Role of the Growth-Associated Protein GAP-43 in NCAM-Mediated Neurite Outgrowth

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Introduction

During development, the neural cell adhesion molecule (NCAM) and growth-associated protein-43 (GAP-43) play key roles in neurite outgrowth and growth cone navigation [1–3]. These processes are vital for axonal growth and formation of highly organized neuronal nets in the brain. In adult organisms, NCAM and GAP-43 participate in the reorganization of synaptic contacts and learning and memory formation [4–6]. Enhanced synthesis of these proteins occurs during development and axonal regeneration. They are localized in detergent-resistant plasma membrane domains (rafts) [7–11]. NCAM was shown to deliver external signals to GAP-43 in growth cones, stimulating both GAP-43 phosphorylation on S41 and neurite outgrowth [1]. Therefore, numerous data suggest functional interdependence between NCAM and GAP-43. In this chapter, we discuss the interactions between GAP-43 and NCAM isoforms in the process of neurite outgrowth.

Role of NCAM in the Nervous System

NCAM appears early in embryonic development during the blastoderm stage [12]. During development, NCAM levels peak at the late embryonic/early postnatal stage and then rapidly decrease [13–16]. Several reports suggest an involvement of NCAM in regenerative processes (for review see [17]). NCAM is expressed in three major

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isoforms (120 kDa, 140 kDa, and 180 kDa) generated by alternative splicing of mRNA transcribed from a single gene. NCAM-180 and NCAM-140 are transmembrane proteins, whereas NCAM-120 is linked to the plasma membrane via a lipid anchor. The formation of different NCAM isoforms is temporally and cell-specifically regulated. NCAM-120 is expressed mainly in glial cells of adult animals [18–20]. NCAM-140 is expressed in neurons, glia [19, 21], and tissues of non-neural origin, such as skeletal muscle [22], cardiac muscle [23, 24], smooth muscle [25], and kidneys [26]. NCAM-180 is present mainly in neurons. Expression of NCAM-180 also has been detected in astrocytes [19, 21, 27], adult epididymis [28], heart [23], pancreatic islets [29], and embryonic muscle [30]. Mice lacking NCAM-180 have been shown to have defects in the development of the olfactory bulb [31] and in circadian rhythms [32].

In contrast to the other two NCAM isoforms, NCAM-180 is not synthesized in the proliferating neuronal precursors and becomes detectable only at later stages of development [33]. These in situ observations are supported by in vitro experiments, in which neuroblastoma cells induced to differentiate with dimethyl sulfoxide or laminine shifted NCAM synthesis toward predominately NCAM-180 [34, 35]. Importantly, neuroblastoma growth cones that establish contacts with target cells contain mainly NCAM-180, whereas NCAM-140 has been found mainly in growth cones at the time they were still in the process of the target search [35, 36]. Moreover, Sytnyk et al. [37] reported that NCAM-180 plays a role in synapse stabilization and rapidly accumulates at sites of contact between axonal growth cones and postsynaptic cells in hippocampal cultures. During aging, a significant selective downregulation of NCAM-180 was observed [38]. However, NCAM-180 synthesis remained significant in some brain regions, such as the hippocampus [39] and olfactory bulb [40], where neuritogenesis, sprouting and synaptic remodeling occur continually.

NCAM mediates neurite outgrowth through several signaling pathways. One pathway depends on the nonreceptor protein-tyrosine kinase Fyn and the focal adhesion kinase (FAK). Another pathway involves signaling mediated by the fibroblast growth factor receptor (FGFR). A third pathway involves signaling through pertussis toxin-sensitive heterotrimeric G-proteins (for review, see [41]). Signaling through the Fyn–FAK pathway is known to be mediated by the NCAM-140 isoform, whereas signaling through FGFR can be mediated by both NCAM-140 and NCAM-180 [42, 43]. Additionally, NCAM has been demonstrated to interact with the main cytoskeleton elements. Both NCAM-140 and NCAM-180 have been shown to associate with α- and β-tubulin [44], and NCAM-180 binds brain spectrin [36], which in turn interacts with multiple targets, including filamentous actin, Na^+, K^+-ATPase, the voltage-gated Na^+ channel, and acidic phospholipids [4, 45]. Therefore, NCAM can directly affect the assembly, disassembly, and stabilization of cytoskeletal elements and neurite outgrowth [46].

Role of GAP-43 in Remodeling of the Actin Cytoskeleton and Neurite Outgrowth

GAP-43 is abundant in growth cones during development. In adulthood, GAP-43 expression is relatively low, with the exception of neurons that are hypothesized to be involved in synaptic remodeling, such as in the associative (cerebral) cortex,

hippocampus, and olfactory bulb [47]. GAP-43 is reexpressed in neurons that have damaged axons [48]. When nervous system tissue architecture is disrupted, GAP-43 expression is increased, not only in neurons, but also in glial cells in situ [47]. In mature neurons, GAP-43 is a presynaptic protein that is localized only in axons, whereas dendrites have no GAP-43 [49–51]. However, at the initial stage of neurite growth, GAP-43 was found in all neurites [47]. GAP-43 knockout mice show disrupted cortical maps, possibly attributable to defective axonal guidance [52, 53]. Conversely, transgenic mice overexpressing GAP-43 show enhanced neurite sprouting in the adult nervous system [54, 55] and improved learning ability [6]. The high correlation between extension of neurite processes and increased GAP-43 synthesis has made GAP-43 a tool often used to mark neurite outgrowth.

The neuritogenic function of GAP-43 depends on its ability to bind to the plasma membrane [56, 57] (for discussion, see [10, 58]). This ability is provided by the presence of cysteines C3 and C4 that are dynamically palmitoylated [59–63]. Rat-1 and 3T3 fibroblasts and PC12E2 cells transfected with plasmids encoding GAP-43 mutated at the C3C4 site display cytosolic localization of this protein and are morphologically indistinguishable from untransfected controls, while expression of a plasmid construct encoding wild-type GAP-43 induces formation of filopodial extensions during spreading [10, 64, 65].

