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Synapse reorganization—a new partnership revealed

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Changes in synaptic function require both qualitative and quantitative reorganization of the synaptic components. Ca^{2+} plays a central role in this process, but the mechanism has not been fully elucidated. Zhang *et al* report a novel mechanism whereby Ca^{2+}/calmodulin (CaM) regulates the stability of the postsynaptic scaffold. Ca^{2+}/CaM interacts with PSD-95, a core protein in the postsynaptic density (PSD) that supports synaptic signaling and structural components. Ca^{2+}/CaM interferes with the palmitoylation of PSD-95, resulting in the dissociation of PSD-95 from the postsynaptic membrane. This process may explain the reduction of surface glutamate receptor observed during synaptic depression and homeostatic regulation of the synaptic response after prolonged neuronal activity.

See also: **Y Zhang *et al*** (June 2014)

The adaptation of synaptic strength is a fundamental process for learning and memory. This form of synaptic plasticity is triggered by influx of Ca^{2+} from NMDA-type glutamate receptor (NMDAR), leading to the synaptic insertion or removal of AMPA-type glutamate receptor (AMPAR) and determines the strength of synaptic transmission. This process is mediated by the gross reorganization of the postsynaptic composition in a qualitative and quantitative fashion (Bosch *et al*, 2014). Importantly, the number of synaptic AMPAR is regulated by the number and affinity of postsynaptic 'slots', a hypothetical receptor binding site within a synapse, which is regulated during synaptic plasticity processes.

PSD-95, a scaffolding protein at excitatory synapses, has been considered as a major candidate for the slot. It interacts with AMPAR through the TARP/stargazin protein family and modulates the synaptic localization of the receptor as well as the strength of synaptic transmission (El-Husseini *et al*, 2000). In turn, the localization of PSD-95 at the synapse is regulated by a constant cycle of palmitoylation by protein palmitoyl acyltransferases (PAT) at cysteines 3 and 5, which is required for efficient synaptic targeting of the protein, and depalmitoylation by palmitoyl protein thioesterases (PPT) (El-Husseini *et al*, 2002; Noritake *et al*, 2009). Activation of glutamate receptors increases depalmitoylated PSD-95 and releases it from the postsynaptic site (El-Husseini *et al*, 2002; Sturgill *et al*, 2009), whereas blockage of neuronal activity by TTX increases palmitoylated PSD-95 and targets it to the synapse (Noritake *et al*, 2009). However, it remains unclear how neuronal activity controls the palmitoylation/depalmitoylation cycle and the subsequent trafficking of PSD-95 to and from the synapse.

In this issue of *The EMBO Journal*, using a combination of structural biological, biochemical, and cell biological approaches, Zhang *et al* (2014) revealed a novel mechanism by which Ca^{2+} regulates the synaptic localization of PSD-95. The authors found that Ca^{2+} complexed to CaM can bind PSD-95 within the first 13 residues—the exact location where palmitoylation takes place. Ca^{2+}/CaM binds preferentially to unmodified PSD-95, thereby blocking the accessibility of the PAT to the palmitoylation sites. In contrast, Ca^{2+}/CaM does not bind to palmitoylated PSD-95, and therefore, palmitoylated PSD-95 is subject to depalymitoylation by PPT. Overall, the net effect of Ca^{2+}/CaM is a reduction of PSD-95 bound to the synaptic membrane (Fig 1). Consistent with this, a PSD-95 mutant that was unable to bind Ca^{2+}/CaM does not leave the synapse following glutamate/glycine treatment. Furthermore, the mutant PSD-95 showed an increased in synaptic accumulation, indicating that the treatment also increases palmitoylation activity but it is normally dominated by the depalmitoylating action of Ca^{2+}/CaM binding (Noritake *et al*, 2009).

The beauty of the work by Zhang *et al* (2014) is that the structure fully explains the biology. However, several important questions remain. Above all, it is still unclear when this mechanism would operate. Generally, the palmitoylation/depalmitoylation cycle is considered to be in the order of minutes. Therefore, for Ca^{2+}/CaM to effectively reduce the palmitoylation of PSD-95, a prolonged influx of Ca^{2+} is required. In contrast, the rise in intracellular Ca^{2+} induced by a single excitatory postsynaptic current (EPSC) or dendritic action potential is in the order of milliseconds to seconds. A slow repetitive stimulation protocol, such as that used to induce long-term depression (LTD) (1 Hz, 15 min), may be effective in increasing Ca^{2+}/CaM for a sufficiently long period of time. Homeostatic scaling induced by a prolonged increase in network activity (for example, via the pharmacological blockade of inhibitory synaptic transmission) may also be a possible mechanism for the Ca^{2+}/CaM-mediated removal of synaptic PSD-95. In contrast, stimulation used to induce long-term potentiation (LTP) (a brief

