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SHORT COMMUNICATION
A cytoplasmic motif targets neuroligin-1 exclusively to dendrites of cultured hippocampal neurons

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Abstract

The formation of neuronal synapses is thought to depend on trans-synaptic interactions between cell adhesion molecules (CAMs) on the surface of axons and dendrites. Synapses are highly asymmetric structures. Pre- and post-synaptic domains might therefore be assembled around heterophilic CAMs which are polarized to axons vs. dendrites. We here investigated the targeting of neuroligin (NLG)-1, a heterophilic CAM, which promotes synapse formation through interaction with its receptor β-neurexin in axons. We demonstrate that NLG-1 is highly polarized to the dendritic plasma membrane. Dendritic targeting relies on a cytoplasmic amino acid motif. By expressing chimeras of NLG-1 and CD8, an unpolarized protein, we show that the cytoplasmic domain of NLG-1 is necessary and sufficient for dendritic targeting. Furthermore, by truncation analysis we isolated a 32-amino-acid targeting motif. When appended to CD8 this cytoplasmic sequence is sufficient to direct exclusively dendritic localization of the protein. Analysis of yellow fluorescent protein-tagged NLG-1 revealed that vesicular structures containing NLG-1 are excluded from the axon indicating that polarized distribution may be achieved by direct dendritic transport. We propose that the strict polarity of NLG-1 contributes to the directional assembly of synapses during development of the central nervous system.

Introduction

Cell polarity is a remarkable feature of neurons that underlies most aspects of their function. Several ion channels, cytoskeletal proteins and cell adhesion molecules are localized exclusively to the axonal or somato-dendritic domain of neurons thereby enabling them to propagate action potentials and carry out synaptic transmission (Craig & Banker, 1994). Previous studies revealed two principally different mechanisms for achieving the polarized distribution of membrane proteins. Proteins are either directly sorted to their appropriate membrane domain or are initially transported without apparent polarity but then selectively stabilized in axons or dendrites (Burack *et al.*, 2000; Sampo *et al.*, 2003; Fache *et al.*, 2004). However, for most neuronal proteins the mechanisms for polarized sorting and the signals that control axo-dendritic targeting are not well understood.

Appropriate polarized targeting is particularly relevant in the case of proteins that contribute to synapse formation. Contact of axons with dendrites initiates the development of synaptic structures whereas axo-axonal contacts generally do not lead to synapse assembly. This implies that there are directional synaptogenic signals provided by the dendrites that are absent from axons. Some of these synaptogenic signals are thought to be provided by cell adhesion molecules that mediate either homo- or heterophilic interactions between the axonal and dendritic cell surfaces (Scheiffele, 2003; Yamagata *et al.*, 2003; Washbourne *et al.*, 2004). Neuroligin (NLG)-1 is a heterophilic cell adhesion molecule which is concentrated at the post-synaptic density of glutamatergic synapses (Ichtchenko *et al.*, 1995; Song *et al.*, 1999). Importantly, NLG-1 mediates heterophilic adhesion with the axonal receptor β-neurexin (βNRX) (Nguyen & Sudhof, 1997) and NLG-1–βNRX interactions can trigger the assembly of functional synaptic terminals between the axon and dendrites (Dean *et al.*, 2003; Prange *et al.*, 2004). Moreover, the NLG-1–βNRX complex can also promote post-synaptic differentiation (Graf *et al.*, 2004; Chih *et al.*, 2005). These studies suggest that the heterophilic NLG-1–βNRX complex might provide a bidirectional signal for the assembly of neuronal synapses and might contribute to the structural asymmetry of neuronal synapses. A prerequisite for such a role in synapse formation is that NLG-1 should be exclusively targeted to the somato-dendritic domain and should be excluded from axons. However, the mechanisms that control polarity and transport of NLG-1 are not known.

Using exogenous expression of epitope-tagged NLG-1 proteins and quantitative, wide-field fluorescence microscopy we here demonstrate that NLG-1 is selectively targeted to the dendritic plasma membrane of cultured hippocampal neurons. Moreover, we show that a cytoplasmic sorting motif in NLG-1 is necessary and sufficient for dendritic targeting. Our results support a model where the polarized distribution of NLG-1 and its heterophilic interactions with βNRX contribute to the asymmetric assembly of pre- and post-synaptic membrane specializations around an axo-dendritic NLG-1–βNRX link.