Another important post-translational modification of GAP-43 – phosphorylation by protein kinase C (PKC) on S41 [47, 66–68] – is considered to be very important for neuronal signal transduction. In particular, phosphorylation-dephosphorylation on S41 regulates the interaction of GAP-43 with the actin cytoskeleton. S41-phosphorylated GAP-43 stabilizes actin filaments and promotes neurite outgrowth. In contrast, unphosphorylated GAP-43 may reduce actin filament length [3, 69]. At the same time, phosphorylation on S41 prevents GAP-43 from binding to the lipid membrane [70, 71] and interacting with calmodulin [72]. This modification also prevents GAP-43 from site-specific proteolysis by μ-calpain [73]. Dephosphorylation of S41 restores the susceptibility of the GAP-43 molecule to these interactions. One of the after-effects may be colocalization of dephosphorylated GAP-43 with a disorganized cytoskeleton in the collapsed area of axonal endings [3, 69]. Additionally, the interaction of dephosphorylated GAP-43 (or its *N*-terminal fragment formed by μ-calpain) with heterotrimeric GTPase G_0 (G_0 protein) may result in activation of signal pathways leading to cytoskeleton destruction and growth cone collapse [56, 74, 75].

Involvement of GAP-43 in Neuronal Adhesion and NCAM-Mediated Neurite Outgrowth

Evidence suggests an involvement of GAP-43 in neuronal adhesion. When dorsal root ganglia (DRG) neurons are depleted of GAP-43 by antisense oligonucleotide treatment, they lose the ability to extend neurites on poly-L-ornithine-coated surfaces and fail to accomplish NGF-induced spreading [76]. However, when GAP-43-depleted DRG neurons are grown on laminin-coated substrates, they

extend long and thin neurites bearing growth cones that adhere poorly to the substrate [77]. In contrast, PC12 cells, which do not express GAP-43 (PC12-B subclone), exhibit reduced adhesion to uncoated plastic culture surfaces and are easily dislodged from culture plates [78]. When these cells are transfected with GAP-43 cDNA, their attachment to untreated substrates increases substantially [79].

A role for GAP-43 in neuronal adhesion and neurite outgrowth was suggested in a model of GAP-43 functionality in NCAM-induced neurite outgrowth [1]. NCAM was unable to stimulate neurite outgrowth in mouse cerebellar granule cells depleted of the GAP-43 gene by homologous recombination, while integrin-mediated neurite outgrowth was unaffected by GAP-43 gene deletion. Similarly, primary hippocampal neurons expressing GAP-43 mutated at C3C4 do not respond to NCAM stimulation [10]. GAP-43 mutated at C3C4, in contrast to wild-type GAP-43, is suggested to be unable to attach to the cytoplasmic membrane. Thus, GAP-43 association with the membrane may be important for NCAM-induced neuritogenesis.

Role of GAP-43 Phosphorylation in NCAM-Mediated Neurite Outgrowth

The role of GAP-43 phosphorylation in the regulation of the cytoskeleton is described above (for details, see "Role of GAP-43 in Remodeling of the Actin Cytoskeleton and Neurite Outgrowth" in this chapter). NCAM-stimulated neurite outgrowth has been shown to be associated with increased GAP-43 phosphorylation on S41 in cerebellar granule cells and hippocampal primary neurons [1, 10]. Meiri et al. [1] demonstrated that both neurite outgrowth and GAP-43 phosphorylation in isolated growth cones are stimulated by the first three immunoglobulin (Ig) modules of NCAM-soluble chimera as efficiently as by the whole molecule. Similarly, peptide P2 (GRILARGEINFK) corresponding to the 12-amino acid sequence localized in the F and G β-strands and interconnecting loop of the second NCAM Ig module [80] also is capable of inducing both GAP-43 phosphorylation on S41 and neurite outgrowth in primary hippocampal neurons [10]. In contrast, mutations on S41 in GAP-43 led to abrogation of NCAM-stimulated neurite outgrowth in hippocampal neurons [10]. Thus, GAP-43 phosphorylation on S41 is required for NCAM-stimulated, GAP-43-mediated neurite outgrowth.

Other sites in GAP-43 (S191 and S192) have been shown to be phosphorylated by casein kinase II (CKII) in vitro [81, 82] and in isolated growth cones [83]. However, this effect has not yet been shown in vivo [68, 84]. Korshunova et al. [10] found that expression of GAP-43 mutated at S191 and S192 in primary hippocampal neurons downregulates both NCAM-mediated and GAP-43-dependent neurite outgrowth. These results suggest that phosphorylation of GAP-43 on S191 and S192 may be important for NCAM-induced neuritogenesis.

FGFR Function Is Required for NCAM-Stimulated GAP-43 Phosphorylation

FGFR activation is involved in the signaling pathway underlying neurite outgrowth stimulated by NCAM [1] (for review, see [41]). Neurite outgrowth stimulated by NCAM-mediated activation of FGFR was shown to be associated with increased GAP-43 phosphorylation on S41 [1]. FGFR activation by NCAM initiates the sequential activation of phospholipase Cγ (PLCγ) and diacylglycerol lipase (DAG lipase) that results in the release of arachidonic acid (AA) that, in turn, activates voltage-gated calcium channels (VGCC). Local increase in submembrane calcium contributes to activation of PKC, which phosphorylates membrane-associated GAP-43 on S41 [1, 71].

