RIM function in short- and long-term synaptic plasticity

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Abstract
RIM1α (Rab3-interacting molecule 1α) is a large multidomain protein that is localized to presynaptic active zones [Wang, Okamoto, Schmitz, Hofmann and Südhof (1997) Nature (London) 388, 593–598] and is the founding member of the RIM protein family that also includes RIM2α, 2β, 2γ, 3y and 4γ [Wang and Südhof (2003) Genomics 81, 126–137]. In presynaptic nerve terminals, RIM1α interacts with a series of presynaptic proteins, including the synaptic vesicle GTPase Rab3 and the active zone proteins Munc13, liprins and ELKS (a protein rich in glutamate, leucine, lysine and serine). Mouse KOs (knockouts) revealed that, in different types of synapses, RIM1α is essential for different forms of synaptic plasticity. In CA1-region Schaffer-collateral excitatory synapses and in GABAergic synapses (where GABA is γ-aminobutyric acid), RIM1α is required for maintaining normal neurotransmitter release and short-term synaptic plasticity. In contrast, in excitatory CA3-region mossy fibre synapses and cerebellar parallel fibre synapses, RIM1α is necessary for presynaptic long-term, but not short-term, synaptic plasticity. In these synapses, the function of RIM1α in presynaptic long-term plasticity depends, at least in part, on phosphorylation of RIM1α at a single site, suggesting that RIM1α constitutes a ‘phosphoswitch’ that determines synaptic strength. However, in spite of the progress in understanding RIM1α function, the mechanisms by which RIM1α acts remain unknown. For example, how does phosphorylation regulate RIM1α, what is the relationship of the function of RIM1α in basic release to synaptic plasticity and what is the physiological significance of different forms of RIM-dependent plasticity? Moreover, the roles of other RIM isoforms are unclear. Addressing these important questions will contribute to our view of how neurotransmitter release is regulated at the presynaptic active zone.

Structure and protein interactions of RIMs (Rab3-interacting molecules)
Active zones are composed of an electron-dense protein network that is attached to the presynaptic plasma membrane at the synaptic cleft of the synapse [1,2]. Active zones perform a central function in neurotransmitter release because this is where synaptic vesicles dock and fuse during release [3,4]. RIM proteins are scaffolding molecules that are composed of multiple independently folded domains and are thought to regulate neurotransmitter release by interacting directly or indirectly with multiple other synaptic proteins (Figure 1a) [4,5].

The mammalian genome contains four RIM genes (Rims1–4 [6,7]) that encode six principal RIM forms (RIM1α, 2α, 2β, 2γ, 3y and 4γ; Figures 1b and 1c). RIM1α and 2α are composed of an N-terminal zinc finger, a central PDZ domain and two C-terminal C2 domains [6,8] that are separated by multiple alternatively spliced sequences. RIM2β (which, like RIM2γ, is expressed from an internal promoter in the Rims2 gene) lacks the N-terminal zinc-finger region but is otherwise identical with RIM2α. RIM2γ, 3y and 4γ consist of only the C-terminal C2 domain with flanking sequences [6].

RIMs were originally identified as effectors of Rab3 [8]. The N-terminal sequence of RIM1α and 2α (that is absent from RIM2β, 2γ, 3y and 4γ; Figures 1b and 1c) binds to two different synaptic proteins: the synaptic vesicle GTPase Rab3 and another active zone protein called Munc13 [4,9,10]. This N-terminal region includes two nested domains that mediate these two binding activities: an α-helical Rab3-binding region and a Munc13-binding zinc-finger domain. Although in vitro binding essays initially suggested that Rab3 and Munc13 compete with each other for RIM1α binding [9], recent results indicate that they bind simultaneously [10a]. Since Rab3 regulates synaptic vesicle fusion [11,12] and Munc13 is critical for priming synaptic vesicles for fusion [13], RIM1α and 2α probably couple these two proteins functionally. In addition to Munc13 and Rab3, RIMs also bind directly to three other active zone proteins and one other synaptic vesicle protein. First, the PDZ domains of RIM1α, 2α and 2β bind to ELKS proteins [named after the amino acids glutamate, leucine, lysine and serine in which it is rich, and also referred to as Rab6-interacting protein or CAST, and also abbreviated as ERC (ELKS/Rab6-interacting protein/CAST)] [14]. In mammals, two ELKS genes produce three different ELKS proteins (ELKS1A, 1B and 2B), of which ELKS1B and 2 are active zone proteins [14–16]. Secondly, RIMs bind via their C-terminal C2B
domain to $\alpha$-liprins [4]. The functions of mammalian liprins have not been addressed, but in Caenorhabditis elegans and Drosophila, liprins regulate the formation, differentiation and maintenance of synaptic termini [17,18]. Thirdly, RIMs bind via a proline-rich region to the SH3 domain (Src homology 3 domain) protein RIM-BP (RIM-binding protein), which in turn may bind to voltage-gated Ca$^{2+}$ channels [19]. Fourthly, RIMs interact, via their C-terminal C2B domain, with synaptotagmin 1 in a Ca$^{2+}$-dependent manner, although the validity of this interaction has not been established. In addition to these interactions, RIMs bind several other proteins in vitro, including SNAP-25 (25 kDa synaptosome-associated protein) [20], N-type Ca$^{2+}$ channels [20], cAMP-GEFII (guanine nucleotide-exchange factor II) [21] and 14-3-3 adaptor proteins (P.S. Kaeser and T.C. Südhof, unpublished work) [22,23]. However, not all of these interactions have been reproducible, and their significance is unclear at present. Finally, RIMs interact indirectly with other synaptic proteins, for example with piccolo and bassoon via ELKS [24], and with receptor-tyrosine phosphatases via liprins [17,25].

