

Axon Initial Segment

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Axon Initial Segment

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Advanced article

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The axon initial segment (AIS) is a specialised region of the axon where the action potential is initiated following integration of excitatory and inhibitory inputs. The AIS contains high density of sodium and potassium channels anchored on scaffold proteins, but it also includes receptors to neurotransmitters and neuromodulators. Besides its role in action potential initiation, the AIS constitutes a diffusion barrier that controls cytoplasmic traffic towards the axon. Activity-dependent plasticity has been recently shown to affect the length and position of the AIS whereas stimulation of ligand-gated receptors alters the ion channel content of the AIS. Thus, the AIS represents a critical element in neuronal excitability and its plasticity.

Introduction

The axon initial segment (AIS) is a unique neuronal domain located at the first 20–60 µm of the axon. It is characterised by a high density of voltage-gated ion channels supported by specific scaffold proteins and a distinctive cytoskeleton (Rasband, 2010). Functionally, it plays a critical role since the AIS is the site of action potential (AP) initiation. If the net integration of inputs from multiple excitatory or inhibitory synapses crosses a certain threshold, an AP will be triggered at the AIS. Besides this main function, the AIS is essential to maintain axon identity (Hedstrom *et al*., 2008). In this sense, AIS serves as a barrier that controls membrane and cytoplasmic traffic towards the axon (Kobayashi *et al*., 1992; Song *et al*., 2009). Given the importance of the AIS in the input–output function, it is easy to understand how perturbations of AIS organisation or integrity can have profound effects on nervous system function. In fact, the AIS has become a promising target regarding neurodevelopmental disorders, neurodegenerative diseases and brain trauma (Schafer *et al*., 2009; Del Puerto *et al*., 2015).

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Structure of the AIS: Scaffold Proteins, Ion Channels and Receptors

AIS cytoskeleton and scaffold proteins

AIS structure is maintained by a highly stable cytoskeleton and AIS-specific scaffold proteins (**Figure 1**). The first characterisation of the AIS showed the presence of fascicules of microtubules that were not present in any other neuronal domain (Palay *et al*., 1968). This observation pointed to a special function of the AIS. Further studies have shown that AIS proteins are characterised by their resistance to detergent extraction (Winckler *et al*., 1999), which confer a high stability to the AIS. This may be due to acetylation and detyrosination of α-tubulin subunits that compose the AIS microtubules (Shea, 1999). However, the regulatory mechanisms that confer AIS microtubules their characteristics are poorly studied. One study has shown that this may be due to the absence of the tubulin deacetylase HDAC6 in the AIS (Tapia *et al*., 2010). Other studies propose that TRIM46 contributes to microtubules organisation at the AIS or may modulate tubulin posttranslational modifications (van Beuningen *et al*., 2015). However, a clear picture of the involvement of microtubules in the structure and function of the AIS is still lacking.

Not only microtubules but also actin cytoskeleton is important to maintain the AIS structure. Recent studies describe a role of actin cytoskeleton in the control of axonal traffic (Song *et al*., 2009), and its structure has been depicted using stochastic optical reconstruction microscopy (STORM) showing a distribution in regularly spaced rings along the AIS (Xu *et al*., 2013). The functional role of these rings remains however largely unknown. The AIS contains actin-binding proteins, such as synaptopodin, that are necessary to maintain the cisternal organelle (Benedeczky *et al*., 1994; Bas Orth *et al*., 2007). This organelle is irregularly distributed in clusters along the AIS associated to submembranous actin, but it is unknown how it contributes to AIS function. The actin cytoskeleton also serves to anchor the scaffold protein βIV-spectrin that is essential to maintain other scaffold proteins, ion channels and membrane proteins at the AIS. New isoforms of βIV-spectrin were recently identified in the