GAP-43 and PKC were found to be within the same lipid raft platform of the plasma membrane [7, 85]. The integrity of lipid rafts was shown to be mandatory for GAP-43 phosphorylation by PKC [85]. Moreover, after phosphorylation by PKC, GAP-43 was suggested to shift from raft to nonraft membranes [85]. However, according to Tejero-Diez et al. [71], S41 phosphorylation is highest in cytoskeleton-associated GAP-43 and lowest in membrane-associated GAP-43. The authors assume a continuous cycling of GAP-43 between intracellular compartments depending on its phosphorylation. S41-phosphorylated GAP-43 molecules were proposed to translocate to the actin cytoskeleton and stabilize newly formed adhesive complexes that are essential for neurite outgrowth [71]. Because the data are controversial, determining where GAP-43 phosphorylation occurs and where phosphorylated GAP-43 is localized needs to be clarified.

PSA-NCAM and GAP-43 Are Coexpressed as Plasticity-Promoting Molecules: Possible Signaling Mechanisms Linking PSA-NCAM to GAP-43 Phosphorylation

NCAM can be glycosylated with polysialic acid (PSA; large homopolymers of the negatively charged molecule α-2,8-sialic acid) on its extracellular domain [86]. The length of the PSA chain (i.e., the number of sialic acid residues) is developmentally regulated. During the late embryonic and early postnatal periods, highly polysialylated NCAM, so-called embryonic NCAM (PSA-NCAM), is abundant throughout the nervous system and associated with morphogenetic changes, such as cell migration, synaptogenesis, and axonal growth. With the establishment of neuronal connections, PSA-NCAM, similar to GAP-43 (for details, see “Role of GAP-43 in Remodeling of the Actin Cytoskeleton and Neurite Outgrowth” in this chapter), rapidly decreases [87]. Importantly, coexpression of PSA-NCAM and GAP-43 have been reported in regions of adult brain structures that display a high degree of structural plasticity [88–90]. Thus, both PSA-NCAM and GAP-43 emerge as plasticity-promoting molecules in the adult nervous system.

NCAM/PSA-NCAM most likely activates tyrosine kinase receptors, such as FGFR, through the induction of dimerization and clustering of receptor molecules [90, 91]. Strong adhesion mediated by nonpolysialylated NCAM through the formation of tight two-dimensional zipper-like adhesion clusters may hamper interactions between NCAM and FGFR [91]. In contrast, the formation of loose clusters of one-dimensional zippers in the presence of PSA favors the association between NCAM and FGFR [91]. Thus, the expression of PSA on NCAM may switch NCAM functions from adhesion to signaling. Consistent with this hypothesis, PSA expression appears to be required for NCAM-induced FGFR signaling [92]. Consequently, FGFR activation was shown to be associated with increased GAP-43 phosphorylation on S41 [1] (for details, see "FGFR Function Is Required for NCAM-Stimulated GAP-43 Phosphorylation" in this chapter). This hypothesis, however, has not been substantiated experimentally, and further studies are needed to elucidate the signaling mechanisms linking PSA-NCAM with GAP-43-phosphorylation.

Differential Role of NCAM Isoforms in GAP-43-Mediated Neurite Outgrowth

By comparing manifestations of GAP-43 and different NCAM isoforms in the organism during development, one might suggest that NCAM-180 is the most likely candidate for interactions with GAP-43. The earliest expression of GAP-43 and NCAM-180 is detected in postmitotic neurons. Both proteins are actively synthesized during development and significantly downregulated in adulthood. Both GAP-43 and NCAM-180 regulate specific mechanisms that control the growth of axons and dendrites, and the establishment of synaptic contacts plays a role in stabilizing newly established synapses. In mature neurons, synthesis of GAP-43 and NCAM-180 is relatively low, with the exception of neurons of associative brain areas (e.g., hippocampus and olfactory bulb) that are involved in synaptic remodeling [39, 40, 47]. NCAM-120 and NCAM-140 synthesis correlates with GAP-43 synthesis to a much lesser degree (for details, see "Role of NCAM in the Nervous System" and "Role of GAP-43 in Remodeling of the Actin Cytoskeleton and Neurite Outgrowth" in this chapter).

NCAM has been shown to be enriched in special microdomains of the plasma membrane, termed lipid rafts, some of which containing GAP-43 [9]. Numerous data suggest that rafts are heterogeneous with regard to lipid and protein composition [9, 58]. Analysis of rafts isolated by immunoprecipitation and containing GAP-43 has revealed that these rafts also are enriched in the NCAM-180 isoform, whereas NCAM-140 predominated in rafts not containing GAP-43 [10]. Thus, GAP-43 was suggested to be involved in NCAM-180-, rather than NCAM-140-mediated neurite outgrowth. This suggestion was confirmed by the observation that in GAP-43-negative PC12E2 cells, NCAM-induced neurite outgrowth depended solely on the function of NCAM-140 [93], whereas in PC12E2 cells transfected

with plasmids carrying the GAP-43 gene, NCAM-induced neurite extension depended on NCAM-180 [10]. This result was reproduced in hippocampal neurons possessing the endogenous GAP-43 gene. In this system, expression of dominant negative NCAM-180, but not dominant negative NCAM-140, abrogated NCAM-stimulated neuritogenesis [10]. These findings appear to contradict the results obtained by Niethammer et al. [11], in which cultured hippocampal neurons of NCAM-deficient mice were transfected with one of the NCAM isoforms and stimulated with the extracellular part of NCAM. Only NCAM-140-expressing neurons exhibited increased neurite outgrowth. The authors concluded that NCAM-140, but not NCAM-180, could serve as a homophylic receptor that induces neuritogenesis. However, only one NCAM isoform at a time was transfected into NCAM-negative neurons, leaving unanswered the question whether the presence of NCAM-140 might be important for NCAM-180 function. Importantly, the level of NCAM-140 expression in cultured hippocampal neurons is significantly higher than that of NCAM-180 [94]. In an experimental system employed by Korshunova et al. [10], transfection of hippocampal neurons with dominant negative NCAM-140 was insufficient for completely "switching off" NCAM-140-mediated signaling. Therefore, although the results obtained by Korshunova et al. [10] strongly suggest the importance of NCAM-180 for NCAM-induced neurite outgrowth in the presence of GAP-43, they do not exclude a neuritogenic role for NCAM-140.

