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Got diversity? Wiring the fly brain with Dscam

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The *Drosophila* gene *Dscam*, encoding Down syndrome cell-adhesion molecule, is required for the development of neural circuits. Alternative splicing of *Dscam* mRNA potentially generates 38 016 isoforms of a cell-surface recognition protein of the immunoglobulin superfamily. These isoforms include 19 008 different ectodomains joined to one of two alternative transmembrane segments. Each ectodomain comprises a unique combination of three variable immunoglobulin domains. Biochemical studies support a model in which each isoform preferentially binds to the same isoform on opposing cell surfaces. This homophilic binding requires matching at all three variable immunoglobulin domains. These findings raise the intriguing possibility that specificity of binding by the Dscam isoforms mediates cell-surface recognition events required for wiring the fly brain.

Cell recognition and the establishment of neural circuits

Neural circuits comprise many different neurons that are interconnected by a network of highly precise and often complex patterns of synaptic connections. These patterns are scripted from developmental programs that give rise to the right type, location and number of neurons, the morphologies of their axons and dendrites, and the synaptic connections that interlink them. The formation of neural circuits relies on interactions among cell-surface recognition proteins. Identifying these proteins, determining their recognition specificities, and understanding the strategies by which they sculpt the developing brain are exciting areas of current study.

Neurons form connections in a stepwise process [1]. Typically, a neuron extends a single axon adorned at its leading edge with a sensorimotor structure called the 'growth cone'. Cell-surface receptors on the growth cone detect signals that, in turn, direct the growth cone along stereotyped pathways in the developing brain to the cells with which they will make synaptic connections. These signals can act at a distance, as diffusible cues, or they can act at short range, as components that are anchored to the extracellular matrix or expressed on the surface of cells along the pathway [1]. Neurons also elaborate dendrites [2], which are often complex and highly branched. During development, neurites (i.e. processes of axons and dendrites) from many different cells intermingle. Interactions between neurites in this 'spaghetti-like' milieu are crucial for regulating wiring patterns by preventing or promoting association between them (Figure 1). These interactions ultimately lead to the formation of precise patterns of synaptic connections.

Cell recognition proteins that mediate interactions among neurites in the developing nervous system have been identified and characterized, and include cadherins [3], ephrins and ephs [4], semaphorins and plexins [5], neurexins and neuroligins [6,7], and immunoglobulin superfamily proteins such as syg1 and syg2 [8] and sidekicks [9]. The interactions mediated by these recognition proteins can be homophilic, whereby the proteins interacting on the two opposing membranes are the same (e.g. sidekicks), or heterophilic, whereby the interacting proteins are different (e.g. syg1 and syg2).

Binding between proteins on opposing cell surfaces can result in different cellular responses. For instance, adhesive interactions between syg1 and syg2 specify the location of synaptic terminals in *Caenorhabditis elegans* [8]. By contrast, binding of ephrin ligands to eph receptors on growth cones often results in repulsion [10]. The notion that binding between proteins on opposing cell surfaces can promote repulsion might seem counterintuitive. In other words, how can two cells bound to each other be repelled from one another? Studies on ephrin–eph interactions have revealed molecular mechanisms by which this repulsion can occur [11,12]. For example, after ephrin A binds to eph A, the ephrin A ligand is cleaved by a metalloprotease and cytoskeletal remodeling pathways in the growth cone are activated, leading to growth cone collapse and retraction [11].

Thus, cell-surface recognition molecules pattern the developing brain by mediating contacts between cells. These contacts elicit signaling events that lead to distinct cellular outputs, which can be broadly characterized as attractive or repulsive (Figure 1). If attractive, they can promote fascicle formation (or bundling) or stimulate synapse formation. If repulsive, by contrast, mechanisms must be engaged to downregulate these contacts and to promote the growth of neurites away from each other or, in principle, to prevent synapse formation.

How do neurites distinguish the staggering number of different cell surfaces that they encounter during development? Given the small number of genes in organisms with highly complex nervous systems, it is clear that each cell recognition event cannot be mediated by the product of a different gene. This realization has led to the view that gradients of cell-surface molecules [10,13,14] and different

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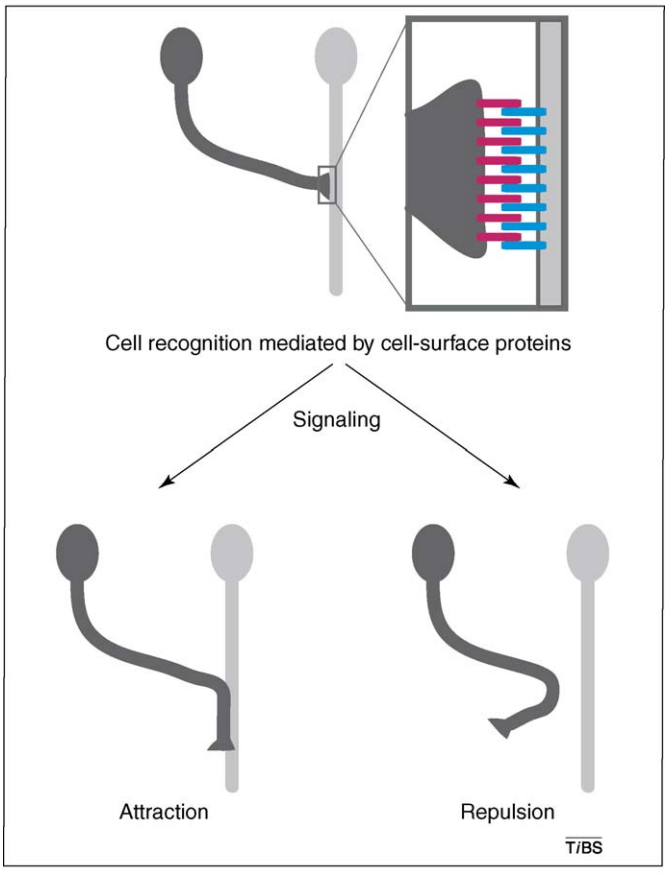

