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Dscam-Mediated Cell Recognition Regulates Neural Circuit Formation

Daisuke Hattori, **S. Sean Millard**, **Woj M. Wojtowicz**, and **S. Lawrence Zipursky***
Department of Biological Chemistry, Howard Hughes Medical Institute, David Geffen School of Medicine, University of California, Los Angeles, California 90095; email: lzipursky@mednet.ucla.edu

Abstract

The Dscam family of immunoglobulin cell surface proteins mediates recognition events between neurons that play an essential role in the establishment of neural circuits. The *Drosophila Dscam1* locus encodes tens of thousands of cell surface proteins via alternative splicing. These isoforms exhibit exquisite isoform-specific binding in vitro that mediates homophilic repulsion in vivo. These properties provide the molecular basis for self-avoidance, an essential developmental mechanism that allows axonal and dendritic processes to uniformly cover their synaptic fields. In a mechanistically similar fashion, homophilic repulsion mediated by *Drosophila* Dscam2 prevents processes from the same class of cells from occupying overlapping synaptic fields through a process called tiling. Genetic studies in the mouse visual system support the view that vertebrate DSCAM also promotes both self-avoidance and tiling. By contrast, DSCAM and DSCAM-L promote layer-specific targeting in the chick visual system, presumably through promoting homophilic adhesion. The fly and mouse studies underscore the importance of homophilic repulsion in regulating neural circuit assembly, whereas the chick studies suggest that DSCA Mproteins may mediate a variety of different recognition events during wiring in a context-dependent fashion.

Keywords

self-avoidance; tiling; immunoglobulin domain; homophilic repulsion; binding specificity

INTRODUCTION

The patterns of synaptic connections between neurons in an animal's brain are key determinants of behavior. How these patterns are established during development remains a central question in neurobiology. More than one hundred years ago, Ramón y Cajal, the father of neuroanatomy, proposed a chemical basis for wiring specificity based on his extensive studies on the structure and organization of the developing nervous system and mature nervous system in a wide variety of species. He argued that a dynamic structure at the leading edge of an extending axon, which he called the growth cone (Ramón y Cajal 1890), detects signals in the developing brain produced by targets that promote directed motility. This chemotactic model fell into disfavor during the 1930s and 1940s with the rise of behaviorally based explanations for circuit

*Authors are listed alphabetically.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

assembly; during this period, circuits were viewed as initially rather coarse, with their refinement being determined by an animal's interaction with the environment.

In the 1960s, Roger Sperry resurrected the notion that neural circuit formation relies on interactions between specific chemical signals expressed by different neurons (Sperry 1963). Working with lower-vertebrate preparations, Sperry damaged nerves, scrambled nerve fibers, and then assessed the restoration of specific behaviors and connection patterns following nerve regeneration. Sperry demonstrated that damaged neurons in the visual system restored patterns of normal connectivity. Importantly, regenerating axons frequently extended over uninnervated, inappropriate targets to form connections in the appropriate location. To explain this specificity, Sperry proposed that each neuron "must carry some kind of individual identification tags, presumably cytochemical in nature, by which they are distinguished one from another almost at the level, in many regions, of the single neuron." He proposed that these tags would provide the basis for synaptic specificity, with "each axon linking only with certain neurons to which it becomes selectively attached by specific chemical affinities." He argued that not only were "synaptic terminals ⋯ selectively determined" but also the "route by which growing fibers reach those terminals, is selectively determined, presumably on the basis of similar or identical chemoaffinity factors." These chemoaffinity factors are what we now call cell recognition molecules. Sperry envisioned that wiring specificity emerges through the combined action of long-range (i.e., secreted) signals, as Ramón y Cajal had proposed, and shortrange "identification tags" (i.e., cell surface molecules) that mediate contact-dependent signals.

Since Sperry proposed his chemoaffinity model some 45 years ago, investigators have identified various cell surface recognition molecules that contribute to the patterning of neural circuits (Tessier-Lavigne & Goodman 1996). These recognition molecules allow neurons to exchange information, thus providing cells with patterning instructions. Recognition molecules include proteins that bind to the same protein (homophilic binding) or to different proteins (heterophilic binding) on opposing membranes (Figure 1a). The interactions between these recognition proteins, in turn, influence the motility of developing axons and dendrites by activating intracellular signaling pathways.In some cases, these interactions promote adhesion between cells, whereas in others they activate repulsion (Figure 1b,c). Both adhesion and repulsion play crucial roles in specifying neural circuitry.

Adhesive and repulsive cellular responses to recognition molecules mediate every aspect of neuronal patterning from the initial neurite outgrowth events through all the guidance and targeting steps that ultimately culminate in the formation of precise synaptic connections. Although we frequently broadly categorize interactions between proteins expressed on two opposing cell surfaces as either adhesive or repulsive, and indeed this categorization can be of considerable value, adhesive and repulsive cellular signaling events can elicit a variety of complex responses during the assembly of neural circuits (Figure 1b). For example, adhesive interactions between recognition molecules expressed on opposing neurites can promote growth of an axon along the surface of another axon, a process commonly called fasciculation. Binding between recognition molecules may also transiently inhibit growth as cells interact with intermediate targets or guidepost cells, and finally, adhesive interactions can lead to the formation of specific synapses. Repulsive interactions can promote defasciculation, thereby directing neurites into divergent paths; can prevent neurites from crossing one another; and can generate boundaries between distinct populations of neurons. We wish to remind the reader that, although considerable progress has been made in identifying cell recognition molecules in the developing nervous system, in no case do we know how neurons choose to make synaptic connections with one target neurite over another.

Perhaps a useful starting point is to ask how many cell recognition molecules there are and what developmental strategies have evolved to achieve specificity in the face of the extensive cellular complexity in the developing nervous system. Sperry's notion that a vast number of specific chemical labels play a role in wiring was criticized at the time as being inconsistent with information theory. It was difficult to envision enough chemical labels being available in the developing brain to regulate the formation of neural circuits on this basis. Indeed, in the human brain there are 10^{12} neurons and some 10^{15} connections, yet, as we now know, our genomes harbor only some 3×10^4 genes. One solution envisioned by Sperry was to use the graded expression of cell surface molecules in target cells and matched graded expression of their receptors on growth cones. The discovery by Bonhoeffer and colleagues (Drescher et al. 1995) and Cheng & Flanagan (1994) that topographic maps in the vertebrate visual system are, indeed, assembled through the use of graded expression of Eph receptors and their ligands, the ephrins, validated this idea (Flanagan & Vanderhaeghen 1998). The vertebrate immune system provides an example of a different mechanism for generating large numbers of recognition proteins on the surface of cells from a limited number of genes. Here gene families, gene rearrangement, and assembly of multisubunit recognition proteins conspire to generate large families of related proteins with different recognition specificities (i.e., antibodies and T cell receptors). Do similar strategies for generating diverse cell recognition proteins exist in the nervous system? The discovery that alternative splicing at the *Dscam1* (*Down syndrome cell adhesion molecule*) locus in *Drosophila* generates literally tens of thousands of molecularly distinct axon guidance receptors of the immunoglobulin (Ig) superfamily with distinct recognition specificities raised the intriguing possibility that large diverse families of chemical labels do exist in the developing nervous system and that they contribute to the assembly of precisely interconnected neural circuits (Schmucker et al. 2000, Wojtowicz et al. 2004).

Although Sperry envisioned that chemoaffinity tags mediate interactions between the neuronal processes of neighboring cells that encounter one another as they migrate through the developing nervous system, the Sperry-like tags encoded by fly *Dscam1* function in an entirely unexpected way. As we discuss here, Dscam1 provides each cell with a unique cell surface identity (Neves et al. 2004). This allows both axonal and dendritic branches to distinguish between branches emanating from the same cell, so-called sister branches, and branches from different cells (Hughes et al. 2007, Matthews et al. 2007, Soba et al. 2007, Zhan et al. 2004, Zhu et al. 2006). Self-recognition leads to a repulsive response between processes of the same cell, a phenomenon known as self-avoidance (Figure 1b) (Kramer et al. 1985). Self-avoidance allows sister branches to spread out over a large target area, thus maximizing the receptive field of each neuron while simultaneously allowing multiple neurons to have overlapping fields. This process is necessary for patterning dendritic and axonal branches in both the peripheral nervous system and the central nervous system. A second *Dscam* gene in flies, *Dscam2*, promotes a related repulsive function called tiling (Millard et al. 2007). Here, processes from different cells of the same class use Dscam2 to repel each other and to promote growth away from one another. Tiling ensures that the receptive fields of neurons from the same class do not overlap with one another, thus restricting connections to specific circuits. Recent studies in mouse demonstrate that DSCAM is selectively expressed in subclasses of cells and suggest that it uses homophilic repulsion to simultaneously promote both self-avoidance and tiling (Fuerst et al. 2008). Thus, Dscam proteins sculpt local circuitry by preventing inappropriate interactions between processes. These studies underscore the importance of homophilic repulsion in establishing neural circuitry.

Dscam1 ISOFORMS EXHIBIT EXQUISITE ISOFORM-SPECIFIC HOMOPHILIC BINDING PROPERTIES

Dscam was identified as an open reading frame from a region of human chromosome 21 implicated in Down syndrome, which arises from triploidy of all or part of this chromosome.

