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Advances in Synapse Formation: Forging Connections in the Worm

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Abstract

Synapse formation is the quintessential process by which neurons form specific connections with their targets to enable the development of functional circuits. Over the past few decades, intense research efforts have identified thousands of proteins that localize to the pre- and postsynaptic compartments. Genetic dissection has provided important insights into the nexus of the molecular and cellular network, and greatly advanced our knowledge about how synapses are formed and function physiologically. Moreover, recent studies have highlighted the complex regulation of synapse formation with the identification of novel mechanisms involving cell interactions from nonneuronal sources. In this review, we cover the conserved pathways required for synaptogenesis and place specific focus on new themes of synapse modulation arising from studies in *C. elegans*.

Introduction

Synapses are specialized connections that allow for information to flow from neurons to their targets. Rapid and precise neurotransmission at synapses underlies nearly all aspects of brain function, and as such, has been the major research focus for decades. Understanding how neurons connect into functional circuits is the first task prior to considering how synapses function to regulate complex processes such as sensory perception, learning and memory, and decision-making. In this review we will focus on the molecular mechanisms that underlie chemical synapse formation, summarizing the recent work utilizing *Caenorhabditis elegans*. For space limitation, we place focuses on the core components assembling synapses and cues and molecules specifying the position and pattern of synapses. For more information concerning the trafficking of synaptic components and receptor function and turnover, a number of recent reviews on these topics are available[1-6].

Overview of the methodology for investigating synaptogenesis in *C. elegans*

Synapses are asymmetric cellular junctions with specialized compartments composed of the presynaptic cell, typically in the form of an axon, and the postsynaptic cell, such as a dendrite or soma, connected in precise registration through a specialized synaptic cleft. Biochemical studies have uncovered over 1,000 proteins present in each compartment, and the majority of these proteins are conserved throughout evolution[7-10]. The *C. elegans*

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nervous system consists of 302 neurons that fall into 118 morphologically defined groups[11]. Synapse formation occurs *en passant* at specific locations within axons that are closely opposed to their targets. The presynaptic compartment in *C. elegans* exhibits an overall structural organization similar to those in vertebrates, with synaptic vesicles clustered around electron-dense projections (Figure 1A-B). Decades of molecular and genetic studies have shown that the core machinery for synaptic vesicle exo- and endocytosis is conserved, including voltage-gated calcium channels, constituents of synaptic vesicles, and the SNARE required for vesicle fusion and exocytosis[12]. The postsynaptic compartment contains voltage-gated channels and receptors for specific neurotransmitters; however, there is a general lack of morphological landmarks[11]. In order for a synapse to form, two events must occur: 1) the presynaptic and postsynaptic cells must recognize each other, most often through target recognition signaling and cell adhesion; 2) the synaptic machinery must be properly assembled and localized to the synaptic compartments.

The major advantage of using *C. elegans* to identify genes that regulate synaptogenesis is the transparency of the body, which enables ready observation of subcellular structures and the stereotypic pattern of synapses. In combination with the powerful genetics of *C. elegans*, the early studies, primarily using chimeric GFP reporters fused to synaptic vesicle proteins, such as synaptobrevin[13, 14], led to the isolation of mutations that disrupted synapse morphology, synapse placement, or trafficking of synaptic vesicles[15]. This cohort of genes is now shown to be conserved throughout evolution and functions to regulate synaptogenesis in many types of neurons.

There are two classes of genes that regulate synapse formation: 1) the core assembly genes, in whose absence synapses are poorly formed or fail to form, and 2) genes that modulate the number, morphology, or placement of synapses. In the next section, we consider the core factors and their regulators that function at nearly all *C. elegans* synapses. In subsequent sections, we will discuss neuron-specific regulators and non-neuronal regulators of synapse formation.

Pre-synaptic assembly machinery and modulatory factors

Formation of the electron dense projection marks the signature, namely, the active zone, of a presynaptic site (Figure 1A, B). *C. elegans* SYD-2 was the first protein identified as a necessary component of synaptic development from a genetic screen[16]. SYD-2 is an ortholog of the Liprin (Lar-interacting-protein-related) -alpha proteins, which share a high degree of sequence conservation in the N-terminal coiled-coils domain and the C-terminal SAM (Sterile-Alpha-Motif) domain [16-18]. Both immunocytochemistry and immuno-electron microscopic analyses show that SYD-2 is localized to the presynaptic dense projection, within the center of synaptic vesicles[16, 19]. A recent technological advance in correlative fluorescence and electron microscopy has further confirmed, with single-molecule imagining, the precise localization of SYD-2 to the electron dense active zone of the presynaptic terminal[20].

Loss-of-function (*lf*) mutations in *syd-2* cause presynaptic domains to become enlarged, as measured by synaptic vesicle components and length of the active zone[15]. Recent findings using high-pressure freezing coupled with electron tomography have revealed nano-

structural units of the active zone, called bays (Figure 1B), in which electron-dense materials form arms that appear to accommodate synaptic vesicle docking[21]. Repeated units of bays may be connected to form large dense projection in some synapses of interneurons. The three-dimensional (3D) reconstruction analysis shows that *syd-2(lf)* results in minimal or smaller bays[21]. The shortened dense projections lead to fewer docked vesicles, and consistently, synaptic function as measured through electrophysiology shows reduced amplitude of evoked post-synaptic currents in *syd-2(lf)* animals [21, 22]. Interestingly, an amino acid substitution in the highly conserved N-terminal coiled-coil domain of SYD-2 results in a gain-of-function *(gf)* mutation[23]. The mutant SYD-2(R187C gf) proteins show a higher tendency to form oligomers[18] and cause enlarged dense projections that appear to disrupt the ordered shape of the bays[21]. While there is no enhancement of evoked post-synaptic amplitude, the *syd-2(gf)* mutants show a decrease in recovery time and in synaptic depression in response to successive stimulation using channelrhodopsin[21]. Together, these studies indicate that SYD-2 functions to regulate the size of the dense projections at the active zone.

The synaptic activity of SYD-2 is positively regulated by SYD-1, a rhoGAP protein that also contains a PDZ domain, a C2 domain, and multiple SH3 domains[23]. SYD-1 itself localizes to active zones, and contributes to the proper localization of SYD-2. SYD-1 and SYD-2 recruit other active zone proteins, such as ELKS-1 (ELKS) and UNC-10 (RIM1) to the presynaptic terminals[23] (Figure 1C). This recruitment is mediated in part by direct protein interactions between SYD-2 and ELKS-1[23, 24]. Another 3D electron tomography study has shown that SYD-2 and UNC-10 function in the same pathway to tether synaptic vesicles to dense projections[22]. UNC-10 also directly interacts with UNC-13, which is required for the docking and priming of synaptic vesicles at the active zone[25]. One negative regulator of SYD-2 is RSY-1, a protein containing a proline-rich domain, a coiled-coils domain, and serine/arginine-rich domain with close homology to the mammalian PNN-interacting serine/arginine-rich protein[24]. RSY-1 reduces synapse formation by decreasing the interaction between SYD-2 and ELKS-1 as assessed in a single-cell protein-protein interaction assay[24] (Figure 1C). SYD-2 has also been shown to exhibit intramolecular interactions and can dimerize in yeast two-hybrid assays; the human liprin-α1 when exogenously expressed in mammalian cells is capable of oligomerization [18, 26]. These data implicate SYD-2 as a presynaptic signaling scaffold, and altering the intramolecular interactions could regulate SYD-2 function by determining its ability to interact with upstream modulators and downstream targets for active zone assembly.

