

Synaptogenesis: insights from worm and fly

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Synapse formation is the ultimate step in wiring a nervous system. Synapses are remarkably diverse in size and shape, and are regulated dynamically. Recently, live observations combined with ultrastructural analysis have revealed many details of the cellular interactions that precede synapse formation. Genetic screens in *Caenorhabditis elegans* and *Drosophila* have implicated signaling pathways that may involve small G-proteins, ubiquitin-mediated protein degradation and selective cell adhesion in target recognition, synaptic assembly and growth.

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Abbreviations

<i>dlg</i>	<i>discs-large</i>
Ena	Enabled
<i>faf</i>	<i>fat facet</i>
Fas	Fasciclin
GABA	γ -amino butyric acid
GEF	guanine nucleotide exchange factor
GFP	green fluorescent protein
hiw	highwire
NMJ	neuromuscular junction
Rim	Rab3a-interacting molecule
RPM	regulator of presynaptic morphology
RPTP	receptor protein tyrosine phosphatase
RRP	readily releasable pool
<i>sad</i>	<i>synapses of the amphid defective</i>
<i>sam</i>	<i>synapse abnormal morphology</i>
SNB	synaptobrevin
SVs	synaptic vesicles
<i>syd</i>	<i>synapse defective</i>
<i>unc</i>	<i>uncoordinated</i>

Introduction

Chemical synapses are specialized subcellular architectures built across intercellular junctions between neurons and partner cells. Although varying in form and size, the presynaptic terminal is hallmarked with a large number of synaptic vesicles (SVs) orderly clustered around electron-dense specializations at the junctional membranes. The postsynaptic site contains neurotransmitter receptors densely positioned by a specialized submembranous cytoskeleton opposing the presynaptic density. Holding presynaptic and postsynaptic partners in precise register usually requires a specialized synaptic extracellular matrix [1,2].

Our understanding of how synaptic structures are formed has always depended on methodological breakthroughs that improve accessibility. Here, I review recent findings on the cellular dynamics at *Drosophila* neuromuscular

junctions and genetic studies of presynaptic assembly in fly and worm. Recent comprehensive discussions on related topics can be found elsewhere [3–7].

Target recognition at fly neuromuscular junctions

About 40 motor neurons innervate 30 muscles in each abdominal hemisegment of *Drosophila* embryos and larvae [8]. Motor neuron axons enter their target territory following stereotyped pathways and differentiate terminal branches, which form synapses with muscles as a chain of varicosities or ‘synaptic boutons’. These synaptic boutons are classified by size, which correlates with neurotransmitter type and function. The shape and location of the synapses are specific to a given muscle fiber, and each muscle can receive inputs from up to four motor axons. A recent study has found an apparent rule of ‘bouton exclusion’ such that a single muscle receives innervations from only one motor axon of a given bouton type [9]; however, the mechanism underlying this process remains to be elucidated.

Molecules for target recognition

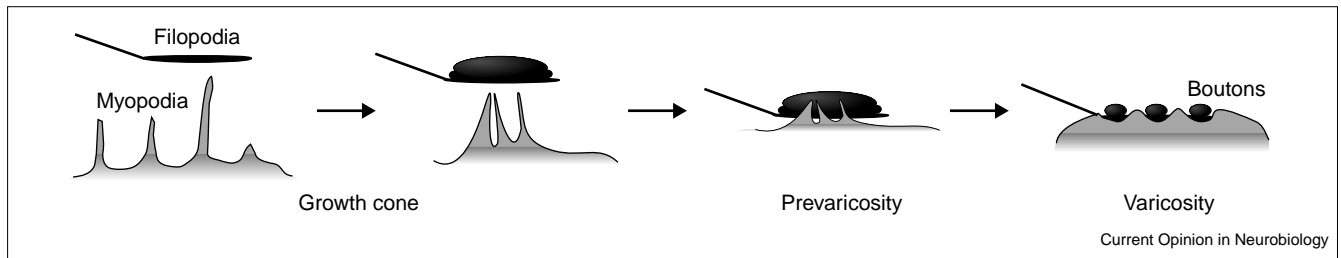
The terminal nerve branches explore muscle surfaces through a distinct process known as ‘target recognition’, in which synaptic partners are matched, highly motile filopodia of the growth cones are transformed into nascent presynaptic terminals, and presynaptic and postsynaptic membranes are brought into close apposition. A series of early studies established that the pairing of synaptic partners at a fly neuromuscular junction (NMJ) does not depend on a single specific molecule, but instead involves a combinatorial assessment of adhesive property — conferred by the expression of the target recognition molecules — between partners [5,10,11].

