

Neuronal activity moves protein palmitoylation into the synapse

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Many neuronal proteins undergo lipid modification that regulates their function and subcellular localization. One such modification is palmitoylation, which is mediated by a large class of protein palmitoyl acyltransferases (PATs). Now, a paper in this issue (Noritake et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200903101) demonstrates that the localization of the PAT DHHC2 is regulated by neuronal activity and thereby selectively controls the palmitoylation and subsequent accumulation of specific proteins in the synapse.

Lipid modification can control the cellular localization, trafficking, interactions, and activity of proteins (el-Husseini and Brecht, 2002; Linder and Deschenes, 2003; Resh, 2006). One prominent lipid modification is palmitoylation, which is the addition of palmitate, a 16-carbon long-chain fatty acid, to Cys residues. Palmitoylation is thought to be particularly important within the nervous system, where many neuronal proteins vital for synaptic plasticity and function undergo this modification. Recently, using proteomic technology, >250 neuronally expressed proteins were shown to undergo palmitoylation (Kang et al., 2008). Notable among these is the postsynaptic scaffolding protein PSD-95 (also known asDlg4). Previous work has demonstrated that neuronal activity regulates the active palmitoylation and depalmitoylation of PSD-95 (El-Husseini et al., 2002; Iwanaga et al., 2009), which in turn regulates the retention of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-type glutamate receptors at neuronal synapses (Chen et al., 2000; Nicoll et al., 2006). The number and localization of AMPA-type glutamate receptors is a principle mechanism controlling the strength and plasticity of synapses (Kessels and Malinow, 2009). Therefore, the activity-regulated palmitoylation of PSD-95 may be an important element in the regulation of synaptic strength.

Given the wide array of proteins that undergo palmitoylation, it is not surprising that there is a large family of acyltransferases that performs this modification. Critical for their enzymatic function is a DHHC (Asp-His-His-Cys) domain. Seminal work first defined a family of seven DHHC domain-containing proteins in

yeast (Bartels et al., 1999; Putilina et al., 1999; Roth et al., 2002). In mammals, there are 23 proteins with DHHC domains that have distinct patterns of tissue and subcellular localization (Fukata et al., 2004; Ohno et al., 2006). Although many of these proteins are localized to the Golgi apparatus when overexpressed in cultured cells, the endogenous localization of most DHHC domain-containing proteins remains to be determined (Ohno et al., 2006). Importantly, different DHHC domain-containing enzymes control the palmitoylation of specific proteins. For instance, DHHC2, -3, -7, and -15 have activity for PSD-95, whereas DHHC17 (HIP14) has activity for SNAP-25 and huntingtin (Fukata et al., 2004; Huang et al., 2004; Keller et al., 2004; Stowers and Isacoff, 2007; Greaves et al., 2009). Yet, how the cellular localization and substrate specificity of DHHC proteins is controlled is not known.

Noritake et al. (see p. 147 of this issue) provide important new insights into this question. Using high resolution live-cell total internal reflection fluorescence microscopy (TIRFM) coupled with fluorescently tagged forms of synaptic proteins and acyltransferases, they examine the role of palmitoylation and the enzymes that perform this modification in the neuronal activity-dependent regulation of PSD-95 in cultured hippocampal neurons. First, the authors address the question of how neuronal activity affects the lipid state of PSD-95. Consistent with previous work (Iwanaga et al., 2009), they demonstrate that PSD-95 accumulates at synapses and becomes palmitoylated after the blockade of neuronal activity. A version of PSD-95 that cannot be palmitoylated fails to accumulate at synapses after activity blockade. Next, Noritake et al. (2009) demonstrate that not all synaptic proteins are equally affected by activity blockade. Thus, although PSD-95 palmitoylation is activity regulated, palmitoylation of other synaptic proteins such as G α q, GluR2, and GRIP1 is unaffected by neuronal activity blockade. Given that specific PATs mediate the addition of palmitate to different proteins (Iwanaga et al., 2009), the authors focus on the subfamily including DHHC2 and -15 because these proteins target PSD-95 and not G α q or GluR2. Consistent with their model, transfection of a dominant-negative DHHC2 disrupts the neuronal activity-sensitive palmitoylation of PSD-95. The authors then conduct a careful series of experiments to demonstrate that DHHC2 is likely to be the PAT responsible for regulating the

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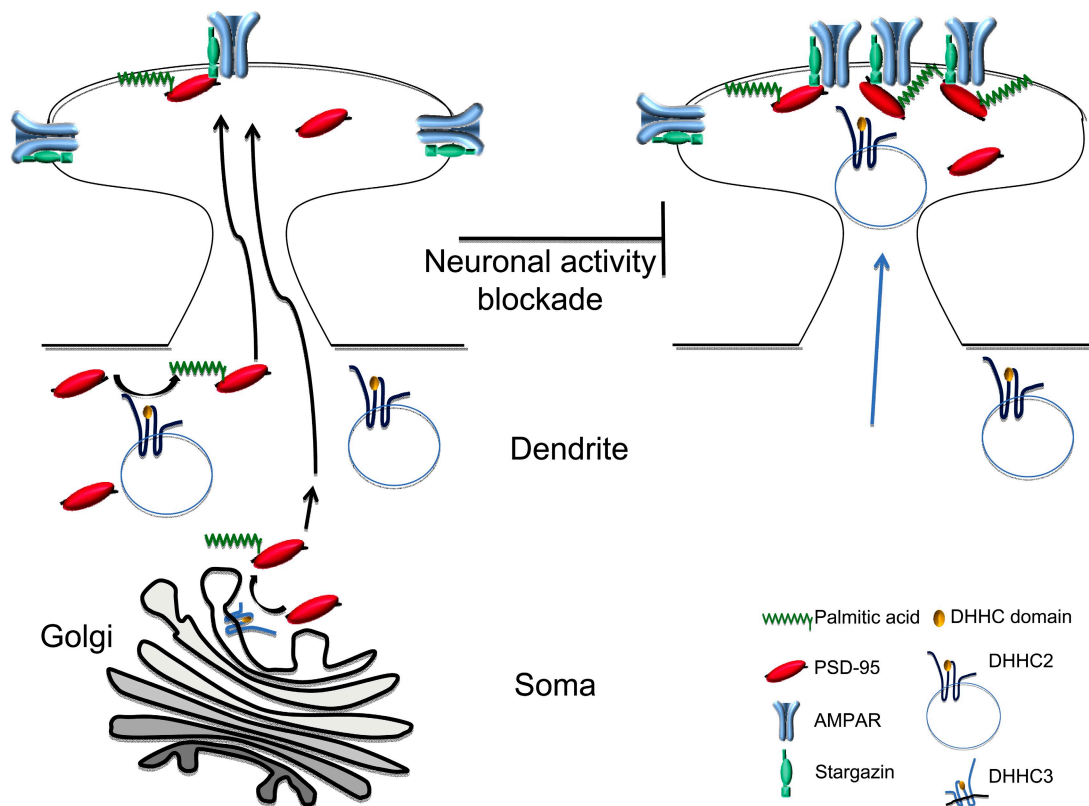