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Methods and materials

Hippocampal cell culture and expression of transgenes

Primary cultures of dissociated neurons from embryonic day 18 rat hippocampi were prepared essentially as described by Banker & Goslin (1998). Animals were killed using CO_2 inhalation as approved by the University Animal Care and Use Committee. The dissociated neurons were plated onto pre-treated glass coverslips and then the coverslips were placed neuron-side down. After 7–8 days *in vitro* the neurons were cotransfected with a plasmid containing the protein of interest and a plasmid JPA (pJPA) containing enhanced green fluorescent protein (Sampo *et al.*, 2003). In preparation for transfection, the DNA (1–2 μg of each plasmid) was mixed with Lipofectamine 2000 (Gibco) according to the manufacturer's directions as demonstrated in Sampo *et al.* (2003). Coverslips were then placed neuron-side down and neurons were allowed to express for either 12 or 18 h at 37 °C under a controlled atmosphere containing 5% CO_2. Animals were treated in accordance with the California State Polytechnic University, Pomona's Animal Care and Use Committee guidelines (protocol no. 03-002).

Constructs

Each NLG construct was subcloned into the pJPA for optimal neuronal expression (Sampo *et al.*, 2003). Except for CD8-NLG and CD8-NLG-32aa, the NLG cDNAs are as described by Scheiffele *et al.* (2000). For pJPA-CD8-NLG, the stop codon of CD8 was changed by polymerase chain reaction to an alanine with the inclusion of a 3′-BamH1 restriction site. The entire cytoplasmic tail of NLG (amino acids 718–841) was amplified with the inclusion of 5′-BamH1 and 3′-NotI restriction sites for subcloning into pJPA-CD8 without the stop codon. For pJPA-CD8-NLG-32aa, the sequence encoding amino acids 772–804 was amplified to include 5′-Age1 and 3′-Xba1 restriction sites for subcloning into pJPA-CD8(Δstop). The yellow fluorescent protein (YFP)-NLG-1 fusion protein was generated by inserting the EYFP coding sequence without a stop codon into the mature extracellular domain of NLG-1. Plasmid composition was confirmed by DNA sequencing.

Immunostaining

To detect expressed proteins on the cell surface of live neurons, the primary antibody against the influenza hemagglutinin epitope tag (1 : 1000, clone 3F10, Roche) or CD8 (1 : 250, DAKO Corp.) was diluted in culture medium and placed on wax in a humidifying chamber at 37 °C. Coverslips containing transfected cells were exposed to the primary antibody-containing medium. After 7 min, the cells were rinsed in phosphate-buffered saline and fixed in a solution of 4% paraformaldehyde/4% sucrose in phosphate-buffered saline. Cells were incubated in fish skin gelatin and bovine serum albumin (0.5 and 10%, Sigma) in phosphate-buffered saline for 1 h at 37 °C to block nonspecific antibody-binding sites. Finally, coverslips were incubated with the appropriate fluorescently conjugated secondary antibody for 1 h at 37 °C and mounted on slides with Elvanol (Banker & Goslin, 1998).

Microscopy and quantitative measurements of immunofluorescence

Images of fluorescently labeled cells were acquired using a Photometrics CoolSNAP fx cooled CCD camera controlled by Metamorph software (Universal Imaging, Downingtown, PA, USA). Images were taken with a 20× 0.75 numerical aperture, plan apochromat or 60× 1.4 plan apochromat (both Nikon) objective. Exposure time was adjusted so that the maximum pixel value did not reach saturation. Only cotransfected cells whose labeled processes did not overlap with other labeled cells were selected for quantification.

All of the image processing was performed using Metamorph software essentially as described in Sampo *et al.* (2003). After acquiring the image, two corrections were performed on the live image before analysing its fluorescence levels. First, a shading correction was applied to compensate for the uneven illumination of the field (based on an image of a uniformly fluorescent specimen). Next, the average background calculated from several regions that did not contain labeled neurites was subtracted and this corrected image was used for analysis. To quantify the fluorescence in the cellular processes of the live-stained cells, several 1-pixel-wide lines (300–500 μm total length) were drawn medially along the axon and dendrites on the green fluorescent protein (GFP) image. The line traces were transferred onto the corrected images and the average fluorescence intensity of those lines was tabulated. An image of that same neuron labeled for microtubule associated protein-2 (MAP-2) (anti-MAP2, 1 : 1000, Chemicon) was used to assist in identifying dendrites. The average intensities from the axon and dendrites of each cell were transferred to an Excel (Microsoft) spreadsheet and used to generate dendrite to axon (D : A) ratios. The D : A ratios for each construct were calculated from several cells from at least three different cultures. In several highly polarized cells, there was no axonal staining above background, which led to D : A ratios in excess of 100 : 1. To prevent introducing a bias into the data set we used a set maximal D : A ratio of 20 : 1 for these cells.

Statistical significance was assessed using the PROC GLM function in the statistical software SAS 9.1.3. First, a one-way ANOVA was used to test whether the means of the D : A ratios for all constructs analysed were equal ($F = 20.29$, d.f. $= 6, 94$, $P = 10^{-15}$). Subsequently, each construct was tested against wild-type NLG using Dunnett's test for comparing a control mean with each other group mean.

