cortex and future studies will be needed to show whether these pathways are required for axon specification in vivo and whether such a feedback loop may also be the driving force of neuronal polarization.

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The Multiple Faces of RIM

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Rab3 interacting molecules (RIMs) are highly enriched in the active zones of presynaptic terminals. It is generally thought that they operate as effectors of the small G protein Rab3. Three recent papers, by Han et al. (this issue of *Neuron*), Deng et al. (this issue of *Neuron*), and Kaeser et al. (a recent issue of *Cell*), shed new light on the functional role of RIM in presynaptic terminals. First, RIM tethers Ca^{2+} channels to active zones. Second, RIM contributes to priming of synaptic vesicles by interacting with another presynaptic protein, Munc13.

A hallmark of synaptic transmission is speed. Although synaptic transmission involves two chemical messengers, Ca^{2+} and the transmitter, the entire signaling process takes place within less than a millisecond under physiological conditions. To minimize delays generated by the diffusion, an ideal synapse would have to be constructed as a point-to-point device, in which the relevant molecules are tightly packed on the nanometer scale at both sides of the synaptic cleft. While a lot of information is available about the molecular composition of postsynaptic densities, little is known about the organization of presynaptic active zones.

Active zones are composed of several different proteins, including Munc13s, Rab3 binding proteins (RIMs), RIM-binding proteins (RIM-BPs), ELKSs, and many others (Wojcik and Brose, 2007; Müller et al., 2010). Among these proteins, RIMs have received particular attention as binding partners of Rab3, a highly abundant protein in synaptic vesicles (Castillo et al., 2002; Takamori et al., 2006). RIMs are multidomain proteins, comprised of a Rab3 binding domain at the N terminus, a Zn^{2+} finger domain, a putative protein kinase A (PKA) phosphorylation site, a PDZ domain, a C2 domain, a proline-rich domain, and another C2 domain at the C terminus (Wojcik and Brose, 2007). The functional significance of these multiple domains, however, is largely unclear. It is generally thought that RIMs operate as Rab3 effectors. Furthermore, RIMs are substrates of PKA and are thought to play important roles in presynaptic forms of synaptic plasticity (Wang et al., 1997; Castillo et al., 2002).

Three recent papers (Kaeser et al., 2011; Han et al., 2011, Deng et al., 2011; the latter two of which can be found in this issue of *Neuron*) shed new light on the function of RIMs, approaching the problem by genetic elimination (knockout). RIM proteins in mammals are highly diverse. They are encoded by four genes (*Rim1–4*) that drive the expression of seven known RIM isoforms: RIM1α and 1β; RIM2α, 2β, and 2γ; RIM3γ; and RIM4γ. Unfortunately, RIM1α and RIM2α double knockout mice die immediately after birth (Schoch et al., 2006), preventing a systematic analysis of the function of RIMs in synaptic transmission. The Südhof group (Kaeser et al., 2011) has now solved this problem by generating

Table 1. The Complex Synaptic Phenotype of Conditional RIM1/RIM2 Knockout Mice

Synaptic Parameter	Hippocampal Inhibitory Synapses (Culture)	Calyx of Held (Acute Slice)	Other Synapses/Systems
Reference	Kaeser et al. (2011) Deng et al. (2011)	Han et al. (2011)	
Miniature PSC frequency	↓ (IPSCs)		
Evoked PSC amplitude	↓ (IPSCs)	↓ (EPSCs)	
Synchrony of release	↓	↓ (EPSC rise time)	
Presynaptic Ca^{2+} inflow	Presynaptic Ca^{2+} concentration transient (Fluo5F) ↓	Presynaptic Ca^{2+} current density ↓	
Presynaptic Ca^{2+} channel gating		=	Removal of inactivation of recombinant Cav2.1, 2.2, and 2.3 by RIM expression (Kiyonaka et al., 2007)
Presynaptic Ca^{2+} channel subtype	= (P/Q and N)	= (P/Q and N)	
Ca^{2+} channel immunoreactivity	↓ (P/Q)		
Releasable pool size	↓ (sucrose pool, stimulus train)	↓ (stimulus train, uncaging)	
Release probability		↓	
Ca^{2+} sensitivity of release		↓	
Ca^{2+} transient at sensor		↓	
Ca^{2+} channel – sensor coupling distance	↑ (EGTA-AM onset kinetics)		
Affinity PSC – $[Ca^{2+}]_o$ curve	↓		
Number of docked vesicles	↓ (electron microscopy)	↓ (serial electron microscopy, < 10 nm)	
Number of outlier vesicles (serial EM)		= (serial electron microscopy)	
Synaptic plasticity			Mossy fiber LTP ↓ (RIM1α knockout; Castillo et al., 2002)

