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Adhesion molecules in establishing retinal circuitry

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Abstract

The formation of neural circuits requires molecular mechanisms to confer cell identity, to establish appropriate dendritic arbors, and to space cell bodies within groups of homotypic neurons. Recent work in a variety of organisms has implicated cell adhesion molecules in these processes. The DSCAMs in particular have functions including cell identity and self-avoidance through repulsion in *Drosophila*, differential adhesion and synaptic pairing in chick retina, and the masking of adhesion within specific cell types in the mouse retina. These differences in molecular function between different organisms, and potentially different cell types within a single tissue, emphasize how seemingly subtle distinctions may be important for deciphering this molecular adhesion code.

Keywords

tiling; mosaics; adhesion code; Dscam; masking

Introduction

The assembly of neural circuits depends on the generation and integration of multiple classes of neurons into a functionally connected network. This requires that each cell adopts its appropriate morphology, for example, the size and shape of its dendritic arbor will largely determine its receptive field properties. In addition, cells distribute themselves with respect to other cells of the same type, such as in mosaic patterning during the formation of functional circuits. Establishing these patterns of cell morphology, cell body spacing, and synaptic pairing requires molecular systems for recognition, adhesion, and repulsion. This is clearly demonstrated in the vertebrate retina, where specific types and subtypes of neurons are organized in both vertical laminae and horizontal mosaics [1–3]. Identification of retinal neuron classes, and many subclasses, along with the availability of antigen and transgenic labels to mark them make the retina an ideal system in which to discover basic principles of neural organization.

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Retinal Architecture

The circuitry of the retina in vertebrates is essentially columnar, with signals passing from the photoreceptors to the interneurons of the inner nuclear layer to the retinal ganglion cells (RGCs) that send retinotopic axonal projections to the brain. The cell bodies of retinal neurons are stacked in three vertical laminae, including the outer nuclear layer (ONL), containing the rod and cone photoreceptors, the inner nuclear layer (INL), containing the horizontal, bipolar, and amacrine cells, and the retinal ganglion layer (RGL), containing the retinal ganglion cells and additional amacrine cells (Figure 1).

In addition to this vertical lamination, individual neuron cell types are distributed horizontally throughout the lamina in which they reside. The non-random, horizontal distribution of neurons is referred to as tiling or mosaic patterning (Figure 2 A and B). In a strict sense, tiling refers to a process in which the neurites of neighboring homotypic cells may abut, but do not overlap, whereas mosaic patterning refers more generally to the even positioning of cell bodies, the neurites of which may tile or may intermingle. The *Drosophila* compound eye may represent the anatomical extreme of tiling, with approximately 800 physically distinct ommatidia, each with its own columnar circuit receiving input from the visual world and projecting to precise targets in the brain. In contrast, the vertebrate retina is composed a mixture of tiled mosaics and intermingled mosaic neuronal populations wherein the neurites of a given cell type overlap extensively (Figure 2 C and D). The latter pattern could be considered “Shingling,” like tiles on the roof of a building, where the cells are evenly spaced but heavily overlapping provided the analogy is not taken to be exact, for instance, many retinal neurons more than doubly overlap their arbors (termed the coverage factor, the average area occupied by a single cell of a given type multiplied by the number of cells of that type in a given area). In both tiled and intermingled mosaics, a neuron occupies a discrete region of space, represented functionally by its receptive field, and anatomically by its cell body position, dendritic arbor, and synaptic connections, but they are not physically separated as in *Drosophila*.

Mechanism of dendrite tiling and mosaic patterning

How tiled or intermingled mosaic patterns of neurons arise is a matter of intense investigation. In the vertebrate retina, there are data in support of mosaics arising from clonal progenitors, through lateral migration, and by sculpting through cell death to eliminate inappropriately spaced cells [1,4–6]. An attractive hypothesis explaining the molecular mechanism underpinning mosaic formation is the use of mechanical forces mediated by adhesion molecules. If a mosaic of neurons with intermingled processes were tacked together by adhesive sites, then tension on this network would result in the neurons being pulled into a single plane and being evenly spaced, as beautifully outlined in Galli-Resta et al., 2008 [7]. By analogy, such a mosaic is like a fishnet in which the knotted intersections represent cell bodies and the connecting webbing represents the cellular processes. Pulling outward on a piled net results in flattening and even spacing of the knots. This is indeed the final organization of the retina, in which homotypic classes of cells are stratified vertically and spaced into mosaics laterally.