Besides active zone core factors, several modulators have been identified that function to enhance synapse formation in multiple neuronal cell types. SAD-1 encodes a serine/threonine kinase that is related to AMPKs and MARKs and is required for proper synaptic vesicles localization[27]. Although kinase function is required for SAD-1 to modulate synaptic vesicle localization, no targets have yet to be identified[27, 28]. Interestingly, SYD-2 properly localizes to synapses in the absence of SAD-1[27], suggesting that SAD-1 signaling is downstream of SYD-2, possibly to assist in cell polarity and vesicle sorting (Figure 1C). Consistent with this hypothesis, overexpression of SAD-1 causes mislocalization of synaptic vesicles to the dendrites, in addition to axonal guidance and neuronal polarity defects[27, 28].

In higher eukaryotes, the AMPK family is regulated by LKB-1 to modulate metabolic states and cellular growth in dividing cells[29]. While LKB-1-related proteins regulate the development of numerous cell types[30], much less is known about the function of LKB-1/AMPK signaling in neurons. A study showed that both SAD-1 and PAR-4 (*C. elegans* LKB-1) regulate neuronal polarity and synapse formation[31]. Unlike non-neuronal cells, in *C. elegans* neurons PAR-4 and SAD-1 mediate synapse formation through parallel pathways[31] (Figure 1C). The actin scaffolding protein NAB-1 (Neurabin) was also identified as a SAD-1 binding protein through a yeast two-hybrid screen[28]. Their interaction involves the NAB-1 PDZ domain and the SAD-1 PDZ-binding motif. While both proteins localize to the presynaptic periactive zone, loss of function in *nab-1* exhibits little or mild effects on synapses in a manner dependent of cell types (see later) [27, 28, 32]. These findings suggest either a neuron-specific requirement of this interaction or the possibility of still unidentified proteins that act redundantly in this pathway.

The RPM-1 E3 ubiquitin ligase complex of the PHR (Pam/Highwire/RPM-1) protein family defines another conserved pathway ensuring proper synapse formation[33-36]. RPM-1 was identified from two independent genetic screens for mutations that disrupted normal synaptic morphology in the motor neurons and in the mechanosensory neurons [35, 36]. RPM-1 has 3766 amino acids encoding two RCC1 domains, two PHR repeats, a B-box zinc finger, and a RING-H2 zinc finger. Through its many interactions, RPM-1 enhances synapse formation (Figure 2). The E3 ligase activity of RPM-1 involves FSN-1, which was identified in a screen for mutants resembling *rpm-1(lf)* using *syd-2(lf)* as sensitized background[33]. Together with FSN-1 and its binding partners, CUL-1 and SKR-1[33], RPM-1 limits the levels of the Dual Leucine-zipper Kinase 1, DLK-1 MAPK cascade, through protein ubiquitination of DLK-1[34]. A proteomic approach has led to the identification of multiple binding partners of RPM-1, several of which are shown to act in parallel to the E3 ligase function. The interaction between RPM-1 and the Rab GEF GLO-4 targets the Rab protein, GLO-1[37]. Through RAE-1 binding, RPM-1 may act to stabilize microtubules[38, 39]. A phosphatase, PPM-1, has been identified as a negative regulator of PMK-3, a downstream kinase in the DLK-1 signaling cascade[40]. Moreover, ANC-1, a member of the Nesprin family that is known to mediate anchorage of nucleus and organelle, is shown to bind to RPM-1 and acts through β-catenin BAR-1[41]. While more work is required to understand how each pathway contributes to RPM-1 function, these interactions place RPM-1 at the hub of a large signaling network to regulate presynaptic terminals (Figure 2).

Finally, extensive genetic double mutant studies among the core components of presynaptic assembly with *rpm-1* pathway indicate that synaptogenesis is regulated by multiple parallel pathways. While removing each gene individually is not detrimental to synapse function and animal behavior, the double mutants of *syd-2* or *syd-1* with *rpm-1(lf)* exhibit severely impaired movement. In these double mutants, synapse number is dramatically reduced, and the remaining synapses are poorly developed[42]. This behavioral synergy was instrumental to further genetic dissection of individual signaling pathways[23, 33, 34].

Postsynaptic differentiation at the neuromuscular junctions

The neuromuscular junctions of *C. elegans* locomotor circuit have long been studied in understanding the transmission mechanisms and assembly of cholinergic and GABAergic postsynaptic receptors. Early studies that used behavioral and drug-resistance screens have identified a set of genes that modulate cholinergic neurotransmission and made important contributions to the discovery of the core components of the presynaptic release machinery[43, 44]. These studies also led to the identification of RIC-3, which regulates acetylcholine receptor maturation in the muscle[44, 45]. In the absence of RIC-3, nicotinic acetylcholine receptors fail to traffic to postsynaptic regions[45]. RIC-3 is a coiled-coil domain protein that contains two transmembrane domains. Interestingly, the coiled-coils domains are dispensable for receptor maturation, since only the transmembrane domains are required for functional expression of the nicotinic acetylcholine receptors[46].

The cholinergic postsynaptic apparatus contains two classes of acetylcholine receptors, the nicotine-sensitive and the levamisole-sensitive receptors, each containing different receptor subunits. Although RIC-3 is required for the function of both classes of receptors, the two classes also use distinct mechanisms to localize to the postsynaptic membrane adjacent to the presynaptic region. The proper localization and clustering of levamisole-sensitive acetylcholine receptor around the synaptic sites has been greatly aided by a set of genes that confer resistance to levamisole when mutated[47]. LEV-10 is a transmembrane protein containing multiple CUB domains that is expressed by postsynaptic muscles and localizes to the postsynaptic region due to its interaction with levamisole sensitive acetylcholine receptors[48]. Subsequently, a secreted protein, LEV-9, is also shown to regulate acetylcholine receptor clustering, likely interacting with LEV-10 via its multiple complement control protein domains[49]. Interestingly, LEV-9 and LEV-10 are interdependent on each other for their localization to the postsynaptic region [49]. Another small, secreted immunoglobulin domain-containing protein, OIG-4, also localizes to the postsynaptic regions and functions to stabilize the interaction between LEV-10 and acetylcholine receptors [50]. Since neither LEV-9 nor LEV-10 correctly localize to postsynaptic regions in the absence of OIG-4, an attractive model is that OIG-4 acts upstream of LEV-9/LEV-10 as a possible recruitment factor for this complex (Figure 3).

