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Developmental Neurobiology: It Takes Nrg to Separate Dendrites

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The development of sensory receptive fields requires the coordinated spatial patterning of neurites from multiple sensory neuron subtypes. A new study identifies a role for neuron–skin cell interactions in preventing the bundling of dendritic arbors from distinct neurons.

Our ability to sense touch critically depends on the proper spatial organization of the sensory receptive fields of many coexisting specialized subtypes of touch-sensing neurons innervating the skin. The sensory endings of these touch-sensing neurons branch extensively in the skin to establish their innervation territories. The spatial patterning of neurite arbors is influenced by interactions both between neighboring neurons and between neurons and epithelial skin cells. For example, growing neurites are repelled by sister branches from the same neuronal arbor, and by neurites of neighboring cells of the same sensory subtype, phenomena known as 'self-avoidance' and 'tiling', respectively [1]. Together, these two mechanisms ensure comprehensive and efficient coverage of the skin by the receptive fields of each type of sensory neuron. In addition to these neurite–neurite interactions, interactions between sensory endings and epithelial cells in the skin play critical roles in regulating sensory neurite arborization and spacing [2–8]. A new study from Yang and colleagues reported in this issue of *Current Biology* identifies a new molecular player with interrelated roles in neuron–neuron and neuron–epithelial cell interactions required for proper dendrite spacing [9].

The larval *Drosophila* skin is a simple epithelium innervated by dendrites of several types of sensory neurons, each of which is specialized to detect specific kinds of mechanical, thermal, or light stimuli. Sensory neurons and epithelial skin cells express different splice isoforms of the immunoglobulin superfamily cell adhesion molecule L1CAM/Neuroglian (Nrg) [10]. These isoforms differ intracellularly, but have the same ectodomain, and are capable of binding to each other to promote adhesion [9]. Yang *et al.* demonstrate that

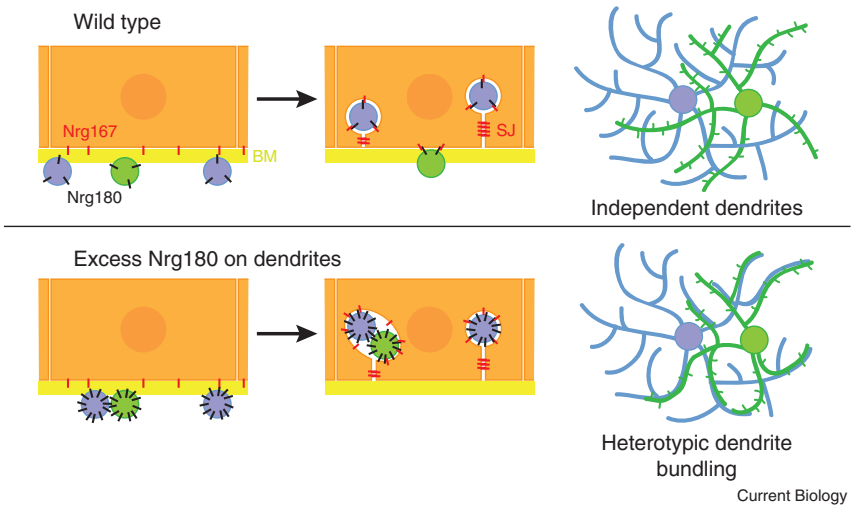

Figure 1. Nrg regulates dendrite enclosure and bundling.
In wild-type *Drosophila* larvae, an epidermal Nrg isoform, Nrg167, interacts with a dendritic isoform, Nrg180, at the basal surface of skin cells. Dendrites of different subtypes (indicated by different colors) innervate the skin independently, and dendrites of some subtypes have a greater propensity than others to become enclosed by epidermal cells. When neurons express excess Nrg180 on dendrites, adhesion between dendrites of different subtypes can lead to heteroneuronal dendrite bundling, and even ensheathment of dendrite bundles in some cases. Cross-sections through the skin (left); en face views of sensory arbors (right). BM, basement membrane; SJ, autotypic septate junction.

Nrg mediates a competition between neuron–neuron and neuron–epithelial adhesion to establish dendrite territories [9]. As dendrites branch underneath the skin, the dendritic isoform, Nrg180, recruits the epidermal isoform, Nrg167, into linear stretches on the basal skin cell surface that run along the length of the underlying dendrite branches. Genetic experiments demonstrated that Nrg167–Nrg180-mediated dendrite–skin interactions oppose Nrg180–Nrg180 dendrite–dendrite interactions: decreasing Nrg167 expression in epithelial cells, overexpressing Nrg180 in sensory neurons, or preventing Nrg180 endocytosis all resulted in inappropriate heteroneuronal dendrite bundling (Figure 1). Thus, proper dendrite spacing underneath the epidermis requires mechanisms ensuring that Nrg167–Nrg180 interactions are favored over Nrg180–Nrg180 interactions. This finding emphasizes that molecular mechanisms regulate not only the tiling of dendritic arbors of the same sensory subtype, but also the spatial organization of arbors of different neuronal subtypes [9].

