

# LAR, liprin $\alpha$ and the regulation of active zone morphogenesis

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## Summary

Active zones are protein-rich regions of neurons that act as sites of synaptic vesicle fusion and neurotransmitter release at the pre-synaptic terminus. Although the discovery that the receptor protein tyrosine phosphatase LAR and its cytoplasmic binding partner liprin  $\alpha$  are essential for proper active zone formation is nearly a decade old, the underlying mechanisms are still poorly understood. Recent studies have identified a number of binding partners for both LAR and liprin  $\alpha$ , several of which play key roles in active zone assembly. These include nidogen, dallylike and syndecan – extracellular ligands for LAR that regulate

synapse morphogenesis. In addition, liprin- $\alpha$ -interacting proteins such as ERC2, RIM and the MALS/Veli-Cask-Mint1 complex cooperate to form a dense molecular scaffold at the active zone that is crucial for proper synaptic function. These studies allow us to propose testable models of LAR and liprin  $\alpha$  function, and provide insights into the fundamental molecular mechanisms of synapse formation and stabilization.

Key words: Synapse development, Liprin  $\alpha$ , Syd-2, LAR, PTP-3, Active zone

## Introduction

The synapse is a highly specialized structure, optimized for the rapid, high-fidelity transfer of information from a presynaptic neuron to a postsynaptic cell. When an action potential arrives at a presynaptic terminus, calcium ions flow into the terminal, triggering the fusion of a small fraction of neurotransmitter-containing synaptic vesicles with the presynaptic membrane. The fusion of this 'readily releasable pool' of synaptic vesicles with the plasma membrane and the release of neurotransmitter effectively converts an electrical signal into a chemical signal that can be detected across the synaptic cleft. Synapse function is critically dependent on the juxtaposition of the neurotransmitter-release machinery at the presynaptic terminus and the neurotransmitter receptor machinery in the postsynaptic cell.

Synaptic vesicle docking and neurotransmitter release are limited to conspicuous subcellular specializations at the presynaptic terminus called active zones (Fig. 1). Although the structure of the active zone varies considerably between species and between synapse types, all active zones are protein rich – they have an electron-dense ultrastructure, and numerous proteins have been identified at the active zone. These include cytoskeletal and scaffolding proteins, synaptic vesicle release machinery, as well as several additional active-zone-enriched proteins, such as piccolo and bassoon, the cytosolic protein liprin  $\alpha$  and the receptor tyrosine phosphatase LAR (Fig. 2) (Zhai and Bellen, 2004).

Prior to synaptogenesis, an unusual class of dense-core vesicle is present in the specialized structure at the tip of the growing axon called the growth cone (Shapira et al., 2003; Zhai et al., 2001). These dense-core vesicles contain a diverse array of active-zone-enriched proteins, and current models suggest that an individual active zone is assembled by the fusion of

only a few of these dense-core vesicles at a specific presynaptic site (Shapira et al., 2003; Zhai et al., 2001). Because these dense-core vesicles are pre-formed in the Golgi apparatus, active zone assembly can be broken down into four key steps: (1) synthesis of these dense-core vesicles; (2) transport of these vesicles to the presynaptic terminus; (3) recruitment of dense-core vesicles to appropriate sites on the presynaptic membrane; and (4) fusion of dense-core vesicles with the membrane at prospective active zone sites. Importantly, following fusion, proteins borne by the vesicles must be stabilized to prevent their lateral diffusion out of the active zone.

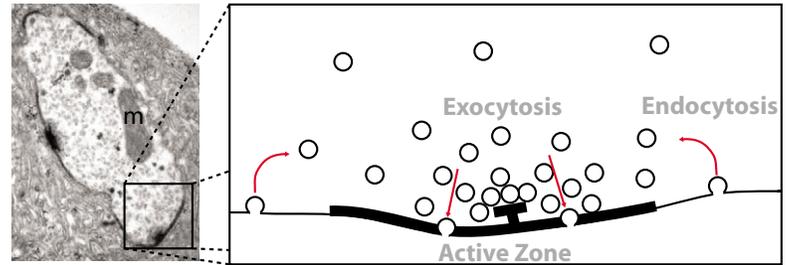
Although the mechanisms that control neurotransmitter release have been relatively well characterized, those that regulate active zone assembly are only beginning to be understood. Recent studies have pointed toward crucial roles for LAR and liprin  $\alpha$  during active zone formation. Here we review this evidence, discuss the proteins that interact with liprin  $\alpha$  and LAR, and propose models for how these proteins cooperatively regulate active zone assembly.