Functional Complex of NCAM-180 with GAP-43 and Spectrin

Spectrin is an actin-binding protein that links membrane domains to the actin cytoskeleton. Pollerberg et al. [35, 36] reported that NCAM-180, but not NCAM-140 or NCAM-120, is bound specifically to brain spectrin. Leshchyns'ka et al. [95] found that all three NCAM isoforms coprecipitated with spectrin, but of the three, NCAM-180 was much more potent in precipitating spectrin. GAP-43 was suggested to interact also with brain spectrin [96]. Additionally, GAP-43 controls the dynamics of the actin cytoskeleton and neurite outgrowth [56, 97]. Korshunova et al. [10] subsequently demonstrated that spectrin may be involved in NCAM-mediated neurite outgrowth in the presence, but not absence, of GAP-43. Thus, NCAM-180 was proposed to be linked to the spectrin-actin network, and GAP-43 appears to regulate this connection. These data together suggest the existence of a functional NCAM-180/spectrin/GAP-43 complex that can transmit NCAM-initiated signals. Korshunova et al. [10] proposed a model in which GAP-43 acts as a "switch" between NCAM-140- and NCAM-180-induced signaling (Fig. 1). According to this model, both NCAM isoforms employ FGFR. In the presence of GAP-43, NCAM-induced neurite outgrowth is crucially dependent on the NCAM-180/spectrin/GAP-43 complex (Fig. 1a). This complex induces an FGFR-dependent signal cascade that leads to PKC activation as a consequence of the successive activities of PLCγ and DAG lipase. One of the prominent outputs of this cascade is GAP-43 phosphorylation by PKC, leading to stabilization of the actin cytoskeleton

and neurite outgrowth. In the absence of GAP-43, the NCAM-140/Fyn/FAK signaling pathway is involved in neurite outgrowth (Fig. 1b).

Open Question: Fyn/RPTPα Association with NCAM-180/Spectrin/GAP-43 Complex

NCAM-induced morphogenic effects depend on activation of Src family tyrosine kinases, particularly activation of Fyn [98]. NCAM-dependent neurite outgrowth is impaired in neurons from Fyn-deficient mice [99] and is abolished by inhibitors of Src kinase family members [43, 100, 101]. Fyn is constitutively associated with NCAM-140, but does not associate significantly with NCAM-180 [42]. He and Meiri [9] reported that GAP-43 and Fyn were found in different membrane raft fractions. Similar results were obtained by Botto et al. [7]. The authors isolated subsets of rafts from cerebellar granule cells and found that one of the subsets contained GAP-43 and PKC, but not Fyn. Thus, one could conclude that Fyn is not involved in NCAM-180/GAP-43-mediated neurite outgrowth. However, Bodrikov et al. [102] demonstrated that the cytoplasmic part of both NCAM-140 and NCAM-180 can be bound to Fyn through receptor protein-tyrosine phosphatase α (RPTPα), a known activator of Fyn. Indeed, Kolkova et al. [43] found that Fyn participates in NCAM-140-dependent neurite outgrowth (Fig. 1b). With regard to the possible interplay between NCAM-180 and RPTPα, the affinity of the NCAM-180–RPTPα interaction was reported to be significantly lower than that of the NCAM-140–RPTPα interaction [102]. This fact may explain the faint co-precipitation of NCAM-180 with Fyn that was demonstrated in earlier studies [42]. Thus, the NCAM-180–Fyn interaction, though not strong, may play a role in NCAM-180-mediated neurite outgrowth. The issue of whether phosphorylated Fyn and phosphorylated GAP-43 and/or NCAM-180 are localized in different raft fractions will need to be clarified further.

NCAM and Growth-Associated Proteins BASP1 and MARCKS

GAP-43 is a member of the family of neuronal growth-associated proteins that also includes brain acid soluble protein-1 (BASP1; also known as CAP-23 and NAP-22) and myristoylated alanine-rich C-kinase substrate (MARCKS). Although these proteins do not share noticeable sequence homologies, they have a number of properties in common [47, 65, 103–105]. All three proteins are characterized by high hydrophilicity, and all are subjected to N-terminal fatty acylation resulting in linkage to the plasma membrane preferentially in lipid rafts [8, 97, 106, 107]. All three proteins also contain a basic domain (termed an effector domain) that binds acidic phospholipids, calmodulin, and actin filaments. These interactions can be modulated

