

Preview

A closer look at the synapse: *dlg1*[4K] enables cell-specific visualization of PSD-95/DLG1

Emily L. Hendricks¹ and Faith L.W. Liebl^{1,*}¹Department of Biological Sciences, Southern Illinois University Edwardsville, Edwardsville, IL 62025, USA

*Correspondence: fliebl@siue.edu

<https://doi.org/10.1016/j.crmeth.2023.100487>

The PSD-95 homolog, DLG1, is important for excitatory synapse structure and function throughout the *Drosophila* nervous system. In this issue of *Cell Reports Methods*, Parisi et al. present a tool, *dlg1*[4K], that enables cell-specific DLG1 visualization without altering basal synaptic physiology. This tool will potentially enhance our understanding of neuronal development and function in both circuits and individual synapses.

Neuronal synapses are sites of bidirectional communication that, when organized into circuits, enable sensory perception, movement, and higher cognitive processes. Understanding the structure, function, and development of individual synapses is essential for larger-scale studies of nervous system function and wiring. The *Drosophila* nervous system is a highly tractable model system for studying the regulatory mechanisms of pre- and postsynaptic structure and function at both developing and mature synapses. Recent advances in mapping the *Drosophila* connectome¹ have prompted the development of genetic tools, like single-cell binary expression systems, that allow for greater experimental accessibility. These resources may be used in tandem with ultrastructural imaging and transcriptomics approaches to examine circuit wiring mechanisms and subsequent behavioral outputs.² Current tools to probe synaptic structure present several limitations that have historically hindered *in vivo* studies. Thus, additional resources are needed to enhance the resolution through which individual pre- and postsynaptic components can be visualized and distinguished throughout the *Drosophila* nervous system.

The formation of functional synaptic connections requires multiphasic, coordinated structural changes. At *Drosophila* peripheral and central synapses, the CAST/ELKS protein family homolog, Bruchpilot (Brp), is an essential structural component of active zones.³ Presynaptic sites are reliably visualized using current antibody-directed approaches toward

endogenous, full-length Brp (nc82) or conditionally tagged isoforms. Similar tools, however, are not available to study the innate architecture of *Drosophila* peripheral and central nervous system postsynapses possibly due to the variability in neurotransmitter receptor identity across synapses. In this issue of *Cell Reports Methods*, Parisi and colleagues⁴ present a newly engineered, conditional marker of excitatory postsynapses, *dlg1*[4K], which can be readily combined with highly accessible binary expression systems in *Drosophila* to visualize the PSD-95 homolog, DLG1, in a cell-specific manner.

PSD-95/DLG1 is an organizational protein within the postsynaptic density, which is a dynamic structure that includes cytoskeletal and scaffolding proteins, neurotransmitter receptors, and signal transducers. At excitatory postsynapses, PSD-95/DLG1 recruits and anchors postsynaptic proteins thereby mediating synaptic structure and function.^{5,6} Specifically, DLG1 directly binds the cell adhesion molecule, Fasciclin II (FasII),⁷ and the cytoplasmic tail of Shaker K⁺ channels⁸ (Figure 1). PSD-95/DLG1 also interacts with and regulates the clustering of AMPA/glutamate receptors in an activity-dependent manner at both vertebrate and the *Drosophila* synapses.^{9,10} These organizational roles enable PSD-95/DLG1 to modulate synaptic strength and plasticity through activity-dependent mechanisms.¹¹ Given its importance, the ability to experimentally distinguish DLG1 across distinct neuronal subtypes and synaptic compartments is highly valuable.

Parisi et al.⁴ use CRISPR-directed editing of the *dlg1* locus in *Drosophila* to generate a conditionally inducible DLG1-V5 construct (Figure 1). In *dlg1*[4K] animals, the *dlg1* locus features a UAA stop cassette flanked by FRT sites just upstream of the furthest downstream translation stop site used by many *dlg1* isoforms. In the absence of FRT recombinase, a slightly truncated (~4 aa) DLG1 that lacks a V5 tag is generated. However, when FRT recombinase is introduced under the control of a binary expression system, excision of the stop cassette enables readthrough of the terminal *dlg1* and V5 coding sequences thereby producing a V5-tagged DLG1 (Figure 1). The authors demonstrate that *dlg1*[4K] knockin is properly localized at the synapse and does not compromise viability, synaptic morphology, or motor function. However, one limitation of this knockin approach is that all *dlg1* isoforms will not be tagged. The *Drosophila* genome encodes 21 *dlg1* isoforms that are developmentally and temporally regulated. These different isoforms are expressed in neuronal and nonneuronal tissues, where they have both unique and partially redundant roles (FlyBase). Despite this limitation, *dlg1*[4K] provides an experimental approach that enables conditional, cell-specific visualization of DLG1 at developing and mature synapses without disrupting basal synaptic physiology. Although several epitope-tagged DLG1 constructs already exist (Bloomington *Drosophila* Stock Center), *dlg1*[4K] is highly favorable over these transgenic lines as *dlg1* is expressed under the control of its



