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SPOTLIGHT

When neuroligin-2 sticks around, astrocytes take shape

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The complex structure of astrocytes allows for nervous system function; however, mechanisms underlying astrocyte morphogenesis remain unclear. Sakers et al. (https://doi.org/10.1083/jcb.202512111) find that astrocyte neuroligins (NLs) are functionally diverse, and intracellular ubiquitination of astrocyte NL2 by Nedd4l promotes astrocyte morphogenesis.

Neurons transmit information within a neural circuit at contact sites called synapses, which are stabilized by intercellular adhesion. Neuroligins (NLs) are conserved cell adhesion transmembrane molecules originally isolated from rat brain tissue as binding partners of neurexins (1, 2). Neurexin–NL adhesion physically bridges two neurons at a synapse, enabling synaptic connectivity. Lesions in these factors are linked to neurodevelopmental conditions like autism (2). Humans express four NLs (NL1–4) that function nonredundantly. NL1 primarily localizes to and modulates excitatory synapses, NL2 does so at inhibitory synapses, NL3 acts on both synapse types, and NL4 affects glycinergic synapses (2). Curiously, triple NL1–3 knockout animals have normal synapse number and structure, but impaired synaptic transmission, suggesting that NLs have roles beyond physically enabling neuronal synapse architecture (2).

Astrocytes also modulate synaptic connectivity and function at tripartite synapses through mechanisms including the secretion of neuroactive factors, ion buffering, and neurotransmitter diffusion/uptake (3). This is enabled by their elaborate, bushy morphology, with fine cellular processes that contact neuronal synapses (3). How astrocytes establish their ramified structures remains poorly understood molecularly. Defects in astrocyte cell shape not only affect neuronal connectivity and function, but are also correlated with genes associated with diseases like Alzheimer's dementia (3). In this issue of *JCB*, Sakers et al. reveal insights into NL biology in astrocytes through a series of elegant molecular studies (4) (Fig. 1). They show that as in neurons, NL1–3 have nonredundant roles in astrocytes. By identifying their cell- and gene-specific interactomes, the authors uncover that ubiquitination of astrocytic NL2 at lysine 749 by Nedd4l/HECT ligase prevents its degradation, enabling it to drive astrocyte morphogenesis, independent of NL roles in astrocyte-driven synaptogenesis.

Rodent NL1–3 and the *Drosophila* ortholog, gliotactin, are expressed in both neurons and glia (2, 5). Previously, the Eroglu lab showed that rodent cortical astrocytes express NL1–3 at levels approximating neurons, and loss of any NL reduces the territory size of bushy astrocytes. They also found that astrocytic NL2 binds neuronal neurexins to promote astrocyte morphogenesis and synaptogenesis and, thereby, synapse function (6). Building on this work, the authors aimed to systematically investigate NL redundancy and regulation in astrocytes. They first leveraged an in vitro astrocyte–neuron coculture system to manipulate individual NLs in an astrocyte-specific manner and quantify astrocyte morphology. Depleting *Nlgn2* by either shRNA or CRISPR/Cas9 mutagenesis reduced astrocyte morphological complexity. This was rescued by co-expressing an shRNA-resistant construct specifically for NL2, but not NL1 or NL3. The authors therefore concluded that the role of NL2 in regulating astrocyte morphology is distinct from NL1 or NL3.

The three NLs have similar extracellular domain (ECD) but divergent intracellular domain (ICD) sequences. In neurons, each NL ICD has unique functions and binding partners (7). To probe if similar biology was at play in astrocytes, Sakers et al. conducted a series of structure–function studies. First, they altered known neuron-relevant ICD motifs (PDZ, polyproline motif, gephyrin-binding region) by mutagenesis but found these irrelevant for astrocyte morphology. Next, they examined chimeras of NL2 ECD fused to either the NL1 or NL3 ICD. While the chimera with NL1 ICD rescued astrocyte complexity defects of *Nlgn2* depletion, a chimera with NL3 ICD did not. They inferred that the role of NL2 in astrocyte morphogenesis required a motif shared with NL1 and absent in NL3. From bioinformatics analyses, they then identified a PPDY (PPxY) motif in the NL2 ICD. Mutating this motif (PPDY>AAAA) in either NL2 or the NL2 ECD/NL1 ICD chimera abrogated the ability of either construct to rescue morphogenesis defects of *Nlgn2* knockdown, indicating that the PPDY motif in the NL ICD mediates astrocyte morphogenesis.

PPDY motifs bind Group I WW domain–containing proteins. To identify the relevant ones, the authors next performed in vivo BioID in the mouse cortex for all NLs in astrocytes and NL2 in neurons. This revealed three interesting facets of NL biology

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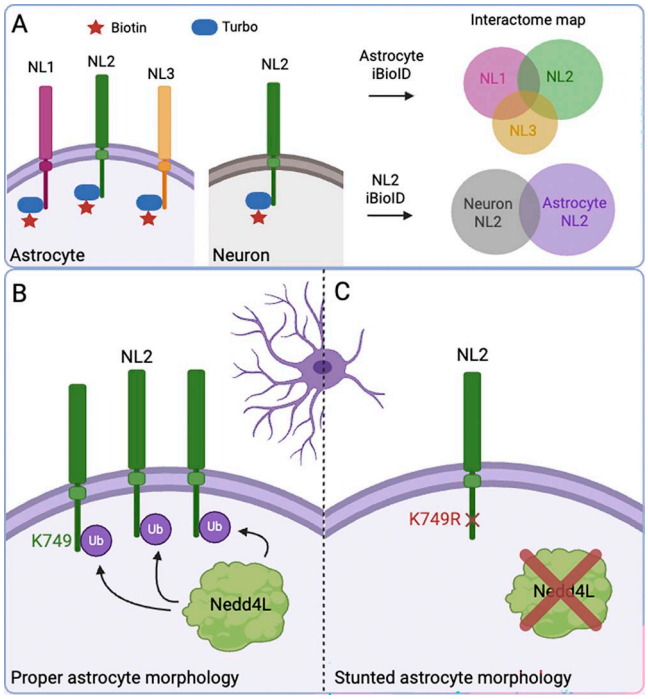