There are four *Dscam* paralogs in *Drosophila* (*Dscam1-4*). We now refer to the founding member of the *Drosophila Dscam* family as *Dscam1* instead of *Dscam*. Diversity in *Dscam* generated through alternative splicing is unique to *Dscam1*, and although highly conserved within arthropods, vertebrate *DSCAM*s do not exhibit this feature. Dscam1 isoforms share a common domain structure with 10 Ig domains and 6 fibronectin type III domains, a single transmembrane segment, and a C-terminal cytoplasmic domain (Figure 2a) (Schmucker et al. 2000, Yamakawa et al. 1998). Three of the Ig domains are encoded by blocks of alternative exons, and as such they contain variable amino acid sequences. There are 12 alternative exons that encode the first half of Ig2, 48 alternative exons that encode the first half of Ig3, and 33 alternative exons that encode Ig7. Splicing at each of the exon blocks is independent of splicing at the other blocks; therefore, alternative splicing of the *Dscam1* gene can potentially give rise to 19,008 different ectodomains (i.e., 12 Ig2s × 48 Ig3s × 33 Ig7s). Each ectodomain is tethered to the membrane by one of two different alternative transmembrane domains to give rise to 38,016 different Dscam1 isoforms (i.e., 19,008 ectodomains × 2 transmembrane domains).

Each Dscam1 ectodomain has a distinct and exquisite binding specificity (Figure 2b). A series of in vitro binding experiments (Wojtowicz et al. 2004, 2007) and cell aggregation assays (Matthews et al. 2007) established that Dscam1 isoforms engage in homophilic binding. By contrast, no heterophilic binding was observed between isoforms that differ at all three variable Ig domains. To assess which of the three variable domains contributes to this binding specificity of Dscam1 isoforms, three pairs of isoforms were tested for binding: (*a*) a pair containing different Ig2 variants but the same Ig3 and Ig7 variants, (*b*) a pair containing different Ig3 variants but the same Ig2 and Ig7, and (*c*) a pair containing different Ig7 variants but the same Ig2 and Ig3 (Wojtowicz et al. 2004). Although each isoform bound homophilically to itself, no binding was observed between any of the three pairs of isoforms differing at only a single variable Ig domain (Wojtowicz et al. 2004). These studies demonstrated that all three variable domains contribute to Dscam1 binding specificity. On the basis of these studies, we proposed that each variable domain binds to an identical domain in an opposing molecule— i.e., Ig2 binds to Ig2, Ig3 binds to Ig3, and Ig7 binds to Ig7 (Figure 2b). In this way, isoform-specific homophilic binding arises from matching of the three self-binding variable domains between molecules expressed on opposing cell surfaces.

If Dscam1 homophilic binding is indeed mediated by matching of three, self-binding domains, then whether or not all 19,008 isoforms exhibit isoform-specific homophilic binding depends upon whether each of the 12 Ig2 variants,48 Ig3 variants, and 33 Ig7 variants exhibits specific self-binding. To analyze the binding properties of these variable domains, a high-throughput ELISA-based binding assay was developed (Wojtowicz et al. 2007). The variants of each variable domain within an otherwise common ectodomain were tested for binding in a grid (i.e., 12 × 12 for Ig2, 47 × 47 for Ig3, and 33 × 33 for Ig7). The grids demonstrated that each variable domain binds to itself (with the exception of Ig7.33) but weakly, if at all, to other variants. As each variable domain preferentially binds to itself, each isoform comprising three self-binding variable domains preferentially binds to itself.

Matching of all three variable domain pairs is required for isoform binding, and thus isoforms identical at two domain pairs but differing at the third domain pair do not bind to one another. Rare exceptions to this rule occur when the differing domain pair exhibits high amino acid sequence identity. Although heterophilic binding is observed in these rare cases, heterophilic binding is always weaker than the homophilic binding of each isoform to itself. On the basis of the self-binding properties of each Ig2, Ig3, and Ig7 variant, the *Dscam1* gene is proposed to give rise to 18,048 proteins that engage in isoform-specific homophilic binding (i.e., 12 Ig2s × 47 Ig3s × 32 Ig7s = 18,048) (Wojtowicz et al. 2007).

THE STRUCTURAL BASIS FOR Dscam1 HOMOPHILIC RECOGNITION

Crystal structures of the Dscam1 ectodomain have confirmed the modular model for Dscam1 isoform–specific homophilic binding (Figure 2d). Meijers et al. (2007) described the structure of the N-terminal four Ig domains ($Dscam1_{1–4}$) for two isoforms, and Sawaya et al. (2008) described the structure of the N-terminal eight Ig domains ($Dscam1_{1–8}$) for one isoform. The $Dscam1_{1–8}$ structure was solved, in part, by molecular replacement through the use of the $Dscam1_{1–4}$ structure. In both $Dscam1_{1–4}$ structures and the $Dscam1_{1–8}$ structure, the molecules associate as dimers with direct contacts between opposing variable domains. Indeed, greater than 80% of the surface area buried by the $Dscam1_{1–8}$ dimer is derived from variable residues. The eight-domain structure comprises a region sufficient for homophilic binding (Wojtowicz et al. 2004), and extensive agreement between the structure and biochemical experiments (Meijers et al. 2007, Wojtowicz et al. 2007) strongly suggests that the eight-domain structure represents the structure formed upon homophilic binding in vivo.

Modular Ig2-Ig2, Ig3-Ig3, and Ig7-Ig7 interactions are observed in the $Dscam_{1–8}$ homophilic dimer (Sawaya et al. 2008). Each of the three variable domains binds to its identical counterpart in an antiparallel fashion, giving rise to twofold symmetric interfaces. Self-binding of Ig2 occurs via an eight-residue peptide along the first strand in the Ig fold (Meijers et al. 2007, Sawaya et al. 2008). Similarly, self-binding of Ig3 occurs via a transition segment along the first strand (Meijers et al. 2007, Sawaya et al. 2008). In contrast to the Ig2-Ig2 and Ig3-Ig3 interfaces, which occur along a single strand located at the edge of the Ig fold, the Ig7 self-binding interface comprises an entire face of the Ig fold involving multiple strands (Wojtowicz et al. 2007, Sawaya et al. 2008). Extensive biochemical studies demonstrated that these self-binding interface regions observed in the crystal structures can be generalized to most, if not all, Ig2, Ig3, and Ig7 variants (Wojtowicz et al. 2007). Interface residues were swapped between Ig2 domains and shown to be sufficient to confer the binding specificity of Ig2 variants. Similar experiments were conducted for Ig3 and Ig7. The crystal structures and additional modeling studies of the Ig2, Ig3, and Ig7 interface regions demonstrate that each self-binding variable domain pair fits snugly together (Figure 2c). Self-binding of each variable domain is mediated by exquisite electrostatic and shape complementarity. This complementarity does not exist between different alternatives of each variable domain, thus preventing binding between them. These studies demonstrate that variants of each domain have evolved a unique self-complementary interface that is incompatible with the interfaces of other variants (Sawaya et al. 2008).

In the crystal structure, $Dscam1_{1–8}$ monomers fold into an S shape, which positions the three variable domains on one side of the molecule poised for self-binding interactions with their counterparts in the opposing molecule (Figure 2d) (Sawaya et al. 2008). These interactions give rise to a double-S homophilic dimer formed by two symmetrically paired S-shaped monomers. The overall S shape is composed of two halves. Ig1–Ig4 fold into a compact horseshoe structure in the top half of the S curve, similar to the Ig1–Ig4 structures of other Ig superfamily cell surface adhesion molecules, including hemolin (Su et al. 1998) and axonin-1/TAG-1 (transiently expressed axonal surface glycoprotein-1) (Freigang et al. 2000). The horseshoe fold is accommodated by a long linker region between Ig2 and Ig3, which allows a sharp bend in the backbone, and extensive intramolecular interactions between Ig1:Ig4 and Ig2:Ig3. The bottom turn of the S is formed by a long linker region between Ig5 and Ig6, which similarly adopts a sharp bend and allows Ig5:Ig6 interactions.

Previous biochemical studies demonstrated that isoforms sharing identity at two variable domains and differing by only a single interface residue at the third variable domain do not bind to one another. The $Dscam1_{1–8}$ structure illuminates how a small number of residue differences can have such a dramatic effect on the overall binding properties of isoforms. For

Ig7, residue differences change the interface slightly, but like perfectly crafted puzzle pieces, a small change in one piece prevents it from fitting with its partner. Although Ig2 and Ig3 engage in modular self-binding with their counterparts, the horseshoe structure constrains Ig2 and Ig3 within the monomer such that the Ig2-Ig2 and Ig3-Ig3 contacts form one composite interface. Therefore, if one-half of the composite Ig2-Ig3 interface does not fit with its counterpart, then the other half cannot fit—i.e., small differences between opposing Ig2 domains lead to the loss of both the Ig2-Ig2 and the Ig3-Ig3 contacts. The homophilic dimer buries 4500 $Å^2$ of surface area, 1300 $Å^2$ of which is contributed by theIg7-Ig7 interface and 1659 $Å^2$ of which is contributed by the combined Ig2-Ig2 and Ig3-Ig3 interfaces. As such, small differences between variants, which result in loss of an entire variable domain interface, lead to loss of binding. These findings illuminate the molecular basis for the extraordinary all-or-none homophilic binding properties of Dscam1 isoforms.

Comparison of electron micrographs of single $Dscam1_{1–8}$ molecules (Meijers et al. 2007) and $Dscam1_{1–8}$ monomers within the dimer in the crystal structure revealed a marked difference in shape. Negative-stained images of single $Dscam1_{1–8}$ molecules revealed that the four N-terminal domains form a rigid horseshoe structure consistent with the crystal structure. By contrast, the rest of the molecule (i.e., Ig5–Ig8) adopts a large range of different shapes, suggesting that in the absence of homophilic binding this region is relatively unstructured. This comparison suggests that the C-terminal half of the structure undergoes a marked conformational change upon formation of the homophilic dimer.