To ensure that overexpression was not influencing protein distribution, all constructs were expressed for the minimum time where cell surface staining could be detected (12 h). Similar D : A ratios were obtained for each protein (data not shown).

Results

Neuroligin is polarized to the dendritic cell surface

To investigate whether NLG-1 might provide a directional signal for synapse formation, we asked whether NLG-1 was exclusively targeted to dendrites of cultured hippocampal neurons or whether it might also be present in axons. We expressed a cDNA encoding a hemagglutinin-tagged NLG-1 in hippocampal neurons for 18 h and then immunostained living cells using an anti-hemagglutinin antibody directed against the extracellular hemagglutinin tag. We coexpressed soluble enhanced green fluorescent protein in order to label the entire axon and dendritic arbor of the cell. Comparison with the distribution of this soluble marker showed that NLG-1 is restricted to the dendrites (Fig. 1A and B). To quantify the polarity of expressed NLG-1, we measured the average fluorescence intensity in dendrites and axons using the method described by Sampo *et al.* (2003). The ratio of fluorescence in dendrites to that in axon (the D : A ratio) was 11.0 : 1 ± 5.8 for wild-type NLG-1 (Fig. 2). This is significantly different from expressed CD8 (D : A, 1.8 ± 0.4, Fig. 2), a lymphocyte transmembrane protein with no known sorting signal that localizes to both the axon and dendrites (Sampo *et al.*, 2003). Importantly, the D : A ratio of NLG-1 is similar to or greater than that of several other dendritic membrane proteins (Cheng *et al.*, 2002; Rivera *et al.*, 2003;

