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Building a synapse: lessons on synaptic specificity and presynaptic assembly from the nematode *C. elegans*

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Abstract

Synapses are specialized sites of cell contact that mediate information flow between neurons and their targets. Genetic screens in the nematode *C. elegans* have led to the discovery of a number of molecules required for synapse patterning and assembly. Recent studies have demonstrated the importance of guidepost cells in the positioning of presynaptic sites at specific locations along the axon. Interestingly, these guideposts can promote or inhibit synapse formation, and do so by utilizing transmembrane adhesion molecules or secreted factors that act over relatively larger distances. Once the decision of where to build a presynaptic terminal has been made, key molecules are recruited to assemble synaptic vesicles and active zone proteins at that site. Multiple steps of this process are regulated by ubiquitin ligase complexes. Interestingly, some of the molecules involved in presynaptic assembly also play roles in regulating axon polarity and outgrowth, suggesting that different neurodevelopmental processes are molecularly integrated.

Introduction

Chemical synapses are the basic communication units between neurons, and their precise patterning and development underlie circuit formation and nervous system function [1]. The nematode *Caernohabditis elegans* has served as an excellent model system for studying synaptic development. Its simple nervous system consisting of 302 neurons, a known wiring diagram [2], and genetic tractability have led to the discovery of a number of novel molecules important for the development and maintenance of synapses [3,4]. In this review, we will focus on the recent advances made in understanding the formation of presynaptic terminals in *C. elegans*, starting with the issues of synaptic partner choice and subcellular distribution of synapses, and proceeding with recent findings related to synapse assembly and maintenance.

Synaptic positioning and specificity

Nervous system function is mediated by a precisely patterned network of synaptic connections. In development, axon guidance provides the basic layout for constructing neural circuits, which is then built upon by subsequent synaptic target selection and maturation [1]. Classically, synaptic specificity is thought to be mediated by adhesive molecules acting between presynaptic and postsynaptic partners to establish connections

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across the synaptic cleft [5]. However, recent studies in *C. elegans* suggest a different way by which precise neural connectivity can arise. Rather than directly pairing appropriate presynaptic and postsynaptic partners through adhesive forces, guidepost cells can be utilized. A guidepost cell initially determines the spatial placement of the presynaptic sites along the axon; then, the postsynaptic partner is guided to these presynaptic sites, either by the same guidepost or by some other mechanism.

Contact-mediated mechanisms of synaptic specificity

The importance of guideposts for synaptic patterning in *C. elegans* was initially demonstrated in the studies of the motor neuron HSNL, which controls egg-laying by forming synapses onto the vulva muscles and the VC interneurons (Figure 1). HSNL achieves this specificity in target choice by positioning presynaptic sites at a specific location along the axon. This precise positioning of synapses is mediated by primary vulval epithelial cells that express SYG-2, an immunoglobulin superfamily transmembrane molecule (Table 1). SYG-2 interacts with SYG-1, another transmembrane molecule that is expressed in HSNL, and thus recruits SYG-1 to the location where presynaptic sites are formed [6,7]. Thus, SYG-2 acts as an attractive guidepost molecule, and whether it also helps to guide HSNL postsynaptic partners is presently unclear.

How does SYG-1 ensure the formation of presynaptic sites at the appropriate location in HSNL? During development, transient presynaptic sites form at multiple locations along the HSNL axon. However, most of these presynaptic sites are eliminated by adulthood and only those where SYG-1 localizes remain. As it turns out, SYG-1 helps achieve this stereotypical presynaptic pattern by playing a protective role. Ding *et al.* recently showed that an E3 ubiquitin ligase, a Skp1-Cullin-F-box (SCF) complex, acts in HSNL to eliminate unwanted presynaptic sites [8••]. Animals with loss-of-function mutations in components of this complex have delayed or incompletely eliminated additional presynaptic sites. Furthermore, the authors show that SYG-1 binds to the Skp1 homolog, SKR-1, preventing it from interacting with the rest of the SCF complex. The interpretation of these results is that SYG-1 plays a protective role by locally inhibiting the SCF complex, thus preventing the degradation of presynaptic sites at the SYG-2-marked location.