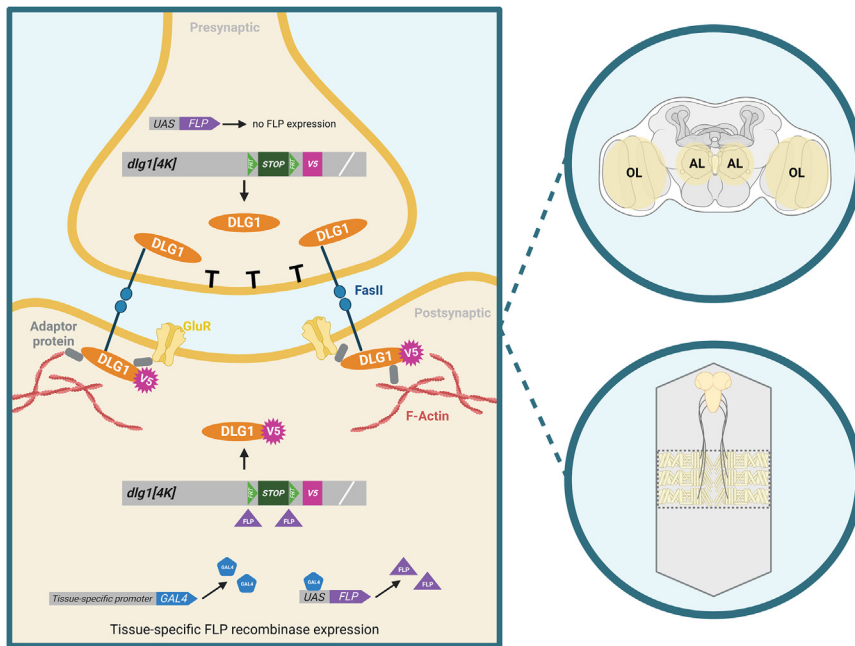


Figure 1. The conditional marker, *dlgl[4K]*, enables cell-specific labeling and visualization of PSD-95/DLG1 at peripheral and central synapses in *Drosophila*

The PSD-95 homolog, DLG1, interacts with several postsynaptic components including glutamate receptors, cell adhesion molecules, and the cytoskeleton. Presynaptic DLG1 interactions remain largely uncharacterized. However, interactions between DLG1 and FasII were observed *in vitro*.⁷ To visualize DLG1 in a cell-specific manner, Parisi et al.⁴ engineered *dlgl[4K]*, which features a conditionally expressed V5 tag. Under the control of binary expression systems, such as the *UAS-GAL4* system, FLP recombinase can be introduced in a cell-specific manner to promote excision of a *STOP* cassette, thereby enabling DLG1-V5 expression in *dlgl[4K]* animals. Parisi et al.⁴ employ this tool at both central and peripheral synapses across the adult optic (OL) and antennal lobes (AL) (shown within the upper right circle) and larval nervous systems (shown in lower right circle). Figure created using BioRender (<https://www.biorender.com>).

endogenous promoter rather than a *UAS* sequence. As such, this strategy circumvents perturbations of basal synaptic physiology that accompany artificial PSD-95/DLG1 overexpression, which may occur with other tagged DLG1 constructs.