On the basis of these structures, we have proposed the following model for Dscam1 binding in vivo (Figure 2d). In the absence of homophilic binding, Ig1–Ig4 exhibit a stable horseshoe structure at the end of a flexible trunk comprising the remaining Ig and fibronectin type III domains. Self-binding of the three variable domain pairs between isoforms expressed on opposing cells induces a conformational change, leading to formation of the sharp bend between Ig5 and Ig6 and formation of the symmetric double-S structure. This double-S shape is formed only when all three pairs of variable domains match. The sharp bend between Ig5 and Ig6 allows intramolecular interactions to form between these constant domains, which stabilize the double-S homophilic dimer structure. This large confor-mational change in the ectodomain may provide a mechanism for transducing the signal of homophilic binding to the cytoplasmic domain, thereby triggering intracellular signaling, which leads to changes in the motility of axonal and dendritic processes.

Together these structural and biochemical studies demonstrate that the *Dscam1* gene gives rise to tens of thousands of isoform-specific homophilic binding proteins through the combinatorial association of different sets of three modular, self-binding variable domains. Finely tuned complementarity at each of the variable domains provides extraordinary specificity, which ensures all-or-none binding of Dscam1 isoforms.

ISOFORM-SPECIFIC HOMOPHILIC RECOGNITION PROVIDES THE MOLECULAR BASIS FOR SELF-AVOIDANCE

How is this extraordinary binding specificity of Dscam1 isoforms used in vivo? Dscam1 is broadly expressed, and many isoforms have been found in the developing nervous system (Schmucker et al. 2000). The elimination of ectodomain diversity leads to defects in wiring, demonstrating that Dscam1 diversity and the vast recognition specificities that it provides contribute to establishing neural circuits (Hattori et al. 2007). Extensive loss-of-function analyses revealed a common function for Dscam1. In many cases, loss of Dscam1 results in defects in the segregation of branches that extend from the same neuron, or sister branches (Hughes et al. 2007, Hummel et al. 2003, Matthews et al. 2007, Soba et al. 2007, Wang et al. 2002, Zhu et al. 2006). This led to the view that Dscam1 proteins promote self-recognition

between sister branches through their isoform-specific homophilic binding properties (Zhan et al. 2004). This recognition and selective binding are followed by disengagement and repulsion of sister branches. Thus, the extraordinary binding specificity of Dscam1 provides the molecular recognition underlying self-avoidance. Below, we review findings that established the central role of Dscam1 in self-avoidance.

Early studies by Lee and colleagues demonstrated that Dscam1 plays a crucial role in the segregation of axon branches in mushroom body (MB)neurons (Wang et al. 2002). The MB is a central brain structure involved in learning and memory. During development, MB neurons project axons through the brain in a large nerve bundle called the peduncle (Figure 3a) (Kurusu et al. 2002).At the base of the peduncle, each axon bifurcates (i.e., splits in two), giving rise to two sister branches. Each sister branch segregates to a different pathway, either dorsally or medially, where again it fasciculates with and extends along other MB axon branches. The MARCM technique was used to generate single mutant cells in an otherwise wild-type background to show that Dscam1 has a discrete function in MB axons. Although *Dscam1* mutantMBaxons bifurcate, the two sister branches often fail to segregate to different pathways and frequently grow in parallel along the same pathway (Figure 3b) (Wang et al. 2002). Thus, Dscam1 is required, not for branch formation itself, but rather for the segregation of the sister branches to different pathways.

How do Dscam1 diversity and isoform-specific homophilic binding contribute to MB sister branch segregation? Gain-of-function studies suggested that Dscam1 isoform–specific homophilic binding mediates repulsion (see below). This raised the possibility that Dscam1-mediated homophilic repulsion promotes sister branch segregation in MB neurons (Zhan et al. 2004). For Dscam1 to serve this function, each MB neuron must express isoforms that enable them to distinguish between sister branches and the branches of neighboring MB neurons. Assessment of alternative exon expression in MB neurons by the use of customized microarrays comprising all alternative exons provided evidence that most alternative exons are expressed in MB neurons, and that each MB neuron expresses multiple Dscam1 isoforms (Zhan et al. 2004). Sequencing analysis of Dscam1 cDNA revealed that most isoforms expressed in MB neurons are distinct from each other (89 distinct isoforms were identified from 93 cDNA sequenced). Quantitative RT-PCR and an independent statistical analysis showed that each MB neuron expresses ~10–30 Dscam1 mRNAs. Collectively, these data indicate that different MB neurons express different sets of Dscam1 isoforms, thereby providing each neuron with a unique Dscam1 identity. Thus, at the branch point where many different MB axons bifurcate, sister branches selectively recognize each other by isoform-specific homophilic binding (i.e., self-recognition) and, in turn, disengage and extend away from each other along different pathways (i.e., self-avoidance). Branches of different neurons do not recognize each other because they express few, if any, common isoforms. Homophilic repulsion therefore occurs only between sister branches. Thus, the unique Dscam1 identity in each MB neuron allows each branch to selectively recognize its sister branch and distinguish it from all other branches and thus to segregate accordingly (Figure 3a).

Two additional observations confirmed that Dscam1 is a self-avoidance receptor. Hattori et al. (2007) generated *Dscam1*^{single} knock-in mutants, in which only one isoform is encoded by the *Dscam1* locus and therefore all neurons share the same Dscam1 identity. As expected, these mutants exhibited defects in MB formation, consistent with loss of sister branch segregation. Even animals carrying a wild-type *Dscam1* allele in *trans* to a *Dscam1*^{single} allele displayed similar defects, exemplifying the necessity for each MB neuron to express different isoforms from its neighbors. In sharp contrast, when a single MB neuron was engineered to express Dscam1^{single} in an otherwise wild-type background, it retained normal sister branch segregation (Hattori et al. 2007) (Figure 3b). This observation confirmed the findings of previous studies in which exogenously supplied single isoforms were sufficient to restore sister

branch segregation at the single-cell level (Wang et al. 2004, Zhan et al. 2004). These findings are consistent with the notion that the identity of the isoforms expressed in a given neuron is not important as long as the isoforms expressed are different from those expressed by neighboring neurons. These data firmly established the model that Dscam1 diversity and its recognition specificity provide the molecular basis for self-avoidance in MB axons.

Studies by Zhu et al. (2006) suggested that Dscam1 also mediates self-avoidance in dendrites in the CNS. Using the MARCM technique, these researchers showed that dendrites of single projection neurons and interneurons in the olfactory system form clumps and fail to uniformly innervate their targets in the absence of Dscam1. Owing to the complexity of the neuropil, it was not possible to determine whether Dscam1 promotes self-avoidance between these dendrites or whether it is required for other aspects of dendritogenesis, such as outgrowth.

A compelling argument for Dscam1's function in regulating dendrite self-avoidance has been made in dendritic arborization (da) neurons in the peripheral nervous system (Hughes et al. 2007, Matthews et al. 2007, Soba et al. 2007). There are four classes of da neurons, classes I–IV, which elaborate dendritic fields in a two-dimensional pattern within the body wall of the larva. Whereas the dendrites of one class of da neurons overlap with the processes of other da classes, the dendrites of the same neuron, or sister dendrites, do not overlap with each other (Figure 3c). Indeed, the dendrites of all four classes of da neurons exhibit self-avoidance properties. Given Dscam1's function in MB neurons, it seemed plausible that Dscam1 might provide the recognition specificity that enables sister dendrites to selectively recognize and segregate from each other while allowing them to overlap with the dendrites of other da neurons.

Genetic studies established that Dscam1 is essential for self-avoidance in all four classes of da neurons. In the absence of Dscam1, sister dendrites adhered to each other and extended across the body wall in fascicles (Figure 3c). The number and length of the dendrites were the same as for wild type; only the dendrites' spatial relationship was altered. Self-avoidance was restored when single arbitrarily chosen isoforms were supplied in single *Dscam1* mutant neurons, showing that, as in MB neurons, the identity of isoforms expressed in these neurons is not important. To assess whether expression of the same isoform on opposing dendrites is sufficient to mediate self-recognition, neurons with overlapping dendritic fields in wild type were engineered to express the same isoform. Expression of the same isoform caused the dendrites of these neurons to repel each other, leading to nonoverlapping dendritic fields (Figure 3c). This finding provided evidence that repulsion ensues when opposing dendrites express the same Dscam1 isoform. In summary, these data suggest that sister dendrites of da neurons exhibit self-avoidance as they express the same Dscam1 isoforms, whereas dendrites of different da neurons overlap as they express different Dscam1 isoforms.

These studies led to the view that self-avoidance is achieved by a molecular mechanism in which Dscam1 isoforms on opposing dendrites bind to each other and then this binding, in turn, activates a repulsive response. The repulsive response is likely mediated by signaling events through the cytoplasmic domain. To test this hypothesis, Matthews et al. (2007) utilized a chimeric Dscam1 isoform, in which the cytoplasmic domain was substituted with GFP, presumably resulting in the loss of cytoplasmic signaling events required for a repulsive response. Using the same genetic strategy described above, Matthews et al. (2007) engineered da neurons with overlapping dendritic fields in wild type to express this chimeric Dscam1 isoform. Whereas dendrites engineered to express a single wild-type Dscam1 isoform repel each other and no longer overlap, dendrites expressing this chimeric Dscam1-GFP not only overlap but extensively bind to each other, in a sense trapping an intermediate in the repulsive pathway. This argues that, during the self-avoidance process, binding of Dscam1 isoforms on opposing sister dendrites activates a repellent response mediated by the cytoplasmic domain.

The repulsive response involves disengagement of the homophilic receptor complex at the cell surface and activation of cytoskeletal reorganization events. In summary, these studies demonstrate that Dscam1 acts as an isoform-specific homophilic repulsive molecule and that this function of Dscam1 is essential for mediating self-avoidance.