Any proposed mechanism of mosaic patterning requires cellular identifiers so that neurons of the same class know if they are interacting with a like (homotypic) neighbor. Such identifiers must be complex enough to identify the many classes of neurons, which even within the retina include at least 25 classes of amacrine cells, 10 classes of bipolar cells and 14 classes of ganglion cells [8–12]. In sparsely spaced cell types, these identifiers could be diffusible, but this is hard to reconcile in populations with intermingled processes, or even in tiled systems, where distal processes may come into very close proximity. Therefore, such cues must be very short range, and are likely to be contact mediated. There are also intrinsic factors within cells

that govern the size and shape of dendritic arbors independent of cell density, even in the absence of extrinsic cues, as seen in cases where cell density is reduced so that contact with homotypic neighbors is lost and in studies of dendrite morphogenesis from *Drosophila* [13, 14].

Dscams and Molecular identity cues

According to the differential adhesion hypothesis [15,16], the organization of the nervous system is mediated by adhesion molecules that act as molecular cues to facilitate recognition of homotypic versus heterotypic cells, as well as synaptic partners. Balancing these adhesive forces with other mechanical forces leads to the sorting of cells into their more energetically favorable positions. For example, a core of strongly adhering cells may be surrounded by a shell of loosely adhering cells, or in the case of the retina, the cell body layers may fall out of the balance of adhesion to the extracellular matrix versus differential adhesion between the cell mosaics. The Immunoglobulin (Ig)-superfamily of cell adhesion molecules is one class of candidate proteins for mediating cell recognition and establishing tiling and mosaics. Current work has established a role for several Ig-superfamily genes in synaptic pairing, tiling and mosaic patterning.

In *Drosophila*, the Ig-superfamily *Dscam* genes confers cellular recognition, but in this case, it is largely mediating self-avoidance rather than adhesion. A repulsive function for fly *Dscam* is perhaps best demonstrated in dendrite arborization, where neurites of the same cell recognize one another and repel, creating an arbor, but each cell is invisible to its neighbors because they express different *Dscam* isoforms and therefore do not interact [17–19]. Similar instances of recognition leading to repulsion are seen in axonal branching and synaptic target recognition [20–24]. Specificity of the recognition system is generated by the vast number of alternative splice forms that are generated (over 19,000 extracellular domains), and their remarkable isoform-specific hemophilic interactions [20,25]. The molecular diversity of *Drosophila* *Dscam* is necessary for its function, but *Dscam2* in flies has a similar role in axonal tiling in the visual system and it is not extensively alternatively spliced [23,26]. These aspects of *Dscam* cellular and molecular biology have been reviewed recently, and it is clear they are important neurodevelopmental molecules that may be functioning differently in different organisms [27,28].

In the chick retina, Sidekick1, and Sidekick2 (SDK1, SDK2) and the Dscams (DSCAM and DSCAML1) are candidates for a cell-type-specific recognition and adhesion code [29,30]. Each protein is a homophilic adhesion molecule that binds specifically with itself and does not cross-react even with its close homologs. In the chick retina, these proteins demarcate specific synaptic laminae in the inner plexiform layer. In these laminae, the neurites of amacrine and bipolar cells arborize and form synaptic connections with the dendrites of ganglion cells. Non-overlapping subsets of both amacrine and ganglion cells express each of the SDKs or DSCAMs, and driving the ectopic expression of one of these proteins causes the misexpressing cell to now arborize in the lamina specified by its ectopic protein. Conversely, reduction in expression causes a diffuse stratification of processes in the inner plexiform layer [29,30].

In the mouse, the loss of *Dscam* results in cell-type-specific fasciculation of neurites and clumping of cell bodies [31]. In this way, the function more closely resembles that of *Drosophila* self-avoidance than chick synaptic lamination. However, the mouse *Dscam* gene (like all vertebrate *Dscams*) is not subject to extensive alternative splicing. An unresolved question is therefore how “Self” can be specified by DSCAM without numerous protein isoforms. One possibility is that classes of cells using *Dscam* for self-avoidance are simply physically separated. For example, dopaminergic amacrine cells and bNOS-positive amacrine cells both rely on *Dscam* to prevent adhesion. The dopaminergic cells stratify in lamina S1 of

the IPL, while the bNOS cells stratify in S3, and both cell types have fairly sparse distribution of cell bodies. Therefore, physical separation may allow the recycling of the identity or avoidance code and intervening cell types could be using other related molecules such as DSCAML1 or the Sidekicks.