Diffusable factors and synaptic specificity

Another example of a prosynaptogenic guidepost cell was revealed in a recent study investigating the formation of the thermotaxis circuit in *C. elegans* [9••]. The authors found that two glia-like sheath cells coordinate the innervation between the interneurons AIY and RIA by secreting the diffusible molecule UNC-6/Netrin (Figure 1). In the presynaptic AIY neurons, the Netrin receptor UNC-40/DCC plays a novel role, promoting assembly of presynaptic terminals in the vicinity of the glia endings. In RIA neurons, UNC-40/DCC plays a conventional axon guidance role, bringing the RIA process ventrally and ensuring that it encounters its presynaptic partner. Thus, glial cells act as attractive guidepost cells, and the signal they use is a diffusible molecule which elicits distinct guidance and synaptogenic responses, depending on the cellular context. It will be interesting to elucidate the molecular pathway acting downstream of *unc-40*/DCC in the AIY neuron that promotes synapse assembly.

A recent study illustrated that guidepost cells can also sculpt neuronal connectivity by secreting anti-synaptogenic factors [10••]. The authors focused on the *C. elegans* tail motor neuron DA9, whose presynaptic terminals are restricted to a specific segment of its axon (Figure 1). They found that the asynaptic domain of the DA9 axon is created by the local inhibitory action of the Wnt receptor, LIN-17/Frizzled. The Wnt ligand, LIN-44, mediates localization of LIN-17/Frizzled to this asynaptic domain. Thus, LIN-44/Wnt (secreted by the

hypodermal cells in the tail) acts as a long-range inhibitory cue to pattern DA9 presynapses. Interestingly, a recent study in *Drosophila* suggests that the inhibitory activity of Wnts in synaptogenesis might be evolutionarily conserved [11•]. Future work will be needed to elucidate the downstream components of this novel, anti-synaptogenic Wnt signaling pathway, and determine whether Wnts play similar synaptic patterning roles in vertebrates.

Transcriptional mechanisms of synaptic specificity

Past studies of the locomotion circuit in *C. elegans* have shown that the specificity of the VA motor neuron synapses can be transcriptionally regulated [12,13]. In *unc-4* mutants, the VA motor neurons display a pattern of synaptic inputs characteristic of their lineal sisters, the VB neurons, while retaining VA morphology and axon trajectory (Figure 1). UNC-4 is a homeodomain protein that, together with its transcriptional corepressor, UNC-37/Groucho, acts in VAs to repress the VB synaptic fate [14]. A recent study used a neuron-specific microarray strategy to identify genes that act downstream of *unc-4* to regulate VA synaptic choice [15••]. The authors show that a homeodomain protein *ceh-12*, which is normally expressed in VBs, is both necessary and sufficient to impose VB-like inputs onto VAs. It will be interesting to determine the downstream mechanisms that regulate VA synaptic partner choice, and see if guidepost cells play a role in this system.

Guidepost cells in vertebrate circuit formation

The importance of guidepost cells in synaptic development is not restricted to *C. elegans*, as guideposts have been shown to play a role in the development of the vertebrate nervous system as well. For instance, subplate neurons act as transient guidepost cells in the maturation of the visual cortical circuit [16], while in the developing hippocampus, Cajal-Retzius cells and a set of GABAergic neurons ensure that two different populations of afferents synapse onto distinct subcellular domains of pyramidal dendrites [17]. In both cases, the guidepost cells transiently receive afferent synaptic input and therefore serve as ‘placeholders’ for the presynaptic terminals until the dendritic processes of postsynaptic neurons grow in. Thus, guidepost cells seem to be particularly important when there is a temporal discrepancy between axonal and dendritic development. Future work will be needed to determine the prevalence of guideposts in vertebrate nervous system development, and elucidate the molecular signals that mediate their ‘guiding’ effect.

