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Role of Leucine-Rich Repeat Proteins in the Development and Function of Neural Circuits

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axon guidance, target selection, synapse formation, cell adhesion, neural connectivity

Abstract

The nervous system consists of an ensemble of billions of neurons interconnected in a highly specific pattern that allows proper propagation and integration of neural activities. The organization of these specific connections emerges from sequential developmental events including axon guidance, target selection, and synapse formation. These events critically rely on cell-cell recognition and communication mediated by cell-surface ligands and receptors. Recent studies have uncovered central roles for leucine-rich repeat (LRR) domain-containing proteins, not only in organizing neural connectivity from axon guidance to target selection to synapse formation, but also in various nervous system disorders. Their versatile LRR domains, in particular, serve as key sites for interactions with a wide diversity of binding partners. Here, we focus on a few exquisite examples of secreted or membrane-associated LRR proteins in *Drosophila* and mammals and review the mechanisms by which they regulate diverse aspects of nervous system development and function.

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Contents

INTRODUCTION

Assembling a functional nervous system requires connecting neurons into circuits with extraordinary precision. To accomplish this specific connectivity, axons and dendrites must navigate toward their target regions, identify their appropriate target cells, and form synaptic contacts with these cells. Along their trajectory toward their synaptic targets, neurites are guided by an array of secreted and membrane-bound factors that help them navigate the complex extracellular environment and establish contacts with other cells. Among the factors regulating the development of neural circuits, proteins containing extracellular leucine-rich repeat (LRR) domains have recently emerged as key organizers of connectivity. The LRR is a protein-interaction motif that regulates axon guidance, target selection, synapse formation, and stabilization of connections. In addition, recent work implicates LRR proteins in disorders of the nervous system.

In this review, we highlight recent advances in our understanding of the role of LRR proteins in the development, function, and disorders of neural circuits. We focus on secreted and membrane-associated LRR proteins with LRRs in their extracellular domain (listed in **Table 1** and **Figure 1**). We have limited the discussion to the fly and mammalian nervous systems because most of the experimental work on the function of extracellular LRR (eLRR) proteins has been done in these two model systems.

THE LEUCINE-RICH REPEAT

The LRR, one of the most common protein domain repeats across species (Bjorklund et al. 2006), is a structural motif of 20 to 30 amino acids in length. The N-terminal part of the repeat consists of a conserved 11-residue sequence rich in leucines at defined positions (LxxLxLxxNxL, where x is any amino acid), although the leucine and asparagine residues can be substituted with other hydrophobic residues. This part of the motif forms a β-strand and a

Table 1 Leucine-rich repeat (LRR) proteins covered in this review[a]

LRR[b] proteins	Membrane topology	Aspects of neural circuit development	Binding partner
Slit, Slit1-3	Secreted	Axon guidance, dendrite arborization, target selection	Robo receptors
TrkA, TrkB, TrkC	Transmembrane	Axon guidance, neuronal survival, synapse formation	Neurotrophins (NGF, NT3, NT4, BDNF), RPTPσ (TrkC)
Linx/Islr2	Transmembrane	Axon guidance	TrkA, Ret
Capricious	Transmembrane	Target selection	Unknown
Tartan	Transmembrane	Target selection	Unknown
Connectin	GPI anchored	Target selection	Self
Toll	Transmembrane	Target selection	Spatzle
NGL1, 2, 3	Transmembrane	Synapse formation	Netrin-G1, -G2 (NGL1, 2, respectively), LAR family RPTPs (NGL3)
LRRTM1, 2, 3	Transmembrane	Synapse formation, synapse function, nervous system disorders	α- and β-neurexins (-S4) (LRRTM1, 2)
SALM2, 3, 5	Transmembrane	Synapse formation	Unknown
LGI1	Secreted	Synapse function, nervous system disorders	ADAM22
LGI4	Secreted	Myelination (PNS)	ADAM22
LINGO-1	Transmembrane	Myelination (CNS)	Unknown
NgR1	GPI anchored	Limiting plasticity, nervous system disorders	Nogo, MAG, OMgp
OMgp	GPI anchored	Limiting plasticity	NgR1
Slitrk1, 2	Transmembrane	Nervous system disorders	Unknown
LRRN3	Transmembrane	Nervous system disorders	Unknown
Tlr4	Transmembrane	Nervous system disorders	Pathogen-associated molecular patterns

[a]Red: *Drosophila* LRR proteins; blue: mammalian LRR proteins.

[b]Abbreviations: ADAM22, a disintegrin and metalloprotease domain 22; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; GPI, glycosylphosphatidylinositol; LAR, leukocyte common antigen-related; LGI, leucine-rich glioma inactivated; LINGO-1, LRR and Ig domain containing, Nogo-receptor-interacting protein 1; LRRN, LRR neuronal; LRRTM, LRR transmembrane; MAG, myelin-associated glycoprotein; NGF, nerve growth factor; NGL, netrin-G ligand; NT, neurotrophin; OMgp, oligodendrocyte-myelin glycoprotein; NgR, Nogo receptor; PNS, peripheral nervous system; RPTP, receptor protein tyrosine phosphatase; SALM, synaptic adhesion-like molecule; Slitrk, Slit and Trk-like; Tlr, Toll-like receptor.

loop region that connects with the C-terminal part of the repeat, which is more variable in sequence and structure (Kajava 1998; Kobe & Deisenhofer 1994, 1995a) (**Figure 2*a***). Individual LRRs are arrayed in tandems of two or more repeats that together constitute the LRR domain. The first crystal structure of a protein consisting entirely of LRRs, ribonuclease inhibitor, revealed that this arrangement in multiple repeats results in a curved, horseshoe-shaped structure (Kobe & Deisenhofer 1993) (**Figure 2*b***). The concave side of this structure is made up of a continuous β-sheet, to which each repeat contributes a β-strand. This is a defining feature of all LRR domains. The convex side of ribonuclease inhibitor consists of α-helices, but composition can vary substantially in other LRR proteins (Bella et al. 2008). The curved structure of the LRR domain and the exposed β-sheet on the concave side form a large binding surface, which makes the LRR domain a very effective protein-binding motif (Kobe & Kajava 2001). The crystal structure of ribonuclease inhibitor in complex with its ligand RNase A first demonstrated that globular ligands can fit in the concave space formed by

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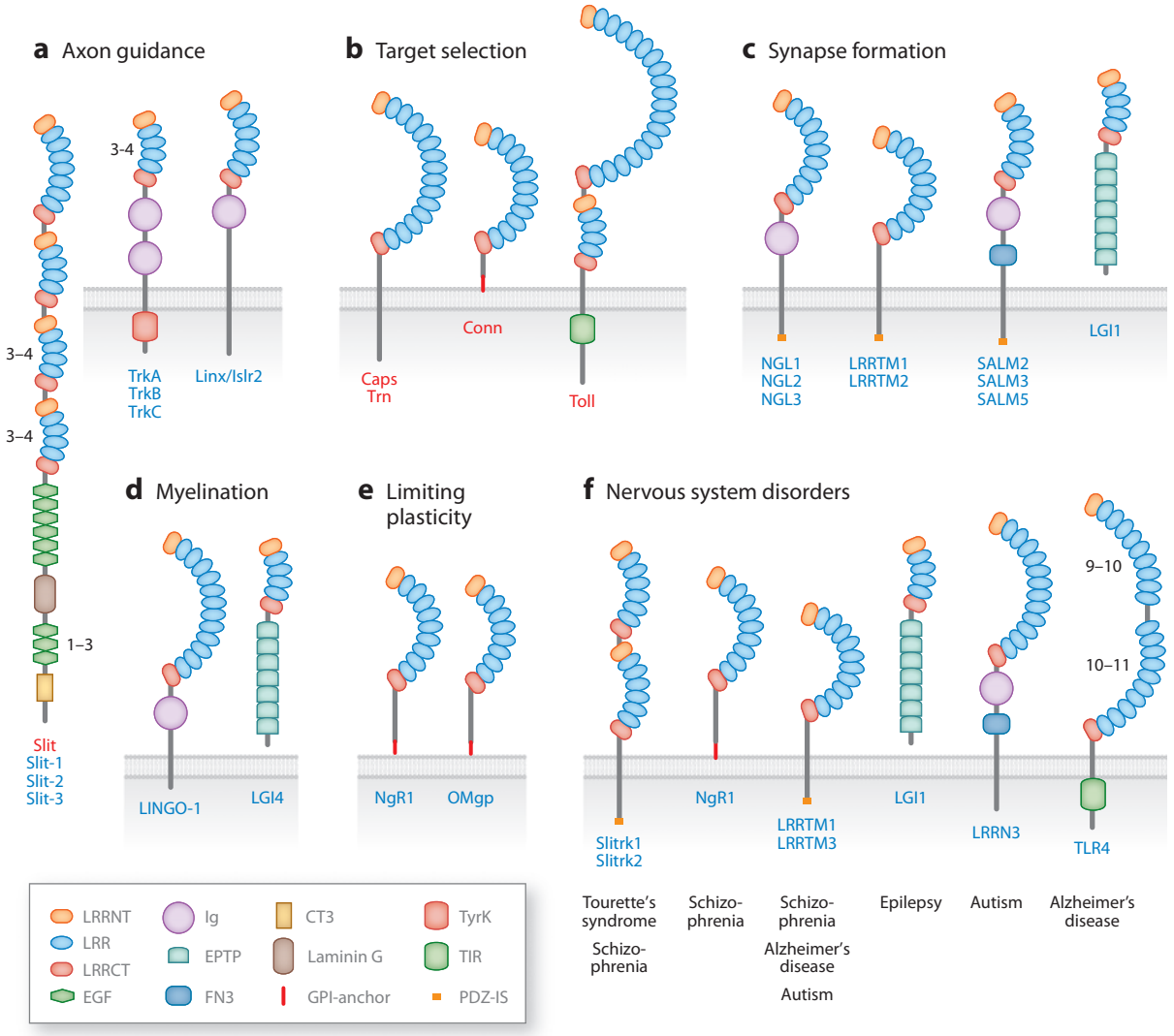

Figure 1

Overview of the domain organization of leucine-rich repeat (LRR) proteins regulating development and function of neural circuits. The schematic overview shows the neural LRR proteins discussed in this review, grouped by the cellular processes that they regulate. Protein names are indicated below the diagrams, in red for fly LRR proteins and in blue for mammalian LRR proteins. Domain abbreviations: CT3, cysteine-knot; EGF, epidermal growth factor-like; EPTP, epitempin; FN3, fibronectin type III; GPI, glycosylphosphatidylinositol; Ig, immunoglobulin-like; laminin G, laminin globular; LRRNT and LRRCT, LRR N- and C-terminal flanking domains; PDZ-IS, postsynaptic density protein (PSD-95), *Drosophila* disc large tumor suppressor (DlgA), and zonula occludens-1 protein (zo-1) interaction site; TIR, Toll/interleukin-1 receptor; TyrK, tyrosine kinase.

the LRR domain (Kobe & Deisenhofer 1995b). Many LRR proteins bind ligands with their concave surfaces (**Figure 2*e***), although exceptions exist (Bella et al. 2008). Variation in the length and number of repeats and in secondary structures on the convex side affects curvature of the LRR domain (**Figure 2*b–f***), and this in turn permits interaction with an enormous

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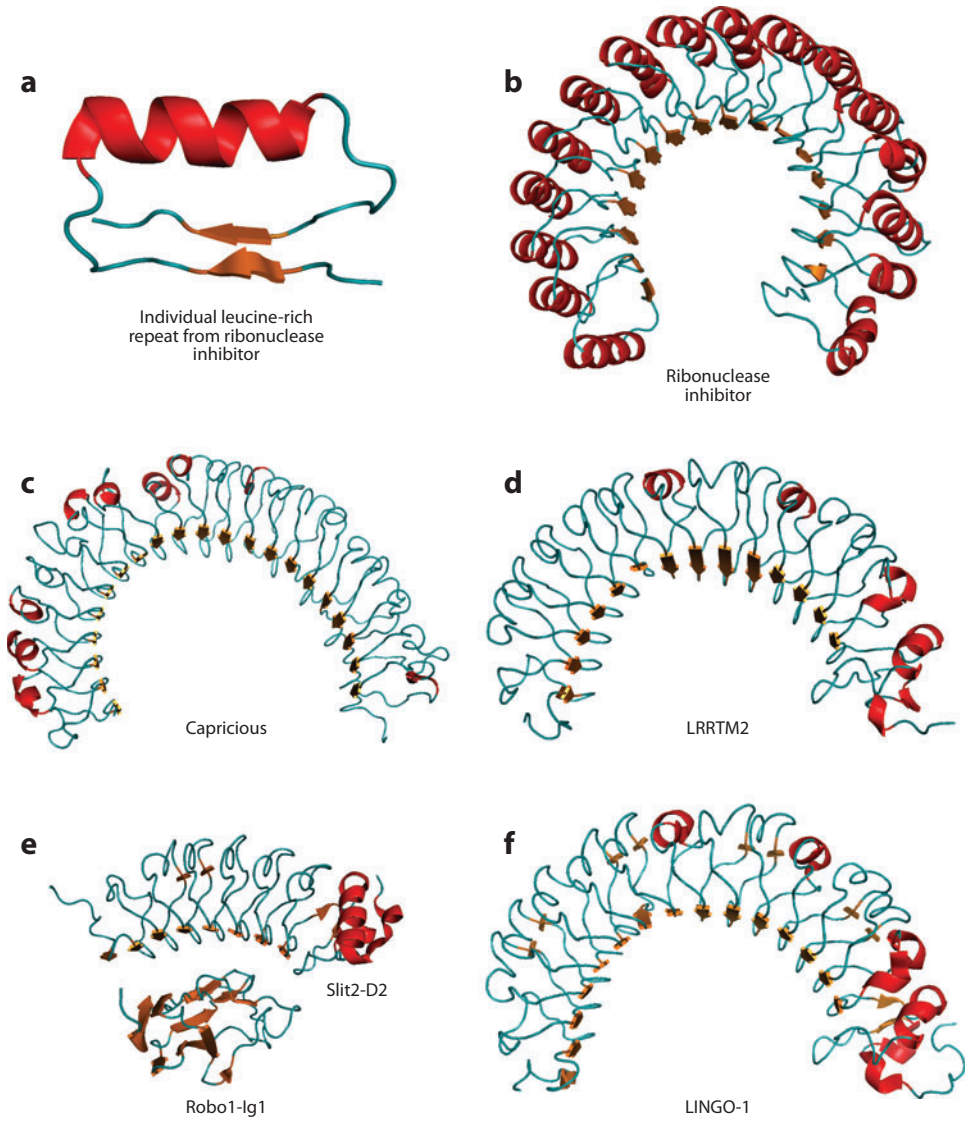

Figure 2

Structure of the leucine-rich repeat (LRR) domain. (*a*) Individual LRR from ribonuclease inhibitor (RI). The LRR is composed of a right-handed β-strand (*orange*) connected by a loop region (*teal*) to an α-helix motif (*red*) roughly parallel to the strand. The β-strand of the consecutive LRR is also shown. The color code is identical for all panels. (*b*) Ribbon diagram of the 3D structure of the porcine RI (PDB ID: 2BNH) (Kobe & Deisenhofer 1993). LRR domains rich in α-helices on the convex side show more pronounced curvature. (*c*) Homology model of the ectodomain of *Drosophila* Capricious. The model was built using the structure of the extracellular domain of the human Toll-like receptor 3 as a template (PDB ID: 1ZIW) (Choe et al. 2005). (*d*) Homology model of the ectodomain of human LRRTM2. The model was built using the structure of the extracellular domain of LINGO-1 as a template (PDB ID: 2ID5) (Mosyak et al. 2006). (*e*) Three-dimensional structure of the second LRR domain of Slit in complex with the first immunoglobulin-like (Ig) domain of Robo1 (PDB ID: 2V9T) (Morlot et al. 2007). (*f*) Three-dimensional structure of the LINGO-1 ectodomain (PDB ID: 2ID5). Online ModWeb version SVN.r1278 was used to search and build the models. Figure courtesy of Davide Comoletti, University of California, San Diego.

diversity of ligands (Bella et al. 2008, Buchanan & Gay 1996). Thus, the LRR domain is not only an efficient but also a very versatile protein-interaction motif.