However, spatial separation encounters several problems in explaining how a single isoform of *Dscam* mediates avoidance of multiple cell types. First, the cell types in the mouse retina expressing *Dscam* have extensive overlap of their distal processes (Figure 3). It is therefore unlikely that the neurites of these cells are truly repellant, that is, actively avoiding contact with one another in the wild type retina. *Dscam* is also very widely expressed in the developing retinal ganglion cell layer (P.G.F., R.W.B. unpublished), making it difficult to conceive of how all *Dscam*-expressing cells could be spatially separated. Therefore, alternative hypotheses need to be considered and for these alternatives, an examination of other retinal cell types may be informative.

In mouse horizontal cells, a mosaic arises in what appears to be a two-step process [32]. Horizontal cells reside in the outer portion of the inner nuclear layer. These cells extend lateral branches that synapse in the outer plexiform layer. In adult mice, the horizontal cell bodies are evenly spaced, but have heavily intermingled neurites with a coverage factor of approximately six [33]. Functionally and morphologically, horizontal cells are a single homogeneous cell population, and can therefore be considered as a single mosaic. However, in their early postnatal development (postnatal day 1 to 3, abbreviated P1 to P3), these cells extend vertical rather than lateral processes and are tiled. Adjacent cells do not intermingle, and if a cell is ablated, surrounding cells grow into the vacated space until they abut, but they still do not intermingle. This tiling effect is consistent with the short-range or contact dependent repulsion discussed previously, and is age dependent. It is not seen after one week of age, when processes have begun to intermix. Therefore, it appears that the horizontal cells undergo a switch from being a tiled mosaic to an intermingled mosaic during development (Figure 4 A and B).

The molecular bases that permit such a switch are not known. If we assume they are using a similar cell surface marker, then there are several possibilities for how this switch may occur. First, the recognition molecule at P1 may be repulsive and its expression may shut off by P7. Alternatively, the intracellular signaling cascade may change after P3, and no longer mediate active repulsion. If the repulsive signal (or its intracellular signaling apparatus) is retained on cell bodies and proximal processes, but is reduced or missing from more distal processes, it may account for the “Exclusion zone” (the region surrounding each cell body in which it is very unlikely to find another cell body of the same type), but still allow the interaction of more distal neurites in the later lateral mosaic phase.

It needs to be determined if *Dscam*-expressing cells like dopaminergic amacrine cells have a similar tiled/repulsive phase before their mosaic pattern is established. One limitation in studying this is identifying the cells earlier in development, before they have assumed their mosaic pattern and elaborated processes. *Dscam* is not required for mosaic patterning of DA cells at early time points (P6), although defects in neurite arborization can begin to be seen at this age [31]. At later time points mosaic patterning is disrupted, likely as a result of extensive neurite fasciculation and lateral movement of the cell bodies into these fascicles (Figure 4 C and D). One interpretation of this is that mosaic patterning of DA cells, and possibly other *Dscam*-expressing cell types, is established independent of *Dscam* in the wild type retina. As the neurites of homotypic cells begin to intersect each other, DSCAM promotes an indifference to an underlying adhesion signal that is unmasked in the absence of DSCAM, leading to adhesion and fasciculation. According to this model, DSCAM is not actively repellant, but instead is simply a “nonstick coating” to mask a cell type-intrinsic adhesion code. This is in contrast to *Drosophila Dscam1*, where it clearly mediates repulsion [17]. One attractive feature

of this model for vertebrates is that it does not require each cell to be uniquely identified, they simply have to recognize, and then ignore, all other cells expressing the same nonstick coating, and therefore, *Dscam* molecular diversity is not required.

Beyond indifference

How DSCAM, an adhesion molecule, functions to prevent adhesion remains to be determined. However, the *Dscam* null phenotype in mice suggests that a cell-type-specific adhesion code must exist [31]. Neurons that express *Dscam* (dopaminergic and bNOS-positive amacrine cells, for example) must retain their cell identities because the clumps and fascicles that form are cell-type-specific. Therefore, it appears that there is indeed an underlying cell-type-specific adhesion code that is unmasked when *Dscam* is lost. It is easiest to imagine this code being homophilic, since it is present on all cells of a given type, but reciprocal heterophilic interactions (neurexin/neurologin type binding for example [34,35]) could also lead to such a phenotype, provided all cells of a given type are expressing both binding partners. The nature of this adhesion code will be exciting to unravel. It potentially constitutes the adhesive tacks that allow mosaics to form through mechanical interactions between homotypic cells, and may even serve to direct synaptic interactions if it functions across different cell types.