Figure 1. **Neuronal activity controls the subcellular localization of DHHC2.** DHHC2 and -3 are protein acyltransferases that palmitoylate PSD-95. This modification is important for synaptic targeting of PSD-95. DHHC3 localizes to the Golgi regardless of neuronal activity. DHHC2 is found in small vesicle-like structures in the dendrite under conditions of normal neuronal activity. Activity blockade leads to the translocation of DHHC2-containing puncta to synapses, increased levels of synaptic PSD-95, synaptic stargazin (a TARP), and surface-localized AMPA-type glutamate receptors. AMPAR, AMPA receptor.

activity-sensitive palmitoylation of PSD-95. They find that of the PATs known to act on PSD-95, only DHHC2 and -3 are highly expressed in the hippocampus. Moreover, only DHHC2 is found in the postsynaptic fraction of neuronal lysates and localized in dendrites in small vesicle-like structures, whereas DHHC3 is localized to the Golgi apparatus, suggesting that DHHC2 is positioned properly to act on PSD-95 at synapses.

Although DHHC2 appears to be important for the activity-regulated effects on palmitoylation of PSD-95, both PATs expressed in the hippocampus are important for the synaptic localization of PSD-95 (Fig. 1). Knockdown of either DHHC2 or -3 using microRNAs reduced the amount of PSD-95 at synapses, but only knockdown of DHHC2 prevented the increase of PSD-95 accumulation at synapses after activity blockade. Moreover, rescue experiments demonstrated that these effects require DHHC2 with an active PAT domain. Thus, although both DHHC proteins expressed in the hippocampus are needed for normal PSD-95 localization, they each have distinct functions.

How is DHHC2 function linked to neuronal activity? The enzyme activity of DHHC2 was unaffected by neuronal activity blockade. Using TIRFM, Noritake et al. (2009) demonstrate that DHHC2 reversibly localizes to sites near synapses when activity was blocked with glutamate receptor blockers (kynurenic acid) or sodium channel blockers (tetrodotoxin). These effects were selective to DHHC2, as the localization of DHHC3 was unaffected even with long-term activity blockade. It will be

interesting to determine how neuronal activity controls DHHC2 trafficking. This may depend on specific domains in these enzymes other than those required for palmitoylation.

What is the consequence of PSD-95 palmitoylation blocking neuronal activity? Activity blockade is known to increase synaptic strength by recruiting additional AMPA-type glutamate receptors, which are important for synaptic plasticity and homeostasis, to synapses. These receptors can be anchored at synapses by PSD-95 through interactions with regulatory proteins of the transmembrane AMPA receptor regulatory protein (TARP) family such as stargazin (Chen et al., 2000; Nicoll et al., 2006). To test whether the increased PSD-95 found at synapses after activity blockade might be associated with increases in AMPA receptors at synapses, the authors monitored the amount of the surface-localized AMPA-type receptor GluR1. Using live-cell TIRFM and microRNA knockdown, the authors establish that the increased surface GluR1 they observe after activity blockade requires PSD-95 and DHHC2 with PAT activity. Moreover these effects are likely to be selective for AMPA-type glutamate receptors, as neither specific *N*-methyl-D-aspartate-type glutamate receptors nor specific glutamate transporters (vesicular glutamate transporter) were affected. Thus, there appears to be remarkable selectivity to DHHC2 palmitoylation of PSD-95 such that only certain interacting proteins are affected. How this selectivity is generated is an important and unresolved question.

Overall, Noritake et al. (2009) show that altering the cellular localization of a DHHC domain-containing protein allows

it to act selectively. Recent work from Huang et al. (2009) has begun to define additional mechanisms for substrate specificity. By examining the activity of fusion proteins comprising different domains of DHHC proteins, Huang et al. (2009) found that regions outside of the DHHC domain of HIP14L (DHHC13) determine the specificity of DHHC13 for the huntingtin protein. Despite these two new studies, the rules and motifs that govern DHHC specificity remain to be fully characterized. The study by Noritake et al. (2009) raises a new question: how is the localization of DHHC2 regulated by neuronal activity? Given the clear substrate specificity of DHHC family members and their links to human disease (Oyama et al., 2000; Mansouri et al., 2005; Raymond et al., 2007; Mukai et al., 2008), determining the mechanisms that enable the selective and specific function of these proteins can only become an increasingly important area of research.

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