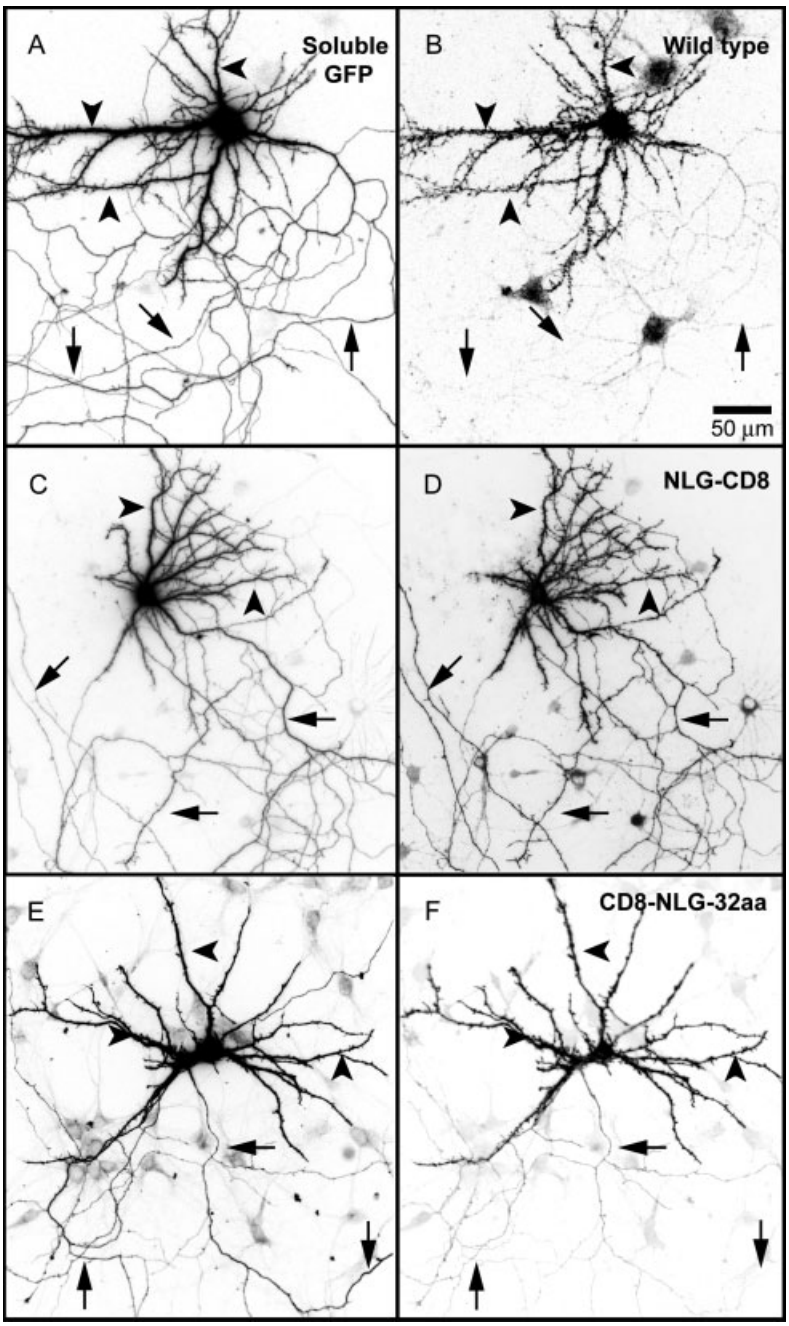

FIG. 1. The distribution of wild-type and mutant neuroligin (NLG) in 8 days *in vitro* cultured hippocampal neurons. Cells were cotransfected with soluble GFP and the appropriate hemagglutinin (HA)-tagged NLG construct and allowed to express for 18 h. Live-cell immunostaining was performed against the extracellular HA epitope or for the extracellular domain of CD8. Soluble GFP was used to fill the entire cell to allow for the identification of axons (arrows) and dendrites (arrowheads; A, C and E). Wild-type NLG is restricted to dendrites (B); NLG-ΔC1 is found in both the axon and dendrites (D) and a chimera with the exracellular portion of CD8 and the intervening 32 amino acids between NLG-ΔC1 and NLG-ΔC2 of the cytoplasmic domain of NLG (CD8-NLG32aa) is highly polarized to dendrites (F).

Silverman *et al.*, 2005), indicating that NLG-1 is highly polarized to the somato-dendritic domain.

Cytoplasmic domain of neuroligin is necessary and sufficient for dendritic targeting

To determine which domain of NLG-1 governs dendritic targeting, we analysed two chimeric molecules, one composed of the extracellular and transmembrane domain of NLG-1 and the cytoplasmic domain of CD8 (NLG-CD8) and one containing the extracellular and transmembrane domain of CD8 but the cytoplasmic domain of NLG-1 (CD8-NLG). CD8-NLG containing the cytoplasmic tail of NLG-1 was localized only to the dendritic plasma membrane with a D : A ratio of 13.1 ± 5.0 (Fig. 2) whereas NLG-CD8 was found on the cell surface of both the axon and dendrites (D : A ratio of 2.5 ± 1.0, Figs 1C and D, and 2). This demonstrates that the C-terminus of NLG-1 is sufficient for dendritic targeting of a heterologous protein.

To define the cytoplasmic sequences required for NLG-1 targeting, we analysed two cytoplasmic truncation mutants, NLG-ΔC1 and