Figure 1. Cell recognition between two neurons mediated by cell-surface proteins. Top, the dark gray neuron extends an axon with a growth cone at its tip, which contacts the axon of the light gray neuron. As shown in the box, this contact is mediated by the binding of cell-surface recognition proteins (red and blue rectangles). This binding can be either homophilic, whereby the same molecules on both cells bind to each other (not shown), or heterophilic, whereby each cell expresses different molecules that bind to each other. Bottom, binding between cell-surface recognition proteins can lead to different outputs. Left, binding can result in attraction: different attractive responses include growth along the axon (fasciculation, as shown here) or synapse formation (not shown). Right, binding can also promote a repulsive response: mechanisms exist that downregulate initial binding between neurites and promote cytoskeletal remodeling, leading to growth cone retraction.

combinations of a limited number of recognition proteins [15] specify patterns of neuronal circuits. Thus, the discovery that the *Drosophila Dscam* gene gives rise to tens of thousands of neuronal cell-surface proteins raised the intriguing possibility that alternative splicing of a single gene can generate numerous different cell-surface proteins with distinct recognition properties [16].

Here we review studies demonstrating that Dscam isoforms show remarkable homophilic binding specificity [17], which suggests that they do indeed possess distinct recognition properties that mediate cell recognition events. We discuss a mechanism by which neurons use this binding specificity for cell recognition during the establishment of neural circuits. Recent studies have suggested that Dscam also contributes to insect immunity [18,19]. Here, however, we focus exclusively on the role of Dscam in nervous system development. These are still early days in analyzing the relationship between the molecular complexity of Dscam and neuronal wiring and, as such, some speculation is required in considering how Dscam functions. Studies so far, however, suggest that Dscam-mediated cell recognition has a crucial, widespread and intriguing role in wiring the fly brain.

Like likes like: Dscam isoform-specific homophilic binding

The *Drosophila* Dscam gene encodes a large family of single-pass transmembrane proteins that contain ten immunoglobulin (Ig) domains and six fibronectin type III repeats [16]. The gene comprises four blocks of tandemly arranged alternative exons, and each transcript contains one alternative exon from each block (Figure 2a). Because splicing in each block seems to be independent of the others (Box 1), alternative splicing has the potential to generate 38 016 isoforms [16], including 19 008 different ectodomains linked to one of the two alternative transmembrane segments. All isoforms share the same domain structure, but differ in amino acid sequence in the transmembrane segments and three extracellular immunoglobulin domains: the N-terminal halves of Ig2 and Ig3, and all of Ig7.

Because many immunoglobulin superfamily proteins engage in homophilic binding [20], we tested whether Dscam isoforms share this property [17]. We used three different assays to explore the binding properties of the ectodomains of different Dscam isoforms. The 29 isoforms tested so far show robust homophilic binding ([17]; and D.H., W.M.W. and S.L.Z., unpublished) (Figure 2b). Binding is localized to the N-terminal seven immunoglobulin domains – a region comprising the three variable immunoglobulin sequences ([17]; and W.M.W., S. Millard and S.L.Z., unpublished). Remarkably, whereas we found robust binding between identical isoforms, we did not detect binding between isoforms that differ in any one of the three variable immunoglobulin domains (Figure 2b), although weak binding was observed for one heterophilic pair in one assay system (see later). These experiments raised the intriguing possibility that not only do all three variable immunoglobulin domains contribute to binding specificity but binding is restricted to isoforms that are identical in all three regions. To assess this possibility, isoforms sharing the same Ig2 and Ig3 sequences, but differing within Ig7 by only seven, nine, 11 or 12 amino acids, were tested for binding. Although all of these isoforms showed strong homophilic binding, no binding was detected between isoforms differing by nine, 11 or 12 amino acids. In one assay, weaker binding was observed between isoforms differing by seven amino acids [17]. The simplest structural model that can account for the preferential binding of proteins sharing identity in all three immunoglobulin domains is one in which Ig2 in one molecule binds to Ig2 in the opposing molecule and likewise for Ig3 and Ig7 (Figure 2c).

