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Cellular and Molecular Mechanisms of Synaptic Specificity

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Abstract

Precise connectivity in neuronal circuits is a prerequisite for proper brain function. The dauntingly complex environment encountered by axons and dendrites, even after navigation to their target area, prompts the question of how specificity of synaptic connections arises during development. We review developmental strategies and molecular mechanisms that are used by neurons to ensure their precise matching of pre- and postsynaptic elements. The emerging theme is that each circuit uses a combination of simple mechanisms to achieve its refined, often complex connectivity pattern. At increasing levels of resolution, from lamina choice to subcellular targeting, similar signaling concepts are reemployed to narrow the choice of potential matches. Temporal control over synapse development and synapse elimination further ensure the specificity of connections in the nervous system.

Contents

INTRODUCTION

The central nervous system (CNS) is arguably the most complex organ of the human body. Its network-like structure consists of an estimated 10^{11} neurons, which connect to each other through over 10^{14} chemical and electrical synapses. Glial cells, as numerous as neurons, play major developmental and homeostatic roles and contribute to the complex environment of the brain. Because the function of neuronal circuits critically depends on connectivity, synaptic specificity is a key aspect of neuronal development.

Wiring specificity emerges gradually, through sequential developmental processes that include cell fate determination, cell migration, axon and dendrite growth and guidance, branch layer formation, and synapse formation. Similar in concept to Waddington's (1959) landscape model, each step restricts the number of potential synaptic partners. Genetically encoded or activity-dependent synapse elimination further sculpts neuronal connectivity.

It is well appreciated that neurons are endowed with exquisite discriminating power to choose their correct synaptic targets in vivo among a dense array of potential partners. This property is evident from serial electron microscopy (EM) reconstructions of *Caenorhabditis elegans* (White et al. 1986) or the visual system of flies (Meinertzhagen & O'Neil 1991). Likewise, the axonal arbor of a retinal ganglion cell (RGC) in the lateral geniculate nucleus (LGN) forms synapses with 4 out of 43 cells it contacts (Hamos et al. 1987).

Although the definition of synaptic specificity traditionally has been examined on a whole-cell basis, the uniquely polarized neuronal morphology implies that specificity also exists on a subcellular level. Subcellular specificity also has functional consequences. For example, inhibitory synapses formed onto distal dendrites will mostly affect dendritic calcium-dependent spikes, whereas perisomatic inhibitory synapses have a far more profound effect on action potential firing by the postsynaptic cell (Miles et al. 1996, Pouille & Scanziani 2004). An increasing number of studies now illuminate mechanisms through which such exquisite subcellular specificity emerges. We first discuss mechanisms that ensure specificity on a whole-cell level. We then detail examples where mechanisms that generate subcellular specificity have been described. Interestingly, similar developmental strategies and signaling pathways are employed in both types of specificity.

At both the cellular and subcellular level, several nonmutually exclusive strategies may cooperatively ensure correct matching of synaptic partners. Many examples exist of positive cues in the form of adhesion molecules, which mediate recognition of partner cells and tethering of their membranes. Positive cues may also be diffusible and could be expressed by nontarget cells, such as guidepost cells. Inhibitory cues on nontarget cells have also emerged as critical regulators of synaptic specificity. Besides preventing aberrant connections, diffusible inhibitory cues also pattern the subcellular distribution of synapses along axons. Additional mechanisms that restrict synapses to the right place include synapse elimination and temporal control over the ability of a cell to form synapses. Below, we detail each strategy and attempt to provide examples from recent literature in vertebrate and invertebrate models.

SYNAPTIC SPECIFICITY BETWEEN PARTNER CELLS

Molecular recognition events occurring on the cell surface may generate specific connections by presenting a cell as being target or nontarget based on its expression of specific molecules. This idea, first introduced by Langley (1895), and later formulated in Sperry's (1963) chemoaffinity hypothesis, posits that recognition occurs through matching pairs of receptors or adhesion molecules. Although the recognition molecules may be expressed only by correct synaptic partners in a lock-and-key fashion, correct target selection may also occur by choosing the highest-affinity partners among a range of possible interactions.

Homophilic Interactions Among Cell Surface Molecules Generate Sublaminar Specificity

The vertebrate retina is organized into layers, which contain cell nuclei (the outer and inner nuclear layer) or synaptic connections between projecting axons and dendrites (the outer and inner plexiform layers). In the inner plexiform layer (IPL), the dendrites of RGCs receive synaptic input from bipolar and amacrine cells (**Figure 1*a***). This connectivity determines the ON or OFF response properties of RGCs to light and is therefore crucial to correct vision (Sanes & Yamagata 2009, Sanes & Zipursky 2010). The IPL is only a few tens of micrometers thick at the time synaptic connections are formed; it thus represents a very crowded environment in which neuronal processes must discriminate their true targets from inappropriate ones. This challenge is partially simplified by organizing the axonal dendritic processes into sublaminae, where specific connections form (Sanes & Yamagata 2009).

Three families of immunoglobulin superfamily (IgSF) adhesion molecules were identified in the chick retina as playing a pivotal role in establishing sublaminar specificity in the IPL: Dscams, Sidekicks (Sdks), and Contactins (Cntns) (Yamagata & Sanes 2008, 2012; Yamagata et al. 2002). These molecules are expressed by nonoverlapping sets of bipolar amacrine and retinal ganglion

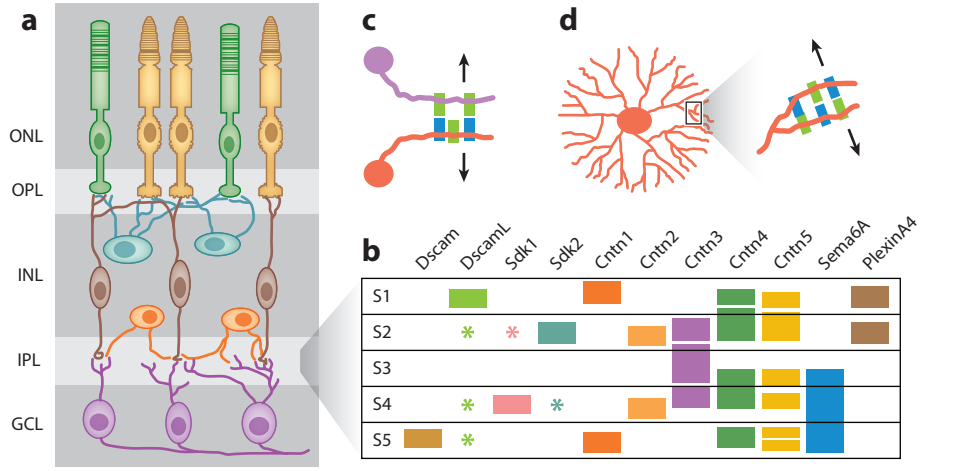

Figure 1

Laminar organization of the vertebrate retina. (*a*) Illustration of the different layers in the retina. Cone (*yellow*) and rod (*green*) photoreceptors are located in the outer nuclear layer (ONL). In the outer plexiform layer (OPL), photoreceptors form synapses with bipolar and horizontal cells (*brown* and *blue*, respectively). In the IPL (inner plexiform layer), the processes of retinal ganglion cells, bipolar cells, and amacrine cells ramify in specific sublaminae to form synapses. (*b*) Schematic illustration of sublaminar expression patterns in the IPL from chick (Dscams, Sdks, and Cntns) (Yamagata & Sanes 2008, 2012; Yamagata et al. 2002) and mouse (Sema6A and PlexA4) (Matsuoka et al. 2011b). Asterisks denote low levels of expression in the cases of Dscams and Sdks. (*c*) Sema6A-PlexA2 *trans* interactions mediate repulsion between ON and OFF starburst amacrine cells (SACs) at an early developmental stage. PlexA2 is expressed by both ON and OFF SACs, whereas Sema6A is expressed only in ON and in deeper layers of the IPL (see *b*). (*d*) Sema6A and PlexA2 mediate self-avoidance of dendrites from ON SACs. Repulsive signaling among dendritic branches between P2 and P10 ensures that ON SACs elaborate symmetric dendritic fields (Sun et al. 2013). Abbreviation: INL, inner nuclear layer.