## Structure of LAR and liprin $\alpha$

Members of the LAR family are transmembrane tyrosine phosphatases that have highly conserved extracellular and cytoplasmic domains (Fig. 2). The regions of highest sequence similarity are the tandem cytoplasmic phosphatase domains. The membrane-proximal phosphatase domain is responsible for the vast majority (>99%) of catalytic activity, whereas the membrane-distal phosphatase domain binds numerous downstream effectors, including trio (Debant et al., 1996), Abelson tyrosine kinase and enabled (Wills et al., 1999),  $\beta$  catenin (Kypta et al., 1996) and liprin  $\alpha$  (Serra-Page et al., 1995). The extracellular region resembles those of cell adhesion molecules, consisting of multiple immunoglobulin

## The presynaptic active zone

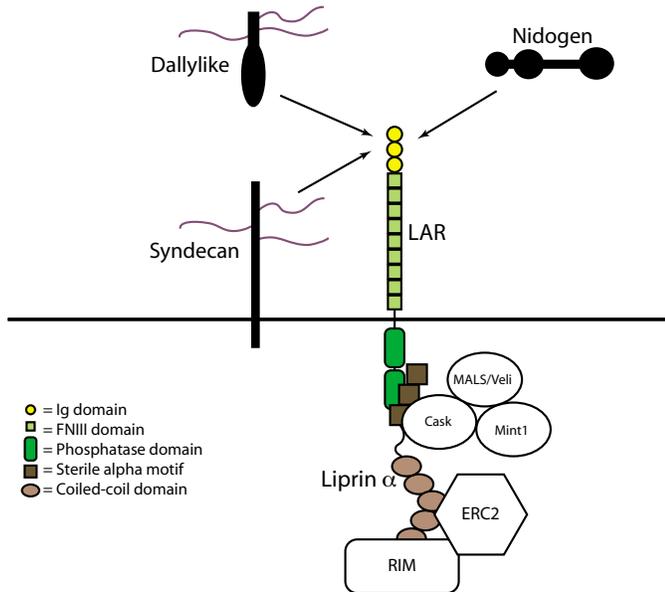
**Fig. 1.** The structure of the active zone. An electron micrograph of a *Drosophila* NMJ [adapted from Atwood (Atwood, 2006)] shows electron-dense active zones, synaptic vesicles and mitochondria (m). The schematized active zone on the right shows the cycling of synaptic vesicles (white circles) at the active zone. Synaptic vesicle endocytosis occurs peripheral to the active zone, and synaptic vesicle exocytosis and neurotransmitter release occur within the active zone.



(Ig) and fibronectin type III (FNIII) domains (Fig. 2) (reviewed in Johnson and Van Vactor, 2003). Vertebrate genomes generally encode three LAR paralogues (LAR, PTP- $\sigma$  and PTP- $\delta$ ) whereas *Drosophila* has two (LAR and PTP69D) and *C. elegans* has one (PTP-3).

The liprin  $\alpha$  family of proteins were first characterized by their ability to bind to the cytoplasmic domains of LAR in a yeast two-hybrid screen (Serra-Pages et al., 1995). They each have N-terminal coiled-coil domains that act as dimerization domains and a C-terminal liprin homology (LH) domain, composed of three sterile alpha motifs (SAMs), that interacts

with LAR (Fig. 2) (Serra-Pages et al., 1998). Liprin  $\alpha$  and LAR can influence one another's subcellular distribution; LAR mislocalization is sufficient to change the distribution of liprin  $\alpha$  (Serra-Pages et al., 1995), and liprin  $\alpha$  can localize LAR to the proximal edges of focal adhesions (Serra-Pages et al., 1995). Despite binding to the second phosphatase domain of LAR, liprin  $\alpha$  does not appear to be tyrosine phosphorylated itself, nor does it appear to influence the catalytic activity of LAR (Serra-Pages et al., 1995). Vertebrate genomes generally encode four liprin  $\alpha$  paralogues (liprin  $\alpha$ 1-4), whereas *Drosophila* and *C. elegans* each have one (liprin  $\alpha$  and Syd-2, respectively).