Synaptic assembly and maintenance

While extracellular signals mark the location for synapse formation, intracellular signals interpret these cues and regulate cellular responses accordingly. Over the past 10 years, unbiased mutagenesis screens in *C. elegans* have identified key molecules that regulate synapse assembly, maturation, stability, and remodeling [3,18]. Mutants have been identified in active zone proteins, such as SYD-2/liprin-α and SYD-1, the SAD-1 protein kinase, regulators of vesicular trafficking (UNC-16, dynein, and kinesin), and the E3 ubiquitin ligase RPM-1 [19–26]. Importantly, these molecules and many of their functions are evolutionarily conserved (Table 1).

Active zone proteins in synapse assembly

Recent genetic and biochemical studies have begun to unravel the mechanisms of how key active zone proteins function to assemble a synapse. Two recent studies have provided molecular insight into the relationship between SYD-1 and SYD-2, which act as master regulators of presynaptic assembly [27•,28•]. The existing evidence supports a model in which guidepost signals trigger SYD-1 accumulation at immature synapses, which facilitates the recruitment of SYD-2/liprin-α (Figure 2), thus leading to synapse assembly. ELKS/ERC/CAST is another important scaffolding molecule that is thought to activate or stabilize

SYD-2 at the active zone [27•]. The role of SYD-2 and ELKS-1 in presynaptic assembly is likely to be evolutionarily conserved, as their *Drosophila* homologs, liprin-α and Bruchpilot, act as important synaptic regulators at the *Drosophila* neuromuscular junction [29,30•, 31,32]. SYD-2 recruitment and active zone formation are further regulated by a number of other molecules in *C. elegans*. These include the transcription factor *unc-3* and the G protein Goa-1 [33], as well as an isoform of the LAR-like phosphatase receptor, *ptp-3A*, which interacts with the extracellular matrix component nidogen to regulate synaptic morphology [34].

Ubiquitination and endocytosis in synapse formation

Another key molecule that regulates synapse formation in *C. elegans* is the E3 ubiquitin ligase RPM-1, which acts in a parallel pathway to *syd-1* and *syd-2* [35,36••]. Recent studies have begun to decipher the mechanism of how RPM-1 functions at the synapse by identifying downstream signaling molecules. Genetic suppressor screens have shown that RPM-1 acts as a negative regulator of a MAP kinase cascade that includes *dlk-1*, *mkk-4*, and the p38 MAPK ortholog, *pmk-3* (Figure 2) [36••]. RPM-1 is a component of an SCF complex E3 ligase [35] that stimulates ubiquitination of DLK-1, leading to its degradation [36••]. In addition, a proteomic screen in combination with genetic analysis has shown that RPM-1 positively regulates a Rab GTPase pathway by binding the Rab GEF, GLO-4 [37••]. *glo-4* functions downstream of *rpm-1* in a linear pathway that also includes the Rab GTPase GLO-1, and the adaptor complex AP-3. Interestingly, this pathway regulates the biogenesis and/or trafficking of late endosomes/lysosomes at presynaptic terminals. Thus, RPM-1 may act as a master switch that regulates synapse formation by coordinating protein ubiquitination, regulation of transcription, and degradation of synaptic proteins by the late endosome/lysosome (Figure 2).

Ion channels and transporters

Many ion channels and transporters are crucial for synaptic function and are localized to synapses. Recent studies have identified suppressors of a lipid phosphatase *unc-26*/ synaptojanin, a critical regulator of presynaptic endocytosis [38]. These *unc-26* suppressors include two novel proteins, *unc-80* and *unc-79*, and the NCA ion channel [39•]. *unc-80* has a specific role in regulating NCA ion channel localization to the plasma membrane. A separate study has also identified a novel role for *unc-46* (a LAMP-like protein) in targeting GABA transporter to synaptic vesicles [40•]. Thus, synapse formation requires specific molecules to target cargo to synaptic vesicles and the plasma membrane, and endocytosis seems to be intimately linked with these processes.

