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Cytoskeletal scaffolding of Na_Vs and K_Vs in neocortical pyramidal neurons: Implications for neuronal signaling and plasticity

Carina C. Elvira[1] and Paul M. Jenkins[2,3]

The initiation and propagation of action potentials (APs) depend on the precise localization of voltage-gated sodium (Na_V) and potassium (K_V) channels in neurons. In neocortical pyramidal neurons, $Na_V1.2$ and $Na_V1.6$ are key at the axon initial segment (AIS) and nodes of Ranvier (noR), driving AP initiation and propagation. $Na_V1.2$ also supports AP backpropagation in the soma and dendrites. Ankyrin-G anchors these channels at the AIS and noR, while new findings reveal that ankyrin-B scaffolds $Na_V1.2$ in dendrites. This review highlights how ankyrins stabilize Na_V and K_V channels across neuronal domains, ensuring proper function crucial for excitability, synaptic plasticity, and signaling. Recent findings explore how ankyrins differentially localize $Na_V1.2$ and $Na_V1.6$, with implications for understanding neurological disorders linked to disrupted channel localization.

Addresses
[1] Cellular and Molecular Biology Program, University of Michigan Medical School, Ann Arbor, MI, USA
[2] Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI, USA
[3] Department of Psychiatry, University of Michigan Medical School, Ann Arbor, MI, USA

Corresponding author: Jenkins, Paul M. (pjenkins@umich.edu)

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Introduction

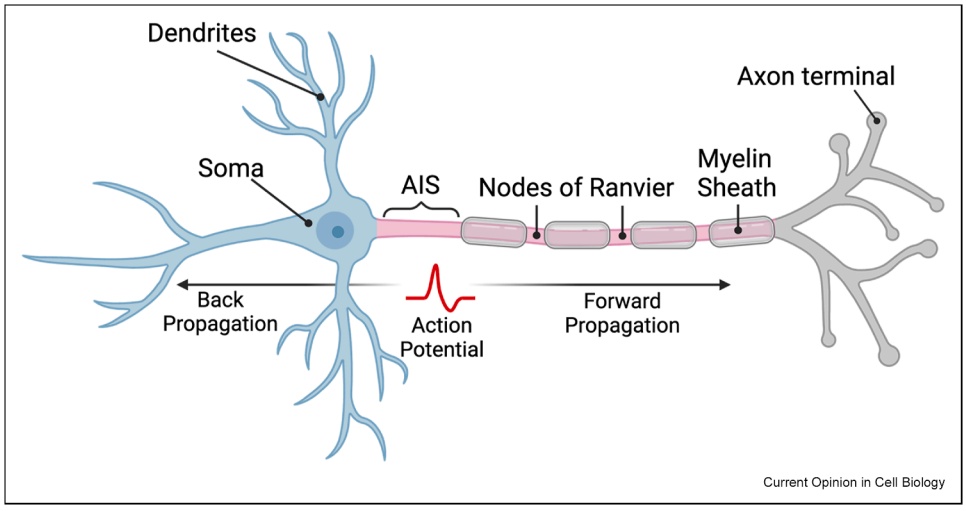
Action potentials (APs) are the fundamental electrical signals used by neurons to communicate across the nervous system. They are initiated at the axon initial segment (AIS), where depolarizing stimuli lead to rapid voltage changes that propagate along the axon, often to distant regions of the brain and beyond (Figure 1) [1]. These electrical signals are crucial for synaptic transmission, sensory processing, and motor control, and they depend on complex interactions between ion channels, scaffolding proteins, and the neuronal cytoskeleton [1]. APs are then propagated down the axon, aided by nodes of Ranvier (noR) in myelinated neurons that allow for rapid saltatory conduction (Figure 1). While much of the focus has been on the forward propagation of APs, a growing body of research highlights the significance of AP backpropagation through the soma and into the dendrites, especially in the context of synaptic plasticity and the integration of incoming synaptic inputs [2].

The initiation and propagation of APs rely heavily on two key families of ion channels: voltage-gated sodium channels (Na_Vs) and voltage-gated potassium channels (K_Vs). Na_Vs, found at high concentrations in the AIS, are responsible for the rapid depolarization that underpins the initiation of an AP, allowing for efficient signal conduction along the axon [1]. K_Vs, on the other hand, modulate repolarization and control the refractory period following an AP, thereby influencing the frequency and timing of neuronal firing [1]. The proper function of these ion channels is not solely determined by their intrinsic properties, but also by their spatial distribution within the neuron.

Ankyrin proteins play a pivotal role in the organization of Na_Vs and K_Vs. Ankyrins act as scaffolding molecules, linking ion channels, cell adhesion molecules, and more to the underlying spectrin-actin cytoskeleton (Figure 2). Three ankyrin genes are found in vertebrates: *ANK1* (encoding ankyrin-R), *ANK2* (encoding ankyrin-B), and *ANK3* (encoding ankyrin-G). These ankyrins can bind different proteins and appear to occupy largely non-overlapping regions of the neuronal membrane. They also undergo alternative splicing events, giving rise to several isoforms that are expressed throughout the neuron [3].