In contrast, much less is known about the postsynaptic differentiation for the GABAergic neuromuscular junctions. Presynaptic regions dictate postsynaptic differentiation; however, GABA transmission is not required for GABA receptor clustering[51]. This suggests that GABAergic neurons may instruct GABA receptor clustering by a secreted or membrane bound protein that has yet to be identified. While the loss of GABA inputs caused GABA receptors to diffuse away from synapses, loss of both GABAergic and cholinergic presynaptic terminals caused the endocytosis and degradation of GABA receptors mediated in part by the autophagy-lysosomal degradation pathway[52]. These studies hint at a possible cross talk between the formation of excitatory and inhibitory synapses through the regulation of postsynaptic receptors.

Determining subtype specificity of cholinergic neuromuscular junctions

Recent studies have uncovered a novel secreted protein MADD-4 and its regulation as an alternative means to modulate synapse formation at the neuromuscular junction. In *C. elegans*, the axon trajectory is restricted by the basement membrane. Instead of forming elaborate presynaptic terminals onto the muscle, the body wall muscles generate small protrusions, called muscle arms, which traverse the basement membrane to form synapses [11]. The muscle arm extension is guided by multiple cues including the DCC protein UNC-40 and MADD-4[53]. MADD-4 is a member of the Punctin proteins, which are secreted proteins containing thrombospondin repeats and Ig domains. The *madd-4* gene encodes a long and a short protein isoform, which are differentially secreted by both cholinergic and GABAergic motor neurons to attract muscle arms to form synapses at the neuromuscular junctions [53]. Moreover, MADD-4 regulates GABA and acetylcholine receptor clustering subsequent to muscle arm extension. In *madd-4* mutants, both GABA receptors and acetylcholine receptors are mislocalized to non-synaptic sites on the muscle arm [54]. Interestingly, the short isoform is required for GABA receptor clustering, and the long isoform is required for acetylcholine receptor clustering, but in the absence of the short isoform, mediates the mislocalization of GABA receptors[54] (Figure 3). Since the long isoform can localize both GABA and acetylcholine receptors adjacent to cholinergic presynaptic regions, this suggests some form of competition between the two MADD-4 isoforms to prevent mislocalization in wild type animals. The homophilic and heterophilic interactions of the long and short isoforms may underlie this mechanism of cross talk between the GABAergic and cholinergic synapses[54] (Figure 3). This study also implies the possibility that secreted proteins from cholinergic and GABAergic neurons directly interact to regulate the localization of excitatory and inhibitory synapses to prevent excitation/inhibition imbalances.

Regionalization and placement of cholinergic DA9 synapses

The *C. elegans* cholinergic motor neurons can be further classified by location of synapses and function within the locomotion circuit. The DA (dorsal type A) cholinergic neurons synapse onto the dorsal body wall muscles and function to drive backwards movement. DA9 is the most posterior neuron, with soma residing in the tail of the worm, extending a dorsally- and anterior-directed axon. The placement of the DA9 synapses is regulated by Wnt signaling arising from non-neuronal cells. LIN-44 (Wnt) is secreted from the tail epidermis and negatively regulates synaptic placement through LIN-17 (Frizzled) expressed in DA9[55]. EGL-20 (Wnt) secreted from the intestine also patterns synapses in DA neurons through both LIN-17 and MIG-1 (Frizzled)[56]. While this Wnt signaling pathway regulates absolute, anterior-posterior placement of synapses, it does not affect the density of synapses. In addition to the Wnt and Frizzled pathway, ectopic expression of UNC-6 can also function as an anti-synaptogenic cue through its receptor, UNC-5, which is expressed in DA9[57]. These studies suggest that both Wnt and UNC-6 gradients emanating from the tail epidermis can alter synapse formation. Indeed the endogenous activity of UNC-6 acts to maintain neuronal polarity by preventing synapses from forming in the dendritic compartment of DA9[57].

Within the dorsal nerve cord the axons of adjacent DA neurons, such as DA8 and DA9, show extensive overlap, yet, their synaptic regions are completely exclusive of each other, a phenomenon known as synaptic tiling[11]. A recent study shows that this tiling is regulated by a plexin homolog, PLX-1 and the semaphorins, SMP-1 and SMP-2[58]. While the function of SMP-1 and -2 are not fully understood, PLX-1 localizes to the boundaries of the DA9 synaptic region, and acts as a RasGAP and regulates synaptic actin to inhibit synapse formation[58]. These observations raise outstanding questions of how the localization of PLX-1 is regulated and further what reciprocal molecules on DA8 ensure proper tiling of DA8. These studies highlight the interplay between neighboring cells in the regulation of synapse placement through anti-synaptogenic cues. A similar interplay between neighboring cells is also found in another set of neurons known as HSN; however, in this case the neighboring cells provide positive synaptogenic cues.

HSN synapse specification by guidepost mechanisms involving SYG proteins

The Hermaphrodite Specific Neuron (HSN) synapses onto vulval muscles to control the egg laying behavior of *C. elegans*[11, 59]. The formation of these synapses is regulated by two immunoglobulin domain proteins, SYG-1 and SYG-2, that mediate an epithelial-neuronal interaction to specify proper presynaptic region localization[60, 61]. SYG-1 is a homolog of NEPH1, and is expressed on the HSN cell surface and specifies the presynaptic region[60]. SYG-2 is a homolog of Nephrin, and is expressed on vulval epithelial cells and specifies the localization of SYG-1 on HSN, and in turn determines where the presynaptic region will form[61] (Figure 4). Recent protein structural studies have identified the first Ig domain in each SYG-1 and SYG-2 as the interaction region that mediates *in vivo* function[62, 63]. The crystal structure predicts that the rigid ectodomains of SYG-1 and SYG-2 are essential for their function, possibly allowing for the interaction to be transduced to intracellular signaling molecules[63]. Via its cytoplasmic tail, SYG-1 interacts with WVE-1 to direct the formation of an F-actin network at the presynaptic region[64]. The actin binding protein NAB-1 then recruits SYD-1 and SYD-2 to the F-actin rich active zone in an actin polymerization-dependent manner[32] (Figure 4). Although actin polymerization is required for synaptic vesicle localization, SYD-1 and SYD-2 recruitment is independent of actin assembly in the AIY neurons, suggesting that actin-mediated recruitment of presynaptic machinery may be cell-type specific[65]. Additionally, SAD-1, is also required for HSN synapse formation[32]; however, it is unclear how the SAD-1 signaling pathway interacts with SYD-1 and SYD-2 recruitment to the active zone.

