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RESEARCH HIGHLIGHT

Dendritic self-avoidance: protocadherins have it covered

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Dendrites exhibit self-avoidance, in which branches of the same neuron repel each other while overlapping with branches from neighboring neurons. A recent paper by Lefebvre and colleagues reveals that clustered protocadherins provide a basis for neuronal recognition during dendrite self-avoidance in vertebrates.

The ability of the mammalian brain to accurately encode and process information depends on the formation of highly precise wiring of dendritic and axonal processes. One important principle of dendrite and axon organization is termed self-avoidance, which refers to the tendency of branches from the same cell (or sister branches) to spread from one another with minimal crossing or overlap [1]. Self-avoidance ensures that dendritic territories are covered completely and yet non-redundantly (Figure 1). Conversely, arbors from different neurons can overlap freely with one another, or co-exist. This simple pair of patterning rules, self-avoidance and co-existence, presents a major problem in cellular recognition, since it requires use of a molecular code that allows individual dendritic branches to identify any other nearby branch as either "self" or "non-self".

Some insights into molecular mechanisms of self-avoidance have come from studies of *Drosophila* dendritic arborization sensory neurons. Sensory dendrite self-avoidance is mediated by Down syndrome cell adhesion molecule (Dscam1), a member of the immunoglobulin superfamily of proteins. Dscam1 is especially compelling because of the complexity of the gene locus. Alternative splicing of large exon cassettes potentially generates 19 008 Dscam1 isoforms that differ in their extracellular domain and are capable of isoform-specific homophilic binding [2, 3]. Loss of *Dscam1* from single sensory neurons leads to excess overlap of isoneuronal branches, indicating a defect in self-avoidance. Single Dscam1 isoforms can support self-avoidance; however, overlapping arbors must express distinct Dscam1 isoforms for robust discrimination of self and non-self and thus dendritic co-existence [4-7].

Numerous studies have explored the molecular basis of self-avoidance in mammals [8]. Although mammalian Dscam participates in self-avoidance, it is unlikely to underlie discrimination of self and non-self since it does not undergo the same complex alternative splicing as *Dscam1* in *Drosophila*. Alternatively, the clustered protocadherins (Pcdhs) have emerged as good candidate mediators of self identity in mammalian neurons [9-11]. Pcdhs are generated from a complex genetic locus in mice, consisting of *Pcdh-α, Pcdh-β* and *Pcdh-γ*, which encode 14, 22 and 22 variant proteins, respectively (Figure 1) [10]. Different isoforms are expressed in stochastic and combinatorial patterns in individual neurons. Pcdh isoforms interact to form hetero-tetramers and show isoform-specific homophilic binding [12]. As a result, many thousands of distinct binding specificities can be generated from the *Pcdh* locus.

Given the similarities between Dscam1 and Pcdhs, they could conceivably function similarly in neuronal self-recognition [11]. The striking findings of Lefebvre *et al*. [13] have supported this view by pinpointing a role for Pcdhs in self-recognition and self-avoidance in mammalian neurons. The authors focused on the *Pcdh-γ* cluster (Pcdhg), which was previously shown to be important for neuronal survival and synapse formation [14, 15]. Mouse retinal starburst amacrine cells (SACs) express *Pcdhg* genes and display clear evidence of dendritic self-avoidance [13, 14]. Using a pan-retina Cre driver to delete all 22 *Pcdhg* genes from all retinal cells, the authors observed increased SAC dendritic self-crossings and fasciculation (Figure 1). Other features of SAC morphology, including size of dendritic arborizations and organization of SAC cell bodies, were not affected, suggesting that *Pcdhg* genes are specifically required for self-avoidance in SACs. In wild-type SACs, self-avoidance follows a period during which dendrites form isoneuronal contacts, but in mutant neonatal retinas, SACs showed excessive neurite crossing and bundling that persisted well past the age when contacts are normally eliminated, suggesting that *Pcdhg* genes are involved in the development of SAC arborizations. The study went on to show that Pcdhg acts cell-autonomously to mediate self-avoidance in SACs since deletion of *Pcdhg* genes only in SACs and not in their neighboring cells recapitulated the self-avoidance defect. Additional studies of mouse cerebellar Purkinje cells argue that the function for Pcdhgs in self-avoidance is not retina-specific.

Next, in an elegant series of manipulations, the authors addressed the role of Pcdhg isoform diversity in self-avoidance. After verifying that all 22 *Pcdhg* variants were expressed in the retina and also in SACs specifi-

cally, they approached the question in two ways. First, they used a *retina-Cre* driver to delete three *Pcdhg* variable exons, C3-C5, thereby reducing Pcdhg isoform diversity in the retina. Second, they expressed a single Pcdhg isoform, PcdhgC3, throughout retina in which the 22 Pcdhg isoforms had been deleted. Using these two complementary approaches, the authors were able to show that full Pcdhg isoform diversity was not necessary for self-avoidance and that expression of a single isoform was enough to restore self-avoidance in SAC dendrites. Similar analysis of another set of variable exons, PcdhgA1-A3, revealed similar effects, indicating that no single isoform is necessary, but any single isoform is sufficient, to mediate SAC dendrite self-avoidance.