The *ANK3* gene gives rise to three main classes of ankyrin-G isoforms: 190 kDa, 270 kDa, and 480 kDa, with expression of the larger two predominantly found in the nervous system [3]. 480 kDa ankyrin-G is a key organizing protein at the AIS and noR, interacting both directly and indirectly with various components

Figure 1



Basic anatomy of a neuron.
The neuron is composed of three main parts: dendrites, the cell body (soma), and the axon. The dendrites receive incoming signals from other neurons. The soma contains the nucleus and processes the received signals. The axon contains the axon initial segment which is the site of action potential initiation. The action potential then propagates in a forward direction down the myelin sheaths, which insulate the axon to increase conduction speed, with gaps called Nodes of Ranvier enabling signal regeneration. The axon terminates in synaptic terminals, which transmit signals to the next neuron or target tissue. Action potentials also propagate through the soma and into the dendrites.

essential for its structure and function (Figure 2) [4]. *ANK2* undergoes similar alternative splicing, generating 220 kDa and 440 kDa ankyrin-B peptides. Notably, the giant isoforms of both ankyrin-G and ankyrin-B are implicated in several neurodevelopmental disorders [5–8]. In cortical pyramidal neurons, the 480 kDa isoform of ankyrin-G links ion channels, structural proteins, and more to the underlying spectrin-actin cytoskeleton, and associates with microtubules to become stabilized at the plasma membrane [9]. However, a separate mechanism for channel localization is required in the dendrites, as the 480 kDa isoform of ankyrin-G is largely absent from this region, except for the most proximal dendrite [10]. Although the 190 kDa isoform of ankyrin-G is present, it is predominantly found in dendritic spines where it regulates spine structure and function [10]. This leads to the question of what is scaffolding these dendritic channels. The answer to this question came from recent work demonstrating that related ankyrin family member, ankyrin-B, scaffolds dendritic $Na_V1.2$, thus expanding the known functions of ankyrin-B.

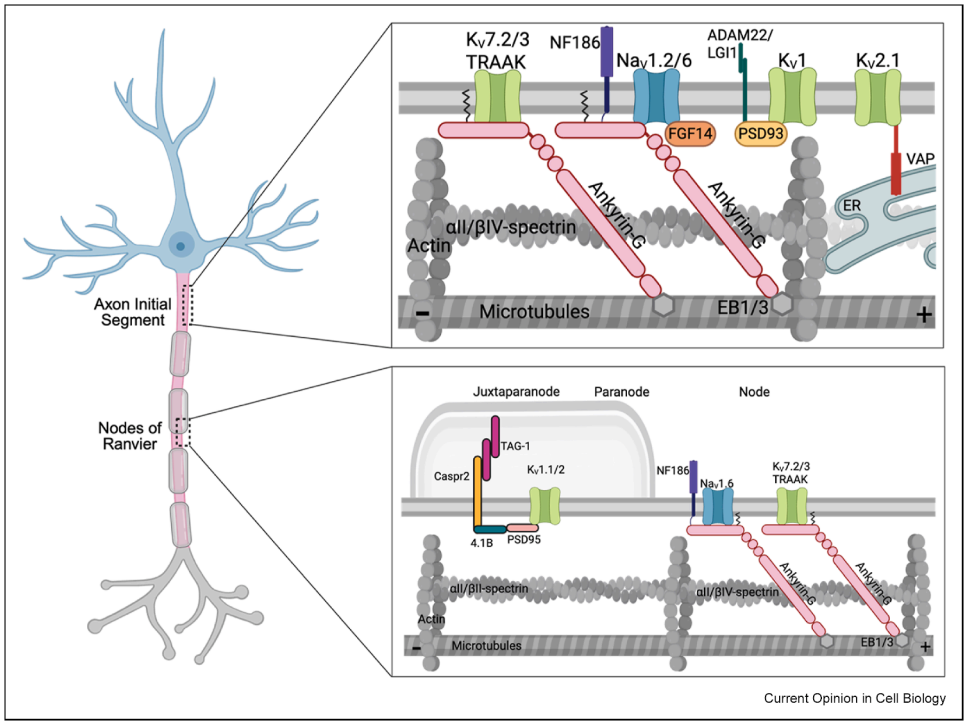
By anchoring Na_Vs and K_Vs to the plasma membrane, ankyrins ensure the precise positioning of these channels, which is crucial for efficient AP propagation and the modulation of synaptic plasticity. As such, the intricate relationship between ion channels, ankyrin proteins, and the cytoskeleton is central to our understanding of how neurons process and transmit electrical signals, and how these processes underlie complex behaviors and cognitive functions. In the following review, we will discuss in greater detail the localization and stabilization of Na_Vs and K_Vs throughout cortical pyramidal neurons via ankyrin-dependent and ankyrin-independent mechanisms.

Action potential initiation at the AIS

Action potentials (APs) are initiated at the axon initial segment (AIS), a specialized region organized primarily by the 480 kDa isoform of ankyrin-G (Figure 1). Loss of ankyrin-G disrupts the formation of the AIS entirely, leading to the absence of all known AIS components [3]. Among the key elements involved in AP initiation and propagation at the AIS are voltage-gated sodium (Na_V) and potassium (K_V) channels. The AIS's high density of these ion channels—especially Na_Vs—combined with its small volume, which requires less charge to reach threshold, makes this region particularly effective for triggering action potentials [11,12].