LEUCINE-RICH REPEAT PROTEINS IN NEURAL DEVELOPMENT

Because the LRR domain is such an efficient structure for protein-ligand interactions, proteins with eLRR domains are well suited to regulate intercellular communication and cell adhesion. With increasing complexity of organisms, the evolutionary need for more molecules involved in adhesion and cell-cell communication arises (Hynes & Zhao 2000). A systematic comparative analysis of all eLRR genes has shown that, compared with worms (29 eLRR proteins), the eLRR superfamily has greatly expanded in mammals (135 eLRR proteins in mouse, 139 in human) and to a lesser extent in flies (66 eLRR proteins) (Dolan et al. 2007). Many of these eLRR genes are expressed in the nervous system and exhibit strikingly specific expression patterns, often labeling distinct subpopulations of neurons (Beaubien & Cloutier 2009, Homma et al. 2009, Hong et al. 2009, Lauren et al. 2003). The binding partners and functions of many of these eLRR proteins remain unknown. Among the eLRR proteins with identified binding partners, a remarkable structural variety in ligands exists, sometimes even within LRR subfamilies (Kim et al. 2006, Lin et al. 2003, Woo et al. 2009). This suggests that they can mediate diverse cellular interactions. The wide variety in expression patterns and ligand-binding specificity between members of the eLRR family strongly suggests that these proteins function in the patterning of neuronal connectivity.

Accumulating evidence from fly and mammalian systems shows that eLRR proteins indeed control key aspects of neural circuit formation (**Table 1**, **Figure 1**). In the early phases of nervous system development, LRR proteins regulate the guidance of axons and dendrites to their target area, mediate the selection of appropriate target cells within that area, and induce the formation of synaptic contacts onto these cells. In the maturing nervous system, LRR proteins regulate the myelination of axons and the stabilization of neuronal circuits. Their essential role in the assembly of neural circuits is further emphasized by the fact that several LRR proteins have been linked to human neurological and psychiatric disorders (Matsushima et al. 2005).

AXON GUIDANCE AND DENDRITE ARBORIZATION

Neurons extend axons that navigate over long distances toward their approximate target regions. Along their trajectories, growth cones are guided by attractive and repulsive cues in the extracellular environment via contact-dependent or -independent mechanisms. Neurons also elaborate dendritic branches to cover an area where they receive sensory and/or synaptic inputs. Extensive studies over the past 20 years have uncovered crucial functions of a number of signaling pathways, which act in a coordinated manner to provide a fine control for growth cone navigation and branching (Jan & Jan 2010, Kolodkin & Tessier-Lavigne 2011). This section discusses the role of LRR proteins in axon guidance and dendrite arborization and particularly focuses on Slit proteins in the *Drosophila* and mammalian nervous system and Trk receptors in the mammalian nervous system.

Slit and Robo Regulate Axon Midline Crossing

The LRR domain-containing Slits and their Robo receptors have been extensively studied as one of the most crucial ligand-receptor pairs regulating axon guidance. In addition to axon guidance, Slits and Robos control not only diverse physiological processes inside the nervous system, such as axon branching, dendrite morphogenesis, synapse formation, and neuronal cell migration, but also processes outside the nervous system, such as lung, mammary gland, and kidney development. The diverse functions of Slit and Robo have

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been reviewed in several articles (Dickson 2002, Dickson & Gilestro 2006, Hinck 2004, Huber et al. 2003, Ypsilanti et al. 2010). Here we focus on the functions of Slit/Robo in axon guidance and dendrite arborization, with an emphasis on recent advances.

The function of the *slit* and *robo* genes in axon pathfinding was first identified in *Drosophila* (Seeger et al. 1993). There is one *slit* gene in *Drosophila* and three in mammals, *slit1–3* (Dickson & Gilestro 2006); all of them encode large secreted proteins with a common domain composition and a high sequence similarity. Slits contain four LRR domains; each domain consists of an array of four to seven LRRs (Hohenester 2008; **Figure 1**). There are three *robo* genes in *Drosophila* (*robo*, *robo2*, and *robo3*) and four in mammals (*robo1–4*) (Dickson & Gilestro 2006). For clarity in this review, we refer to *Drosophila* Robo as Robo1, and use Robo as a generic name for any members in the family.

Genetic and biochemical evidence has established Slit as the ligand for the Robo receptors (Battye et al. 1999, Brose et al. 1999, Kidd et al. 1999, Li et al. 1999). The primary Slit-Robo binding sites are mapped to the first two Ig domains in Robo proteins and the second LRR domain in Slit proteins. All three *Drosophila* Robo proteins exhibit comparable binding affinity to the same LRR domain of Slit (Howitt et al. 2004, Liu et al. 2004, Morlot et al. 2007).

In the *Drosophila* ventral nerve cord and vertebrate spinal cord, both ipsilateral and commissural axons initially extend toward the midline, and only commissural axons cross to the contralateral side. Upon reaching the contralateral side, many commissural axons turn longitudinally, extending in parallel to the midline without recrossing it. During the initial extension step, the growing axons are attracted toward the midline by the chemoattractant Netrin, which signals through its DCC receptor. The subsequent steps, midline crossing and longitudinal extension, are controlled by the midline repellent Slit, which signals through the Robo family receptors (**Figure 3**; Dickson & Gilestro 2006).

In the *Drosophila* ventral nerve cord, Slit is expressed and secreted by midline cells (Rothberg et al. 1988, 1990) and signals through Robo1 to antagonize the action of the midline attractant Netrin and to prevent ipsilateral axons from crossing the midline (**Figure 3*a***; Battye et al. 1999, Brose et al. 1999, Kidd et al. 1999, Li et al. 1999). Interestingly, Robo1 is downregulated in the commissural axons, rendering these axons insensitive to the Slit repellent before crossing (**Figure 3*a***). This downregulation requires Commissureless (Comm), a putative endosomal sorting receptor that prevents most Robos from reaching the cell surface (Keleman et al. 2002, 2005; Kidd et al. 1998). After axons reach the contralateral side, Robo1 is upregulated as a result of downregulation of Comm, and axons become sensitive to Slit, which prevents them from stalling at the midline or recrossing it (**Figure 3*a***; Keleman et al. 2002).

The function of Robo1 in midline crossing requires its unique structural features, which were mapped to the CC1-2 motif in its cytoplasmic domain (Spitzweck et al. 2010). The CC2 motif in Robo1, which is absent in Robo2 and Robo3, interacts with several cytoplasmic factors, including Ena/VASP, the GTPase activating protein (GAP) Vilse/CrGAP, and the SH2-SH3 adaptor Dock (Bashaw et al. 2000, Fan et al. 2003, Hu et al. 2005, Lundström et al. 2004). Robo/Dock complexes also recruit Sos, a Rac guanine nucleotide exchange factor (GEF) (Yang & Bashaw 2006). Thus, coordinated actions of GEF and GAP modulate Rac-dependent cytoskeletal rearrangement in response to the Slit ligand.

Similar to the *Drosophila* Robos, mammalian Robo1–3 are also expressed in spinal cord neurons in a regionally restricted pattern (**Figure 3*b***; Brose et al. 1999, Kidd et al. 1998, Long et al. 2004, Sabatier et al. 2004). Robo1 and Robo2 are present at low levels before crossing but are upregulated after crossing. Surprisingly, Robo3.1, a splice isoform of Robo3, is localized in an opposite pattern: high before crossing and low after crossing (Chen et al. 2008, Sabatier et al. 2004). Analogous

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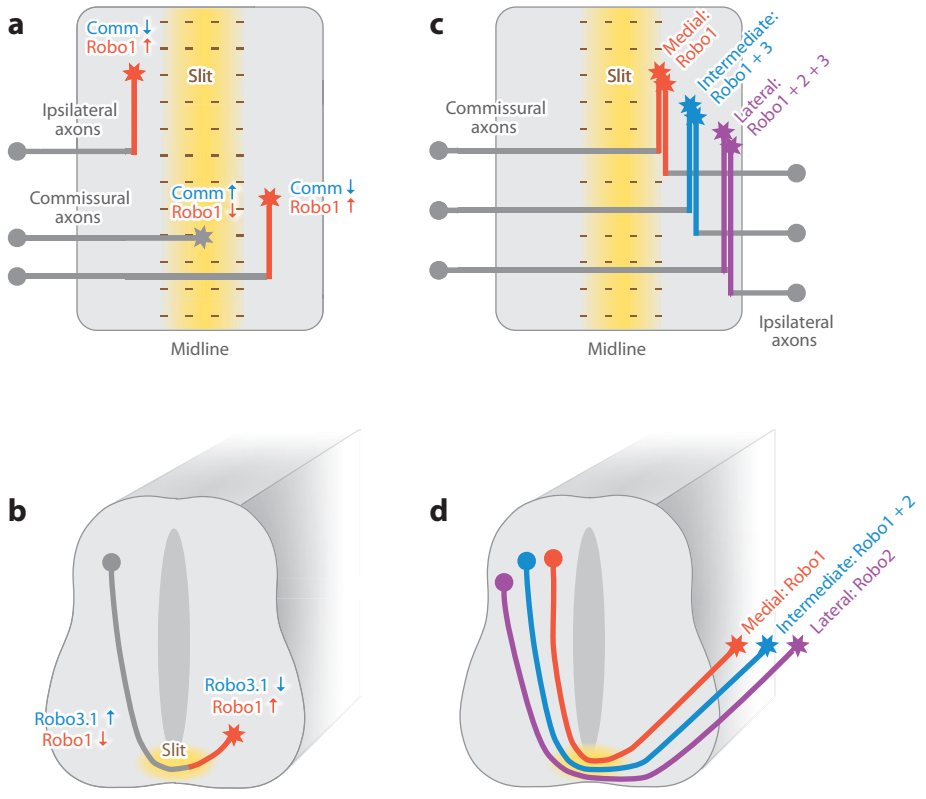

Figure 3

Slit/Robo regulates midline crossing and axon tract positioning in *Drosophila* and vertebrates. (*a*) Schematic view showing midline crossing of commissural axons in the *Drosophila* ventral nerve cord. Both ipsilateral and commissural axons initially extend toward the midline. Ipsilateral axons express the Robo1 receptor and are repelled by its Slit ligand to stay within the ipsilateral side. Commissural axons, however, downregulate Robo1 via a Comm-mediated mechanism. In the absence of Robo1, the axons are insensitive to the Slit repellent and are able to cross the midline. After commissural axons reach the contralateral side, Robo1 is upregulated as a result of downregulation of Comm, and axons become sensitive to Slit, preventing them from stalling at the midline or recrossing it. (*b*) Schematic view showing midline crossing of commissural axons in the vertebrate spinal cord. Robo1 regulates midline crossing of commissural axons in a similar manner. Analogous to *Drosophila* Comm, mammalian Robo3.1, a splice isoform of Robo3, downregulates Robo1 before midline crossing. (*c*) A Robo code for axon tract positioning in the *Drosophila* ventral nerve cord. Both ipsilateral and commissural axons extend longitudinally alongside the midline. These longitudinal tracts are divided into three discrete zones: a medial zone expressing only Robo1, an intermediate zone expressing both Robo1 and Robo3, and a lateral zone expressing all Robos. (*d*) A Robo code for axon tract positioning in the vertebrate spinal cord. Longitudinal axons extending along the lateral funiculus are separated into a medial zone expressing only Robo1, a lateral zone expressing only Robo2, and an intermediate zone expressing both.

to *Drosophila* Comm, Robo3.1 serves as a negative regulator of Robo1 before crossing the midline, rendering these axons insensitive to Slit repellent. Robo3.1 is downregulated upon crossing the midline, leading to an upregulation of Robo1 and Robo2 and making these axons sensitive to Slit (**Figure 3*b***).

Slit and Robo Regulate Axon Tract Positioning

In the *Drosophila* ventral nerve cord, both ipsilateral and commissural axons extend longitudinally alongside the midline, regardless of whether they cross the midline or not. Antibody staining against each Robo protein shows differential expression of three Robo proteins in these axon tracts, which can be divided into three discrete zones: a medial zone expressing only Robo1, an intermediate zone expressing both Robo1 and Robo3, and a lateral zone expressing all Robos (**Figure 3*c***; Rajagopalan et al. 2000, Simpson et al. 2000). Loss of function of *robo2* and *robo3* shifts the lateral axons medially, whereas overexpression of Robo2 and Robo3 shifts the medial axons laterally, suggesting that three Robos form a "Robo code" to instruct positioning of three distinct bundles (Rajagopalan et al. 2000, Simpson et al. 2000).

What is the underlying mechanism of this Robo code? Is each axon bundle specified by a unique combination of Robos, a single Robo with unique structural features, or the total level of all Robos? Positioning of the medial ($Robo1^+$) and intermediate ($Robo1^+/3^+$) bundles does not require unique structural features of Robo3, but it seems to respond to the total amount of Robo proteins, with axons expressing higher levels localized more laterally (Spitzweck et al. 2010). Positioning of lateral bundles ($Robo1^+/2^+/3^+$), however, does require unique structural features of Robo2 that were mapped to its extracellular Ig1 and Ig3 domains (Evans & Bashaw 2010, Spitzweck et al. 2010). Thus, distinct regulatory mechanisms involving differential expression levels and unique structural features likely contribute to positioning of different axon bundles.

All these proposed mechanisms involve a role for Slit as a repellent gradient so that axons expressing different Robos can respond differently to it. Indeed, removal of Slit abolishes the effect of Robo2 overexpression on shifting axons laterally, and removal of the Slit-binding domain in Robo2 exhibits a similar disruption (Evans & Bashaw 2010). This indicates a direct requirement for Slit in Robo2-dependent lateral positioning.

Lateral positioning of longitudinal axons also occurs in the vertebrate spinal cord and involves the differential expression of mammalian Robo1 and Robo2 (**Figure 3*d***; Long et al. 2004). Longitudinal axons extending along the lateral funiculus are separated into a medial zone expressing only Robo1, a lateral zone expressing only Robo2, and an intermediate zone expressing both. Loss of function of *robo1* or *robo2* causes a lateral or medial shift of axons, respectively, which supports an instructive Robo code mechanism in lateral positioning of these axons. Thus, Slit and Robo signaling regulate both midline crossing and axon tract positioning via mechanisms that are conceptually similar between *Drosophila* and mammals.

Slit and Robo Regulate Dendritic Arborization

Dendritic branches from distinct types of neurons form arborization patterns with specific size, density, and shape, allowing proper sampling and processing of diverse inputs (Jan & Jan 2010). Slit/Robo signaling also regulates dendrite branching in mammalian neurons (Whitford et al. 2002). Overexpression of Robo with a truncated cytoplasmic domain inhibits neurite branching in cultured neurons. The in vivo functions of Slit and Robo in dendrite arborization were shown in *Drosophila* motor neurons (Furrer et al. 2007) and dendritic arborization neurons (Dimitrova et al. 2008). Interestingly, loss of *robo1* in aCC motor neurons leads to a reduction of dendritic branching, whereas loss of *robo1* in dendritic arborization neurons results in a dendrite overbranching phenotype. Recent studies found

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that Robo1 is also involved in determining the specific branching patterns of *Drosophila* motor neuron dendrites (Brierley et al. 2009, Mauss et al. 2009). *Drosophila* motor neurons of different morphological classes elaborate dendrites at distinct positions in the ventral nerve cord along the medial-lateral axis. Loss of function of *robo1* leads to a medial expansion of dendritic branches, which suggests that Robo1 is required for specifying branching patterns at distinct positions.

Trk Receptors Regulate Axon Guidance

TrkA, TrkB, and TrkC, the receptors for neurotrophins, are a family of LRR domain-containing proteins (**Figure 1**). Neurotrophin and Trk receptor signaling play central roles in axon targeting, neuronal survival, and synaptogenesis in the vertebrate peripheral nervous system (PNS). TrkA is the primary receptor for nerve growth factor (NGF); TrkB is the primary receptor for both BDNF (brain-derived neurotrophic factor) and NT4; and TrkC is the primary receptor for NT3, although NT3 also activates TrkA and TrkB (Segal 2003). The functions of neurotrophins and their receptors, especially concerning their roles in neuronal survival, have been extensively reviewed (Bibel & Barde 2000, Huang & Reichardt 2001, Segal 2003). Here, we focus on recent studies on the functions of Trk receptors in axon extension and guidance.