Trafficking and sorting

The formation of a presynaptic terminal requires accurate trafficking of presynaptic components from their site of synthesis in the cell body to the axon. Although molecular motors such as kinesins and dynein [22,23,41] were known to play a role in this process for some time, a recent study has begun to unravel the mechanism of how synaptic vesicles are linked to kinesin motors [24•]. In this study, the authors showed that the JIP ortholog, *unc-16*, and the RUN-domain protein, *unc-14*, are critical cargo adaptors that allow kinesin-1 to transport synaptic vesicles to the mature synapse. A recent study has also found that the protein kinase LRK-1, a homolog of the familial Parkinsonism gene PARK8/ LRRK2, is required for sorting synaptic vesicle proteins to the axonal compartment [42•]. Localization and genetic interaction studies suggest that LRK-1 may be acting at the *trans*-Golgi network to prevent synaptic vesicle proteins from being mis-sorted to the dendrites. Interestingly, in addition to these general axonal trafficking mechanisms, cell-specific pathways also exist. A recent study found that the inositol-producing enzyme *myo*-inositol

monophosphatase (IMPase) is required for polarized localization of presynaptic and postsynaptic proteins in the RIA neurons in *C. elegans* [43•]. It is presently unclear how depletion of inositol causes polarity maintenance defects in the RIA neurons, or why this defect is exquisitely cell-specific.

Molecular crosstalk between synapse formation, axon polarity, and termination

While synapse formation has been considered a distinct event in neuronal development, recent work has suggested that presynaptic molecules also participate in other events in developing neurons. For example, the SAD-1 protein kinase [21,44], and the coil–coil domain protein, UNC-69 [45], regulate synapse formation as well as axon polarity and outgrowth. A recent study suggested that the scaffolding protein NAB-1/Neurabin provides specificity to SAD-1 function [46•]. Like *sad-1* animals, *nab-1* mutants have dramatic axon polarity defects in the D-type neurons, but their synapse morphology is normal. Thus, the NAB-1/SAD-1 physical interaction is necessary only for the development of neuronal polarity in this cell type. Interestingly, *nab-1* mutants have synaptic assembly defects in other *C. elegans* neurons, suggesting that *nab-1* can regulate both processes depending upon the cellular context ([47••] and K. Shen, unpublished observation). Similarly, *rpm-1* regulates both synapse formation and axon termination through a common set of downstream mediators, which suggests that these processes are also molecularly linked [37••,48•,49•,50]. Thus, intracellular signals may be integrated to coordinate synapse formation with the establishment of neuronal polarity and axon termination.

Large-scale screens

Understanding how synapses are formed through assembly, maturation, stabilization, and remodeling remains a challenging and important biological problem. Although we have begun to identify the players involved, developing a sophisticated and thorough understanding of these processes remains an immense task. Recently, using a large-scale RNA interference screen, Sieburth *et al.* have shown that at least 12 different molecules function in synapse formation in *C. elegans* [47••]. These molecules fall into several broad categories, including protein kinases, Ras GTPases, protein scaffolds, as well as known and putative regulators of endocytosis and ubiquitination-mediated protein trafficking. Given that this screen was not genome-wide and it involved only one type of neuron, it is probable that the number of players required to successfully assemble a synapse is considerably larger.

Conclusions

Conceptually, synaptic specificity can be achieved through positive selection between appropriate partners, negative selection between inappropriate partners, selective elimination of aberrant synapses, or a combination of these processes. Studies in *C. elegans* suggest that all of these mechanisms can potentially be utilized *in vivo*, and that guidepost cells play a crucial role in patterning synaptic connectivity. Once the synaptic location has been determined, the coordinated activity of several key molecules leads to the assembly of a presynaptic terminal. In particular, two parallel pathways, mediated by *syd-1/syd-2* and *rpm-1*, act as master switches to regulate synapse formation. In the future, integrating genetic, RNAi, biochemical, and proteomic studies in *C. elegans* will continue to be highly informative as we move toward a mature, comprehensive understanding of how to build and maintain a synapse.