These molecules are mostly homophilic cell-adhesion molecules, and are grouped by function into two general categories: synaptogenic, which includes Fasciclin III (FasIII), connectin, capricious and netrin B; and anti-synaptogenic, which includes semaphorins, Toll and Beaten path [5]. Complete loss of function of any one of these molecules results, at most, in a mild effect on NMJ formation, indicating that there is considerable functional redundancy. Misexpression of synaptogenic molecules in inappropriate partners promotes ectopic formation of synapses; and, likewise, misexpression of antisynaptogenic molecules inhibits synapse formation.

Cellular interactions during presynaptogenesis

Recent studies combining live observation with ultrastructural preservation have shown that complex cellular interactions take place during target recognition, identifying a ‘presynaptogenesis’ period. During this process, the nerve endings undergo three morphologically defined changes [12] (Figure 1). Initially, the nerve terminals are thin and flat

Figure 1



Presynaptogenesis at fly NMJ. At the growth cone stage, both nerve endings (black) and muscles (gray) extend thin, motile, actin-rich filopodia and myopodia; myopodia show nerve-dependent clustering. By the prevaricosity stage, nerve endings develop broad thick swellings that contain rudimentary synaptic components. The muscle continues to

exhibit a specialized reaction to the presence of the nerve terminal in the form of ruffles around its periphery. By the varicosity stage, increased complexity of the postsynaptic structures and the localized accumulations of presynaptic active zones have led to constriction of the prevaricosity to form individual boutons. Modified with permission from [12,13**].

with long filopodia. Filopodia from several growth cones often overlap and explore muscle surfaces broadly. Over time, filopodia decrease in length, and growth cones acquire strand-like structures and sheet-like lamellipodia. Subsequently, smoothly contoured enlargements, called 'prevaricosities', arise from the planar region of the growth cone. Prevaricosities contain dense-core vesicles, irregularly shaped SV-like vesicles and immature presynaptic specializations. Last, in contrast to a general view that synapses develop at the end of nerve terminals, mature synaptic boutons are formed through constrictions of the prevaricosities.

Muscle membranes show equally dynamic cellular changes during the presynaptogenesis period (Figure 1). Newly formed muscle cells are covered with 'myopodia' — filopodia-like microprocesses, which can be more than 5 μm long and have rounded heads, and are rich in actin filaments and randomly distributed [13**]. As motor axons approach their targets, myopodia become progressively clustered at the site of neuron contacts and intermingle with presynaptic filopodia. Ultrastructural analyses show frequent mutual wrappings of membrane protrusions from the growth cones and the muscles, accompanied by the accumulation of electron-dense cytoplasmic material on both sides of the sites of membrane association [14*]. Myopodia are proposed to contribute to long-distance cellular communication in synaptic target recognition [13**].

The presynaptogenesis cellular events at fly NMJs are remarkably similar to the events that occur in adherens junction formation between epithelial cells, which are mediated by E-cadherin and α -catenin [15]. On stimulation with calcium, epithelial cell filopodia penetrate and embed into neighboring cells, generating a two-rowed puncta structure that has electron-dense adherens junctions anchored to F-actin at the tip of the filopodia.

The potential role of cadherin at fly NMJs has not yet been addressed because of its early involvement in motor axon guidance [16]; however, cadherin is involved in

synaptic target recognition in the fly retina [17**]. Other target recognition molecules can influence presynaptogenesis interactions between nerve and muscle [14*]. For example, ectopic expression of FasIII, a synaptogenic molecule, promotes membrane contacts between the growth cones of the RP3 motor neuron with both its usual targets and other FasIII-expressing muscles that RP3 would normally avoid. Similarly, ectopic expression of Toll, an anti synaptogenic molecule, in RP3 target muscles discourages membrane contacts, and RP3 growth cones are unable to proceed with synaptogenesis. It is likely that the selective adhesion mediated by target recognition molecules stabilizes the initial cellular contacts between myopodia and filopodia, and leads to 'zippering' of the presynaptic and postsynaptic membranes at NMJs.