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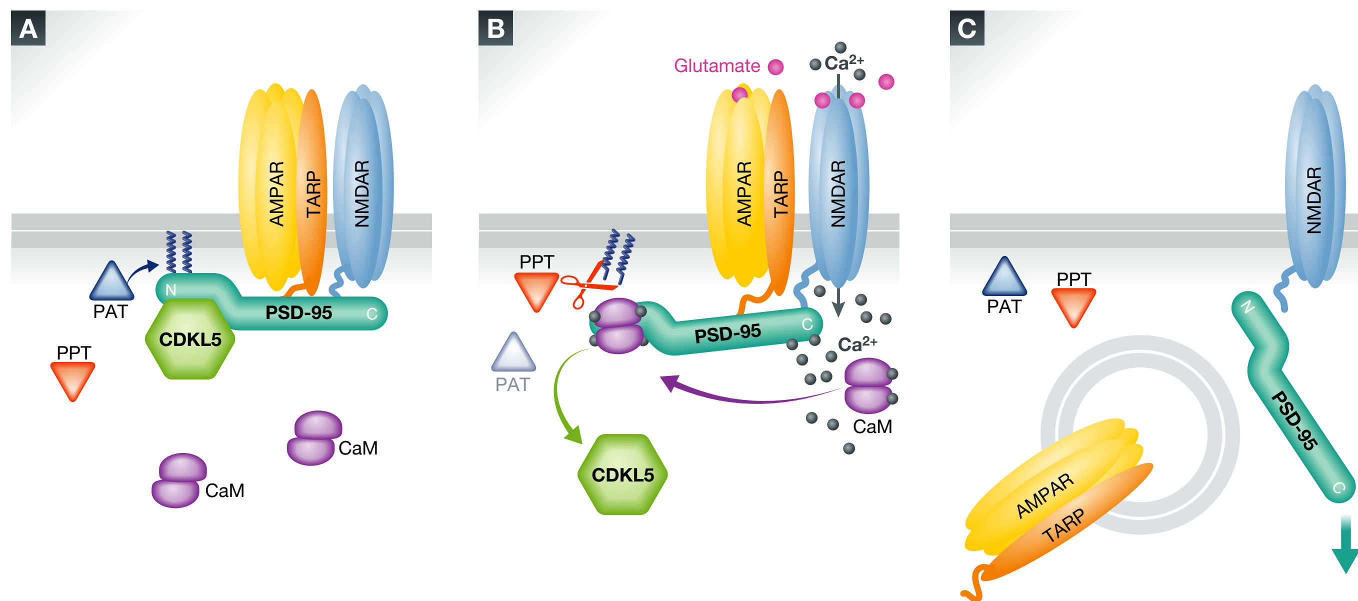

Figure 1. Calcium influx-induced release of PSD-95 and glutamate receptor from the synapse.
At a synapse, cysteine residues of PSD-95 (C3 and C5) are under a continuous cycle of palmitoylation by protein palmitoyl acyltransferase (PAT), and depalmitoylation by palmitoyl protein thioesterase (PPT). A. Palmitoylated PSD-95 associates with the synaptic membrane and CDKL5 and serves as a 'slot' for AMPAR at the synapse through the interaction with TARP/stargazin. B. Upon glutamate stimulation, Ca^{2+} influx through NMDARs induces binding of Ca^{2+}/CaM to PSD-95. Ca^{2+}/CaM blocks the accessibility of PAT, thereby facilitating the depalmitoylation of PSD-95, which subsequently allows PSD-95 to dissociate from the synaptic membrane and CDKL5. C. The dissociation of PSD-95 from the synaptic membrane reduces the number of available 'slots' for AMPAR on the postsynaptic membrane, leading to a reduction of AMPAR-mediated synaptic transmission.

high-frequency stimulation such as 100 Hz, 1 s) may not be effective. Indeed, Bosch *et al* (2014) found that the induction of LTP at single dendritic spines in hippocampal CA1 pyramidal neurons does not decrease or increase PSD-95 during the first hour after induction even if the dendritic spine enlarges during this period.

In this context, it is important to understand what impact Ca^{2+}/CaM-mediated removal of synaptic PSD-95 has on synaptic transmission. If PSD-95 is indeed the slot for AMPAR, the Ca^{2+}/CaM-mediated removal of synaptic PSD-95 is expected to reduce the synaptic transmission. The stimulation protocol used here by Zhang *et al* (bath application of glutamate/glycine) is similar to previously reported approaches to induce 'chemical' LTD and hence can provide an explanation for the decrease in PSD-95 from the synapse for 10–15 min after stimulation. The PSD-95 mutant that is unable to bind Ca^{2+}/CaM will be useful to further analyze the link between the observed PSD-95-Ca^{2+}/CaM interaction and synaptic plasticity.

PSD-95 is also known to interact with the cyclin-dependent protein kinase-like kinase 5 (CDKL5) at the first 19 residues in a palmitoylation-dependent manner (Zhu *et al*, 2013). As expected, Ca^{2+}/CaM binding also regulates CDKL5 association with PSD-95. The treatment of neurons with NMDA reduces the palmitoylation of PSD-95 and, concomitantly, the association with CDKL5. Mutations of CDKL5 and netrin-G1 gene have been reported in patients with an atypical form of Rett syndrome. Netrin-G1 ligand (NGL1) has been identified as an interaction partner and substrate of CDKL5 (Ricciardi *et al*, 2012). CDKL5 phosphorylates NGL1, and this phosphorylation stabilizes the interaction of NGL1 with PSD-95. Given that the Ca^{2+} signal only lasts a few milliseconds to seconds, it is important to investigate the spatiotemporal interaction between CaM, PSD-95, CDKL5, and NGL1 in dendritic spines during physiological and pathological conditions. In addition, CDKL5 can function as an upstream modulator of Rac signaling during development (Chen *et al*, 2010). Together with the fact that Rac also plays an important role in long-term potentiation, the PSD-95/CDKL5 complex might regulate Rac activity in the vicinity of the PSD.

Other neuronal proteins including AMPAR subunit GluR1/2, glutamate receptor interacting protein (GRIP), G-protein-coupled receptors, δ-catenin, and small and trimeric G-proteins can also undergo palmitoylation, suggesting that this process plays an essential role in subcellular targeting and that these proteins can be regulated by activity (Kang *et al*, 2008). The question of whether Ca^{2+}/CaM can regulate the palmitoylation of these proteins remains. Interestingly, the Ca^{2+}/CaM interaction site of PSD-95 does not conform to a canonical IQ-motif, a CaM binding motif found in many other proteins (Zhang *et al*, 2014). Therefore, a bioinformatic approach is not possible at this point in time. This opens further directions of research.

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Conflict of interest

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