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AKAP5 Keeps L-type Channels and NFAT on Their Toes

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Abstract

In this issue of *Cell Reports*, Murphy et al. and Dittmer et al. present exciting new insight into the regulation of Ca^{2+} influx via the L-type Ca^{2+} channel $Ca_V1.2$ and how increased Ca^{2+} influx translates into localized activation of the nuclear transcription factor NFAT upon depolarization in neurons.

Ca^{2+} influx via $Ca_V1.2$ is especially potent in stimulating the dephosphorylation of NFAT by the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin/PP2B (CaN) for nuclear translocation and gene expression (Nystoriak et al., 2014; Oliveria et al., 2007). The A kinase anchor protein AKAP5 links PKA and CaN to $Ca_V1.2$ for localized activation and release from this complex (Figure 1). Both of these processes are important for NFAT activation (Li et al., 2012; Oliveria et al., 2007). A new mouse model with a deletion of the PKA binding segment of AKAP5 (rodent AKAP150 and human AKAP79) in the *AKAP5* gene (AKAP5ΔPKA, residues 709–718) now surprises us by revealing that removal of PKA from the $Ca_V1.2$/AKAP5 complex impairs NFAT activation (Murphy et al., 2014, this issue of *Cell Reports*). This study shows that phosphorylation of S1700 and S1928, the two main PKA sites in $Ca_V1.2$, is decreased in AKAP5ΔPKA mice, thus reducing channel activity (Fu et al., 2013; Oliveria et al., 2007). Remarkably, removing CaN from the $Ca_V1.2$/AKAP5 complex by mutating the CaN binding site in AKAP5 increases S1928, but not S1700, phosphorylation. Accordingly, AKAP5-anchored CaN effectively dephosphorylates S1928 but not S1700. It is tempting to speculate that their differential phosphorylations have divergent regulatory roles in $Ca_V1.2$ function.

The PKA/CaN/$Ca_V1.2$ docking requirement implies localized CaN/NFAT activation. In fact, NFATc3-GFP is concentrated in dendritic spines (similar to $Ca_V1.2$) and released upon Ca^{2+} influx (Murphy et al., 2014). Disruption of PKA or CaN binding to AKAP5 prevents NFAT nuclear accumulation. Now, intriguing questions are whether NFAT also forms part of the AKAP5/$Ca_V1.2$ signaling complex and whether NFAT moves from the spine to the nucleus in order to mediate gene regulation.

Fitting the reduced S1700 and S1928 phosphorylation, depolarization-induced L-type Ca^{2+} transients are nearly abolished in AKAP5ΔPKA neurons. Accordingly, AKAP5-anchored PKA maintains a certain level of L-type currents under basal conditions. Because PKA inhibition does not abrogate L-type currents (e.g., Dittmer et al., 2014), this loss is

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unexpected. However, Ca^{2+} transients are a reflection of L-type currents and subsequent Ca^{2+} accumulation, which is a function of influx, clearance, and potentially Ca^{2+} release from intracellular stores. Any of these events could be affected by impairing PKA activity, although the apparent elimination of L-type-mediated Ca^{2+} transients in AKAP5ΔPKA mice suggests that PKA exerts its effect on or near $Ca_v1.2$.

This is where the work by Dittmer et al. (2014), also published in this issue of *Cell Reports*, on the role of AKAP5-anchored PKA in regulating $Ca_v1.2$ function comes into play. The authors show that AKAP5ΔPKA reduces, but does not eliminate, L-type currents. Accordingly, loss of Ca^{2+} transients described above could result from reduced L-type currents, which would substantially decrease the overall Ca^{2+} accumulation or other effects affecting overall Ca^{2+} levels over the time course of the transients.