RIM proteins and synaptic plasticity

RIM1$\alpha$ KO (knockout) mice exhibit severe changes in synaptic transmission, whose nature varies dramatically with different types of synapses. A subset of central excitatory synapses, such as mossy fibre synapses in the CA3 region of the hippocampus [26] (Figure 2a), parallel fibre synapses in the cerebellum [27,28], corticostriatal synapses [29] and corticothalamic synapses [30] undergo LTP (long-term potentiation) or LTD (long-term depression) when presynaptic PKA (protein kinase A) is activated. PKA-dependent presynaptic long-term plasticity in mossy fibre synapses is abolished in RIM1$\alpha$ and in Rab3A KO mice (Rab3A is one of the Rab3 proteins with which RIM1$\alpha$ interacts; Figures 3a and 3b). At the same time, the basic properties of synaptic transmission, including short-term synaptic plasticity, appear to be unchanged in these synapses [31–33]. In contrast with synapses capable of PKA-dependent presynaptic LTP, in other excitatory synapses such as the CA3 to CA1 Schaffer-collateral synapses in the hippocampus that are incapable of PKA-dependent LTP (Figure 2a), deletion of RIM1$\alpha$ and of Rab3 proteins caused impairments in basic release properties and in short-term plasticity (Figures 2b and 2c) [4,34]. In these synapses, RIM1$\alpha$ KO mice have a more severe phenotype than Rab3 KO mice even when
all four Rab3 isoforms are deleted [12], consistent with the notion that RIM1α is an effector of Rab3 but performs additional functions beyond acting as a downstream effector for Rab3. RIM1α is not required for the induction of classical NMDA (N-methyl-D-aspartate) receptor-dependent LTP in Schaffer-collateral synapses, but recently was found to be essential for ‘late’ NMDA receptor-dependent LTP in these synapses [35]. Late LTP is maintained beyond 2 h and depends on PKA activation, similar to presynaptic PKA-dependent LTP. Thus, although late LTP is initially induced in a postsynaptic NMDA receptor-dependent manner that does not require RIM1α, its maintenance may involve presynaptic PKA activation and RIM1α similar to presynaptic LTP. This extension of RIM function beyond presynaptically induced forms of synaptic plasticity suggests that RIM proteins may be involved in more general mechanisms to change the presynaptic release machinery, probably other forms of long-term plasticity that require post-synaptic induction with presynaptic feedback mechanisms.

How does PKA trigger long-term plasticity in synapses such as mossy fibre synapses in the hippocampus or parallel fibre synapses in the cerebellum? A potential avenue to addressing this question is to test whether PKA might act via either Rab3A or RIM1α (Figure 3c). Direct phosphorylation experiments showed that Rab3A is not a substrate for PKA [33], but RIM1α is phosphorylated at least at two sites: Ser413, which is in between the zinc-finger domain and the PDZ domain, and Ser1548, which is at the very C-terminus [36]. Identification of these sites allowed testing of whether phosphorylation of one or both of these sites is essential for presynaptic PKA-dependent LTP. Using rescue experiments in cultured cerebellar RIM1α-deficient neurons, it was found that serine phosphorylation at Ser413 is essential for presynaptic LTP and, moreover, that mutant RIM1α containing a serine-to-alanine substitution at residue 413 inhibits presynaptic LTP when introduced into wild-type cultured cerebellar neurons [36]. These observations suggest that parallel fibre LTP – and by extension, mossy fibre LTP and other long-term forms of PKA-dependent presynaptic plasticity – is regulated by the cAMP/PKA pathway at a single phosphorylation site: Ser413 of RIM1α. It suggests a model whereby signalling by Rab3A and PKA converges on to RIM1α during presynaptic LTP (Figure 3d).