Emerging themes of non-neuronal regulation of synaptogenesis

The *C. elegans* nerve ring is an ensemble of synapse connections formed by many nerve processes. An excellent model to study synaptogenesis in the nerve ring is those formed between AIY (Amphid Interneuron Y) and RIA (Ring Interneuron A) (Figure 5A). AIY is an interneuron that integrates olfactory, gustatory, and thermal sensory inputs from the amphid sensory neurons. It synapses onto RIA, which integrates AIY information with that from other interneurons to instruct behavioral outputs in the form of locomotion. The synapses between AIY and RIA form at very early larval stages and persist throughout adulthood, and those synapses are reliably visualized by the use of cell-specific markers.

Unlike the other circuits discussed thus far, the amphid sensory neurons and the nerve ring have glial cells that associate with axons and dendrites in the nerve ring. Not only do these glial cells provide support and guidance during sensory neuron development, but they also promote synaptogenesis between AIY and RIA. Specifically, glial cells secrete UNC-6 (Netrin), which interacts with UNC-40 on the AIY neuron, to instruct AIY to form synapse onto RIA in a precise location[66]. UNC-6 binding to UNC-40 causes activation of CED-10 (Rac1) through the CED-5 (DOCK180)[65]. CED-10 then activates MIG-10 (Lamellipodin), which interacts with ABI-1, to enhance actin polymerization at the active zone[67] (Figure 4). Although loss of function mutants in this pathway lead to reductions in actin polymerization, they do not affect SYD-1 or SYD-2 localization[65, 67]. These findings suggest that SYD-1 and SYD-2 may be recruited to the active zone through an alternative mechanism downstream of UNC-40, independent of actin polymerization[67].

More recently, CIMA-1, a lysosomal transporter, was identified as a non-neuronal regulator of synaptogenesis[68]. As previously noted, glia cells regulate the synaptogenesis of AIY onto RIA. Interestingly, the neighboring epidermis influences the glia cell morphology. In this case, loss of CIMA-1 function in the epidermis disrupted glial morphology, which in turn caused aberrant synaptogenesis between AIY and RIA interneurons[37]. One hypothesis is that the perturbed glial morphology may lead to mislocalization of secreted UNC-6, thereby causing aberrant distribution of AIY synapses. Although it is unclear how CIMA-1 affects glial morphology, one suggestion is that it could act by negatively regulating the EGL-15 (FGFR), which may be functioning as an adhesion molecule between the epidermis and glial cells rather than as a signal transducing receptor[68] (Figure 5B, C). While the mechanism requires further investigation, a few pertinent questions are: how is EGL-15 localization regulated at the cell surface and how does increased expression perturb this? What are the interacting molecule(s) on glial cells that interact with EGL-15? What molecule does CIMA-1 transport? Overall, these studies suggest that glia promote and direct synaptogenesis. Moreover, the regulation of glial cells by neighboring cells may play an important role in maintaining proper synapses especially during periods of growth throughout development.

The regulation of secreted factors in the vicinity of the nerve ring is not limited to glial cells. Secreted UNC-6 signaling also regulates the presynaptic regions of the serotonergic neuron, NSM. In this case the source appears to be neighboring neurons in the nerve ring[69]. UNC-6 secreted from these neurons signals in an UNC-40 dependent mechanism to instruct the synaptogenesis of the NSM[69]. Overall, these studies highlight the complex, overlapping cell-signaling pathways that regulate synaptogenesis and suggest how non-neuronal cells and neurons may interact to direct synaptogenesis.

Conservation of presynaptic assembly and modulatory mechanisms

The core components discussed at the outset are well conserved throughout evolution and play similar roles in higher eukaryotes. For example, the Drosophila dLiprin and dSYD-1 regulate synapse formation and active zone size at the neuromuscular junction[5, 17, 21]. In mammalian hippocampal neurons Liprin-alpha 2 is also required at glutamatergic synapse formation [70]. The mammalian mSYD1A regulates synaptic vesicle docking, in part via

interaction with Munc18[71]. The SYG-1 and SYG-2 homologs in the *Drosophila* visual system provide an example of conservation of the synaptic regulators, where Hibris (Nephrin/SYG-2) and Roughest (NEPH1/SYG-1) mediate synapse formation[72]. Unlike *C. elegans* that use SYG-1 and SYG-2 as a signal from non-neuronal cells to neurons, *Drosophila* uses the Nephrin/NEFPH1 homologs trans-synaptically to instruct synapse formation. Additionally, fly and vertebrate PHR proteins are shown to play broad roles in synaptogenesis and axon outgrowth and patterning, largely through control of the DLK kinases [39]. Furthermore, downstream components, such as actin polymerization via GTPases[73, 74] and protein degradation[75-77], have all been implicated in the formation of synapses in other species. While the list is not exhaustive, these few examples highlight the idea that synaptic structure and organization are maintained throughout evolution through the use and reuse of a common set of synaptic proteins.

Although the synaptic protein pools have been expanded and the interactions may vary, the general role of non-neuronal cells in regulating synaptogenesis is well conserved. In mammals and *Drosophila*, glia can enhance synapse formation, similar to the glia and epithelial cells in *C. elegans*[78-81]. Most notable are the recent findings in mammalian astrocytes that regulate synapse formation through both secreted and membrane-associated proteins[78], reminiscent of the roles of SYG-1/SYG-2 and UNC-6/UNC-40 interactions in *C. elegans*.

Conclusion

Synaptogenesis is a key event during the development of the nervous system. The major components and mechanisms are conserved throughout evolution from *C. elegans* to humans. From target specifying proteins to active zone organizers, the identification of both positive and negative regulators has highlighted the importance of generating a discrete number of connections. Exciting new studies have also identified how nonneuronal cells can modulate synaptogenesis. Overall, the identification of synaptogenesis regulators lays the groundwork for investigating the complex biology underlying nervous system function, including the molecular mechanisms that underlie learning/memory, decision-making, and synaptic maintenance during development/aging.