Because the binding properties of only 29 of the 19 008 potential ectodomains have been tested and, of these, only ten were selected at random, it is premature to argue that all Dscam isoforms show isoform-specific homophilic binding. We have recently developed efficient assays for assessing the binding properties of large numbers of isoforms, which will enable us to address critically whether isoform-specific homophilic binding is a general property of Dscam isoforms. For the remainder of this review, however, we

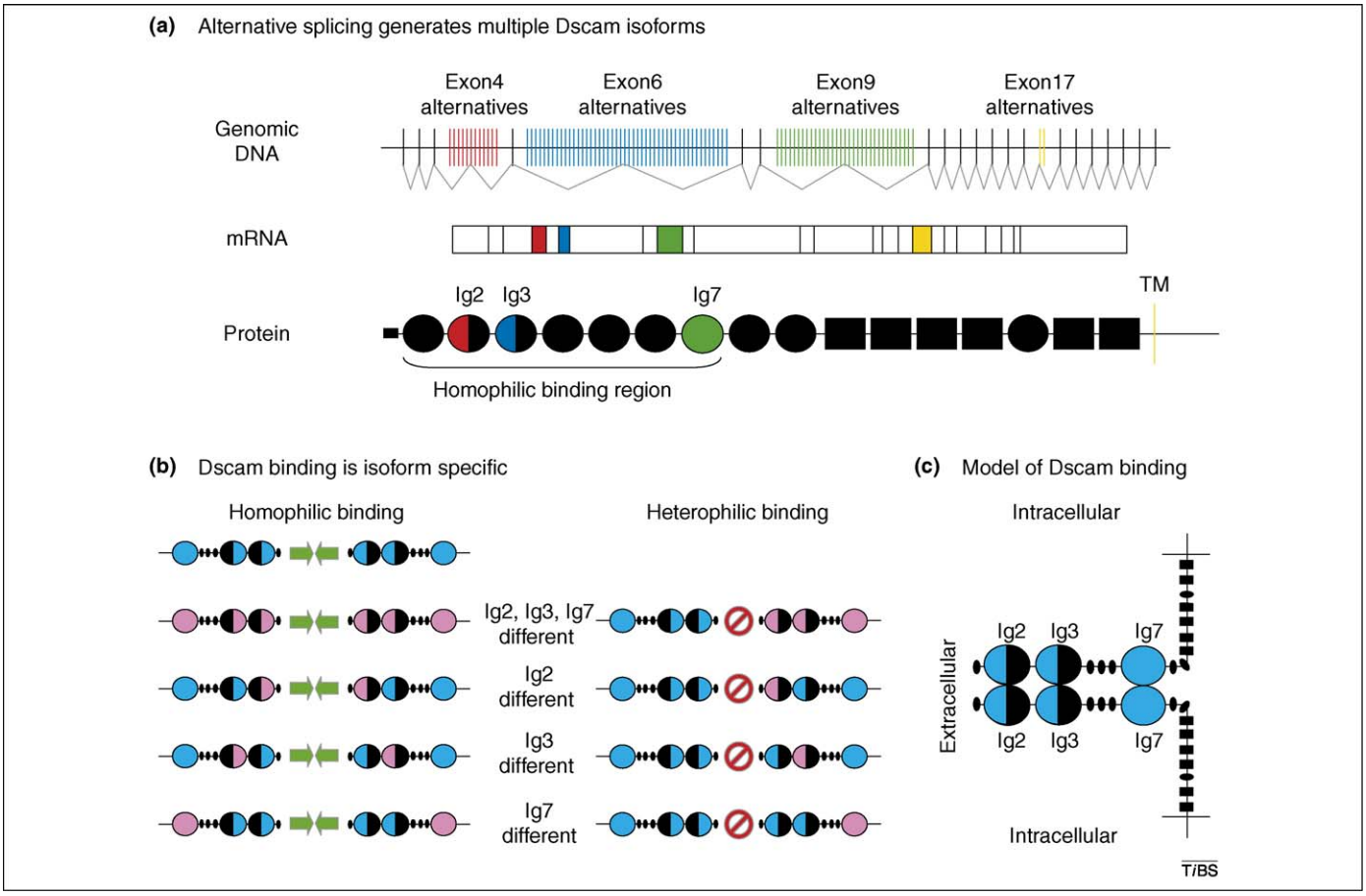

Figure 2. Dscam encodes a large family of homophilic binding proteins. **(a)** Alternative splicing of the *Dscam* gene generates numerous cell-surface proteins of the immunoglobulin superfamily. There are four blocks of alternatively used exons in the *Dscam* gene: the exon-4 block contains 12 alternatives; the exon-6 block contains 48 alternatives; the exon-9 block contains 33 alternatives; and the exon-17 block contains two alternatives. Each transcript contains one alternative exon from each block. The proteins generated by alternative splicing share the same domain structure: immunoglobulin (Ig) domains (spheres); fibronectin type III repeats (rectangles). Isoforms differ in amino acid sequence in the N-terminal halves of Ig2 (red) and Ig3 (blue), in all of Ig7 (green) and in the transmembrane domain (yellow). Exon 4 encodes the variable region of Ig2, exon 6 encodes the variable region of Ig3, exon 9 encodes the whole of Ig7, and exon 17 encodes the transmembrane domain. Because alternative splicing in each block seems to be independent, the *Dscam* gene has the potential to generate 38 016 cell-surface proteins [16]. **(b)** Dscam proteins show isoform-specific homophilic binding. Shown is a summary of the results obtained in Dscam-binding studies [17]. Only the seven N-terminal immunoglobulin domains of Dscam, a region sufficient for homophilic binding, are shown. The domains that contain variable amino acid sequences as a result of alternative splicing are disproportionately sized to emphasize the sequence differences in these regions. Two different isoforms are shown in pink and turquoise. Each contains a unique combination of variable immunoglobulin domains. The Ig2, Ig3 and Ig7 domains are different in the two isoforms. The hybrid isoforms, containing both pink and turquoise domains, were generated by swapping pink domains into the turquoise molecule. Green arrows indicate that binding was observed between Dscam isoforms on opposing surfaces, whereas the red 'no' symbol indicates that binding was not detected. All Dscam isoforms showed strong homophilic binding (left). Heterophilic binding was not detected between isoforms differing in all three variable immunoglobulin domains (right, top row). In addition, heterophilic binding was not detected between isoforms differing in any one of the three variable immunoglobulin domains (right, bottom three rows) with one exception; two isoforms differing by seven amino acids in Ig7 showed weak binding in one assay. These data demonstrate that binding between isoforms is dependent on sequences in all three variable immunoglobulin domains and that isoforms bind preferentially to themselves. **(c)** Structural model of isoform-specific homophilic binding of Dscam. Binding data suggest a simple model in which Ig2 of one molecule binds to Ig2 of an opposing molecule, and likewise for Ig3 and Ig7. Although not shown, it is possible that the constant regions of the protein are directly involved in intermolecular protein–protein interactions or intramolecular interactions that properly orient the variable immunoglobulin domains to facilitate Ig2–Ig2, Ig3–Ig3 and Ig7–Ig7 interactions, or both.

assume that all isoforms of Dscam show preferential homophilic binding.

The biochemical studies described above raise the exciting possibility that the vast repertoire of Dscam isoforms mediates cell-surface recognition events underlying the formation of complex neural circuits.

Finding yourself attractive is repulsive: Dscam mediates self-repulsion