Neurotrophins not only promote general axon outgrowth but also have chemotropic effects in steering directional growth of axons (Markus et al. 2002, McAllister et al. 1999, Song & Poo 1999). Recently, various genetically modified mouse models have provided a better understanding of the roles of neurotrophin signaling in the establishment of vertebrate peripheral neural circuitry.

In vertebrates, primary somatosensory neurons are located in the dorsal root ganglia and send both peripheral axon projections to specific targets, such as the skin and muscles, and central axon projections to second-order neurons in the central nervous system (CNS). Cutaneous nociceptive sensory neurons, for instance, express TrkA during development and project axons to both the skin and the spinal cord. NGF is secreted primarily from peripheral targets and is required for nociceptor innervation of the skin (Patel et al. 2000) and the establishment of a correct pattern of axonal projections in the spinal cord (Guo et al. 2011). By contrast, proprioceptive sensory neurons express TrkC and project axons to both muscle spindles and spinal motor neurons. In the absence of NT3-TrkC signaling, proprioceptive axons do not reach muscle targets or spinal motor neurons (Patel et al. 2003). Lastly, many cutaneous low-threshold mechanoreceptors express TrkB, and BDNF-TrkB signaling is required for proper axonal innervation of several types of cutaneous mechanosensory end organs (Perez-Pinera et al. 2008). Thus, evidence from multiple subtypes of mammalian somatosensory neurons has revealed a common requirement of target-derived neurotrophins during projection of sensory axons toward their respective central and peripheral targets.

In addition to their roles in the peripheral sensory system, signaling through Trk receptors is also essential for axon target innervation of postganglionic sympathetic neurons (Glebova & Ginty 2004, Kuruvilla et al. 2004). Here, NT3 is expressed by the intermediate targets of sympathetic neurons (the vasculature) and promotes extension of axons along their initial trajectory. NGF, by comparison, is expressed by the final targets (e.g., the kidney) and is necessary for axons of sympathetic neurons to reach their final targets. Interestingly, although both NGF and NT3 locally activate the receptor TrkA in sympathetic neuronal growth cones, only NGF initiates retrograde transport of signaling endosomes, which is required for neuronal survival (Kuruvilla et al. 2004). These observations illustrate how distinct neurotrophins can signal through a common receptor to achieve different functions.

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NGF and other neurotrophins both induce local signaling events at the growth cone and exert transcriptional control in the nucleus (Segal 2003). Although local signaling events such as phosphorylation of MEK/ERK are involved in axonal targeting (Zhong et al. 2007), in many cases regulation of gene expression is also important for establishment of neural circuitry. For instance, the transcription factor SRF (serum response factor), one downstream effector in the MEK/ERK pathway, mediates NGF-dependent axonal outgrowth through regulating gene expression (Wickramasinghe et al. 2008). In another intriguing example, NGF induces the transcription factor Hoxd1 in mammalian but not avian nociceptive neurons, and Hoxd1 instructs a pattern of nociceptive projections that is observed in mammals but not in other vertebrate species (Guo et al. 2011). Finally, in proprioceptive neurons, NT3-TrkC signaling promotes expression of the ETS transcription factor ER81, which plays an important role in axonal targeting of these neurons (Arber et al. 2000, Patel et al. 2003).

Recent data suggest that the Trk receptors may physically interact with other LRR proteins that can modulate the outcomes of Trk signaling. Linx (alias Islr2) was recently identified as a TrkA signaling component (Mandai et al. 2009). Linx is an LRR and Ig domain–containing transmembrane protein structurally related to Trk receptors (**Figure 1**), and many peripheral nociceptive neurons coexpress Linx and TrkA. Linx physically interacts with TrkA and modulates its activity to control axonal extension and targeting. Interestingly, several other LRR and Ig domain family members, including LINGO-1, Lrrc4b, AMIGO1, and LRIG1, are expressed in nonoverlapping subsets of sensory and motor neurons (Mandai et al. 2009). This raises the intriguing possibility that different members of LRR and Ig domain proteins encode target specificity for functionally distinct neuronal subsets in vertebrates, analogous to the role of LRR proteins in regulating neural connectivity in *Drosophila* (discussed below).

TARGET SELECTION

After navigating over long distances, neuronal growth cones reach their target regions and make final connections. In many brain regions, synaptic connections between dendrites and axons are organized into anatomically identifiable structural units, such as layers, columns, and glomeruli, where convergence and divergence of neuronal projections and specificity of connections emerge. Accumulating evidence suggests that the targeting of axons and dendrites usually arises from two sequential strategies: a global, approximate projection to broad target zones involving molecular gradients, followed by a local, precise targeting to distinct structural units involving discrete cues. Depending on individual contexts, specific neural circuits may predominantly utilize one strategy or the other, or a combination of both (Luo & Flanagan 2007). Both strategies rely heavily on cellular responses to extracellular cues mediated by cell-surface molecules. This section focuses on a few exquisite examples of LRR proteins in target selection, including Slit as a gradient cue in the mammalian olfactory system and Capricious (Caps) as a discrete cue in the *Drosophila* neuromuscular, olfactory, and visual systems.

Slit Controls Global Directional Targeting

Slit/Robo signaling controls directional targeting of mouse olfactory sensory axons (Cho et al. 2007). In the mouse olfactory system, axons that belong to the same class of olfactory receptor neurons (ORNs) converge onto two specific glomeruli in the olfactory bulb, the first olfactory processing center in the brain, which contains ~2,000 glomeruli (Luo & Flanagan 2007, Sakano 2010). Thus, selecting two among the ~2,000 glomeruli represents a striking specificity. Mammalian Robo2 is expressed in a dorsal-high and ventral-low gradient in ORN axon termini (Cho et al. 2007). Correspondingly, the Robo2 ligands, Slit1 and Slit3, are expressed in the ventral olfactory bulb, where ORN axons expressing low levels of Robo2

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terminate. Removal of Robo2 or Slit1 specifically causes a ventral shift of the axonal projections along the dorsal-ventral axis, which suggests that Slit/Robo repulsive signaling is involved in a dorsal-to-ventral segregation of ORN axons.

Capricious Regulates Discrete Targeting

Two well-known *Drosophila* LRR proteins involved in target selection are Caps and Tartan (Trn). Both are transmembrane proteins with 14 LRRs in their highly similar extracellular domains (65% identical and 79% similar) (**Figure 1**). Accumulating evidence has uncovered their critical roles in regulating targeting specificity of both axons and dendrites in a variety of places, including the *Drosophila* neuromuscular, visual, and olfactory systems (**Figure 4**).

Caps was first identified in the *Drosophila* neuromuscular junction, where it regulates muscle-specific targeting of motor neuron axons (Shishido et al. 1998). In each abdominal hemisegment of the *Drosophila* larval neuromuscular system, 36–40 identified motor neurons innervate 31 body-wall muscle fibers; the axons of each motor neuron stereotypically innervate a specific muscle or muscle group (Hoang & Chiba 2001, Ruiz-Cañada & Budnik 2006). Caps is expressed in a subset of muscle groups and the corresponding motor neurons that innervate them (**Figure 4*a***). In wild-type flies, muscle 12 expresses Caps and is innervated by the Caps-expressing ($Caps^+$) motor neuron RP5. In *caps* mutants, RP5 exhibits an ectopic innervation of Caps-negative ($Caps^-$) muscle 13 (Shishido et al. 1998) (**Figure 4*b***). Ectopic expression of Caps in all muscles or only muscle 13 attracts RP5 axons to form ectopic innervation (Shishido et al. 1998, Taniguchi et al. 2000) (**Figure 4*c***). These results indicate an important role of Caps in regulating neuromuscular connectivity. A recent overexpression screen identified the close paralog of Caps, Trn, which shows a similar axon mistargeting phenotype in its loss-of-function mutants (Kurusu et al. 2008). Interestingly, these mistargeting phenotypes in *caps* or *trn* single mutants are greatly enhanced in *caps trn* double mutants, which suggests that they are partially redundant (Kohsaka & Nose 2009, Kurusu et al. 2008).

The matching expression patterns of Caps in neurons and their target muscles, together with loss- and gain-of-function data, suggest a homophilic axon-target interaction of Caps in regulating motor axon targeting. Indeed, live-imaging experiments observed a concentration of Caps proteins at the tips of muscular filopodia (Kohsaka & Nose 2009). *caps trn* double mutants show a significant reduction of contacts between muscular filopodia and neuron growth cones, which supports a role of Caps and Trn in mediating direct interactions between pre- and postsynaptic partners.

Caps was later found to regulate layer-specific targeting of *Drosophila* photoreceptor axons (Shinza-Kameda et al. 2006). The *Drosophila* visual system is organized into parallel layers, where neurons of different hierarchical orders make specific connections (Sanes & Zipursky 2010). For instance, the photoreceptor cells R7 and R8 project their axons directly to one of the two distinct layers within the medulla, where they connect with specific interneurons and transmedullary neurons. Caps is expressed in R8 photoreceptor cells and their target layer M3 in medulla, but not in R7 cells and their target layer M6 (Shinza-Kameda et al. 2006) (**Figure 4*d***). Loss of *caps* in $Caps^+$ R8 cells causes their axons to invade inappropriate layers (**Figure 4*e***), whereas misexpression of Caps in $Caps^-$ R7 cells causes their axons to mistarget to the $Caps^+$ M3 layer (**Figure 4*f***). Together, these findings imply a homophilic interaction of Caps between R8 and its postsynaptic neurons in the target layer M3, although this has not been tested directly.

Caps and Trn also instruct glomerulus-specific targeting of dendrites in the *Drosophila* olfactory system (Hong et al. 2009). Similar to its mammalian counterpart, the *Drosophila* olfactory system is organized into discrete neural pathways (Luo & Flanagan 2007). The axons of ORNs and dendrites of projection neurons

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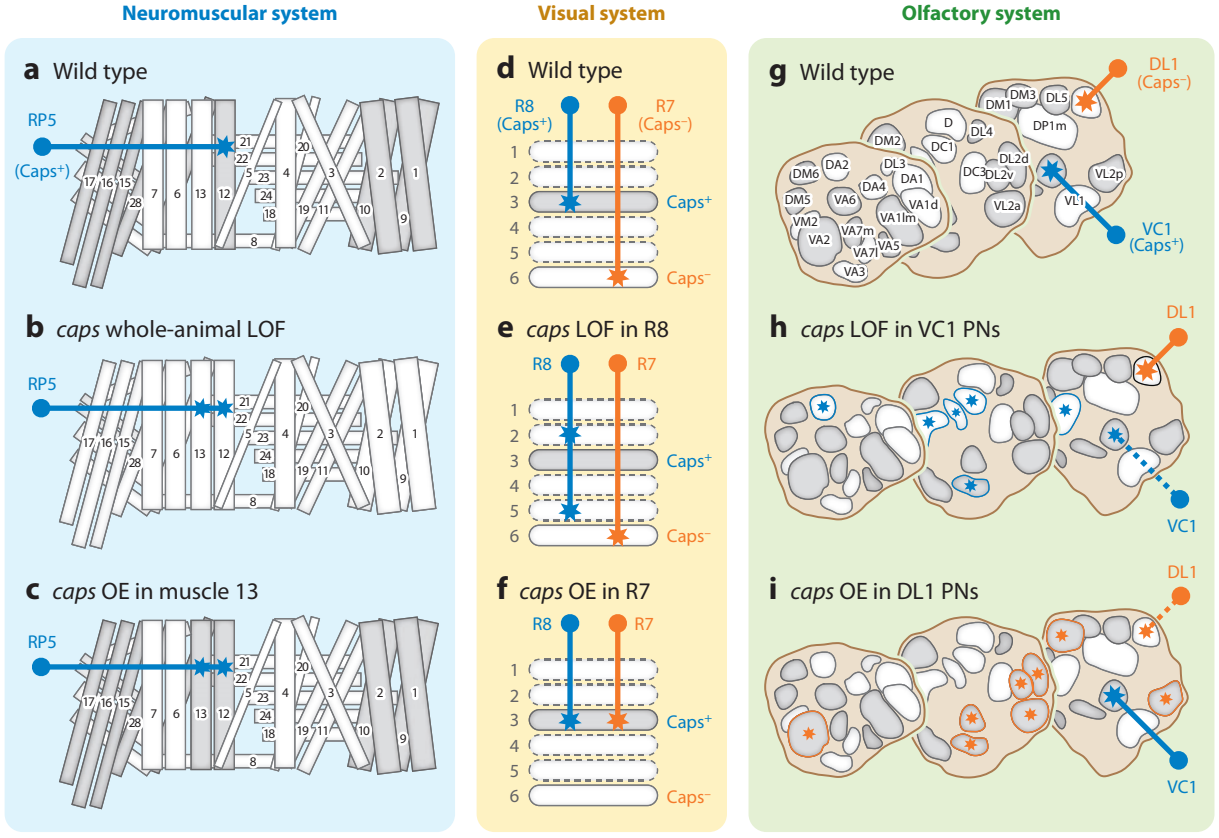

Figure 4

Discrete expression of Capricious (Caps) regulates target selection in the *Drosophila* neuromuscular, visual, and olfactory systems. (*a–c*) Caps regulates muscle-specific targeting of motor neuron axons. (*a*) Schematic view showing Caps expression pattern (*gray*) in fly larval body-wall muscles. In wild-type flies, Caps$^+$ motor neuron RP5 innervates muscle 12. (*b*) In *caps* whole-animal mutants, RP5 shows an ectopic innervation onto Caps$^-$ muscle 13. LOF, loss of function. (*c*) Ectopic expression of Caps in all muscles or only muscle 13 attracts RP5 to form ectopic innervation. OE, overexpression. (*d–f*) Caps regulates layer-specific targeting of *Drosophila* photoreceptor axons. (*d*) Schematic view showing Caps expression pattern in different medulla layers in *Drosophila* visual system. Caps is expressed in R8 photoreceptor cells and their target layer M3 (*gray*) but not in R7 cells and their target layer M6. (*e*) *caps* LOF in Caps$^+$ R8 cells causes their axons to invade inappropriate layers. (*f*) Caps misexpression in Caps$^-$ R7 cells causes their axons to mistarget to the Caps$^+$ M3 layer. (*g–i*) Caps instructs glomerulus-specific targeting of dendrites in the *Drosophila* olfactory system. (*g*) Schematic view showing differential expression of Caps in a subset of projection neurons (PNs) that innervate intercalated glomeruli (*gray*) in the antennal lobe (e.g., VC1 is Caps$^+$ and DL1 is Caps$^-$). (*h*) *caps* LOF in Caps$^+$ VC1 PNs causes their dendrites to invade glomeruli innervated by Caps$^-$ PNs. (*i*) Caps misexpression in Caps$^-$ DL1 PNs causes their dendrites to invade glomeruli innervated by Caps$^+$ PNs. Blue and orange stars in panels *a–i* indicate the targets of axons or dendrites.

(PNs) that belong to the same class make specific one-to-one connections in a single one of ~50 glomeruli in the *Drosophila* antennal lobe. Caps is differentially expressed in the dendrites of a subset of PNs that innervate intercalated glomeruli in the antennal lobe (Hong et al. 2009; **Figure 4*g***). Loss of *caps* in Caps$^+$ PNs causes their dendrites to invade glomeruli normally innervated by Caps$^-$ PNs (**Figure 4*h***), whereas misexpression of Caps in Caps$^-$ PNs causes their dendrites to invade glomeruli normally innervated by Caps$^+$ PNs (**Figure 4*i***). Caps, therefore, serves as part of a cell-surface code to provide a discrete identity to distinct PNs. Furthermore, the expression patterns of Caps in PNs and ORNs do not match each

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other, and genetic ablation of ORNs during development does not suppress the Caps misexpression phenotype, which suggests that Caps-mediated PN dendrite targeting is independent of ORNs.