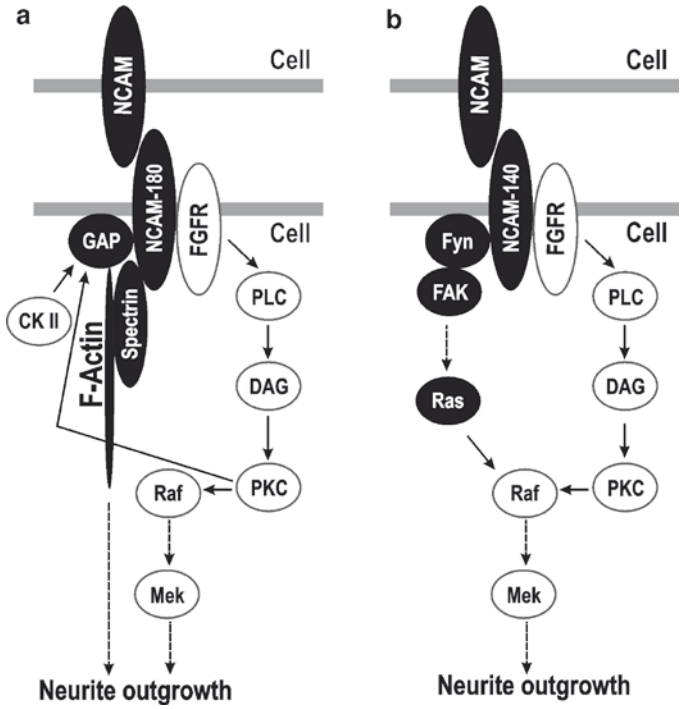

Fig. 1 Schematic representation of the functional relationship between GAP-43 and the two NCAM isoforms, NCAM-180 and NCAM-140. (**a**) In the presence of GAP-43, neurite outgrowth is mediated through the NCAM-180/spectrin/GAP-43 complex (modified from [10]). (**b**) In the absence of GAP-43, neurite outgrowth is mediated through the NCAM-140/Fyn/FAK complex (modified from [43]). FAK, focal adhesion kinase; Fyn, p59 fyn kinase; FGFR, fibroblast growth factor receptor; DAG, diacylglycerol; PKC, protein kinase C; CKII, casein kinase II; Mek, mitogen-activated protein kinase

by PKC-mediated phosphorylation within the effector domain [2, 47, 70, 108]. Numerous data implicate a role for BASP1 and MARCKS in regulating actin cytoskeleton dynamics and neurite outgrowth. However, no information has been provided regarding their participation in NCAM-triggered pathways. Recently, Korshunova et al. [109] found that BASP1 is not involved in NCAM-induced signaling.

Further investigation of NCAM, in particular NCAM-180, interactions with growth-associated proteins in axon endings will be useful for elucidating the participation of NCAM in the processes of neuronal net formation and learning and memory.

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References

1. Meiri KF, Saffell JL, Walsh FS et al (1998) Neurite outgrowth stimulated by neural cell adhesion molecules requires growth-associated protein-43 (GAP-43) function and is associated with GAP-43 phosphorylation in growth cones. J Neurosci 18:10429–10437
2. Sasaki Y (2003) New aspects of neurotransmitter release and exocytosis: Rho-kinase-dependent myristoylated alanine-rich C-kinase substrate phosphorylation and regulation of neurofilament structure in neuronal cells. J Pharmacol Sci 93:35–40
3. Dent EW, Meiri KF (1998) Distribution of phosphorylated GAP-43 (neuromodulin) in growth cones directly reflects growth cone behavior. J Neurobiol 35:287–299
4. Hirokawa N (1998) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279:519–526
5. Popov VI, Medvedev NI, Kraev IV et al (2008) A cell adhesion molecule mimetic, FGL peptide, induces alterations in synapse and dendritic spine structure in the dentate gyrus of aged rats: a three-dimensional ultrastructural study. Eur J NeuroSci 27:301–314
6. Routtenberg A, Cantallops I, Zaffuto S et al (2000) Enhanced learning after genetic overexpression of a brain growth protein. Proc Natl Acad Sci USA 97:7657–7662
7. Botto L, Masserini M, Cassetti A et al (2004) Immunoseparation of Prion protein-enriched domains from other detergent-resistant membrane fractions, isolated from neuronal cells. FEBS Lett 557:143–147
8. Caroni P (2001) New EMBO members' review: actin cytoskeleton regulation through modulation of PI(4, 5)P(2) rafts. EMBO J 20:4332–4336
9. He Q, Meiri KF (2002) Isolation and characterization of detergent-resistant microdomains responsive to NCAM-mediated signaling from growth cones. Mol Cell Neurosci 19:18–31
10. Korshunova I, Novitskaya V, Kiryushko D et al (2007) GAP-43 regulates NCAM-180-mediated neurite outgrowth. J Neurochem 100:1599–1612
11. Niethammer P, Delling M, Sytnyk V et al (2002) Cosignaling of NCAM via lipid rafts and the FGF receptor is required for neuritogenesis. J Cell Biol 157:521–532
12. Crossin KL, Chuong CM, Edelman GM (1985) Expression sequences of cell adhesion molecules. Proc Natl Acad Sci USA 82:6942–6946
13. Chuong CM, Edelman GM (1985) Expression of cell-adhesion molecules in embryonic induction. I. Morphogenesis of nestling feathers. J Cell Biol 101:1009–1026
14. Jacque CM, Jorgensen OS, Bock E (1974) Quantitative studies on the brain specific antigens S-100, GFA, 14–3-2, D1, D2, D3 and C1 in Quaking mouse. FEBS Lett 49:264–266
15. Jorgensen OS (1981) Neuronal membrane D2-protein during rat brain ontogeny. J Neurochem 37:939–946
16. Linnemann D, Bock E (1989) Cell adhesion molecules in neural development. Dev Neurosci 11:149–173
17. Ronn LC, Pedersen N, Jahnsen H et al (1997) Brain plasticity and the neural cell adhesion molecule (NCAM). Adv Exp Med Biol 429:305–322
18. Gennarini G, Hirsch MR, He HT et al (1986) Differential expression of mouse neural cell-adhesion molecule (N-CAM) mRNA species during brain development and in neural cell lines. J Neurosci 6:1983–1990
19. Minana R, Sancho-Tello M, Climent E et al (1998) Intracellular location, temporal expression, and polysialylation of neural cell adhesion molecule in astrocytes in primary culture. Glia 24:415–427
20. Nybroe O, Albrechtsen M, Dahlin J et al (1985) Biosynthesis of the neural cell adhesion molecule: characterization of polypeptide C. J Cell Biol 101:2310–2315
21. Kobayashi S, Vidal I, Pena JD et al (1997) Expression of neural cell adhesion molecule (NCAM) characterizes a subpopulation of type 1 astrocytes in human optic nerve head. Glia 20:262–273
22. Covault J, Sanes JR (1986) Distribution of N-CAM in synaptic and extrasynaptic portions of developing and adult skeletal muscle. J Cell Biol 102:716–730