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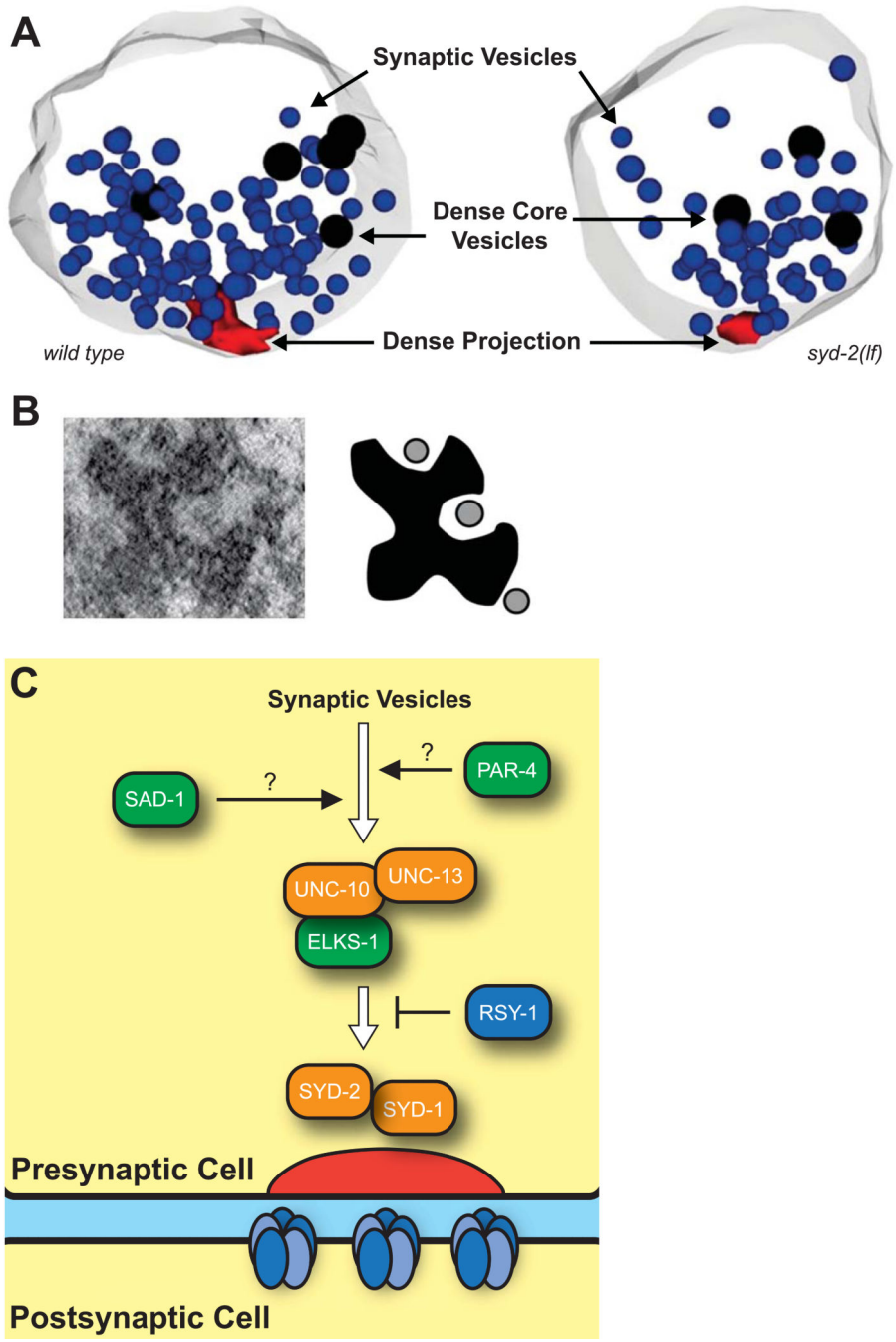

Figure 1. The active zone and core components of presynaptic terminals

A) 3D electron tomography reconstructions of a presynaptic bouton at the neuromuscular junction in wild type and *syd-2(lf)* backgrounds. Synaptic vesicles (blue) are docked at the membrane adjacent to the dense projection (red). Dense core vesicles (black) are mixed among the reserve pool of synaptic vesicles. Images were adapted from Kittelman et al [21] ,with permission.

B) Electron tomography and schematic rendering of bays formed by the presynaptic density and associated synaptic vesicles[21]. The view of bays is shown as parallel to the plasma membrane. Images were adapted from Kittelman et al [21] ,with permission.
C) Schematics of active zone assembly: SYD-1 and SYD-2 are recruited to the active zone. SYD-2 binding to ELKS-1 directs UNC-10 and UNC-13 to the active zone. RSY-1 exerts a negative role on SYD-2 and ELKS-1 interaction. Through UNC-10 and UNC-13, synaptic vesicles are then docked at the active zone. SAD-1 and PAR-4 enhance proper synaptic organization likely by regulating vesicle localization. NAB-1 has a facilitating role through protein interaction with SAD-1 and SYD-1. Orange proteins are core components, green proteins are positive regulators of synapse formation, and the blue protein is a negative regulator of synapse formation. Black arrows and lines represent signaling; white arrows represent protein complex recruitment.