Two key Na_Vs are found at the AIS of cortical pyramidal neurons: $Na_V1.2$ and $Na_V1.6$. Early in development, $Na_V1.2$ is the predominant Na_V at the AIS. As the neuron matures, $Na_V1.6$ largely replaces $Na_V1.2$ at the distal AIS and $Na_V1.2$ localizes to the proximal AIS as well as the somatodendritic region [13]. Although $Na_V1.2$ and $Na_V1.6$ share high structural homology, they differ in their biophysical properties—$Na_V1.6$ activates at more hyperpolarized membrane potentials and supports more persistent and resurgent currents than

Figure 2



Na_V and K_V channel localization at the AIS and Nodes of Ranvier.
The figure provides a magnified view of the axon initial segment, Nodes of Ranvier, Dendrites, and soma. *(Axon Initial Segment)* Ankyrin-G is maintained at the membrane via interactions with the underlying actin/spectrin cytoskeleton and microtubules. It is further stabilized via an interaction with neurofascin-186 (NF186). At the AIS, ankyrin-G stabilizes Na_Vs (blue) and K_Vs (green). Other proteins, such as fibroblast growth factor 14 (FGF14) and postsynaptic density protein 93 (PSD93) are also involved in the proper clustering of Na_Vs and K_Vs. Meanwhile, K_V2.1 does not directly interact with ankyrin-G, rather it is maintained at the AIS by forming endoplasmic reticulum/plasma membrane junctions via interactions with VAMP-associated proteins (VAPs). (*Nodes of Ranvier)* At the nodes, ankyrin-G is maintained at the membrane via interactions with the underlying actin/spectrin cytoskeleton and microtubules. It is further stabilized via an interaction with NF186. Here, ankyrin-G anchors Na_V1.6, K_V7.2, K_V7.3, and TRAAK at the membrane. In the juxtaparanodal region, K_V1.1 and K_V1.2 are stabilized at the membrane via a protein complex including Caspr2, TAG-1, protein 4.1B, and PSD95. Interactions with the extracellular matrix and myelinating glial cells contribute to the maintenance of this architecture.

Na_V1.2 [2]. Despite their unique distribution and function, both channels are secured at the AIS via an interaction with the ankyrin-G repeats (Figure 2). Ankyrin-G/Na_V binding requires a sequence of nine amino acids known as the ankyrin binding domain (ABD) in the Na_V intracellular loop connecting domains II and III (II-III loop) [14–16]. While deletion of the giant exon of ankyrin-G results in the loss of Na_V clustering at the AIS, western blot analysis shows that protein levels remain unchanged, suggesting that the channels are being redistributed at low levels across the plasma membrane below the limit of detection [3]. What exactly contributes to the distinct interactions between Na_V1.2 and Na_V1.6 with ankyrin-G remains unknown, but may be explained by sequence differences in the II-III loop of the channels. Findings suggest that the entire II-III loop contributes to the strong binding between Na_Vs and ankyrins [17]. Thus, residues surrounding the ABD could result in differences in the interactions between Na_V1.2 and Na_V1.6 with ankyrin-G, although further study is necessary. For a more detailed discussion of the distinct localizations of Na_V1.2 and Na_V1.6 throughout development and their contribution to disease, refer to a recent review focusing on AIS structure and function in health and disease [12].

Ankyrin-G does not work alone to stabilize Na_Vs to the AIS. Neurofascin186 (NF186), a transmembrane cell-adhesion molecule, interacts with ankyrin-G to stabilize it at the membrane (Figure 2). This NF186/ankyrin-G complex is crucial for the maintenance of the AIS and allows for the proper anchoring of Na_Vs [18,19]. Furthermore, this complex allows ankyrin-G to recruit βIV-spectrin, further aiding in the stabilization of the AIS and clustering of Na_Vs (Figure 2) [20,21]. Loss of either NF186 or βIV-spectrin impacts the ability of neurons to generate APs [18–21]. For more information on the critical role of axonal spectrins in the maintenance of AIS integrity, refer to the following review [22]. Meanwhile, fibroblast growth factor 14 (FGF14)

interacts directly with the C-terminal tail of Na_V to control channel gating and channel expression (Figure 2). FGF14 loss-of-function decreases Na^+ currents and reduces Na_V expression at the AIS, ultimately impairing neuronal excitability [23,24].

$K_V7.2$ and $K_V7.3$, encoded by *KCNQ2* and *KCNQ3*, are also found at high densities in the distal AIS, where they play a critical role in setting the resting membrane potential and restoring the membrane potential after an AP [25–27]. Ankyrin-G scaffolds $K_V7.2$ and $K_V7.3$ at the AIS via an interaction with the K_V C-terminal domain (Figure 2) [28–30]. PSD-93 also acts as a scaffolding protein to mediate K_V clustering at the AIS (Figure 2). Knock-down of PSD-93 in culture or silencing in PSD-$93^{-/-}$ mice disrupts K_V1 clustering at the AIS in cortical neurons [31]. This could have significant consequences, as alterations in the K_V1 channel subunit composition at the AIS influence the excitability of the AIS. For example, K_V1 channels modulate the AP waveform in the AIS of layer 5 pyramidal neurons, facilitating transmitter release at intracortical synapses [32]. Furthermore, $K_V1.1^{-/-}$ mice exhibit lifelong seizure activity [33], and autoantibodies targeting K_V1 channels have been identified in some patients with chronic epilepsy [34].