Key liprin  $\alpha$  and LAR interactors

**Fig. 2.** Key molecular interactions of LAR and liprin  $\alpha$  at the synapse. The extracellular domains of LAR have three identified ligands, which regulate the function and/or localization of LAR at the synapse. Nidogen genetically interacts with PTP-3 in *C. elegans* and may regulate active zone morphogenesis through its ability to localize PTP-3 to the synapse. Dallylike and syndecan both bind to the extracellular domains of LAR in *Drosophila* and have distinct effects on synapse formation; syndecan positively regulates LAR to promote synapse growth, whereas Dallylike inhibits its ability to limit active zone size. Inside the presynaptic terminus, liprin  $\alpha$  binds the MALS/Veli-Cask-Mint1 complex, ERC2, and RIM. Through these interactions, liprin  $\alpha$  is able to promote the formation of an electron-dense, tightly clustered, robust active zone.

Initial studies on LAR and liprin  $\alpha$  in synapse formation

Several studies in invertebrates have defined crucial functions for LAR and liprin  $\alpha$  in the regulation of active zone assembly. First, Zhen and Jin demonstrated that mutations in *C. elegans* *syd-2* caused the mislocalization of a synaptobrevin-GFP fusion protein that labels the active zone (Zhen and Jin, 1999). In *syd-2* mutants, although there is no change in the total number of synapses, there is a significant increase in the size of active zones, and a concomitant decrease in active zone electron density (Zhen and Jin, 1999). The behavioral phenotypes of *syd-2* mutants (sluggish movement and egg-laying defects) can be rescued by the presence of a *syd-2*<sup>+</sup> extrachromosomal array in neurons, but not in muscles, which indicates that SYD-2 has a presynaptic role (Zhen and Jin, 1999); this finding has been confirmed by transgenic rescue experiments (Dai et al., 2006). On the basis of the localization of SYD-2 to the active zone, Zhen and Jin proposed that liprin  $\alpha$  acts as a cytoplasmic anchor that recruits or stabilizes other molecules at the active zone (Zhen and Jin, 1999). This model is consistent with previous studies that demonstrated an important role for liprin  $\alpha$  in the localization of LAR family members (Serra-Pages et al., 1998), and raised the possibility that LAR tyrosine phosphatases are involved in synapse formation.

The interaction between LAR and liprin  $\alpha$  is conserved in *Drosophila* (Kaufmann et al., 2002). Fly liprin  $\alpha$  mutants display dramatic reductions in the size and branch complexity of neuromuscular junctions (NMJs). LAR mutants have a nearly identical phenotype (Kaufmann et al., 2002), which is consistent with a model in which liprin  $\alpha$  and LAR work together to control synapse formation. Pre-synaptic overexpression of LAR is sufficient to increase synaptic size. This effect depends on the presence of liprin  $\alpha$ , demonstrating that LAR requires liprin  $\alpha$  to function at the NMJ. In addition,

both *LAR* and *liprin  $\alpha$*  mutant larvae have enlarged active zones, 2.5 and 2.4 times the size of wild-type active zones, respectively (Kaufmann et al., 2002). This phenotype is highly similar to that of the *C. elegans* *syd-2* mutant. Both *liprin  $\alpha$*  and *LAR* mutants have reduced evoked synaptic responses and a nearly 50% reduction in quantal content (Kaufmann et al., 2002), which suggests a defect in the function of the synaptic-vesicle-release machinery in both mutants.

The *C. elegans* orthologue of LAR has two splice variants: PTP-3A has the full-length extracellular domain whereas PTP-3B lacks the Ig domains and the first four FNIII repeats (Harrington et al., 2002). Mutants lacking *ptp-3A* have approximately twofold larger synaptobrevin-GFP-positive puncta, reflecting a doubling of active zone size (Ackley et al., 2005). Again, this phenotype is highly similar to that of the *syd-2* mutant (Zhen and Jin, 1999) and those of the *liprin  $\alpha$*  and *LAR Drosophila* mutants (Kaufmann et al., 2002), which indicates these proteins have an evolutionarily conserved function in active zone morphogenesis. The observations that PTP-3A localization is severely disrupted in *syd-2* mutants (Ackley et al., 2005), that liprin  $\alpha$  can influence the subcellular localization of LAR (Serra-Pages et al., 1998), and that liprin  $\alpha$  is required for LAR activity (Kaufmann et al., 2002) suggested a model in which liprin  $\alpha$  functions upstream of LAR, possibly acting to localize LAR to the synapse.