With which ligand does Caps interact? Caps and Trn lack the capability to promote homophilic S2 cell aggregation in vitro (Milán et al. 2001, Shishido et al. 1998); however, a more recent study detected aggregation of Caps-expressing S2 cells (Shinza-Kameda et al. 2006), but only when the expression level of Caps is very high (A. Nose, personal communication). These contradictory results probably indicate a very weak, if any, homophilic interaction of Caps in vitro. Although expression patterns and genetic evidence in the neuromuscular system imply a Caps-Caps homophilic interaction in vivo, they cannot rule out a possible involvement of heterophilic interaction partners. Indeed, in the olfactory system, Caps seems to function through heterophilic ligands (Hong et al. 2009). Studies of Caps in boundary formation in the *Drosophila* wing imaginal disc also favor a heterophilic mechanism (Milán et al. 2001). Further identification of Caps ligand(s) will be essential to understand mechanistically how Caps functions across a variety of different systems.

Caps is one of the best examples of how the same molecule can be reused in multiple systems through a common underlying strategy. In the *Drosophila* neuromuscular, visual, and olfactory systems, Caps is differentially expressed in a discrete fashion and instructs targeting specificity of axons or dendrites in anatomically discrete structural units (muscles, layers, or glomeruli). On top of this common strategy, detailed cellular and molecular mechanisms vary depending on individual contexts. In the neuromuscular system, Caps likely mediates an axon-target interaction, whereas in the olfactory system, Caps likely mediates a dendrite-dendrite interaction.

The molecular identity in target selection could be encoded by either a single unique molecule or a combinatorial set of molecules expressed in distinct and partially overlapping patterns. The discrete expression of Caps in multiple classes of neurons and targets in the olfactory and neuromuscular systems favors the latter model (**Figure 4*a*,*g***). In the olfactory system, Caps is expressed in approximately half of the PN classes. Because Caps distinguishes only between Caps$^+$ PNs and Caps$^-$ PNs (Hong et al. 2009; **Figure 4*g***), additional cell-surface molecules must be involved in determining the targeting specificity among different Caps$^+$ PNs or among different Caps$^-$ PNs. Caps/Trn and additional cell-surface molecules likely form a combinatorial code to specify dendrite targeting of ~50 different PN classes in a 3D olfactory map.

Other Leucine-Rich Repeat Proteins Involved in Target Selection

Besides Caps and Trn, Connectin also regulates connectivity in the *Drosophila* neuromuscular system (Nose et al. 1992). Connectin is a membrane-anchored extracellular protein with 11 eLRR domains (**Figure 1**) and is expressed in a subset of muscles and the motor neurons that innervate them (Nose et al. 1992). Loss of *connectin* exhibits no phenotype in neuromuscular connectivity, although ectopic expression of Connectin in muscles alters the targeting of presynaptic axons (Nose et al. 1994, Raghavan & White 1997). This overexpression mistargeting phenotype can be suppressed by removing Connectin expression in neurons, which suggests that Connectin mediates homophilic interaction between neurons and muscles (Nose et al. 1997). The lack of a loss-of-function phenotype is likely due to a high redundancy of molecules involved in regulating connectivity.

In addition, Toll, a transmembrane protein with 22 eLRRs (**Figure 1**), was first identified in a genetic screen for genes involved in *Drosophila* embryo patterning (Nüsslein-Volhard & Wieschaus 1980). Since its original discovery, 8 other Toll-related receptors in *Drosophila*, 13 Toll-like receptors in mice, and 11 Toll-like receptors in humans have been identified (Valanne et al. 2011, West et al. 2006). Toll-like receptors utilize their LRR domains

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to recognize an extremely diverse repertoire of ligands (West et al. 2006). Although Toll and Toll-like receptors in both *Drosophila* and mammals are required primarily for the immune response, they are also expressed in the nervous system and are involved in both neural development and neurological diseases (Kielian 2009, Okun et al. 2009). One role of the *Drosophila* Toll receptor during development is to serve as a repulsive cue in the neuromuscular system (Inaki et al. 2010, Rose et al. 1997). Toll is differentially expressed in a subset of muscles and locally inhibits the innervation of motor neuron axons onto these muscles. In wild-type flies, Toll is preferentially expressed in muscle 13 but not in the neighboring muscle 12. In *toll* loss-of-function mutants, motor neurons that normally innervate muscle 12 form smaller synapses on muscle 12 and instead ectopically innervate muscle 13. Conversely, overexpression of Toll in muscle 12 reduces synapse formation of the motor neurons that innervate them. This inhibitory role of Toll is negatively regulated by the transcription factor Tey, which is specifically expressed in muscle 12 (Inaki et al. 2010). Because Toll is expressed only in muscles but not in motor neurons, it appears to interact with a heterophilic ligand (Rose et al. 1997). Spatzle has been identified as a ligand for Toll in both embryonic patterning and innate immunity (Belvin & Anderson 1996, Hoffmann 2003), and both Spatzle and Toll have been shown to have neurotrophic functions in *Drosophila* (Zhu et al. 2008). However, it is unclear whether Spatzle-Toll signaling is also involved in target selection.

A recent systematic overexpression screen of cell-surface and secreted molecules further identified a group of *Drosophila* LRR cell-surface proteins involved in neuromuscular system development, highlighting the crucial roles of LRR proteins in regulating target selection (Kurusu et al. 2008). In this study, 53 LRR proteins were screened, and 16 of these produced mistargeting defects, neuromuscular-junction defects, or both. These identified LRR proteins include not only known molecules such as Caps, Trn, and 18-wheeler but also novel genes such as *CG14351/Haf*, *CG8561*, and *CG3413/windpipe*.

The mechanisms for ensuring proper neural connectivity tend to be highly redundant. In both the neuromuscular and olfactory systems, for instance, *caps* or *trn* single mutants show a relatively mild mistargeting phenotype, which is greatly enhanced in *caps trn* double mutants (Hong et al. 2009, Kohsaka & Nose 2009, Kurusu et al. 2008). This kind of redundancy exemplified by Caps and Trn seems to be a general mechanism for complex neural circuits to increase their wiring fidelity and robustness. Identifying additional molecules will help us to better understand how neural circuits are specified and assembled during development.

SYNAPSE FORMATION AND FUNCTION

Following selection of the appropriate target cell, the initial contact between two synaptic partners is transformed into a functional synapse. Neurotransmitter release machinery is recruited to the presynaptic membrane, and neurotransmitter receptors and scaffold proteins are assembled into a dense protein network on the postsynaptic side, which results in precisely aligned pre- and postsynaptic membrane specializations to ensure reliable neurotransmission (reviewed in Jin & Garner 2008, McAllister 2007, Waites et al. 2005). Recent evidence indicates that LRR proteins, both membrane-bound and secreted, are key regulators of these processes.

SALMs, NGLs, and LRRTMs Regulate Synapse Formation in Vertebrates

Three eLRR protein families have recently been implicated in vertebrate synapse formation: the SALMs (synaptic adhesion-like molecules), NGLs (netrin-G ligands), and LRRTMs (leucine-rich repeat transmembranes). All are eLRR-containing type I transmembrane synaptic adhesion molecules. The extracellular domain of LRRTMs exclusively

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contains LRRs, whereas SALMs and NGLs contain additional protein domains (**Figure 1**). Proteins in all three families are predominantly found on the postsynaptic membrane of excitatory synapses (**Figure 5*a***). Interestingly, no invertebrate homologs exist for these proteins, which suggests that they serve functions specific to the vertebrate nervous system.

SALMs and NGLs were identified in yeast two-hybrid screens for proteins interacting with postsynaptic density PDZ protein SAP97 or PSD-95 (Kim et al. 2006, Ko et al. 2006, Wang et al. 2006). LRRTMs were identified in an expression screen for proteins that can induce presynaptic differentiation in axons (Linhoff et al. 2009). Overexpression and knockdown studies in cultured hippocampal neurons have shown that SALM2 (Ko et al. 2006), NGL-2 (Kim et al. 2006) and NGL-3 (Woo et al. 2009), and LRRTM2 (de Wit et al. 2009, Ko et al. 2009) all selectively regulate the density of excitatory synapses. In the case of LRRTM2, the LRR domain is required for these effects (de Wit et al. 2009). Overexpression of SALM3 and SALM5 in hippocampal neurons increases the density of both excitatory and inhibitory synapses, and SALM5 knockdown decreases excitatory as well as inhibitory synapse density (Mah et al. 2010), which suggests that some LRR-containing synaptic adhesion molecules may also act at inhibitory synapses.

Although SALMs, NGLs, and LRRTMs appear to have overlapping functions in synapse formation in culture, functional differences among these three protein families exist. SALMs, NGLs, and LRRTMs regulate postsynaptic differentiation by recruiting key components of the synaptic machinery to the postsynaptic density. All contain PDZ-interaction sites at their C termini that bind the scaffold protein PSD-95 and related MAGUK family proteins (de Wit et al. 2009, Kim et al. 2006, Ko et al. 2006, Linhoff et al. 2009, Morimura et al. 2006; **Figure 5*a***). Artificial aggregation of overexpressed SALMs, NGLs, or LRRTMs on the surface of dendrites results in the clustering of an overlapping complement of postsynaptic proteins that, besides PSD-95, also consists of glutamate receptor subunits

Figure 5

Transmembrane and secreted leucine-rich repeat (LRR) proteins regulate synapse formation and function in the vertebrate nervous system. (*a*) Schematic view of the postsynaptic adhesion molecules NGL, LRRTM, and SALM and their known presynaptic binding partners. NGL-3 binds to the first two FN3 repeats in LAR (Kwon et al. 2010). NGL-1 and NGL-2 bind Netrin-G1 and Netrin-G2, respectively. LRRTM1 and LRRTM2 bind to both α- and β-neurexins that lack a small insert at splice site 4 (S4) in the LNS domain (Ko et al. 2009, Siddiqui et al. 2010). Only β-neurexins are shown. The presynaptic partners for SALM proteins are not known. The postsynaptic LRR proteins and their presynaptic partners form a transsynaptic complex that bridges the synaptic cleft and recruits essential scaffolding molecules to both sides of the junction as well as other components of the synaptic machinery, such as neurotransmitter receptors. (*b*) Schematic view of LGI1 and its binding partner ADAM22. On the postsynaptic side, LGI1 binds ADAM22 in a complex with PSD-95, Stargazin (STG), and AMPARs and enhances AMPAR-mediated synaptic transmission. On the presynaptic side, LGI1 blocks rapid inactivation of Kv1.1 channels by the Kvβ1 subunit through a mechanism that is not well understood. LGI1 is depicted here as secreted from the presynaptic terminal; little is known about the location and mode of LGI1 secretion. Domain abbreviations: C′, C-terminal domain; CaMK, Ca^{2+}/calmodulin-dependent kinase; CC, coiled coil; CHO, carbohydrate attachment; CR, cysteine-rich; D1 and D2, membrane-proximal (catalytically active) and -distal (inactive) tyrosine phosphatase domains; DI, disintegrin; GPI, glycosylphosphatidylinositol; GuK, guanylate kinase domain; Ig, immunoglobulin-like; LEGF, laminin epidermal growth factor-like motifs 1–3; LGI1, leucine-rich glioma inactivated 1; LNS, laminin-α/neurexin/sex-hormone-binding globulin (also known as laminin G domain); LRRNT and LRRCT, LRR N- and C-terminal flanking domains; LRRTM, LRR transmembrane; MP, metalloprotease domain (inactive); NGL, netrin-G ligand; PDZ-IS, postsynaptic density protein (PSD-95), *Drosophila* disc large tumor suppressor (DlgA), and zonula occludens-1 protein (zo-1) interaction site; Pro, propeptide; SALM, synaptic adhesion-like molecule; SAM, sterile α motif; SH3, Src homology 3; VI, laminin N-terminal.

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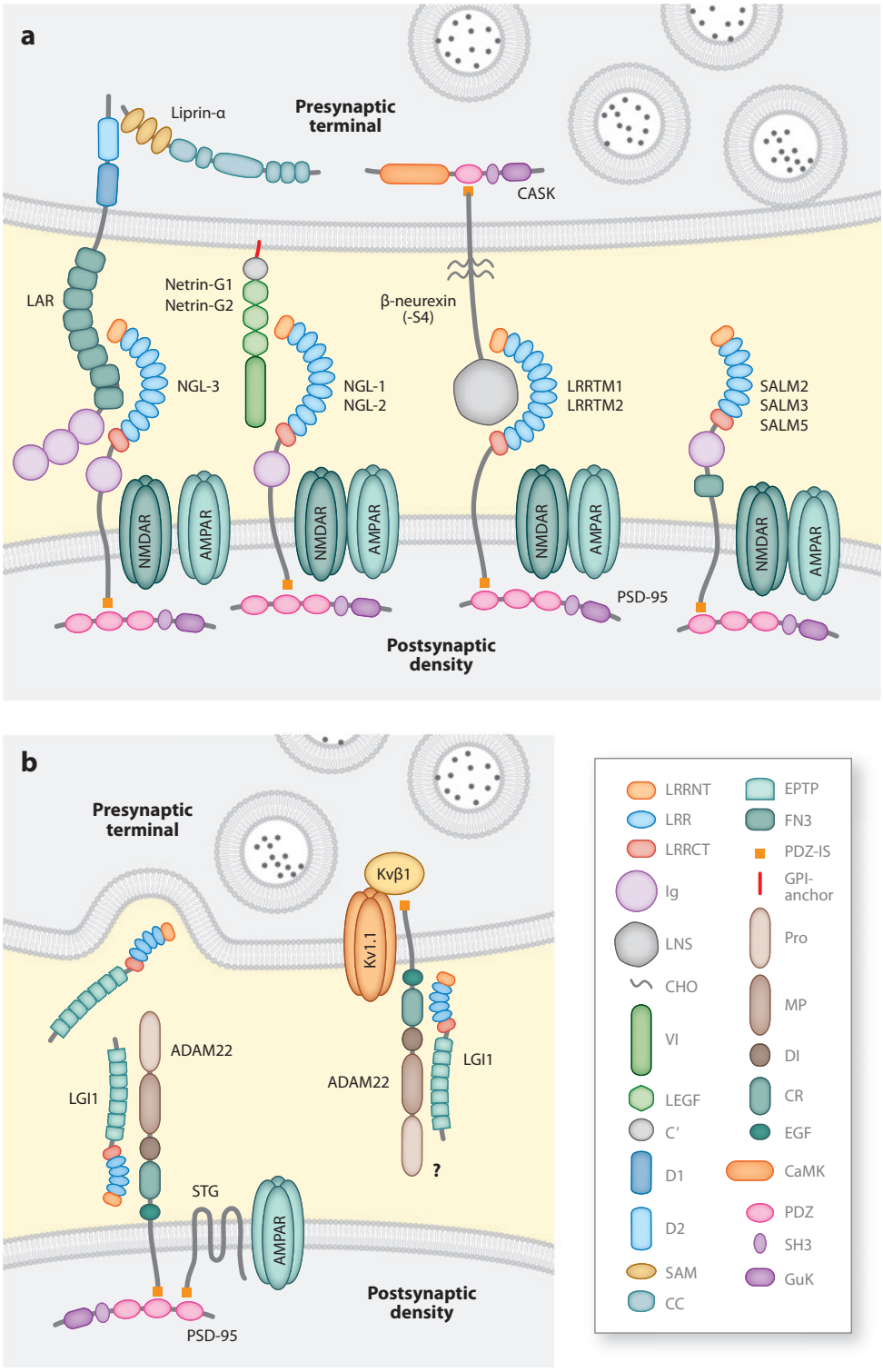

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such as NR1 (Kim et al. 2006, Ko et al. 2006, Linhoff et al. 2009, Woo et al. 2009). It appears likely that these proteins are recruited through intracellular interactions of the LRR proteins' cytoplasmic tails, but experiments in heterologous cells suggest that SALM1 and LRRTM2 can also bind to glutamate receptor subunits through their extracellular domains (de Wit et al. 2009, Wang et al. 2006). Subtle differences in the sets of recruited proteins exist. Aggregation of NGL-3 induces clustering of the AMPA receptor (AMPAR) subunit GluR2, whereas aggregation of NGL-2 does not (Kim et al. 2006, Woo et al. 2009). Aggregation of SALM3 recruits PSD-95, whereas SALM5 does not (Mah et al. 2010). This suggests that individual LRR-containing synaptic adhesion molecules may differ in their ability to recruit specific components of the postsynaptic protein complex.

