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Two Distinct Secretory Pathways for Differential Kv2.1 Localization in Neurons

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Review of Jensen et al.

The subcellular localization of ion channels in neurons is tightly controlled to restrict membrane currents within specific domains, which is crucial for proper neuronal function. Regulation of ion channel localization is important during development, learning, and response to injuries (Lai and Jan, 2006), and channel mislocalization has been linked to several neurological disorders, including epilepsy and neuropathic pain (Spillane et al., 2016). Investigating how ion channels are targeted to specific subcellular regions might therefore provide insights into potential therapies for these conditions.

The distinct localization of ion channels in neurons depends on specialized mechanisms to traffic and stabilize these molecules. These mechanisms include selective cargo transport, exclusion by a cytoskeleton-based physical barrier, retention by binding to scaffolding proteins, and removal from the plasma membrane by endocytosis (Leterrier et al., 2010; Vacher and Trimmer, 2012). All of these mechanisms rely on the existence of particular motifs on ion channels. For example, a conserved intracellular loop on voltage-gated sodium channels is required for axonal targeting (Garrido et al., 2003; Lemaillet et al., 2003), whereas a C-terminal dileucine motif in Kv4.2 potassium channels is necessary for dendritic localization (Rivera et al., 2003).

Most ion channels are localized to a specific compartment to facilitate synaptic integration, action potential initiation and propagation, or vesicle release. One exception to this rule is the delayed-rectifier potassium channel Kv2.1: it is located both in the somatodendritic domain, where it helps to process synaptic input (Misonou et al., 2004), and at the axon initial segment (AIS), where it is thought to modulate axonal excitability (Sarmiere et al., 2008). In both domains, Kv2.1 may be a key player in regulating neuronal firing (Misonou et al., 2004; Aras et al., 2009; Thiffault et al., 2015). However, considering its unique dual localization, it is unknown whether Kv2.1 is trafficked to these two domains by the same mechanism or distinct mechanisms.

A recent study in *The Journal of Neuroscience* (Jensen et al., 2017) elucidated how the trafficking and clustering of Kv2.1 differ between somatodendritic and AIS domains. Previous studies showed that interacting proteins, phosphorylation, and a proximal restriction and clustering (PRC) motif of Kv2.1 are critical for its somatodendritic localization and clustering (Lim et al., 2000; Misonou et al., 2004; Jensen et al., 2014). Jensen et al. (2017) confirmed that the PRC motif is necessary for somatodendritic delivery of Kv2.1, but demonstrated that it is dispensable for localizing Kv2.1 to the AIS. In contrast, loss of the C-terminal tail (amino acids 536–853) was sufficient to disrupt AIS clustering of Kv2.1. The fact that different protein motifs are required for dendritic and AIS localization of Kv2.1 suggests that the channel is targeted to the two subcellular domains via different mechanisms. Jensen et al. (2017) therefore sought to determine the mechanisms responsible for targeting Kv2.1 to the AIS.

Theoretically, Kv2.1 channels could arrive at the AIS either by diffusing laterally from the soma or by being transported intracellularly. To test the former possibility, Jensen et al. (2017) conducted fluorescence recovery after photobleaching experiments. These indicated that Kv2.1 is not trafficked to the AIS via lateral diffusion. Because the authors previously showed that Kv2.1 is trafficked to the somatodendritic domain through a conventional secretory pathway (Jensen et al., 2014), they asked whether disrupting this pathway prevented targeting of Kv2.1 to the AIS. Specifically, they disrupted the ability of the Golgi apparatus to process and release membrane proteins. Although this disrupted trafficking of Kv2.1 to the somatodendritic domain, Kv2.1 channels still appeared at the AIS. This suggests that Kv2.1 destined for the AIS bypasses the