All data are from Kaeser et al. (2011), Deng et al. (2011), and Han et al. (2011), unless stated differently. ↓, reduction; ↑, increase; = , no change.

a new mouse line in which both RIM1 and RIM2 genes are flanked by loxP sites (floxed). Because RIM3 and RIM4 are selectively expressed in short γ versions (composed of only a single C2 domain), this allows conditional elimination of all long forms of RIM.

Kaeser et al. (2011) have addressed the function of RIMs in an elegant series of biochemical and electrophysiological experiments. The starting point of the analysis was the finding that RIMs directly and specifically interact with P/Q- and N-type Ca^{2+} channels. Kaeser et al. then systematically examined the functional significance of this molecular interaction, measuring synaptic currents in cultured hippocampal neurons. To eliminate RIMs from these synapses, lentiviral infection followed by Cre recombinase expression was used. Multiple pieces of evidence suggested that genetic elimination of RIMs changed the coupling between Ca^{2+} channels and transmitter release (Table 1). First, the amplitude of evoked inhibitory postsynaptic currents (IPSCs) was reduced. Second, evoked release was desynchronized. Third, the onset of the blocking effects of the Ca^{2+} chelator EGTA-AM was prolonged, suggesting a loosening of the coupling between Ca^{2+} channels and Ca^{2+} sensors of exocytosis (Neher, 1998; Bucurenciu et al., 2008). Fourth, the dependence of release on the external Ca^{2+} concentration was shifted to higher concentrations. Finally, the amplitude of presynaptic Ca^{2+} concentration transients measured by fluorescent Ca^{2+} indicators was reduced. Taken together, these results suggest that conditional knockout of RIMs impairs the tethering of presynaptic Ca^{2+} channels to the active zone of inhibitory synapses.

Han et al. (2011) have used the same mouse line to examine the function of RIMs at the calyx of Held, a glutamatergic synapse in the auditory brainstem accessible to quantitative biophysical analysis of transmitter release. To eliminate RIMs from these synapses, the new RIM1 and RIM2 floxed mouse line (Kaeser et al., 2011) was crossed with a previously generated driver line expressing Cre recombinase under the control of the Krox20 promoter (a transcription factor selectively expressed in the brainstem). Similar to that in hippocampal synapses, several lines of evidence suggested that genetic elimination of RIMs interfered with the coupling between Ca^{2+} channels and transmitter release (Table 1). First, the presynaptic Ca^{2+} channel density was reduced. Because the gating properties of Ca^{2+} channels were unchanged, this suggests a reduction in the density of presynaptic Ca^{2+} channel proteins (see Kaeser et al., 2011). Second, the intrinsic Ca^{2+} sensitivity of transmitter release measured by Ca^{2+} uncaging was diminished. Third, the amplitude of the Ca^{2+} concentration transient at the Ca^{2+} sensor, estimated from a comparison of synaptic data and uncaging data, was altered, again consistent with a loosening

of the coupling between Ca^{2+} channels and Ca^{2+} sensors of exocytosis.