### LAR-interacting proteins at the synapse

How do LAR and liprin  $\alpha$  influence the structure of the active zone and the function of the synaptic vesicle release machinery? The LAR family are best known for their roles in axon guidance (reviewed in Johnson and Van Vactor, 2003), and numerous downstream effectors have been identified in this context. Unfortunately, the roles of many of these in synaptic function [for example, the guanucleotide exchange factor trio (Bateman et al., 2000), the Abelson tyrosine kinase (Wills et al., 1999), and the LAR and Abelson substrate, enabled (Wills et al., 1999)] have not yet been examined. The identification of extracellular binding partners for LAR, however, has begun to illuminate how it functions at the presynaptic terminus.

In *C. elegans*, the two PTP-3 isoforms exhibit dramatic differences in localization: PTP-3A-GFP concentrates at presynaptic termini, whereas PTP-3B-GFP is more diffusely localized (Ackley et al., 2005). Because the cytoplasmic domains of these splice variants are identical, the localization of PTP-3A to the synapse must be influenced by an interaction specific to its extracellular domains. SYD-2 alone thus cannot localize PTP-3A properly; isoform-specific interactions with an extracellular binding partner must also play a role (Ackley et al., 2005). Studies showing that transgenic constructs lacking the Ig domains cannot rescue the *LAR* synaptic phenotype (Johnson et al., 2006) – whereas wild-type, or constructs lacking the FNIII domains can – support this idea. Moreover they suggest the Ig domains of LAR have an important function at the synapse. So, what interacts with the Ig domains of LAR?

One candidate ligand for a LAR ligand is nidogen. Nidogen is a sulfated glycoprotein that can bind to the Ig domains of certain LAR splice forms (O'Grady et al., 1998). *C. elegans* *nid-1* mutants have mislocalized PTP-3, and *nid-1* shows potent genetic interactions with *ptp-3* (Ackley et al., 2005). In

addition, *nid-1* mutants have expanded active zones, as in *syd-2* or *ptp-3* mutants (Ackley et al., 2005). Whether nidogen regulates the catalytic activity of PTP-3, or simply engages in adhesive interactions with it remains to be seen.

Syndecan (Sdc) and Dallylike (Dlp) are two heparan sulfate proteoglycans (HSPGs) that bind with nanomolar affinity to the Ig domains of *Drosophila* LAR (Fox and Zinn, 2005; Johnson et al., 2006). *Sdc* loss-of-function mutants have small synapses (Johnson et al., 2006). This phenotype can be rescued by presynaptic, but not by postsynaptic, expression of an *sdc* transgene. Since LAR is exclusively presynaptic at the *Drosophila* NMJ (Krueger et al., 1996), and since presynaptic but not postsynaptic expression of LAR can rescue the LAR mutant phenotype (Johnson et al., 2006), these data suggest that Sdc works in cis with LAR to promote synapse growth. Although mutations in *dlp* do not affect synapse size, they produce significantly smaller active zones (Johnson et al., 2006). Post-synaptic *dlp* overexpression is sufficient to double active zone size, which suggests that Dlp opposes LAR in the regulation of active zone size (Johnson et al., 2006). Knocking down Dlp in vitro demonstrates that Dlp directly inhibits the catalytic activity of LAR. Because Dlp can compete with Sdc for binding to LAR, LAR may therefore act as a molecular switch at the synapse: initially Sdc-LAR interactions promote synapse growth; then Dlp-LAR interactions inhibit growth and promote active zone assembly (Johnson et al., 2006).

LAR thus appears to allow extracellular matrix components such as nidogen and Dallylike to signal across the membrane to regulate active zone morphogenesis or stability. These LAR ligands act through LAR to either increase or decrease active zone size, but at present it is unclear whether they require the function of liprin  $\alpha$ .