Another shared feature of these LRR-containing synaptic adhesion molecules is the ability of some family members to trigger presynaptic differentiation in contacting axons of neurons when these proteins are expressed on the surface of cocultured heterologous cells (Biederer & Scheiffele 2007, Scheiffele et al. 2000). However, the underlying molecular mechanisms are very different between subfamilies. In the NGL family, for instance, NGL-3 is a much more potent inducer of presynaptic differentiation than are NGL-1 and NGL-2 (Woo et al. 2009), and this is reflected in their respective presynaptic binding partners. NGL-3 binds the receptor protein tyrosine phosphatase (RPTP) LAR (leukocyte common antigen-related) (Woo et al. 2009; **Figure 5*a***), and it also interacts with LAR family members RPTPδ and RPTPσ (Kwon et al. 2010). The NGL-3 LRR domain is required for these interactions (Kwon et al. 2010). LAR family RPTPs bind the cytoplasmic protein liprin-α (Pulido et al. 1995, Serra-Pages et al. 1995), a synaptic scaffolding protein critical for synapse formation (reviewed in Spangler & Hoogenraad 2007, Stryker & Johnson 2007), suggesting one potential mechanism by which LAR family RPTPs can couple transsynaptic adhesion to presynaptic differentiation. NGL-1 and NGL-2, by contrast, bind Netrin-G1 and Netrin-G2, respectively, through their LRR domains (Kim et al. 2006, Lin et al. 2003; **Figure 5*a***). Netrin-Gs are glycosylphosphatidylinositol (GPI)-anchored proteins expressed on the surface of distinct axonal subsets (Nakashiba et al. 2000, 2002; Yin et al. 2002). Binding of NGL-2 to Netrin-G2 by itself is not sufficient to trigger presynaptic differentiation (Kim et al. 2006), which suggests the existence of additional signal-transducing coreceptors for Netrin-Gs that are yet to be identified. Thus, even within a single subfamily, LRR proteins can show remarkable diversity in their binding partners.

Three labs independently identified the presynaptic adhesion molecule neurexin as the binding partner for LRRTM1 and LRRTM2, using affinity chromatography (de Wit et al. 2009, Ko et al. 2009) or a candidate screening approach (Siddiqui et al. 2010; **Figure 5*a***). Neurexins are best known for their binding to the neuroligin family of postsynaptic adhesion molecules (Ichtchenko et al. 1995, Song et al. 1999) to form a transsynaptic adhesion complex critical for synapse development and function (Sudhof 2008). The finding that neurexins also form a transsynaptic complex with LRRTMs came as a surprise, because LRRTMs are structurally unrelated to neuroligins. The neurexin genes undergo extensive alternative splicing, potentially generating thousands of isoforms with different extracellular domains that may be involved in establishing specific synaptic connectivity (Ullrich et al. 1995). The neurexin cytoplasmic tail interacts with the synaptic PDZ protein and neurexin kinase CASK (Hata et al. 1996, Mukherjee et al. 2008), which likely serves as a key scaffold molecule to mediate assembly of the presynaptic machinery. Whether LRRTM3, which has a very limited capacity to induce presynaptic differentiation (de Wit et al. 2009, Linhoff et al. 2009), also binds neurexins or whether additional receptors exist for LRRTMs remains to be determined.

Presynaptic binding partners for SALMs have not yet been identified. Of the five SALM

family members, only SALM3 and SALM5 induce presynaptic differentiation in cocultured axons (Mah et al. 2010). SALM5 displays homophilic and transcellular adhesion, but SALM3 does not (Seabold et al. 2008), which suggests the presence of additional receptors on the presynaptic membrane. Whether these are LAR family RPTPs, neurexins, or other proteins is not known.

Interestingly, TrkC, the receptor for the neurotrophin NT3 (see above), was recently found to induce excitatory presynaptic differentiation in cocultured hippocampal neurons (Takahashi et al. 2011). The synaptogenic activity of TrkC is unique among the Trk neurotrophin receptors, requires its LRR domain and Ig1 domain (**Figure 1**), and is independent of the NT3-binding Ig2 domain. Postsynaptic TrkC transsynaptically binds to presynaptic RPTPσ and is required for excitatory synapse formation in vitro and in vivo (Takahashi et al. 2011). These findings highlight a dual role for TrkC as a neurotrophin receptor and a transsynaptic adhesion molecule as well as show that RPTPs, similar to neurexins, interact with multiple postsynaptic ligands.

The role of SALM, NGL, and LRRTM proteins in regulating synapse function is still largely unknown. Knockdown of *LRRTM2* in hippocampal granule cells in vivo decreases the strength of glutamatergic synaptic transmission at the perforant path-granule cell synapse (de Wit et al. 2009), which indicates that LRRTM2 is required for synaptic transmission at this synapse. Analysis of synapse function in *NGL-2* (Zhang et al. 2008) or *LRRTM1* (Linhoff et al. 2009) knockout mice has not yet been reported. Assessing the role of these proteins in regulating synaptic function remains a major goal for future studies.

What could be the purpose of such a large array of LRR-containing synaptic adhesion molecules? As in the fly nervous system, part of the answer may lie in functional redundancy to ensure reliable synaptic connectivity (Brose 2009). However, the differential recruitment of postsynaptic proteins and coupling to distinct presynaptic adhesion systems by postsynaptic LRR proteins strongly suggest that the diversity of synaptic LRR proteins has functional significance. Analogous to the specific matching of axons with select targets in the fly nervous system, LRR proteins could also function to establish connectivity between specific synaptic partners in the mammalian CNS. There is enormous structural and functional variety in CNS synapses, and the eLRR-containing protein family appears well suited to contribute to the control of synaptic specificity in the brain. Evidence for such synapse-specific roles of synaptic LRR proteins is still scarce, but there are some intriguing hints. NGL-1, NGL-2, and LRRTM2 all show lamina-specific distributions in the hippocampus, and loss of *LRRTM1* alters the distribution of presynaptic VGlut1 puncta in a lamina-specific manner (Linhoff et al. 2009, Nishimura-Akiyoshi et al. 2007). As hippocampal laminae differ with respect to the subtypes of synapses they contain, these observations suggest that at least some synaptic LRR proteins may function at specific subsets of synapses. It will be interesting to determine how loss of LRR proteins at these synapses affects the formation and function of neural circuitry.

LGI1 Regulates Synapse Function

Whereas characterization of the role of transmembrane LRR proteins in synapse function awaits further study, a secreted LRR protein, LGI1 (leucine-rich glioma inactivated 1), has recently emerged as an important regulator of excitatory synaptic transmission. The LGI family consists of four members, LGI1–4, all of which are secreted neuronal glycoproteins (Senechal et al. 2005, Sirerol-Piquer et al. 2006; **Figure 1**). LGI1 was identified as a component of affinity-purified pre- and postsynaptic protein complexes using mass spectrometry (Fukata et al. 2006, Schulte et al. 2006). LGI1 copurifies with immunoprecipitated PSD-95, together with the catalytically inactive transmembrane metalloprotease ADAM22 (a disintegrin and metalloprotease domain 22) and the AMPAR regulatory protein Stargazin

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(Fukata et al. 2006). Secreted LGI1 serves as a ligand for ADAM22, which in turn binds PSD-95 through its C-terminal PDZ-interaction site (**Figure 5*b***). Exogenously applied LGI1 increases the strength of AMPAR-mediated synaptic transmission in hippocampal slices, whereas loss of *LGI1* in knockout mice reduces AMPAR-mediated transmission (Fukata et al. 2006, 2010). This suggests that binding of LGI1 to ADAM22 may enhance AMPAR-mediated synaptic transmission by stabilizing a PSD-95 scaffolded ADAM22/Stargazin/AMPAR complex at the synapse.

A second study identified LGI1 in a complex with affinity-purified Kv1.1 (Schulte et al. 2006), a voltage-gated presynaptic potassium channel subunit that modulates neuronal excitability and neurotransmitter release probability. Rather than LGI1 serving as an extracellular ligand, however, the authors proposed a cytosolic mechanism for LGI1 in blocking rapid inactivation of Kv1.1 channels by the cytoplasmic regulatory Kvβ1 subunit. Consistent with a role for LGI1 in regulating presynaptic function in a Kv1.1-dependent manner, transgenic mice overexpressing *LGI1* show a decrease in presynaptic release probability at hippocampal perforant path-granule cell synapses that can be reversed with a Kv1-specific blocker (Zhou et al. 2009).

LGI1's proposed intracellular role in modulating presynaptic Kv1 channel activation seems difficult to reconcile with LGI1's extracellular role in regulating postsynaptic AMPAR-mediated transmission. A naturally occurring, C-terminally truncated isoform of LGI1 is retained intracellularly (Sirerol-Piquer et al. 2006). However, this isoform does not seem capable of blocking Kv1.1 inactivation, given that experimental C-terminal truncations of LGI1 fail to block Kvβ1-mediated Kv1.1 inactivation (Schulte et al. 2006). Alternatively, extracellular LGI1 could be part of the presynaptic Kv1.1 complex through an interaction with an intermediate transmembrane protein. ADAM22 and ADAM23 are candidates, as both proteins interact with Kv1 (Fukata et al. 2010, Ogawa et al. 2010) and bind LGI1 (Fukata et al. 2006, Owuor et al. 2009, Sagane et al. 2008; **Figure 5*b***). However, the exact subcellular localization (pre- versus postsynaptic) of ADAM22 and ADAM23 has not been unequivocally demonstrated. Taken together, recent evidence indicates that LGI1 acts both pre- and postsynaptically to regulate synaptic function and maturation, but fundamental questions regarding the precise site and mode of action remain to be answered. It will be important to identify the exact mechanisms by which the LGI1/ADAM complex controls synaptic function, as mutations in *LGI1* cause an inherited form of epilepsy (see below).

MYELINATION

A hallmark of vertebrate nervous system maturation is the formation of a myelin sheath around nerve fibers, which allows the rapid propagation of action potentials along the axon. Myelin is critical for normal functioning of the nervous system, and defects in myelin sheath formation result in neurological disorders. The process of myelination requires tightly regulated cell-cell signaling between the axon and myelin-producing glial cells: Schwann cells in the PNS and oligodendrocytes in the CNS (Emery 2010, Nave 2010). Cell-surface and secreted LRR proteins have been identified as important regulators of myelination in the PNS and CNS.

The LGI/ADAM signaling module is also employed in the PNS, but here it regulates an entirely different cellular process. *LGI4* was originally identified as the affected gene in the *claw paw* strain of mutant mice, which show hypomyelination in their PNS, but not their CNS (Bermingham et al. 2006). The *claw paw* mutation in *LGI4* results in an internal deletion in the protein and abolishes secretion of LGI4. The resulting hypomyelination in mutant Schwann cell–neuron cocultures can be rescued by exogenous application of LGI4 (Bermingham et al. 2006), which indicates that LGI4 acts extracellularly to regulate myelin formation by Schwann cells. A subsequent

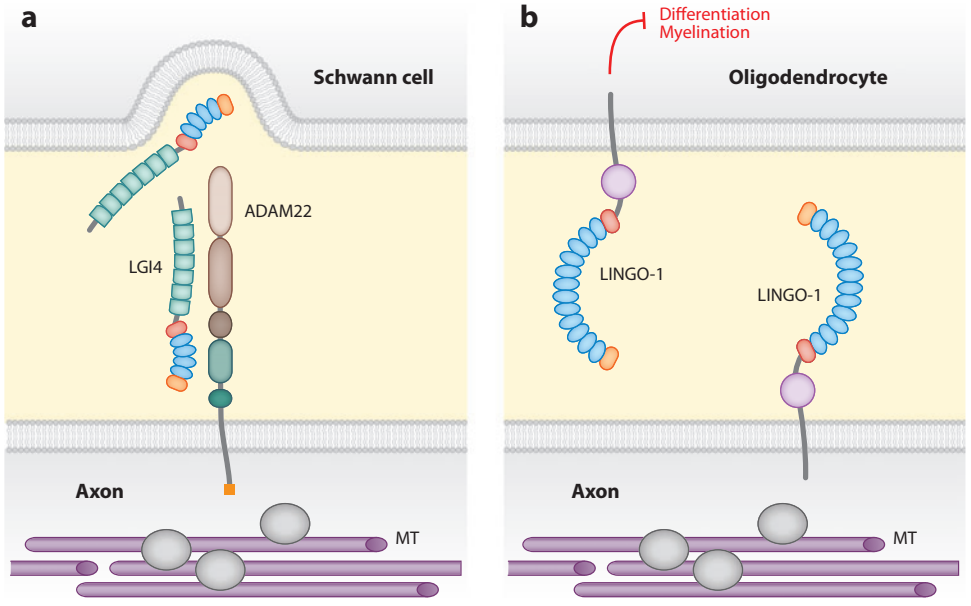

Figure 6

Transmembrane and secreted leucine-rich repeat (LRR) proteins regulate myelination in the vertebrate nervous system. (*a*) LGI4 (leucine-rich glioma inactivated 4) regulates myelination in the peripheral nervous system. LGI4 is secreted from Schwann cells and binds to ADAM22 on axons. This directs myelination of axons by Schwann cells through an unidentified mechanism. (*b*) LINGO-1 is a negative regulator of myelination in the central nervous system. LINGO-1 expressed on oligodendrocytes inhibits oligodendrocyte differentiation and myelination through the downstream signaling molecules RhoA and Fyn kinase. LINGO-1 expressed on axons can also inhibit differentiation and myelination of oligodendrocytes through an unidentified mechanism. The extracellular binding partner for LINGO-1 is not known. Domain structures are identical to those in **Figure 5**. Abbreviation: MT, microtubule.

study using mouse conditional genetics identified Schwann cells as the principal source of LGI4 and showed that LGI4's binding partner ADAM22 (Sagane et al. 2008) is required on the axon for proper myelination (Ozkaynak et al. 2010; **Figure 6*a***). Exactly how LGI4 binding to axonal ADAM22 directs myelin formation is still unclear. Perhaps LGI4 binding to ADAM22 stabilizes signaling complexes involved in directing Schwann cell differentiation at the axonal surface, analogous to LGI1 and the ADAM22/Stargazin/AMPAR complex in the CNS (Fukata et al. 2006). Alternatively, LGI4 binding to ADAM22 may activate intracellular signaling cascades in the axon that drive myelin formation by Schwann cells.

In the CNS, the transmembrane LRR protein LINGO-1 (LRR and Ig domain-containing, Nogo-receptor-interacting protein 1) (**Figure 1**) is a key negative regulator of myelination. *LINGO-1* is expressed in neurons and oligodendrocytes. Interference with LINGO-1 function in cultured oligodendrocytes leads to increased oligodendrocyte differentiation and myelination of cocultured axons (Mi et al. 2005). *LINGO-1* knockout mice show an early onset of myelination in the CNS, thus supporting a role for LINGO-1 as an inhibitor of central myelination (Mi et al. 2005). Inhibition of oligodendrocyte differentiation requires LINGO-1's cytoplasmic domain, which controls the activity of the downstream signaling molecules RhoA and Fyn kinase (Mi et al. 2005) (**Figure 6*b***). Whether LINGO-1 binding to a secreted or membrane-bound ligand, in *cis* or in *trans*, is required to initiate intracellular

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signaling is currently unknown. Interestingly, LINGO-1 expressed on the axonal surface also inhibits oligodendrocyte differentiation and myelination, both in oligodendrocyte-neuron cocultures and in transgenic mice overexpressing LINGO-1 under a neuronal promoter (Lee et al. 2007; **Figure 6*b***). This suggests that axonal LINGO-1 signals from axon to oligodendrocyte to inhibit oligodendrocyte differentiation. The nature of this signal is not yet clear, but it does not seem to involve the LINGO-1 binding proteins NgR1 (see below) and $p75^{NTR}$ (Lee et al. 2007, Mi et al. 2004).