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SAD-1 protein kinase via its PDZ domain. NAB-1 is expressed in neurons and co-localizes with SAD-1 at synapses. *nab-1* mutants have defects in axon polarity, and *nab-1* acts in the same genetic pathway as *sad-1*. However, *nab-1* mutants have normal synapses. Thus, *nab-1* mediates the function of *sad-1* in axon polarity but not synapse formation in the D-type neurons.

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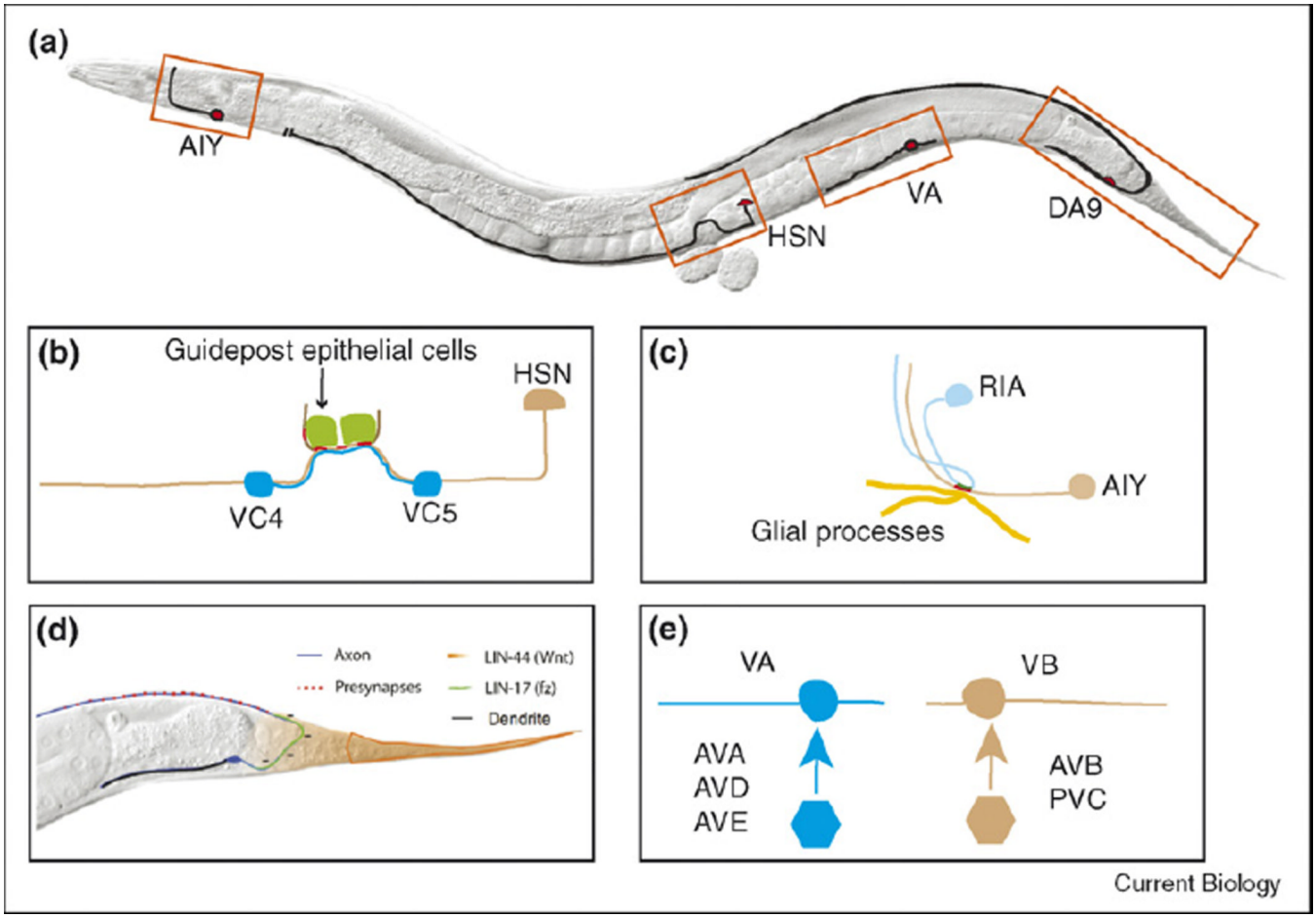