cells and concentrate at synaptic sites (**Figure 1*b***). Pre- and postsynaptic cells expressing the same molecule arborize in the same sublamina, suggesting that these patterns form a sublaminar specificity code based on homophilic interactions. Indeed, Dscam, DscamL, Sdk1 and Sdk2, and Cntn2 display homophilic interactions, and reducing their levels in RGCs leads to the dendrites losing their sublaminar confinement. Furthermore, RGC dendrites and afferent processes mistarget to a new sublamina following forced expression of a new adhesion protein (Yamagata & Sanes 2008, 2012; Yamagata et al. 2002). Although the precise mechanism through which these extracellular homophilic interactions control specificity in process arborization or synaptic connectivity is still unknown, and may differ between the GPI-anchored contactins and the transmembranal Dscams/Sdks, the results illustrate how specific matching of surface molecules can ensure synaptic partner specificity.

A general design principle of the nervous system, exemplified by the retina, is the organization of synaptic connections into laminae, which may facilitate the task of matching the appropriate pre- and postsynaptic cells. Hence, laminar specificity is often a prerequisite for synapse specificity. Examples of laminar organization are abundant in the vertebrate and invertebrate nervous systems and include the *Drosophila* visual system as well as the mammalian hippocampus (Clandinin & Zipursky 2002, Forster et al. 2006).

Besides the theme of laminar organization, another concept that emerges from this and other examples is the repeated use of the same molecules to control synaptic connectivity in various

systems. Interestingly, the same molecules can achieve the same effect, i.e., synaptic specificity, through entirely different mechanisms. For example, Dscam1 and Dscam2 are required for the specific composition of postsynaptic cells in tetrad synapses of the *Drosophila* lamina (**Figure 2*c***), a function that probably requires repulsive homophilic interactions (Millard et al. 2010). In this context, the homophilic recognition of the extracellular domain of Dscams is likely coupled to a repulsive downstream pathway, and the intracellular signaling pathways must be different for the homophilic fasciculation activity in the retina. In a different example, Cntn5 with its coreceptor Caspr4 controls the exquisite synaptic specificity of GABApre interneurons onto sensory axonal terminals in the sensory-motor circuit in the mouse spinal cord. Unlike the homophilic interactions, which mediate sublaminar specificity in the retina, here heterophilic interactions between Cntn5/Caspr4 and postsynaptic NrCAM/CHL1 ensure recognition of synaptic partners (Ashrafi et al. 2014, Betley et al. 2009). Therefore, diverse receptor-ligand interactions of the same adhesion molecules can provide additional flexibility to chemoaffinity systems.

Expression Levels of Receptors Can Be Instructive in Matching Synaptic Partners

A variation on the lock-and-key mode of recognition is matching not only the type of cell surface molecules but their relative levels in the pre- and postsynaptic elements. Two examples where the levels of cell surface molecules are instructive in generating specificity come from the *Drosophila* antennal lobe. Olfactory receptor neurons (ORNs) expressing a specific odorant receptor project their axons to discrete glomeruli in the antennal lobe, where they form connections with dendrites of first-order interneurons, the projection neurons (PNs). This organization ensures that PNs carry information from a single class of olfactory neurons to higher processing centers in the brain (Hong & Luo 2014). The transmembrane Semaphorin Sema-1a is expressed in a dorsolateral-to-ventromedial gradient by the PNs and is required for the targeting of their dendrites to discrete glomeruli before the arrival of ORN axons. Removal of Sema-1a from dorsolateral PNs caused mistargeting of their dendrites to medioventral positions, suggesting that the levels of Sema-1a are instructive in generating this coarse projection pattern (Komiyama et al. 2007). Similar phenotypes are observed when Sema-2b and Sema-2a are removed from the antennal lobe. These secreted Semaphorins are expressed in an opposing gradient to Sema-1a, and Sema-1a can bind to cells expressing Sema-2a (Sweeney et al. 2011). Together, these results suggest that opposing gradients of secreted and transmembrane Semaphorins can generate discrete coarse projections in a way that depends on their relative levels.

Once PN dendrites and ORN axons have reached particular glomeruli, matching of the pre- and postsynaptic elements must take place. A forward genetic screen has identified the Teneurins, Ten-a and Ten-m, two EGF repeat–containing transmembrane proteins that are required for matching of synaptic partners. Teneurins mediate homophilic interactions in vitro and in vivo. Furthermore, Ten-a and Ten-m are highly expressed by matching ORN and PN pairs and seem to determine connectivity based on their expression levels. Thus, overexpression of Teneurin in Teneurin-low PNs caused their dendrites to mismatch with high-Teneurin-expression ORNs, instead of the low-Teneurin ORNs with whom they usually connect. Teneurin overexpression in already high-Teneurin PN had no effect (Hong et al. 2012). These findings suggest that unlike classical synaptic pairing through adhesion molecules, which relies on their mere presence, PNs and ORNs pair by comparing and matching relative Teneurin levels. This mechanism may broaden the spectrum of specific synaptic connections that can be generated by a given molecule in the same neuropil.