Intracellular signaling of target recognition

How is target recognition linked to intracellular events in the presynaptic and postsynaptic cells? A candidate molecule at fly NMJs is Dock, a Src homology domain 2–3 adapter protein (Table 1). Dock is concentrated in growth cones, and loss of Dock function causes a selective delay in synapse formation by RP3 onto its muscles [18]. The mammalian homolog of Dock, Nck, binds activated receptor tyrosine kinases and interacts with Rho-family GTPase signaling pathways [19]. Thus, Dock may regulate cytoskeleton remodeling during the transition of a growth cone into a presynaptic terminal.

Late bloomer, a tetraspanin-like molecule that is transiently expressed in the membranes of motor axon terminal arbors, shares similar mutant phenotypes with Dock [20] (Table 1). The double mutant of Dock and Late bloomer resembles each single mutant, consistent with the possibility that they function in a common pathway [18]. In vertebrates, tetraspanins interact with integrins [21]. In integrin mutant larvae of fly, the terminal arbors of motor axons are overdeveloped [22,23] (Table 1), suggesting that they negatively regulate synaptic growth. But whether they affect early events during target recognition remains to be examined.

Table 1

Selected genes and their loss-of-function effects on synaptic organization and growth.

Protein	Worm gene and phenotype	Fly gene and phenotype	Candidate vertebrate homolog
Liprin	<i>syd-2</i> Altered presynaptic density [35]	CG11199	Liprin- α [48]
Kinase	<i>sad-1</i> Broad SV distribution [34**]	CG6114	STK29
GEF and E3	<i>rpm-1</i> Absent and abnormal SV clusters, disorganized presynaptic terminal [36**,37**]	<i>hiw</i> Overgrowth of NMJ; smaller but normal bouton [64**]	Pam [74]
UBP	?	<i>faf</i> Suppresses the physiological, but not morphological, defects in <i>hiw(lf)</i> [67**]	?
Rim	<i>unc-10</i> Normal synapse [47**]	?	Rim [43]
β -spectrin	<i>unc-70</i> Normal synapse [52*,53*]	β -spectrin Normal synapse [52*,53*]	β -spectrin
α -spectrin	<i>spc-1</i>	α -spectrin: Normal synapse [52*,53*]	α -spectrin
Synapsin	?	Synapsin Inconclusive	Synapsin [56,57]
MAP	–	Futsch Disruption of MT loop; fewer but larger boutons [60]	MAP1B
α -integrin	<i>ina-1</i>	Volado Overgrowth of terminal arbors [22]	α -integrin
β -integrin	<i>pat-3</i>	Myospheroid Overgrowth of terminal arbors [23]	β -integrin
RPTP	<i>ptp-3</i> Unknown	Dlar Target recognition [75,76**,77**]	Lar-RPTP
Synaptojanin	<i>unc-26</i> Collapsed presynaptic cytoskeleton and few SVs[61**] ?		Synaptojanin [73]
SH2–SH3 adaptor	CeNck Unknown	Dock Delayed synapse formation [18]	Nck
Tetraspanin	?	Late bloomer Delayed synapse formation [20]	tetraspanin
RacGEF	?	SIF NMJ growth [63]	?
CAM	?	FasII NMJ growth	CAM
Cadherin ?	N-cadherin	Target recognition [17**]	?

Genetic approaches to synaptic assembly

Despite differences in size and form, all synapses have morphologically and functionally defined domains at both the presynaptic and postsynaptic sites. A presynaptic terminal contains at least three domains: an active zone for vesicle release, a vesicle reserve domain, and a periaxial zone for synaptic stabilization and growth. A postsynaptic site has both a receptor clustering domain and intermediate regions for receptor targeting and signal transduction.

