane resulting in degradation (80, 81). It is noteworthy that the human CHL1 gene (termed "CALL") is closely linked with another syndromic form of mental retardation known as the 3p-syndrome (82).

Different isoforms of L1 are generated by alternative splicing and yield functionally distinct proteins

that are expressed in different cell types. L1 isoforms retaining exons 2 and 27 are expressed by neurons, whereas forms lacking these sequences are expressed in certain nonneuronal cells including Schwann cells (83), hematopoietic cells (T-cells, B cells, granulocytes; see references in (84)), and epithelial cells (85). Insertion of amino acids encoded by exon 2 into the L1 Ig2 domain causes enhanced homophilic binding between L1 molecules (81, 86). Insertion of the RSLE sequence encoded by exon 27 into the cytoplasmic domain enables L1 to recruit the AP2/clathrin adapter important for receptor-mediated endocytosis (87).

4.2. L1 - Ankyrin Interactions

The cytoplasmic domain of L1 contains the sequence SFIGQY that is highly conserved among L1-CAMs. This motif is part of a binding site for ankyrin, an adapter protein that links L1 to the actin cytoskeleton through spectrin (88-91). The physiological role of L1-ankyrin binding is not yet clear but the similar phenotypes displayed by ankyrin B and L1-minus mice showing dysplastic axon tracts and ventricular enlargement support a role for their interaction in stabilizing axonal interactions (92-95). Reversible phosphorylation of the tyrosine residue within the SFIGQY sequence is achieved by an unidentified kinase but serves to reversible regulate ankyrin binding to L1-CAMs (96, 97). Two pathological mutations of L1 situated within the SFIGQY sequence (S1224L and Y1229H) reduce L1-ankyrin interactions (98) and may destabilize neuronal interactions necessary for normal neurodevelopment.

4.3. L1 Signaling in Axonal Growth and Neuronal Migration

Besides acting as an adhesive molecule providing strength for intercellular connections, L1 functions as a signal-transducing receptor providing neurons with cues from their environment for axonal growth and guidance. Clustering of cell surface L1 (containing RSLE) with L1 antibodies or L1-Fc fusion protein activates a MAP kinase signaling cascade (99). This rapid and transient activation of MAP kinase is required for L1-dependent neurite outgrowth (100, 101). However, inhibition of the MAPK cascade reduces neurite outgrowth by 50-60% when primary neurons are cultured on L1, suggesting the existence of additional mechanisms contributing to axon growth. L1-stimulated MAP kinase is achieved through activation of the nonreceptor kinase pp60c-src, Rac1 GTPase, p21-associated kinase (PAK1), mitogen-and-extracellular signal-regulated kinase (MEK), and the guanine nucleotide exchange factor Vav-2 (101, 102). L1 clustering in growth cone membranes has been shown to activate tyrosine phosphatases, which probably accounts for the transient nature of the phosphorylation events in the MAP kinase cascade (23, 103). Another signaling intermediate of the L1 pathway, the phosphatidylinositol-3-kinase (PI3 kinase) (101) is well known for its role in promoting neuronal survival in response to neurotrophins and growth factors (104). This might therefore contribute to the ability of L1, as well as CHL1, to foster the survival of neuronal cells in culture (105).