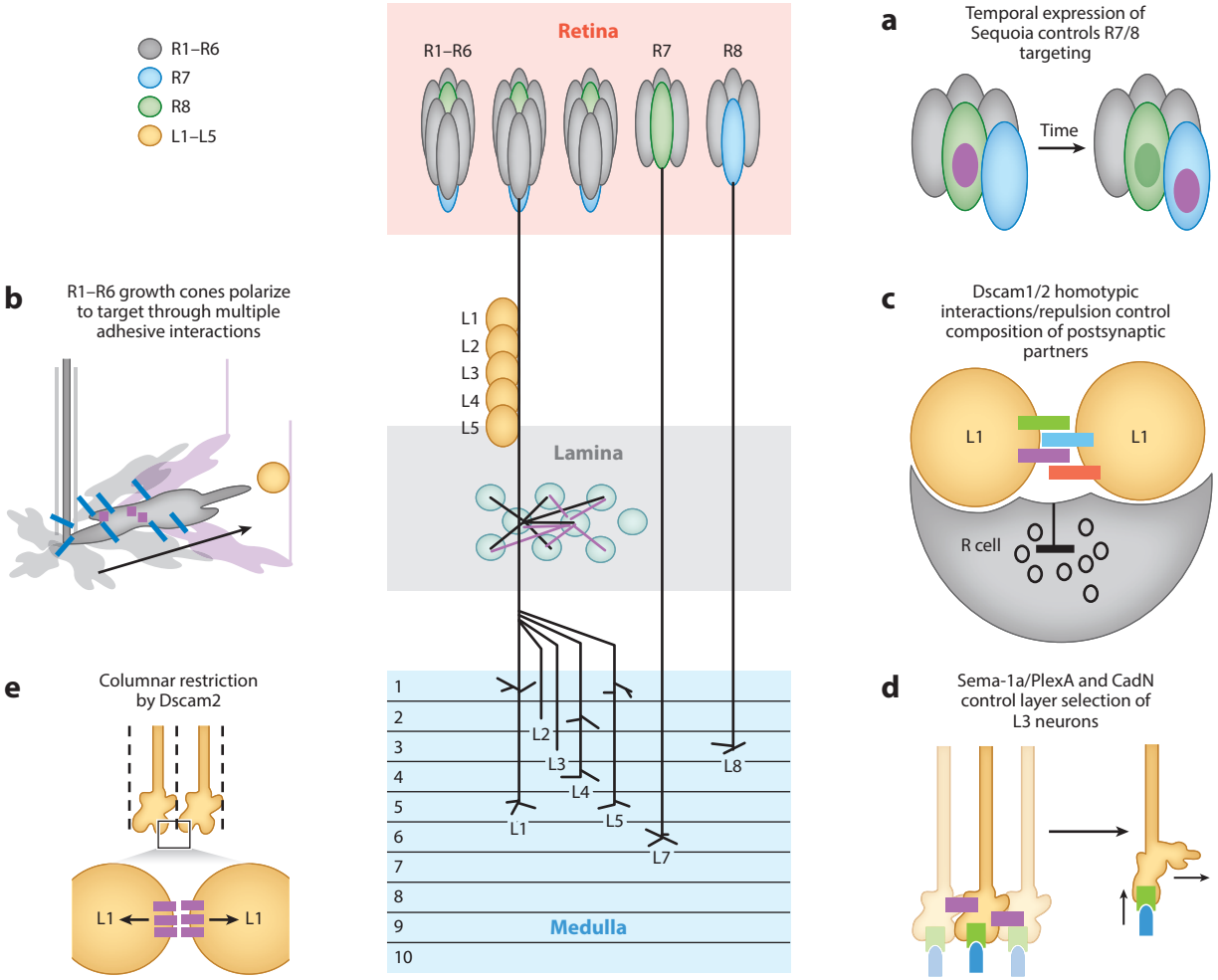

Figure 2

Mechanisms of synaptic specificity in the *Drosophila* visual system. The first three components of the fly visual system are the retina, lamina, and medulla. Photoreceptors in the eye are clustered into ~700 ommatidia, each containing six outer photoreceptors (R1–R6, *gray*) and two inner photoreceptors (R7 and R8, *blue and green*). R1–R6 project axons to the lamina, where they synapse with lamina neurons L1–L5 (*yellow*) in columns called cartridges. Black lines in the lamina show the projections of R1–R6 from a single ommatidium, and purple lines show the projection patterns of several R cells that receive visual input from the same point in space. Lamina neurons project to the medulla, where they terminate in one of ten different layers. R7 and R8 send their axons directly to the medulla. (*a*) Sequoia (*purple*) is expressed initially in R8 and then in R7. This temporal sequence determines the competence of the inner photoreceptors to target to the appropriate medulla layer through regulation of CadN levels (Petrovic & Hummel 2008). (*b*) Incoming R1–R6 growth cones (*gray*) polarize toward their future site of synaptic contact with lamina neurons (*yellow*) through a network of adhesive interactions among axonal growth cones. These interactions are controlled by Flamingo and CadN, which are differentially localized in the growth cones (Schwabe et al. 2013). (*c*) At tetrad synapses in the lamina, the invariant presence of single L1 and L2 lamina neurons as postsynaptic partners is controlled by Dscam1 and Dscam2. Repulsive interactions between similar Dscam isoforms prevent the incorporation of two L1 or two L2 neurons into the synapse (Millard et al. 2010). (*d*) L3 lamina neurons project to a temporary layer in the outer medulla while maintaining contact with L1 and L5 lamina neurons through CadN (*purple*). PlexA, expressed by tangential fibers, interacts with Sema-1a on the growth cone to halt growth. At a second stage, L3 segregates into the M3 layer by extending in the layer while retracting from the temporary layer (Pecot et al. 2013). (*e*) Repulsive interactions between Dscam2 molecules on the surface of L1 lamina neurons restrict these neurons to the appropriate axonal column (Millard et al. 2007).

Adhesion Among Afferent Growth Cones Mediates Synaptic Target Selection

In many cases, axons project to their target area as a bundle, and therefore, adhesive interactions are expected to exist among them. Can afferent-afferent interactions generate targeting specificity?

In the *Drosophila* visual system, the axons of R1–R6 photoreceptor neurons project to the first optic neuropil in the brain, the lamina, as bundles. Upon arrival at the lamina, the bundle defasciculates, and individual R cells form stereotypic connections with postsynaptic lamina neurons, such that specific R cell subtypes that receive visual information from a single point in space will converge on the same postsynaptic lamina neurons to form a cartridge (Hadjieconomou et al. 2011) (**Figure 2**). This generates a repeated, patterned organization that is genetically hardwired (Hiesinger et al. 2006) and is carried out in an extremely robust and reproducible manner (Schwabe et al. 2013). Indications that interactions among R cell axons are critical for their targeting initially came from the analysis of mutants in which specific R cell classes are missing. In these mutants, the remaining R cells often show mistargeting defects (Clandinin & Zipursky 2000). Two adhesion molecules, N-Cadherin (CadN) and the atypical Cadherin Flamingo (Fmi), are required for generating correct connections between R cell axons and lamina neurons (Chen & Clandinin 2008; Lee et al. 2001, 2003). Whereas CadN is expressed by both pre- and postsynaptic cells, Fmi is expressed only by the incoming axons. Recently, Clandinin and coworkers showed that as R1–R6 axons defasiculate, they polarize toward their correct target (**Figure 2*b***), suggesting that correct target selection occurs before contact with the postsynaptic membrane. Strikingly, it is the adhesive network of interactions between R cell growth cones in the same bundle and in neighboring bundles that mediates this polarization and hence target-cell selection. Compromising CadN and Fmi function in single cells leads to failures in growth cone extension, but the polarization remains intact. Conversely, when a large population of axons is depleted of Fmi and CadN, growth cones often polarize in an aberrant manner (Schwabe et al. 2013). This suggests that adhesion among an ensemble of R cell axons mediates the polarization of individual axons toward their postsynaptic target. The redundancy of interactions among afferents makes target selection in this system extremely robust and reproducible. Although the nature of the actual polarization cue is not clear, the authors speculate that could arise from the asymmetrical localization of Fmi in the growth cone and its varying levels of expression among different R cell types.

Place Holder and Guidepost Cells Direct Pre- and Postsynaptic Matching Specificity

Future synaptic partners are contacted by a large number of nontarget cells before matching. Several examples in both vertebrate and invertebrate systems support the notion that nontarget cells act as guidepost cells in the generation of specific synaptic connections. One role that nontarget cells play is as place holders. When one of the synaptic partners reaches the future synaptic site before its partner, it may form transient connections with place holder cells. These connections are subsequently lost, and new connections form with the final synaptic partner.

