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Cell autonomy of DSCAM function in retinal development

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ABSTRACT

Cell adhesion molecules (CAMs) provide identifying cues by which neural architecture is sculpted. The Down Syndrome Cell Adhesion Molecule (DSCAM) is required for many neurodevelopmental processes in different species and also has several potential mechanisms of activity, including homophilic adhesion, homophilic repulsion and heterophilic interactions. In the mouse retina, *Dscam* is expressed in many, but not all neuronal subtypes. Mutations in *Dscam* cause the fasciculation of dendrites of neighboring homotypic neurons, indicating a role in self-avoidance among cells of a given type, a disruption of the non-random patterning of their cell bodies, and a decrease in developmental cell death in affected cell populations. In order to address how DSCAM facilitates retinal pattering, we developed a conditional allele of *Dscam* to use alongside existing *Dscam* mutant mouse strains. Conditional deletion of *Dscam* reproduces cell spacing, cell number and dendrite arborization defects. Inducible deletion of *Dscam* and retinal ganglion cell depletion in *Brn3b* mutant retinas both indicate that these DSCAM-mediated phenotypes can occur independently. In chimeric retinas, in which wild type and *Dscam* mutant cells are comingled, *Dscam* mutant cells entangle adjacent wild type cells of the same type, as if both cells were lacking *Dscam*, consistent with DSCAM-dependent cell spacing and neurite arborization being mediated through homophilic binding cell-to-cell. Deletion of *Dscam* in specific cell types causes cell-type-autonomous cell body spacing defects, indicating that DSCAM mediates arborization and spacing by acting within given cell types. We also examine the cell autonomy of DSCAM in laminar stratification and find that laminar disorganization can be caused in a non-cell autonomous fashion. Finally, we find *Dscam* dosage-dependent defects in developmental cell death and amacrine cell spacing, relevant to the increased cell death and other disorders observed in Down syndrome mouse models and human patients, in which *Dscam* is present in three copies.

Introduction

The neural retina offers an ideal system in which to determine how genes mediate the organization of neurons into functional circuits. The retina is organized in both radial and circumferential space (vertical and horizontal planes in whole mount preparations). Vertically, the retina consists of three nuclear layers, containing the cell bodies of neurons, separated by two synaptic layers, which contain the chemical synapses and gap junctions that connect the estimated fifty-five basic types of retinal neurons into the functional circuitry of visual detection (Masland, 2001). Both the nuclear and synaptic layers can be further subdivided into different laminae that contain the cell bodies or processes of specific neuronal subtypes. Retinal neurons are also organized in the horizontal plane of the retina. Different aspects of visual processing are performed by different retinal circuits, many of which are spaced in a roughly even manner within the retinal nuclear layers, in patterns called mosaics (Wassle and Riemann, 1978). This organization is thought to ensure that different regions of the retina will contain representation from all of the various circuits by which different aspects of vision, such as motion detection or color discrimination, are detected (Sanes and Zipursky, 2010).

In vertebrates, the Down Syndrome Cell Adhesion Molecule (DSCAM) is required for regulation of cell number, neurite arborization, lamination and segregation of ipsilateral projections in the lateral geniculate nucleus (Blank et al., 2011; Fuerst et al., 2008; Fuerst et al., 2009; Yamagata and Sanes, 2008). In the absence of *Dscam*, cells within the mouse retina that would normally express the gene are not organized in evenly spaced horizontal mosaics, but are arranged in cell type specific clumps, knotted together by densely fasciculated neurites (Fuerst et al., 2008, 2009). In the chick retina, DSCAMs, and the closely related Sidekick cell adhesion molecules, are required for the laminar specificity of dendrite arborization in the plexiform layers (Yamagata and Sanes, 2008). In addition to functioning in self-avoidance, vertebrate DSCAMs can also bind the heterologous ligands netrin and draxin and function in axon guidance (Ahmed et al., 2011; Liu et al., 2009; Ly et al., 2008).