Another K_V channel, $K_V2.1$, does not directly interact with ankyrin-G to cluster at the AIS [35,36]. Rather, this channel contains an AIS trafficking motif with putative phosphorylation sites within its C-terminus, critical for the clustering of $K_V2.1$ at the AIS [37]. $K_V1.2$ channels are maintained at the AIS by forming endoplasmic reticulum/plasma membrane junctions via interactions with VAMP-associated proteins (VAPs) (Figure 2) [38]. Although these channels do not bind directly to ankyrin-G, their overall AIS localization still requires ankyrin-G, as deletion of ankyrin-G leads to the loss of AIS targeting of PSD-93, which is responsible for K_V1 scaffolding at the AIS [31].

The TRAAK (TWIK-related Arachidonic Acid-Stimulated K+) channel, which is a member of the K_{2P} family, was also recently shown to be scaffolded at the AIS. TRAAK is a K_V leak channel that is critical for rapid repolarization of the membrane. Recent findings show that TRAAK forms a complex with ankyrin-G via an ankyrin-G binding motif in the TRAAK C-terminus, allowing for clustering of TRAAK at the AIS of forebrain pyramidal neurons (Figure 2) [39,40]. Another K_V leak channel, TREK-1, forms a heterodimer with TRAAK, stabilizing TREK-1 at the AIS [39].

Together, these findings reveal several mechanisms through which Na_Vs and K_Vs are anchored at the AIS. Most of these processes are dependent, directly or indirectly, on the presence of ankyrin-G, highlighting the critical role this protein plays maintaining proper neuronal signaling. Further discussion of the importance of AIS integrity in health and disease can be found in a recent publication [12].

Forward propagation of action potentials

The noR are short, myelin-free segments of the axonal membrane that are distributed at regular intervals along myelinated axons and allow for saltatory conduction of the AP (Figure 1). The noR can be categorized into three distinct regions: (1) the node, where Na_V channels are concentrated; (2) the paranodes, which border the nodes and serve as the attachment sites for each layer of the myelin sheath to the axon; and (3) the juxtaparanodes, located adjacent to the paranodes, rich in K_V1s, and covered by the myelin sheath.

Node

Nodes are enriched in Na_V channels, allowing APs to propagate down the axon in a saltatory manner. A reduction of $Na_V1.6$ at the nodes results in a significant decrease in AP conduction velocities, underscoring the importance of proper Na_V localization [41]. The processes and players involved in nodal formation differs between the central and peripheral nervous systems (CNS; PNS). While this review focuses on the CNS, a useful discussion of the differences can be found at the following reference [42].

At the nodes, ankyrin-G binds to βIV-spectrin and NF186, creating a stable scaffold and ensuring proper clustering of Na_Vs (Figure 2). While NF186 interacts with the repeats of ankyrin-G, βIV-spectrin interacts with the first ZU5 domain, which is shared by all ankyrin-G isoforms [3]. Interestingly, specific knock-down of 480 kDa ankyrin-G in cultured hippocampal neurons results in a loss of clustering of βIV-spectrin at the AIS, despite the maintained presence of the ZU5 domain in the remaining isoforms, suggesting that 480 kDa ankyrin-G recruits βIV-spectrin via mechanisms unique to the giant isoform [3]. In fact, the study went on to show that 480-kDa ankyrin-G recruits β4-spectrin through an interaction regulated by phosphorylation at S2417, which is missing from the smaller ankyrin-G isoforms, suggesting that additional contacts within the giant exon-encoded sequence may be necessary for full interaction between ankyrin-G and βIV-spectrin [3].Deletion of either NF186 or βIV-spectrin causes severe deficits in node formation as well as Na_V and ankyrin-G localization, further impairing node integrity and function [21,43]. Interestingly, ankyrin-R has been found to compensate for the loss of ankyrin-G in mature, fully myelinated axons, rescuing Na_V clustering [44].

In addition to localizing to the AIS, TRAAK and TREK-1 are also clustered at the nodes of myelinated axons, where they are required for the quick and efficient propagation of APs down the axon as well as rapid AP

repolarization [45]. Like AIS localization, the C-terminus of TRAAK interacts with ankyrin-G to cluster at the nodes. Here, TREK-1 and TRAAK can form heterodimers, stabilizing TREK-1 at the nodes (Figure 2) [39].

Paranodes

Paranodes are the regions of the axon adjacent to the node, where the axonal membrane interacts with the myelin sheath. These regions are crucial for ensuring proper myelin-axon attachment and facilitating efficient saltatory conduction. While the paranodes themselves do not contain high concentrations of ion channels, they are crucial for organizing and maintaining the ion channels at the node, particularly Na_Vs [46]. This importance stems from the role of paranodal junctions in forming a physical and molecular barrier that restricts the lateral diffusion of nodal proteins, thereby preserving the distinct molecular composition of the node [47]. Paranodal axo-glial junctions, formed by interactions between axonal Caspr/contactin complexes and glial neurofascin-155, act as diffusion fences that prevent the encroachment of juxtaparanodal K_Vs into the node and help retain Na_Vs in their correct position [46]. Moreover, the loss or disruption of these junctions results in mislocalization or reduced clustering of Na_Vs at the node, highlighting their specific reliance on an intact paranodal structure for stable nodal localization [47].

