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Long-term potentiation: two pathways meet at neurogranin

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Since the discovery of hippocampal long-term potentiation (LTP) as a cellular equivalent of learning and memory, hundreds of molecules belonging to different signal transduction pathways have been implicated in the mechanism underlying LTP. To understand LTP, it is therefore important to elucidate how different signal transduction pathways intersect with each other. Neurogranin/RC3, a calmodulin-trapping protein kinase C (PKC)-substrate featuring in new work reported in this issue of *The EMBO Journal* (Zhong *et al*, 2009), seems ideally situated to serve as such a point of crosstalk between two major signalling pathways involved in LTP.

There are two classes of glutamate receptors, ionotropic and metabotropic. Although both are crucial for LTP, just how these two pathways interact with each other to signal LTP was not known. Metabotropic glutamate receptors (mGluRs) couple to heterotrimeric G proteins, which subsequently activate intracellular second messenger systems including the phospholipase C/PKC/IP_3 pathway. Ionotropic glutamate receptors, on the other hand, trigger a chain of Ca^{2+}-dependent events, including activation of kinases such as CaMKII, eventually leading to postsynaptic insertion of AMPA receptors. One of the major players in this pathway is calmodulin, a Ca^{2+}-binding protein with a canonical EF-hand that binds many different proteins including CaMKII. Importantly, the amount of calmodulin in a cell is limited compared with the amount of its target molecules (Zhabotinsky *et al*, 2006). Therefore, availability of calmodulin can serve as a point of regulation in calmodulin-dependent signalling. One of the mechanisms determining the availability of calmodulin is binding to neurogranin, a 78 amino acid-polypeptide of the calpacitin family (see Gerendasy and Sutcliffe, 1997 for review). Neurogranin contains an IQ domain that interacts with and sequesters apo-calmodulin (the Ca^{2+}-free form of calmodulin). On rise of the intracellular Ca^{2+} concentration, neurogranin releases calmodulin, freeing it to bind Ca^{2+} and activate downstream signalling molecules.

In their new study, Zhong *et al* show that neurogranin is concentrated in dendritic spines, the sites of most excitatory synapses in the brain, especially beneath the postsynaptic membrane (Zhong *et al*, 2009). Through this localization, apo-calmodulin is also sequestered beneath the postsynaptic membrane, where it is ideally placed to sense Ca^{2+} influx through NMDA receptors. The authors further find that the number of neurogranin molecules available determines the efficiency of calmodulin signalling in the synapse and the strength of AMPA receptor transmission (Zhong *et al*, 2009). Exogenous overexpression of neurogranin sequesters more calmodulin at the synapse and lowers the threshold of Ca^{2+} signalling. Neurons in circuit are always firing and, in those overexpressing neurogranin, such basal neuronal activity is sufficient to trigger a Ca^{2+} signal that normally induces LTP. This leads to synaptic trafficking of AMPA receptor and, therefore, a potentiation in AMPA receptor-mediated synaptic transmission. This neurogranin-induced synaptic potentiation and LTP share common signalling mechanism: like LTP, neurogranin-induced potentiation requires the activity of NMDA receptors and CaMKII. Furthermore, in such neurons that overexpress neurogranin and where the AMPA receptor response is therefore already potentiated, LTP can no longer be electrophysiologically induced. Taken together, these observations in turn imply the involvement of neurogranin in the signalling pathway of LTP.

The most intriguing aspect of neurogranin is its phosphorylation at serine 36, in the midst of the IQ domain, by PKC, a downstream signalling protein not only of mGluRs but also of other G protein-coupled receptor (GPCR) systems such as monoaminergic, cholinergic, or peptidergic receptors projecting to the hippocampus from other parts of the brain. Ser36 phosphorylation decreases neurogranin's ability to interact with apo-calmodulin, putting neurogranin in a unique position to integrate signal crosstalk between PKC-mediated mGluR (or other GPCR) pathways and NMDA receptor/Ca^{2+}/CaMKII signalling. LTP-inducing stimuli, as well as a pharmacological mGluR activation, induce PKC phosphorylation of neurogranin; the resulting decrease in apo-calmodulin affinity increases the availability of free apo-calmodulin in the spine and thereby enhances the activity of the CaMKII-mediated pathway. In support of this model, a neurogranin mutant that can neither be phosphorylated by PKC nor release calmodulin on Ca^{2+} influx was unable to enhance AMPA receptor transmission in overexpression experiments (Zhong *et al*, 2009). Likewise, a mutant that mimics the phosphorylated state and never binds calmodulin was also unable to enhance AMPA receptor transmission. In line with these findings, neurogranin knockout animals display modified mGluR-mediated metaplasticity (Krucker *et al*, 2002). Of special note, a genome-wide analysis of single nucleotide

polymorphisms recently even identified neurogranin as a risk factor in schizophrenia (Stefansson *et al*, 2009).

One remaining question is how neurogranin is localized to the postsynaptic membrane. This may be linked to the presence of N-terminal cysteines that could be potentially palmitoylated, as homologous cysteines in another member of the calpacitin family, GAP43, are known to be palmitoylated and involved in membrane targeting. In addition, the precise time course of the neurogranin-calmodulin association remains to be demonstrated *in vivo*. Live imaging techniques such as fluorescent resonance energy transfer may be a powerful method to gain further insight into this question.

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