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Studies of Drosophila have established that DSCAM1-mediated self-avoidance and repulsion occur through isoform-specific homophilic binding (Schmucker et al., 2000; Wojtowicz et al., 2007). Drosophila *Dscam1* undergoes extensive alternative splicing, resulting in isoform diversity that allows the protein to mediate avoidance between the dendrites or axon branches of individual neurons, each of which expresses a different complement of *Dscam1* isoforms and is thus uniquely identified (Hattori et al., 2007; Hughes et al., 2007; Wang et al., 2002; Zhan et al., 2004; Zhu et al., 2006). Because each individual neuron expresses a different subset of *Dscam1* isoforms, different neurons are able to overlap and *Dscam* is able to specify repulsion of axon or dendrite branches in each individual cell (isoneuronally). The molecular diversity of *Dscam1* is essential for its normal function in Drosophila (Hattori et al., 2007; Hughes et al., 2007; Matthews et al., 2007; Soba et al., 2007). Unlike Drosophila *Dscam1*, vertebrate *Dscams* are not subject to extensive alternative splicing and the neurites of *Dscam*-expressing cell types overlap to a considerable degree, even within a single cell type, complicating a repulsive mechanism (Keeley and Reese, 2010). Surprisingly, despite the reduced molecular complexity of vertebrate *Dscams*, the vertebrate gene seems to mediate a similar repulsion or self-avoidance to like neurites, and the loss of *Dscam* results in the clumping and fasciculation phenotypes described.

Here we seek to better understand how vertebrate Dscams achieve this function by testing the cell autonomy of DSCAM activity in retinal development to determine if the protein acts through homophilic or heterophilic binding and by determining if the protein mediates soma spacing and dendrite arborization within a single cell type through self-avoidance or between cell types through adhesion. Results indicate that arborization and spacing are indeed mediated by homophilic DSCAM interactions that act within cell-types to facilitate self-avoidance, while DSCAM-dependent regulation of laminar specificity can occur in a non-cell autonomous manner.

Results

Dscam is required for a range of developmental processes in the mouse retina. The *Dscam* loss of function phenotype has first been reported at late embryonic stages of development, when retinal ganglion cells in mutant mice begin to develop hyper-fasciculated processes and clumped cell bodies (Fuerst et al., 2009). Fasciculation and clustering of cells that would normally express *Dscam* are observed in a cell-type-specific fashion. For example, a given type of amacrine or ganglion cell will fasciculate with other cells of the same type, but generally not with other cell types (Figs. 1A and B) (Fuerst et al., 2008, 2009). The affected cell types are also overly abundant, due to a decrease in developmental cell death. Outside of the retina, *Dscam*-deficient retinal ganglion cell axons project through the optic nerve to appropriate CNS targets, but defects in segregation of ipsilateral projections to the lateral geniculate nucleus (LGN) occur in a dose dependent manner (Blank et al., 2011).

Conditional allele of *Dscam*

To better understand whether these functions of Dscam in retinal development depend on homophilic interaction between DSCAMs, whether the phenotypes observed have separate etiologies or stem from a common defect, and whether there is a specific period during development in which *Dscam* is required, a conditional allele of *Dscam* was developed. The *Dscam* conditional allele was generated by flanking exon 27, encoding the *Dscam* transmembrane domain, with loxP sites using standard ES cell homologous recombination (Fig. 1C and Supplementary Fig. 1 for allele genotyping). The homozygous floxed allele, referred to as $Dscam^{F/F}$, is viable and has no overt neurological phenotypes prior to Cre expression. In the retina, these mice have normal spacing and arborization of retinal dopaminergic amacrine (DA) cells and other assayed neuron types (Figs. 1D and E and data not shown). To test if deletion of the transmembrane domain would recapitulate other *Dscam*-mutant alleles, the $Dscam^{F/F}$ mice were bred to transgenic mice expressing Cre recombinase under the control of the *Pax6α* promoter (Marquardt et al., 2001). *Pax6α* Cre is broadly expressed in most types of retinal neurons, except a swath in the central–dorsal retina in which only amacrine cells are targeted (Stacy et al., 2005.). DA cells in the $Dscam^{F/F}$ *Pax6α* Cre retina fasciculate and aggregate, comparable to other *Dscam* mutant strains (Fuerst et al., 2008, 2010). This confirms that deletion of the DSCAM transmembrane domain reproduces the *Dscam* spacing and arborization phenotypes seen in other strains of *Dscam*-mutant mice, despite leaving the coding sequence in frame (Fig. 1F).