23. Gaardsvoll H, Krog L, Zhernosekov D et al (1993) Age-related changes in expression of neural cell adhesion molecule (NCAM) in heart: a comparative study of newborn, adult and aged rats. Eur J Cell Biol 61:100–107
24. Wharton J, Gordon L, Walsh FS et al (1989) Neural cell adhesion molecule (N-CAM) expression during cardiac development in the rat. Brain Res 483:170–176
25. Akeson RA, Wujek JR, Roe S et al (1988) Smooth muscle cells transiently express NCAM. Brain Res 464:107–120
26. Klein G, Langegger M, Goridis C et al (1988) Neural cell adhesion molecules during embryonic induction and development of the kidney. Development 102:749–761
27. Ricard CS, Kobayashi S, Pena JDO et al (2000) Selective expression of neural cell adhesion molecule (NCAM)-180 in optic nerve head astrocytes exposed to elevated hydrostatic pressure in vitro. Mol Brain Res 81:62–79
28. Moller CJ, Byskov AG, Roth J et al (1991) NCAM in developing mouse gonads and ducts. Anat Embryol (Berl) 184:541–548
29. Moller CJ, Christgau S, Williamson MR et al (1992) Differential expression of neural cell adhesion molecule and cadherins in pancreatic islets, glucagonomas, and insulinomas. Mol Endocrinol 6:1332–1342
30. Figarella-Branger D, Pellissier JF, Bianco N et al (1992) Expression of various NCAM isoforms in human embryonic muscles: correlation with myosin heavy chain phenotypes. J Neuropathol Exp Neurol 51:12–23
31. Tomasiewicz H, Ono K, Yee D et al (1993) Genetic deletion of a neural cell adhesion molecule variant (N-CAM-180) produces distinct defects in the central nervous system. Neuron 11:1163–1174
32. Shen H, Watanabe M, Tomasiewicz H et al (2001) Genetic deletions of NCAM and PSA impair circadian function in the mouse. Physiol Behav 73:185–193
33. Persohn E, Schachner M (1987) Immunoelectron microscopic localization of the neural cell adhesion molecules L1 and N-CAM during postnatal development of the mouse cerebellum. J Cell Biol 105:569–576
34. Pollerberg EG, Sadoul R, Goridis C et al (1985) Selective expression of the 180-kD component of the neural cell adhesion molecule N-CAM during development. J Cell Biol 101:1921–1929
35. Pollerberg GE, Schachner M, Davoust J (1986) Differentiation state-dependent surface mobilities of two forms of the neural cell adhesion molecule. Nature 324:462–465
36. Pollerberg GE, Burridge K, Krebs KE et al (1987) The 180-kD component of the neural cell adhesion molecule N-CAM is involved in cell-cell contacts and cytoskeleton-membrane interactions. Cell Tissue Res 250:227–236
37. Sytnyk V, Leshchyns'ka I, Delling M et al (2002) Neural cell adhesion molecule promotes accumulation of TGN organelles at sites of neuron-to-neuron contacts. J Cell Biol 159:649–661
38. Linnemann D, Gaardsvoll H, Olsen M et al (1993) Expression of NCAM mRNA and polypeptides in aging rat brain. Int J Dev Neurosci 11:71–81
39. Kramer I, Hall H, Bleistein U et al (1997) Developmentally regulated masking of an intracellular epitope of the 180 kDa isoform of the neural cell adhesion molecule NCAM. J Neurosci Res 49:161–175
40. Touyarot K, Venero C, Sandi C (2004) Spatial learning impairment induced by chronic stress is related to individual differences in novelty reactivity: search for neurobiological correlates. Psychoneuroendocrinology 29:290–305
41. Walmod PS, Kolkova K, Berezin V et al (2004) Zippers make signals: NCAM-mediated molecular interactions and signal transduction. Neurochem Res 29:2015–2035
42. Beggs HE, Baragona SC, Hemperly JJ et al (1997) NCAM140 interacts with the focal adhesion kinase p125fak and the SRC-related tyrosine kinase p59fyn. J Biol Chem 272:8310–8319
43. Kolkova K, Novitskaya V, Pedersen N et al (2000) Neural cell adhesion molecule-stimulated neurite outgrowth depends on activation of protein kinase C and the Ras-mitogen-activated protein kinase pathway. J Neurosci 20:2238–2246