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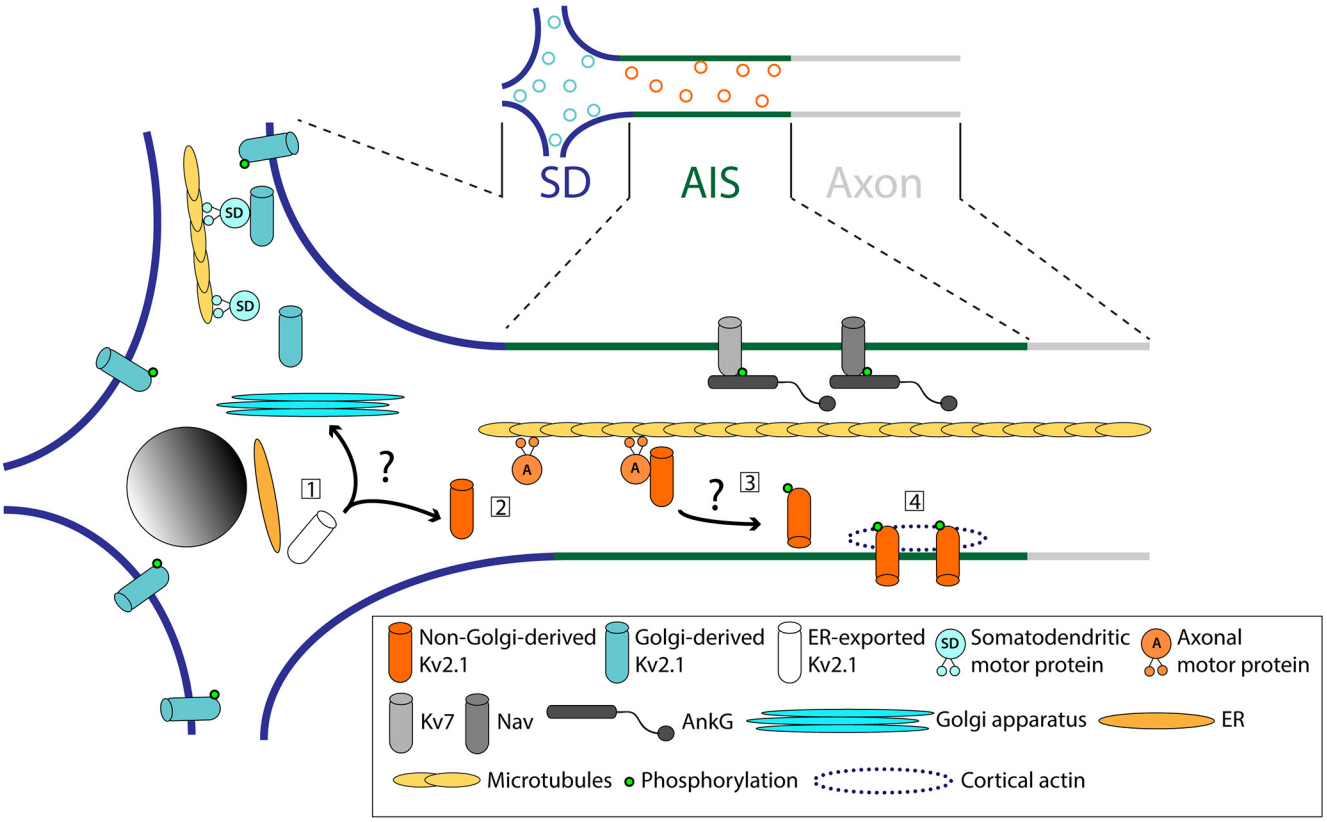

Figure 1. Proposed trafficking and clustering mechanism of Kv2.1 and other ion channels at the AIS. ***1***, After being synthesized in ER, Kv2.1 is either exported to Golgi-mediated or non–Golgi-mediated pathways by an unknown process. ***2***, Golgi-derived Kv2.1 acquires post-translational modifications that are recognized by motor proteins targeted to the somatodendritic (SD) domain, whereas non–Golgi-derived Kv2.1 interacts with axonally targeted motor proteins. ***3***, Axonally targeted Kv2.1 is phosphorylated by an unknown kinase at specific residues in its AIS motif, which induces clustering. ***4***, Kv2.1 clusters at AIS subdomains bounded by cortical actin and deficient in AnkG. This mechanism of Kv2.1 clustering at the AIS is distinct from the mechanisms for Nav and Kv7 channels. Nav channels are thought to be directly trafficked to the AIS and/or selectively endocytosed from non-AIS domains. Furthermore, the effect of phosphorylation of Nav and Kv7 is to promote binding to the scaffolding protein AnkG.

Golgi and does not use the conventional secretory pathway.

How does Kv2.1 cluster at the AIS via a Golgi-independent route? Jensen et al. (2017) determined that the AIS localization sequence is contained within the Kv2.1 C-terminal tail; grafting the Kv2.1 C-terminal tail onto the Kv4.2 potassium channel (not normally present at the AIS) was sufficient to force the resulting chimera to the AIS. To distinguish the regions necessary for AIS clustering, the authors generated a series of internal deletion mutants at the C terminus of Kv2.1 and transfected them into cultured neurons. They found that the PRC domain (aa 583–599) cooperates with a downstream region (aa 720–745), which they termed "the AIS motif," to cluster Kv2.1 at the AIS. Double-deletion mutants lacking these regions failed to cluster at the AIS and showed uniform surface distribution in both somatodendritic and axonal compartments. Furthermore, this mutant did not traffic to the surface in the presence of the Golgi inhibitor brefeldin A, suggesting that these two regions are required for Kv2.1 to use the nonconventional secretory route.

