

Role of Dscam mediated self-avoidance and tiling in neural branching

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ABSTRACT

Dscams (Down syndrome cell adhesion molecules) play an important role in the formation of neural circuits. Various studies have highlighted the role of Dscams in two major wiring strategies, self-avoidance and tiling, leading to broad and uniform branching. The *Drosophila* Dscam1 protein, which has thousands of isoforms formed by alternative splicing, has been shown to confer unique identities to cells and mediate homotypic recognition, homophilic repulsion and consequently self-avoidance behavior between neurites of a single neuron. The *Drosophila* Dscam2 protein mediates homophilic repulsion between projections from the same class of cells, in a process called tiling. The vertebrate Dscam has been shown to mediate both tiling and self-avoidance. However, the mechanisms by which this is accomplished in the absence of homotypic recognition are unclear. This review provides an overview of functional similarities and differences between Dscam homologues in invertebrate and vertebrate species, and describes some mechanisms proposed to account for these differences.

KEYWORDS

Self-avoidance, tiling, neural circuitry, Down syndrome

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INTRODUCTION

The formation of neural circuitry occurs under the direction of many molecules that guide axons to form proper synapses. Dscam (Down syndrome cell adhesion molecule), a cell surface protein first identified by Yamakawa *et al.*, belongs to a class of the immunoglobulin (Ig) family of molecules. Dscam in particular is involved in recognition processes between neurons and plays an essential role in mediating the formation of extensive and complex connections in the brain. The human *Dscam* gene was isolated from the chromosome band 21q22.2-22.3, a region implicated in many neurological phenotypes observed in Down syndrome (1). The invertebrate *Dscam* gene, a homologue of the vertebrate Dscam, was isolated by Schmucker *et al.* in *Drosophila melanogaster* (fruit fly) (2). The Dscam protein has been implicated in a variety of functions in different species. It confers unique identities to cells in fruit flies (3) and mediates dendritic and axonal synaptic targeting in fruit flies, chicks and mice (4), as well as synaptogenesis in *Aplysia* (sea slug) (5). Dscam's role in innate immunity in flies is under investigation (6).

While the vertebrate *Dscam* gene appears to have shared a common ancestor with the invertebrate *Dscam*, they have very different functional characteristics. *Drosophila*, in particular, has four classes of *Dscam* genes (*Dscam 1-4*). The *Dscam1* gene has 24 exons, and four of these exons (4, 6, 9, 17) contain cassettes of genes that undergo mutually exclusive alternative splicing (7). Exon six, for instance, has forty-eight variants within its array, but only one of these variants will contribute to the mature transcript. This form of alternative splicing in the fly *Dscam1* produces as many as 38,016 unique isoforms of the protein. This trait is not shared by other *Dscam* classes in flies or by the vertebrate *Dscam* (2, 7). Nonetheless, all *Dscams* have conserved molecular functions required for neural wiring. These “core molecular mechanisms” (8) allow for self-avoidance and tiling leading to generation of structured axonal and dendritic pathways.

REVIEW

Self-avoidance allows the axons and dendrites extending from a single neuron to repel one another, thereby branching widely and uniformly covering the synaptic field. Tiling allows neurites, axonal and dendritic projections, of different cells of the same functional class to repel one another, thereby preventing overlapping of synaptic domains (9). In this review I examine how *Dscam* is involved in these mechanisms that allow for neural branching.

Kramer and Stent (10) first characterized self-avoidance in neurons in a study of the giant Amazon leech, *Haementeria ghilianii*. They found that branches from different neurons innervating the organism overlapped, whereas branches rising from the same neuron did not overlap. To account for this observation, Kramer and Stent proposed that molecular cues conferred unique identities to neurons and allowed for homotypic recognition, that is recognition that they possess the same identity (10). Self-avoidance has been observed as a universal mechanism in neuronal branching (9).

Tiling of neurons was first characterized by Wassle *et al.* (11) in retinal ganglion cells (RGCs) of cats. They found that ganglion cells consisted of subpopulations whose dendritic field size—the breadth of the area with which dendrites extending from the cell interact—was limited by interactions with neighboring cells of the same class. This finding was corroborated in rat RGCs by Perry and Linden (12), who identified different classes of RGCs and found that if an area in the developing rat retina was depleted of a class of RGCs, neighboring cells of the same class extended dendrites into the area, recovering a uniform dendritic receptive field.

HOMOTYPIC RECOGNITION, HOMOPHILIC REPULSION AND SELF-AVOIDANCE MEDIATED BY DSCAM1

Dscams are thought to guide neuronal branching largely via homophilic repulsion, a process in which after some recognition event molecules of the same type repel one another. Of the four classes of Dscams in *Drosophila*, Dscam1 mediates self-avoidance while Dscam2 mediates tiling, both via homophilic repulsion (13). In vertebrates, only two Dscam molecules exist (DSCAM and DSCAML1). These have been observed to mediate both self-avoidance and tiling. The exact mechanism is currently the subject of debate (14).