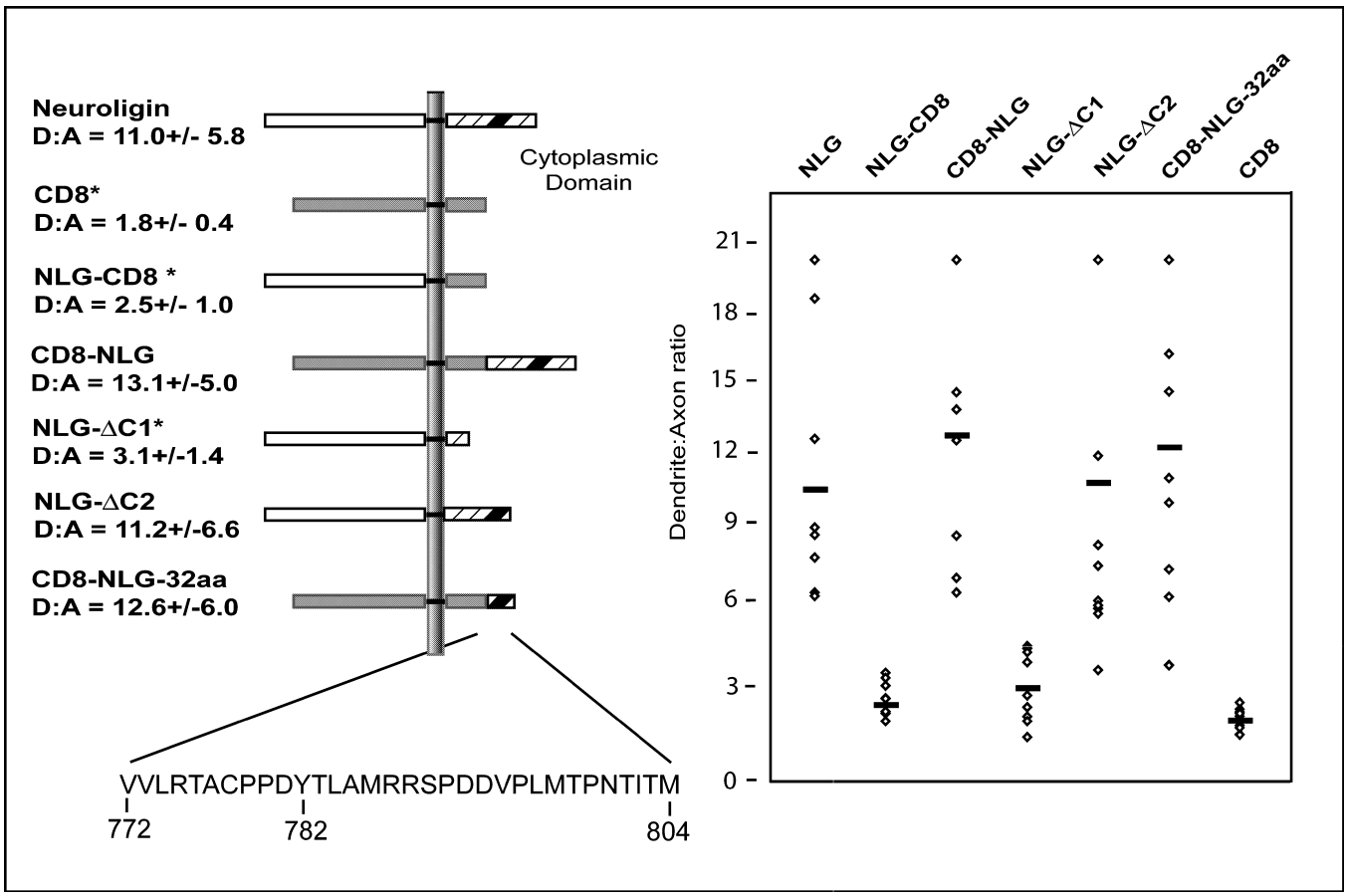

FIG. 2. Schematic representation and cell surface quantification of wild-type neuroligin (NLG), truncations and chimeras with CD8. NLG is restricted to dendrites and deletion or alteration of the cytoplasmic tail of NLG disrupts dendritic targeting. Based on the expression of the uniform protein, CD8, fused to the 32-amino-acid sequence between NLG-ΔC1 and NLG-ΔC2, demonstrates that the cytoplasmic tail contains a 32-amino-acid region that is necessary and sufficient for polarization. The dendrite to axon (D : A) ratios are provided for each construct. The whisker plot displays the data for each construct with the black bar representing the mean. A minimum of 10 cells was analysed for each condition. *Significantly different from wild type, $P < 0.05$.

NLG-ΔC2, lacking the C-terminal 72 and 40 amino acids, respectively. While NLG-ΔC1 was on the cell surface of both the axon and dendrites (D : A ratio of 3.1 ± 1.4), the NLG-ΔC2 mutant was polarized to dendrites with a similar D : A ratio (11.2 ± 6.6) to that of wild-type NLG-1 (Fig. 2). This suggests that the 32 amino acids included in NL-ΔC2 but absent in NLG-ΔC1 might contain a dendritic sorting signal. To directly test whether this putative signal is sufficient for dendritic sorting, we analysed the distribution of a chimera of CD8 and the 32 amino acids identified in the truncation analysis. The chimeric protein CD8-NLG32aa was polarized to dendrites with a D : A ratio (12.6 ± 6.0) similar to that of wild-type NLG-1 (Figs 1E and F, and 2). This demonstrates that the cytoplasmic tail of NLG-1 contains a critical cytoplasmic targeting sequence, which is sufficient for the exclusive dendritic localization of a transmembrane protein.

Neuroligin-yellow fluorescent protein is restricted to the dendritic domain

The targeting signal in NLG-1 might either confer selective stabilization of NLG-1 at the dendritic cell surface or direct polarized transport of NLG-1-containing membrane carriers into the dendrite. To distinguish these possibilities, we expressed a YFP-tagged NLG-1 (NLG-YFP) along with soluble cyan fluorescent protein. Comparing the distribution of the soluble marker with that of NLG-YFP in fixed cells revealed that NLG-YFP is restricted to dendrites (Fig. 3A, green) similar to the hemagglutinin-tagged NLG-1. Higher magnification revealed potential transport intermediates found only in dendrites (Fig. 3B and C) and absent from the axon. This demonstrates that the polarized distribution of NLG-1 is probably achieved by selective trafficking to the somato-dendritic domain as has been shown for the dendritic protein the transferrin receptor (Burack *et al.*, 2000).

Discussion

This report provides three important new findings: (1) NLG-1 is highly polarized to the dendritic domain; (2) dendritic targeting relies on a cytoplasmic motif of 32 amino acids and (3) this signal is sufficient to direct dendritic polarization of a heterologous protein.