Perhaps reflecting the late onset nature of synaptogenesis and functional redundancy, early genetic screens had only limited success in identifying genes that function specifically at synapse formation [24]. The recent development of green fluorescent protein (GFP) fusion reporter genes

has markedly enhanced our ability to observe synapses in living animals [25–27], providing indispensable tools for studying synapse formation.

Drosophila

Recently, to circumvent the pleiotropy and functional redundancy of genes that are involved in NMJ formation, a systematic approach has been taken in *Drosophila* to look for abnormal NMJ morphology caused by overexpressing or misexpressing a given gene, using existing collections of Gal4–enhancer and promoter (EP) lines [28,29*]. Although such an approach has an inherent flaw in that overexpression or misexpression of a gene often produces effects that do not necessarily reflect the normal function of that gene, it can in part provide insights into how such a process is regulated.

When overexpressed, 76 genes were found to cause defects in the terminal branching pattern and NMJ morphology [29•]. Among these, 41 are known genes, 19 of which cause axon pathfinding, target recognition and synapse defects as loss-of-function mutations; and 35 are new genes, whose protein products seem to function in all cellular aspects and include, for example, G-proteins, kinases, membrane and secreted proteins, and RNA-binding proteins. Elucidating the roles of these new genes in NMJ formation will depend on further analysis of the loss-of-function mutant phenotypes.

C. elegans

The simple nervous system of the worm, combined with its transparent body, allows observation of synaptic abnormalities at the resolution of a single synapse. Moreover, the fact that much of the nervous system function is not required for viability or fertility makes it easy to maintain homozygous mutants. In contrast to the NMJ of fly and mammals, in which synapses form at terminal branches of motor neurons, most synapses in the worm are formed *en passant* along the nerve processes [30]. At the ultrastructural level, the presynaptic terminals of worm synapses resemble many synapses in the vertebrate central nervous system, whereas the postsynaptic density is usually less prominent.

Synaptic target recognition is generally thought to be the result of axon guidance, which takes place through evolutionarily conserved mechanisms [31]. The observation that misguided axons form normal NMJs suggests, however, that some kinds of chemotrophic interaction may exist for target recognition [32].

Three independent genetic screens using a synaptobrevin-1-GFP (SNB-1:GFP) chimeric gene [33] to label SV clusters in chemosensory, mechanosensory and motor neurons of the worm have identified sets of overlapping genes, named *synapses of the amphid defective (sad)*, *synapse abnormal morphology (sam)* and *synapse defective (syd)*, respectively [34••,35,36••,37••]. These mutations seem to affect all synapses, but the terminal synaptic phenotypes differ in a manner specific to synapse type.

The frequency of detecting synaptic abnormalities caused by a given gene also varies depending on the type of neurons [34••,36••] (M Zhen, Y Jin, unpublished data), raising such questions as does the same gene participate in different cellular processes in different synapses? And do different types of synapses have different sensitivity to the perturbation of the same gene?

Moreover, although these mutations were isolated on the basis of abnormal patterns of SNB-1:GFP clusters, the molecular characterizations of the three genes have shown that they are not SV-associated proteins, but instead seem to define distinct subdomains in presynaptic terminals. The analysis of these mutants, together with other existing

mutants in fly and worm (Table 1), has given us a glimpse of the complexity underlying synaptic assembly.

Presynaptic active zone

The presynaptic active zone usually refers to the electron-dense presynaptic density, which is positioned immediately adjacent to the membrane apposite to the postsynaptic cell, and the SVs that are closely associated with the density and the immediate surrounding membranes [38]. Electron microscopy tomography on frog NMJs reveals that the presynaptic density has a sophisticated structure made of molecular blocks of distinct shapes [39]. Presynaptic density in fly glutamatergic type Ib boutons has a T-shape and is therefore referred to as a 'T-bar', whereas that in worms is usually plaque-like. Several T-bars are often present in a single bouton of fly NMJs [7,8], but usually there is only one density in a presynaptic varicosity in worm [30].