Dscam protein is widely expressed in the developing nervous system and is largely localized to axons and dendrites [16,21]. Mutations that remove all Dscam isoforms greatly reduce viability and result in massive defects in brain structure [16,21–23]. These defects are difficult to interpret at the single-cell level because they represent the direct consequence of loss of Dscam in the neuron of interest, in addition to the indirect effects arising from loss of Dscam in neighboring cells. Insights into how Dscam mediates cell recognition during brain wiring have therefore relied on the analysis of single mutant neurons in an otherwise wild-type fly using a genetic mosaic technique called MARCM (see Ref. [24]). These analyses have established that Dscam has a role in axon guidance, patterning of both axonal and dendritic branches, and targeting of axons [21,22,25,26]. Because the role of Dscam diversity on branching has been studied in most detail, it is this facet of the Dscam phenotype that we focus on here.

The role of Dscam in axon branching was first reported for a subset of central brain neurons, called mushroom body (MB) neurons, by Lee and colleagues [22]. During normal development, each MB axon bifurcates, giving rise to two branches that segregate from each other and extend

Box 1. How is Dscam splicing controlled?

Dscam presents a unique and challenging set of questions about RNA splicing. Analysis of hundreds of cDNAs from many different cell and tissue sources isolated at various developmental stages has established that each mRNA includes one, and only one, exon from each block of alternative exons (Figure 2a). Thus, mechanisms must ensure that an exon from each alternative block is spliced into an mRNA and, furthermore, that only one exon from each block is included. Work from Graveley and colleagues [39–42] suggests that the mechanisms that control splicing might be unique for each block. For instance, a specific sequence in the intron separating exon 3 and the first alternative exon 4 (exon 4.1) is essential in specifying the inclusion of one exon 4 in each transcript. Deleting this sequence results in transcripts lacking exon 4. By contrast, Graveley [39] has identified sequences in the block of alternative versions of exon 6 that are conserved in all insect species and has proposed an elegant model by which these sequences have a crucial role in preventing more than one alternative exon from being incorporated into the same mRNA.

There is some evidence of developmental and cell-type-specific regulation of Dscam alternative splicing. This evidence comes from studies that assessed the use of alternative exons through single-strand conformation polymorphism (SSCP) analysis for exon 4 in both cell culture and *in vivo* [42], and from microarray analysis of alternative exons 4, 6 and 9 [23,27]. For example, these studies have demonstrated temporal and spatial control of alternative exon 4.2 usage. In addition, biases have been observed in the use of exon 9 alternatives in different cell populations. Graveley and colleagues [40,41] have identified specific *cis*-acting sequences and *trans*-acting splicing factors that regulate splicing of exon 4.2. The mechanisms leading to biases in exon 9 use, and whether these splicing biases are biologically relevant, remain unknown.

along orthogonal pathways (Figure 3a). We refer to these two branches as 'sister branches' because they come from the same neuron. Like wild-type axons, an MB axon lacking Dscam bifurcates, but the sister branches fail to segregate and instead project along the same pathway (Figure 3b). These results suggest that Dscam provides a mechanism that enables sister branches to segregate from each other. The simplest mechanism to account for this function is that homophilic binding of Dscam between sister branches promotes repulsion [17,23] (Figure 4).

The notion that homophilic binding of Dscam promotes repulsion is consistent with overexpression studies. For instance, expression of a single isoform of Dscam selectively on axons that normally form a tight fascicle (or bundle) leads to defasciculation because axons are apparently repelled by one another [16]. In a different developmental context, axons that normally cross glial cells are prevented from doing so when the same Dscam isoform is expressed in both the axons and the glial cells [17].