The dendrite endings of *Drosophila* sensory neurons do not just course below the skin, but some branches become enveloped by skin cells (Figure 1), which wrap them into tunnel-like channels [4,5,11,12], a process termed ‘enclosure’ or ‘ensheathment’. Dendrite enclosure by *Drosophila* epidermal cells regulates the tiling [4,5], branching [9,11,12], and function [12] of sensory neurons. *Drosophila* epidermal ensheathment channels share ultrastructural features with similar structures in the skin of *Caenorhabditis elegans* [13], zebrafish [14], and humans [15], all of which resemble ensheathment channels made by non-myelinating Schwann cells [16]. Recent work in *Drosophila* and zebrafish has revealed that epidermal ensheathment proceeds through a conserved sequence of morphogenetic events: Neurites induce the accumulation of specialized lipid microdomains underneath axons, which recruit F-actin to the cortex surrounding invaginating neurites [12]. Finally, opposing membranes at the ‘neck’ of ensheathment channels form junctions, apparently to seal the channels [12]. In zebrafish, these junctions contain components of adherens junctions and desmosomes, whereas in *Drosophila* they contain components of septate and adherens junctions. Moreover, in *Drosophila*, junctional components mediate interactions not only between epidermal cell membranes, but also between dendrites and skin cells [11]. Using super-resolution and electron microscopy, Yang *et al.* have provided further support for the formation of autotypic contacts between epidermal membranes, and report that Nrg is also required for dendrite enclosure, likely as a component of septate junctions [9].

L1CAM/Nrg adds to the growing list of junctional proteins that regulate ensheathment [5,11,12], but Yang *et al.* suggest that Nrg’s role in regulating heteroneuronal bundling is separable from its role in ensheathment [9]. Septate junction proteins, whether in autotypic epithelial junctions or neuron–epithelial junctions, could sequester dendritic Nrg180 to prevent it from establishing homophilic interactions with non-sister dendrites. However, reducing ensheathment by depleting the septate junction protein Coracle did not appear to cause heteroneuronal bundling, like Nrg167 knockdown, despite a requirement for Coracle in maintaining proper levels of Nrg167 in the epidermis [9]. Moreover, Yang *et al.* showed that dendrite bundling and ensheathment are not mutually exclusive, since electron microscopy revealed that heteroneuronal dendrite bundles caused by excess surface Nrg180 can become enclosed as a multi-dendrite unit (Figure 1) [9]. This finding suggests that ensheathment *per se* is not sufficient to prevent bundling. Curiously, Nrg167 prevents bundling of non-sister dendrites [9], whereas Coracle may be required to limit heteroneuronal repulsion [11], though both Nrg167 and Coracle are required for ensheathment. More studies are needed to determine if ensheathment of neurites using junctional proteins directly regulates heteroneuronal dendrite spacing.

The current study by Yang *et al.* expands the list of roles that L1CAM family members play in dendrite development in worms [2,3], flies [10], and mice [17]. Similar to *Drosophila* Nrg, epidermal expression of the *C. elegans* L1CAM homolog, SAX-7, regulates dendrite morphogenesis in

the skin-associated PVD sensory neurons [2,3]. SAX-7 and another cell surface protein expressed by skin cells, MNR-1, interact with LECT-2, a protein secreted by muscle [18,19], and a receptor on PVD dendrites called DMA-1 [2,3]. DMA-1 functions as a coincidence detector, stabilizing dendrites only when they encounter all three ligands together. Mutants in components of the SAX-7/MNR-1/LECT-2/DMA-1 complex exhibit misrouted dendrites and frequently overlapping dendrite branches. Thus, in both worms and flies, neuron–epithelial interactions mediated by L1CAM homologs regulate branching and spacing of sensory dendrites. However, critical distinctions between the functions of L1CAM homologs in worms and flies suggest that their roles have diverged. First, whereas epidermal Nrg in *Drosophila* appears to directly interact with another Nrg isoform on dendrites, and is a component of septate junctions, in *C. elegans* SAX-7 functions as part of a heterophilic, multi-protein complex. Second, pre-patterned SAX-7 forms stereotyped 'foundations' that guide dendrites in *C. elegans* [2,3], whereas in *Drosophila,* dendrites dictate the accumulation of epidermal Nrg at dendrite–skin cell contact sites [9]. Finally, whereas *Drosophila* sensory dendrites regulated by Nrg become enclosed, PVD dendrites are not enclosed, but are instead sandwiched between muscle and epidermal cells [20]. It is nonetheless an intriguing possibility that Sax-7-mediated molecular interactions fulfill functions equivalent to Nrg-mediated structural enclosure in the larval *Drosophila* skin.

The work by Yang *et al.* adds to growing evidence for the importance of neuron–epithelial interactions during innervation of the invertebrate and vertebrate skin. Neuron–skin interactions act at multiple stages to regulate innervation of the skin, including pathfinding into the epidermis [8], neurite branching after reaching the skin [2,3,5,6,11,12], and regulating neurite–neurite interactions [4,5,11]. The precise mechanisms by which ensheathment of sensory endings regulate branching are still unknown. Multiple groups have reported that ensheathment limits neurite branching [5,11,12], whereas Yang *et al.* describe a positive effect of ensheathment on branching [9]. It is unclear what technical or experimental differences account for these divergent results, but both emphasize a role for skin in regulating dendritic morphology. Enclosure may affect access to, or signaling by, extrinsic cues regulating branching, or impose mechanical constraints on branching. The work by Yang and colleagues makes the intriguing observation that contact between skin cells and dendrites locally stabilizes interactions between epidermal Nrg167 and dendritic Nrg180 [9]. By stabilizing molecular interactions between dendrites and skin cells, ensheathment could pattern the distribution of cell surface receptors along neurites, helping to create distinct branching patterns at different positions along complex arbors.

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