Despite its unmistakable ultrastructural appearance, little is known about the exact molecular components of the presynaptic density. Although calcium channels and vesicle fusion complexes are thought to be located in the presynaptic density, disrupting the vesicle fusion machinery has no effect on the integrity of the presynaptic density [40–42].

The Rab3a-interacting molecule (Rim) family of proteins, which consist of conserved zinc-finger, PDZ and C2 domains, are possible candidates for tethering SVs to the presynaptic density [43,44]. The vertebrate Rims interact with Rab3GTP, cAMPGEFII and Munc-13 [43–46], and Rim1 is localized to the presynaptic density [43]. The vertebrate Rims interact with Rab3GFP, cAMPGEFII and Munc-13, all of which are involved in regulated vesicle release. In *uncoordinated (unc)-10*, the *C. elegans* Rim, the presynaptic density and vesicle docking are normal, but vesicle release is impaired. This defect is suppressed by an open form of syntaxin, indicating that UNC-10/Rim regulates vesicle priming in a post-docking step, but not formation of the presynaptic active zone [47••].

The first gene that was shown to affect presynaptic density is the *C. elegans syd-2* gene [35], also identified as *sad-2* [34••] (K Shen, C Bargmann, personal communication). In *syd-2* loss-of-function mutants, presynaptic densities are lengthened and appear to have less electron density than normal. The localization of UNC-10/Rim is also less discrete in *syd-2* mutants than in wild type (M Nonet, personal communication). SYD-2 is a member of the Liprin protein family, which contains coiled-coil and sterile alpha motif domains [48], and is localized very close to the presynaptic density [35]. Liprins can interact with the Lar family of receptor protein tyrosine phosphatases (RPTPs), and cluster Lar-type RPTPs to focal adhesions [48,49]. SYD-2 may recruit components of the presynaptic density and facilitate its assembly. Supporting an evolutionarily conserved function of Liprin, the *Drosophila* Liprin- α is also localized to presynaptic terminals of the NMJ, and, in

mutant flies for Liprin and DLar, the morphology and size of the presynaptic active zones are affected (D Van Vactor, personal communication).

Synaptic vesicle clustering

Synaptic vesicles are generated both from precursors that are synthesized at the cell body and transported to the terminals and from recycling through endocytosis at the terminals. How precursors mature into vesicles and how the initial clusters of vesicles form are unclear. In *sad-1* mutants of *C. elegans*, presynaptic densities are normal but SVs are distributed more broadly than in wild-type [34**]. SAD-1 defines a family of protein kinases that are related to PAR-1 kinase, which regulates microtubules to generate cell polarity [50]. SAD-1 partially colocalizes with an SV protein synaptotagmin, but this localization does not depend on SVs, suggesting that SAD-1 may associate with the presynaptic cytoskeleton to facilitate vesicle clustering. In support of this notion, the overexpression of SAD-1 can induce clustering of synaptic vesicle markers or vesicle precursors at non-synaptic regions [34**].

At mature presynaptic terminals, vesicles are distributed into at least two distinct pools: the readily releasable pool (RRP), corresponding to the docked vesicles; and the reserve pool, in which most vesicles are tethered to the presynaptic cytoskeleton [51]. The nature and function of the presynaptic cytoskeleton are subjects of intense study. Spectrins are found at fly NMJs [52*], but mutations in spectrins of both fly and worm do not alter the number and localization of vesicles, indicating that spectrins are not essential for preserving the vesicle clustering domain [52*,53*].

Disrupting filamentous actin at fly NMJ using cytochalasin D eliminates the reserve pool but not the RRP (as shown by labeling experiments with a FM1-43 fluorescence probe [54]), which supports a possible role of F-actin in the SV reserve domain. But the precise functions of actin at synapses are controversial [55]. Synapsins, a family of actin-associated phosphoproteins, are required for maintaining the RRP in vertebrate synapses [56,57]. Mutation analysis of synapsin homologs in fly and worm are not yet available [58,59].

At fly NMJs, special microtubule loops are present at select presynaptic terminals where new boutons are added [60]. In mutants of *futsch*, which encodes a MAP1B-like molecule, the microtubule loops are absent, synaptic growth is blocked, and the size of synaptic boutons is increased. MAP1B regulates microtubule dynamics. Microtubules are pivotal in vesicle transport, but it is unclear whether the microtubule loops have a role in establishing or maintaining distribution of the vesicle pool at new synapses.