In summary, we have proposed that homophilic binding of Dscam between sister branches of MB neurons promotes repulsion [17,23]. The molecular mechanism of Dscam-mediated repulsion is not known, but this repulsion enables sister branches to segregate from each other [23]. Notably, branch segregation does not occur in isolation; indeed, many MB axons branch in close proximity to one another (Figure 3a). To ensure that sister branches segregate from each other along orthogonal pathways, each branch must recognize its sister as distinct from all other branches. If homophilic binding of Dscam promotes sister branch segregation, then each MB neuron must express a different Dscam isoform or set of them.

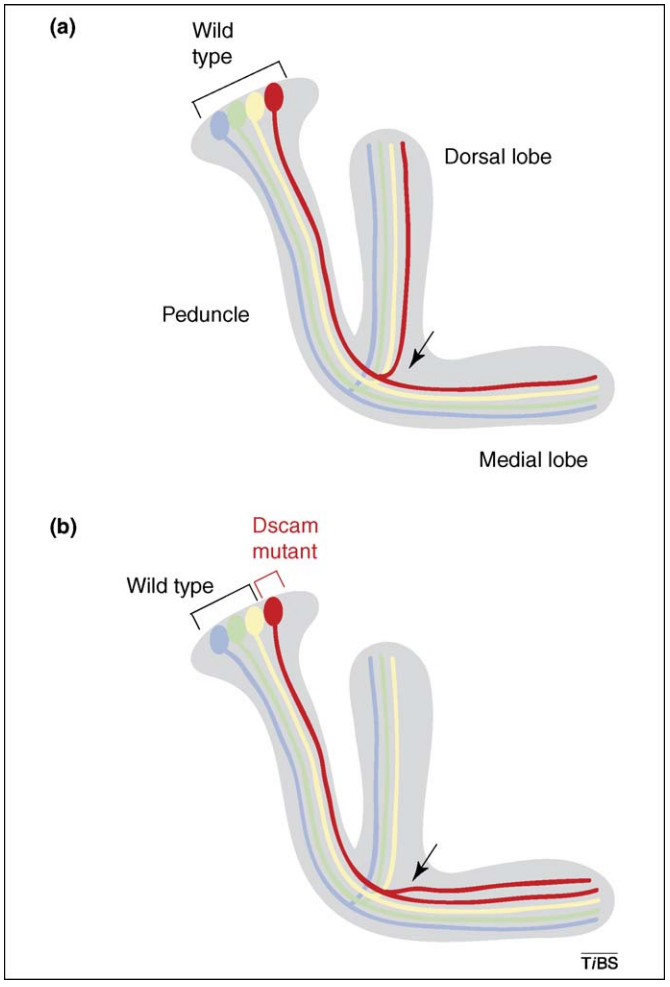

Figure 3. Dscam is required for the segregation of MB sister branches. Each MB (shaded in gray) comprises a region called the peduncle and two lobes (dorsal and medial). Each MB comprises on the order of 2500 neurons. Four neurons are shown in different colors (red, yellow, green and blue) and the cell bodies are represented as filled ovals. **(a)** Wild-type MB neurons. The axons grow as a bundle into the peduncle. Each axon bifurcates (arrow) and the resulting two branches, called sister branches, segregate from one another and project along two orthogonal pathways into the dorsal and medial lobes. **(b)** A single Dscam mutant MB neuron (red) in an otherwise wild-type background (i.e. the yellow, green and blue axons are wild type). This mutant axon projects through the peduncle and bifurcates like the wild-type axons; however, the two sister branches fail to segregate to different lobes, and instead both sister branches project into the same lobe [22].

Dscam barcoding: the ultimate scan

Expression of the Dscam isoforms in MB neurons has been assessed in a study using mRNA isolated from fluorescence-activated cell sorting (FACS)-separated populations of MB neurons [23]. The identity of the isoforms was determined by cDNA sequencing. Although a limited number of cDNAs were analyzed (i.e. 93), most alternative exons were expressed in MB neurons and were found in many combinations. The relative expression level of all of the alternative exons was assessed with a customized microarray [23,27]. These studies found little bias in the expression levels of alternative exons encoding the variable Ig2 and Ig3 domains. By contrast, a bias was observed in the spectrum of variable Ig7 exons expressed (see later).