Current approaches for visualizing postsynaptic compartments largely rely on the labeling of postsynaptic receptors, which limits the visualization of all postsynapses in brain regions with heterogeneous neuronal subtypes. For example, the adult mushroom body is a widely studied structure that facilitates *Drosophila* learning and memory and is comprised of several neuronal cell types with different neurotransmitter identities. *dlgl[4K]* is particularly relevant for future studies of circuitry in complex brain regions like the mushroom body. DLG1 is not only present at glutamatergic synapses of the mushroom body but also functionally interacts with nicotinic acetyl-

choline receptors in this structure and is required for odor avoidance behavior in adult flies.¹² The mushroom body receives upstream inputs from the optic and antennal lobes where Parisi et al.⁴ employ *dlgl[4K]* to label postsynaptic connections. At DM8-R7 synapses in the visual system, *dlgl[4K]*, coupled with postsynaptic FRT expression under the control of *DIP-γ-GAL4*, produces specific and robust DLG1 labeling that stereotypically apposes R7 axons in the medulla. In the antennal lobe, olfactory receptor neurons (ORNs) synapse onto projection neurons (PNs) and local interneurons (LNs) with PNs and LNs also synapsing onto one another. The authors show *dlgl[4K]*-mediated DLG1 labeling can be modulated through driver-specific FRT expression to label these distinct postsynaptic connections and enable the discernment of antennal lobe circuitry. Furthermore, the authors highlight how *dlgl[4K]* enhances the resolution through which cir-

cuit connections can be probed by characterizing the three-dimensional architecture and wiring of ORN, PN, and LN connections in the DA1 glomerulus of the antennal lobe. Thus, *dlgl[4K]* circumvents the limitation of labeling individual postsynaptic receptor types since DLG1 is a component of both cholinergic and glutamatergic synapses. These results demonstrate how *dlgl[4K]* can be reliably employed as a valuable experimental tool in future circuit-level studies.

There is limited knowledge of the functions of presynaptic DLG1 due to difficulties in visualizing distinct pools of DLG1. Current understandings of presynaptic function rely on indirect observations from *dlgl* loss-of-function studies. These studies implicate DLG1 in the regulation of evoked neurotransmitter release, localization of presynaptic voltage-gated Ca^{2+} channels, and active zone density.^{7,10} These functions might be mechanistically explained through transsynaptic regulation of active zone architecture by cell adhesion molecules, specifically by DLG1-FasII interactions.⁷ Alternatively, DLG1 could directly exert these presynaptic functions through PDZ domain-mediated interactions as the PDZ domains of other synaptic proteins, including RIM,¹³ directly bind Ca^{2+} channels at active zones. However, direct interactions between presynaptic DLG1 and other presynaptic proteins have not been characterized *in vivo*. *dlgl[4K]* enables, for the first time, endogenously expressed presynaptic DLG1 to be labeled independent of postsynaptic DLG1 pools. Interactions between DLG1 and other presynaptic components, which are largely unknown (Figure 1), could also be uncovered via co-immunoprecipitation using a V5 antibody. Therefore, *dlgl[4K]* has the potential to advance the *in vivo* resolution through which individual synaptic connections can be discerned throughout the *Drosophila* nervous system and is highly relevant for future investigations into the previously uncharacterized roles of presynaptic DLG1. In addition, Parisi et al.⁴ highlight the modularity of *dlgl[4K]* as they successfully employ postsynaptic *dlgl[4K]*-mediated DLG1 labeling along with presynaptic Brp-Short-mStraw labeling. The use of simultaneous binary expression systems with *dlgl[4K]* successfully retains cell

specificity, thereby allowing for multiplexing and the discernment of pre- and post-synaptic proteins at individual peripheral and central nervous system synapses.

