Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

CaMKK–CaMKI Signaling Pathways Differentially Control Axon and Dendrite Elongation in Cortical Neurons

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Review of Ageta-Ishihara et al.

Axonal and dendritic outgrowth is an integral component of both initial neural development and neuronal regeneration. Both axon and dendrite outgrowth are activity dependent, and recent evidence has accumulated that both are regulated by calcium (Ca^{2+}) (Zheng and Poo, 2007; Wayman et al., 2008). Furthermore, block of calmodulin-dependent kinases (CaMKs) by KN-62 or KN-63 reduced both axonal and dendritic growth, suggesting a role for CaMK in Ca^{2+}-dependent neurite outgrowth (Wayman et al., 2008). However, because KN-62 and KN-63 inhibit CaMK kinase (CaMKK), as well as CaMKI, CaMKII, and CaMKIV (Wayman et al., 2008), these studies were unable to determine which kinase was responsible for axonal or dendritic outgrowth. Although Takemoto-Kimura et al. (2007) established that dendritogenesis in cortical neurons was regulated specifically by CaMKIγ, the pathway responsible for axonal elongation remained unknown.

In a recent issue of *The Journal of Neuroscience*, Ageta-Ishihara et al. (2009) extended this earlier work to demonstrate that another member of the CaMKI family, CaMKIα, is responsible for axon elongation. Using an elegant series of genetic studies, including knockdown, knock-out, and overexpression studies, the authors established that CaMKK activation of CaMKIα leads to axonal growth, whereas CaMKK activation of CaMKIγ promotes dendritic outgrowth [Ageta-Ishihara et al. (2009), their Fig. 1, Fig. 2, Fig. 3]. This work provides clear evidence for a switch in CaMKK–CaMKI signaling to control either dendritic or axonal elongation. Figure 1 provides a simple summary of the CaMKK–CAMKI signaling pathways derived from Takemoto-Kimura et al. (2007) and Ageta-Ishihara et al. (2009).

Given that CaMKIα and CaMKIγ likely have similar catalytic domain structures (Swulius and Waxham, 2008), how do they exert different effects on axonal and dendritic growth? One possibility is that they are targeted to different cellular locations. CaMKIγ has several residues that can be palmitoylated and/or prenylated, targeting it to lipid rafts (Takemoto-Kimura et al., 2007). CaMKIα is instead found in the cytoplasm. To address whether this different localization is important for the differential effects on process growth, the authors performed a series of mutagenesis studies [Ageta-Ishihara et al. (2009), their Fig. 4] to cause CaMKIγ to be retained in the cytosol and CaMKIα to be targeted to lipid rafts. Surprisingly, the two enzymes could not substitute for each other: when CaMKIγ was expressed in the cytosol, it did not remediate the effects of CaMKIα knockdown on axonal growth, and when CaMKIα was targeted to lipid rafts, it did not remediate the effects of CaMKIγ knockdown on dendrite growth. These studies indicate that despite the structural similarity between CaMKIα and CaMKIγ, a high degree of substrate specificity may still exist. To extend this conclusion, it would be useful to determine whether CaMKIα can activate Rac, a small GTPase activated by CaMKIγ, to facilitate dendrite elongation (Takemoto-Kimura et al., 2007). Furthermore, enzymatic activity assays should be performed to confirm that the mutant forms of CaMKIα and CaMKIγ exhibited normal enzymatic activity and that the results of the mutagenesis studies were not spuriously caused by a lack of enzymatic activity.

Ageta-Ishihara et al. (2009) showed that whereas BDNF promotes dendritic growth in agreement with previous work (Takemoto-Kimura et al., 2007), muscimol, a $GABA_A$ receptor ($GABA_AR$) agonist, promoted axonal growth [Ageta-Ishihara et al. (2009), their Fig. 5]. The authors established that, as shown previously for immature neurons, $GABA_AR$ activation is depolarizing in the cortical cultures used in this study. Instead of causing hyperpolarization, $GABA_AR$ activation in immature neurons results in efflux of Cl^-, which causes cell depolar-

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ization, activation of voltage-gated Ca^{2+} channels, and Ca^{2+} influx (Fig. 1). This reversal of polarization occurs because immature neurons lack the potassium-chloride cotransporter, KCC2, which maintains the Cl^- gradient in mature neurons (Rivera et al., 1999). Ageta-Ishihara et al. demonstrate that Ca^{2+} influx is increased after application of muscimol [Ageta-Ishihara et al. (2009), their Fig. 6], and that forced expression of KCC2 in these cultures impairs axonal growth under control conditions, as well as following exposure to muscimol [Ageta-Ishihara et al. (2009), their supplemental Fig. 5]. These data support their hypothesis that $GABA_AR$ activation and subsequent Ca^{2+} influx is one of the underlying mechanisms responsible for axonal outgrowth in developing cortical neurons.

