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Mission CaMKIIγ: Shuttle Calmodulin from Membrane to Nucleus

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Abstract

Neuronal plasticity depends on plasma membrane Ca^{2+} influx, resulting in activity-dependent gene transcription. Calmodulin (CaM) activated by Ca^{2+} initiates the nuclear events, but how CaM makes its way to the nucleus has remained elusive. Ma et al. now show that CaMKIIγ transports CaM from cell surface Ca^{2+} channels to the nucleus.

Learning and memory depend on long-term neuronal plasticity and the ability of neurons to weaken or strengthen synapses in response to changes in electrical activity. A long-lasting decrease in neuronal network activity leads to an overall increase in the average synaptic strength to maintain homeostasis of excitatory inputs into the neurons. This process is initiated at the cellular membrane, where Ca^{2+} influx through surface Ca_V1 (L-type) channels starts a series of events culminating in the activation of nuclear CREB and gene transcription. Though many of the players in these signaling events are known, surprisingly, the mechanism through which electrical activity at the cell surface gets transmitted to the nucleus has remained a mystery. Richard Tsien and colleagues now show that γCaMKII, a member of the CaMKII family of kinases, acts as a shuttle and, independent of its kinase activity, conveys the Ca-dependent events from the membrane to the nucleus.

The molecular details of this shuttle mission are as intriguing as the voyage of CaMKIIγ through the neuronal microcosm. In hippocampal neurons, L-type Ca^{2+} channels $Ca_v1.2$ and $Ca_v1.3$ couple neuronal excitation to Ca^{2+}-controlled gene expression via the transcription factor NFAT (Murphy et al., 2014; see also Nystoriak et al., 2014) through the direct association of key signaling elements with $Ca_v1.2$ and $Ca_v1.3$. For instance, the anchor protein AKAP5 links PKA (Hall et al., 2007; Oliveria et al., 2007) and the Ca^{2+}/CaM-activated phosphatase calcineurin to $Ca_v1.2$ for localized activation of NFAT (Figure 1) (Murphy et al., 2014). Moreover, CaMKII binds to a specific motif in $Ca_v1.2$ (called the IQ motif) and likely to the identical motif in $Ca_v1.3$, to Ca^{2+} channel β subunits, and to $Ca_v1.3$-associated densin-180 (Hell, 2014). Work in superior cervical ganglion neurons shows that CaMKIIβ is recruited to $Ca_v1.3$ upon Ca^{2+} influx through this channel and, less effectively, non-L-type channels (Wheeler et al., 2012). This recruitment turns out to be critical for activation of nuclear CaMKK and CaMKIV by Ca^{2+}/CaM and for the ensuing phosphorylation of CREB. Ma et al. now reveal the missing link between these two events: CaMKIIγ, activated by CaMKIIβ upon Ca influx, carries Ca^{2+}/CaM to the nucleus for the

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initiation of the CaMKK-CaMKIV-CREB cascade. Indeed, Ca^{2+} influx through $Ca_v1.3$ plasma membrane channels triggers CaMKIIγ accumulation at $Ca_v1.3$ clusters, where CaMKIIγ becomes loaded with the Ca^{2+}/CaM cargo. Calcineurin-mediated dephosphorylation then launches CaMKIIγ from $Ca_v1.3$ to the nucleus.

To determine whether depolarization-induced Ca^{2+} influx via $Ca_v1.3$ results in nuclear accumulation of CaMKIIγ, the authors analyzed the effects of a mutation mimicking constitutive phosphorylation of the residue S334. Not only did this mutation block nuclear import as expected, it also unmasked activity-driven accumulation of CaMKIIγ at $Ca_v1.3$ clusters, which apparently is only fleeting for wild-type CaMKIIγ. Inhibition of calcineurin (but not PP1/PP2A) had the same effects, indicating that calcineurin dephosphorylates S334 for both release of CaMKIIγ from $Ca_v1.3$ and nuclear translocation. Furthermore, mutating A303 to arginine and application of KN93 (both of which block Ca^{2+}/CaM binding to CaMKII) inhibited CaMKIIγ clustering at $Ca_v1.3$. Accordingly, CaMKIIγ accumulation at $Ca_v1.3$ requires Ca^{2+}/CaM binding to its A303/T306/T307 segment (Ma et al., 2014), which activates CaMKII through displacement of the partially overlapping pseudo-substrate segment from the catalytic center (Hell, 2014). Such a requirement also explains why CaMKIIγT287E and CaMKIIγT287E/S334E did not accumulate at $Ca_v1.3$ clusters; analogous to the autophosphorylation of CaMKIIα(T286D) on T305/T306 that prevents Ca^{2+}/CaM binding (Hell, 2014; Pi et al., 2010), the T287E mutation in CaMKIIγ likely abrogates Ca^{2+}/CaM binding by corresponding autophosphorylation at T306/T307.