### Liprin- $\alpha$ -interacting proteins at the synapse

Liprin  $\alpha$  interacts with a diverse array of proteins that regulate the formation of the active zone (Zhen and Jin, 2004). Two such proteins are RIM1- $\alpha$  (Schoch et al., 2002) and RIM-interacting proteins of the ERC (ELKS-Rab6-interacting protein-CAST) family (Ko et al., 2003b). RIM proteins are concentrated on the dense core vesicles that give rise to active zones (Shapira et al., 2003), and interact with numerous active zone proteins. They are essential for regulated synaptic vesicle release and interact with the synaptic vesicle trafficking protein Rab3A. ERC family members form large oligomeric complexes with other known active zone proteins and influence the subcellular localization of both liprin  $\alpha$  (Ko et al., 2003b) and RIM (Ohtsuka et al., 2002), targeting these proteins to presynaptic sites. Liprin  $\alpha$  is essential for active zone localization of RIM1 (Schoch et al., 2002). ERC2 may therefore function to localize liprin  $\alpha$ , which in turn recruits RIM1 to the active zone.

Liprin  $\alpha$  also binds the MALS/Veli-Cask-Mint1 scaffolding complex, a ternary complex of PDZ-domain-containing proteins that regulates cycling of reserve synaptic vesicles into the readily releasable pool of synaptic vesicles that are primed to fuse with the plasma membrane (Olsen et al., 2005). Although MALS mutants have no visible defects in liprin  $\alpha$  localization, they do have larger synaptic areas and decreased excitatory post-synaptic currents – which is highly similar to liprin  $\alpha$  mutants (Olsen et al., 2005). Given that liprin  $\alpha$  mutants have defects in evoked responses (Kaufmann et al.,

2002), that RIM1- $\alpha$  regulates synaptic vesicle release and that the MALS/Veli-Cask-Mint1 complex primes synaptic vesicles, together these proteins may establish a molecular scaffold that facilitates synaptic vesicle release at the active zone.

In vertebrates, piccolo and bassoon are massive proteins that are concentrated at the active zone and interact with RIM1 and ERCs to regulate active zone assembly (Fenster et al., 2000; Shapira et al., 2003; tom Dieck et al., 1998). Liprin  $\alpha$ , presumably via interactions with RIM1 and ERC, partly colocalizes with piccolo clusters at the active zone (Ko et al., 2003a). Invertebrates lack both piccolo and bassoon, but it appears that bruchpilot may assume some of their functions during active zone morphogenesis. Bruchpilot is localized to active zones and has regions with sequence similarity to ERC, as well as numerous coiled-coil domains (Wagh et al., 2006). *bruchpilot* mutants have severe electrophysiological, behavioral and morphological defects (Kittel et al., 2006; Wagh et al., 2006); they lack the conspicuous electron-dense T-bar structures found in *Drosophila* active zones, have improper calcium channel localization, and diminished evoked responses (Kittel et al., 2006).

The phenotypic differences between *bruchpilot* and *liprin  $\alpha$*  mutants suggest that, although both proteins regulate active zone formation, these proteins function in distinct pathways. Nevertheless, the observation that the localization of bruchpilot to the active zone is reduced almost 30% in liprin  $\alpha$  mutants (Miller et al., 2005) suggests some kind of functional interaction between bruchpilot and liprin  $\alpha$  during active zone assembly. If the enlarged active zones in liprin  $\alpha$  mutants are caused by reduced bruchpilot levels at the NMJ, then *bruchpilot* mutants should logically have increased active zone areas. Indeed, postsynaptic receptor field sizes are increased in *bruchpilot* mutants (Kittel et al., 2006), which suggests that presynaptic active zone areas are increased, but this has yet to be quantified.

In addition to binding a number of proteins at the active zone, liprin  $\alpha$  interacts with the kinesin motor protein Kif1a (Shin et al., 2003). It might therefore have a role in the transport of cargo destined for the presynaptic terminus, which would be consistent with its ability to target LAR to distinct subcellular locations (Ackley et al., 2005; Serra-Pages et al., 1995; Serra-Pages et al., 1998). In *liprin  $\alpha$*  mutants, synaptic vesicles accumulate along the length of the axon (Miller et al., 2005); a similar pattern can also be seen in *kinesin-1* mutants (Hurd and Saxton, 1996). This supports a model in which liprin  $\alpha$  functions in the transport of synaptic components to the presynaptic terminus. However, the transport of the dense-core vesicles that contain active zone components has not been described in *liprin  $\alpha$*  mutants. In fact, the increased size of active zones in *liprin  $\alpha$*  mutants suggests that liprin  $\alpha$  is not required for trafficking dense core vesicles to the presynaptic terminus. Whether LAR is present at the *Drosophila* NMJ in *liprin  $\alpha$*  mutants is not known, although it is clear that liprin  $\alpha$  is present at the NMJ in *LAR* mutants (Kaufmann et al., 2002). If liprin  $\alpha$  is essential for the anterograde transport of LAR to the NMJ, this is clearly not a universal model, because localization of LAR to photoreceptor growth cones does not depend on liprin  $\alpha$  (Hofmeyer et al., 2006).