LIMITING STRUCTURAL PLASTICITY IN THE ADULT CENTRAL NERVOUS SYSTEM

Myelin, and the proteins associated with it, serves an additional role besides acting as an insulator to facilitate the propagation of action potentials. As the nervous system matures, neuronal connections are stabilized, and the potential for structural remodeling or regrowth after injury becomes severely restricted. The development of ocular dominance in the visual cortex is a classic example of postnatal structural plasticity that is limited to a set developmental time window, termed the critical period. During this period, monocular deprivation induces a shift in ocular dominance toward the nondeprived eye, but the same manipulation no longer alters cortical wiring after the end of the critical period. The closing of the critical period coincides with myelination, and myelin-associated proteins are considered to be major factors in limiting structural plasticity in the adult mammalian CNS (although other myelin-independent mechanisms exist). The Nogo receptor (NgR), a GPI-anchored LRR protein (**Figure 1**), plays a central role in transducing the inhibitory effects of myelin components. Here we focus on the role of the NgR complex in structural and synaptic plasticity of neuronal connections. The role of NgR in inhibiting axon regeneration in the injured CNS has been the topic of several recent reviews (Giger et al. 2008, Schwab 2010).

NgR1 appears to interact with a surprising number of coreceptors and ligands. The Nogo receptor complex is thought to consist of the ligand-binding subunit NgR1 and the signal-transducing subunits p75, LINGO-1, and TROY (Mi et al. 2004, Park et al. 2005, Shao et al. 2005, Wang et al. 2002a, Wong et al. 2002). Not all components of this complex are present in every cell type. NgR1 binds the inhibitory myelin components Nogo and MAG (myelin-associated glycoprotein) as well as the LRR protein OMgp (oligodendrocyte-myelin glycoprotein) (**Figure 1**; Fournier et al. 2001, Liu et al. 2002, Wang et al. 2002b). In addition, NgR1 binds several non-myelin-associated ligands (Schwab 2010). The first demonstration of NgR1's role in restricting the plasticity of neural circuits was the finding that ocular dominance plasticity in *NgR1* or *Nogo-A/B* knockout mice is extended until well after the end of the critical period (McGee et al. 2005). This suggests that myelin-derived factors stabilize wiring in the visual cortex. Although the underlying mechanisms are not clear, NgR1 may function at the synapse to restrict plasticity. NgR1 localizes to pre- and postsynaptic sites of asymmetric (presumptive excitatory) synapses at the ultrastructural level (Wang et al. 2002c), and loss of *NgR1* function in hippocampal pyramidal neurons by gene deletion or RNA interference induces a shift in the morphology of dendritic spines toward more immature phenotypes (Lee et al. 2008, Zagrebelsky et al. 2010). Although basal synaptic transmission and long-term potentiation (LTP) are normal in *NgR1* knockout mice, acute application of the NgR1 ligands Nogo-66 or OMgp suppresses LTP at Schaffer collateral-CA1 synapses in a *NgR1*-dependent manner (Lee et al. 2008, Raiker et al. 2010). This suggests that NgR1 ligands may act as negative regulators of synaptic plasticity. Together, these results suggest that NgR1 is required for proper maturation of dendritic spines and may function at synapses to limit synaptic plasticity and stabilize neuronal wiring.

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LEUCINE-RICH REPEAT PROTEINS AND DISORDERS OF THE NERVOUS SYSTEM

Given the fundamental role of LRR proteins in the development of neural circuits, it is perhaps not surprising that human genetic studies have linked LRR proteins to psychiatric and neurological diseases such as obsessive-compulsive disorder (OCD), schizophrenia, and epilepsy. Here, we focus on those LRR proteins for which some molecular or cellular function has been identified in culture assays or transgenic mouse models.

Slitrks in Tourette's Syndrome and Obsessive-Compulsive Disorder

Recent evidence indicates that Slitrks, transmembrane LRR proteins with resemblance to Slit in their LRR domain and Trk neurotrophin receptors in their intracellular domain (**Figure 1**), are involved in Tourette's syndrome (TS) and OCD. TS is a psychiatric disorder characterized by involuntary physical and vocal tics; OCD is an anxiety disorder characterized by intrusive thoughts (obsessions) and repetitive behaviors (compulsions). *SLITRK1*, the gene encoding the transmembrane LRR protein Slitrk1 (Slit and Trk-like family member 1), was identified as a candidate gene for TS on the basis of rare sequence variants found in TS patients, including a frameshift mutation that results in a truncation of the protein (Abelson et al. 2005). Although other genetic studies have failed to find a clear association of *SLITRK1* with TS (reviewed in O'Rourke et al. 2009), *Slitrk1* knockout mice do display elevated anxiety-like behavior (Katayama et al. 2010). Moreover, *Slitrk5* mutant mice show excessive self-grooming and increased anxiety-like behavior (Shmelkov et al. 2010), characteristics of obsessive-compulsive-like behavior. Although *Slitrk5* is widely expressed in the brain, analysis of *Slitrk5* knockout mice suggests that corticostriatal circuitry, which is associated with OCD in humans, is specifically affected by loss of *Slitrk5*. Reductions in dendritic complexity were found in striatal neurons but not in hippocampal neurons of *Slitrk5* mutants, and *Slitrk5*-null mice exhibited a decrease in corticostriatal neurotransmission that could be attributed to reductions in glutamate receptor subunit expression levels (Shmelkov et al. 2010). Together, these studies in knockout mice suggest that Slitrks are important for the development of the neural circuitry associated with anxiety disorders. Little is thus far known about the molecular and cellular mechanisms of Slitrks in neural development besides regulating neurite outgrowth in newly plated dissociated neurons (Abelson et al. 2005, Aruga & Mikoshiba 2003), a feature shared by many LRR proteins. Slitrks contain a PDZ-interaction site at their C terminus, which suggests that Slitrks are synaptic proteins. Moreover, Slitrk2 induces presynaptic differentiation in coculture assays (Linhoff et al. 2009), although its presynaptic binding partner is not known. Thus, there seem to be striking parallels between the Slitrks and other transmembrane synaptic LRR proteins such as NGLs, LRRTMs, and SALMs. It will be of interest to determine whether Slitrks also engage in transsynaptic adhesion and regulate pre- and postsynaptic differentiation to control the development of neural circuits associated with anxiety disorders.

LGI1 in Epilepsy

Strong genetic evidence links *LGI1* to an inherited form of epilepsy called autosomal dominant lateral temporal lobe epilepsy (ADLTE). *LGI1* was identified as the causative gene in ADLTE after systematic sequencing of candidate genes in the affected chromosomal region in patients (Kalachikov et al. 2002, Morante-Redolat et al. 2002). Many *LGI1* mutations resulting in amino acid changes or protein truncations have since been found in ADLTE patients, and mutations in *LGI1* are prevalent among ADLTE patients (reviewed in Nobile et al. 2009). Similar to the *claw paw* mutation in *LGI4* (Bermingham et al. 2006), ADLTE-associated mutations in *LGI1* abolish

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secretion of the protein (Chabrol et al. 2007, Senechal et al. 2005, Sirerol-Piquer et al. 2006, Striano et al. 2008). This suggests that loss of extracellular LGI1 in ADLTE leads to abnormal glutamatergic synapse development or function. Studies in knockout and transgenic mouse models support this view. Mice lacking *LGI1* die of severe epileptic seizures, a phenotype that could be rescued by transgenic neuron-specific expression of *LGI1*, but not of the related family member *LGI3* (Fukata et al. 2010). Expression of a truncated ADLTE mutant form of *LGI1* in transgenic mice impairs the postnatal decrease in presynaptic release probability that normally occurs in development (Zhou et al. 2009). The switch from NR2B to NR2A subunits normally associated with postsynaptic maturation is also impaired in these mice. Together, these results suggest that mutant *LGI1* prevents the normal maturation of pre- and postsynaptic function. In addition to functional defects, dentate granule cells in mutant *LGI1* transgenic mice retained excess dendritic branches and spines, which suggests that mutant *LGI1* also inhibits the pruning of excess branches and synaptic contacts during postnatal development. Finally, mutant *LGI1* transgenic mice showed increased AMPAR- and NMDAR-mediated synaptic transmission and were more susceptible to seizures (Zhou et al. 2009). These results suggest that mutated *LGI1* prevents the functional and structural maturation of glutamatergic synapses normally occurring during postnatal development. Interestingly, these mutant *LGI1* transgenic mice still express wild-type *LGI1*, which suggests that mutated LGI1 can have dominant-negative effects through an unidentified mechanism.

NgR in Schizophrenia

Many genetic (and other) factors are likely to contribute to a complex mental disorder such as schizophrenia, and these include genes encoding LRR proteins. Mutations in the Nogo receptor 1 gene (*NGR* or *RTN4R*) have been associated with schizophrenia, although these variants are rare and are not found in all ethnic populations (for a review see Voineskos 2009). Interestingly, four NgR1 variants associated with schizophrenia failed to transduce the growth-inhibitory effects of Nogo, MAG, and OMgp when expressed in chick neurons and showed dominant-negative activity on NgR1-mediated growth cone collapse in response to myelin ligands (Budel et al. 2008). This suggests that a disruption in signaling by myelin-derived factors that restrict plasticity could be a risk factor in the development of schizophrenia. Such a hypothesis is consistent with the general notion that myelin dysfunction is implicated in schizophrenia (Karoutzou et al. 2008). How the mutations in GPI-anchored NgR1 disrupt signaling is not yet clear. Ligand binding to the four variants tested was largely intact, and association with the signaling coreceptors $p75^{NTR}$, TROY, and LINGO-1 was not altered (Budel et al. 2008). Behavioral experiments using *NgR1* mutant mice have produced mixed results but generally do not show a strong effect of *NgR1* loss of function in schizophrenia-related behavioral tasks (Budel et al. 2008, Hsu et al. 2007).

Additional LRR proteins identified as susceptibility factors for schizophrenia are *SLITRK2* (Piton et al. 2011) and *LRRTM1* (Francks et al. 2007, Ludwig et al. 2009), for which rare missense variants have been found in schizophrenic patients. Deletions in the LRRTM binding partner neurexin 1 (*NRXN1*) have also been linked to schizophrenia (reviewed in Kirov et al. 2009), suggesting that impaired glutamatergic synaptic connectivity could be a contributing factor to the development of schizophrenia.

Other Leucine-Rich Repeat Proteins in Nervous System Disorders

Other recent studies have begun to explore the association of other LRR proteins with neurodevelopmental disorders. Polymorphisms in the transmembrane LRR genes *LRRTM3* and *LRRN3* (leucine-rich repeat neuronal 3) (**Figure 1**) are associated with autism spectrum

disorder (Sousa et al. 2010). *LRRTM3* was also identified as a candidate gene for late-onset Alzheimer's disease (Majercak et al. 2006). Toll-like receptors, transmembrane LRR proteins that bind a wide molecular variety of pathogen-associated ligands and are involved in the immune response (**Figure 1**), have been implicated in neurodegenerative diseases such as multiple sclerosis, stroke, and Alzheimer's disease (Okun et al. 2009, Kielian 2009). Little is yet known about the role of these proteins in neural development.

In summary, genetic studies have associated LRR proteins with several nervous system disorders. Although these studies often identify rare mutations that are not representative of the majority of affected patients for a given disorder, they provide a starting point for mechanistic studies and support the notion that altered connectivity lies at the base of many neurological and psychiatric diseases. Many questions remain to be answered. For example, how LRR proteins such as Slitrk5, which have broad expression patterns in the nervous system, affect the function of specific circuits and behaviors is unclear. Modeling the effects of altered LRR protein function in mouse models, through either conventional gene knockout approaches or overexpression of mutated LRR proteins in transgenic mice, will be an essential tool to answer these questions. In combination with molecular and cellular analysis of LRR protein function, this approach begins to identify the mechanisms underlying complex neurological disorders and may eventually suggest potential avenues for treatment.

CONCLUDING REMARKS

The LRR is an extremely versatile protein-binding motif that allows interaction with a wide diversity of partners. LRR domains have been used throughout evolution to regulate cell-cell interactions in increasingly complex organisms. The fly and mammalian nervous systems are two excellent examples of highly complex multicellular systems in which developing neurons face many challenges to connect into functional neural circuits. As we discussed, LRR proteins regulate key aspects of neural circuit development, from axon pathfinding and target selection to synapse formation. Some of these mechanisms are conserved from fly to vertebrate, such as the role of Slit in midline guidance; others appear to have evolved specifically in vertebrates, such as the role of LRR-containing synaptic adhesion molecules in excitatory synapse formation. For some LRR proteins we are now beginning to understand the mechanistic details involved, but for many, their role in the development of neural circuits is still unsolved. The extracellular binding partners for many LRR proteins remain to be discovered. For almost all, the downstream signaling mechanisms are unidentified. Why so many different LRR proteins evolved with at least partially redundant functions, such as Caps and Trn in flies or SALMs, NGLs, and LRRTMs in vertebrates, is currently not well understood. Nor is it understood why a key protein involved in target selection such as Caps is not conserved in vertebrates, where neurons face similar problems. Perhaps some of the LRR proteins that specifically evolved in vertebrates fulfill analogous functions. Finally, it appears likely that more genetic variants of LRR proteins associated with human nervous system disorders will be discovered. An increased understanding of the role of LRR proteins in the development and function of neural circuits will yield insight into these disorders and may help to find cures.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

Abelson JF, Kwan KY, O'Roak BJ, Baek DY, Stillman AA, et al. 2005. Sequence variants in *SLITRK1* are associated with Tourette's syndrome. *Science* 310:317–20

Arber S, Ladle DR, Lin JH, Frank E, Jessell TM. 2000. ETS gene *Er81* controls the formation of functional connections between group Ia sensory afferents and motor neurons. *Cell* 101:485–98

Aruga J, Mikoshiba K. 2003. Identification and characterization of Slitrk, a novel neuronal transmembrane protein family controlling neurite outgrowth. *Mol. Cell Neurosci.* 24:117–29

Bashaw GJ, Kidd T, Murray D, Pawson T, Goodman CS. 2000. Repulsive axon guidance: Abelson and Enabled play opposing roles downstream of the Roundabout receptor. *Cell* 101:703–15

Battye R, Stevens A, Jacobs JR. 1999. Axon repulsion from the midline of the *Drosophila* CNS requires slit function. *Development* 126:2475–81

Beaubien F, Cloutier JF. 2009. Differential expression of Slitrk family members in the mouse nervous system. *Dev. Dyn.* 238:3285–96

Bella J, Hindle KL, McEwan PA, Lovell SC. 2008. The leucine-rich repeat structure. *Cell Mol. Life Sci.* 65:2307–33

Belvin MP, Anderson KV. 1996. A conserved signaling pathway: the *Drosophila* Toll-Dorsal pathway. *Annu. Rev. Cell Dev. Biol* 12:393–416

Bermingham JR Jr, Shearin H, Pennington J, O'Moore J, Jaegle M, et al. 2006. The *claw paw* mutation reveals a role for *Lgi4* in peripheral nerve development. *Nat. Neurosci.* 9:76–84

Bibel M, Barde YA. 2000. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. *Genes Dev.* 14:2919–37

Biederer T, Scheiffele P. 2007. Mixed-culture assays for analyzing neuronal synapse formation. *Nat. Protoc.* 2:670–76

Bjorklund AK, Ekman D, Elofsson A. 2006. Expansion of protein domain repeats. *PLoS Comput. Biol.* 2:e114

Brierley DJ, Blanc E, Reddy OV, VijayRaghavan K, Williams DW. 2009. Dendritic targeting in the leg neuropil of *Drosophila*: the role of midline signalling molecules in generating a myotopic map. *PLoS Biol.* 7:e1000199

Brose K, Bland KS, Wang KH, Arnott D, Henzel W, et al. 1999. Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. *Cell* 96:795–806

Brose N. 2009. Synaptogenic proteins and synaptic organizers: "many hands make light work." *Neuron* 61:650–52

Buchanan SG, Gay NJ. 1996. Structural and functional diversity in the leucine-rich repeat family of proteins. *Prog. Biophys. Mol. Biol.* 65:1–44

Budel S, Padukkavidana T, Liu BP, Feng Z, Hu F, et al. 2008. Genetic variants of Nogo-66 receptor with possible association to schizophrenia block myelin inhibition of axon growth. *J. Neurosci.* 28:13161–72

Chabrol E, Popescu C, Gourfinkel-An I, Trouillard O, Depienne C, et al. 2007. Two novel epilepsy-linked mutations leading to a loss of function of LGI1. *Arch. Neurol.* 64:217–22

Chen Z, Gore BB, Long H, Ma L, Tessier-Lavigne M. 2008. Alternative splicing of the Robo3 axon guidance receptor governs the midline switch from attraction to repulsion. *Neuron* 58:325–32