Few studies have examined the *in vivo* effects of disrupting CaMK signaling. Therefore, Ageta-Ishihara et al. (2009) knocked down CaMKIα expression *in utero* and examined corpus callosal axons on postnatal day 16 (P16) [Ageta-Ishihara et al. (2009), their Fig. 7]. In control animals, callosal axons originating in layers II/III of the somatosensory (S1/S2) cortex crossed the corpus callosum and terminated in layers II/III of the contralateral S1/S2 cortex. However, in animals that were electroporated with a short hairpin RNA targeted against CaMKIα (shKIα), the axons did not extend substantially beyond the white matter layer of the contralateral S1/S2 cortex. Thus, knockdown of CaMKIα activity seemed to affect terminal axon extension, suggesting that *in vivo* CaMKIα may only be involved in the final steps of axon arborization. An important alternative hypothesis, however, is that knockdown of CaMKIα delays axon midline crossing. The progression of axons in CaMKIα knockdown animals at P16 described by Ageta-Ishihara et al. (2009) is comparable to that of wild-type P8 animals in a study by Wang et al. (2007). This latter study, which used similar electroporation methods, demonstrated that hyperpolarization of developing somatosensory axons through overexpression of Kir2.1, an inward rectifying K^+ channel, delayed midline crossing (Wang et al., 2007). The results of Ageta-Ishihara et al. (2009) suggest that hyperpolarization would block CaMKIα signaling, thus, it is possible that the two studies produce similar effects on axon growth. It would be interesting to determine whether the CaMKIα knockdown animals exhibited a slower rate of midline crossing, as well as or rather than having deficits associated with terminal axon extension.

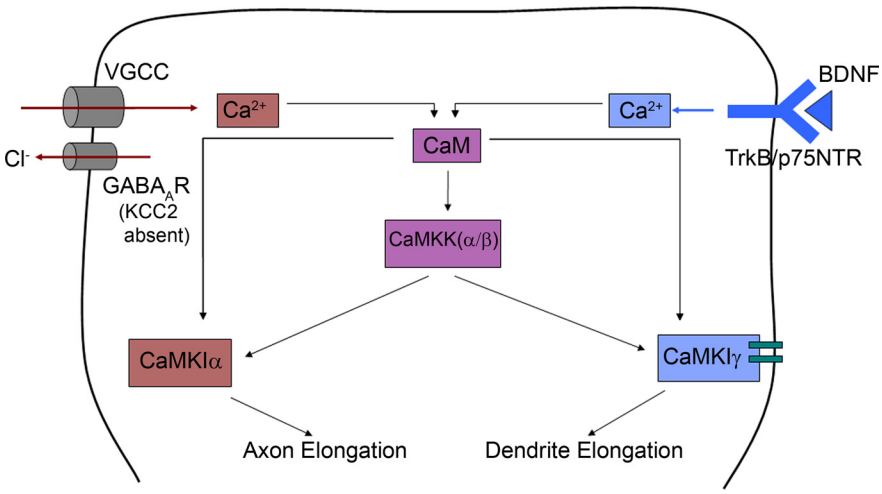

Figure 1. CaMKK–CaMKI signaling in developing cortical neurons. Immature cortical neurons lack KCC2, a key transporter that normally causes chloride influx and thus hyperpolarization upon $GABA_A$ receptor ($GABA_AR$) activation (Rivera et al., 1999). As a result, $GABA_AR$ activation in immature neurons causes depolarization and subsequent activation of voltage-gated Ca^{2+} channels (VGCC) and Ca^{2+} influx. Ca^{2+} binds calmodulin (CaM), allowing CaM to bind both CaMKK and CaMKI. Binding of CaM to CaMKI results in a conformational change in the activation loop, allowing phosphorylation by CaM-CaMKK and subsequent enzymatic activation (Wayman et al., 2008). CaMKIα activation results in axon outgrowth, while BDNF signaling in dendrites results in a rise of intracellular Ca^{2+}, which cumulates in activation of CaMKIγ and dendrite outgrowth. This scheme incorporates combined results from Takemoto-Kimura et al. (2007) and Ageta-Ishihara et al. (2009).