Further insight has come from studies examining synapse formation by the HSNL neuron in *C. elegans*. SYD-1, a PDZ-domain-containing protein that has a RhoGAP domain,

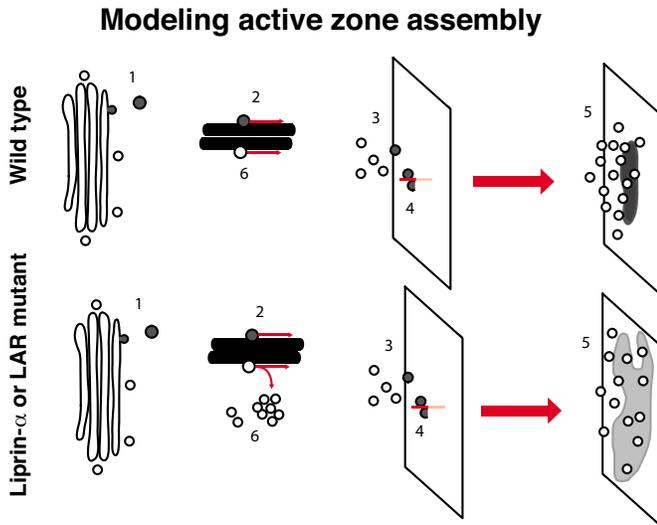
localizes several presynaptic proteins, including SYD-2, to the active zone (Dai et al., 2006; Hallam et al., 2002). Mutations in *syd-1* or *syd-2* cause the mislocalization of numerous active zone proteins (Patel et al., 2006), including the ERC family member ELKS-1, the RIM orthologue unc-10 and the GTPase-activation-domain-containing, G protein coupled receptor binding protein GIT. The localization of SYD-1 and SYD-2, however, does not require the presence of ELKS, unc-10 or GIT (Ackley et al., 2005; Dai et al., 2006; Patel et al., 2006). These data suggest a defect in either the targeting and/or the stabilization of active zone components in both SYD-1 and SYD-2 mutants. In addition, the *syd-2* hypermorphic phenotype (an Arg184Cys mutation in the coiled-coil domain of SYD2 that can suppress the *syd-1* mutant phenotype) requires the presence of ELKS-1 for its effect (Dai et al., 2006). This suggests a model in which SYD-1 and SYD-2 act upstream of ELKS, RIM and GIT to promote active zone consolidation. But how does SYD-1, and therefore SYD-2, get localized specifically to synaptic sites, and is this a general mechanism?

The answer to the first question, at least in HSNL, appears to involve the Ig superfamily proteins SYG-1 and SYG-2 (Dai et al., 2006; Patel et al., 2006). SYG-2 is expressed on the guidepost epithelial cells, specialized cells near the vulval muscle that initiate the process of presynaptic specialization in HSNL prior to the innervation of their normal postsynaptic target. SYG-1 is expressed in a subset of neurons, including HSNL. In *syg-1* or *syg-2* mutants, much like in *syd-1* or *syd-2* mutants, components of the presynaptic active zone do not localize properly (Dai et al., 2006; Patel et al., 2006). SYG-1 appears to recruit SYD-1 and SYD-2 to synaptic sites, which suggests a hierarchical process of active zone assembly in which trans-synaptic SYG-1 and SYG-2 interactions define sites of active zone assembly, recruiting SYD-1 and SYD2, which in turn localize other proteins such as ELKS-1, RIM and GIT to the active zone (Patel et al., 2006). Interactions between SYG-1 and SYD-1/SYD-2 have not yet been demonstrated, which leaves open the question of how SYG-1 recruits SYD-2 to synaptic sites. The observation that SYD-2 is also mislocalized in *ptp-3* mutants (Ackley et al., 2005) suggests the appealing possibility that, in HSNL, SYG-1 cooperates with nidogen to recruit PTP-3A to the active zone, which in turn recruits SYD-2 and thereby other active zone components.