Figure 1. **NLs are functionally diverse, and astrocytic NL2 is ubiquitinated by Nedd4l for proper astrocyte morphogenesis. (A)** The authors used in vivo BioID to identify the interactomes for astrocyte NL1–3 and neuronal NL2. **(B)** In wild-type animals, Nedd4l ubiquitinates NL2 for protein stability, which allows for proper astrocyte morphology. **(C)** When Nedd4l is knocked down or in NL2 K749R Ub mutants, astrocytes show stunted morphology. Ub, ubiquitination. Created in BioRender. Ray, S. (2026) https://BioRender.com/wkjy20h.

(Fig. 1 A). First, the astrocyte interactome of NL2 was surprisingly distinct from its known neuronal interactors, for example, NL2 interacts with actin-binding proteins in astrocytes but not neurons. This supports past work indicating that NL2 has cell type–specific activity. Second, the identified WW domain–containing protein interactors were unique to each NL, consistent with their nonredundant astrocyte functions. Third, this dataset identified Nedd4l as a WW domain protein that interacts with astrocytic NL2 and NL1, but not NL3.

Nedd4l is a HECT family E3 ubiquitin ligase that ubiquitinates astrocytic membrane proteins like Kir4.1/K channel and Glt1/glutamate transporter for degradation (8, 9). Independently, it is known that NL2 is ubiquitinated at lysine 749 (K749), although the relevance of this posttranslational modification had so far been unclear (10). The authors thus hypothesized that NL2 is a Nedd4l ubiquitination substrate. To test this, they first knocked down Nedd4l in vitro, and found that it phenocopied NL2 depletion, and did not exacerbate it. Nedd4l depletion similarly reduced cortical astrocyte morphological complexity in vivo (Fig. 1, B and C). Thus, Nedd4l regulates astrocyte shape likely in the same pathway with NL2. Interestingly, distinct from *Nlgn2* loss, sparse knockdown of Nedd4l in vivo did not alter synaptogenesis. Thus, NL2 requires Nedd4l-dependent stabilization to regulate astrocyte morphogenesis but not astrocyte-driven synaptogenesis.

To test the mechanism of Nedd4l function in astrocyte morphogenesis, the authors did biochemistry studies in primary rat astrocytes and HEK293T cell assays which showed that the NL2 PPDY motif bound Nedd4l, and Nedd4l ubiquitinated NL2 at K749 (Fig. 1 B). However, posttranslational modification of $NL2^{K749}$ by Nedd4l stabilized, not degraded, the protein. As a corollary, expressing the nonubiquitinable variant $NL2^{K749R}$ in astrocyte cocultures impaired astrocyte morphology and did not rescue NL2 knockdown defects. The authors further showed this held true also in vivo: unlike wild-type NL2, expressing $NL2^{K749R}$ in astrocytes did not rescue astrocyte morphology defects in the developing cortex of homozygous *Nlgn2* mutant mice (Fig. 1 C).

In summary, Sakers et al. provide significant insight into the biology of disease-relevant NLs, particularly NL2, and developmental morphogenesis of astrocytes. This work also expands the functional role of Nedd4l in astrocytes, and underscores the need to interrogate factors in glia and neurons with cell- and paralog-specific resolution. Finally, their shared interactome data of astrocytic NLs will be a valuable resource for future studies. This study also raises important mechanistic questions. (1) Does K749 ubiquitination also stabilize neuronal NL2 or astrocytic NL1? And (2) how does this developmental mechanism apply to animal aging, astrocyte heterogeneity, other glia types, or disease-state glioma cells? Finally, NLs form both homo- and heterodimers (2). As such, (3) how might stabilizing one NL (like NL2) alter the ratio of NL dimers that an astrocyte expresses? Tackling these open questions will be exciting avenues of future inquiry into NL biology, astrocyte development, and synapse biology.

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References

1. Ichtchenko, K., et al. 1995. *Cell*. https://doi.org/10.1016/0092-8674(95)90396-8
2. Südhof, T.C. 2017. *Cell*. https://doi.org/10.1016/J.CELL.2017.10.024
3. Barres, B.A. 2008. *Neuron*. https://doi.org/10.1016/j.neuron.2008.10.013
4. Sakers, K., et al. 2026. J. Cell Biol. https://doi.org/10.1083/jcb.202512111

5. Schulte, J., et al. 2003. *J. Cell Biol.* https://doi.org/10.1083/JCB.200303192
6. Sakers, K., and C. Eroglu. 2019. *Curr. Opin. Neurobiol.* https://doi.org/10.1016/j.conb.2019.03.007
7. Nguyen, Q.A., et al. 2016. *Elife*. https://doi.org/10.7554/ELIFE.19236
8. Altas, B., et al. 2023. *J. Cell Biol.* https://doi.org/10.1083/JCB.201902050
9. Zhang, Y., et al. 2017. *Cell Death Dis.* https://doi.org/10.1038/CDDIS.2016.454
10. Wagner, S.A., et al. 2012. *Mol. Cell. Proteomics*. https://doi.org/10.1074/MCP.M112.017905