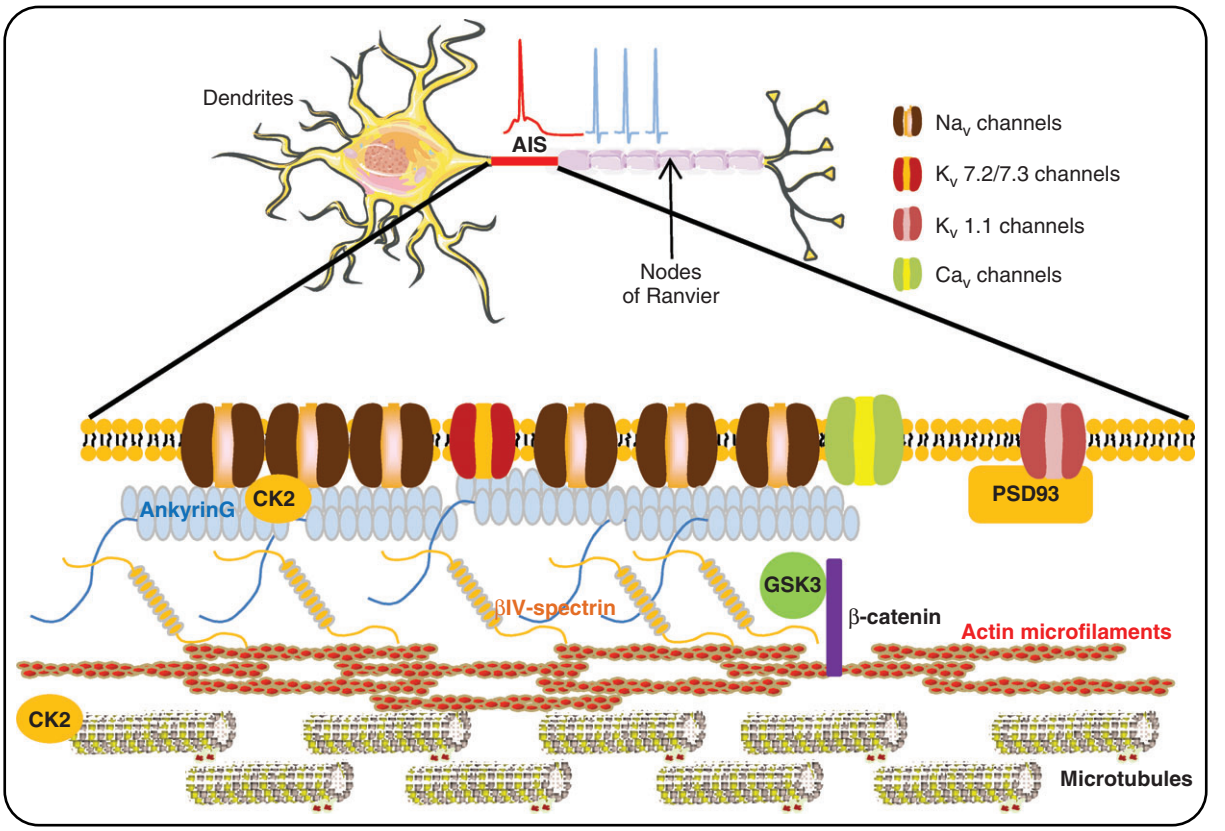


Figure 1 Structure and composition of the AIS. Inputs originated from the dendrites are integrated at the AIS where the AP is triggered. The propagation of the spikes to the presynaptic terminal is made possible by nodes of Ranvier, which composition is similar to AIS. The membrane of AIS is characterised by a high concentration of sodium channels (Na_V), 50 times higher than in soma. Other potassium and calcium voltage-gated ion channels (K_V and Ca_V) contribute to modulate excitability. These channels are anchored and concentrated through scaffold proteins like AnkyrinG and PSD-93. AnkyrinG is anchored to the AIS actin microfilaments by βIV-spectrin. Both actin microfilaments and microtubules have distinctive characteristics at the AIS and its contribution to AIS structure and function is still mostly unknown. All this complex structure is modulated through recently identified kinases (such as CK2 or GSK3) and phosphatases (such as calcineurin).

