

## A lipid kinase controls the maintenance of dendritic spines

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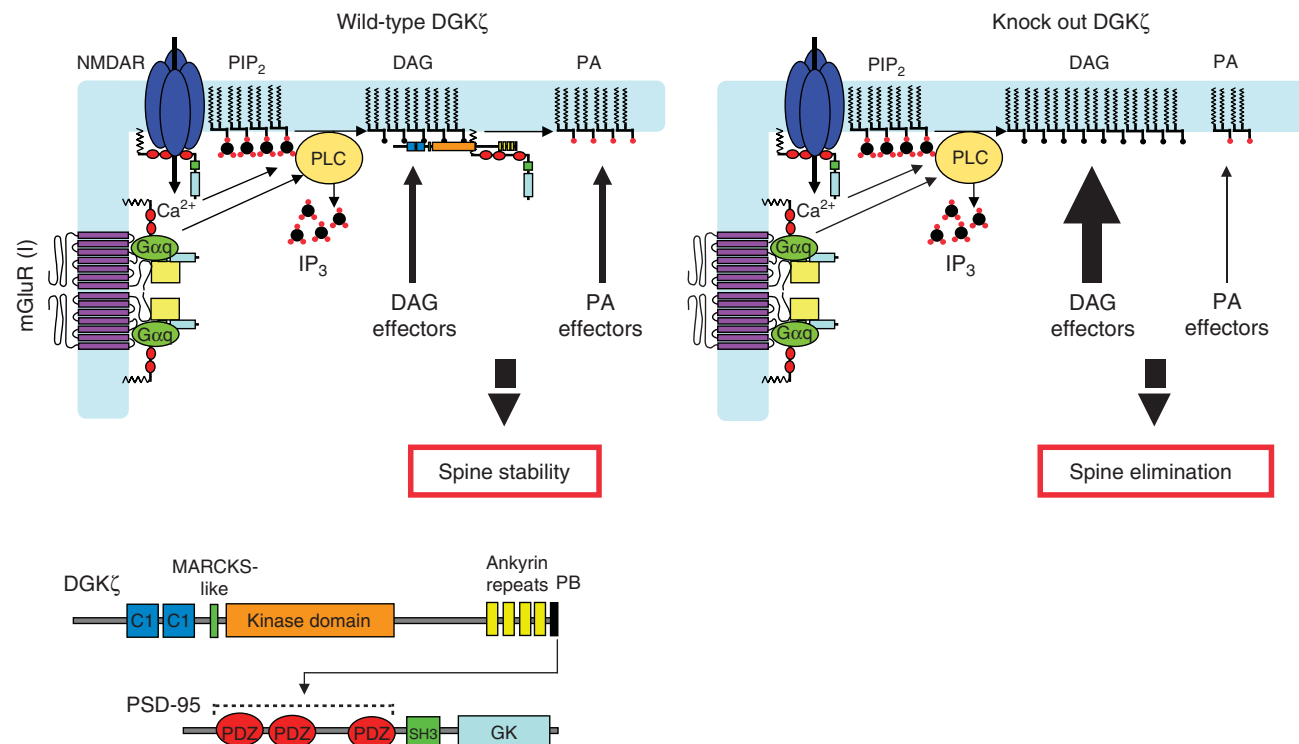
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Dendritic spines are tiny membranous protrusions from neuronal dendrites that receive inputs from other neurons' nerve terminals and are believed to provide an anatomical substrate for memory storage and synaptic transmission. A new study in this issue suggests that a diacylglycerol (DAG) kinase isoform interacts with a major scaffolding protein of the spines, PSD-95, and regulates the dynamics of these structures as well as synaptic transmission.

Intracellular signalling lipids are key regulators of cell physiology. At neuronal synapses, mounting evidence implicates lipids,

such as phosphoinositides, as critical regulators of synaptic transmission (Di Paolo and De Camilli, 2006). One of the best characterized reactions in phosphoinositide-based signalling is the hydrolysis of PIP<sub>2</sub> to DAG and IP<sub>3</sub> by PLC (Di Paolo and De Camilli, 2006) (Figure 1). Whereas soluble IP<sub>3</sub> mediates the release of Ca<sup>2+</sup> from intracellular stores, membrane-bound DAG stimulates PKC (Di Paolo and De Camilli, 2006). In addition, DAG regulates a variety of proteins harbouring PKC domain 1 (i.e. C1 domains), such as members of the Munc13 (i.e. factors involved in vesicle 'priming'), chimaerin (i.e. RacGAP) and DAG kinase (DGK) families (Brose and Rosenmund, 2002; Buttery *et al.*, 2006; Topham, 2006). On the basis



**Figure 1** Hypothetical scheme of the role of DGK $\zeta$  in the regulation of dendritic spine morphology. Based on Kim *et al.* (this issue of *EMBO J*), the recruitment of DGK $\zeta$  to the postsynaptic density of dendritic spines occurs through an interaction of its COOH-terminus (PB, PDZ domain-binding) with the PDZ domains of PSD-95 and related family members. These proteins are anchored to the membrane through a lipid modification and localize to the PSD through a variety of direct or indirect interactions with neurotransmitter receptors. PLC activation downstream of NMDAR or group I mGluR activation leads to hydrolysis of PIP<sub>2</sub> to DAG and IP<sub>3</sub>. DAG can be further converted to PA by the kinase domain of DGK $\zeta$ , following recruitment of the lipid kinase to DAG-rich membranes through its C1 domain. In the absence of DGK $\zeta$ , accumulation of DAG and increased recruitment of DAG effectors are believed to cause spine loss. Alternatively, this phenotype can result from reduced production of PA and recruitment of PA effectors. GK, guanylate kinase-like domain.

