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Fly Dscams Can Also Help You Find the Right Partners

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The modular reiterative pattern of the fly visual system makes it an ideal model to study axon guidance and synaptogenesis. In this issue of *Neuron*, Tadros et al. (2016) show that Dscam2/4 promote the anchoring of dendrites to their targets.

The connectome of the *Drosophila* optic lobe has been extensively described using Golgi staining, genetic labeling methods, and serial electron microscopy to describe the stereotyped wiring between photoreceptors (R1–R8, R-cells) and their respective targets (Figure 1A) and serves as an ideal system to identify molecular codes for axon and dendrite targeting. The optic lobes are comprised of four ganglia: the lamina, the medulla, the lobula, and lobula plate (Hadjieconomou et al., 2011). Anatomical and physiological studies (Hadjieconomou et al., 2011; Tadros et al., 2016) have shown that the lamina is a mosaic of about 750 modules, called cartridges, forming a retinotopic map. Adjacent cartridges process visual information coming from adjacent points in the fly visual field. The lamina monopolar neurons (L1–L5) are the main post-synaptic targets of the outer-cell R1-R6 photoreceptor axons and therefore key components of the cartridge (Figure 1B). Their cell bodies are localized at the cortex and they extend unipolar axons through the lamina up to the medulla, with the intriguing case of the L4 monopolar neuron attracting attention for many years as the exception to this rule (Meinertzhagen and O'Neil, 1991). First, until recently it was thought to completely lack presynaptic inputs from photoreceptors (though recent SEM data have challenged this view and suggested that R6 projects to L4; see Rivera-Alba et al., 2011). Second, unlike other monopolar cells, L4 synaptic partners extend beyond the limit of a single cartridge. Every L4 cell establishes reciprocal cholinergic synaptic connections with L2 cell axons in the underlying cartridge and with two additional L2 processes located in adjacent ventroposterior and anteroposterior cartridges (Figures 1B and 1C). Thus, each L2 cell in a cartridge is connected to three different L4 cells to potentially help integrate visual inputs from multiple ommatidia and detect motion. Surprisingly, how L4 neurons recognize their target cells in the lamina was completely unknown. Tadros et al. (2016) used a variety of genetic tools and identified two molecules controlling the selective targeting of L4 branches.

Almost 30 years ago, the first *Drosophila* immunoglobulin superfamily member (IgSF), Fasciclin II, was identified as a homophilic cell-adhesion molecule promoting the selective fasciculation of a subset of motor axons and their targeting to specific body wall muscles (Harrelson and Goodman, 1988). Recent analyses (Özkan et al., 2013) estimated that at least 130 distinct IgSF are encoded in the *Drosophila* genome (not taking into account splice variants). Forward genetic screens and biochemical approaches have shown that IgSFs play a pivotal role in regulating axonal extension, fasciculation, and target recognition in the developing fly optic lobes (Tan et al., 2015). One of the most striking discoveries was the identification of the fly ortholog of the mammalian Down syndrome Cell adhesion molecule (Dscam1) and its about 38,000 splice variants (Schmucker et al., 2000). Three additional Dscam genes (Dscam2–4) exist in *Drosophila* and all are expressed in the developing visual system (Millard et al., 2007). However, they do not undergo massive splicing and there are only two isoforms of Dscam2, Dscam2A and Dscam2B (Millard et al., 2007). While in vitro assays showed that fly Dscams are homophilic adhesion molecules, previous in vivo data suggest that in fly neurons, homophilic binding of Dscams only promoted repulsion—a developmental process underlying self-avoidance between sister neuritic branches of the same neuron in various *Drosophila* neuronal systems (Zipursky and Sanes, 2010). In the medulla, Dscam2 expression on L1 axons prevents them from adhering to other L1 axons in neighboring columns (Millard et al., 2007). Subsequently, it was shown that in the nascent lamina, Dscam1 and Dscam2 exert selective homophilic repulsion ensuring avoidance between dendrites from the same cell type (L1/L1 and L2/L2 pairs). This leads to each of the 50 R-Cells' presynaptic terminals exclusively contacting one L1 and one L2 dendrite per cartridge (Millard et al., 2010). Whereas self-avoidance happens in individual cells, homophilic repulsion between cells of the same type also controls tiling of receptive target fields. In this new study, Tadros et al. (2016) show that Dscam2 and Dscam4 control the lamina tiling of L4 monopolar dendrites—but in an unexpected manner: by homophilic adhesion.