In *C. elegans* mutants of *unc-26/synaptojanin*, the presynaptic cytoskeleton collapses to a lamina, and fewer SVs are present and arranged in an abnormal pattern [61*], supporting the notion that SV endocytosis has a role in the maintenance of the presynaptic cytoskeleton.

Presynaptic periaxial zone

The 'periaxial zone' is a newly identified subsynaptic domain that is further away from the active zone and devoid of SVs. In fly NMJs, this domain contains several molecules that function in synaptic growth [62*], such as Still-life (a guanine nucleotide exchange factor [GEF] for Rac [63]), FasII and Highwire (Hiw) [64**], a putative E3 ligase and GEF. The worm regulator of presynaptic morphology (RPM)-1, a Hiw homolog, is also localized to the periaxial zone [36**]. Partly reflecting the less-defined morphology of the periaxial zone, defects resulting from its disruption vary greatly depending on the synapse type [34**,36**,37**,64**].

Mutations in the worm *rpm-1* gene cause diverse abnormalities in synaptic morphology. Mechanosensory neurons frequently fail to cluster SVs in the proper synaptic sites, and instead extend axons ectopically [37**]. In chemosensory neurons, the vesicle clusters appear disorganized [34**] (G Crump, C Bargmann, personal communication). In motor neurons, individual presynaptic terminals contain several presynaptic densities with disorganized vesicle clusters [36**]. As RPM-1 has a candidate E3 ubiquitin ligase domain, it may possibly interact with different substrates in different neurons. Alternatively, RPM-1 may function in two steps: first to stabilize the initial synaptic contacts and then to regulate synaptic organizations once synaptic assembly is underway. Different types of synapses may have different sensitivity to the *rpm-1* signaling at each step.

In fly larval development, new synaptic boutons are added in a regulated manner to accompany muscle growth [8,27,65]. Mutations in fly *hiw* cause excessive numbers of synaptic boutons to be added continuously throughout larval development [64**], suggesting that the periaxial zone contains information to inhibit synaptic growth, possibly through ubiquitin-mediated protein degradation. In support of this possibility, *hiw(lf)* mutant phenotypes are enhanced by the overexpression of *fat facet (faf)* — a deubiquitin enzyme that antagonizes ubiquitin-mediated protein degradation [66] — and partially suppressed by *faf(lf)* [67**].

The partial suppression of *hiw* by *faf(lf)* mutations suggests either that *hiw* has different substrates functioning in different aspects of synaptic growth, or that some events are more sensitive than others to the disruption of *hiw*-regulated ubiquitin-dependent protein degradation in synaptic growth — consistent with the interpretation of the worm *rpm-1*. Thus, identifying the essential substrates and elucidating the signal pathways of Hiw/RPM-1 at different types of synapses are crucial for understanding the function of this family of proteins.

Crosstalk between presynaptic and postsynaptic partners

Many rudimentary aspects of synaptic assembly can happen independently of each other. Growth cones release SVs

spontaneously, presumably independently of the presynaptic density [68]. Primitive presynaptic specializations form in the absence of postsynaptic cells [69]. Postsynaptic cells express neurotransmitter receptors before nerve contacts [68]; however, precise positioning of presynaptic specializations requires the postsynaptic cell [69].

In *syd-2*, *sad-1* and *rpm-1* mutants of *C. elegans*, the localizations of SVs and a postsynaptic receptor are affected equally, and both are rescued by the presynaptic expression of these genes [36**] (M Zhen, personal communication). The muscle cells at glutamatergic type Ib boutons in *Drosophila* develop a specialized membranous organelle called the subsynaptic reticulum to surround and separate individual boutons. Mutations in *discs-large* (*dlg*), a member of the membrane-associated guanylate kinase family, affect both the numbers of presynaptic active zones and the subsynaptic reticulum morphology, which also can be rescued by the presynaptic Dlg expression [70]. Presynaptic terminals seem to provide the initial instructive signals, but synaptic maturation is a coordinated event involving cross-talk between partners. Agrin and its receptors mediate the cross-talk at vertebrate NMJs [71]. At invertebrate synapses, such molecules are yet to be identified.