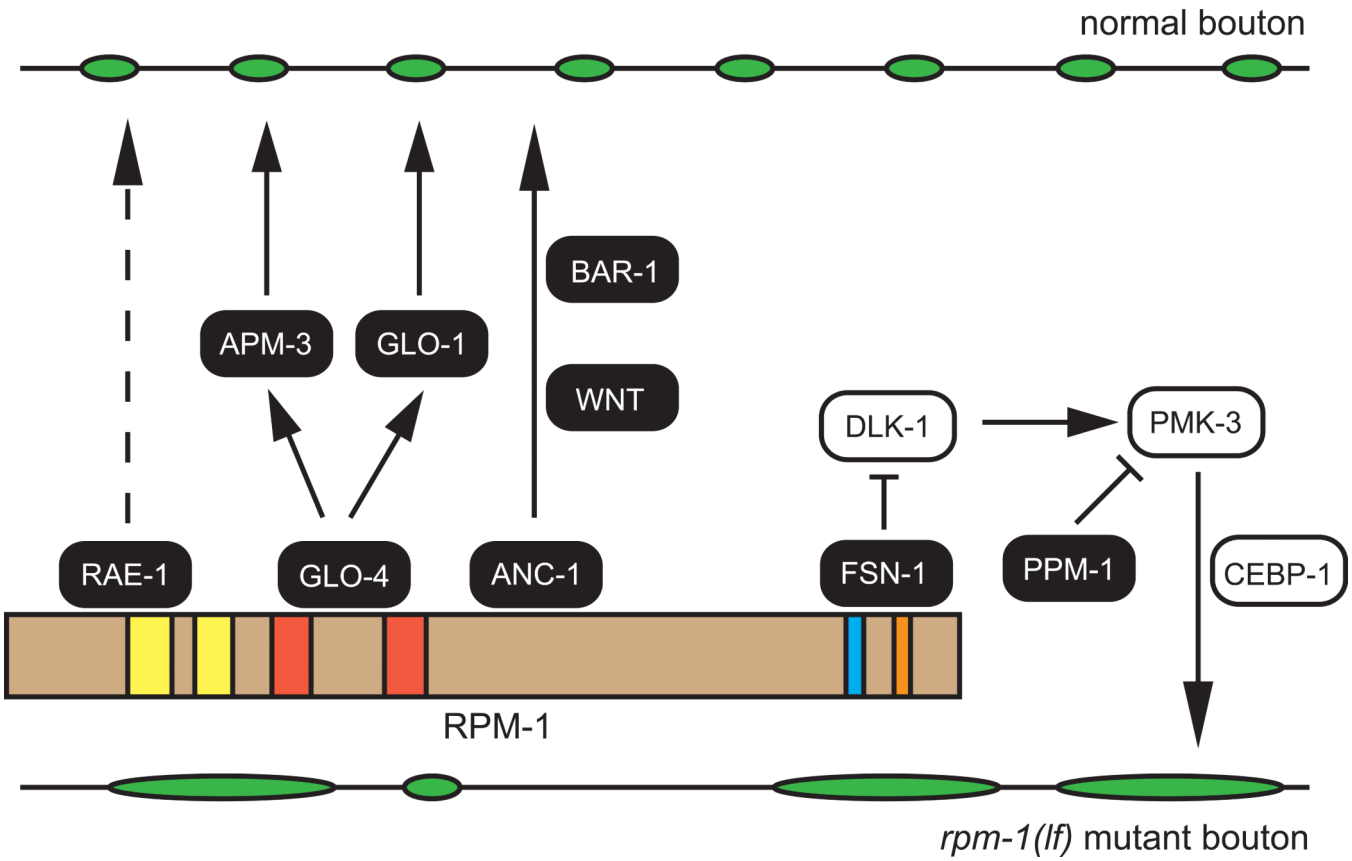

Figure 2. Regulation of synapse formation by the RPM-1 signaling network

RPM-1 ensures proper synapse formation by signaling through multiple binding partners through its RCC1 (yellow), PHR(red), B-Box(blue), and RING(orange) domains. RPM-1 E3 ligase activity, via binding to FSN-1, inhibits the DLK-1 MAPK cascade, which in the absence of RPM-1 disrupts proper synapse formation. ANC-1 binds to RPM-1 and functions downstream through WNT signaling and BAR-1 (beta-catenin) to maintain proper synapse morphology. PPM-1 may prevent aberrant synapse formation through the dephosphorylation of PMK-3 substrates, such as CEBP-1. Through GLO-4 binding, RPM-1 may activate GLO-1 and APM-3, directly or indirectly, to regulate synaptic endosomes. RPM-1 binding to RAE-1 may regulate synapse formation by stabilizing microtubules. Solid arrows and lines represent activating or inhibiting signaling events, respectively. Dashed line represents unknown signaling events that regulate synaptic morphology.

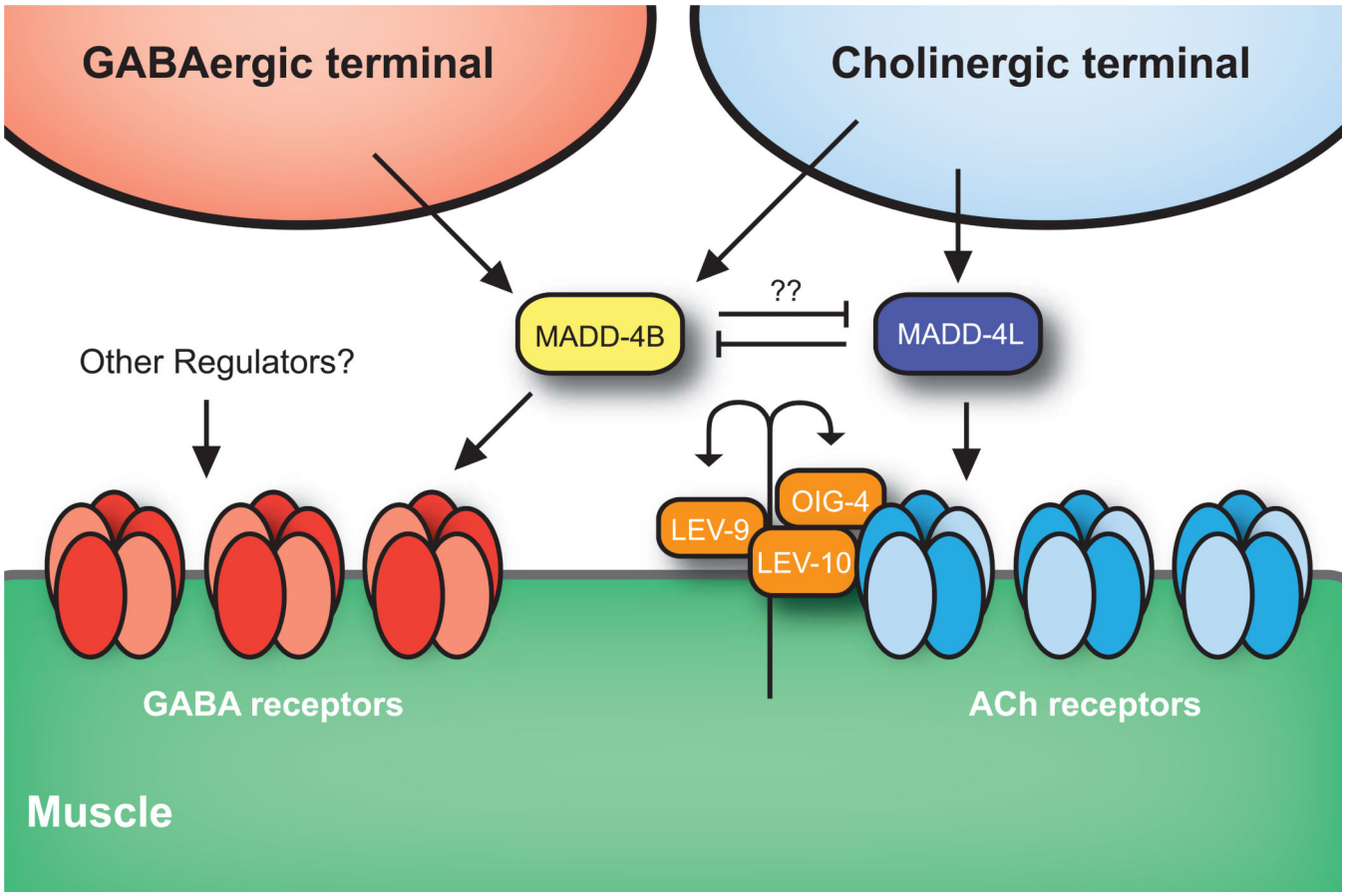

Figure 3. Synapse specification at the neuromuscular junction

Acetylcholine receptors are clustered at the neuromuscular junction through at least two different pathways. First, the muscles can cell-autonomously enhance acetylcholine receptor clustering through the expression of LEV-10 and its secreted binding protein LEV-9. This complex is also aided by OIG-4, secreted by muscle. A second pathway requires the cholinergic terminals and MADD-4 to enforce acetylcholine receptor clustering. Cholinergic neurons secrete the MADD-4 long isoform (MADD-4L), which through an unknown mechanism causes acetylcholine receptor clustering at the neuromuscular junction. Cholinergic neurons also assist with the clustering of GABA receptors. Through the secretion of MADD-4B short isoform, both GABAergic neurons and cholinergic neurons increase GABA receptor clustering. It is thought that the proper postsynaptic clustering of receptors adjacent to their respective presynaptic terminals is mediated through some heterophilic interaction between MADD-4 isoforms. Since GABA receptors cluster in the absence of MADD-4B, albeit adjacent to cholinergic terminals, this suggests that other molecules are required for the clustering of GABA receptors.