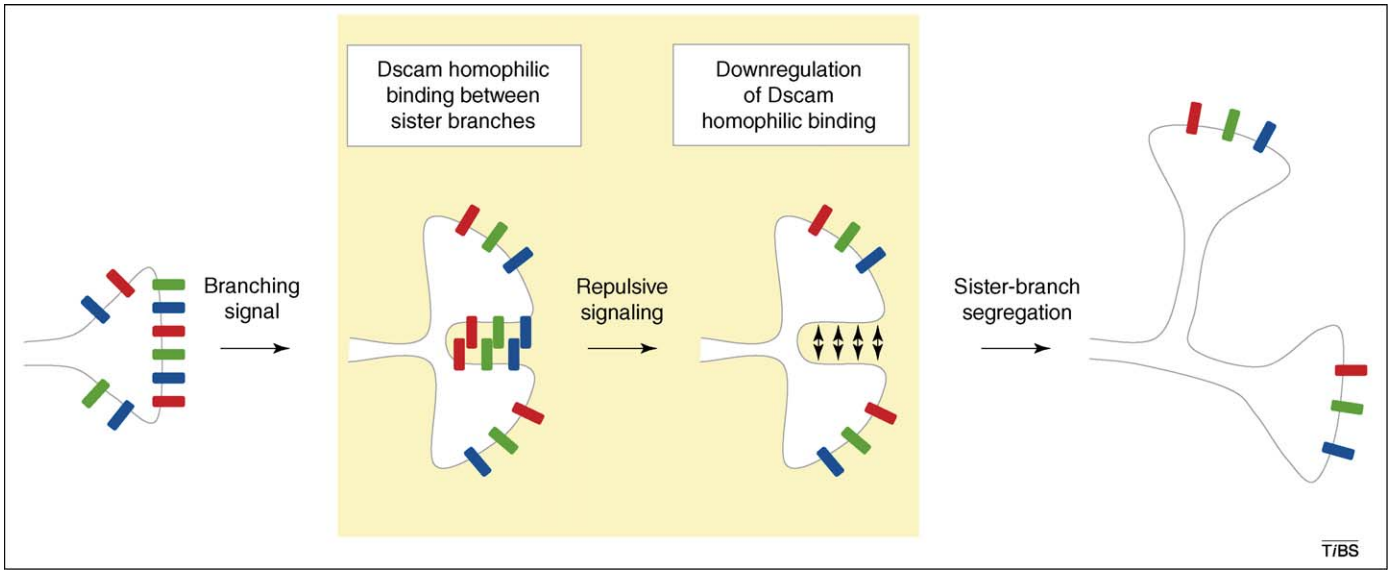

Figure 4. A model of Dscam-mediated self-recognition and segregation of sister branches in MB development. Shown are the extending axon and growth cone of an MB neuron expressing three different Dscam isoforms (red, blue and green rectangles). When the growth cone reaches the base connecting the dorsal and medial lobes (Figure 3), it bifurcates, giving rise to two sister branches. This branching event is not mediated by Dscam. After branching, homophilic binding of Dscam occurs between identical sets of isoforms expressed on the surface of opposing sister branches. Homophilic binding generates repulsive signaling, which results in subsequent downregulation of Dscam homophilic binding and cytoskeletal rearrangements leading to growth of the sister branches away from one another (double-headed arrows represent repulsion). The molecular mechanisms of this downregulation and repulsion are not known. As the sister branches segregate, they grow orthogonally with each sister branch growing into a separate MB lobe (Figure 3). This model emphasizes the role of homophilic binding of Dscam between sister branches. Homophilic binding enables sister branches to recognize each other as self. The repulsive signaling that results from homophilic binding promotes segregation of sister branches.

Taken together, these population studies demonstrate that many different isoforms are expressed in MB neurons.

The population data are consistent with two possible modes of expression at the single-cell level. Each MB neuron could express a single isoform that is different from the isoforms expressed by other MB neurons. Alternatively, each MB neuron could express several isoforms. To distinguish between these two possibilities, the expression of exons encoding Ig7 in single MB neurons was assessed. Each neuron was found to express several Ig7 exons, and no two neurons expressed an identical set [23]. On the basis of MB population analysis and single-cell analysis in other neurons [27], it is likely that each MB neuron also expresses many variable Ig2 and Ig3 domains.

Because single MB neurons express several isoforms and each MB neuron has been estimated to express 8–30 mRNAs out of thousands of potential alternatives, branches from different MB neurons will share, at most, a few isoforms in common. In a sense, this confers a unique cell-surface identity on each MB neuron [23,27], thereby ensuring that only sister branches share identical sets of isoforms. Homophilic binding, and subsequent repulsion, is therefore restricted to sister branches. Thus, Dscam isoform diversity can provide a recognition mechanism by which branches distinguish between sister (self) branches and the branches of other (non-self) neurons (Figure 5).

A Dscam code: how chaos leads to order

We have proposed that Dscam diversity endows MB neurons with the ability to recognize self, thereby ensuring proper segregation of sister branches [17,23]. This self-recognition model requires that each MB neuron expresses different isoforms from its neighbors. It does not, however, require the expression of specific isoforms in any given MB neuron, which gives rise to two predictions.

First, expression of the same isoform in all MB neurons should lead to marked defects in MB development. Overexpression of the same isoform in all MB neurons did, indeed, cause a severe phenotype with axons projecting out from the MB [23]. Although this phenotype is consistent with a failure to recognize self as distinct from non-self, it is important to note that this phenotype could also result from overexpression of Dscam rather than a loss of Dscam isoform identity. To address this issue, it will be necessary to generate flies in which the sole source of Dscam is a single ectodomain-containing isoform expressed at the same level and spatiotemporal pattern as the whole array of isoforms expressed from the wild-type locus.