Juxtaparanodes

The juxtaparanodes are specialized regions adjacent to the paranodes and nodes in myelinated axons that house a high concentration of K_Vs. They are involved in the structural organization of the myelin sheath through the action of adhesion molecules like Caspr2 and Contactin, ensuring the integrity of the noR and efficient signal transmission. Proper function of the juxtaparanodal region is essential for normal neuronal signaling, and dysfunction in this area can lead to various neurological disorders [48]. In multiple sclerosis, demyelinated lesions often exhibit altered distributions of nodal, paranodal, and juxtaparanodal proteins [49]. This misdistribution has been found to impair nerve conduction and contribute to neurological deficits. Moreover, patients with disorders such as chronic inflammatory demyelinating polyneuropathy and Morvan syndrome have autoantibodies that target protein complexes throughout the nodes, including Caspr2 and Tag1 (contactin-2), which accumulate at the juxtaparanode [50]. These disruptions in protein complexes can destabilize K_V channels, adversely affecting nerve transmission.

$K_V1.1$ and $K_V1.2$ lay underneath the compact myelin sheath just proximal to paranodes [51,52]. Here, $K_V1.1$ and $K_V1.2$ are linked to Caspr2 via the PDZ binding domain, a common structural domain of 80–90 amino acids found in signaling proteins (Figure 2). This domain plays a key role in anchoring receptor proteins in the membrane to cytoskeletal components. Importantly, TAG-1 was found to be required for the proper accumulation of Caspr2 at the juxtaparanodes [53,54]. Deletion of TAG-1 results in failure of Caspr2 to localize to the juxtaparanodal region and exclusion of $K_V1.1$ and $K_V1.2$ from the juxtaparanodal axonal membrane [53,54]. These findings provide a critical role for the Caspr2/TAG-1 complex in clustering K_V1s in the juxtaparanodes. In fact, Caspr2 deficient mice exhibit $K_V1.1$ and $K_V1.2$ mislocalization to the paranodal region, which has significant implications for neuronal development, excitability, and neurological function, and has been associated with several disorders such as autism, epilepsy, and language impairment [55,56].

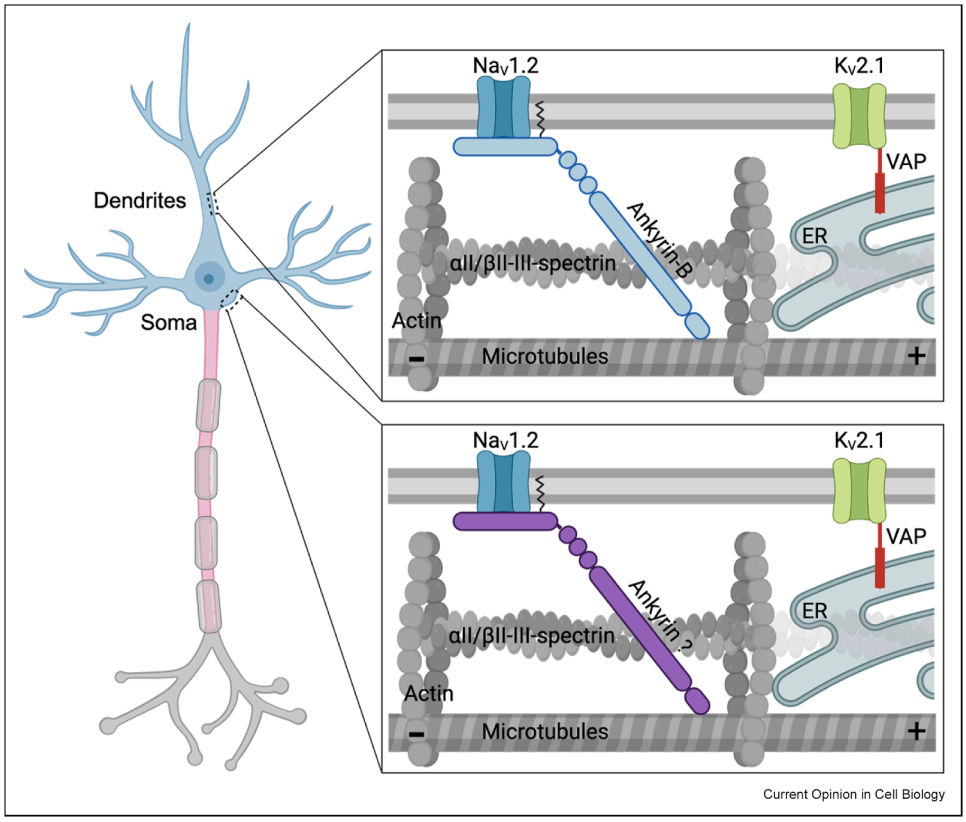
Action potential backpropagation

Soma

Neuronal backpropagation refers to the process in which the AP travels back through the somatic membrane into dendrites, where it delivers crucial signals to the dendrites that are essential for sustaining synaptic strength, integration, and plasticity. $Na_V1.2$ at the proximal AIS of mature cortical pyramidal neurons promotes backpropagation to the soma, where other $Na_V1.2$ channels in the soma are triggered, leading to somatic depolarization [2,57]. Thus, the soma not only acts as a transition zone between the axonal and dendritic compartment, but also as a major source of sodium current necessary for normal synaptic activity. Deficits in backpropagating action potentials (bAPs) can impair learning, memory, and dendritic computation, a hallmark of many brain disorders including autism spectrum disorder and intellectual disability [2,58]. bAPs are most commonly observed in pyramidal neurons, particularly those in the cerebral cortex and hippocampus. However, bAPs are not exclusive to pyramidal neurons and have also been identified in other cell types, including cerebellar Purkinje cells and certain types of GABAergic interneurons [59,60]. These cell types exhibit varying propagation dynamics. For example, Purkinje cells in the cerebellum show bAPs, but their dendritic morphology and ion channel composition result in different propagation characteristics compared to pyramidal neurons [60]. Specifically, the amplitude of APs decreases with increasing distance from the soma into the dendrites, suggesting that Purkinje cell dendrites do not actively propagate APs as pyramidal cell dendrites do, but rather passively conduct them [60].