Going back to the membrane effects, the authors found that, upon excitation, CaMKIIβ accumulates in parallel with CaMKIIγ at $Ca_v1.3$ clusters (Wheeler et al., 2012) for trans-phosphorylation of CaMKIIγ at T287. This phosphorylation causes CaM trapping by dramatically increasing CaMKIIγ affinity for Ca^{2+}/CaM (Hell, 2014). Following knockdown of CaMKIIβ, CaMKIIγ shuttles to the nucleus but without Ca^{2+}/CaM on board, failing to activate CREB. These observations suggest that CaMKIIβ phosphorylates CaMKIIγ on T287 upon their encounter at $Ca_v1.3$, but CaMKIIβ knockdown could also act by, e.g., prohibiting CaMKIIβ from facilitating Ca^{2+} influx via $Ca_v1.3$, as previously seen during repetitive, frequent depolarization (Jenkins et al., 2010). This effect could limit Ca^{2+} influx and thereby CaMKIIγ autophosphorylation on T287 and consequently loading of its cargo. Nevertheless, replacing endogenous CaMKIIγ with a catalytically inactive CaM KIIγK43R mutant rescued nuclear translocation of both CaMKIIγ and Ca^{2+}/CaM. These findings suggest that CaMKIIβ has at least the potential to phosphorylate CaMKIIγ, although CaMKIIγ autophosphorylation could become necessary as the one remaining mechanism of T287 phosphorylation when CaMKIIβ is absent.

Accumulation of CaMKIIγS334E at $Ca_v1.3$ clusters indicates that CaMKIIγ obtains temporarily heightened affinity for $Ca_v1.3$, which likely requires some sort of release mechanism for sending CaMKIIγ on its journey to the nucleus. In fact, inhibition of calcineurin prevents the CaMKIIγ release. Analogous to $Ca_v1.2$, calcineurin could be associated with $Ca_v1.3$ via AKAP5 for localized, selective, and effective signal transduction (Figure 1). Perhaps CaMKIIγS334 dephosphorylation by $Ca_v1.3$-associated calcineurin fulfills dual function by facilitating release from $Ca_v1.3$ as well as enabling nuclear import. However, accumulation of CaMKIIγS334E at $Ca_v1.3$ clusters could also simply be because

abrogation of nuclear import increases Ca^{2+}/CaM-loaded CaMKIIγ S334E in the cytosol, thereby shifting the equilibrium toward $Ca_v1.3$ binding. Dephosphorylation of T287 would not be useful for release of CaMKIIγ from $Ca_v1.3$ because it would result in loss of the Ca^{2+}/CaM cargo. In fact, CaMKIIγT287A accumulates in the nucleus but without Ca^{2+}/CaM. Although this finding implies that CaMKIIγT287A can transiently associate with $Ca_v1.3$, as required for S334 dephosphorylation, it also suggests that T287 phosphorylation is not required for $Ca_v1.3$ association.

How is Ca^{2+}/CaM released from CaMKIIγ once it arrives in the nucleus? Inhibition of the serine/threonine phosphatase PP2A, which can dephosphorylate CaMKII, increased T287 phosphorylation and decreased CREB phosphorylation without affecting total CaMKIIγ or Ca^{2+}/CaM inside the nucleus. It thus appears that activation of the CaMKK-CaMKIV-CREB cascade requires nuclear PP2A dephosphorylation of T287 to trigger Ca^{2+}/CaM release from CaMKIIγ (Figure 1).

Important questions now arise from the current work. For instance, it still remains to be understood how Ca^{2+}/CaM can remain associated with CaMKIIγ after release from $Ca_v1.3$ when CaM usually dissociates rather quickly from CaMKIIs upon falling Ca^{2+} levels. Perhaps prolonged stimulation by elevated K^+ or high-frequency field stimulations as used by Ma et al. maintains high enough $[Ca^{2+}]_i$ to sustain Ca^2/CaM-CaMKIIγ association (especially as CaM binding to targets typically increases affinity of CaM for Ca^{2+}), but this issue warrants further detailed investigation. Moreover, an intriguing point to consider is how does the large CaMKIIγ-Ca^2/CaM complex enter the nucleus? Is this translocation facilitated through interactions with the nuclear pore complex? Along these lines, how is dephosphorylation of T287 by PP2A regulated so that it occurs in the nucleus, but not cytosol? And finally, are different transcriptional pathways selectively activated and how? This is an especially fascinating question, as Ca_v1-associated calcineurin plays a central role in activation of both NFAT and CREB upon Ca^{2+} influx.

In summary, this study identifies a long sought-after mechanism of communication between the membrane and the nucleus upon excitation, which mediates the ability of neurons to sustain long-term plasticity. Future studies will undoubtedly expand on these findings to reveal additional mechanisms that mediate selective activation of transcriptional pathways in health and disease.