Second, an arbitrarily chosen isoform should rescue the sister branch segregation phenotype in an otherwise wild-type MB, because it is highly unlikely that an arbitrarily chosen isoform will be expressed on neighboring neurons. Indeed, any one of three arbitrarily selected isoforms rescued single mutant neurons in an otherwise wild-type background [23,28]. These rescue data are consistent with the analysis of a set of deletion mutants, in which each deletion removes a different cluster of alternative Ig2 exons [28]. As a collection, these deletions remove all variable Ig2 domains, thereby providing a way to assess critically whether there is a requirement for any of the 19 008 different ectodomains in MB development! The branch segregation of single mutant MB neurons was indistinguishable from that of wild-type MB neurons for all of the Ig2 deletions [28]. This finding demonstrates that MB branch segregation does not require the expression of any specific Dscam isoforms. Because the largest of these

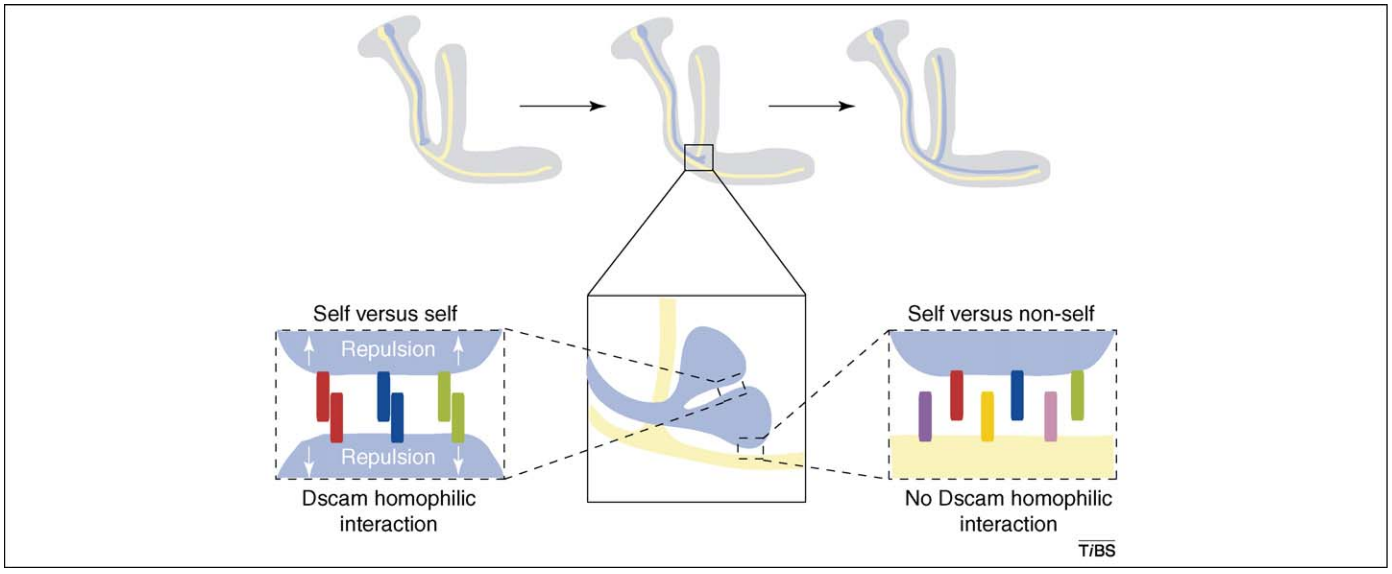

Figure 5. Differential expression of Dscam isoforms enables branches of MB neurons to distinguish between sister branches and branches of other neurons. Top, the MB (gray) is shown with two neurons highlighted (blue and yellow). The yellow MB neuron has already extended branches into the dorsal and medial lobes. The blue neuron extends an axon through the peduncle along the path previously pioneered by the yellow neuron (left). At the base of the peduncle, the growth cone bifurcates, giving rise to two sister branches (middle). After branching, the sister branches segregate from each other, growing orthogonally into separate MB lobes (right). Bottom, immediately after growth cone bifurcation the sister branches of the blue neuron are in close proximity to the previously branched yellow axon (middle). Different Dscam isoforms (colored rectangles) are expressed on the surface of each neuron (left and right). Expression studies support the view that each neuron expresses a unique combination of Dscam isoforms [23]. Sister branches or self neurites (blue) express identical combinations of Dscam isoforms, whereas non-self neurites (blue and yellow) express different combinations. Homophilic binding of Dscam occurs between the identical combination of isoforms expressed on the surface of sister branches (left). This binding results in repulsion, thereby causing segregation of sister branches into different MB lobes. By contrast, the blue sister branches express a different combination of Dscam isoforms from the yellow neuron (right) and homophilic binding does not occur. Thus, no contact-dependent repulsion ensues between the blue sister branches and the branches of the yellow neuron.

deletions reduces the potential diversity from 38 016 to 9504 isoforms, this degree of diversity is apparently sufficient to enable MB neurons to distinguish between self and non-self. These observations are not altogether surprising because the *Dscam* locus in the bee, *Apis mellifera*, encodes only 12 240 isoforms [29], even though the bee MB is considerably more complex than the fly MB [30].

These studies support the idea that the unique Dscam identity of each MB neuron enables each branch to distinguish between its sister and all other branches. Homophilic binding between identical Dscam isoforms on sister branches promotes a repulsive response, leading to segregation of sister branches (Figure 5). Single-cell Dscam mutant phenotypes in other neurons are also consistent with the idea that Dscam has a role in promoting repulsion between sister branches. It should be noted, however, that it is much more difficult to interpret phenotypes in these systems, owing to the increased complexity of the wild-type branching patterns. In the absence of Dscam, dendrites of interneurons and projection neurons in the fly olfactory system do not extend away from each other and form clumps [25]. Similar phenotypes have been observed for branched terminal axonal processes of olfactory receptor [21] and ellipsoid body [22] neurons. We thus propose that Dscam is important in mediating self-recognition and repulsion in many regions of the fly brain.