It is still not clear which proteins are scaffolding $Na_V1.2$ on the somatic membrane, but it does seem likely that the process is dependent on ankyrins (Figure 3). Deletion of the ankyrin-binding motif from the $Na_V1.2$ II-III loop causes a complete loss of $Na_V1.2$ localization not only to the AIS and dendrites, but also to the somatic plasma membrane [61].

Figure 3

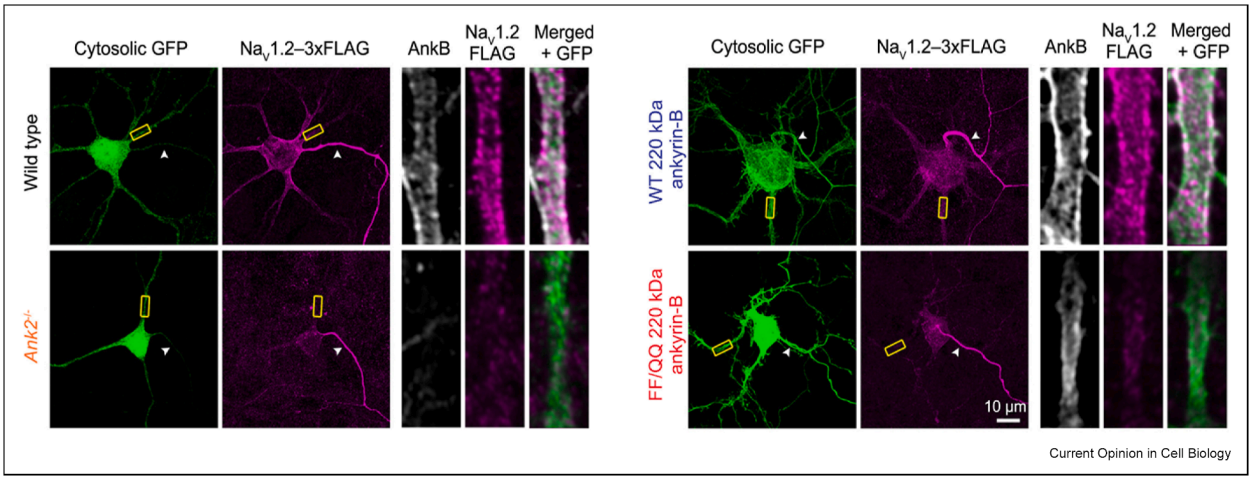


Na_V and K_V channel localization at the soma and dendrites.
(*Dendrites*) $K_V2.1$ is maintained at the dendrites by forming endoplasmic reticulum/plasma membrane junctions via interactions with VAMP-associated proteins (VAPs). Ankyrin-B scaffolds $Na_V1.2$ to the dendrites of forebrain pyramidal neurons. (*Soma*) $K_V2.1$ is maintained at the soma by forming endoplasmic reticulum/plasma membrane junctions via interactions with VAPs. It is currently unknown what anchors $Na_V1.2$ at the soma. It is possible that ankyrin-G, ankyrin-B, or ankyrin-R are responsible, though further study is required.

The 480 kDa isoform of ankyrin-G does accumulate on the soma of mature neocortical pyramidal neurons [62], suggesting that it could be the scaffold for these somatic channels. However, APs evoked through somatic current injection persisted in recordings from the motor cortex of mice lacking giant ankyrin-G, which may be a result of compensation from the smaller 190 kDa isoform of ankyrin-G that is upregulated upon giant ankyrin-G knockout [3]. Interestingly, a study assessing all isoform deletion of *Ank3* in cortical pyramidal neurons showed significant increases in staining for the activity-dependent marker c-Fos, supporting the idea that a complete deletion of *Ank3* isoforms does not fully abolish AP firing [63]. These findings suggest that a different ankyrin may be scaffolding these somatic channels. While ankyrin-B is also found at high levels on the somatic membrane, deletion of *Ank2* had no effect on $Na_V1.2$ somatic localization [61]. One intriguing possibility is that both ankyrin-G and ankyrin-B are capable of scaffolding $Na_V1.2$ on the soma in a functionally redundant way, which could provide a backup source of scaffolding in case of deleterious mutations. Likewise, there may be compensation from ankyrin-R as is seen in the NoR [44]. It would be particularly interesting to investigate the effects of deleting both ankyrin-G and ankyrin-B.

$K_V2.1$ is present in large clusters on the soma and proximal dendrites [36]. Interestingly, the channel clusters in the somatodendritic region in a manner distinct from its localization to the AIS [37]. The somatodendritic localization of $K_V2.1$ is determined by a proximal restriction and clustering (PRC) motif within its cytoplasmic C-terminal tail [37]. This motif binds to vesicle-associated membrane protein (VAMP)-associated proteins (VAPs), facilitating the formation of endoplasmic reticulum/plasma membrane junctions, which are essential for maintaining $K_V2.1$ localization in the somatodendritic region (Figure 3) [38,64].