Self-avoidance critically depends upon the acquisition of a unique Dscam1 identity in each neuron. How is Dscam1 isoform expression controlled to ensure this? In principle, a unique Dscam1 identity can arise if each neuron expresses isoforms in a deterministic fashion, reliably making one neuron different from another. This requires strictly regulated expression of isoforms. Microarray expression studies revealed that different types of neurons (e.g., photoreceptor neurons and MB neurons) express different spectra of alternative exons (Neves et al. 2004, Zhan et al. 2004). Consistent with this notion, Graveley and colleagues identified specific splicing factors regulating the expression of particular alternative exons (Park et al. 2004). At least one of the splicing factors is known to be expressed in a tissue-specific manner, suggesting that these splicing factors may contribute to regulating the expression of specific isoforms in vivo. Alternatively, unique Dscam1 identities may be obtained through stochastic expression of multiple isoforms from the large number of isoforms. In theory, if a given neuron expresses 20 isoforms at random from 19,008 possible isoforms, more than 10^{67} unique Dscam1 identities, which far exceeds the total number of neurons in a fly, can be generated. The stochastic nature of isoform expression may arise from the intrinsic variability in the splicing machinery from one neuron to another. Additionally, some sequence elements within alternative exon clusters of the *Dscam1* gene may contribute to stochastic expression. Sequence analysis of the Ig3 alternative exon cluster by Graveley identified a docking site in the intron preceding the cluster that is partially complementary to selector sequences immediately upstream of every alternative exon (Graveley 2005). The random pairing of this docking site and a selector sequence in pre-mRNA may contribute to generating the randomness of the alternative exon utilization. In summary, although it is clear in the case of MB neurons that each neuron expresses a unique set of Dscam1 isoforms, it remains unclear how the isoform expression is controlled to ensure self-avoidance. Analysis of the isoform expression in the same cell identifiable from one animal to another is necessary to address whether Dscam1 splicing is regulated in a deterministic or a stochastic fashion.

How much Dscam1 diversity is required for self-avoidance? We envision that different neural tissues require different degrees of diversity depending upon the complexity of the tissue. For example, neurons in a less complex tissue (e.g., da neurons in the body wall) that encounter processes of only a small number of nonself neurons would likely require fewer isoforms than those in an environment with the processes of many different nonself neurons (e.g., MBs). Studies on deletion mutants in which the ectodomain diversity was reduced from 19,008 to 4752 did not identify defects in self-avoidance in either the MB (Wang et al. 2004) or the da systems (Matthews et al. 2007). Thus, although *Dscam1*^{single} studies clearly showed that Dscam1 diversity is essential for self-avoidance in many tissues, how much Dscam1 diversity is required to provide a robust system for self-avoidance in each system is not known.

In summary, studies in the central and the peripheral nervous systems provide strong evidence that Dscam1 diversity endows neurites with the ability to distinguish between self and nonself neurites. Dscam1 isoform– specific homophilic repulsion between sister branches provides a molecular basis for self-avoidance. Kramer, Stent, and colleagues first described the phenomenon of self-avoidance some 20 years ago on the basis of studies in the leech peripheral nervous system (Kramer et al. 1985). They proposed that self-avoidance would play a more general role in patterning circuits in both vertebrate and invertebrate brains. However, its general importance in regulating the patterning of neural circuits had remained unclear, largely because the molecular basis for self-avoidance remained to be identified and additional experimental evidence for the existence of self-avoidance was lacking. Studies on fly

Dscam1 underscore the widespread requirement for self-avoidance in establishing neural circuits. We propose that self-avoidance is an essential step in wiring, providing an initial driving force for the formation of intricate branch patterns in both the peripheral and the central nervous systems.

Dscam2 IS A TILING RECEPTOR IN THE FLY VISUAL SYSTEM

The prominent role played by Dscam1 in self-avoidance raised the possibility that homophilic repulsion might be a more general property of Dscam proteins. This was supported by the finding that Dscam2 mediates tiling between processes of a subset of neurons in the fly visual system (Millard et al. 2007) (Figure 4a).

In contrast to *Dscam1*, the three fly *Dscam1* paralogs, *Dscam2*, *Dscam3*, and *Dscam4*, do not undergo extensive alternative splicing. *Dscam2* encodes two alternative Ig7 domains, but *Dscam3* and *Dscam4* encode only a single ectodomain (Millard et al. 2007). Like Dscam1 isoforms, the two different Dscam2 proteins exhibit isoform-specific homophilic binding. In addition, Dscam3 and Dscam4 bind homophilically but not heterophilically to other family members. As a first step toward understanding how the binding properties of Dscam paralogs function in the developing brain, Millard et al. (2007) generated mutants in *Dscam2*. These mutants showed marked defects in the organization of the visual system.

The fly visual system contains the compound eye, comprising an array of photoreceptor neurons that detect light, and four neuropil regions that process visual information, including the lamina and medulla (Meinertzhagen & Hanson 1993). The medulla is organized into ~750 reiterated columns. Columnar organization preserves the spatial quality of information received at the retina. This information can then be transferred to cells that connect multiple columns and interpret the combined data as a specific visual stimulus, such as motion. Each column contains axons from R7 and R8 photoreceptor neurons, 5 different types of lamina neurons called L1–L5, and ~50 other neuronal cell types (Fischbach & Dittrichi 1989).

Using MARCM techniques, Millard et al. (2007) assessed the role of Dscam2 in R7, R8, L1, and L2 neurons. In wild type, the synaptic connections made by these cells are restricted to a single column and form at specific layers. Dscam2 was not required in R7, R8, or L2 cells for either layer specificity or columnar restriction. By contrast, although the terminal arbors and interstitial branches of L1 formed in the appropriate layers, they aberrantly extended into neighboring columns (Figure 4a). Thus, Dscam2 is required for restricting L1 axons to a single column. Because Dscam2 mediates interactions between cells, loss of Dscam2 homophilic binding should generate phenotypes in both mutant cells and the nonmutant cells with which they interact. We exploited this bio chemical property to determine how Dscam2 restricts L1 axons to columns.

Columnar restriction may reflect either adhesion between L1 axons and another cell within the same column or, alternatively, repulsion between the processes of L1 neurons in adjacent columns. To distinguish between these possibilities, we focused on the directionality of the L1 phenotype. *Dscam2* mutant L1 axons extend bidirectionally into adjacent columns the majority of the time. If another lamina neuron within the same column anchors L1 through Dscam2-mediated adhesive interactions, then the loss of Dscam2 in this cell should lead to a bidirectional phenotype in the wild-type L1 cell. Alternatively, if L1 cells in adjacent columns restrict L1 axons through homophilic repulsive interactions, then the loss of Dscam2 in one of the adjacent columns should lead to unidirectional growth toward that mutant column. The technique of reverse MARCM (Lee et al. 2000) provided a way to distinguish between these two possibilities. Here wild-type neurons were labeled, and the consequence of having neighboring mutant neurons was assessed. Consistent with the repulsion model, wild-type neurons with mutant cells in adjacent columns extended uni-directionally (Figure 4a). These

studies established that Dscam2 mediates cell-type-specific avoidance between L1 axons in adjacent columns.

In summary, Millard et al. (2007) argue that Dscam2 promotes repulsion between processes of adjacent cells, thus preventing inappropriate connections from being formed in neighboring columns. Because each column contains similar L1 target cells, cell-type-specific avoidance provides a mechanism for restricting connections to a single circuit. The restriction of axonal or dendritic processes from the same cell types to nonoverlapping spatial domains is referred to as tiling (Perry & Linden 1982, Wassle et al. 1981). Tiling is mechanistically similar to self-avoidance. Interestingly, self-avoidance maximizes the number of connections that an individual neuron can make, whereas tiling limits the extent of neuronal connections. Both of these mechanisms ensure precise wiring of the brain. Thus, Dscam1 and Dscam2 proteins regulate two very different aspects of neural connectivity through a common, homophilic repulsive mechanism.

MOUSE DSCAM PROMOTES HOMOPHILIC REPULSION

A recent study by Burgess and coworkers supports the notion that mammalian DSCAMs also mediate homophilic repulsion (Fuerst et al. 2008). There are two *DSCAM* homologs in vertebrates (Agarwala et al. 2001, Yamakawa et al. 1998). These proteins mediate homophilic binding, but neither gene is alternatively spliced. Fuerst et al. (2008) identified a spontaneous mutation disrupting the mouse *DSCAM* locus that resulted in an overt neurological phenotype. These animals exhibit anatomical defects in the cerebellum and retina. In the vertebrate retina, visual input is processed by interneurons, including amacrine cells. Different classes of amacrine cells (~30 classes) extend dendrites into specific layers of the inner plexiform layer (Masland 2004), where they make connections with retinal ganglion cells. Amacrine cells of the same class appear to be tiled across the layer, generating a sheet of evenly spaced cells. In addition, sister dendrites from the same amacrine cell are patterned by self-avoidance. The *DSCAM* mutant has profound defects in dendritic organization in distinct subsets of amacrine cells (Fuerst et al. 2008) (Figure 4b).

DSCAM expression is restricted to two subclasses of amacrine cells: tyrosine hydroxylase (TH)-expressing, dopaminergic amacrine cells and nitric oxide synthase (bNOS)-expressing amacrine cells. TH and bNOS amacrine cells arborize in different layers. In *DSCAM* mutant animals, a quantitative measurement of spacing between cells of the same type demonstrated that tiling is lost in the TH and bNOS cells at stage P13. Importantly, two other cell types that do not express DSCAM did not exhibit defects in *DSCAM* mutants. In addition to the tiling defect, examination of mutant TH and bNOS cells revealed that sister neurites are fasciculated, indicative of a deficit in self-avoidance. Thus, both self-avoidance and tiling are disrupted, and the combination of these two defects results in a hyperfasciculation phenotype in the mutant animals. The simplest interpretation of these findings is that DSCAM promotes repulsive interactions between sister neurites and between processes of the same cell types, an amalgam of the *Drosophila* Dscam1 and Dscam2 functions.