To uncover how L4 dendrites are tiled, Tadros et al. (2016) used elegant genetic tricks, such as MARCM and fluorescent reporters, to show that developing L4 neurons express both Dscam2 isoforms. They next generated flies in which either one or both Dscam2A or Dscam2B were mutated in a few L4 cells and found that in both cases Dscam2-deficient L4 cells developed supernumerary dendritic branches innervating additional posterior

cartridges (Figure 1C). Using confocal microscopy and live imaging, they found that in wild-type, developing L4 dendrites first go through a phase of overt protrusion dynamics ("exploratory phase") during which transient contacts to the target fascicles occur, followed by a "consolidation phase" when filopodia stabilize on the target fascicles prefiguring the two posterior dendrites. Dscam2 mutant L4 neurons also initially grow two dendrites but they do not stop in the nearest posterior cartridges and further extend to more distal cartridges (Figure 1C). Live imaging revealed that the exploratory phase is unaltered, but these dendrites fail to anchor to the target fascicles and instead deviate toward dorsal and ventral poles.

Tadros et al. (2016) showed that in addition to L4, Dscam2A is expressed by L2, L3, and L5 monopolar neurons, whereas Dscam2B is expressed by L1 and all R-cells, except R4. To determine which Dscam2-expressing cells provide the stop-signal for L4 dendrites, Tadros et al. (2016) again use genetic mosaicism to sequentially silence Dscam2 expression in different lamina cells and analyze the consequence on dendritic patterning of a few wild-type L4 neurons. Additionally, they developed a brilliant, novel genetic method to simultaneously visualize individual morphology and interaction of a small number of wild-type dendrites with dendrites from Dscam2 mutant L1, L2, or L4 neurons or R-cell axons. This new method allowed Tadros et al. (2016) to demonstrate that the absence of Dscam2 in L1 or L2 neurons, but not in L4 or R-cells, perturbs targeting of L4 dendrites.

In a genetic screen designed to identify additional genes involved in L4 dendritic patterning, Tadros et al. (2016) surprisingly uncovered mutations in Dscam4, the only Dscam family member with hitherto unknown biological function, that phenocopied L4 branching defects observed in Dscam2 mutants (Figure 1C). Dscam4 is expressed in all lamina neurons (and also in R3) and analysis of Dscam2;Dscam4 compound mutant cells showed they act in the same pathway.

Together, this suggests that Dscam2 and Dscam4 cooperate to stabilize L4 dendritic growth cones to the closest adjacent posterior cartridges and to prevent invasion of additional ones. This is the first evidence of a "positive" effect of Dscam homophilic binding, instead of the classical repulsive role, in the fly. Interestingly, in the mouse retina, DSCAM, the ortholog of Dscam1, promotes homophilic adhesion of a subset of retinal ganglion cell dendrites to their target amacrine cells in a specific sublamina of the inner plexiform layer (Zipursky and Sanes, 2010). These new findings thus suggest that the mechanism of action for Dscam in visual circuit patterning may be more conserved in evolution than initially envisaged.

Quite strikingly, two recent studies (Carrillo et al., 2015; Tan et al., 2015) show that two further families of IgSFs, the Dpr (Defective in proboscis extension response; Özkan et al., 2013) and their ligands the Dpr-interacting protein (DIPs) control synaptic specificity in the layers of the medulla by promoting the recognition of medulla target neurons (expressing specific DIPs or combination of DIPs) by lamina monopolar axons expressing their corresponding Dpr receptors. Tan et al. (2015) also performed RNA-seq of the transcriptome or each lamina neurons (including L4), an analysis harboring large potential to further decipher molecular mechanisms controlling development of optic lobe connectivity.

As always with such ground-breaking studies, exciting new findings bring many unanswered questions. For instance, what is the identity of the signaling pathways downstream of Dscam2 underlying either its adhesive or anti-adhesive (i.e., repulsive) function? Dscam1 signaling involves components of the actin cytoskeleton including the serine-threonine kinase Pak1, the small GTPase Cdc42, Abl tyrosine kinase, the adaptor protein DOCK, and JNK1 (Schmucker et al., 2000; Sterne et al., 2015). However, nothing is known about Dscam2 or Dscam4 molecular partners.