of its pivotal signalling role and, particularly, its capacity to amplify signals, DAG must be tightly regulated. One of the mechanisms involved in the control of DAG levels is the DGK pathway, which converts DAG to phosphatidic acid (PA), another bioactive signalling lipid (Topham, 2006). This conversion has two functional implications—the termination of DAG signalling and the initiation of PA signalling. Consistent with the daunting complexity of DAG/PA actions, multiple DGK-encoding genes are present in the genome of higher eukaryotes (Topham, 2006). In the fly phototransduction cascade, a DGK member (RdGA) mediates light response inactivation (Hardie, 2007). In the worm, mutations in DGK-1 modulate presynaptic release of acetylcholine, likely reflecting the effects of DAG accumulation on vesicle priming factor, Unc13 (Nurrish *et al.*, 1999). However, evidence for synaptic roles for mammalian DGKs has been scant before the study by Kim *et al.* published in this issue of *EMBO J.*

Kim *et al.* report the identification of DGK $\zeta$  as an interactor of the postsynaptic density-associated protein PSD95, which is a major scaffolding protein concentrated in dendritic spines. These structures are dynamic actin-rich protrusions of neuronal dendrites that are characteristic of excitatory neurons and receive inputs from other neurons' presynaptic terminals (Newpher and Ehlers, 2008). Spines are, thus, primary sites of signal integration, in which a variety of receptors and receptor-channels modulate synaptic strength and plasticity, with intracellular Ca<sup>2+</sup> as a major player. PSD-95 family members regulate the function, traffic and signalling downstream of glutamate receptors, such as AMPA, NMDA and PLC-coupled metabotropic glutamate receptors 1 and 5 (AMPA, NMDAR, mGluR1 and mGluR5, respectively). After demonstrating the dependency of DGK $\zeta$  on PSD-95 for its proper targeting at dendritic spines, Kim *et al.* examined the relevance of this interaction for synaptic physiology. They show that overexpression of DGK $\zeta$  in primary hippocampal neurons increases dendritic spine density and that this phenotype requires its catalytic activity. Conversely, silencing DGK $\zeta$  and genetic ablation of this enzyme lead to a decrease in the spine density in mouse hippocampal cultures and slices, respectively (Figure 1). Importantly, lack of DGK $\zeta$  significantly impaired PA formation in knock out slices upon stimulation of PLC-coupled group I mGluR. By monitoring the dynamics of dendritic spines of hippocampal neurons expressing an shRNA vector directed to DGK $\zeta$ , the authors found that DGK $\zeta$  is required for the maintenance of spines, but not their formation. The decreased spine density in pyramidal neurons from DGK $\zeta$  knock out hippocampi correlated with functional deficits in AMPAR-mediated synaptic transmission. Altogether, this study by Kim *et al.* identifies

DGK $\zeta$  as a key regulator of synaptic morphology and excitatory neurotransmission through its ability to modulate DAG/PA signalling at synapses as part of the PSD-95 protein network (Figure 1).

What are the physiologically relevant signalling pathways that involve PLC and are regulated by DGK $\zeta$  in spines? A partial answer may be provided by earlier studies showing a role for PLC in the induction of long-term depression (LTD) [see Horne and Dell'Acqua (2007) and references therein], which is a form of plasticity correlating with the loss of dendritic spines (Newpher and Ehlers, 2008), a feature also observed in DGK $\zeta$ -deficient neurons. PLC activation, achieved either through group I mGluR stimulation or Ca<sup>2+</sup> elevation after NMDAR activation, seems to be critical for LTD (Figure 1). The latter pathway was shown to lead to the disassembly of F-actin and the internalization of the AMPAR by the loss of AKAP79/150, a protein that stabilizes AMPAR phosphorylation and is targeted to spine membranes by PIP<sub>2</sub> (Horne and Dell'Acqua, 2007). Although PIP<sub>2</sub> loss, *per se*, may be a primary signal for the initiation of this LTD pathway, DAG production may significantly contribute to this phenomenon. Exacerbation of this pathway through a failure to eliminate DAG in the DGK $\zeta$  mutant mouse (Figure 1) may thus predispose synapses for an LTD mode. In addition, as PA stimulates PIP kinases and is an important precursor for the synthesis of inositol lipids, failure to convert DAG to PA may hamper the resynthesis of PIP<sub>2</sub> following its consumption by the PLC pathway (Rodriguez de Turco *et al.*, 2001; Hardie, 2007).

This study by Kim *et al.* also raises the need for the identification of the molecular effectors of DAG (and potentially PA) that are involved in the regulation of dendritic spine maintenance. Surprisingly, the PKC pathway does not appear to be implicated. Members of the chimaerin family are likely DAG effectors at spines, based on the ability of these C1 domain-containing proteins to regulate actin dynamics through their RacGAP domain (Brose and Rosenmund, 2002; Buttery *et al.*, 2006). Accordingly,  $\alpha$ 1-chimaerin loss of function leads to an overgrowth of dendritic spines, a phenotype reminiscent of that observed upon DGK overexpression (Buttery *et al.*, 2006). Other potential effectors of DAG and PA are discussed in Kim *et al.*

Finally, the findings reported by the authors may be relevant for brain disorders that are associated with synaptic dysfunction and cognitive deficits. For instance, amyloid beta, a major synaptotoxic agent in Alzheimer's disease, has been shown to decrease spine density in various instances, which, along with recent studies indicating that this cytotoxic peptide activates the PLC pathway and DAG production (Berman *et al.*, 2008), may be related to the phenomena occurring in DGK $\zeta$ -deficient synapses.

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