This L1 signaling pathway closely resembles an early integrin signaling pathway producing lamellipodia and membrane ruffling (106-108) (see Figure 1). Accordingly, a novel role for L1 has been identified in potentiating haptotactic migration of neural and nonneural cells toward extracellular matrix (ECM) proteins that is mediated by functional interaction of L1 with beta 1 integrins (109) (110). A conserved RGD sequence in the Ig6 domain of L1 is necessary for enhancing migration, suggesting that L1 might physically associate with beta 1 integrins through their extracellular domains. The activity of MAP kinase and pp60c-src are both necessary for L1-potentiated migration to ECM proteins (110), and an intact RGD in the L1 Ig6 domain is essential for signaling to MAP kinase (110). However, an association between L1 and beta 1 integrins must be transient or low in affinity as it has not been demonstrated by direct binding assays or co-immunoprecipitation in these studies. Other studies have revealed an interaction between L1 and specific subclasses of integrins in nonneuronal cells: alpha 5 beta 3 (111, 112),alpha 5 beta 1 (113), alpha v beta 1 and alpha 2b beta 3(114), alpha 9 beta 1 (115). Such interactions are also mediated through the RGD sequence in the 6th Ig domain of L1, and additionally through a dibasic sequence within the 3rd FNIII domain (115). The third FNIII domain of L1 has been shown to be critical for neurite outgrowth by cerebellar neurons (116). L1/integrin engagement may be effective not only in *trans* but also in *cis* when both molecules are present on the plasma membrane of the same cell. An interesting mechanism for L1 potentiated migration was suggested (109) in which ectodomain cleavage of L1 by ADAMs (A Disintegrin and Metalloproteinase) promotes migration by autocrine stimulation of integrins. This mechanism may depend on particular cell types, as it was suppported by results of ADAM inhibitors in transfected CHO cells, but were not effective in B35 neuroblastoma cells (Maness, PF, unpublished results).

Endocytosis of L1 occurs during active cell migration and growth cone motility, which may be important in cycles of cell attachment/detachment necessary for directed locomotion. Antibody-mediated clustering of L1 induces rapid dynamin I- and clathrin-dependent internalization of the neuronal form of L1 (with RSLE) (87, 100, 101). A slower form of internalization occurs for the nonneuronal form of L1 lacking RSLE (117). Internalization of neuronal L1 requires a functional pp60c-src kinase (101). pp60c-src specifically regulates neurite outgrowth on L1 as shown in cerebellar cultures from Src-minus mice (102). Other members of the Src family of tyrosine kinases that are expressed in growth cones, namely p59fyn, and p62c-yes (118, 119), do not serve redundant functions in L1-dependent neurite growth (102). Inhibition of dynamin I or pp60c-src as a result of expression of dominant negative mutants or treatment with Src kinase inhibitors such as PP2, block L1-induced endocytosis and MAP kinase activation, supporting the interpretation that Src-mediated L1 endocytosis is required for downstream signaling to MAP kinase (100, 101). The target of pp60c-src that regulates L1 endocytosis has not been identified, but this kinase is known to phosphorylate dynamin I (120)

and clathrin (121). pp60c-src can also phosphorylate L1 *in vitro* on tyrosine residue 1176, which precedes the RSLE motif of L1, and this modification prevents L1 binding to the AP2/clathrin adaptor (122). Inhibition of dynamin I or pp60c-src blocks L1-potentiated cell migration toward ECM proteins in addition to reducing neurite outgrowth on L1 substrates (110), hence endocytosis appears to be critical for promoting cell migration in addition to growth cone motility. It is important to note that clustering of L1 in HEK293 cells on fibronectin induces rapid endocytosis of not only L1 but also of beta 1 integrins (110) . Thus it is not clear whether it is the internalization of L1, beta 1 integrin, or both molecules that is essential for potentiating cell migration to ECM.

4.4. Calcium as a Second Messenger in L1 Signaling

There is much evidence that intracellular signaling with calcium as a second messenger occurs in neurons and contributes to neurite extension (123-128). Direct pharmacological activation of calcium channels is sufficient to enhance neurite outgrowth in cell monolayers (126, 129), leading to the hypothesis that calcium-activated second messenger systems triggered by binding of L1 rather than adhesion per se is a key factor in axon growth. Indeed, clustering of L1, as well as NCAM, causes rapid Ca2+ influx in neuronal cells (29) and the L1-induced calcium increase is inhibited by N- and L-type calcium channel antagonists (28, 130). Pharmacological experiments have indicated that L1, like NCAM, may interact with an FGF receptor through the putative CAM homology domain on L1 leading to activation of PLC-gamma (131) and (132). Consecutive stimulation of PLC-gamma and DAG lipase is proposed to result in the production of arachidonic acid and subsequent opening of voltage-gated calcium channels (25, 133). A recent finding has shown that binding of L1 to sensory neurons leads to a local increase in submembranous calcium but not to an overall cellular calcium rise (133, 134), pointing to a localized activation of calcium signaling. If calcium transients occur at specific sites within the growth cone or leading edge of the cell, they would be expected to cause specific types of actin cytoskeletal rearrangements (lamellipodia, filopodia) that could facilitate directional motility.