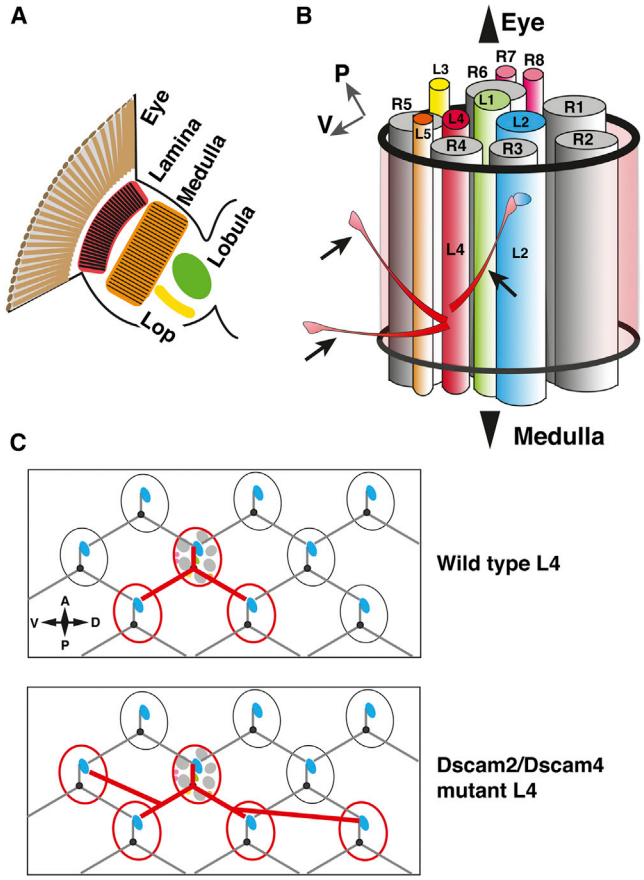

Figure 1. Organization of the Lamina in Wild-Type and Dscam2 or Dscam4 Mutant

(A) Schematic of the fly visual system.

(B) 3D representation of a lamina cartridge only showing processes from the five lamina neurons (L1–L5) and eight photoreceptors (R1–R8). A portion of R3 and R4 was removed to show the inner part of the cartridge. The L4 neuron has three main dendrites (arrows) innervating its own cartridge and two adjacent ones (data not shown).

(C) Wild-type L4 dendrites, in red (top), extend into three cartridges (indicated by the red circles): the home cartridge and the nearest ventroposterior and dorsoposterior ones. The blue disks correspond to L2. L4 dendrites mutated for Dscam2 or Dscam4 (bottom) project to additional cartridges.

Abbreviations: A, anterior; P, posterior; D, dorsal; V, ventral; Lop, Lobula plate.

In terms of circuit function, additional new questions arise from the study at hand. Does the presence of supernumerary L4 branches have any consequence on the total number of L4 branches innervating a single cartridge? Furthermore, is synaptic specificity affected in Dscam2 mutants?

Importantly, these novel mutant flies could help to better understand the role of L4 cells and their tiling of the lamina in visual processing. The lamina is known to be involved in motion detection and L4 and L2 neurons belong to the so-called OFF pathway. A recent study showed that L4 neurons play a key role motion processing, resolving previous contradictory studies on its function (Meier et al., 2014). It was also found that visual circuit miswiring in the absence of Dscam2 leads to specific and conditional differences in visual acuity (Bosch et al., 2015). Strikingly, in three of the behavioral paradigms tested, Dscam2 mutants showed an opposing response as wild-type flies. Therefore, disrupted modularity in the Dscam2-deficient optic lobe leads to aberrant cross-wiring of visual columns resulting in deficits in light and motion detection. The new Dscam2 and Dscam4 mutant lines generated by Tadros et al. (2016) should help to assess and model the function of L4 dendritic tiling in visual processing.

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Can You Hear Me Now?

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Auditory communication is central to the social interactions of many animals. In fruit flies, males sing to court females. Coen et al. (2016) demonstrate that males can dynamically adjust the loudness of their songs according to the distance to a female.

The animal kingdom abounds with examples of strange mating rituals. Bowerbirds decorate elaborate nests; blue-footed boobies kick up their brilliant blue feet and dance; many humans post carefully curated representations of their lives online and wait for prospective mates to swipe right. Fruit flies, too, engage in elaborate courtship rituals that involve, among other things, male flies serenading females with songs. Central to all of these rituals is communication: prospective mates evaluate the suitability of a potential partner by the sensory cues they provide. As a result, careful calibration and delivery of these signals is critical to successful courtship. How does one implement an effective signaling strategy that maximizes one's chances of mating? The fruit fly brain is relatively simple, and powerful genetic tools and experimental techniques facilitate dissection, manipulation, and quantification of neural circuit activity and behavioral responses. Thus, song production by male flies provides an ideal model for investigating the neural underpinnings not only of courtship rituals in particular, but also of complex sensorimotor transformations in general. In this issue of *Neuron*, Coen et al. describe a new level of sophistication in how male flies tune their songs in response to female movements.

Fly songs, produced by the extension and vibration of a wing, are composed of two modes: sine song, a low-frequency humming, and pulse song, a train of high-frequency, high-amplitude pulses separated by inter-pulse intervals (IPIs)