In contrast to Ageta-Ishihara et al. (2009), another recent publication in *The Journal of Neuroscience* reported that CaMKIγ is responsible for axonal rather than dendritic elongation in hippocampal cultures (Davare et al., 2009). In this study, transient receptor potential canonical 5 channel (TRPC5) activation stimulated axon elongation via CaMKIγ (Davare et al., 2009). Some of the findings of Davare et al. (2009) are consistent with those of Ageta-Ishihara et al. (2009); both groups observed that pharmacological inhibition of CaMKK or knockdown of CaMKI or CaMKK blocked axon elongation and that deficits in axon elongation could be rescued by expression of constitutively active CaMKI. However, the studies disagree on the specific CaMKI isoform responsible for axon elongation. Davare et al. (2009) observed that knockdown of CaMKIγ and to a lesser extent CaMKIα each significantly affected axonogenesis, but only CaMKIγ activity affected total axon length.

What underlies the differences in the findings of these studies? Axon elongation in hippocampal neurons may be regulated differently from that in cortical neurons. For example, Ko et al. (2005) demonstrated that the cholesterol content (and lipid raft composition) differed in hippocampal versus cortical neurons, and these differences could account for differences in the timeline of axonal and dendritic morphogenesis in the two neuronal cultures [Ko et al. (2005) and references therein]. CaMKIγ is targeted to lipid rafts (Takemoto-Kimura et al., 2007), so perhaps differences in lipid raft composition alters the abundance or signaling cascade of CaMKIγ. The mutagenesis studies by Ageta-Ishihara et al. (2009) suggest that CaMKIα and CAMKIγ signaling cascades are divergent at the level of substrate specificity [Ageta-Ishihara et al. (2009), their Fig. 4], so it is also possible that different intracellular targets exist in cortical versus hippocampal cultures. Another possibility for the difference in the two studies is that Ageta-Ishihara et al. (2009) used high-density cortical cultures while Davare et al. (2009) used low-density hippocampal cultures. Neuronal activity has a large influence on neuritogenesis [Wayman et al. (2008) and references therein], and one would expect a difference in the level of activity between high- and low-density cultures. Thus, the differences in the findings may also be a result of different degrees of network activity.

Overall, the study by Ageta-Ishihara et al. (2009) provides strong evidence that CaMKIα plays a critical role in cortical axon elongation, and it greatly extends our understanding of the differential control of axon and dendritic elongation by the CaMKK–CaMKI signaling pathway (Fig. 1). The major strengths of the article lie in the carefully designed genetic manipulations, as well as the first characterization of the effects of *in vivo* knockdown

of CaMKIα on axon elongation. However, apparent conflict with the results of Davare et al. (2009) calls for examination of whether CaMKK–CaMKI signaling pathways differ in cortical versus hippocampal neurons due to differences in CaMKIγ signaling or cell density-dependent network activity.

References

Ageta-Ishihara N, Takemoto-Kimura S, Nonaka M, Adachi-Morishima A, Suzuki K, Kamijo S, Fujii H, Mano T, Blaeser F, Chatila TA, Mizuno H, Hirano T, Tagawa Y, Okuno H, Bito H (2009) Control of cortical axon elongation by a GABA-driven Ca^{2+}/calmodulin-dependent protein kinase cascade. J Neurosci 29:13720–13729.

Davare MA, Fortin DA, Saneyoshi T, Nygaard S, Kaech S, Banker G, Soderling TR, Wayman GA (2009) Transient receptor potential canonical 5 channels activate Ca^{2+}/calmodulin kinase Iγ to promote axon formation in hippocampal neurons. J Neurosci 29:9794–9808.

Ko M, Zou K, Minagawa H, Yu W, Gong JS, Yanagisawa K, Michikawa M (2005) Cholesterol-mediated neurite outgrowth is differently regulated between cortical and hippocampal neurons. J Biol Chem 280:42759–42765.

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K (1999) The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251–255.

Takemoto-Kimura S, Ageta-Ishihara N, Nonaka M, Adachi-Morishima A, Mano T, Okamura M, Fujii H, Fuse T, Hoshino M, Suzuki S, Kojima M, Mishina M, Okuno H, Bito H (2007) Regulation of dendritogenesis via a lipid-raft-associated Ca^{2+}/calmodulin-dependent protein kinase CLICK-III/CaMKIγ. Neuron 54:755–770.

Wang CL, Zhang L, Zhou Y, Zhou J, Yang XJ, Duan SM, Xiong ZQ, Ding YQ (2007) Activity-dependent development of callosal projections in the somatosensory cortex. J Neurosci 27:11334–11342.

Wayman GA, Lee YS, Tokumitsu H, Silva AJ, Soderling TR (2008) Calmodulin-kinases: modulators of neuronal development and plasticity. Neuron 59:914–931.

Zheng JQ, Poo MM (2007) Calcium signaling in neuronal motility. Annu Rev Cell Dev Biol 23:375–404.