Finally, the authors identified two highly conserved potential phosphorylation sites (T728 and S732) within the AIS motif of the Kv2.1 C-terminal tail. Because phosphorylation is critical for subcellular localization of Kv2.1 (Misonou et al., 2004; Shah et al., 2014), Jensen et al. (2017) hypothesized that these phosphorylation sites could regulate AIS clustering. To study the role of phosphorylation independent of the cooperative PRC motif, they used Kv2.1-Δ583–599, which still clusters at the AIS; they then mutated the two sites (T728A/S732A) to block phosphorylation and verified its localization. While Kv2.1-Δ583–599 clustered at the AIS, nonphosphorylatable Kv2.1-Δ583–599 T728A/S732A displayed axonal localization but did not form clusters. These data indicated that phosphorylation at these two conserved residues is necessary for the formation of Kv2.1 clusters at the AIS.

Jensen et al. (2017) make an important contribution to the literature regarding ion channel trafficking and localization by demonstrating that Kv2.1 can take two distinct routes to the plasma membrane. However, the study raises two important questions. First, what mechanism dictates whether newly synthesized Kv2.1 enters the conventional or nonconventional secretory pathway after leaving the endoplasmic reticulum (ER)? Second, how are Golgi-derived Kv2.1 and non–Golgi-derived Kv2.1 transported to the somatodendritic domain and AIS, respectively? We speculate that post-translational modifications of Kv2.1 that occur in the Golgi and interactions with trafficking machinery are crucial (Fig. 1). Previous studies showed that these factors regulate export of other potassium channels from the ER, thus influencing channel surface expression and neuronal excitability (Vacher and Trimmer, 2012). The presence of post-translational modifications might allow Kv2.1 to selectively interact with one set of motor proteins to be trafficked to the somatodendritic domain, whereas the absence might allow Kv2.1 to interact with a different set of proteins that target it to the

AIS (Fig. 1, step 2). Because the interactome of Kv2.1 is not well known, it would be informative to screen for Kv2.1 binding proteins to find essential regulators involved in sorting and trafficking to the different domains. The amino acid residues that Jensen et al. (2017) identified as important for compartment-specific targeting might be involved and could be the starting point for a screen. This approach could help to determine how neurons coordinate synaptic inputs and action potential initiation by distributing the surface pool of Kv2.1 between somatodendritic and axonal domains.

After Kv2.1 reaches the proximal axon, how does it cluster at the AIS? Phosphorylation of ion channel subunits has been shown to regulate AIS clustering of other channel complexes (Vacher and Trimmer, 2012). For example, CK2-mediated phosphorylation of a conserved motif in the intracellular II-III loop of Nav channels or of a conserved C-terminal motif in Kv7 channels promotes their binding to the AIS scaffolding protein Ankyrin G (AnkG) (Bréchet et al., 2008; Xu and Cooper, 2015). Moreover, phosphorylation state is an important activity-dependent mechanism for controlling the level of surface Kv2.1 (Park et al., 2006; Shah et al., 2014). However, the role of phosphorylation in clustering Kv2.1 at the AIS is poorly understood (Fig. 1, step 3). Cerda and Trimmer (2011) reported that phosphorylation by CDK5 is required for maintenance of steady-state somatodendritic and AIS clusters of Kv2.1; these effects appear specific to phosphorylation at S603, upstream from the AIS motif described by Jensen et al. (2017). It is also possible that, unlike for Nav and Kv7 channels, phosphorylation of Kv2.1 at the AIS motif does not facilitate direct binding to scaffolding proteins. While Jensen et al. (2017) demonstrated that this phosphorylation was required for cluster formation, prior studies show that individual Kv2.1 channels within a cluster are surprisingly mobile, suggesting that Kv2.1 may not interact with scaffolding proteins (O'Connell et al., 2006; Sarmiere et al., 2008). Additionally, Kv2.1 clusters occur in AIS subdomains deficient in AnkG and are often adjacent to GABAergic synapses and cisternal organelles (King et al., 2014). Therefore, the mechanism of clustering of Kv2.1 at the AIS may be significantly different from that for Nav and Kv7 channels (Fig. 1, step 4). Further work is necessary to sort out the precise mechanism of clustering and function of Kv2.1 clusters at the AIS.

In conclusion, Jensen et al. (2017) found that two distinct secretory pathways determine the unique subcellular localization of Kv2.1 in neurons. Additionally, the authors identified critical regions and post-translational modifications that regulate AIS clustering of Kv2.1. This study provides insight into the ability of neurons to maintain specific ion channel repertoires in distinct compartments, which is critical for regulating neuronal excitability.

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