Figure 1.

A diagram of the spatial relations between neurons and guidepost cells discussed in the text. **(a)** An anatomically approximate schematic depicting the location of selected neurons in the body of the worm. For simplicity, only 1 of 12 VA neurons is shown. **(b)** Synapse formation between HSNL neurons and its VC targets is mediated by HSNL cell contact with guidepost epithelial cells. Red puncta represent presynaptic specializations in HSNL. **(c)** Glial processes guide synapse formation between presynaptic AIY neurons and postsynaptic RIA neurons by secreting Netrin. The red and green patches represent the presynaptic terminals and the postsynaptic specializations of the AIY-RIA synapses, respectively. **(d)** Wnts secreted in the tail inhibit synapse formation in the posterior part of the DA9 axon. The red puncta represent the presynaptic terminals of DA9. The green puncta shows the distribution pattern of LIN-17/Fz. **(e)** VA and VB motor neurons receive distinct synaptic inputs, and this synaptic choice is transcriptionally regulated.

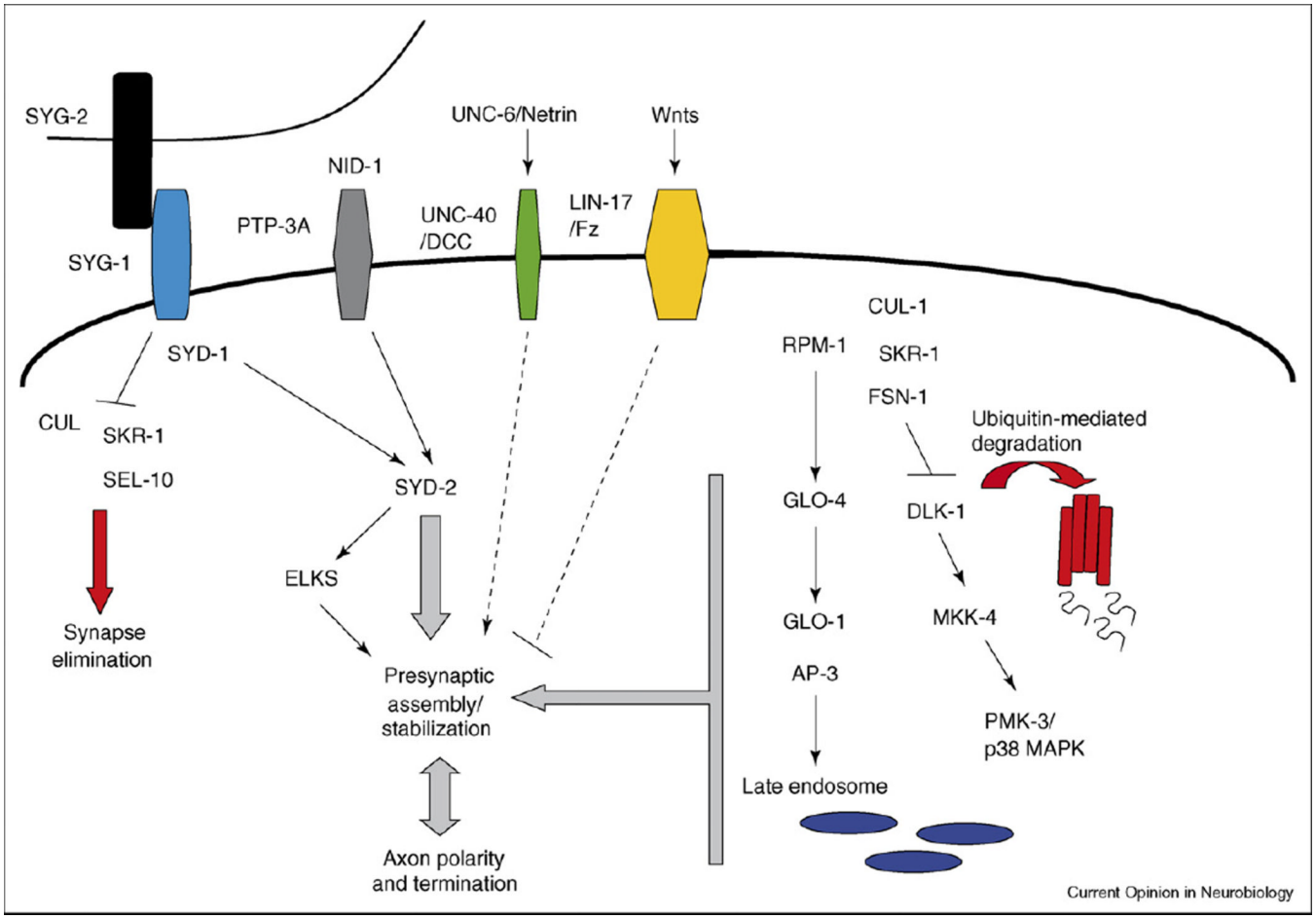

Figure 2.

Flow diagram of molecules and pathways that are integrated to regulate synapse formation in *C. elegans*. Signals activated downstream of guidepost cues, such as SYD-1 and SYD-2, act in parallel with the regulation of protein degradation and late endosomes by RPM-1.