In the cat visual system, LGN axons from the thalamus form transient synaptic connections with subplate cells while they wait a few weeks for the arrival of their mature postsynaptic partners, layer-4 neurons. After the formation of the mature connection, subplate cells are eliminated by cell death. Shatz and colleagues examined the consequences of ablating subplate cells early in development and found that this leads to severe targeting defects of thalamic axons (Ghosh et al. 1990). Furthermore, subcortical plate ablation after LGN axons have arrived at cortical layer 4 results in loss of ocular dominance columns and orientation columns, as well as thalamocortical synaptic transmission defects (Ghosh & Shatz 1992, Kanold et al. 2003). These results demonstrate

the importance of the transient interactions between subplate cells and thalamic axons in the establishment of synaptic targeting and later maturation of the thalamocortical visual circuit.

Besides their extensive effects on synapse development and their well-established role in neuronal excitability, glial cells can also regulate the specificity of synaptic matching by acting as transient place holders or guidepost cells. Glia are more numerous than neurons in the mammalian CNS and are intimately associated with synapses, such that they are well positioned to contribute to the specificity of synaptic connections. A role for glia in the matching of synaptic partners was elegantly described in the cerebral cortex, where stellate cell axonal arbors specifically innervate Purkinje cell dendrites (Ango et al. 2008). Huang and colleagues identified an intermediate scaffold of Bergmann glia (BG) fibers, which interact with stellate cell axons and guide them to the Purkinje cell dendrites. They further identified the Ig family protein CHL1 as a mediator of the interactions between BG fibers and stellate cell axons. Loss of CHL1 led to deviations of axonal arbors from the BG fibers and a reduction in synapse formation (Ango et al. 2008). This example illustrates how the close association between glia and neurons enables glial cells to actively control synapse specificity.

A similar role for glia has been described in *C. elegans*, where two glia-like cells, the sheath cells, direct synapse formation between AIY and RIA interneurons of the thermotaxis circuit. Sheath cell processes are in close association with the area where AIY and RIA form synapses. Release of the diffusible cue UNC-6/Netrin by the sheath cells promotes AIY-RIA synapse formation through the UNC-40/DCC receptor. Interestingly, UNC-40/DCC signaling regulates different functions in RIA and AIY: axon guidance in the first and synapse formation in the latter (Colón-Ramos et al. 2007). This exemplifies the ability of glia to act as guidepost cells through the release of diffusive signals. Additional examples of how nonneuronal cells regulate synapse specificity via secreted signaling molecules are discussed below, when we consider subcellular synaptic specificity.

Inhibitory Interactions Refine Synaptic Specificity

Besides acting as positive cues, cell surface and secreted molecules from nontarget cells are often employed to prevent inappropriate formation of synapses. Such inhibitory cues may act locally, at sites adjacent to the place where synapses form, to refine specificity. Alternatively, they can act at a distance and impact a large area to pattern connectivity.

One of the most common repulsive cues that is repeatedly employed throughout vertebrate and invertebrate neuronal development to prevent mismatching of nonsynaptic partners is Semaphorin-Plexin signaling. In the IPL of the vertebrate retina, the transmembrane Semaphorin Sema6A and its ligand PlexA4 are expressed in a nonoverlapping fashion, with Sema6A in most ON sublaminae and PlexA4 in most OFF sublaminae (**Figure 1*b***). Loss of Sema6A or PlexA4 leads to dramatic defects in the stereotypic lamination of amacrine and RGC cells (Matsuoka et al. 2011b). Thus, Sema6A–PlexA4 signaling is essential for the control of specificity through a repulsive mechanism that is a mirror image of the positive regulation exerted by Dscams, Sdks, and Cntns.

Semaphorin-Plexin signaling also controls broader lamination specificity in the lamina: Transmembrane Sema5A and Sema5B, together with PlexA1 and PlexA3, act as ligand-receptor pairs to confine the processes of several types of neurons within the IPL (Matsuoka et al. 2011a). Similar to Sema6A and PlexA4, Sema5A/B and PlexA1/3 are expressed in a complementary fashion in the developing retina, with Semaphorins in the outer neuroblast layer and Plexins in the inner neuroblast layer. In the absence of these molecules, neurites fail to correctly stratify in the IPL and also project to the inner nuclear layer and as far as the outer plexiform layer (Matsuoka et al.

2011a). These results indicate that Semaphorin-Plexin signaling is repeatedly used to control synaptic specificity through correct lamination.

More refined control over synaptic specificity by Semaphorin-Plexin signaling was described in the sensory–motor circuit reflex arcs. Here, afferents of proprioceptive sensory neurons, which detect changes in muscle length, form homonymous monosynaptic connections with motorneurons innervating the same muscle. These circuits display a high degree of specificity, which arises during development and is independent of synaptic activity (Maro et al. 2009). Jessell and coworkers studied two adjacent reflex arcs that connect to triceps (Tri) and cutaneous maximus (CM) muscles. Whereas the Tri motoneurons receive homonymous synaptic input from Tri proprioceptive neurons, no synapses form between CM motor and sensory neurons. Both CM and Tri sensory neurons express PlexD1, but only Tri motorneurons express the secreted Sema3E. Loss of Sema3E or PlexD1 led to synapse formation between CM sensory and motorneurons but did not cause ectopic connections between Tri sensory neurons and CM motorneurons. Ectopic expression of Sema3E in Tri motorneurons prevented their sensory neurons from synapsing onto them (Pecho-Vrieseling et al. 2009). These results identified Sema3E and PlexD1 as specific inhibitory cues that mediate synapse specificity, independently of motor pool identity. In the gluteus and hamstring reflex arcs, the repellent activity of Sema3E and PlexD1 similarly mediates synaptic specificity. However, in this case, signaling also determines pool specificity, as loss of signaling caused aberrant connections of hamstring proprioceptive afferents with gluteus motor neurons (Fukuhara et al. 2013). Although in the above examples the ligand is expressed by the postsynaptic cell and the receptor by the axon, opposite signaling logic also exists (Ding et al. 2012). Together, these results illustrate the use of inhibitory signaling through secreted Semaphorins in establishing synaptic specificity.

A common theme raised by the above examples is that inhibitory signaling may occur before contact between nontarget cells (as is probably the case with secreted Semaphorins) but may also require contact when transmembrane molecules are involved. Although counterintuitive, contact-dependent repulsion is widespread in the nervous system and occurs during different developmental processes.

Well-known examples include dendritic self-avoidance mediated by Dscam in *Drosophila* or by protocadherins in mammals: When dendritic processes explore their receptive fields during development, homophilic interactions between Dscams or protocadherins on branches from the same cell mediate branch retraction (Zipursky & Grueber 2013). Corroborating the analogy between contact-mediated dendritic retraction and contact-mediated inhibitory signaling in synapse specificity is the role of Dscam1 and Dscam2 inhibitory signaling in ensuring correct partner choice in tetrad synapses of the *Drosophila* lamina (**Figure 2*c***). Dscam2 homotypic interactions also generate contact-dependent repulsion that confines L1 axonal arbors to specific columns in the medulla (Millard et al. 2007, 2010) (**Figure 2*e***).