Characterizing the distribution of PTP-3 in *syg-1* or *syg-2* mutants may shed light on this model. Importantly, the high sequence conservation of both SYG-1 and SYG-2 from *C. elegans* to humans (Shen, 2004) suggests that these proteins play a conserved role in synapse formation. However, the presence of SYG1 in only a subset of neurons indicates that, even if conserved, this mechanism is unlikely to be ubiquitous.

## Conclusions and perspectives

There is compelling evidence that LAR and liprin  $\alpha$  regulate the formation of a functional presynaptic active zone by recruiting or stabilizing a vast array of proteins to this structure (Fig. 3). At present, there is no evidence that liprin  $\alpha$  or LAR play roles in the assembly, or in the trafficking of dense-core vesicles, although a role for liprin  $\alpha$  in trafficking synaptic vesicles is now well established (Miller et al., 2005). In fact, the presence of functional active zones in liprin  $\alpha$  or LAR mutants suggests that dense-core vesicle assembly and



**Fig. 3.** Modeling the function of liprin  $\alpha$  and LAR during active zone assembly. Active zone formation can be divided into five steps: (1) the synthesis of dense core vesicles (dark circles) in the Golgi complex; (2) the transport of dense core vesicles to the presynaptic terminus; (3) the targeting of dense core vesicles to appropriate sites on the membrane; (4) the fusion of dense core vesicles with the plasma membrane; and (5) the stabilization of active zone proteins. The LAR and liprin  $\alpha$  mutant phenotypes suggest that these proteins are essential for stabilizing numerous proteins in a condensed and functional active zone. In addition, proper neurotransmitter release at the active zone depends on the proper transport (6) of synaptic vesicles (white circles) to the presynaptic terminus. In *Drosophila*, liprin  $\alpha$  mutants have severe defects in synaptic vesicle transport, in which synaptic vesicles accumulate in axons.

trafficking do not require liprin  $\alpha$  or LAR. Nevertheless, because dense-core vesicle proteins such as RIM are mislocalized in liprin  $\alpha$  mutants, liprin  $\alpha$  must function either to bring active zone proteins to appropriate sites on the presynaptic membrane (a targeting model) or to maintain active zone proteins in a condensed form after fusion occurs (a stabilization model).

Although an *in vivo* analysis of the dynamics of active zone morphogenesis in liprin  $\alpha$  or LAR mutants may be necessary to discriminate between these models, existing active zone phenotypes seem to support the stabilization model. If liprin  $\alpha$  and LAR stabilize proteins at the active zone, then liprin  $\alpha$  or LAR mutants should have larger active zones that are less electron dense but no change in the number of active zones. By contrast, if liprin  $\alpha$  and LAR were to function strictly in targeting, then liprin  $\alpha$  or LAR mutants would have smaller active zones owing to compromised dense core vesicle fusion, and/or an increase in the number of active zones, owing to inappropriate dense core vesicle fusion events. In *C. elegans*, the *syd-2* mutation does not affect the number of active zones, but increases active zone size and decreases electron density, supporting the stabilization model. Moreover, *Drosophila* liprin  $\alpha$  and LAR mutants exhibit increased active zone sizes and have neither an increase in the number of active zones per synapse nor extrasynaptic active zones (Miller et al., 2005). The recent studies in *C. elegans* demonstrating a hierarchical assembly process leave open the possibility that, at least in a

subset of neurons, liprin  $\alpha$  plays a role targeting proteins to the active zone, possibly through interactions with the RIM-containing dense core vesicles. However, no physical or functional interaction between these vesicles and either liprin  $\alpha$  or LAR has yet been shown and, without evidence for this interaction, support for the targeting model remains purely circumstantial.

In this review, we have discussed the roles of LAR and liprin  $\alpha$  in the formation of the presynaptic active zone, focusing on the invertebrate NMJ. In these systems, most data thus support a model in which liprin  $\alpha$  and LAR function to maintain presynaptic proteins (such as ERC2, RIM, the MALS/Veli-Cask-Mint1 complex) in a dense molecular scaffold at the active zone. In their absence, the tight association of active zone components crucial for optimal synaptic vesicle release is severely disrupted. At synapses in the central nervous system, LAR and liprin  $\alpha$  are also expressed postsynaptically, and may play very different roles on either side of the synaptic cleft. Given the recent identification of ligands for LAR, and the functional characterization of several liprin  $\alpha$  interactors, the generation of more detailed models of liprin  $\alpha$  and LAR function at the active zone will soon be possible.

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