Cho JH, Lépine M, Andrews W, Parnavelas J, Cloutier J-F. 2007. Requirement for Slit-1 and Robo-2 in zonal segregation of olfactory sensory neuron axons in the main olfactory bulb. *J. Neurosci.* 27:9094–104

Choe J, Kelker MS, Wilson IA. 2005. Crystal structure of human Toll-like receptor 3 (TLR3) ectodomain. *Science* 309:581–85

de Wit J, Sylwestrak E, O'Sullivan ML, Otto S, Tiglio K, et al. 2009. LRRTM2 interacts with Neurexin1 and regulates excitatory synapse formation. *Neuron* 64:799–806

Dickson BJ. 2002. Molecular mechanisms of axon guidance. *Science* 298:1959–64

Dickson BJ, Gilestro GF. 2006. Regulation of commissural axon pathfinding by slit and its Robo receptors. *Annu. Rev. Cell Dev. Biol.* 22:651–75

Dimitrova S, Reissaus A, Tavosanis G. 2008. Slit and Robo regulate dendrite branching and elongation of space-filling neurons in *Drosophila*. *Dev. Biol.* 324:18–30

Dolan J, Walshe K, Alsbury S, Hokamp K, O'Keeffe S, et al. 2007. The extracellular leucine-rich repeat superfamily; a comparative survey and analysis of evolutionary relationships and expression patterns. *BMC Genomics* 8:320

Emery B. 2010. Regulation of oligodendrocyte differentiation and myelination. *Science* 330:779–82

Evans TA, Bashaw GJ. 2010. Functional diversity of Robo receptor immunoglobulin domains promotes distinct axon guidance decisions. *Curr. Biol.* 20:567–72

Fan X, Labrador JP, Hing H, Bashaw GJ. 2003. Slit stimulation recruits Dock and Pak to the Roundabout receptor and increases Rac activity to regulate axon repulsion at the CNS midline. *Neuron* 40:113–27

Fournier AE, GrandPre T, Strittmatter SM. 2001. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. *Nature* 409:341–46

Francks C, Maegawa S, Lauren J, Abrahams BS, Velayos-Baeza A, et al. 2007. *LRRTM1* on chromosome 2p12 is a maternally suppressed gene that is associated paternally with handedness and schizophrenia. *Mol. Psychiatry* 12:1129–39

Fukata Y, Adesnik H, Iwanaga T, Bredt DS, Nicoll RA, Fukata M. 2006. Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. *Science* 313:1792–95

Fukata Y, Lovero KL, Iwanaga T, Watanabe A, Yokoi N, et al. 2010. Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. *Proc. Natl. Acad. Sci. USA* 107:3799–804

Furrer M-P, Vasenkova I, Kamiyama D, Rosado Y, Chiba A. 2007. Slit and Robo control the development of dendrites in *Drosophila* CNS. *Development* 134:3795–804

Giger RJ, Venkatesh K, Chivatakarn O, Raiker SJ, Robak L, et al. 2008. Mechanisms of CNS myelin inhibition: evidence for distinct and neuronal cell type specific receptor systems. *Restor. Neurol. Neurosci.* 26:97–115

Glebova NO, Ginty DD. 2004. Heterogeneous requirement of NGF for sympathetic target innervation in vivo. *J. Neurosci.* 24:743–51

Guo T, Mandai K, Condie BG, Wickramasinghe SR, Capecchi MR, Ginty DD. 2011. An evolving NGF-Hoxd1 signaling pathway mediates development of divergent neural circuits in vertebrates. *Nat. Neurosci.* 14:31–36

Hata Y, Butz S, Sudhof TC. 1996. CASK: a novel *dlg*/PSD95 homolog with an N-terminal calmodulin-dependent protein kinase domain identified by interaction with neurexins. *J. Neurosci.* 16:2488–94

Hinck L. 2004. The versatile roles of "axon guidance" cues in tissue morphogenesis. *Dev. Cell* 7:783–93

Hoang B, Chiba A. 2001. Single-cell analysis of *Drosophila* larval neuromuscular synapses. *Dev. Biol.* 229:55–70

Hoffmann JA. 2003. The immune response of *Drosophila*. *Nature* 426:33–38

Hohenester E. 2008. Structural insight into Slit-Robo signalling. *Biochem. Soc. Trans.* 36:251–56

Homma S, Shimada T, Hikake T, Yaginuma H. 2009. Expression pattern of LRR and Ig domain-containing protein (LRRIG protein) in the early mouse embryo. *Gene Expr. Patterns* 9:1–26

Hong W, Zhu H, Potter C, Barsh G, Kurusu M, et al. 2009. Leucine-rich repeat transmembrane proteins instruct discrete dendrite targeting in an olfactory map. *Nat. Neurosci.* 12:1542–50

Howitt JA, Clout NJ, Hohenester E. 2004. Binding site for Robo receptors revealed by dissection of the leucine-rich repeat region of Slit. *EMBO J.* 23:4406–12

Hsu R, Woodroffe A, Lai WS, Cook MN, Mukai J, et al. 2007. Nogo Receptor 1 (*RTN4R*) as a candidate gene for schizophrenia: analysis using human and mouse genetic approaches. *PLoS ONE* 2:e1234

Hu H, Li M, Labrador J-P, McEwen J, Lai EC, et al. 2005. Cross GTPase-activating protein (CrossGAP)/Vilse links the Roundabout receptor to Rac to regulate midline repulsion. *Proc. Natl. Acad. Sci. USA* 102:4613–18

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Huang EJ, Reichardt LF. 2001. Neurotrophins: roles in neuronal development and function. *Annu. Rev. Neurosci.* 24:677–736

Huber AB, Kolodkin AL, Ginty DD, Cloutier J-F. 2003. Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. *Annu. Rev. Neurosci.* 26:509–63

Hynes RO, Zhao Q. 2000. The evolution of cell adhesion. *J. Cell Biol.* 150:F89–96

Ichtchenko K, Hata Y, Nguyen T, Ullrich B, Missler M, et al. 1995. Neuroligin 1: a splice site-specific ligand for β-neurexins. *Cell* 81:435–43

Inaki M, Shinza-Kameda M, Ismat A, Frasch M, Nose A. 2010. *Drosophila* Tey represses transcription of the repulsive cue Toll and generates neuromuscular target specificity. *Development* 137:2139–46

Jan Y-N, Jan LY. 2010. Branching out: mechanisms of dendritic arborization. *Nat. Rev. Neurosci.* 11:316–28

Jin Y, Garner CC. 2008. Molecular mechanisms of presynaptic differentiation. *Annu. Rev. Cell Dev. Biol.* 24:237–62

Kajava AV. 1998. Structural diversity of leucine-rich repeat proteins. *J. Mol. Biol.* 277:519–27

Kalachikov S, Evgrafov O, Ross B, Winawer M, Barker-Cummings C, et al. 2002. Mutations in *LGI1* cause autosomal-dominant partial epilepsy with auditory features. *Nat. Genet.* 30:335–41

Karoutzou G, Emrich HM, Dietrich DE. 2008. The myelin-pathogenesis puzzle in schizophrenia: a literature review. *Mol. Psychiatry* 13:245–60

Katayama K, Yamada K, Ornthanalai VG, Inoue T, Ota M, et al. 2010. *Slitrk1*-deficient mice display elevated anxiety-like behavior and noradrenergic abnormalities. *Mol. Psychiatry* 15:177–84

Keleman K, Rajagopalan S, Cleppien D, Teis D, Paiha K, et al. 2002. Comm sorts Robo to control axon guidance at the *Drosophila* midline. *Cell* 110:415–27

Keleman K, Ribeiro C, Dickson BJ. 2005. Comm function in commissural axon guidance: cell-autonomous sorting of Robo in vivo. *Nat. Neurosci.* 8:156–63

Kidd T, Bland KS, Goodman CS. 1999. Slit is the midline repellent for the Robo receptor in *Drosophila*. *Cell* 96:785–94

Kidd T, Brose K, Mitchell KJ, Fetter RD, Tessier-Lavigne M, et al. 1998. Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. *Cell* 92:205–15

Kielian T. 2009. Overview of Toll-like receptors in the CNS. *Curr. Top. Microbiol. Immunol.* 336:1–14

Kim S, Burette A, Chung HS, Kwon SK, Woo J, et al. 2006. NGL family PSD-95-interacting adhesion molecules regulate excitatory synapse formation. *Nat. Neurosci.* 9:1294–301

Kirov G, Rujescu D, Ingason A, Collier DA, O'Donovan MC, Owen MJ. 2009. Neurexin 1 (*NRXN1*) deletions in schizophrenia. *Schizophr. Bull.* 35:851–54

Ko J, Fuccillo MV, Malenka RC, Sudhof TC. 2009. LRRTM2 functions as a neurexin ligand in promoting excitatory synapse formation. *Neuron* 64:791–98

Ko J, Kim S, Chung HS, Kim K, Han K, et al. 2006. SALM synaptic cell adhesion-like molecules regulate the differentiation of excitatory synapses. *Neuron* 50:233–45

Kobe B, Deisenhofer J. 1993. Crystal structure of porcine ribonuclease inhibitor, a protein with leucine-rich repeats. *Nature* 366:751–56

Kobe B, Deisenhofer J. 1994. The leucine-rich repeat: a versatile binding motif. *Trends Biochem. Sci.* 19:415–21

Kobe B, Deisenhofer J. 1995a. Proteins with leucine-rich repeats. *Curr. Opin. Struct. Biol.* 5:409–16

Kobe B, Deisenhofer J. 1995b. A structural basis of the interactions between leucine-rich repeats and protein ligands. *Nature* 374:183–86

Kobe B, Kajava AV. 2001. The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct. Biol.* 11:725–32

Kohsaka H, Nose A. 2009. Target recognition at the tips of postsynaptic filopodia: accumulation and function of Capricious. *Development* 136:1127–35

Kolodkin AL, Tessier-Lavigne M. 2011. Mechanisms and molecules of neuronal wiring: a primer. *Cold Spring Harb. Perspect. Biol.* 3:a001727

Kurusu M, Cording A, Taniguchi M, Menon K, Suzuki E, Zinn K. 2008. A screen of cell-surface molecules identifies leucine-rich repeat proteins as key mediators of synaptic target selection. *Neuron* 59:972–85

Annu. Rev. Cell Dev. Biol. 2011.27:697-729. Downloaded from www.annualreviews.org
by Stanford University - Main Campus - Robert Crown Law Library on 11/06/11. For personal use only.

Kuruvilla R, Zweifel LS, Glebova NO, Lonze BE, Valdez G, et al. 2004. A neurotrophin signaling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signaling. *Cell* 118:243–55

Kwon SK, Woo J, Kim SY, Kim H, Kim E. 2010. Trans-synaptic adhesions between netrin-G ligand-3 (NGL-3) and receptor tyrosine phosphatases LAR, protein-tyrosine phosphatase δ (PTPδ), and PTPσ via specific domains regulate excitatory synapse formation. *J. Biol. Chem.* 285:13966–78

Lauren J, Airaksinen MS, Saarma M, Timmusk T. 2003. A novel gene family encoding leucine-rich repeat transmembrane proteins differentially expressed in the nervous system. *Genomics* 81:411–21

Lee H, Raiker SJ, Venkatesh K, Geary R, Robak LA, et al. 2008. Synaptic function for the Nogo-66 receptor NgR1: regulation of dendritic spine morphology and activity-dependent synaptic strength. *J. Neurosci.* 28:2753–65

Lee X, Yang Z, Shao Z, Rosenberg SS, Levesque M, et al. 2007. NGF regulates the expression of axonal LINGO-1 to inhibit oligodendrocyte differentiation and myelination. *J. Neurosci.* 27:220–25

Li HS, Chen JH, Wu W, Fagaly T, Zhou L, et al. 1999. Vertebrate Slit, a secreted ligand for the transmembrane protein roundabout, is a repellent for olfactory bulb axons. *Cell* 96:807–18

Lin JC, Ho WH, Gurney A, Rosenthal A. 2003. The netrin-G1 ligand NGL-1 promotes the outgrowth of thalamocortical axons. *Nat. Neurosci.* 6:1270–76

Linhoff MW, Lauren J, Cassidy RM, Dobie FA, Takahashi H, et al. 2009. An unbiased expression screen for synaptogenic proteins identifies the LRRTM protein family as synaptic organizers. *Neuron* 61:734–49

Liu BP, Fournier A, GrandPre T, Strittmatter SM. 2002. Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. *Science* 297:1190–93

Liu Z, Patel K, Schmidt H, Andrews W, Pini A, Sundaresan V. 2004. Extracellular Ig domains 1 and 2 of Robo are important for ligand (Slit) binding. *Mol. Cell. Neurosci.* 26:232–40

Long H, Sabatier C, Ma L, Plump A, Yuan W, et al. 2004. Conserved roles for Slit and Robo proteins in midline commissural axon guidance. *Neuron* 42:213–23

Ludwig KU, Mattheisen M, Muhleisen TW, Roeske D, Schmal C, et al. 2009. Supporting evidence for LRRTM1 imprinting effects in schizophrenia. *Mol. Psychiatry* 14:743–45

Lundström A, Gallio M, Englund C, Steneberg P, Hemphälä J, et al. 2004. Vilse, a conserved Rac/Cdc42 GAP mediating Robo repulsion in tracheal cells and axons. *Genes Dev.* 18:2161–71

Luo L, Flanagan JG. 2007. Development of continuous and discrete neural maps. *Neuron* 56:284–300

Mah W, Ko J, Nam J, Han K, Chung WS, Kim E. 2010. Selected SALM (synaptic adhesion-like molecule) family proteins regulate synapse formation. *J. Neurosci.* 30:5559–68

Majercak J, Ray WJ, Espeseth A, Simon A, Shi XP, et al. 2006. LRRTM3 promotes processing of amyloid-precursor protein by BACE1 and is a positional candidate gene for late-onset Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 103:17967–72

Mandai K, Guo T, St Hillaire C, Meabon JS, Kanning KC, et al. 2009. LIG family receptor tyrosine kinase–associated proteins modulate growth factor signals during neural development. *Neuron* 63:614–27

Markus A, Patel TD, Snider WD. 2002. Neurotrophic factors and axonal growth. *Curr. Opin. Neurobiol.* 12:523–31

Matsushima N, Tachi N, Kuroki Y, Enkhbayar P, Osaki M, et al. 2005. Structural analysis of leucine-rich-repeat variants in proteins associated with human diseases. *Cell Mol. Life Sci.* 62:2771–91

Mauss A, Tripodi M, Evers JF, Landgraf M. 2009. Midline signalling systems direct the formation of a neural map by dendritic targeting in the *Drosophila* motor system. *PLoS Biol.* 7:e1000200

McAllister AK. 2007. Dynamic aspects of CNS synapse formation. *Annu. Rev. Neurosci.* 30:425–50

McAllister AK, Katz LC, Lo DC. 1999. Neurotrophins and synaptic plasticity. *Annu. Rev. Neurosci.* 22:295–318

McGee AW, Yang Y, Fischer QS, Daw NW, Strittmatter SM. 2005. Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. *Science* 309:2222–26

Mi S, Lee X, Shao Z, Thill G, Ji B, et al. 2004. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nat. Neurosci.* 7:221–28

Mi S, Miller RH, Lee X, Scott ML, Shulag-Morskaya S, et al. 2005. LINGO-1 negatively regulates myelination by oligodendrocytes. *Nat. Neurosci.* 8:745–51

Milán M, Weihe U, Pérez L, Cohen SM. 2001. The LRR proteins Capricious and Tartan mediate cell interactions during DV boundary formation in the *Drosophila* wing. *Cell* 106:785–94

Annu. Rev. Cell Dev. Biol. 2011.27:697-729. Downloaded from www.annualreviews.org
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Morante-Redolat JM, Gorostidi-Pagola A, Piquer-Sirerol S, Saenz A, Poza JJ, et al. 2002. Mutations in the *LGI1/Epitempin* gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Hum. Mol. Genet.* 11:1119–28

Morimura N, Inoue T, Katayama K, Aruga J. 2006. Comparative analysis of structure, expression and PSD95-binding capacity of Lrfn, a novel family of neuronal transmembrane proteins. *Gene* 380:72–83