Table 1

Genes involved in synapse formation in *C. elegans*, and their *Drosophila* and vertebrate homologs

C. elegans	***Drosophila***	**Vertebrates**	**Description**
Specificity			
SYG-1	IrreC	NEPH1	IgSF transmembrane protein
SYG-2	SNS	Nephrin1	IgSF transmembrane protein
SKR-1	SkpA	Skp1	E3 ubiquitin ligase component
UNC-6	NetA, NetB	Netrin-1	Axon guidance ligand
UNC-40	Frazzled	DCC	Netrin receptor
LIN-44	Wnt2	Wnt10	Secreted morphogen
LIN-17	Frizzled3	Frizzled10	Wnt receptor
UNC-4	Unc4	Uncx4.1	Homeodomain protein
UNC-37	Groucho	TLE/Grg	Transcriptional corepressor
CEH-12	Hb9	HB9	Homeodomain protein
Assembly and polarity			
SYD-2	Dliprin-alpha	Liprin-alpha 1–4	Active zone protein
SYD-1	DSyd1	SYDE1	Active zone protein
SAD-1	CG6114	SAD-A, SAD-B	Active zone protein
ELKS-1	Bruchpilot	ELKS/ERC/CAST	Active zone protein
RPM-1	Highwire	Phr1/Pam	E3 ubiquitin ligase
DLK-1	Wallenda	Dlk	MAP KKK
MKK-4	Mkk4	MKK4	MAP KK
PMK-3	p38 MAPK	p38 MAPK	Mitogen-activated protein kinase
GLO-4	Claret	RPGR, DelGEF	GEF
GLO-1	Lightoid	Rab32, Rab38, Rab7L1	Rab GTPase
LRK-1	Lrrk	LRRK2/PARK8	Protein kinase
NAB-1	D-Spinophilin	Neurabin, Spinophilin	Scaffolding protein