Figure 4



Ankyrin-B scaffolds Na$_V$1.2 to the dendritic membrane of cortical pyramidal cells.
The figure, adapted from Ref. [61], shows representative confocal images of DIV21 *Ank2$^{flox/flox}$* cultured neocortical neurons co-transfected with NaV1.2-3xFLAG-IRES-EGFP and TagBFP (WT) or Cre-2A-BFP (*Ank2* null), or rescued with WT or FF/QQ mutant 220 kDa ankyrin-B. The yellow boxes indicate zoomed in dendritic regions, shown to the right. FF/QQ is an ankyrin-B mutant that can localize to the dendritic membrane, but loses its ability to interact with Na$_V$1.2. Na$_V$1.2 localization to the AIS is maintained in all conditions due to the presence of 480 kDa ankyrin-G.

Dendrites

Backpropagating APs in dendrites provide feedback signals that influence synaptic strength and contribute to learning and memory processes [2]. In cortical pyramidal cells, Na$_V$1.2 is crucial for this process, with their precise localization and function in dendrites ensuring proper propagation of the AP into the soma and dendritic regions [2,13]. This interaction between axonal and dendritic regions highlights the importance of spatial organization of ion channels in regulating overall neuronal activity and plasticity.

Previous work has detailed the specific localization of Na$_V$1 subtypes throughout the dendrites of hippocampal neurons [13]. However, Na$_V$ localization in neocortical neurons is less clear. Recent advancements in labeling and imaging techniques have contributed significantly to the ability to observe the subcellular localization and dynamics of the homologous Na$_V$1.2 and Na$_V$1.6 channels [61,65]. For example, an epitope-tagged Na$_V$1.2 channel was used to help overcome the limitations of visualizing Na$_V$s outside of the AIS and showed Na$_V$1.2 localization along the dendritic shaft of neocortical neurons [61]. Interestingly, recent work demonstrated that, just as was seen in the soma, Na$_V$1.2 lacking its ankyrin-binding motif failed to localize to dendrites, suggesting that this localization was ankyrin-dependent [61], leading to the question of which ankyrin is scaffolding these dendritic channels.

190 kDa ankyrin-G is found in dendrites, but is primarily localized to the dendritic spines, while 480 kDa is absent from the region, except for the most proximal segments of dendrite [10]. Ankyrin-B, on the other hand, is localized strongly to the dendritic shaft in neocortical pyramidal cells, where it would be well-positioned to bind Na$_V$1.2 [61,65]. In addition, the ankyrin repeats from ankyrin-B are able to bind the Na$_V$1.2 II-III loop with high affinity [17], though the importance of this interaction had not yet been tested. However, Nelson et al. recently showed that ankyrin-B scaffolds Na$_V$1.2 in the dendrites of mature cortical pyramidal neurons (Figure 3) [61]. Homozygous and heterozygous ankyrin-B deletion or expression of a mutated form that cannot interact with Na$_V$1.2 results in the loss of Na$_V$1.2 localization in dendrites (Figure 4) [61]. These findings establish an exciting novel role for ankyrin-B in the dendrites of neocortical pyramidal neurons. One challenge for understanding the role of ankyrin-B in neurons is its growing list of cellular functions. For example, ankyrin-B is also involved in promoting spine regulation in the prefrontal cortex, regulating axonal branching, and controlling microtubule-dependent axonal transport [66–68]. Homozygous deletion of forebrain *Ank2* during development alters the synaptic proteome and causes seizure-related phenotypes [67,68]. How exactly different disease-associated *ANK2* variants affect each of these processes remains to be determined, and the extent of those effects is likely to affect the resulting patient phenotype.

Concluding thoughts

The precise localization of voltage-gated ion channels is essential for maintaining neuronal function, with ankyrin proteins serving as pivotal regulators of this process.

While ankyrin/channel interactions have been studied extensively in axonal domains, much less is known about the somatic and dendritic roles of these proteins. Additionally, the mechanisms underlying differential Na_V localization remain poorly understood. Future studies will aim to further elucidate how Na_V and K_V channels are differentially localized to specialized domains during neuronal development. Moreover, additional investigation is required to understand how ankyrin-G and ankyrin-B, despite their high homology, fulfill distinct roles in Na_V scaffolding. One particularly exciting area of research is the characterization of ankyrin and Na_V post-translational modifications. Studies have identified crucial roles for post-translational modifications, including phosphorylation and palmitoylation, in regulating ankyrin localization, function, and interactions with binding partners [3,69,70]. Similarly, $Na_V1.2$ and $Na_V1.6$ have been shown to undergo both phosphorylation and palmitoylation, among other post-translational modifications [71–73]. Interestingly, alignment of the $Na_V1.2$ and $Na_V1.6$ II-III loops reveals unique potential phosphorylation sites near the ankyrin binding domain, as well as shared palmitoylation sites. Differences in post-translational modification activity could impact ankyrin and Na_V function, localization, and interactions with one another. Furthermore, recent findings show that $Na_V1.2$ and $Na_V1.6$ are sorted into distinct pools of trafficking vesicles via signals residing in the I-II loop of these channels [65]. Although further investigation is needed to identify the specific residues involved and the underlying molecular mechanisms, it is possible that variations in post-translational modifications play a role in this process.

Looking ahead, continued investigation into the complex localization patterns of Na_V and K_V channels will be essential for uncovering the mechanisms that govern neuronal compartmentalization, with promising implications for developing targeted therapies for neurological disorders rooted in channel mislocalization.