Dendritic sorting signal in the cytoplasmic domain of neuroligin

Using NLG-1/CD8 chimeras and truncation mutants of NLG-1, we identified a 32-amino-acid sequence which is required for dendritic sorting. Comparison of this motif in NLG-1 with the motifs that direct dendritic targeting of other membrane proteins, such as the transferrin receptor (West *et al.*, 1997), low-density lipoprotein receptor (Jareb & Banker, 1998) and potassium channel (Kv4.2; Rivera *et al.*, 2003), revealed several interesting parallels. A tyrosine at position 782 and the surrounding residues in NLG-1 are similar in composition to the

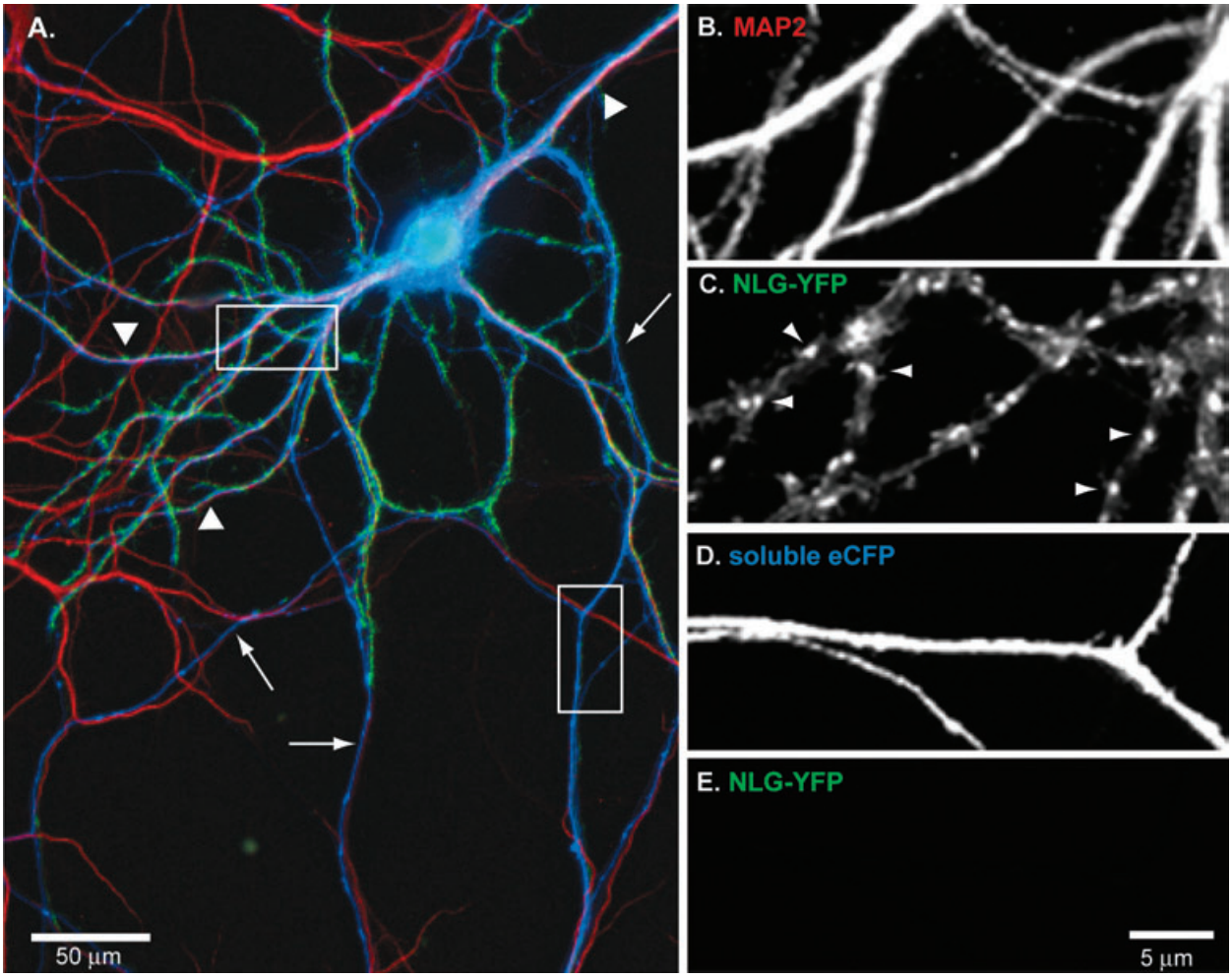

FIG. 3. Neuroligin (NLG)-yellow fluorescent protein (YFP) is polarized to dendrites and contained in potential transport intermediates. (A) Soluble cyan fluorescent protein (CFP) (blue) fills the entire cell, while NLG- YFP (green) is restricted to the dendrites as identified by MAP2 staining (red). The arrows indicate the axon and arrowheads indicate the dendrites. (B and C) High magnification (60×) of a portion of the dendrite reveals a vesicular staining pattern of NLG-YFP. (D and E) However, the axon, filled with soluble CFP, is still void of NLG-YFP staining.