These data argue that the role of Dscam in mediating homophilic repulsion is evolutionarily conserved. In insects, Dscam1 is broadly expressed. Diversity has evolved to ensure that each neuron expresses a unique Dscam1 identity, thereby allowing sister neurites to selectively recognize and repel each other. This Dscam1 identity enables the protein to function throughout the nervous system because repulsion only occurs between processes of the same cell. By contrast, mouse DSCAM is expressed in subsets of neurons. As these subsets arborize in nonoverlapping layers, DSCAM functions in both self- and cell-type-specific avoidance. Thus, self-avoidance does not require extraordinary diversity if the repulsive receptors are restricted to cell subtypes. This argues that many cell recognition molecules that mediate homophilic

repulsion in the vertebrate nervous system remain to be identified. It is also possible, however, that diversity in other families of cell recognition molecules contributes to self-avoidance. One notable example is the protocadherin family (Kohmura et al. 1998, Wu & Maniatis 1999). Stochastic expression of these genes occurs in Purkinje cells, thereby providing each neuron with a unique identity (Esumi et al. 2005). Whether these proteins promote homophilic recognition and repulsion is not yet known.

CHICK DSCAM PROMOTES LAYER-SPECIFIC TARGETING

Many axon guidance molecules can both attract and repel neurons, depending upon the nature of the signaling pathways operating within the growth cone.Even the heterophilic interactions between Eph and ephrin, which have been classically defined as repulsive molecules (Flanagan & Vanderhaeghen 1998), signal attractive or adhesive functions when these molecules are expressed at lower levels (Hansen et al. 2004). Thus, it is not surprising that recent results suggest that DSCAM may also function in an adhesive fashion.

Yamagata & Sanes (2008) used the chick retina as a model system for analyzing cell recognition molecules that play a role in layer-specific targeting. As in the mouse, interneurons (i.e., amacrine and bipolar cells) form synaptic connections with retinal ganglion cells in specific layers. DSCAM and DSCAM-L appear to play a role in this layer-specific targeting (Figure 4c). DSCAM and DSCAM-L are expressed in different subsets of interneurons and ganglion cells. Interestingly, interneurons and ganglion cells that target to the same layer express the same DSCAM protein. Knocking down DSCAM levels through retrovirally mediated expression of interfering RNAs disrupted layer-specific targeting of DSCAM-expressing neurons. Conversely, misexpression of DSCAM in retinal cells led to respecification of neurites to the layers expressing the same DSCAM protein. The simplest model for DSCAM function in regulating layer-specific targeting is one in which DSCAM promotes adhesion between the dendrites of the retinal ganglion cells and the processes of interneurons.

On the basis of these and previous studies demonstrating that Sidekick1 and Sidekick2 proteins play a similar role in layer-specific targeting (Yamagata et al. 2002) (Figure 4c), Yamagata & Sanes (2008) proposed that layer recognition is controlled, in part,by an Ig family homophilic recognition code. The finding that vertebrate DSCAMs may act instructively by mediating adhesive interactions between processes of different cells raises the question of whether the extraordinary isoform diversity of fly Dscam1 could be used in a similar manner in some developmental contexts.

DOES Dscam1 MEDIATE SELECTIVE INTERACTIONS BETWEEN PROCESSES OF DIFFERENT CELLS?

Many lines of evidence support the view that self-avoidance, mediated by isoform-specific homophilic repulsion, is a major function of Dscam1 diversity. However, given the extraordinary complexity of the fly brain and the handful of neuronal cell types analyzed so far, it remains plausible that in some developmental contexts isoform-specific homophilic binding may mediate recognition events other than self-avoidance, such as adhesive interactions between different neurons. In this scenario, specific isoforms should be required in specific neurons to form appropriate connections. In this context, a study by Schmucker and coworkers (Chen et al. 2006) is particularly provocative. They generated two deletion mutants in which 5 of the 12 Ig2 alternative exons were removed. Because different subsets of alternative exons were deleted in each mutant, these mutants encode different isoforms, although they have the potential to generate the same degree of diversity (i.e., ~11,000 ectodomain isoforms). Intriguingly, each of these two mutants showed a different spectrum of abnormalities in the complex branching patterns of a mechanosensory neuron in the thorax.

These defects included an increase in the frequency of branches that occurred at low frequency in wild type and the appearance of ectopic branches not seen in wild type. Thus, different isoforms deleted in each mutant may play distinct roles in regulating the pattern of branching in this neuron.

These findings raise the possibility that, in addition to mediating self-avoidance, Dscam1 diversity may also play an instructive role in which specific isoforms mediate interactions between neurons. It remains unclear, however, which neurons require these deleted isoforms because the analysis was performed in mutant animals in which isoforms were deleted in all neurons rather than in genetically mosaic animals. This also raises the possibility that neurons that normally selectively express the deleted isoforms lose Dscam1 expression altogether and thus are rendered null mutant. Indeed, this phenomenon occurred for the alternatively spliced cell surface protein N-cadherin (Nern et al. 2005). If this is the case in these *Dscam1* deletion mutants, then the deletion-specific defects observed in the mechanosensory neuron may represent an indirect consequence of defects in target neuropil organization resulting from a loss of self-avoidance in specific subsets of target neurons. Thus, additional studies are necessary to demonstrate whether neurons utilize specific isoforms to mediate interactions with different cells in establishing neural circuits.

PERSPECTIVE

A Combinatorial Mechanism Generates Dscam1 Binding Specificities

By the end of the 1990s it was generally believed that, although cell recognition is an integral part of neural circuit assembly, it was highly unlikely that the vast number of individual identification tags envisioned by Sperry existed. It was thus a considerable surprise that, in the course of assessing the role of Dscam1 in axon guidance, we serendipitously discovered that alternative splicing generates an array of some 19,000 different ectodomains each comprising three variable domains embedded in a common scaffold. This molecular theme is reminiscent of antibodies and T cell receptors and raised the intriguing notion that these different isoforms would exhibit different recognition specificities that, in turn, would regulate the assembly of neural circuits. Genetic analysis demonstrated that Dscam1 diversity plays a crucial and widespread role in neural circuit formation, and biochemical studies established that different Dscam1 isoforms exhibit exquisite isoform-specific recognition. Together these studies make a compelling case that Dscam1 proteins function as Sperry-like identity tags.

Although vertebrate DSCAMs share a common function with fly Dscam1 in promoting contact-dependent repulsion and self-avoidance, vertebrate genomes do not encode multiple isoforms of DSCAM. In fact, blocks of tandemly arranged alternative exons as are present in fly *Dscam1* have not been observed in any vertebrate gene characterized so far. Therefore, if similar recognition diversity exists in vertebrates, then other types of combinatorial mechanisms must generate large families of recognition proteins. Similar levels of diversity may be generated by combining modules contained within polypeptide chains encoded by different genes, rather than within a single polypeptide as in fly Dscam1. Indeed, the combinatorial association of different polypeptides contributes to the diverse recognition specificities in vertebrate antibodies and T cell receptors. Here, heavy- and light-chain regions come together in three-dimensional space to generate a unique binding site. Because there are families of related polypeptides in small gene clusters expressed in the developing vertebrate nervous system [e.g., protocadherins (Kohmura et al. 1998, Wu & Maniatis 1999)], it will be interesting to see whether they associate in a combinatorial fashion to give rise to a large number of neuronal recognition molecules.

Repulsive Interactions Between Other Homophilic Molecules May Function in Neural Circuit Assembly

The pioneering studies of Edelman and Takeichi and their colleagues on N-CAM and cadherins led to the view that homophilic adhesive interactions regulate the interactions between neuronal processes in the developing nervous system (Rutishauser 1984, Takeichi 1988). These contact-dependent mechanisms for intercellular communication may drive different types of adhesive interactions, including growth along the surface of axon fascicles in some contexts and, in others, the stable association of cell surfaces to form synaptic contacts. These classic studies led to the implicit assumption that homophilic adhesion in vitro will lead inexorably to adhesive interactions in vivo.

That Dscam-mediated homophilic repulsion plays a key role in patterning neural circuits raises the question of whether other proteins characterized as homophilic adhesion molecules on the basis of in vitro assays promote patterning in vivo through repulsive signaling mechanisms. Many cell culture systems used to characterize adhesion molecules, such as the S2 cell system in flies, may not express the signaling or cytoskeletal proteins that promote repulsion. For example, whereas Dscam1 isoforms mediate repulsion in vivo, in cultured S2 cells they promote aggregation. Thus, other classically defined homophilic adhesion proteins that are characterized by their ability to mediate aggregation in cultured cells in vitro may act in vivo to promote repulsion. Indeed, two other homophilic proteins, the protocadherin Flamingo in *Drosophila* (Lee et al. 2003, Usui et al. 1999) and the Lar phosphatase in the leech (Baker & Macagno 2000), which promote aggregation in vitro, have been proposed to mediate homophilic repulsion in vivo. These studies underscore the importance of both gain-and loss-of-function studies in different developmental contexts to explore the mechanisms by which homophilic binding between proteins on opposing cellular processes mediates neural circuit assembly.

Tiling and Self-Avoidance Are Key Regulators of Neural Circuit Assembly

When, in the early 1980s, the phenomenon of self-avoidance was discovered (see above) (Kramer & Stent 1985, Kramer et al. 1985), it was speculated that self-avoidance is a general property of neurons in the central and peripheral nervous systems in both vertebrate and invertebrate species. Studies of Dscam1 function in flies revealed that, indeed, many neuronal cell types in both the central and the peripheral nervous systems require self-avoidance to elaborate their branches. In addition, mouse studies support the view that vertebrate DSCAM also promotes self-avoidance. On the basis of these findings, we propose that self-avoidance is a universal mechanism regulating neural circuit formation and that the Dscam family of proteins plays a crucial role in this process.