Dittmer et al. (2014) also make remarkable observations concerning the role of AKAP5-anchored PKA in Ca^{2+}-dependent inactivation (CDI) of $Ca_v1.2$. These channels undergo fast inactivation during prolonged depolarizations by two different mechanisms. Whereas voltage-dependent inactivation is independent of Ca^{2+} influx, CDI is triggered by it and requires calmodulin preassociation with the IQ motif in the C terminus of $Ca_v1.2$ (Ben Johny et al., 2013). Dittmer et al. (2014) show that inhibition of PKA activity or binding to AKAP5 impairs CDI. Accordingly, PKA primes $Ca_v1.2$ for CDI likely by increasing channel open probability (P_o). Upon Ca^{2+} influx Ca^{2+}/calmodulin might cause CDI by changing its precise interaction with $Ca_v1.2$ and thereby the conformation of the IQ-harboring region (Ben Johny et al., 2013) in order to promote channel closing. Alternatively, Ca^{2+}/calmodulin might induce dephosphorylation of $Ca_v1.2$ by AKAP5-anchored CaN within the time scale of CDI (hundreds of milliseconds) in order to reduce channel P_o (Oliveria et al., 2012). The authors favor the latter possibility, suggesting that PKA phosphorylation increases $Ca_v1.2$ activity by promoting an open conformation and that CaN acutely opposes this increase during CDI by dephosphorylating pS1928 and potentially other sites.

Nonetheless, it is conceivable that enhanced $Ca_v1.2$ activity upon phosphorylation by AKAP5-anchored PKA could augment CDI via enhanced direct calmodulin action independent of acute CaN activity. As the authors point out, the fact that the voltage dependences of peak current and of CDI parallel each other argues against this hypothesis. For instance, overall Ca^{2+} currents and CDI at −10 mV for wild-type (WT) AKAP5 are both comparable to Ca^{2+} currents and CDI at +10 mV for AKAP5ΔPKA. However, $Ca_v1.2$ can exist in three modes, with modes 0, 1, and 2 showing no, brief, and long openings, respectively (Hess et al., 1984). Thus, the overall Ca^{2+} currents at −10 mV in WT AKAP5 could be mediated by long openings of a smaller number of channels, whereas the Ca^{2+} currents at +10 mV in AKAP5ΔPKA could be mediated by a larger number of channels in mode 1. Ca^{2+} at least partially acts locally to mediate CDI, as indicated by its relative insensitivity to intracellular BAPTA (data not shown; Oliveria et al., 2012), a fast Ca^{2+} chelator. Accordingly, WT AKAP5-associated channels could experience stronger CDI just because the Ca^{2+} influx through them is larger than through channels associated with AKAP5ΔPKA, which is an interesting question for the future.

However, CDI is more complicated. Dittmer et al. (2014) find that stimulating PKA as well as inhibiting CaN activity or CaN binding to AKAP5 decreases CDI. These findings argue against the notion that PKA augments CDI solely by enhancing Ca^{2+} influx, leading to a structural change of the calmodulin/IQ region of $Ca_V1.2$ as both manipulations increase the Ca^{2+} influx (Dittmer et al., 2014; Oliveria et al., 2007, 2012). Rather, PKA may have some sort of primacy for preventing CDI possibly by averting shifting channel from modes 2 to 1 or 0. Here, CaN is required in order to reverse the relevant PKA-mediated channel phosphorylation. Manipulations that enable PKA activity to outpace CaN activity could promote rearrangement of calmodulin that facilitates $Ca_V1.2$ channel activity by altering CDI and its gating modality (Navedo et al., 2010). Dittmer et al. (2014) imply AKAP5-anchored CaN as an immediate effector dephosphorylating relevant sites during CDI rather than a priming factor in this process. Defining the precise mechanisms remains a daunting task, but the current work should encourage future studies examining the molecular mechanisms integrating AKAP5-anchored PKA/CaN and IQ-associated calmodulin in mediating CDI of $Ca_V1.2$.