tyrosine motif required for the targeting of transferrin receptor and low-density lipoprotein receptor. The 32-amino-acid stretch also contains a Val-Val and Val-Leu that conform to the dihydrophobic motif similar to that required for the dendritic sorting of the Kv4.2 potassium channel (Rivera *et al.*, 2003). This indicates that similar targeting signals and sorting receptors might govern polarized exocytosis of transferrin receptor, low-density lipoprotein receptor, Kv4.2 and NLG-1 although further work will be required to identify the receptors recognizing these sorting signals. It is notable that the NLG-1 truncation mutant NLG-ΔC1, in which the 32-amino-acid signal had been deleted, was not completely nonpolarized as compared with CD8 (D : A, 3.1 ± 1.4 vs. 1.8 ± 0.4, statistically significant according to ANOVA). This indicates that additional targeting signals may exist in the NLG-1 sequence.

Dendritic sorting and synaptic concentration depend on separate signals

Interestingly, the signals required for dendritic sorting of NLG-1 were different from those previously suggested to be relevant for its concentration at synapses. This confirms that dendritic targeting and synaptic concentration are separable processes as has also been seen with glutamate transporter excitatory amino acid transporter 3 (Cheng *et al.*, 2002). Prange *et al.* (2004) reported that post synaptic density-95 (PSD-95) binding through the PDZ-binding domain was required for synaptic concentration of over-expressed NLG-1. Other studies have suggested that synaptic targeting of NLG-1 was independent of the PDZ-binding domain requiring either the juxtamembrane domain of NLG-1 or more distal cytoplasmic regions (Dresbach *et al.*, 2004; Iida *et al.*, 2004). Regardless of these discrepancies with respect to synaptic concentration of NLG-1, our data clearly demonstrate that dendritic sorting of NLG-1 is not mediated through either of these signals but rather a 32-amino-acid signal in the central portion of the cytoplasmic tail. Therefore, dendritic sorting and synaptic concentration are two independent events, with the amino acid motif identified in our study facilitating selective sorting of NLG-1 to the somato-dendritic domain followed by the concentration of NLG-1 at synapses through additional cytoplasmic interactions.

Relevance of neuroligin-1 polarity for synapse formation

Our finding that NLG-1 is exclusively sorted to dendrites has important implications for synapse formation. Previous work

demonstrated that contact of axonal βNRXs with NLG-1-containing cells or membranes leads to the formation of pre-synaptic structures (Scheiffele *et al.*, 2000; Dean *et al.*, 2003). The exclusion of NLG-1 from axons makes these axonal membranes unable to serve as post-synaptic targets for other axons, i.e. the formation of heterophilic βNRX–NLG-1 complexes between two axons. Such interactions are restricted to axo-dendritic contacts instead. The exclusively dendritic localization of NLG-1 therefore provides a polarizing activity for axo-dendritic recognition during synapse formation. Importantly, such an activity can only be encoded by a heterophilic adhesion system such as the βNRX–NLG-1 complex but not by a homophilic adhesion system such as SynCAM, cadherins or nectins (Biederer *et al.*, 2002; Fannon & Colman, 1996; Nakanishi & Takai, 2004). Future studies will aim at elucidating the cellular machinery that mediates dendritic sorting and transport of NLG-1 and ultimately define the role and regulation of protein trafficking in synapse formation.

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Abbreviations

D : A, dendrite to axon ratio; NLG, neuroligin; βNRX, β-neurexin; GFP, green fluorescent protein; MAP-2, microtubule-associated protein; PDZ, PSD-95/DLG/ZO-1; pJPA, plasmid JPA; YFP, yellow fluorescent protein.

References

Banker, G. & Goslin, K. (1998) *Culturing Nerve Cells*, 2nd Edn. MIT Press, Cambridge, MA, USA.

Biederer, T., Sara, Y., Mozhayeva, M., Atasoy, D., Liu, X., Kavalali, E.T. & Sudhof, T.C. (2002) SynCAM, a synaptic adhesion molecule that drives synapse assembly. *Science*, **297**, 1525–1531.

Burack, M.A., Silverman, M.A. & Banker, G. (2000) The role of selective transport in neuronal protein sorting. *Neuron*, **26**, 465–472.

Cheng, C., Glover, G., Banker, G. & Amara, S.G. (2002) A novel sorting motif in the glutamate transporter excitatory amino acid transporter 3 directs its targeting in Madin-Darby canine kidney cells and hippocampal neurons. *J. Neurosci.*, **22**, 10 643–10 652.