Overall, *dlg1[4K]*, constructed by Parisi et al.,⁴ offers the potential to advance the study of neuronal development and function by improving the resolution through which individual synaptic connections can be visualized. *dlg1[4K]* also overcomes many of the limitations that have historically hindered the labeling of post-synaptic regions and cell-specific study of DLG1 at the synapse. The authors demonstrate that *dlg1[4K]* can be used across different neuronal populations to label DLG1 in a cell-specific manner (Figure 1). As such, the adaptability of binary expression systems makes *dlg1[4K]* a highly relevant tool for a wide array of future studies in the *Drosophila* larval and adult nervous systems.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Winding, M., Pedigo, B.D., Barnes, C.L., Patolic, H.G., Park, Y., Kazimiers, T., Fushiki, A., Andrade, I.V., Khandelwal, A., Valdes-Aleman, J., et al. (2023). The connectome of an insect brain. *Science* 379, eadd9330. <https://doi.org/10.1126/science.add9330>.
- Deveneni, A.V., and Scaplen, K.M. (2021). Neural circuits Underlying behavioral Flexibility: Insights from *Drosophila*. *Front. Behav. Neurosci.* 15, 821680. <https://doi.org/10.3389/fnbeh.2021.821680>.
- Wagh, D.A., Rasse, T.M., Asan, E., Hofbauer, A., Schwenkert, I., Dürbeck, H., Buchner, S., Dabauvalle, M.C., Schmidt, M., Qin, G., et al. (2006). Bruchpilot, a protein with homology to ELKS/CAST, is required for structural integrity and function of synaptic active zones in *Drosophila*. *Neuron* 49, 833–844. <https://doi.org/10.1016/j.neuron.2006.02.008>.
- Parisi, M.J., Aimino, M.A., and Mosca, T.J. (2023). A conditional strategy for cell-type-specific labeling of endogenous excitatory synapses in *Drosophila*. *Cell Rep. Methods* 3, 100477.
- Budnik, V., Koh, Y.H., Guan, B., Hartmann, B., Hough, C., Woods, D., and Gorczyca, M. (1996). Regulation of synapse structure and function by the *Drosophila* tumor suppressor gene *dlg*. *Neuron* 17, 627–640. [https://doi.org/10.1016/s0896-6273\(00\)80196-8](https://doi.org/10.1016/s0896-6273(00)80196-8).
- Chen, X., Nelson, C.D., Li, X., Winters, C.A., Azzam, R., Sousa, A.A., Leapman, R.D., Gainer, H., Sheng, M., and Reese, T.S. (2011). PSD-95 is required to sustain the molecular organization of the postsynaptic density. *J. Neurosci.* 31, 6329–6338. <https://doi.org/10.1523/jneurosci.5968-10.2011>.
- Thomas, U., Kim, E., Kuhlendahl, S., Koh, Y.H., Gundelfinger, E.D., Sheng, M., Garner, C.C., and Budnik, V. (1997). Synaptic clustering of the cell adhesion molecule fasciclin II by discs-large and its role in the regulation of presynaptic structure. *Neuron* 19, 787–799. [https://doi.org/10.1016/s0896-6273\(00\)80961-7](https://doi.org/10.1016/s0896-6273(00)80961-7).
- Tejedor, F.J., Bokhari, A., Rogero, O., Gorczyca, M., Zhang, J., Kim, E., Sheng, M., and Budnik, V. (1997). Essential role for *dlg* in synaptic clustering of Shaker K⁺ channels in vivo. *J. Neurosci.* 17, 152–159. <https://doi.org/10.1523/jneurosci.17-01-00152.1997>.
- Elias, G.M., Funke, L., Stein, V., Grant, S.G., Bretz, D.S., and Nicoll, R.A. (2006). Synapse-specific and developmentally regulated targeting of AMPA receptors by a family of MAGUK scaffolding proteins. *Neuron* 52, 307–320. <https://doi.org/10.1016/j.neuron.2006.09.012>.
- Astorga, C., Jorquera, R.A., Ramírez, M., Kohler, A., López, E., Delgado, R., Córdova, A., Olguin, P., and Sierralta, J. (2016). Presynaptic DLG regulates synaptic function through the localization of voltage-activated Ca²⁺ Channels. *Sci. Rep.* 6, 32132. <https://doi.org/10.1038/srep32132>.
- Guan, B., Hartmann, B., Kho, Y.H., Gorczyca, M., and Budnik, V. (1996). The *Drosophila* tumor suppressor gene, *dlg*, is involved in structural plasticity at a glutamatergic synapse. *Curr. Biol.* 6, 695–706. [https://doi.org/10.1016/s0960-9822\(09\)00451-5](https://doi.org/10.1016/s0960-9822(09)00451-5).
- Pribbenow, C., Chen, Y.-c., Heim, M.M., Laber, D., Reubold, S., Reynolds, E., Balles, I., Fernández-d V Alquicira, T., Suárez-Grimalt, R., Scheunemann, L., et al. (2022). Postsynaptic plasticity of cholinergic synapses underlies the induction and expression of appetitive and familiarity memories in *Drosophila*. *Elife* 11, e80445. <https://doi.org/10.7554/eLife.80445>.
- Kaesler, P.S., Deng, L., Wang, Y., Dulubova, I., Liu, X., Rizo, J., and Südhof, T.C. (2011). RIM proteins tether Ca²⁺ channels to presynaptic active zones via a direct PDZ-domain interaction. *Cell* 144, 282–295. <https://doi.org/10.1016/j.cell.2010.12.029>.