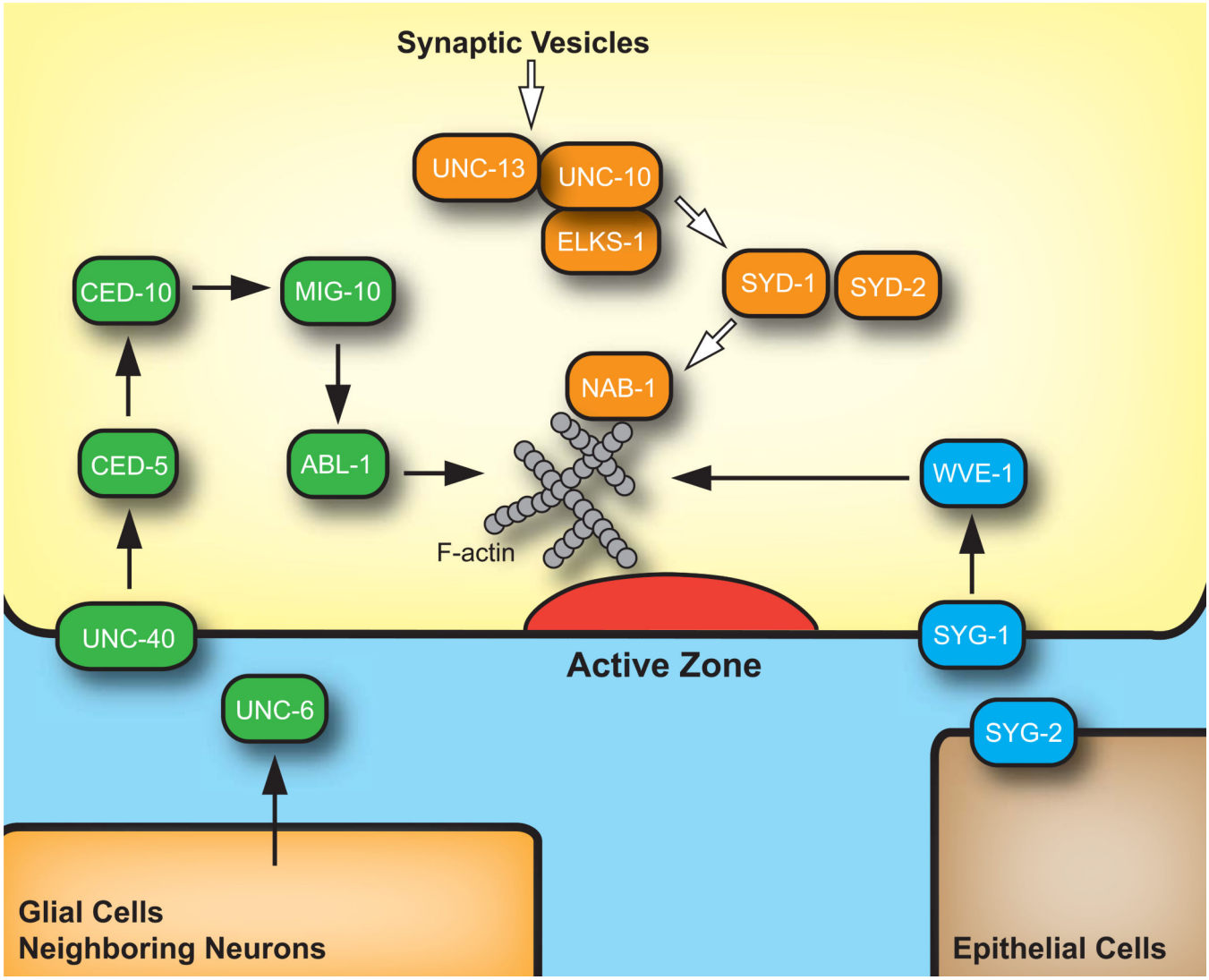

Figure 4. Non-neuronal cells modulate synaptogenesis through secreted and cell-adhesion molecules

Upon binding to epithelial SYG-2, SYG-1 localized in the HSN signals through WVE-1 to increase actin polymerization. In an analogous pathway, in the nerve ring, secreted UNC-6 from glial cells stimulates UNC-40 expressed in AIY, which then actives a cascade of CED-5, CED-10, and MIG-10. MIG-10 interacts with ABI-1, a member of the WAVE complex that directs cytoskeletal remodeling. In the case of the HSN pathway (blue proteins), actin polymerization facilitates the recruitment of SYD-1, SYD-2, and downstream active zone proteins to the dense projection (red) through the actin-binding protein, NAB-1. The glial/nerve ring pathway (green proteins) induces actin polymerization, which is required for synaptic vesicle localization; however, actin polymerization appears to be dispensable for the recruitment of SYD-1 and SYD-2 to the active zone in this case, suggesting an alternative mechanism. Black arrows represent signaling events; white arrows represent protein complex recruitment.