Chih, B., Engelman, H. & Scheiffele, P. (2005) Control of excitatory and inhibitory synapse formation by neuroligins. *Science*, **307**, 1324–1328.

Craig, A.M. & Banker, G. (1994) Neuronal polarity. *Annu. Rev. Neurosci.*, **17**, 267–310.

Dean, C., Scholl, F.G., Choih, J., DeMaria, S., Berger, J., Isacoff, E. & Scheiffele, P. (2003) Neurexin mediates the assembly of presynaptic terminals. *Nat. Neurosci.*, **6**, 708–716.

Dresbach, T., Neeb, A., Meyer, G., Gundelfinger, E.D. & Brose, N. (2004) Synaptic targeting of neuroligin is independent of neurexin and SAP90/PSD95 binding. *Mol. Cell Neurosci.*, **27**, 227–235.

Fache, M.P., Moussif, A., Fernandes, F., Giraud, P., Garrido, J.J. & Dargent, B. (2004) Endocytotic elimination and domain-selective tethering constitute a potential mechanism of protein segregation at the axonal initial segment. *J. Cell Biol.*, **166**, 571–578.

Fannon, A.M. & Colman, D.R. (1996) A model for central synaptic junctional complex formation based on the differential adhesive specificities of the cadherins. *Neuron*, **17**, 423–434.

Graf, E.R., Zhang, X., Jin, S.X., Linhoff, M.W. & Craig, A.M. (2004) Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell*, **119**, 1013–1026.

Ichtchenko, K., Hata, Y., Nguyen, T., Ullrich, B., Missler, M., Moomaw, C. & Sudhof, T.C. (1995) Neuroligin 1: a splice site-specific ligand for beta-neurexins. *Cell*, **81**, 435–443.

Iida, J., Hirabayashi, S., Sato, Y. & Hata, Y. (2004) Synaptic scaffolding molecule is involved in the synaptic clustering of neuroligin. *Mol. Cell Neurosci.*, **27**, 497–508.

Jareb, M. & Banker, G. (1998) The polarized sorting of membrane proteins expressed in cultured hippocampal neurons using viral vectors. *Neuron*, **20**, 855–867.

Nakanishi, H. & Takai, Y. (2004) Roles of nectins in cell adhesion, migration and polarization. *Biol. Chem.*, **385**, 885–892.

Nguyen, T. & Sudhof, T.C. (1997) Binding properties of neuroligin 1 and neurexin 1beta reveal function as heterophilic cell adhesion molecules. *J. Biol. Chem.*, **272**, 26 032–26 039.

Prange, O., Wong, T.P., Gerrow, K., Wang, Y.T. & El-Husseini, A. (2004) A balance between excitatory and inhibitory synapses is controlled by PSD-95 and neuroligin. *Proc. Natl Acad. Sci. U.S.A.*, **101**, 13 915–13 920.

Rivera, J.F., Ahmad, S., Quick, M.W., Liman, E.R. & Arnold, D.B. (2003) An evolutionarily conserved dileucine motif in Shal K+ channels mediates dendritic targeting. *Nat. Neurosci.*, **6**, 243–250.

Sampo, B., Kaech, S., Kunz, S. & Banker, G. (2003) Two distinct mechanisms target membrane proteins to the axonal surface. *Neuron*, **37**, 611–624.

Scheiffele, P. (2003) Cell-cell signaling during synapse formation in the CNS. *Annu. Rev. Neurosci.*, **26**, 485–508.

Scheiffele, P., Fan, J., Choih, J., Fetter, R. & Serafini, T. (2000) Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell*, **101**, 657–669.

Silverman, M.A., Peck, R., Glover, G., He, C., Carlin, C. & Banker, G. (2005) Motifs that mediate dendritic targeting in hippocampal neurons: a comparison with basolateral targeting signals. *Mol. Cell Neurosci.*, **29**, 173–180.

Song, J.Y., Ichtchenko, K., Sudhof, T.C. & Brose, N. (1999) Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. *Proc. Natl Acad. Sci. U.S.A.*, **3**, 1100–1105.

Washbourne, P., Dityatev, A., Scheiffele, P., Biederer, T., Weiner, J.A., Christopherson, K.S. & El-Husseini, A. (2004) Cell adhesion molecules in synapse formation. *J. Neurosci.*, **24**, 9244–9249.

West, A.E., Neve, R.L. & Buckley, K.M. (1997) Identification of a somatodendritic targeting signal in the cytoplasmic domain of the transferrin receptor. *J. Neurosci.*, **17**, 6038–6047.

Yamagata, M., Sanes, J.R. & Weiner, J.A. (2003) Synaptic adhesion molecules. *Curr. Opin. Cell Biol.*, **15**, 621–632.