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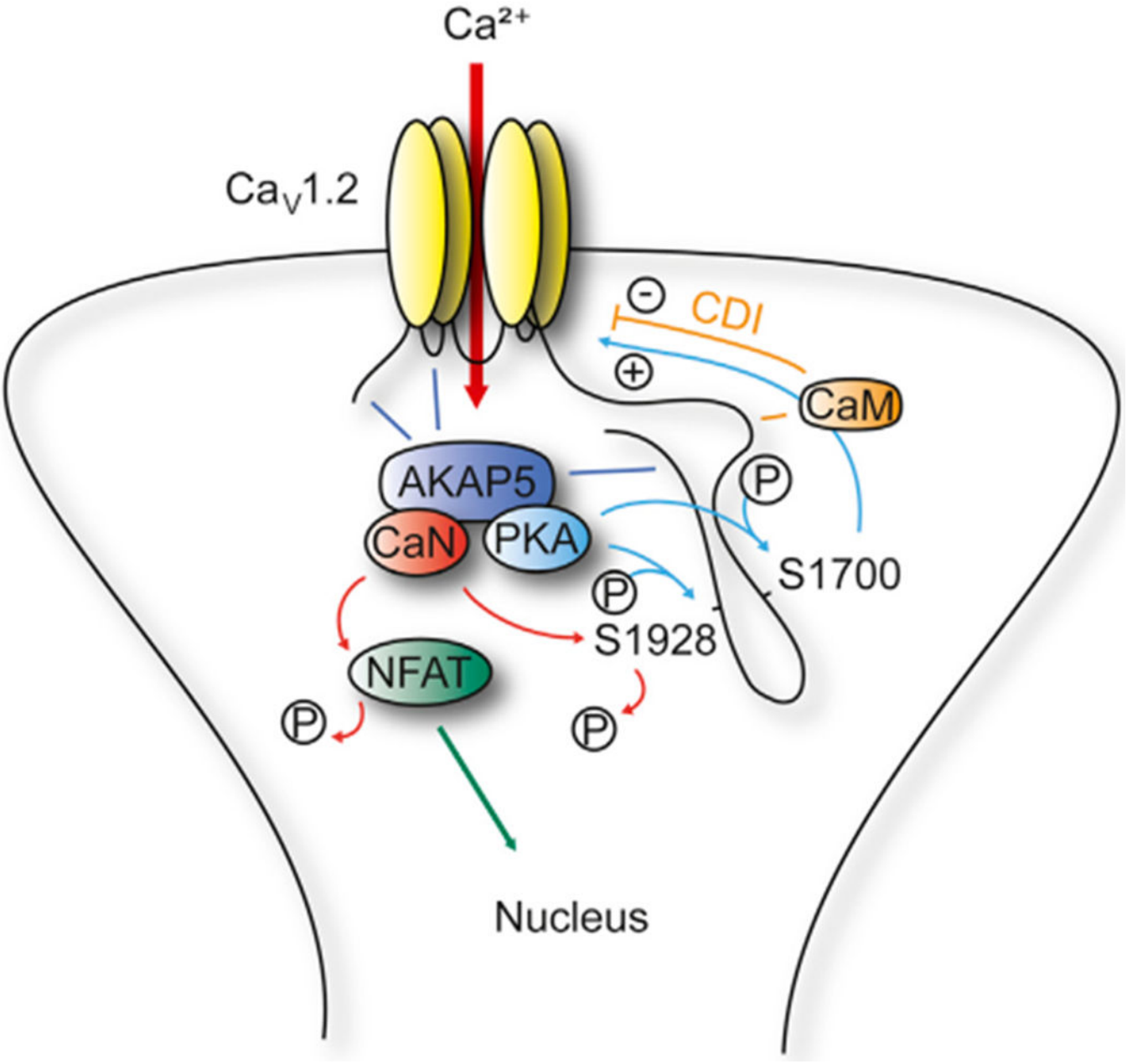

Figure 1. The $Ca_V1.2$/PKA/CaN/NFAT Signaling Complex

AKAP5 interacts with the N terminus, loop between domains I and II, and distal C terminus (dark blue bars) of $Ca_V1.2$ (Hall et al., 2007; Oliveria et al., 2007). AKAP5-anchored PKA phosphorylates $Ca_V1.2$ on S1700 and S1928 in orderto augment channel activity. AKAP5-anchored CaN readily dephosphorylates pS1928 (but not pS1700) and NFAT for nuclear translocation. Calmodulin (CaM) preassociates with the IQ motif of $Ca_V1.2$ for CDI either via a conformational change or by activating CaN for acute dephosphorylation of $Ca_V1.2$. In either case, PKA and CaN affect CDI in an opposing and permissive manner, respectively.