Author contributions

CCE wrote the manuscript and generated figures. CCE and PMJ edited the manuscript.

Declaration of competing interest

PMJ is a consultant for Flux Therapeutics.

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Data availability

No data was used for the research described in the article.

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** of outstanding interest

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This paper explores how a neurodevelopmental mutation in the *ANK3* gene, which encodes giant ankyrin-G, affects the assembly of the axon initial segment (AIS)—a critical structure for action potential initiation in neurons. Using patient-derived data and mouse models, the authors show that the mutation impairs the organization and stabilization of the AIS by disrupting interactions with microtubules and membrane proteins. This disruption leads to significant changes in neuronal polarity and excitability. The findings provide important mechanistic insights into how mutations in scaffolding proteins like ankyrin-G can lead to altered brain function and contribute to neurodevelopmental disorders, including autism and intellectual disability.

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Escobedo et al. investigate the mechanisms behind the clustering of K2P K+ channels at axonal sites. The study identifies an evolutionarily conserved ankyrin-G-dependent motif that plays a central role in organizing and anchoring K2P K+ channels at the axon initial segment (AIS) and nodes of Ranvier. By using a combination of genetic manipulation, imaging, and electrophysiological approaches, the authors demonstrate that this motif is essential for proper channel clustering, which is vital for regulating resting membrane potential and neuronal excitability. The clustering of K2P K+ channels facilitates precise neuronal signaling, influencing action potential initiation and propagation. This study enhances our understanding of ion channel localization in neurons, emphasizing the role of ankyrin-G in organizing K2P K+ channels. By identifying the ankyrin-G-binding motif, it provides insights into how precise channel clustering impacts neuronal function, with implications for action potential propagation, neuronal signaling, and disorders related to ion channel dysfunction.

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Luque-Fernández and colleagues investigate the mechanisms underlying the periodic localization of TRAAK (a two-pore domain potassium channel) in the axon initial segment (AIS) of hippocampal pyramidal neurons. The study reveals that TRAAK channels are dynamically organized along the AIS through an interaction with an ankyrin-G–binding motif. Using a combination of molecular biology, imaging, and electrophysiological techniques, the authors demonstrate that this ankyrin-G-dependent mechanism is essential for the proper clustering of TRAAK channels at periodic intervals along the AIS. This clustering is crucial for modulating neuronal excitability and action potential generation. This study advances our understanding of ion channel localization in neurons, highlighting the role of ankyrin-G in organizing TRAAK channel clusters. By identifying the ankyrin-G-binding motif, it sheds light on how potassium channels are spatially regulated, impacting neuronal excitability and signaling. The findings have implications for neurophysiological disorders and provide a framework for exploring the role of ankyrin-G in axonal membrane organization and neuronal network activity.

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Nelson et al. investigate the interaction between the autism risk genes *SCN2A* (encoding $Na_V1.2$) and *ANK2* (encoding ankyrin-B) in neocortical pyramidal cell dendrites. The study reveals that these two genes physically and functionally converge at dendritic sites, where ankyrin-B organizes and anchors $Na_V1.2$. This convergence plays a pivotal role in shaping dendritic excitability and synaptic signaling. Through a combination of genetic manipulation, electrophysiology, and imaging, the authors demonstrate that the loss of either gene disrupts dendritic function, providing insights into the mechanisms by which $Na_V1.2$ and ankyrin-B contribute to neural network activity and are implicated in neurodevelopmental disorders like autism. This research reveals how *Scn2a* and *Ank2* converge at dendritic sites to regulate dendritic excitability and synaptic plasticity in neocortical pyramidal neurons. It provides insights into how disruptions in these genes may contribute to autism spectrum disorders (ASD) and highlights the role of sodium channels and scaffolding proteins in neuronal function. The findings suggest potential therapeutic strategies targeting *SCN2A* and *ANK2* interactions for treating ASD and other neurodevelopmental disorders.

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This paper investigates the spatial distribution and dynamic behavior of voltage-gated sodium (Na_V) channels in different neuronal compartments. Using advanced imaging approaches such as super-resolution microscopy and single-particle tracking, the authors provide direct evidence of the compartment-specific localization of $Na_V1.2$ and $Na_V1.6$ channels in dendrites and axons, respectively. Specifically, $Na_V1.2$ channels are primarily localized in dendrites, while $Na_V1.6$ channels are concentrated in axonal regions, including the axon initial segment. The study highlights the importance of the precise spatial distribution and dynamic regulation of Na_V channels in shaping neuronal excitability, synaptic integration, and action potential propagation. It also provides insights into the molecular mechanisms that govern neural computation and emphasizes the relevance of these findings for understanding channelopathies associated with NaV channel dysfunction.

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Yoon et al. investigate the effects of early developmental deletion of *Ank2* (ankyrin-B) in the forebrain, focusing on its impact on synaptic function and seizure susceptibility. The study reveals that the loss of *Ank2* alters the synaptic proteome, leading to changes in protein expression that affect neuronal excitability and synaptic signaling. These alterations are associated with seizure-related phenotypes, providing a link between *Ank2* dysfunction and seizure susceptibility. Using proteomic analysis and electrophysiology, the authors demonstrate that ankyrin-B is crucial for maintaining synaptic integrity and preventing abnormal neuronal firing. This research reveals how *Ank2*

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