Furthermore, contact-mediated dendritic self-avoidance and sublamination of ON starburst amacrine cells (SACs) in the mouse retina are mediated by the same molecules: Sema6A and PlexA2 coexpression in ON SACs mediates self-avoidance of dendritic branches through *cis* interactions, and interactions in *trans* with PlexA2 expressed in OFF SACs ensures that the branches of these two cell types elaborate in different sublaminae of the IPL (Sun et al. 2013) (**Figure 1*c*,*d***). These results highlight the importance of contact-mediated repulsion in establishing neuronal circuits.

Despite the well-established role of inhibitory signals in regulating synaptic specificity, less is known about the mechanisms through which such signals act. Although negative regulation could operate as a very-short-distance axon guidance cue, signaling to the growth cone to continue exploring the environment, other mechanisms likely exist. This is especially true for en passant synapses, which form along the axon and not at its terminus. Indeed, downstream effectors through

which inhibitory cues control en passant synapse specificity in *C. elegans* remain elusive (see, for example, Klassen & Shen 2007, Poon et al. 2008). One mechanism through which inhibitory cues might act to sculpt circuits is synapse elimination, which is discussed below. Another clue, which comes from studies of Semaphorin signaling in the mouse CNS, suggests the involvement of the cytoskeleton. Dendrites of DG granule cells and pyramidal neurons in cortical layer 5 show an increase in spine number and size in Sema3F/PlexA2/Npn-2 mutants. Interestingly, Npn-2 is restricted in layer-5 cortical neurons to apical dendrites, where defects arise in the mutants, and the growth-inhibiting activity of signaling requires the cytoplasmic SEA domain of the Npn-2 (Tran et al. 2009). These phenotypes suggest that Sema3F signaling controls synaptic connectivity through an effect on spine growth. Because spine growth and size regulation are heavily dependent on the actin cytoskeleton (Sala & Segal 2014), and actin regulators are known downstream effectors of Semaphorin signaling (Wang et al. 2012), these results suggest that inhibitory cues refine neuronal circuits through a local effect on the cytoskeleton. Similarly, presynaptic actin is a target for Plexin signaling, which restricts synapse formation in *C. elegans* (Mizumoto & Shen 2013a).

To end the discussion of inhibitory signals in the generation of whole-cell synaptic specificity, we note that inhibitory cues from nontarget synaptic partners are evidently part of a larger set of signals, which should include positive interactions. We have used the IPL of the vertebrate retina as an example in which the stratification of neuronal processes to the correct sublamina requires positive cues in the form of homophilic interactions between Dscams, Sdks, and Cntns, along with inhibitory cell-cell interactions mediated by Semaphorins (6A, 5A, and 5B) and Plexins (A4, A2, A3, and A1) (Matsuoka et al. 2011a,b; Yamagata & Sanes 2008, 2012; Yamagata et al. 2002). Likewise, we described positive regulation of synapse formation in the *Drosophila* lamina by Fmi- and CadN-mediated adhesion between photoreceptor growth cones and CadN-mediated interactions with postsynaptic target cells (Lee et al. 2001, 2003; Schwabe et al. 2013). This is coupled to negative cues in the form of Dscam1 and Dscam2, which mediate repulsive homophilic interactions between potential postsynaptic cells, thereby ensuring the correct composition of postsynaptic partners at tetrad synapses (Millard et al. 2010). Another example from the *Drosophila* visual system is the coordinate regulation of lamina neuron L3 targeting to layer 3 in the medulla through combined positive regulation by cadN and repulsive Sema-1a/PlexA signaling from deeper lamina layers (Pecot et al. 2013). Thus, a combination of positive and negative signals refines synapse specificity in multiple developmental scenarios.

SUBCELLULAR SYNAPTIC SPECIFICITY

The polarized structure and complex geometry of neurons imply that synapses can form in very distinct locations, which may consequently endow them with different properties. For example, subcellular specificity could refer to proximal versus distal dendritic arbors with which the presynaptic terminal may connect, or to the location of presynaptic en passant release sites along the length of an axon. Because subcellular localization could profoundly impact synapse function, most synapse formation likely occurs with subcellular precision. Therefore, in the previous examples where specificity was considered on a whole-cell basis, the reason is likely a lack of experimental resolution rather than promiscuous matching. This notion is supported by the similarity of the developmental mechanisms that sculpt subcellular synapse specificity to the ones described in the previous examples.

The functional importance of subcellular synaptic specificity is best illustrated by measuring the impact of inhibitory synapses on hippocampal pyramidal cells. In cortex and hippocampus, multiple types of inhibitory neurons form synaptic inputs on distinct subcellular domains of excitatory pyramidal neurons. The inhibitory effects of these synapses are different despite use of

the same GABA neurotransmitter. Whereas perisomatic inhibition had a profound effect on action potentials elicited in the postsynaptic cells, inhibitory synapses onto distal dendrites affected mostly dendritic calcium-dependent spikes (Miles et al. 1996). These two inhibitory synaptic populations also differ in their feedback responses to activation of the pyramidal cell: Perisomatic synapses function immediately at the onset of action potentials, whereas inhibition later shifts to apical dendrites in response to the rate of action potentials (Pouille & Scanziani 2004). These studies raise the question of how subcellular specificity is generated during development.

Genetic Hardwiring and Experience-Dependent Subcellular Synaptic Specificity

Subcellular specificity may be genetically hardwired or modulated by synaptic activity. The former seems to be the case in the *C. elegans* neuromuscular junctions (NMJs), where developmental cues affect subcellular specificity, but mutations that impair synaptic transmission have little effect (Klassen & Shen 2007). Similarly, mutations that disrupt neuronal transmission in the visual system have little effect on synapse formation in the *Drosophila* lamina (Hiesinger et al. 2006). In mammals, there is also evidence for genetically hardwired subcellular synaptic specificity. Huang and colleagues (Di Cristo et al. 2004) found that bitufted and basket GABA interneurons preferentially target the dendritic and perisomatic domains of pyramidal neurons in the visual cortex, respectively. This subcellular specificity was maintained in the absence of thalamic or visual inputs in cortical organotypic cultures. These results suggest that subcellular specificity in this system is activity independent and may be genetically hardwired.

At the other end of the spectrum, subcellular synaptic specificity may arise in response to synaptic activity. The activity-dependent transcription factor NPAS4 has been shown to regulate inhibitory synapse number in cell culture (Lin et al. 2008). In vivo, NPAS4 was upregulated in pyramidal cells in the hippocampus when mice were placed in an enriched environment. NPAS4 activity elicited two changes in synaptic inputs to pyramidal cells: an increase in perisomatic inhibitory synapses and a decrease in synapse number at distal dendrites (Bloodgood et al. 2013). Thus, exposure to novel environments may modulate neuronal circuits through changes to subcellular synaptic specificity.

Cell Surface Molecules Control Subcellular Synaptic Specificity

In an analogous fashion to the regulation of whole-cell synaptic specificity, positive regulation of synapse formation by appropriately localized cell surface molecules can control where on the cell membrane synapses form. Evidently, the distribution of such molecules on the surface of target cells must be tightly controlled, and it is reasonable to expect that it correlates with cellular landmarks. However, the mechanisms that control the subcellular pattern of such molecules are known in only a handful of cases.

