PKC Defends Crown Against Munc13

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Protein kinase C has long been thought to mediate DAG signaling at the synapse. Recently PKC has been supplanted by members of the Unc13 family as the predominant effectors of DAG signaling. Thanks to a study by Wierda and colleagues in this issue of *Neuron*, PKC returns to reclaim part of the kingdom: both pathways must be active to activate presynaptic potentiation.

Synaptic strength can be turned up by activating G protein-coupled receptors. One of the key outputs of these G protein pathways is the activation of phospholipase C, which cleaves PIP_2 to generate diacylglycerol (DAG). The standard bearer for diacylglycerol function in the synapse has long been protein kinase C (PKC). In 2002 PKC was overthrown and disgraced by Munc13, the young pretender for the throne. In this issue of *Neuron*, studies from Matthijs Verhage's laboratory (Wierda et al., 2007) restore PKC as coregent with Munc13; in fact, their data indicate that these proteins converge on the machinery for synaptic vesicle release in a collaborative and mutually dependent manner.

Phorbol esters act as stable analogs of the endogenous lipid DAG. When applied to neuronal brain slices, phorbol esters mimicked long-term potentiation protocols, suggesting that modulation of synaptic function required the action of DAG (Malenka et al., 1986). What are the endogenous targets of DAG in the brain? It was known that phorbol esters bound tightly to the kinase PKC (Kikkawa et al., 1984). Subsequent studies identified the so-called C1 domain as the phorbol ester binding region in PKC (Ono et al., 1989). It was proposed that phorbol esters mediated synaptic potentiation by activating PKC at the synapse. Pharmacological disruptions of PKC confirmed this view (Majewski et al., 1997). Specifically, inhibitors of PKC, such as bisindolylmaleimide (BIS), eliminated the phorbol esters-dependent potentiation. It was believed that PKC was in fact a unique target of phorbol esters; thus, it was believed that phorbol ester-mediated synaptic potentiation occurred solely through the activation of PKC. This view dominated for 10 years.

However, the usurper was already on the scene. The *unc-13* gene was originally identified in the nematode *C. elegans* based on the uncoordinated phenotype of mutant animals. The protein contained a C1 domain that bound phorbol esters with an affinity equal to that of PKC (Ahmed et al., 1992; Maruyama and Brenner, 1991). Unc13 family members are found in all organisms with nervous systems. Importantly, it was shown that disruption of the C1 domain of Unc13 proteins could prevent or greatly diminish phorbol ester-stimulated potentiation in both *C. elegans* and mice (Betz et al., 1998; Lackner et al., 1999). Finally, in a groundbreaking study, Rhee et al. (2002) showed that Munc13 was itself the sole target of phorbol ester action for synaptic potentiation. A knockin mutation of Munc13 that did not bind phorbol esters exhibited normal baseline synaptic activity, but lacked potentiation by phorbol esters. Perhaps PKC was not the relevant target of DAG after all.

Wierda et al. have now restored PKC as an essential target of phorbol esters at the synapse. First, they confirm that the PKC inhibitors completely block augmentation. However, pretreatment with phorbol esters (or by high-frequency stimulation) 2 min before a second stimulation with phorbol esters makes cells immune to PKC inhibitors. This result was previously observed by Rhee et al., and these authors concluded that the late target of phorbol esters was Munc13. How could these results be reconciled? Surely, both cannot be right.

In an elegant series of experiments, Wierda et al. show that both pathways, Munc13 and PKC, cooperate to potentiate transmission. A downstream target of PKC is a syntaxin binding protein known as Munc18. The authors expressed a Munc18 without the conserved PKC phosphorylation sites in hippocampal cultures lacking the endogenous Munc18 gene. Basal synaptic activity was normal, but the neurons lacked synaptic potentiation when phorbol esters were added, similar to the result with Munc13 mutants lacking phorbol ester binding—and in seeming contradiction. Wierda et al. argue that DAG activates PKC, which opens a window during which a second DAG signal can then act on Munc13—a coincidence detector for two potentiation signals coming in close succession (Figure 1). The PKC-inert Munc18 blocks all synaptic potentiation, and activation of Munc13 by phorbol esters is ineffective. By contrast, a phosphomimetic Munc18 permanently opens the window, and is therefore resistant to the block by the PKC inhibitor BIS. This model reconciles the apparently incompatible observations that both Munc13 and PKC are absolutely required for potentiation.