44. Buttner B, Kannicht C, Reutter W et al (2003) The neural cell adhesion molecule is associated with major components of the cytoskeleton. Biochem Biophys Res Commun 310:967–971
45. De Matteis MA, Morrow JS (2000) Spectrin tethers and mesh in the biosynthetic pathway. J Cell Sci 113:2331–2343
46. Dent EW, Gertler FB (2003) Cytoskeletal dynamics and transport in growth cone motility and axon guidance. Neuron 40:209–227
47. Oestreicher AB, De Graan PNE, Gispen WH et al (1997) B-50, the growth associated protein-43: modulation of cell morphology and communication in the nervous system. Prog Neurobiol 53:627–686
48. Frey D, Laux T, Xu L et al (2000) Shared and unique roles of CAP23 and GAP43 in actin regulation, neurite outgrowth, and anatomical plasticity. J Cell Biol 149:1443–1454
49. Gispen WH, Leunissen JLM, Oestreicher AB et al (1985) Presynaptic localization of B-50 phosphoprotein: the (ACTH)-sensitive protein kinase substrate involved in rat brain polyphosphoinositide metabolism. Brain Res 328:381–385
50. Goslin K, Banker G (1990) Rapid changes in the distribution of GAP-43 correlate with the expression of neuronal polarity during normal development and under experimental conditions. J Cell Biol 110:1319–1331
51. Goslin K, Schreyer DJ, Skene JH et al (1990) Changes in the distribution of GAP-43 during the development of neuronal polarity. J Neurosci 10:588–602
52. Maier DL, Mani S, Donovan SL et al (1999) Disrupted cortical map and absence of cortical barrels in growth-associated protein (GAP)-43 knockout mice. Proc Natl Acad Sci USA 96:9397–9402
53. Strittmatter SM, Fankhauser C, Huang PL et al (1995) Neuronal pathfinding is abnormal in mice lacking the neuronal growth cone protein GAP-43. Cell 80:445–452
54. Aigner L, Arber S, Kapfhammer JP et al (1995) Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. Cell 83:269–278
55. Caroni P (1997) Intrinsic neuronal determinants that promote axonal sprouting and elongation. Bioessays 19:767–775
56. Strittmatter SM, Igarashi M, Fishman MC (1994) GAP-43 amino terminal peptides modulate growth cone morphology and neurite outgrowth. J Neurosci 14:5503–5513
57. Sudo Y, Valenzuela D, Beck-Sickinger AG et al (1992) Palmitoylation alters protein activity: blockade of G(o) stimulation by GAP-43. EMBO J 11:2095–2102
58. Mosevitsky MI (2005) Nerve ending "signal" proteins GAP-43, MARCKS, and BASP1. Int Rev Cytol 245:245–325
59. Aarts LH, van der Linden JA, Hage WJ et al (1995) N-terminal cysteines essential for Golgi sorting of B-50 (GAP-43) in PC12 cells. NeuroReport 6:969–972
60. Liang X, Lu Y, Neubert TA et al (2002) Mass spectrometric analysis of GAP-43/neuromodulin reveals the presence of a variety of fatty acylated species. J Biol Chem 277:33032–33040
61. Liu J, Hughes TE, Sessa WC (1997) The first 35áamino acids and fatty acylation sites determine the molecular targeting of endothelial nitric oxide synthase into the Golgi region of cells: a green fluorescent protein study. J Cell Biol 137:1525–1535
62. Skene JH, Virag I (1989) Posttranslational membrane attachment and dynamic fatty acylation of a neuronal growth cone protein, GAP-43. J Cell Biol 108:613–624
63. Zuber MX, Strittmatter SM, Fishman MC (1989) A membrane-targeting signal in the amino terminus of the neuronal protein GAP-43. Nature 341:345–348
64. Aarts LH, Schrama LH, Hage WJ et al (1998) B-50/GAP-43-induced formation of filopodia depends on Rho-GTPase. Mol Biol Cell 9:1279–1292
65. Wiederkehr A, Staple J, Caroni P (1997) The motility-associated proteins GAP-43, MARCKS, and CAP-23 share unique targeting and surface activity-inducing properties. Exp Cell Res 236:103–116
66. Di LM, Pastorino L, Raverdino V et al (1996) Determination of the endogenous phosphorylation state of B-50/GAP-43 and neurogranin in different brain regions by electrospray mass spectrometry. FEBS Lett 389:309–313

67. Huang KP, Huang FL, Chen HC (1999) Hypoxia/ischemia induces dephosphorylation of rat brain neuromodulin/GAP-43 in vivo. J Neurochem 72:1294–1306
68. Spencer SA, Schuh SM, Liu WS et al (1992) GAP-43, a protein associated with axon growth, is phosphorylated at three sites in cultured neurons and rat brain. J Biol Chem 267:9059–9064
69. He Q, Dent EW, Meiri KF (1997) Modulation of actin filament behavior by GAP-43 (neuromodulin) is dependent on the phosphorylation status of serine 41, áthe protein kinase C site. J Neurosci 17:3515–3524
70. Hayashi N, Matsubara M, Titani K et al (1997) Circular dichroism and 1H nuclear magnetic resonance studies on the solution and membrane structures of GAP-43 calmodulin-binding domain. J Biol Chem 272:7639–7645
71. Tejero-Diez P, Rodriguez-Sanchez P, Martin-Cofreces NB et al (2000) bFGF stimulates GAP-43 phosphorylation at ser41 and modifies its intracellular localization in cultured hippocampal neurons. Mol Cell Neurosci 16:766–780
72. Gamby C, Waage MC, Allen RG et al (1996) Analysis of the role of calmodulin binding and sequestration in neuromodulin (GAP-43) function. J Biol Chem 271:26698–26705
73. Zakharov VV, Mosevitsky MI (2007) M-calpain-mediated cleavage of GAP-43 near Ser41 is negatively regulated by protein kinase C, calmodulin and calpain-inhibiting fragment GAP-43–3. J Neurochem 101:1539–1551
74. Igarashi M, Li WW, Sudo Y et al (1995) Ligand-induced growth cone collapse: amplification and blockade by variant GAP-43 peptides. J Neurosci 15:5660–5667
75. Strittmatter SM, Valenzuela D, Kennedy TE et al (1990) G0 is a major growth cone protein subject to regulation by GAP-43. Nature 344:836–841
76. Aigner L, Caroni P (1993) Depletion of 43-kD growth-associated protein in primary sensory neurons leads to diminished formation and spreading of growth cones. J Cell Biol 123:417–429
77. Aigner L, Caroni P (1995) Absence of persistent spreading, branching, and adhesion in GAP-43-depleted growth cones. J Cell Biol 128:647–660
78. Baetge EE, Hammangt JP (1991) Neurite outgrowth in PC12 cells deficient in GAP-43. Neuron 6:21–30
79. Meiri KF, Hammang JP, Dent EW et al (1996) Mutagenesis of ser41 to ala inhibits the association of GAP-43 with the membrane skeleton of GAP-43-deficient PC12B cells: effects on cell adhesion and the composition of neurite cytoskeleton and membrane. J Neurobiol 29:213–232
80. Soroka V, Kiryushko D, Novitskaya V et al (2002) Induction of Neuronal differentiation by a peptide corresponding to the homophilic binding site of the second Ig module of the neural cell adhesion molecule. J Biol Chem 277:24676–24683
81. Apel ED, Litchfield DW, Clark RH et al (1991) Phosphorylation of neuromodulin (GAP-43) by casein kinase II. Identification of phosphorylation sites and regulation by calmodulin. J Biol Chem 266:10544–10551
82. Pisano MR, Hegazy MG, Reimann EM et al (1988) Phosphorylation of protein B-50 (GAP-43) from adult rat brain cortex by casein kinase II. Biochem Biophys Res Commun 155:1207–1212
83. Edgar MA, Pasinelli P, DeWit M et al (1997) Phosphorylation of the casein kinase II domain of B-50 (GAP-43) in rat cortical growth cones. J Neurochem 69:2206–2215
84. Taniguchi H, Suzuki M, Manenti S et al (1994) A mass spectrometric study on the in vivo posttranslational modification of GAP-43. J Biol Chem 269:22481–22484
85. Botto L, Masserini M, Palestini P (2007) Changes in the composition of detergent-resistant membrane domains of cultured neurons following protein kinase C activation. J Neurosci Res 85:443–450
86. Yang P, Yin X, Rutishauser U (1992) Intercellular space is affected by the polysialic acid content of NCAM. J Cell Biol 116:1487–1496
87. Rutishauser U, Landmesser L (1996) Polysialic acid in the vertebrate nervous system: a promoter of plasticity in cell-cell interactions. Trends Neurosci 19:422–427
88. Alonso G, Prieto M, Legrand A et al (1997) PSA-NCAM and B-50/GAP-43 are coexpressed by specific neuronal systems of the adult rat mediobasal hypothalamus that exhibit remarkable capacities for morphological plasticity. J Comp Neurol 384:181–199