AIS (Yoshimura *et al*., 2016), but their role is still unknown. βIV-spectrin anchors ankyrin G, probably the most important protein at the AIS. The relevance of ankyrin G for AIS and neuronal function is reflected in the fact that ankyrin G elimination leads to a loss of axonal identity characterised by the appearance of dendritic spines in formal axonal structures and loss of neuronal polarity (Hedstrom *et al*., 2008). Ankyrin G is essential to cluster voltage-gated sodium channels, βIV spectrin and other AIS membrane proteins (Jenkins and Bennett, 2001). Several studies demonstrated that sodium channels are targeted to the AIS by an ankyrin G-interacting motif (Garrido *et al*., 2003), found later in Kv7.2/7.3 channels (Pan *et al*., 2006). Other membrane proteins, such as neurofascin, are also concentrated at the AIS by ankyrin G interaction (Zhang *et al*., 1998).

Little is known regarding the regulatory mechanisms that modulate these interactions and the concentration of ion channels at the AIS. Some studies have pointed to a role of some kinases, phosphatases and proteases. Casein kinase 2 (CK2) phosphorylates sodium channels at the AIS modulating their interaction with sodium channels (Brechet *et al*., 2008), but it also modulates microtubules posttranslational modifications at the AIS. Moreover, decrease in CK2 expression levels disrupts the binding of AIS proteins, including ankyrin G, thus affecting AIS structure (Sanchez-Ponce *et al*., 2011). Another important kinase, the glycogen synthase kinase 3 (GSK3), acts by phosphorylating beta-catenin at the AIS and its inhibition decreases ankyrin G concentration (Tapia *et al*., 2013). Regarding phosphatases, calcineurin (PP3) and PP1 also play a role in AIS regulation (Evans *et al*., 2013; Berger *et al*., 2018). Recently, myosin II light chain phosphorylation and myosin V have been related to AIS modulation and protein clustering (Janssen *et al*., 2017; Berger *et al*., 2018). Further work is necessary to understand AIS regulatory mechanisms that allow a better understand of how to modulate the targeting of voltage-gated channels, AP and neuronal function. **See also**: **Axons**

AIS voltage-gated ion channels and receptors

The property of AP initiation is conferred by the high concentration of Na^+, K^+ and Ca^{2+} voltage-gated ion channels in the AIS. During AIS development, ankyrin G and sodium channels

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are detected first at the AIS (Jenkins and Bennett, 2001), whereas potassium channels appear sequentially during AIS maturation (Sanchez-Ponce *et al*., 2012). As mentioned earlier, AIS sodium channels and some potassium channels (Kv7.2/7.3) are targeted to the AIS by an ankyrin G binding motif (Garrido *et al*., 2003; Pan *et al*., 2006). However, other potassium channels, such as Kv1.1, are anchored at the AIS by the scaffold protein PSD-93 (Ogawa *et al*., 2008). Recently, another alternative AIS targeting motif was proposed for Kv2.1 channels (Jensen *et al*., 2017). This motif is located at the C-terminus of Kv2.1, it contains phosphorylation sites and acts in conjunction with the PRC motif that determines dendritic localisation of Kv2.1. Sodium channel opening is responsible for AP initiation and conduction along the axon. Their concentration at AIS was estimated to be approximately 50 times higher than in the somatodendritic membrane (Kole *et al*., 2008). In central nervous system neurons, the main sodium channels at the AIS are $Na_v1.2$ and $Na_v1.6$. Interestingly, $Na_v1.2$ is found at the proximal AIS, whereas $Na_v1.6$ is concentrated at the distal AIS (Hu *et al*., 2009), having a differential contribution to AP initiation. While sodium channels are a common component of AIS in all neurons, neurons are characterised by the presence of specific combinations of potassium channels (Vacher *et al*., 2008). Regarding calcium channels, information on their distribution and localisation in the AIS is limited. Using Ca^{2+} imaging, it has been shown that the AIS contains T-type (Ca_{V3}) and R-type ($Ca_{V2.3}$) Ca^{2+} channels (Bender *et al*., 2010). **See also**: **Sodium Channels**

Besides voltage-gated ion channels, the AIS also contains $GABA_A$ receptors (Nusser *et al*., 1995; Christie and De Blas, 2003), 5-HT1A serotonin receptors (Ko *et al*., 2016), and probably dopamine D3 receptors and 5-HT2A receptors (Bender *et al*., 2010; Bender and Trussell, 2012). These receptors contribute to the modulation of AIS by neurotransmitters released by other neurons that target the AIS. However, AIS modulation can be achieved by other not AIS expressed receptors, such as glutamatergic and purinergic receptors.