Huang and coworkers described a particularly striking example in the cerebellum, where GABAergic basket cells form specific connections onto the AIS of Purkinje neurons (Ango et al. 2004). Basket cell processes initially contact the soma of their future synaptic partner and then move along it to the AIS, where they form pinceau synapses. In the Purkinje neuron, a gradient of the immunoglobulin L1CAM cell adhesion neurofascin 186 (NF186) directs basket cells to the AIS, where ankyrinG mediates the enrichment of NF186. Perturbation of the NF186 gradient or the interaction between ankyrinG and NF186 rerouted basket axons to ectopic locations following NF186 and reduced pinceau synapse formation (Ango et al. 2004). This illustrates the use of a cellular landmark, such as the AIS, for controlling the subcellular localization of synapses.

Two other examples in which adhesion molecules control the subcellular specificity of synaptic connections are the DG-CA3 synapses in the hippocampus and the axo-axonal connections that GABApre interneurons make onto the axonal termini of sensory neurons in the sensory-motor circuit in the spinal cord (Ashrafi et al. 2014, Betley et al. 2009, Williams et al. 2011). In the first example, specific expression of Cadherin-9 in the DG and CA3 is necessary for the formation of the extremely large synapses of mossy fibers onto CA3. Regular synapses onto CA3 from other sources do not require Cadherin-9 (Williams et al. 2011). In the second example, a complex of NB2/Caspr4 on sensory neurons interacts with NrCAM/CHL1 expressed by GABApre interneurons to control synapse formation specifically at the axonal termini of sensory afferents (Ashrafi et al. 2014, Betley et al. 2009). Unlike the ankyrinG-NF186 interaction in the AIS of Purkinje cells, in these two examples it is unknown how the subcellular targeting of adhesion molecules is achieved. For DG-CA3 synapses, the targeting mechanisms may be independent of inputs from the cellular environment, as correct connectivity is maintained in cell culture and seems to be independent of axon guidance cues (Williams et al. 2011). Together, these data suggest that adhesion molecules play important roles in establishing subcellular synaptic specificity.

Inhibitory Interactions Between Surface Molecules Shape Subcellular Synapse Localization

Cell surface molecules can also prevent synapse formation at inappropriate subcellular sites. *C. elegans* motorneurons of the same class show extensive overlap of their axons in the dorsal cord. Despite this overlap, the location where a given axon forms synapses with dorsal muscles is spatially restricted and does not overlap with synapses from neighboring axons of the same class (White et al. 1986). This phenomenon was termed synaptic tiling, in reference to the well-established phenomenon of dendritic tiling. Recent work has shown that the border between the DA8 and DA9 presynaptic regions depends on contact between the two axons, as well as on transmembrane Semaphorins and PLX-1/Plexin (**Figure 3**). PLX-1 localizes to the synapse-free zone of DA9 axon and inhibits local synapse formation through regulation of presynaptic actin. Interestingly, both receptor and ligand are required only in DA9, where they interact in *cis*, suggesting that additional components mediate synaptic tiling in DA8 (Mizumoto & Shen 2013a). This study

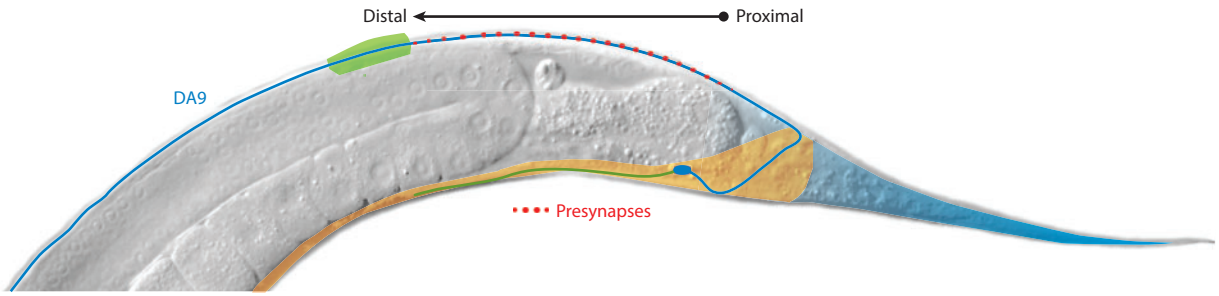

Figure 3

The presynaptic domain of the *Caenorhabditis elegans* DA9 motor neuron is carved out by multiple repulsive cues. Two diffusible cues and transmembrane molecules spatially restrict presynapses (*red dots*) to a specific location along the axon. The Wnt ligand LIN-44, emanating from the tail, forms a posterior-to-anterior gradient (*blue*), which prevents presynapses from forming on the posterior axon (Klassen & Shen 2007). UNC-6/Netrin (*yellow*), expressed by pioneering axons in the ventral nerve cord, prevents synapse formation in the ventral DA9 process (Poon et al. 2008). PLX-1 interacts with two Semaphorins at the anterior border of the synaptic domain (*green*) to restrict DA9 synapses from the anterior axon (Mizumoto & Shen 2013a).

illustrates the use of cell surface molecules to restrict synapse formation to a subcellular location. Furthermore, it demonstrates that transmembrane molecules that control subcellular synaptic specificity do not need to be associated with a preexisting cellular structure. Rather, extrinsic signals, such as nearby processes or secreted cues, can target their localization. Examples of such cues are discussed below.

Guidepost Cells in Subcellular Synaptic Specificity

When no internal landmark, such as the AIS, is present to specify the precise subcellular localization of synapses, external cues can provide spatial information. These may be secreted molecules (discussed below) or cell surface molecules expressed by guidepost cells. Cells neighboring the future synaptic site are ideally situated to specify synapse localization, as previously exemplified in the localization of sheath glia near AIY-RIA synapses in *C. elegans* (Colón-Ramos et al. 2007). Another case in point where a cell surface molecule on guidepost cells precisely controls the localization of en passant synapses along the axon is from the *C. elegans* egg-laying circuit. The presynaptic neuron HSNL makes synapses onto VC2 neurons and vm2 vulval muscles at precise locations along its axon. Surprisingly, the localization of presynapses in HSNL is instructed not by postsynaptic cells but by vulval epithelial cells, which are situated at the site of the future synapse (Shen & Bargmann 2003). Expression of SYG-2, an IgSF adhesion molecule homologous to mammalian nephrin on the surface of vulval epithelial cells, recruits a NEPH1 homolog, SYG-1, in the HSNL axon to localize the future synapses. Ablation of vulval epithelial cells or mutations in SYG-1 and SYG-2 result in mispositioned presynaptic components along the HSNL axon (Shen & Bargmann 2003, Shen et al. 2004). These results show how cell surface molecules expressed by guidepost cells can direct subcellular synaptic specificity.

Long-Rage Signaling Molecules Regulate Local Connectivity Through Inhibition of Synapse Formation

The role of long-range signals emanating from organizing centers in tissue patterning is well established. Developmental morphogenes, which specify cell fates in a concentration-dependent manner, have also been shown to act in the process of axonal navigation (Kolodkin & Tessier-Lavigne 2011). More recently, secreted molecules have also been shown to pattern local synaptic connectivity by inhibiting or promoting synapse formation.