The results (Kaeser et al., 2011; Han et al., 2011) converge on the conclusion that RIMs change the coupling between Ca^{2+} channels and transmitter release. However, the paper of Han et al. (2011), and in particular another extensive study by Deng et al. (2011), suggests that this is only one side of the coin. At both the hippocampal synapses and the calyx of Held, the size of the releasable pool of synaptic vesicles is reduced in the RIM double knockout mouse. In the hippocampal synapses, pool size was measured by application of hypertonic sucrose solution (Deng et al., 2011). At the calyx of Held, the size of the readily releasable pool was elegantly probed in Ca^{2+} uncaging experiments (Han et al., 2011). Based on serial electron microscopy analysis of calyx synapses, Han et al. suggest a reduction in the number of docked vesicles in RIM-deficient synapses. Thus, a docking deficit may underlie the reduction in pool size. In contrast, in the hippocampal synapses, genetic elimination of RIM appears to involve Munc13, a classical priming factor (Betz et al., 2001). This suggests that RIM regulates pool size via effects on priming.

How do the different domains of RIM mediate these diverse functions? For the tethering function of RIM, rescue experiments suggest that both the PDZ domain and the proline-rich domain of RIM are necessary and sufficient for the effects (Kaeser et al., 2011). In contrast, the Rab3 binding domain seems to be dispensable. Because the proline-rich region of RIM represents the site of interaction with RIM-BPs (Hibino et al., 2002), the results suggest that a tripartite complex of RIMs, RIM-BPs, and Ca^{2+} channels is formed during tethering (Figure 1). For the priming function, the Zn^{2+} finger domain of RIM is necessary and sufficient (Deng et al., 2011). Because this site interacts with Munc13, this suggests that the effects on priming are mediated by Munc13 (Betz et al., 2001). Interestingly, the synaptic phenotype in RIM-deficient synapses is rescued by a mutant Munc13 that fails to form homodimers, but not by wild-type Munc13 that dimerizes readily (Deng et al., 2011). This suggests that RIM promotes priming by preventing homodimerization of Munc13 within the active zone, thus disinhibiting Munc13.

Initial studies showed that RIMs act as Rab3 effectors and represent targets for phosphorylation by PKA (Wang et al., 1997; Castillo et al., 2002). The new results demonstrate two additional functions of RIM. First, it tethers presynaptic Ca^{2+} channels to the active zone. Second, it prevents the homodimerization of Munc13, and therefore disinhibits the priming function of Munc13. These different functions are not mutually exclusive, but raise the interesting possibility that the tethering of Ca^{2+} channels or the priming of synaptic vesicles could be altered during presynaptic plasticity (Castillo et al., 2002). Furthermore, it is tempting to speculate that differential expression of RIM could coregulate Ca^{2+} channel-transmitter release coupling and vesicular pool size in parallel, as required to match efficacy and stability of synaptic transmission during repetitive activity. This may be important at both GABAergic and auditory synapses, which release transmitter at high rates during repetitive presynaptic activity in vitro and in vivo (Hefft and Jonas, 2005; Bucurenciu et al., 2008).

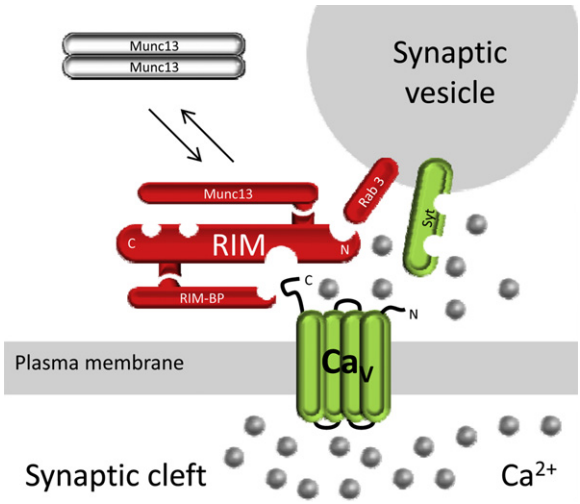

Figure 1. The Multiple Functions of RIM
Red: RIM and its interacting proteins Rab3, RIM-BP, and Munc13. Green: presynaptic Ca^{2+} channel (Cav) on the plasma membrane and Ca^{2+} sensor synaptotagmin (syt) on the synaptic vesicle.

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