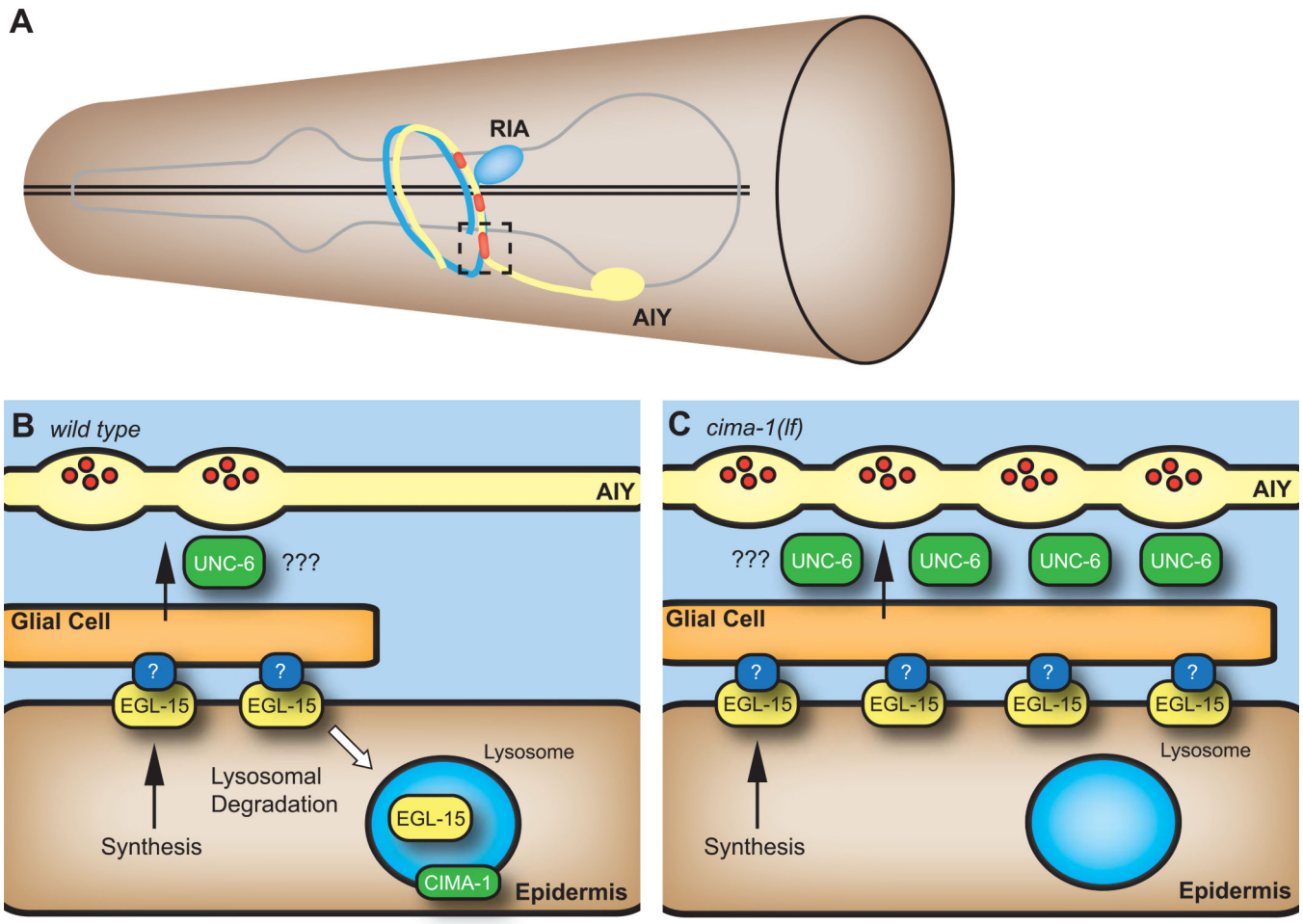

Figure 5. Epidermal regulation of synapse formation

A) Illustration of AIY synapses in the head (brown). AIY and RIA reside in the nerve ring anterior to the posterior bulb of the pharynx (gray outline). AIY makes synapses (red) onto RIA at reliable, discrete locations. A zoomed-in view is illustrated in B and C.

B) Synapses from AIY onto RIA are modulated by adjacent glial cells, which limit the placement of presynaptic terminals. The epidermis, which is in close proximity to the glial cells, regulates their morphology through EGL-15. Through the interaction of EGL-15 on the epidermis and a yet to be identified binding partner in glial cells, the epidermis is able to restrict the morphology into a discrete region adjacent to AIY presynaptic terminals. In wild type animals, EGL-15 expression is mediated by CIMA-1, most likely through lysosomal degradation.

C) In *cima-1(lf)* mutants, EGL-15 is more highly expressed compared to wild type, possibly leading to an elongated region of interaction. In turn, the contact between AIY and the glial cells is also expanded, leading to an increase in presynaptic terminals from AIY onto RIA. On potential explanation for this alteration of presynaptic terminals could be through an increased signaling of UNC-6.

Table 1

C. elegans Gene Abbreviations.

Gene Name	Definition
ABI-1	ABl interactor homolog-1
ANC-1	abnormal nuclear Anchorage-1
BAR-1	Beta-catenin/Armadillo-related-1
CED-10	Cell Death abnormality-10
CED-5	Cell Death abnormality-5
CIMA-1	Circuit Maintenance abnormal-1
CUL-1	Cullin-1
DLK-1	Dual-Leucine zipper Kinase-1
EGL-15	Egg Laying defective-15
EGL-20	Egg Laying defective-20
ELKS-1	glutamine (E), leucine (L), lysine (K), serine (S)-rich protein-1
FSN-1	F-box Synaptic Protein-1
GLO-1	Gut granule Loss-1
LEV-10	Levamisole resistant-10
LEV-9	Levamisole resistant-9
LIN-17	abnormal cell Lineage-17
LIN-44	abnormal cell Lineage-44
MADD-4	Muscle Arm Development Defective-4
MIG-1	abnormal cell Migration-1
MIG-10	abnormal cell Migration-10
NAB-1	Neurabin-1
OIG-4	One Immunoglobulin domain-4
PAR-4	abnormal embryonic Partitioning of cytoplasm-4
PLX-1	Plexin-1
PPM-1	Protein Phosphatase, Magnesium/Manganese-dependent-1
RAE-1	Ribonucleic Acid Export factor-1
RIC-3	Resistance to Inhibitors of Cholinesterase-3
RPM-1	Regulator of Presynaptic Morphology-1
RSY-1	Regulator of Synapse Formation-1
SAD-1	Synapses of Amphids Defective-1
SMP-1	Semaphorin-related-1
SMP-2	Semaphorin-related-2
SYD-1	Synapse Defective-1
SYD-2	Synapse Defective-2
SYG-1	Synaptogenesis abnormal-1
SYG-2	Synaptogenesis abnormal-2
UNC-10	Uncoordinated-10

Gene Name	Definition
UNC-40	Uncoordinated-40
UNC-5	Uncoordinated-5
UNC-6	Uncoordinated-6
WVE-1	WASP family Verprolin-homologous protein-1

Table 2

C. elegans neuron nomenclature.

***C. elegnans* Neuron**	**Definition**
AIY	Amphid Interneuron Y
DA	Dorsal Projecting A type motorneuron
HSN	Hermaphrodite Specific Neuron
NSM	pharyngeal Neurosecretory motorneuron
RIA	Ring Interneuron A

Table 3

General abbreviations for molecules.

Abbreviation	Definition
AMPK	Adenosine Monophosphate-activated Kinase
CUB	complement C1r/C1s-urchin epidermal growth factor-Bone morphogenesis protein-1 domain
DCC	Deleted in Colorectal Cancer protein
DOCK180	Dedicator of Cytokinesis 180
GABA	Gama-aminobutyric acid
GAP	GTPase Activating Protein
GEF	Guanidine nucleotide Exchange Factor
GFP	Green Fluorescent Protein
LKB-1	LKB-1
MAPK	Mitogen-activated Protein Kinase
MARK	Microtubule-associated Protein/microtubule-Affinity Regulating Kinase
NEPH1	NEPH1, also kin of IRRE-like protein 1
PDZ	Postsynaptic Densitv-95/Drosophila disc large tumor suppressor-1/zonula occludens-1 protein domain
PHR	Pam/Highwire/RPM-1 domain
PNN	Pinin
Rac1	Ras-related C3 botulinum toxin substrate 1
RCC1	Regulator of Chromosome Condensation-1 domain
RIM1	Rab-3 interacting molecule 1
RING-H2	Really Interesting New Gene-H2 domain
SNARE	soluble N-ethylmaleimide-sensitive factor (NSF)- attachment protein receptors