In the *C. elegans* DA9 motorneuron, presynaptic release sites onto postsynaptic muscles are restricted to a short fragment of the dorsal axon, which extends to approximately two-thirds of the worm's body length. At least two diffusible molecules, secreted from the tail ventral cells, inhibit presynapse formation from the ventral process and from the posterior-most region of the axon (Klassen & Shen 2007, Poon et al. 2008). At the anterior border of the presynaptic region, the tiling mechanism described previously prevents anterior spreading of presynapses (Mizumoto & Shen 2013a) (**Figure 3**). The Wnt homolog LIN-44 is expressed in a small group of skin cells in the tail and forms a posterior-to-anterior gradient. In DA9, the LIN-44 gradient, transduced through its receptor LIN-17, prevents synapse formation in the posterior axon through an unknown mechanism (Klassen & Shen 2007). Inhibition of synapse formation by Wnts is not restricted to DA9 but also occurs in other neurons, including the more anterior DA8, which reads both the LIN-44 gradient emanating from the tail and the presence of another Wnt, EGL-20, which is secreted from DA9 (Mizumoto & Shen 2013b). Furthermore, in *Drosophila*, secretion of Wnt4 inhibits synapse formation onto M13 muscle but not onto M12, where Wnt4 is not expressed (Inaki et al. 2007). Interestingly, Wnts can also promote different aspects of synapse formation (Koles & Budnik 2012), suggesting that they provide mostly spatial information, which is then

interpreted by receiving cells according to the developmental context. For example, presynaptic secreted Wnt facilitates the differentiation of the postsynaptic specialization in *Drosophila* NMJ (Koles & Budnik 2012, Packard et al. 2002).

The second diffusible cue that affects DA9 synaptic pattern through inhibition of synapse formation is UNC-6/Netrin, acting through its receptor, UNC-5. UNC-6 forms a ventral-to-dorsal gradient, which is known to be required for axonal pathfinding. In *unc-6* mutants, synaptic vesicles and presynaptic active zone proteins accumulate ectopically in the ventral DA9 dendrite, indicating that UNC-6 participates in regulating synapse localization through local inhibition (Poon et al. 2008). Although the mechanisms through which LIN-44/LIN-17 and UNC-6/UNC-5 signaling inhibit synapse formation are unknown, they may be similar. This is suggested from the observation that ectopically expressed UNC-6 can also inhibit synapse formation on the dorsal process and can even replace LIN-44 when expressed from the tail (Poon et al. 2008). These observations suggest that spatial cues that inhibit synapse formation converge on similar targets, which may be part of the basic cellular machinery.

To conclude the discussion about subcellular synaptic specificity, we note again how the mechanisms and molecules that allow such exquisite specificity are similar to the ones that generate specificity at the level of a whole cell or a lamina. This similarity suggests that a given developmental signaling pathway could operate at different scales of resolution. In addition, it suggests that many synaptic connections are patterned with subcellular precision, which may be more or less apparent with different experimental systems. Lastly, it also hints that there might be a core synapse formation program that is regulated by these different cues.

TEMPORAL CONTROL OF SYNAPSE FORMATION

By definition, development occurs over time, and hence developmental processes are temporally regulated. In neurons, the final pattern of spatial organization and circuit connectivity emerges through orderly sequential steps, from the birth of the neuron, through cell migration and process extension, to synapse formation and elimination. Evidence accumulated over the past decade points to the timing of neuronal birth as a determining factor in its future development, including its projection pattern. A striking example of the importance of timing in neurogenesis comes from analysis of *Drosophila* neuroblasts, which sequentially express different transcription factors. The differentiated progeny of a neuroblast maintain expression of the transcription profile present in the neuroblast at their time of birth, and that transcription factor often determines their cell fate (Isshiki et al. 2001). These results demonstrate how temporal control of transcription factor expression sets in motion a program that determines cell fate. Neurogenesis timing also controls projection patterns. For example, in the mouse olfactory system, early- and late-generated mitral cells become differentially distributed in the dorsoventral positions of the odorant receptor map. Furthermore, the late-born mitral cells extend stronger projections to the olfactory tubercule than the early-born cells do (Imamura et al. 2011). Timing of neurogenesis also separates premotor interneurons and their projections in the spinal cord (Tripodi et al. 2011). In the hippocampus, subpopulations of principal neurons that share the timing of neurogenesis also share the timing of synaptogenesis and show preferential connectivity with each other (Deguchi et al. 2011). Together, these studies underscore the impact of the timing of neurogenesis on later neuronal development.

Besides a neuron's birth time, synapse formation or elimination may be coupled to organismal development or to neuronal activity. This is illustrated by remodeling of synapses of DD neurons in *C. elegans*, which are eliminated from the ventral process at the end of the L1 stage and reform on the dorsal process. Mutations in *lin-14*, which controls the timing of cell-specific lineages, result in precocious DD remodeling (Hallam & Jin 1998). Neuronal activity also controls the timing of DD

remodeling, as it leads to increases or decreases in the expression of HBL-1, a transcription factor that controls remodeling and its timing (Thompson-Peer et al. 2012). Together, these studies reveal how temporal control over circuit connectivity is integrated into circuit function and into the developmental timing of the organism.

Temporal control over synapse specificity may act through the restriction of synaptic partner choices. For example, neurons may not be competent to assemble synapses during the phase of axonal migration, thereby avoiding ectopic connections while still establishing many cellular contacts. This hypothesis is supported by the timed expression of synapse priming factors by glial cells, which promote synapse formation in the CNS (Christopherson et al. 2005). One case in which the temporal control over synaptic partner choice is understood at the transcriptional level is in the *Drosophila* visual system, where the zinc-finger protein Sequoia (Seq) determines the competence of R7 and R8 to innervate their target layers in the medulla. R8 and R7 show consecutive peaks of elevated Seq expression, which correlate with the time of target layer innervations (**Figure 1*a***). Prolonged Seq expression in R8 redirected the R8 axons to the R7 recipient layer, and loss of Seq in R7 rerouted R7 axons to the R8 target layer. The effect of Seq on synapse formation is mediated through control of N-Cad expression. This example shows how precisely timed competence can generate specific synaptic connections (Petrovic & Hummel 2008).

After the initial contact between pre- and postsynaptic partners, synaptic maturation takes place. The importance of temporal regulation over the maturation process has recently been underscored by examination of synGAP mutant mice. synGAP is a RasGAP that localizes to postsynaptic sites (Chen et al. 1998, Kim et al. 1998), and haploinsufficiency for synGAP in humans leads to intellectual disability (Hamdan et al. 2009, Krepischi et al. 2010). In synGAP heterozygous mice, dendritic spine development in the hippocampus occurs prematurely, such that in the early postnatal period mutant spines are enlarged and show aberrant dynamics. Consequently, neurons from these animals are hyperexcitable, leading to behavioral abnormalities (Clement et al. 2012). Although before and after this critical period synGAP heterozygous neurons are similar to wild type, the behavioral deficits persist and cannot be rescued by a late expression of synGAP (Clement et al. 2012). This study underscores the importance of temporal regulation over synapse maturation and demonstrates how an early imbalance of excitatory/inhibitory transmission alters brain function irreversibly.