With RIM1α, we now have a molecular handle on PKA-dependent long-term plasticity. However, why does this form of plasticity not operate in synapses such as
Schaffer-collateral synapses? And why does the RIM1α deletion so dramatically alter synaptic strength and short-term synaptic plasticity in Schaffer-collateral synapses, but not in synapses competent for PKA-dependent presynaptic LTP? RIM1α is clearly present, and also phosphorylated on Ser413, in Schaffer-collateral synapses, indicating that PKA may be active in these termini. Several hypotheses may be advanced that answer these questions. First, it is possible that, in Schaffer-collateral synapses, RIM1α is constitutively phosphorylated on Ser413, maybe via other kinases, such that RIM1α is always ‘turned on’. This would explain why the RIM1α KO diminishes synaptic strength and why changing PKA activity does not lead to changes in synaptic strength. Alternatively, it is also possible that the downstream effectors for phospho-RIM1α are different in these synapses, resulting in a situation whereby phosphorylation of Ser413 is regulatory in all synapses, but the endpoints of regulation are different. A third possibility is based on the fact that, up to now, the functions of other RIM isoforms have not been considered. It is thus possible that RIM1α is co-expressed with distinct additional RIM isoforms in the various synapses and that the pattern of RIM expression determines the contribution of RIM1α to overall synaptic strength and plasticity. In this regard, it is noteworthy that the Ser413 phosphorylation site of RIM1α is conserved in RIM2α and 2β, suggesting that PKA might operate in synaptic plasticity on multiple RIMs depending on which RIMs are expressed. To differentiate between these possibilities, it will be necessary to test the effect of deleting other RIM isoforms on synaptic transmission and of mutating the phosphorylation site of RIM1α in vivo. Moreover, it will be important to understand the biochemical properties of different RIM isoforms and, in particular, the molecular consequences of RIM phosphorylation.

Finally, RIM1α is not only involved in excitatory but also in inhibitory synapses. Whereas regulation of synaptic release at excitatory synapses has been intensively studied, markedly less is known about GABAergic inhibitory synapses (where GABA is γ-aminobutyric acid). Generally, inhibitory synapses have a high release probability and express paired-pulse depression instead of paired-pulse facilitation when two action potentials are applied with a short interpulse interval [37]. Unexpectedly, RIM1α KO mice exhibit increased paired-pulse depression [4], indicating that the RIM1α deletion may increase the release probability at GABAergic synapses, opposite to the decrease in release probability at excitatory synapses. This important finding suggests that RIM1α may act differently in excitatory and inhibitory synapses, a possibility that raises a host of additional questions.

**Physiological significance of RIM1α function**

When RIM1α KO mice were tested behaviourally, they exhibited impairments in fear conditioning and spatial learning and an increase in locomotor responses to novelty [38]. At the same time, tests of motor co-ordination using the rotord assay did not uncover an abnormality, indicating that the RIM1α KO mice did not simply show incapacitated nervous system function. Moreover, although some of the behavioural changes in RIM1α KO mice are compatible with a deficit of PKA-dependent LTP, this loss of synaptic plasticity by itself probably does not explain these behavioural changes because Rab3A KO mice that also lack presynaptic PKA-dependent LTP (at least in hippocampal mossy fibre synapses) did not exhibit any behavioural change. Thus, at present, it is clear that RIM1α function has a profound role in animal behaviour, but the precise role of different facets of RIM1α function in behaviour remains to be explored.

**Summary and outlook**

The currently available data establish that RIM1α is an active zone protein with a central function in regulating neurotransmitter release and furthermore suggest that other RIM isoforms may also act in regulating neurotransmitter release. Together with work on other active zone proteins such as Munc13 and bassoon [13,24,39–41], a picture emerges whereby the protein network that makes up the active zone performs two basic functions: first, it organizes the docking and priming of synaptic vesicles for exocytosis. Secondly, it mediates use-dependent changes in release during short- and long-term synaptic plasticity. RIMs are clearly central components of this protein network and may in fact be like the proverbial spider in the net in that RIMs interact with most other active zone proteins directly or indirectly. However, it is also clear that research on RIMs has only started; major questions remain to be addressed before even a rudimentary understanding can be claimed. These questions can be grouped into two classes. First, what are the fundamental mechanisms by which RIMs act? For example, how does RIM1α mediate presynaptic LTP and how does it work in short-term plasticity? Secondly, what is the physiological significance of RIM functions? For example, what is the importance of RIM1α’s role in presynaptic LTP and what do other RIM isoforms do? Addressing these questions will provide a fundamental insight into how synapses work and how their use-dependent plasticity shapes synaptic networks.

**References**


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