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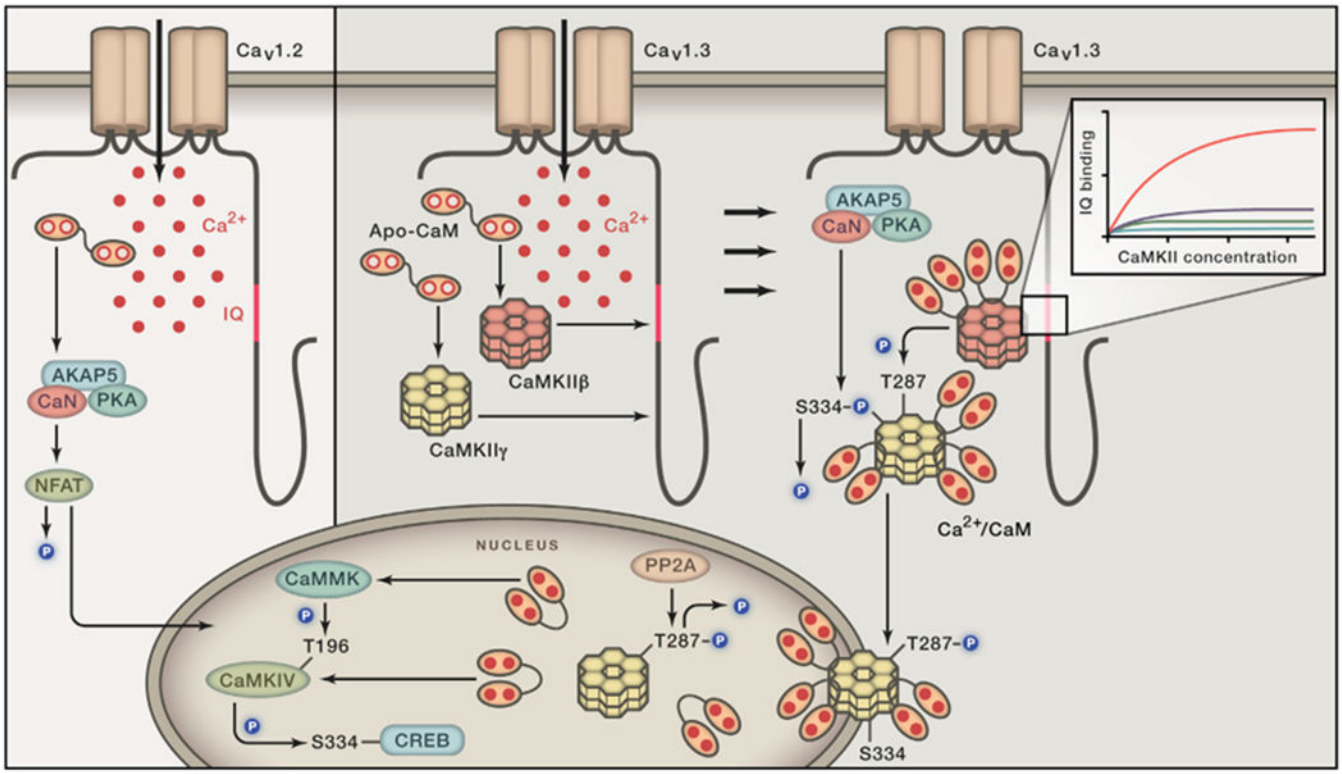

Figure 1. Excitation-Transcription Coupling by Ca^{2+}/CaM-CaMKIIγ Shuttling

In hippocampal neurons (left), the depolarization-triggered Ca^{2+} influx via prevalent $Ca_V1.2$ leads to dephosphorylation and thereby activation of NFAT by AKAP5-anchored calcineurin, which is activated by Ca^{2+}/CaM (Murphy et al., 2014). In SCG neurons, which are thought to lack $Ca_V1.2$, Ca^{2+} influx via $Ca_V1.3$ first results in clustering of Ca^{2+}/CaM-CaMKIIγ and CaMKIIβ at or near clusters of $Ca_V1.3$ and calcineurin, which is probably linked to $Ca_V1.3$ by AKAP5 analogous to $Ca_V1.2$. The IQ motif (red segment) of $Ca_V1.2$ and $Ca_V1.3$ constitute docking sites for CaMKIIα and β, but not CaMKIIγ (Hudmon et al., 2005). The insert shows specific binding of increasing amounts of CaMKIIα to the IQ peptide TVGKFYATFLIQEYFR (red), but not to peptides upstream and downstream of IQ, in $Ca_V1.2$/$Ca_V1.3$ by fluorescence anisotropy assays (Z.A.M. and J.W.H., unpublished data), as seen earlier (Hudmon et al., 2005) (Kd here is 31 ± 7 nM). CaMKIIγ could bind to the Ca^{2+}channel β subunit or $Ca_V1.3$-associated densin-180 (not depicted) (Hell, 2014). Effective delivery of Ca^{2+}/CaM to the nucleus occurs upon phosphorylation of CaMKIIγT287 by CaMKIIβ for trapping of Ca^{2+}/CaM and dephosphorylation of CaMKIIγS334 by calcineurin for nuclear import. Dephosphorylation of pT287 in CaMKIIγ by PP2A in the nucleus releases Ca^{2+}/CaM for stimulation of CaMKK and CaMKIV. CaMKIV requires both Ca^{2+}/CaM binding and phosphorylation of T196 by CaMKII before it can phosphorylate CREB on S133.