The balance of the adhesive code and the repellant or non-adhesive properties of molecules like DSCAM will also be interesting to understand. For example, is this the result of localization or signaling, or both? If DSCAM uniformly coats the neurites, then the adhesive code must overpower the DSCAM's repulsion at points of contact. Alternatively, DSCAM may be physically excluded from points of contact while the adhesive molecules would be concentrated at such points. One protein may even be responsible for both adhesion and repulsion at specific points along the neurite if different intracellular signaling cascades are initiated in different subcellular domains. Many candidate molecules have been proposed to underlie this adhesion code. Cadherins are logical candidates, but loss of N-Cadherin largely results in the dissociation of the retina with the loss of adherens junctions in the outer limiting membrane [36]. Other candidate adhesion molecules, such as protocadherins, may also be able to provide the molecular diversity needed for cell identity [37,38]. The gamma-protocadherins are known to be in the retina, but the elimination of the entire gamma-protocadherin locus results in widespread cell death in the retina [39,40]. The function of individual protocadherin isoforms and their homophilic adhesive properties remain to be determined.

Conclusions

Thus, a cell type specific adhesion code may well explain at least some aspects of mosaic formation in the retina. However, this adhesion code cannot be acting unopposed, and factors such as DSCAM appear to be necessary to balance adhesion, through repulsion or at least cellular indifference, to prevent fasciculation and clumping of homotypic cells. Seeming subtleties, such as the differences between repulsion and indifference or between intermingled and tiled mosaic populations, may turn out to explain apparent discrepancies between different cell types. Just how generalizable these principles will be to other sensory systems and to other parts of the brain will be important to determine, but the relative conservation of these mechanisms through insects, other invertebrates, and mammals suggests that they are fundamental processes in neurodevelopment.

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Figure 1. Retinal Architecture

The retina is organized in three nuclear laminae and two synaptic laminae. The outer nuclear layer (ONL) is composed of rod and cone photoreceptors (1). The inner nuclear layer (INL) is composed of bipolar cells (2), horizontal cells (3), Mueller glia and amacrine cells (4). The retinal ganglion cell layer (RGL) is composed of amacrine cells and retinal ganglion cells (5). The outer plexiform layer (OPL) contains the synapses between photoreceptors and horizontal and bipolar cells. The inner plexiform layer (IPL) contains the synapses between amacrine, bipolar cells and retinal ganglion cells. The inner plexiform layer can be broadly divided into an ON portion, proximal to the RGL, and an OFF portion, proximal to the INL, or into five synaptic bands (S1–S5). The image is a mature mouse retina cut in cross-section (6 μ m) and stained with hematoxylin and Eosin (H&E)

Figure 2. Mosaic patterning, tiling and intermingling

A, Cartoon of a horizontal section (wholemount) of retina. The soma of various cell types, represented by colored circles, are spread across the tissue. **B**, The cells of a particular cell subtype are non-randomly distributed such that cells of the same type are excluded from a zone around other cells of the same type (the “Exclusion zone”). **C**, Neurite self-avoidance prevents the neurites of a given cell from crossing other neurites of the same cell (isoneuronal self-avoidance) or neurites of the same cell type (heteroneuronal self-avoidance and tiling). **D**, Neurite intermingling occurs when the cell bodies of cells of the same type are organized in a mosaic pattern, but the neurites overlap within this population (intermingling).

Figure 3. Intermingled processes of amacrine cell types in the mouse retina

A, B, Examples of bNOS-positive amacrine cells (A), identified using an antibody to bNOS, and dopaminergic amacrine cells (DA), identified with an antibody to tyrosine hydroxylase (B). The neurites of both cell types overlap extensively with the neurites of neighboring cells of the same type, following the pattern shown in Figure 2D, but the intermingled mosaics are independent of one another. Both images are from wild-type, adult mouse retinas and are confocal Z-series projections through the inner plexiform and inner nuclear layers of the wholemount retinas. The scale bar in (B) is equivalent to 193.75 μm .

Figure 4. Mosaic patterning in mouse horizontal and dopaminergic amacrine cells

A, Mouse horizontal cells undergo a stage during which cells are in tiled mosaics. **B**, By P7 the transient tiled neurites have given way to intermingled neurites. **C**, At P6 mouse dopaminergic amacrine (DA) cell bodies are spaced in a mosaic pattern in both the wild type and *Dscam*^{-/-} (C) retina. The neurites of DA cells are beginning to fasciculate in the *Dscam*^{-/-} retina at P6, but remain indifferent to each other in the wild type retina. **D**, By P10 DA neurites are heavily fasciculated in the *Dscam*^{-/-} retina and mosaic spacing of soma is lost.