The notion that fly Dscam1 promotes self-avoidance raised the obvious possibility that regulated splicing could provide neurons with a code to promote tiling, a process that, analogous to self-avoidance, promotes repulsion between processes of different cells rather than between processes of the same cell. The finding that fly Dscam2 and mouse DSCAM serve this function in subsets of neurons in the fly and vertebrate visual systems, respectively, reinforced the notion that the *Dscam* family evolved to promote repulsive interactions between processes of the same cell and processes of the same neuronal subclass.

Self-avoidance and tiling sculpt circuits in complementary ways. Self-avoidance promotes the extension of processes away from one another and allows a cell to maximize its connections. By contrast, tiling provides a means of restricting connections to specific circuits. In the fly visual system, for instance, visual information is initially confined to individual columns for signal processing prior to integrating signals from adjacent columnar units. Restriction of L1 synapses to single columns by Dscam2 prevents the elaboration of synapses in inappropriate

columns; if not prevented, such elaboration would lead to abnormalities in processing visual information. The study of Dscam proteins not only has provided a molecular basis for self-avoidance and tiling but has helped to establish them as fundamental developmental processes contributing to the establishment of precise neural circuitry in both vertebrate and invertebrate species.

SUMMARY POINTS

1. Alternative splicing at the *Dscam1* locus has the potential to generate 38,016 different isoforms. This includes 19,008 ectodomains tethered to the membrane by one of two alternative transmembrane domains.
2. Biochemical experiments reveal that 18,048 different ectodomains exhibit isoform-specific homophilic binding. Identical isoforms bind to themselves. Homophilic binding requires matching of three variable Ig domains. A small fraction of heterophilic pairs exhibit binding, but this is always weaker than their corresponding homophilic binding.
3. Dscam1 diversity is essential for the patterning of neural circuits, as demonstrated in mutant animals expressing only a single ectodomain from the endogenous locus.
4. Molecular experiments support a model in which each neuron expresses a unique combination of Dscam1 isoforms, thereby endowing each neuron with a unique cell surface identity.
5. Homophilic binding between identical Dscam1 isoforms on opposing membranes signals repulsion and results in branch segregation. This homophilic repulsion promotes a phenomenon called self-avoidance.
6. A Dscam1 paralog in fly, Dscam2, promotes tiling. Homophilic binding between Dscam2 molecules on two opposing membranes from different cells promotes repulsion, thereby preventing overlap of their processes.
7. Mouse DSCAM is expressed in discrete sets of amacrine cells that arborize in different layers. Loss-of-function studies reveal that DSCAM promotes both self-avoidance and tiling within these layers.
8. Chick DSCAM and DSCAM-L are expressed in different populations of neurons that form synapses in discrete layers in the inner plexiform layers in the retina. Both gain- and loss-of-function studies support a model in which DSCAM and DSCAM-L contribute to layer-specific targeting. It is likely that in this developmental context DSCAM and DSCAM-L promote adhesion rather than repulsion.

FUTURE ISSUES

1. Although a number of studies argue that Dscam1 diversity plays a key role in promoting self-avoidance, it remains unclear whether Dscam1 also promotes recognition between processes of different cells. It will be important to assess whether Dscam1 diversity, in some developmental contexts, specifies interactions between processes of different cells.
2. The characterization of Dscam1 isoform expression in individual cells is challenging and so far has been assessed with customized microarrays on single cells isolated by fluorescent sorting. It will be important in future experiments to develop more robust methods for isoform expression analysis. Such methods may include splicing reporter constructs. This would facilitate the characterization of

splicing patterns in single cells at multiple stages of development. RNA isolation from single identified cells and methods to characterize the isoforms expressed in them should address the question of whether specific neurons express the same sets of isoforms in different individuals.

3. Dscam1 and Dscam2 promote contact-dependent repulsion. Genetic and biochemical approaches should provide effective ways of dissecting the mechanisms by which homophilic binding promotes this process through interactions with the cytoplasmic signaling domain.

4. Dscam1 diversity is not present in its mammalian homologs. If a similar degree of diversity in cell surface recognition molecules exists in vertebrates, these molecules remain to be identified.

Glossary

Homophilic adhesion or repulsion, interactions between the same protein on two opposing membranes. This can result in adhesion or repulsion

Heterophilic binding, binding between two different proteins on opposing membranes

Fasciculation, the assembly of axons into bundles

Ephrins and Ephs, cell surface recognition molecules that bind to each other in a heterophilic fashion and that can promote repulsion and attraction

Dscam, *Down syndrome cell adhesion molecule*

Sister branches, axonal or dendritic neurites extending from the same cell

Self-avoidance, mediates recognition between sister branches and promotes repulsion

Receptive field, the region of tissue innervated by a neuron

Dscam2, *Drosophila* paralog of *Dscam1*. It promotes tiling in a subset of visual system neurons in *Drosophila*

Tiling, phenomenon in which axonal or dendritic processes of different cells of the same class repel each other

DSCAM, there are two DSCAM genes in vertebrates: *DSCAM* and *DSCAM-L*

Immunoglobin (Ig) domain, a β-sheet sandwich structure found in many cell surface proteins both within and outside the immune system

Ectodomain, the region of a transmembrane protein that is exposed on the extracellular face of the plasma membrane

ELISA (Enzyme-Linked ImmunoSorbent Assay), measures binding between proteins or other molecules

Mushroom body (MB), a structure in the fly brain comprising thousands of neurons

MARCM (mosaic analysis with a repressible cell marker), facilitates the generation and visualization of single mutant cells in an otherwise wild-type background

Dendritic arborization (da) neurons, sensory neurons whose dendrites form highly branched structures in a two-dimensional array in the fly body wall

Medulla, a region of the *Drosophila* visual system that is organized into reiterated columns and multiple layers

Lamina 1 (L1) neurons, receive synaptic input from photoreceptor neurons and make connections in the medulla

Reverse MARCM, facilitates the generation and visualization of single wild-type cells near unlabeled mutant cells

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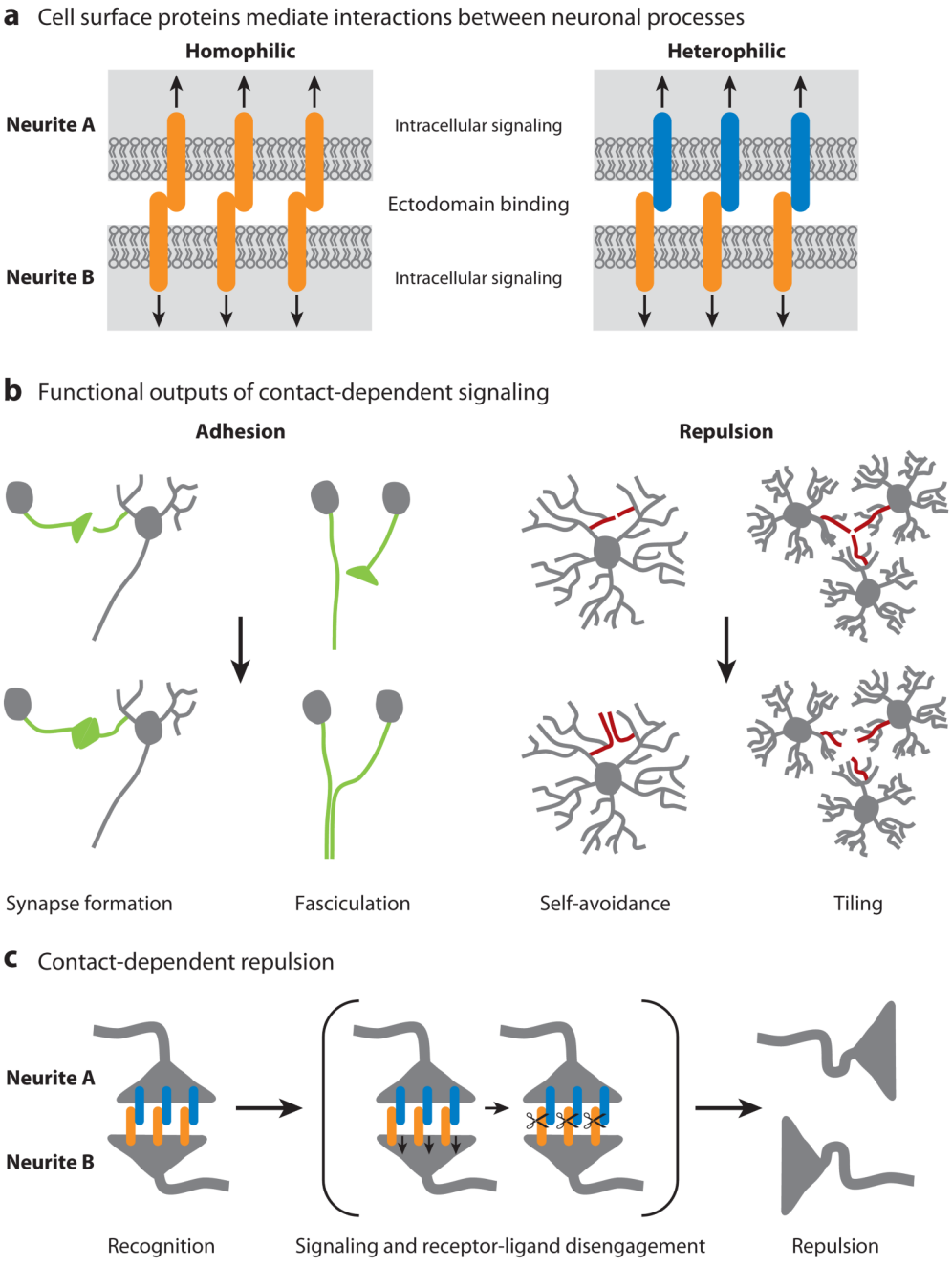

Figure 1.