SYNAPSE ELIMINATION IN THE REFINEMENT OF SYNAPTIC CONNECTIVITY

Besides the precision of synaptic connections that are established during initial development, subsequent synapse elimination often plays a major role in shaping the final circuit connectivity. In principle, synapse elimination may be accompanied by axon loss, as is often the case in terminal boutons, or, in the case of en passant boutons, may modify the synaptic connectivity without altering the axonal projection pattern. Examples of the first case include synapse elimination and axon loss in the vertebrate NMJ (Buffelli et al. 2003, Colman et al. 1997, Sanes & Lichtman 1999), the climbing fiber-Purkinje neuron synapses (Crepel et al. 1976), thalamocortical axon–layer 4 neuron synapses (Hubel et al. 1977), and infrapyramidal mossy fiber axon-CA3 synapses in the hippocampus (Bagri et al. 2003, Liu et al. 2005). A known example for synaptic elimination without changes to the axonal pattern are the DD neurons in the *C. elegans* motor circuit, which eliminate their dorsal synapses before establishing connections on the ventral side (Hallam & Jin 1998, Thompson-Peer et al. 2012).

Synapse elimination may be dependent on synaptic activity, which seems to be the most prominent case in vertebrates, or may be genetically hardwired, as is seen more often in invertebrates.

Surprisingly, despite the fact that synapse elimination has been long recognized, molecular mechanisms have begun to emerge only recently. The best-studied case of synapse elimination involves the vertebrate NMJ, where during development each myotube is innervated by many axons, and through a process of synapse elimination and axon loss, the final one-to-one connectivity is established. During the process, the many inputs gradually decrease in synaptic strength, whereas a single input increases its strength (Colman et al. 1997). In situations where active and inactive axons compete, the active axon is always retained, suggesting that axons must be effective in innervating the muscle to be maintained (Buffelli et al. 2003). Blockade of synaptic activity throughout the junction prevents synapse elimination, as did forced synchronous activity in all fibers innervating the NMJ (Busetto et al. 2000, Sanes & Lichtman 1999). These results underscore the critical role of unequal ability across axons as a driving force in synapse eliminations. At present, molecular insight into the mechanisms that control synapse elimination in the NMJ is lacking. Activity-induced processing of BDNF has been suggested to be involved, with proBDNF promoting synapse loss and preBDNF promoting maintenance. However, BDNF knockout mice have a normal NMJ innervation pattern (Je et al. 2013).

Another well-studied scenario of synapse elimination occurs in the visual circuit, where a multitude of RGC axons initially innervate each dLGN (dorsal lateral geniculate nucleus) neuron. As the circuit matures, synapse elimination reduces the connectivity to one or two RGCs per dLGN neuron. This elimination is activity dependent, with a spontaneous retinal wave being responsible for the bulk of synapse elimination and sensory experience refining and maintaining connections (Hooks & Chen 2006). Surprisingly, the glia-secreted complement pathway proteins C1q and C3 are required for RGC-dLGN synapse elimination. In C1q mutants or C3-deficient mice, the eye-specific segregation of RGC axons in the LGN is defective, and LGN neurons maintain multiple innervations (Stevens et al. 2007). Microglia and astrocytes also actively engulf eliminated synapses, a process that requires the activity of phagocytic pathways in astrocytes (Chung et al. 2013, Schafer et al. 2012). These studies demonstrate the importance of nonneuronal cells in shaping neuronal connectivity through synapse elimination. In the cortex, regulation of synaptic glutamate levels by astrocytes also impacts activity-dependent spine elimination (Yu et al. 2013).

Interestingly, engulfment of excitatory and inhibitory synapses by astrocytes also occurs in the adult brain (Chung et al. 2013). Furthermore, C1q expression, which is associated with synapses during development and is low in the first postnatal week, increases dramatically during aging (Stephan et al. 2013). Thus, synapse elimination is not restricted to development and may play a role in cognitive decline during aging.

Regulation of synapse elimination by secreted or transmembrane molecules is not restricted to the dLGN. In the hippocampus, the infrapyramidal bundle (IPB) of the mossy fiber pathway undergoes significant pruning between birth and P30. This pruning was mediated by PlexA3 and neuropilin-2, as in their absence the IPB persists. Sema3F seems to be the relevant ligand in this signaling event (Bagri et al. 2003). Surprisingly, subsequent EM studies found that IPB pruning is intimately associated with synapse elimination: The IPB forms transient synapses onto basal dendrites of CA3 pyramidal cells in early postnatal development. In mice that lack PlexA3 signaling, these synapses are not removed, and thus pruning fails. These results underscore the link between synapse elimination and axonal pruning (Liu et al. 2005).

Molecular mechanisms operating in the pre- and postsynaptic compartments to mediate synapse elimination involve proteasomal degradation. As previously described, the *C. elegans* HSNL neuron makes en passant presynapses at the center of the vulva region. During development, the presynaptic material is distributed in a broad segment of the HSNL axon. This is followed by a stereotypic elimination process that eliminates all the anterior synapses and gives rise

to the mature synaptic pattern. The synapse-localizing adhesion molecules, SYG-1 and SYG-2, also control synapse elimination: In *syg-1* and *syg-2* mutants, synapse elimination is defective, and the anterior synapses persist. Furthermore, synapses that colocalize with SYG-1 are maintained, and those that do not are removed. Also, overexpression of SYG-1 protects the anterior synapses. These results indicate that SYG-1 provides a spatial cue in the HSNL neuron that marks where synapses are spared from elimination. Downstream of SYG-1, an E3 ubiquitin ligase complex consisting of SKR-1, Cullin, and the F-box protein sel-10 mediates synapse elimination, as shown by the persisting synapses in loss-of-function analysis. Interestingly, SYG-1 binding to SKR-1 disrupts the assembly of the complex (Ding et al. 2007). Thus, synapse elimination activity is distributed throughout the HSNL axon, and synapse protection localizes the future synapse to a spatial landmark.

At the postsynaptic side, synapse elimination also occurs through the proteasome pathway. In mouse hippocampal neurons, the transcription factor MEF-2 is required for activity-dependent synapse elimination (Flavell et al. 2006). The translation of MEF-2 transcripts is regulated by FMRP, as FMRP mutants show excess synapses, and FMRP is required for MEF-2-dependent synapse elimination (Pfeiffer et al. 2010). The effect of FMRP and MEF-2 on synapse elimination is mediated by Protocadherin-10, a target of both regulators. Protocadherin-10 facilitates the proteasomal degradation of ubiquitinated PSD-95, a major component of the postsynaptic density (Tsai et al. 2012). These studies identify a regulatory mechanism for activity-dependent postsynapse elimination by the proteasome.

CONCLUSION

The development of the mammalian nervous system entails the precise matching of trillions of synaptic partners. In this review, we presented a few developmental strategies and molecular pathways that pattern synaptic connections in select model systems. Evidently, development of even the simplest neuronal circuits necessitates the use of several mechanisms. Each of the mechanisms contributes to one aspect of connection specificity. For example, the synaptic domain of DA8 and DA in *C. elegans* is carved out by a combination of inhibitory signals in the form of long-range diffusible molecules and interactions of transmembrane molecules. Each cue prevents synapse formation in one segment of the axons based on its own gradient distribution. Likewise, development of the *Drosophila* visual system involves temporal regulation, contact-mediated repulsion, adhesive interactions, and inhibitory interactions of transmembrane molecules. These examples suggest that complex circuits can be finely patterned by the repeated use of a relatively small number of molecules and conceptually simple signaling mechanisms. In addition to the discovery of novel developmental strategies and signaling molecules, a major future challenge will be to understand their integration in the creation of complex neuronal circuits.

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