Mechanistically, this model is still in its infancy. In the simplest version, phosphorylated Munc18 could interact with Munc13, and the complex would potentiate the synapse.

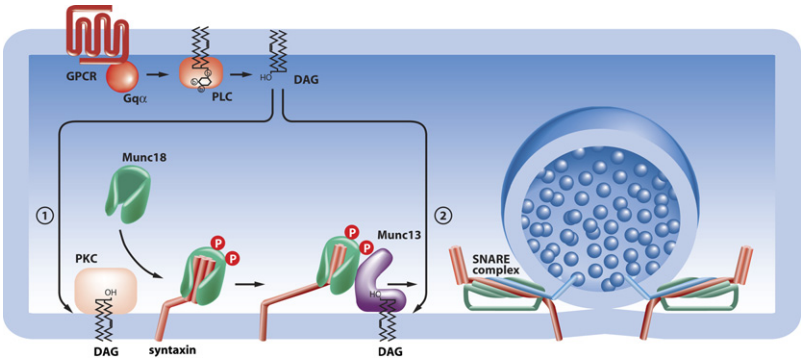

Figure 1. Codependent Targets of DAG
DAG activation of PKC has a long-acting effect by phosphorylating Munc18. DAG activation of Munc13 is short-acting. Together, phosphoMunc18 and Munc13 can stimulate the probability of vesicle fusion (right). The interaction of Munc18 and Munc13 shown is functional and is not known to be direct.

However, two pieces of data argue that Munc18 does not act directly on Munc13. Phorbol ester-dependent Munc13 relocalization to the plasma membrane still occurs in PKC-inert Munc18, arguing that the pathways intercept downstream of Munc13—perhaps at the level of syntaxin, a molecule that binds to both Munc13 and Munc18. Moreover, biochemical studies of *C. elegans* homologs indicate that these proteins have an antagonistic relationship; that is, UNC-13 displaces UNC-18 from syntaxin (Sassa et al., 1999).

There are also published results that contradict these results. First, in contrast to Stevens and Sullivan (1998) but in agreement with Basu et al. (2007), the authors observe that phorbol ester application does not increase the size of the ready-releasable vesicle pool (RRP). Rather, PKC phosphorylation of Munc18 increases the efficacy of synaptic vesicle release. This is true of both calcium-independent priming mechanisms (as assayed by subthreshold sucrose stimulation) and calcium-dependent mechanisms (as measured during electrical stimulation). Second, studies in *C. elegans* suggest that DAG activation of PKC and UNC-13 affect neurotransmission independently and may even act on separate synaptic processes (dense core vesicle and synaptic vesicle exocytosis, respectively) (Sieburth et al., 2007). These issues will need to be resolved in future studies.

Is this the whole story? Well, as with everything, wrinkles come up. First, the PKC-inert Munc18 is completely resistant to phorbol ester application, suggesting that all PKC function acts through Munc18. By contrast, the phosphomimetic Munc18 is only partially resistant to the effects of BIS, suggesting that PKC has other targets in addition to Munc18. Previous studies have implicated different targets, such as voltage-gated channels and other presynaptic proteins, as potential agents of PKC function at the synapse. Second, DAG may have additional targets in augmentation. For example, PKD contains C1 domains, is expressed in neurons, and has movement defects when knocked out in *C. elegans* (Feng et al., 2006). Undoubtedly, the usurpers will have more punches to throw. Nevertheless, Wierda et al. have proposed a very appealing model for reconciling the roles of PKC and Munc13 in potentiation. And it's good to have the king back.

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