(*a*) Cell surface proteins mediate interactions between neurons. Interactions between neuronal processes are mediated by direct contact between cell surface molecules. Two interacting neurites (i.e., neurite A and neurite B) are shown. Binding between the extracellular regions of cell surface proteins is translated to changes in the cytoplasmic domain and elicits intracellular signaling events (*black arrows*), which lead to directed changes in neurite motility through cytoskeletal rearrangements. Binding between identical proteins is referred to as homophilic, whereas binding between different proteins is referred to as heterophilic.
(*b*) Cell surface protein interactions have different signaling outputs. Contact-dependent interactions between cell surface proteins elicit signal transduction cascades within the

cytoplasm that lead to two different outputs: adhesion (also called attraction) and repulsion. (*Left*) The neurites of two different neurons expressing adhesive molecules (*green*) encounter one another. Adhesion can lead to various responses, two of which, synapse formation and fasciculation, are depicted. (*Right*) Neurites of the same neuron (*left*) or two different neurons (*right*) expressing repulsive molecules (*red*) encounter one another. Repulsion causes the neurites to grow away from one another and mediates patterning events such as self-avoidance and tiling.

(*c*) Discrete steps underlying contact-dependent repulsion. First, recognition cell surface molecules expressed on opposing neurites bind to each other. Next, intracellular signaling promotes downregulation of receptor binding and activation of cytoskeletal rearrangements that promote repulsion. Two mechanisms have been described to mediate dissociation: proteolytic cleavage of the interacting molecules (shown) and endocytosis (not shown).

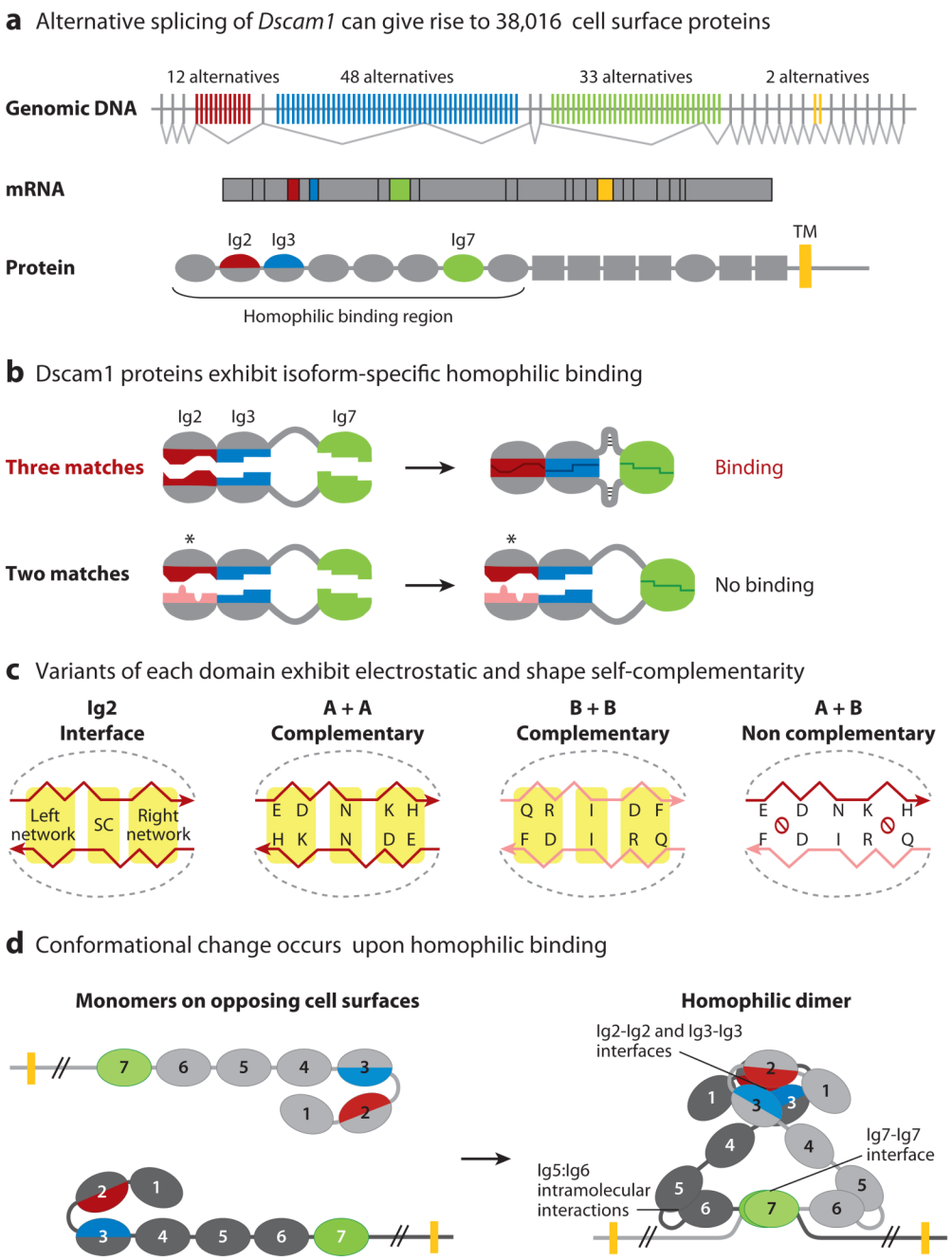

Figure 2.

(*a*) *Drosophila Dscam1* encodes a vast repertoire of cell surface recognition proteins. The *Drosophila Dscam1* gene encodes a large family of single-pass transmembrane proteins of the immunoglobulin (Ig) superfamily. *Dscam1* contains four blocks of alternative exons that encode 12 different variants for the N-terminal half of Ig2 (*red*), 48 different variants for the N-terminal half of Ig3 (*blue*), 33 different variants for Ig7 (*green*), and two different variants for the transmembrane domain (TM) (*yellow*). Splicing leads to the incorporation of one alternative exon from each block, and as such, *Dscam1* encodes 19,008 (i.e., 12 × 48 × 33) different ectodomains linked to one of two different transmembrane domains. (*b*) Dscam1 proteins exhibit isoform-specific homophilic binding. Each isoform binds to itself but rarely,

if at all, to other isoforms. The three variable Ig domains mediate homophilic binding specificity. Variants of each domain engage in self-binding but do not bind to other variants (with rare exceptions). Therefore, homophilic binding occurs between identical isoforms that match at all three variable Ig domains. Isoform pairs that contain only two matches and differ at the third variable domain do not bind to one another. The quaternary structure of the first four Ig domains constrains the Ig2 and Ig3 domains, literally tethering them to one another. As such, if opposing Ig2 domains do not match, Ig3 self-binding is sterically inhibited, even if the Ig3 domains match, and vice versa. Additional intramolecular interactions between constant domains in the linker region between Ig3 and Ig7 are formed when the three variable domains match; such interactions play a crucial role in stabilizing the homophilic dimer (see panel *d*). An asterisk indicates that Ig2 difference is shown. (*c*) Electrostatic and shape complementarity underlies self-binding of each variant. Complementarity is illustrated by the Ig2 interface as an example. The Ig2 self-binding interface occurs between identical segments in opposing Ig2 domains. These segments are oriented in an antiparallel fashion. The interface comprises a symmetry center (SC) residue and flanking left and right networks. The interface segments of two different Ig2 variants (i.e., A and B) are shown in red and pink, respectively. Each self-binding interface exhibits electrostatic and shape complementarity at the SC and the left and right networks (complementarity is illustrated by the *yellow boxes*). The heterophilic interface formed between these two Ig2 variants does not exhibit complementarity at the SC, the left network (note the three negatively charged residues), or the right network (note the three positively charged residues), and thus these different Ig2 variants do not bind to one another. (*d*) A conformational change occurs upon homophilic binding. (*Right*) The $Dscam1_{1–8}$ crystal structure reveals a dimer of two S-shaped monomers with direct contacts between opposing Ig2, Ig3, and Ig7 variable domains. Electron micrographs of $Dscam1_{1–8}$ demonstrated that, whereas the first four Ig domains form a compact horseshoe structure, the remainder of the domains are highly flexible. (*Left*) These differences in structure suggest that the bottom half of the S shape observed in the crystal structure forms upon homophilic binding as opposing Ig2, Ig3, and Ig7 domains interact. Stabilizing intramolecular contacts are formed between regions within constant domains Ig5 and Ig6. This large conformational change that occurs within the Dscam ectodomain upon homophilic binding may provide a molecular mechanism for transducing the signal of homophilic binding to the cytoplasmic domain, where subsequent signaling events occur.