Deletion of the floxed allele was also performed in the germ-line, referred to as $Dscam^{FD}$, to assay the effect of global deletion of the DSCAM transmembrane domain. In addition to phenocopying other *Dscam*-mutant alleles, a dosage dependent phenotype was observed in DA cells. DA cells in wild type and $Dscam^{F/F}$ retina were rarely located in close proximity to other DA cells. The number of DA cells directly abutting other DA cells was counted in $Dscam^{F/F}$ and $Dscam^{FD/F}$ retinas. A significant increase in the number of DA cells abutting other DA cells was observed in $Dscam^{FD/F}$ retinas compared to $Dscam^{F/F}$ retinas (Figs. 1G–I). This indicates that a previously reported DSCAM-dosage phenotype observed in melanopsin-positive RGCs (mRGCs) can be generalized across multiple types of neurons (Blank et al., 2011). The extensive fasciculation between $Dscam^{FD/FD}$ DA cells was not observed in $Dscam^{FD/+}$ *or* $Dscam^{FD/F}$ retinas, suggesting that at intermediate *Dscam* dosage regulation of cell spacing is disrupted without disruption of arborization (Figs. 1G–I and data not shown).

Independence of fasciculation and cell number phenotypes

An increase in cell number through a decrease in cell death is observed in the *Dscam* mutant retina in conjunction with fasciculation and mispatterning of cell bodies. These phenotypes could all derive from a common defect, for instance, decreased cell death leading to increased cell number and therefore increased interaction between processes, resulting in fasciculation and misplaced cell bodies. However, the observation of DA cell spacing defects in the absence of fasciculation or increased cell number suggested the phenotypes might occur independently of each other (Fig. 1H). We therefore tested whether the defects observed in the *Dscam* null retinas are independent of each other, or if a single primary defect can give rise to the other phenotypes secondarily. We reasoned that temporally controlled deletion of *Dscam* could separate phenotypes, and *Dscam* was therefore targeted for deletion at various time points during development. Tamoxifen was given to activate a Cre-estrogen receptor fusion (Esr Cre) in a transgenic mouse strain with the ESR Cre driven ubiquitously by the CAGGS-promoter (Hayashi and McMahon, 2002). Tamoxifen administration at all tested ages resulted in similar widespread activation of the Thy1-Stop-YFP Cre reporter (data not shown) (Campsall et al., 2002). Induction of Cre activity at E13 and E16 reproduced a null phenotype in both DA cells and mRGCs when assayed at postnatal days 0 (mRGCs; P0) and 20 (mRGCs and DA cells; P20) (Figs. 2A and B and data not shown). Induction of Cre activity at P0 resulted in 2–3 fasciculated groups of mRGCs in each retina and no apparent change in cell number (Figs. 2C, D). In DA cells, induction of Cre activity at P0 resulted in a statistically significant increase (T-test<0.05) in DA cell number, but no fasciculation (Figs. 2E and G). Density Recovery Profile (DRP) analysis, a measure of how likely a cell of a given type will be located a given distance from another cell of the same type, was performed to determine if deletion of *Dscam* at P0 influenced the spacing of DA cells. DRP analysis is performed by plotting the location of all cells of a given type within a field. The number of cells at a given distance from a given cell, termed the reference soma, is counted for every cell in the field. The number