89. Emery DL, Royo NC, Fischer I et al (2003) Plasticity following injury to the adult central nervous system: is recapitulation of a developmental state worth promoting? J Neurotrauma 20:1271–1292
90. Gascon E, Vutskits L, Kiss JZ (2007) Polysialic acid-neural cell adhesion molecule in brain plasticity: from synapses to integration of new neurons. Brain Res Reviews 56:101–118
91. Kiselyov VV, Soroka V, Berezin V et al (2005) Structural biology of NCAM homophilic binding and activation of FGFR. J Neurochem 94:1169–1179
92. Dityatev A, Dityateva G, Sytnyk V et al (2004) Polysialylated neural cell adhesion molecule promotes remodeling and formation of hippocampal synapses. J Neurosci 24:9372–9382
93. Kolkova K, Pedersen N, Berezin V et al (2000) Identification of an amino acid sequence motif in the cytoplasmic domain of the NCAM-140 kDa isoform essential for its neuritogenic activity. J Neurochem 75:1274–1282
94. Kiryushko D, Kofoed T, Skladchikova G et al (2003) A synthetic peptide ligand of neural cell adhesion molecule (NCAM), C3d, promotes neuritogenesis and synaptogenesis and modulates presynaptic function in primary cultures of rat hippocampal neurons. J Biol Chem 278:12325–12334
95. Leshchyns'ka I, Sytnyk V, Morrow JS et al (2003) Neural cell adhesion molecule (NCAM) association with PKCβ2 via βI spectrin is implicated in NCAM-mediated neurite outgrowth. J Cell Biol 161:625–639
96. Riederer BM, Routtenberg A (1999) Can GAP-43 interact with brain spectrin? Mol Brain Res 71:345–348
97. Laux T, Fukami K, Thelen M et al (2000) GAP43, MARCKS, and CAP23 modulate PI(4, 5) P2 at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. J Cell Biol 149:1455–1472
98. Schmid RS, Graff RD, Schaller MD et al (1999) NCAM stimulates the Ras-MAPK pathway and CREB phosphorylation in neuronal cells. J Neurobiol 38:542–558
99. Beggs HE, Soriano P, Maness PF (1994) NCAM-dependent neurite outgrowth is inhibited in neurons from Fyn-minus mice. J Cell Biol 127:825–833
100. Cavallaro U, Niedermeyer J, Fuxa M et al (2001) N-CAM modulates tumour-cell adhesion to matrix by inducing FGF-receptor signalling. Nat Cell Biol 3:650–657
101. Crossin KL, Krushel LA (2000) Cellular signaling by neural cell adhesion molecules of the immunoglobulin superfamily. Dev Dyn 218:260–279
102. Bodrikov V, Leshchyns'ka I, Sytnyk V et al (2005) RPTPα is essential for NCAM-mediated p59fyn activation and neurite elongation. J Cell Biol 168:127–139
103. Hartwig JH, Thelen M, Rosen A et al (1992) MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. Nature 356:618–622
104. Mosevitsky MI, Novitskaya VA, Plekhanov AY et al (1994) Neuronal protein GAP-43 is a member of novel group of brain acid-soluble proteins (BASPs). Neurosci Res 19:223–228
105. Mosevitsky MI, Capony JP, Skladchikova GY et al (1997) The BASP1 family of myristoylated proteins abundant in axonal termini. Primary structure analysis and physico-chemical properties. Biochimie 79:373–384
106. Golub T, Caroni P (2005) PI(4, 5)P2-dependent microdomain assemblies capture microtubules to promote and control leading edge motility. J Cell Biol 169:151–165
107. Maekawa S, Taguchi K (2004) Localization of the Cl–ATPase activity on NAP-22 enriched membrane microdomain (raft) of rat brain. Neurosci Lett 362:158–161
108. Maekawa S, Murofushi H, Nakamura S (1994) Inhibitory effect of calmodulin on phosphorylation of NAP-22 with protein kinase C. J Biol Chem 269:19462–19465
109. Korshunova I, Caroni P, Kolkova K et al (2008) Characterization of BASP1-mediated neurite outgrowth. J Neurosci Res 86:2201–2213