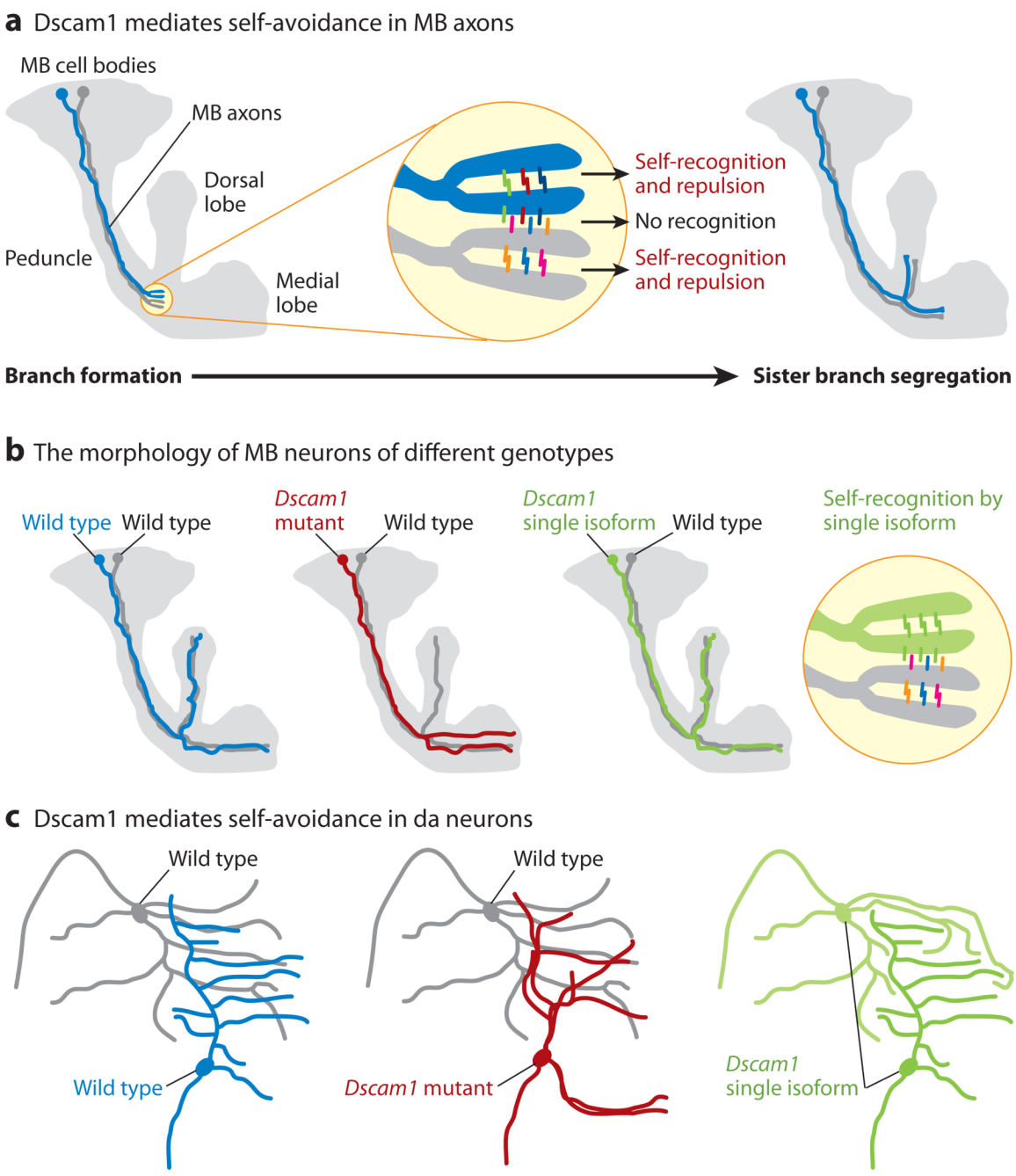

Figure 3.

Dscam1 regulates self-avoidance. (*a*) Dscam1 mediates self-avoidance in mushroom body (MB) neurons. The MB is a central brain structure comprising thousands of neurons. Each MB extends a single axon within a nerve bundle called the peduncle. At the base of the peduncle MB axons bifurcate and extend one branch medially and the other dorsally. Each MB neuron expresses a unique combination of isoforms. As a consequence, sister branches recognize each other through Dscam1 matching. This signals repulsion and subsequent segregation of axons to separate pathways. (*b*) Axon branching patterns of MB neurons of different genotypes. (*Left*) Wild-type MB axon branches segregate with high fidelity. (*Middle*) The branches of a single *Dscam* mutant neuron in a wild-type background frequently do not segregate

appropriately. (*Right*) Expression of a single arbitrarily chosen isoform promotes branch segregation in a single *Dscam1* null mutant cell. These and other experiments demonstrated that, although it is unimportant which isoform a single MB neuron expresses for appropriate branch segregation, it is crucial that each MB neuron express isoforms different from its neighbors. (*c*) Dscam1 mediates self-avoidance in dendritic arborization (da) neurons. Different classes of da neurons elaborate overlapping dendritic fields in the body wall of *Drosophila* larva. (*Left*) Two wild-type neurons are shown. (*Middle*) The dendrites of a *Dscam1* null mutant cell form fascicles, supporting the notion that Dscam1 binding promotes repulsion. (*Right*) Overexpression of the same Dscam1 isoform in both neurons leads to nonoverlapping receptive fields, consistent with homophilic binding inducing repulsion.

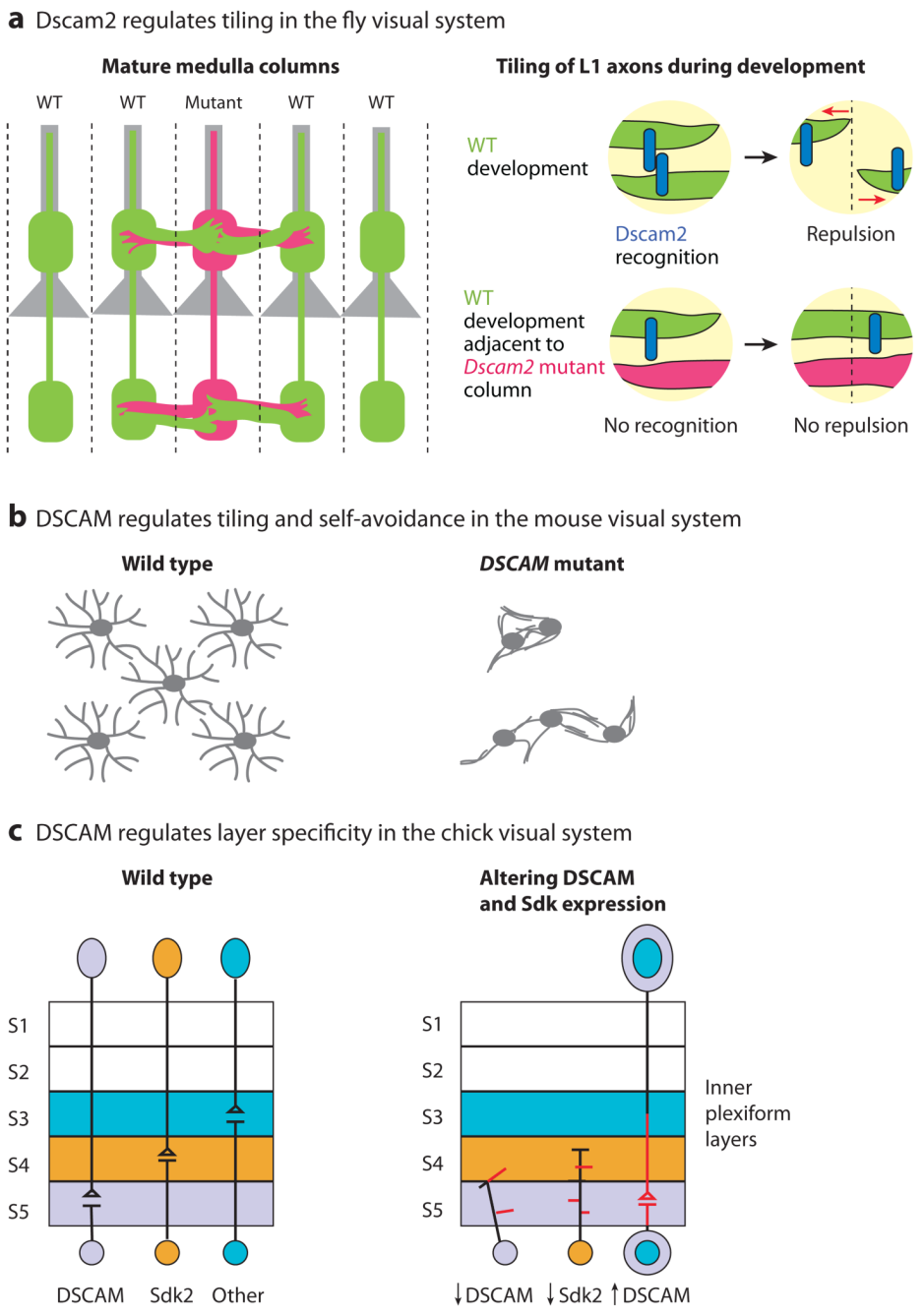

Figure 4.

Dscam proteins participate in several aspects of neuronal wiring. (*a*) *Drosophila* Dscam2 is a tiling receptor. (*Left panel*) Schematic of Dscam2 phenotypes in lamina 1 (L1) axons in the fly medulla region of the visual system. L1 axons (*pink* and *green*) elaborate processes in two distinct layers. Interactions between these processes mediated by Dscam2 restrict the formation of connections to a single column, as indicated in the far left and right columns. Mutant (*pink*) L1 axons are not restricted to a single column. Similarly, the processes of wild-type (WT) (*green*) axons extend axons into columns with a mutant L1. Columns are delineated with dashed lines. (*Right panel*) Schematic of L1 column development. Dscam2 homophilic binding (*blue bars*) occurs between wild-type L1 neurites during pupal development. This induces a

repulsive signal that results in the retraction of neurites back to their column of origin and the formation of columnar boundaries. Mutant neurites (*pink*) cannot interact with wild-type L1 neurites (*green*) because the former lack Dscam2. Without Dscam2 homophilic binding, neither mutant nor wild-type L1 neurites are restricted to their column of origin, and both can form connections in neighboring columns. (*b*) Mouse DSCAM mediates both self-avoidance and tiling. (*Left panel*) DSCAM-positive amacrine cells exhibit both self-avoidance and tiling properties. (*Right panel*) In the absence of DSCAM, both self-avoidance and tiling are lost. Branches from individual amacrine cells fasciculate with one another and with other cells of the same type. (*c*) Chick DSCAM contributes to layer-specific targeting. (*Left panel*) Ganglion cells (*bottom*) and amacrine cells (*top*) in the chick retina, which express the same DSCAM protein or a related Ig superfamily protein in the Sidekick (Sdk) family, target to the same layer. (*Right panel*) When either DSCAM or Sdk2 is knocked down in ganglion cells that normally express these proteins, dendritic targeting is less precise. Similarly, when cells that do not normally express DSCAM are engineered to misexpress it, they target to the DSCAM-positive S5 layer.