Conclusions

Recent technical advances have greatly improved our ability to resolve the cellular and molecular details of synapse formation. The functions of genes are being addressed by forward and reverse genetic approaches. Because several interconnected events precede synaptic contacts, interpreting the function of a gene function can be complicated. Moreover, defects resulting from chronic functional deprivation of a gene may overshadow its role in early and transient events during synapse formation. Despite all the caveats, genetic screens in worm and fly have yielded new molecules that seem to define as yet unidentified signaling pathways in distinct steps during synapse formation (Table 1). For example, Hiw and RPM-1 are expressed in similar subsynaptic domains, and some candidate vertebrate homologs are enriched in the nervous system, although their functions are unknown.

A few differences are apparent on comparing the current studies of synaptogenesis in worm, fly and mammals. First, many *C. elegans* mutants have been isolated and examined for their effects on synapse organization, whereas studies of many *Drosophila* mutants have emphasized their roles in target recognition, terminal aboration and synaptic growth. This difference in mutational effect, and hence the analyses, might be due to the accessibility and the resolution that each organism offers, and might also imply the differential sensitivity of synapse formation to genetic perturbation in worm and fly.

Second, there seems to be little overlap between the molecules identified biochemically in vertebrate synapses and the molecules defined genetically in worm and fly.

Genome expansion can account for some of the differences, because some vertebrate molecules do not have homologs in invertebrates [72]. Moreover, biochemically identified molecules are usually present in large quantity, whereas genetically identified molecules are defined by function, not quantity.

Last, in contrast to the high degree of sequence and function conservation among the molecules that are involved in neurotransmitter release, the genes that are so far implicated in synapse formation seem to show considerable variability in their effects. This is probably a reflection of the diversity of synaptic morphology and the different ways of synapse formation in different neurons and organisms.

The function of a gene must be interpreted in a synapse-specific context. Observations that homologous molecules, such as Hiw and RPM-1, are present in similar spatial domains imply that the signaling pathways involving these genes are likely to be evolutionarily conserved. A key question for future study is how do cells set up boundaries to maintain a local, dynamic and self-renewing subcellular organization at synapses? To answer this question, one must combine genetic, cellular and biochemical approaches.

Update

Recent studies in the *Drosophila* retina have demonstrated that the Lar RPTP is specifically required for retinal axon target recognition. Mutations in fly *lar* were identified in three independent genetic screens for visual system connectivity: one was a behavioral-based screen for mutant flies that failed the optomotor response test [76**]; the other two used visual inspection of retinal axon projections [77**,78].

In the *lar* mutant eye, the retinal axons project normally toward their target zones. However, the axons of the R1–R6 neurons fail to exit the axon bundle upon reaching their targets and some even overshoot [76**]. The growth cones of the R7 neuron retract upon reaching the target layer [76**,77**] and develop presynaptic terminals of aberrant morphology [76**]. LAR is required cell autonomously in the R1–R7 retinal axons for their target recognition [76**,77**]. The *lar* mutant eye phenotype is enhanced by reducing the dosage of Enabled (Ena), or Trio, a Rho family GEF, and can also be suppressed by increasing the dosage of Ena or Trio [77**]. Trio interacts with Dock during retinal axon guidance [78]. Thus, an exciting possibility is that the Lar RPTP functions at the growth cone to link target recognition to multiple intracellular signaling events in the presynaptic terminal, such as the active zone formation through Liprin and cytoskeleton remodeling through Ena, Trio and Dock. Moreover, Lar can also function cell non-autonomously in the R8 axon to influence the targeting of R7 growth cone [77**], hinting at another possible role of Lar in mediating cross-talk between partners during synaptic assembly. The retinal axon phenotypes in *lar* mutant are very similar to those in N-cadherin mutants [17**,76**]. Lar may modulate the homophilic interactions

between cadherins and the intracellular cadherin–catenin signaling pathway to facilitate synaptic contacts.

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