The notion that neurites can distinguish self from non-self was first proposed in the early 1980s on the basis of studies of the complex axon-branching patterns of peripheral sensory neurons in leech [31]. This phenomenon has been also described in multidendritic neurons in the *Drosophila* peripheral nervous system [32]. The molecular basis of self-recognition and repulsion in these systems remains unknown.

Beyond self: does Dscam mediate interactions among different neurons?

The complexity of neural circuit organization in the fly brain, the binding specificity of different Dscam isoforms, and the vast number of isoforms encoded at the *Dscam* locus lead to the attractive notion that this binding specificity could also form the molecular basis of the selective association that occurs between neurites of different cells. Although we have emphasized that homophilic binding of Dscam mediates repulsion, it is plausible that it could also promote attraction between cells. Indeed, other cell-surface molecules are bifunctional: in some contexts they promote repulsion, whereas in others they act in an attractive fashion [33]. As such, it is possible that Dscam homophilic interactions might specify synaptic partners or selective association of specific subsets of axons into distinct fascicles. For Dscam to function in this way, specific subsets of isoforms must be expressed in different neurons and be required in them to form specific neural circuits.

Microarray analysis has demonstrated that different classes of neurons do, indeed, preferentially express different subsets of isoforms [27]. These subsets comprise many isoforms, which has led Chess and colleagues [27] to propose that neurons express Dscam isoforms in a stochastic but biased fashion. This bias might simply be an indirect consequence of the expression of different splicing factors in different neurons. More interestingly, the bias could reflect a functional requirement for a distinct subset of isoforms.

Schmucker and colleagues [26] have recently proposed that specific isoforms are required to establish proper branching patterns of somatosensory neurons. These neurons project from identified bristles in the thorax to targets in the thoracic ganglion, where they elaborate a highly branched morphology. To assess whether specific isoforms of Dscam are required to generate these patterns, Schmucker and colleagues [26] analyzed two different deletion mutants, each of which removes five alternative versions of variable Ig2 exons. The potential diversity is reduced from 38 016 to 22 176 isoforms in these mutants. In contrast to similar deletion studies in MB neurons (see earlier), the removal of these isoforms resulted in morphological defects in somatosensory neurons.

The branching patterns in these isoform deletion mutants deviated from those in wild-type flies in two ways. First, the inherent variability in the wild-type branching pattern of these neurons was enhanced. Second, in some neurons, additional branches not seen in the wild type were formed. Different deletion mutants showed distinct branching pattern defects, raising the intriguing notion that specific isoforms might be required to prevent inappropriate branching. Whether Dscam is acting in the somatosensory neuron, in cells in the environment, or in both, is not known because the effects of these deletions have not been assessed in genetically mosaic flies. In summary, these studies raise the exciting possibility that expression of Dscam isoforms in some neurons is under tight developmental control and that specific isoforms are required for particular cell recognition events.

These data suggest that Dscam diversity functions in different ways to pattern neural circuits. We propose that diversity provides each MB neuron with a unique identity that enables it to distinguish between self and non-self. In these cells, the specific isoforms that are expressed are unimportant. What is crucial, however, is that MB neurons express isoforms that are different from those expressed by their neighbors. By contrast, specific isoforms might function to control branching patterns in somatosensory neurons. We anticipate that extending the analysis of the function of Dscam diversity to other neurons will provide further insights into how diversity mediates cell recognition events that pattern neural circuits.

Concluding remarks

Uncovering the cell recognition mechanisms that underlie the formation of neural circuits remains a considerable challenge. The extraordinary diversity and the remarkable binding specificity of *Drosophila* Dscam have raised the intriguing possibility that Dscam isoforms function to mediate cell recognition events during the wiring process. Indeed, genetic studies support the view that diversity and isoform-specific homophilic binding of Dscam do contribute to the establishment of neural circuits. Furthermore, Dscam diversity is conserved among all insect species for which genome sequences are available [29]. Taken together, these findings and observations suggest that diverse families of cell-surface molecules with distinct recognition specificities are essential for wiring highly complex nervous systems. It is therefore surprising that, although vertebrate and *Drosophila* Dscam proteins share the same domain structure [16,34,35] and homophilic binding properties [17,36], the vertebrate *Dscam* genes do not encode multiple isoforms [35,36]. Presumably, vertebrates have evolved different molecular strategies for generating vast recognition specificity.

We favor the view that early in evolution a set of cell recognition molecules evolved to assemble simple neural circuits. New cell recognition molecules emerged by gene or exon duplication and divergence, contributing to the increasing complexity of circuits during evolution. We speculate that the potential for increased organizational complexity in insects was achieved, in part, by massive diversification at the *Dscam* locus. By contrast, the increase in cell recognition specificities in vertebrates might have emerged through gene duplication and divergence of other loci (e.g. cadherin-related neuronal receptors [37,38]), via graded expression of recognition proteins (e.g. ephrins and ephs [4]) or by combinatorial association of different recognition systems.

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