Evidence for homophilic repulsion leading to self-avoidance was shown by Matthews *et al.* (15). They observed that in neurites extending from the same neuron, after contact and homotypic recognition the neurites withdrew and segregated in a manner consistent with homophilic repulsion. They postulated that homophilic repulsion is mediated by the ability of *Drosophila* Dscam molecules to confer unique identities to cells by generating of thousands of isoforms. Possessing the same isoforms allows neurites to recognize other neurites extending from the same cell. Studies have found that inducing the expression of the same *Dscam1* isoforms in different classes of cells leads to self-avoidance between these cells (16). Examining an olfactory ganglion called the mushroom body in the *Drosophila* brain, Zhan *et al.* (17) concluded that the composition of the isoform is not important in establishing circuitry; rather, the difference between the isoforms—the diversity—is critical.

As an additional safeguard to prevent binding between similar proteins, Dscam1 has “all-or-none” structural and biochemical binding properties. The homophilic binding region of Dscam1 is composed of eight immunoglobulin (Ig) domains. Three of these domains, making up about 80% of the region, are highly variable because of alternative splicing of the gene as described earlier. All these variable protein domains must match in order for binding between isoforms to occur creating an S-shaped homodimer (18). This configuration of Dscam1 ensures that isoforms with slight variations neither bind nor homotypically recognize one another.

TILING AND SELF-AVOIDANCE MEDIATED BY DSCAM HOMOLOGUES

The contribution of other Dscam class members is critical to the formation of neural circuitry. The *Drosophila* Dscam2 presents a framework for understanding vertebrate Dscam function, as neither undergoes the extensive alternative splicing of Dscam1. Furthermore, while the majority of neurons in *Drosophila* express Dscam1, Dscam2 expression is limited and cell-type specific, as is Dscam expression in vertebrates (9).

In flies, Dscam2 is thought to be involved in allowing projections from different cells of the same functional classes to avoid each other, otherwise known as a process called tiling (15). Millard *et al.* (13) examined lamina (L1) neurons in the *Drosophila* retina which receive input from eye photoreceptors. L1 neurons normally form highly discrete vertical columns. Mutant L1 neurites lacking Dscam2 were shown to laterally invade adjacent neighboring columns and were no longer able to properly tile (13).

Vertebrate Dscams have been found to regulate both self-avoidance and tiling. Loss-of-function experiments in DSCAM-expressing mouse amacrine cells, whose dendrites are normally evenly spaced in the internal plexiform layer (IPL) of the retina, led to fasciculation dendrites from different cells of the same class (19). This is consistent with the process of tiling described by Wassle *et al.* in cat retinas (11). The study also found that without properly functioning DSCAM, processes extending from the same amacrine neurons which normally did not overlap with one another now overlapped (19). This indicates that the vertebrate Dscam is also involved in self-avoidance.

Given the preservation of core functional mechanisms of the fly Dscam1 in vertebrate Dscams, it is perplexing that vertebrate Dscams lack the isoform diversity considered critical to the neuronal self-avoidance mechanism in *Drosophila*. Although it is clear that vertebrate Dscams mediate self-avoidance; they do not appear to confer unique identities to neurites (8). The mechanisms by which vertebrate Dscams function without homotypic recognition is unclear (19).

Although structurally vertebrate Dscams are homophilic adhesion molecules, congruent with *Drosophila* Dscams, certain functional incongruities between these have led to speculation about the possibility of alternative pathways mediating self-avoidance. One hypothesis described by Fuerst *et al.* suggests that vertebrate Dscams act via passive repulsion; that is, they act as a “non-stick coating” (14) in a small subset of cells masking these cells’ intrinsic adhesion properties. Establishing such “exclusion zones” around cells would negate the need for molecular diversity and, by extension, homotypic recognition.

Given that the *Drosophila* Dscam and vertebrate Dscam proteins share the same structure and general binding properties, it is possible that the evolution of other recognition systems may have provided vertebrates with a different strategy for homotypic recognition than that of invertebrates. This recognition system may be under the guidance of Dscam co-receptors that, during vertebrate evolution, took over the functional role of isoform specificity seen in invertebrate Dscam (8). The existence and function of such co-receptors has yet to be confirmed.

PERSPECTIVES

Dscam in vertebrates and its arthropod homologue Dscam have been implicated in mediating self-avoidance and tiling via homophilic interactions. Alternative splicing of Dscam1 confers unique identities to neurons, which is used for homotypic recognition, binding and homophilic repulsion between axons and dendrites extending from these neurons. Dscam2, though not alternatively spliced to the extent of Dscam1, also uses homophilic repulsion to mediate tiling. However, the mechanisms by which vertebrate Dscam guide development have yet to be delineated. Studies elucidating self-recognition mechanisms in vertebrate neurons and different intracellular pathways that Dscam molecules can activate will lead to greater understanding of the formation and development of neural circuitry.

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