Morlot C, Thielens NM, Ravelli RBG, Hemrika W, Romijn RA, et al. 2007. Structural insights into the Slit-Robo complex. *Proc. Natl. Acad. Sci. USA* 104:14923–28

Mosyak L, Wood A, Dwyer B, Buddha M, Johnson M, et al. 2006. The structure of the Lingo-1 ectodomain, a module implicated in central nervous system repair inhibition. *J. Biol. Chem.* 281:36378–90

Mukherjee K, Sharma M, Urlaub H, Bourenkov GP, Jahn R, et al. 2008. CASK functions as a Mg^{2+}-independent neurexin kinase. *Cell* 133:328–39

Nakashiba T, Ikeda T, Nishimura S, Tashiro K, Honjo T, et al. 2000. Netrin-G1: a novel glycosyl phosphatidylinositol-linked mammalian netrin that is functionally divergent from classical netrins. *J. Neurosci.* 20:6540–50

Nakashiba T, Nishimura S, Ikeda T, Itohara S. 2002. Complementary expression and neurite outgrowth activity of netrin-G subfamily members. *Mech. Dev.* 111:47–60

Nave KA. 2010. Myelination and support of axonal integrity by glia. *Nature* 468:244–52

Nishimura-Akiyoshi S, Niimi K, Nakashiba T, Itohara S. 2007. Axonal netrin-Gs transneuronally determine lamina-specific subdendritic segments. *Proc. Natl. Acad. Sci. USA* 104:14801–6

Nobile C, Michelucci R, Andreazza S, Pasini E, Tosatto SC, Striano P. 2009. *LGI1* mutations in autosomal dominant and sporadic lateral temporal epilepsy. *Hum. Mutat.* 30:530–36

Nose A, Mahajan VB, Goodman CS. 1992. Connectin: a homophilic cell adhesion molecule expressed on a subset of muscles and the motoneurons that innervate them in *Drosophila*. *Cell* 70:553–67

Nose A, Takeichi M, Goodman CS. 1994. Ectopic expression of Connectin reveals a repulsive function during growth cone guidance and synapse formation. *Neuron* 13:525–39

Nose A, Umeda T, Takeichi M. 1997. Neuromuscular target recognition by a homophilic interaction of connectin cell adhesion molecules in *Drosophila*. *Development* 124:1433–41

Nüsslein-Volhard C, Wieschaus E. 1980. Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287:795–801

O'Rourke JA, Scharf JM, Yu D, Pauls DL. 2009. The genetics of Tourette syndrome: a review. *J. Psychosom. Res.* 67:533–45

Ogawa Y, Oses-Prieto J, Kim MY, Horresh I, Peles E, et al. 2010. ADAM22, a Kv1 channel-interacting protein, recruits membrane-associated guanylate kinases to juxtaparanodes of myelinated axons. *J. Neurosci.* 30:1038–48

Okun E, Griffioen KJ, Lathia JD, Tang S-C, Mattson MP, Arumugam TV. 2009. Toll-like receptors in neurodegeneration. *Brain Res. Rev.* 59:278–92

Owuor K, Harel NY, Englot DJ, Hisama F, Blumenfeld H, Strittmatter SM. 2009. LGI1-associated epilepsy through altered ADAM23-dependent neuronal morphology. *Mol. Cell Neurosci.* 42:448–57

Ozkaynak E, Abello G, Jaegle M, van Berge L, Hamer D, et al. 2010. Adam22 is a major neuronal receptor for Lgi4-mediated Schwann cell signaling. *J. Neurosci.* 30:3857–64

Park JB, Yiu G, Kaneko S, Wang J, Chang J, et al. 2005. A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. *Neuron* 45:345–51

Patel TD, Jackman A, Rice FL, Kucera J, Snider WD. 2000. Development of sensory neurons in the absence of NGF/TrkA signaling in vivo. *Neuron* 25:345–57

Patel TD, Kramer I, Kucera J, Niederkofler V, Jessell TM, et al. 2003. Peripheral NT3 signaling is required for ETS protein expression and central patterning of proprioceptive sensory afferents. *Neuron* 38:403–16

Perez-Pinera P, García-Suarez O, Germanà A, Díaz-Esnal B, de Carlos F, et al. 2008. Characterization of sensory deficits in TrkB knockout mice. *Neurosci. Lett.* 433:43–47

Piton A, Gauthier J, Hamdan FF, Lafreniere RG, Yang Y, et al. 2011. Systematic resequencing of X-chromosome synaptic genes in autism spectrum disorder and schizophrenia. *Mol. Psychiatry* 16:867–80

Annu. Rev. Cell Dev. Biol. 2011.27:697-729. Downloaded from www.annualreviews.org
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Pulido R, Serra-Pages C, Tang M, Streuli M. 1995. The LAR/PTPδ/PTPσ subfamily of transmembrane protein-tyrosine-phosphatases: multiple human LAR, PTPδ, and PTPσ isoforms are expressed in a tissue-specific manner and associate with the LAR-interacting protein LIP.1. *Proc. Natl. Acad. Sci. USA* 92:11686–90

Raghavan S, White RA. 1997. Connectin mediates adhesion in *Drosophila*. *Neuron* 18:873–80

Raiker SJ, Lee H, Baldwin KT, Duan Y, Shrager P, Giger RJ. 2010. Oligodendrocyte-myelin glycoprotein and Nogo negatively regulate activity-dependent synaptic plasticity. *J. Neurosci.* 30:12432–45

Rajagopalan S, Vivancos V, Nicolas E, Dickson BJ. 2000. Selecting a longitudinal pathway: Robo receptors specify the lateral position of axons in the *Drosophila* CNS. *Cell* 103:1033–45

Rose D, Zhu X, Kose H, Hoang B, Cho J, Chiba A. 1997. Toll, a muscle cell surface molecule, locally inhibits synaptic initiation of the RP3 motoneuron growth cone in *Drosophila*. *Development* 124:1561–71

Rothberg JM, Hartley DA, Walther Z, Artavanis-Tsakonas S. 1988. *slit*: an EGF-homologous locus of *D. melanogaster* involved in the development of the embryonic central nervous system. *Cell* 55:1047–59

Rothberg JM, Jacobs JR, Goodman CS, Artavanis-Tsakonas S. 1990. *slit*: an extracellular protein necessary for development of midline glia and commissural axon pathways contains both EGF and LRR domains. *Genes Dev.* 4:2169–87

Ruiz-Cañada C, Budnik V. 2006. Introduction on the use of the *Drosophila* embryonic/larval neuromuscular junction as a model system to study synapse development and function, and a brief summary of pathfinding and target recognition. *Int. Rev. Neurobiol.* 75:1–31

Sabatier C, Plump AS, Ma L, Brose K, Tamada A, et al. 2004. The divergent Robo family protein Rig-1/Robo3 is a negative regulator of Slit responsiveness required for midline crossing by commissural axons. *Cell* 117:157–69

Sagane K, Ishihama Y, Sugimoto H. 2008. LGI1 and LGI4 bind to ADAM22, ADAM23 and ADAM11. *Int. J. Biol. Sci.* 4:387–96

Sakano H. 2010. Neural map formation in the mouse olfactory system. *Neuron* 67:530–42

Sanes JR, Zipursky SL. 2010. Design principles of insect and vertebrate visual systems. *Neuron* 66:15–36

Scheiffele P, Fan J, Choih J, Fetter R, Serafini T. 2000. Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell* 101:657–69

Schulte U, Thumfart JO, Klocker N, Sailer CA, Bildl W, et al. 2006. The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kvβ1. *Neuron* 49:697–706

Schwab ME. 2010. Functions of Nogo proteins and their receptors in the nervous system. *Nat. Rev. Neurosci.* 11:799–811

Seabold GK, Wang PY, Chang K, Wang CY, Wang YX, et al. 2008. The SALM family of adhesion-like molecules forms heteromeric and homomeric complexes. *J. Biol. Chem.* 283:8395–405

Seeger M, Tear G, Ferres-Marco D, Goodman CS. 1993. Mutations affecting growth cone guidance in *Drosophila*: genes necessary for guidance toward or away from the midline. *Neuron* 10:409–26

Segal RA. 2003. Selectivity in neurotrophin signaling: theme and variations. *Annu. Rev. Neurosci.* 26:299–330

Senechal KR, Thaller C, Noebels JL. 2005. ADPEAF mutations reduce levels of secreted LGI1, a putative tumor suppressor protein linked to epilepsy. *Hum. Mol. Genet.* 14:1613–20

Serra-Pages C, Kedersha NL, Fazikas L, Medley Q, Debant A, Streuli M. 1995. The LAR transmembrane protein tyrosine phosphatase and a coiled-coil LAR-interacting protein co-localize at focal adhesions. *EMBO J.* 14:2827–38

Shao Z, Browning JL, Lee X, Scott ML, Shulga-Morskaya S, et al. 2005. TAJ/TROY, an orphan TNF receptor family member, binds Nogo-66 receptor 1 and regulates axonal regeneration. *Neuron* 45:353–59

Shinza-Kameda M, Takasu E, Sakurai K, Hayashi S, Nose A. 2006. Regulation of layer-specific targeting by reciprocal expression of a cell adhesion molecule, Capricious. *Neuron* 49:205–13

Shishido E, Takeichi M, Nose A. 1998. *Drosophila* synapse formation: regulation by transmembrane protein with Leu-rich repeats, CAPRICIOUS. *Science* 280:2118–21

Shmelkov SV, Hormigo A, Jing D, Proenca CC, Bath KG, et al. 2010. Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. *Nat. Med.* 16:598–602

Siddiqui TJ, Pancaroglu R, Kang Y, Rooyakkers A, Craig AM. 2010. LRRTMs and neuroligins bind neurexins with a differential code to cooperate in glutamate synapse development. *J. Neurosci.* 30:7495–506

Annu. Rev. Cell Dev. Biol. 2011.27:697-729. Downloaded from www.annualreviews.org
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Simpson JH, Bland KS, Fetter RD, Goodman CS. 2000. Short-range and long-range guidance by Slit and its Robo receptors: a combinatorial code of Robo receptors controls lateral position. *Cell* 103:1019–32

Sirerol-Piquer MS, Ayerdi-Izquierdo A, Morante-Redolat JM, Herranz-Perez V, Favell K, et al. 2006. The epilepsy gene *LGI1* encodes a secreted glycoprotein that binds to the cell surface. *Hum. Mol. Genet.* 15:3436–45

Song HJ, Poo MM. 1999. Signal transduction underlying growth cone guidance by diffusible factors. *Curr. Opin. Neurobiol.* 9:355–63

Song JY, Ichtchenko K, Sudhof TC, Brose N. 1999. Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. *Proc. Natl. Acad. Sci. USA* 96:1100–5

Sousa I, Clark TG, Holt R, Pagnamenta AT, Mulder EJ, et al. 2010. Polymorphisms in leucine-rich repeat genes are associated with autism spectrum disorder susceptibility in populations of European ancestry. *Mol. Autism* 1:7

Spangler SA, Hoogenraad CC. 2007. Liprin-α proteins: scaffold molecules for synapse maturation. *Biochem. Soc. Trans.* 35:1278–82

Spitzweck B, Brankatschk M, Dickson BJ. 2010. Distinct protein domains and expression patterns confer divergent axon guidance functions for *Drosophila* Robo receptors. *Cell* 140:409–20

Striano P, de Falco A, Diani E, Bovo G, Furlan S, et al. 2008. A novel loss-of-function *LGI1* mutation linked to autosomal dominant lateral temporal epilepsy. *Arch. Neurol.* 65:939–42

Stryker E, Johnson KG. 2007. LAR, liprin α and the regulation of active zone morphogenesis. *J. Cell Sci.* 120:3723–28

Sudhof TC. 2008. Neuroligins and neurexins link synaptic function to cognitive disease. *Nature* 455:903–11

Takahashi H, Arstikaitis P, Prasad T, Bartlett TE, Wang YT, et al. 2011. Postsynaptic TrkC and presynaptic PTPσ function as a bidirectional excitatory synaptic organizing complex. *Neuron* 69:287–303

Taniguchi H, Shishido E, Takeichi M, Nose A. 2000. Functional dissection of *Drosophila* Capricious: its novel roles in neuronal pathfinding and selective synapse formation. *J. Neurobiol.* 42:104–16

Ullrich B, Ushkaryov YA, Sudhof TC. 1995. Cartography of neurexins: more than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. *Neuron* 14:497–507

Valanne S, Wang J-H, Rämet M. 2011. The *Drosophila* Toll signaling pathway. *J. Immunol.* 186:649–56

Voineskos AN. 2009. Converging evidence for the Nogo-66 receptor gene in schizophrenia. *J. Neurosci.* 29:5045–47

Waites CL, Craig AM, Garner CC. 2005. Mechanisms of vertebrate synaptogenesis. *Annu. Rev. Neurosci.* 28:251–74

Wang CY, Chang K, Petralia RS, Wang YX, Seabold GK, Wenthold RJ. 2006. A novel family of adhesion-like molecules that interacts with the NMDA receptor. *J. Neurosci.* 26:2174–83

Wang KC, Kim JA, Sivasankaran R, Segal R, He Z. 2002a. p75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature* 420:74–78

Wang KC, Koprivica V, Kim JA, Sivasankaran R, Guo Y, et al. 2002b. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature* 417:941–44

Wang X, Chun SJ, Treloar H, Vartanian T, Greer CA, Strittmatter SM. 2002c. Localization of Nogo-A and Nogo-66 receptor proteins at sites of axon-myelin and synaptic contact. *J. Neurosci.* 22:5505–15

West AP, Koblansky AA, Ghosh S. 2006. Recognition and signaling by Toll-like receptors. *Annu. Rev. Cell Dev. Biol.* 22:409–37

Whitford KL, Marillat V, Stein E, Goodman CS, Tessier-Lavigne M, et al. 2002. Regulation of cortical dendrite development by Slit-Robo interactions. *Neuron* 33:47–61

Wickramasinghe SR, Alvania RS, Ramanan N, Wood JN, Mandai K, Ginty DD. 2008. Serum response factor mediates NGF-dependent target innervation by embryonic DRG sensory neurons. *Neuron* 58:532–45

Wong ST, Henley JR, Kanning KC, Huang KH, Bothwell M, Poo MM. 2002. A $p75^{NTR}$ and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. *Nat. Neurosci.* 5:1302–8

Woo J, Kwon SK, Choi S, Kim S, Lee JR, et al. 2009. *Trans*-synaptic adhesion between NGL-3 and LAR regulates the formation of excitatory synapses. *Nat. Neurosci.* 12:428–37

Yang L, Bashaw GJ. 2006. Son of sevenless directly links the Robo receptor to Rac activation to control axon repulsion at the midline. *Neuron* 52:595–607

Yin Y, Miner JH, Sanes JR. 2002. Laminets: laminin- and netrin-related genes expressed in distinct neuronal subsets. *Mol. Cell. Neurosci.* 19:344–58

Ypsilanti AR, Zagar Y, Chédotal A. 2010. Moving away from the midline: new developments for Slit and Robo. *Development* 137:1939–52

Zagrebelsky M, Schweigreiter R, Bandtlow CE, Schwab ME, Korte M. 2010. Nogo-A stabilizes the architecture of hippocampal neurons. *J. Neurosci.* 30:13220–34

Zhang W, Rajan I, Savelieva KV, Wang CY, Vogel P, et al. 2008. Netrin-G2 and netrin-G2 ligand are both required for normal auditory responsiveness. *Genes Brain Behav.* 7:385–92

Zhong J, Li X, McNamee C, Chen AP, Baccarini M, Snider WD. 2007. Raf kinase signaling functions in sensory neuron differentiation and axon growth in vivo. *Nat. Neurosci.* 10:598–607

Zhou YD, Lee S, Jin Z, Wright M, Smith SE, Anderson MP. 2009. Arrested maturation of excitatory synapses in autosomal dominant lateral temporal lobe epilepsy. *Nat. Med.* 15:1208–14

Zhu B, Pennack JA, McQuilton P, Forero MG, Mizuguchi K, et al. 2008. *Drosophila* neurotrophins reveal a common mechanism for nervous system formation. *PLoS Biol.* 6:e284

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