4.5. Role of L1 Mutations in X-linked Mental Retardation (L1 syndrome)

Impaired migration of neural precursors and altered axon growth or guidance due to L1 mutations that abrogate cell signaling might contribute to the pathology of the L1 syndrome of mental retardation. L1-induced MAP kinase activation and enhanced migration to ECM proteins are coordinately reduced in cells expressing the 3 known pathological L1 mutations within its cytoplasmic domain (S1194L, S1224L, Y1229H) (110). L1 and beta 1 integrins cooperate for migration of neuronal precursors in the cerebellum (110) raising the possibility that these L1 mutations might alter the migration of neural precursors in development. Accordingly, migration of dopaminergic neuron precursors in L1 knockout mice was shown to be aberrant (135). Certain pathological mutations in the L1 extracellular region that disrupt homophilic binding

strikingly impair neurite outgrowth *in vitro* (136), while many pathological missense mutations of L1 affect homophilic binding and heterophilic association with the GPI-linked cell surface molecules TAG-1 and F3/F11/contactin (137). Thus, L1 may participate in a fundamental way in normal neurodevelopmental mechanisms requiring cell location and axon growth or guidance, and mutation of critical residues within extracellular and intracellular L1 domains may alter neuronal motility and axon growth resulting in developmental abnormalities and altered mental function in the L1 syndrome.

5. PERSPECTIVES

A number of findings connect integrins and cytoskeletal machinery to NCAM and L1 signaling, yet the mechanism by which they are coupled is not clearly elucidated and needs further study. Coupling of signaling between CAMs and integrins could result in signal amplification allowing neurons to integrate information from distinct points in the cell or growth cone due to positional cues on adjacent cells (CAMs) and ECM substrates to achieve directional motility important for development or synaptic plasticity. Rho family GTPases are key components of these CAM signaling cascades with known importance in actin cytoskeletal organization necessary for normal neuron morphology and motility. NCAM and L1 activate RhoA and Rac1 and thus, recognizing their specific downstream effectors would shed light on how different CAMs alter actin cytoarchitecture in growth cones to cause their uniquely different morphologies. It is also important to identify the molecular interactors specific for NCAM140 and 180 isoforms to better understand their functions in axon growth and plasticity, respectively. NCAM140 and L1 signaling culminates in MAP kinase activation, presumably causing transcriptional activation of genes required for neuronal migration and neurite outgrowth. Gene profiling using DNA microarray technology will prove useful to identify those genes induced or down regulated by specific CAMs.

To understand the role of abnormal secretion of NCAM associated with schizophrenia, the NCAM transgenic mouse strain expressing the extracellular fragment in brain should prove to be informative. In particular it will be important to learn whether such altered NCAM expression affects cortical neuron development, consistent with the neurodevelopmental hypothesis of schizophrenia, and adult synaptic function. Epigenetic factors implicated in the etiology of schizophrenia (viruses, social isolation, maternal care) can also be evaluated in these mice. Further, the mice can be intercrossed with other strains bearing mutations in other schizophrenia susceptibility genes to assess the combined load of gene mutations in the phenotypes of the disease. Transgenic mice expressing different L1 mutations corresponding to those identified in human families with X-linked mental retardation will be valuable in delineating the effect of each mutation on pathological consequences in the highly pleiotypic spectrum of neurological defects found in the L1 syndrome.

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