Although Pcdhg diversity was not required for self-avoidance, the authors wanted to know whether there was a role for Pcdhg diversity in self/non-self discrimination. Indeed, by visualizing two nearby SACs with two different fluorescent dyes, the authors were able to show that dendritic overlap of the two SACs was significantly decreased in retinas expressing a single Pcdhg isoform compared to wild-type retinas expressing the full complement of Pcdhg isoforms. Thus, Pcdhg isoform diversity is not required for self-avoidance, but rather allows SACs to distinguish self from non-self dendrites and initiate repulsive interactions only with the former.

Several important issues are resolved and raised by the present study. One remarkable implication of this work is that the principles governing the discrimination between self and non-self during self-avoidance appear to be conserved between invertebrates and vertebrates, despite the use of two very different molecules. Also, notably, although SACs show robust self-avoidance, overlap between neighboring SACs is very extensive, resulting in many individual SACs co-existing in the same retinal field. The requirement for robust self-avoidance and Pcdhg diversity may therefore be particularly important in neurons with extensive overlapping coverage in which many dendrites have to distinguish between self and non-self for proper circuit connectivity. Another important question that should be addressed for both Pcdhs and Dscam1 is how recognition between dendrites is

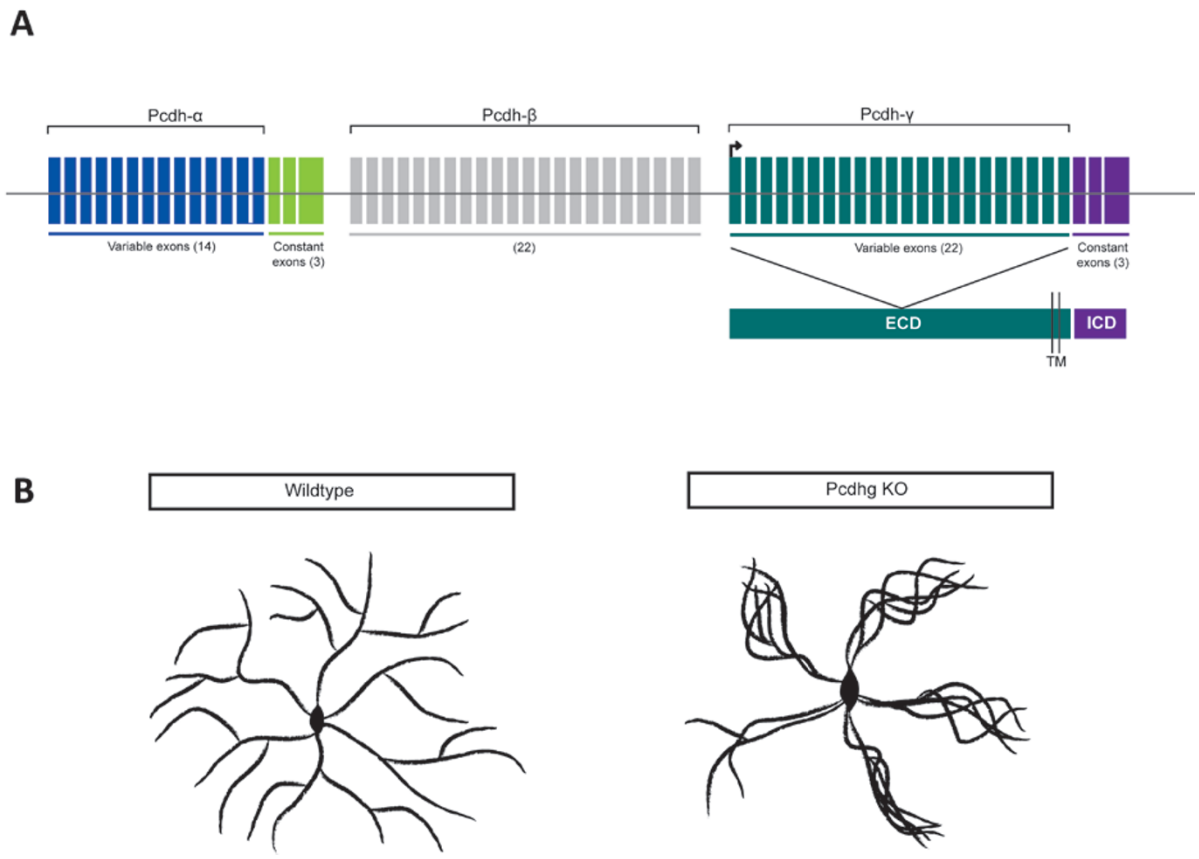

Figure 1 Clustered protocadherins mediate self-avoidance in vertebrates. **(A)** The *Pcdh* locus consists of three exon clusters, *Pcdh-α, Pcdh-β and Pcdh-γ*. Splicing of one *Pcdh-γ* variable exon to three constant exons generates Pcdh-γ isoforms with unique extracellular domains (ECD) but common intracellular domains (ICD). **(B)** Wild-type SAC dendrites exhibit self-avoidance while SACs from *Pcdh-γ* knockout (KO) animals have self-avoidance defects, including self-crossing and fasciculation of dendritic branches.

converted into separation and repulsion. So far, downstream pathways have remained elusive. Finally, this work raises the question of the functional importance of self-avoidance in SACs. One prediction is that the uniform territory coverage that arises from self-avoidance would be important for circuit function. The findings of Lefebvre and colleagues now open the door to understanding the functional reasons for the remarkably similar self-avoidance strategies used by invertebrate and vertebrate species.

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