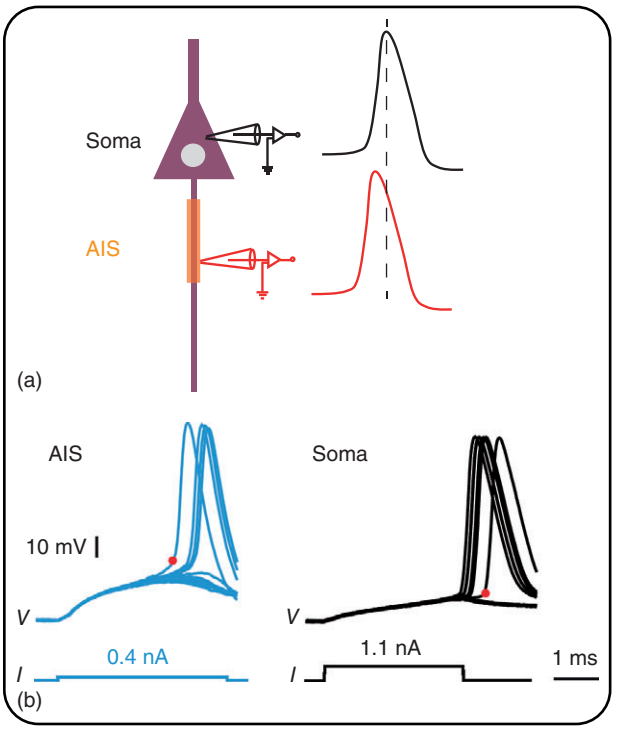


Figure 2 Function of the AIS. (a) Spike initiation in the AIS. Dual AIS-soma recording from a pyramidal neuron. Left, recording configuration. Right, action potential measured in the soma is slightly delayed compared to that measured in the AIS. (b) Spike threshold at the AIS and the cell body. Simultaneous recording from L5 pyramidal neuron in the AIS (left) and the soma (right). While the rheobase is lower in the AIS compared to the soma, the voltage threshold is higher. Reproduced with permission from Kole and Stuart (2008). © Springer Nature.

Function of the AIS: Action Potential Initiation

Action potential initiation at the AIS

Determining the spike initiation zone is particularly important in neuron physiology. The AP classically represents the final step in the integration of synaptic messages at the scale of the neuron (Bean, 2007). Pioneering work in spinal motoneurons in the 1950s revealed that APs are generated in the AIS or possibly the first nodes of Ranvier. In fact, it was shown that the AP consisted of two main components: an 'initial segment' (IS) component was found to precede the full AP originating in the soma (i.e. the somato-dendritic (or SD) component). These two components could be isolated whatever the mode of AP generation (i.e. antidromic stimulation, direct current injection or synaptic stimulation), but the best resolution was obtained with the first derivative of the voltage. The IS component is extremely robust and can be isolated from the SD component by antidromic stimulation of the axon in a double shock paradigm. For very short inter-stimulus intervals, the SD component fails but not the IS component.

The recent development of patch-clamp techniques allowing whole-cell recording from single axons of mammalian neurons (Kole *et al*., 2008; Hu *et al*., 2009) together with the use of voltage-sensitive dyes (Palmer and Stuart, 2006; Popovic *et al*., 2011) or sodium imaging (Kole *et al*., 2008) provide useful means to precisely determine the spike initiation zone. It was shown that sodium spike occurs first in the AIS and then in the soma (**Figure 2a**).

In myelinated axons, APs are initiated at the AIS. Depending on the cell type, the initiation zone is located between 15 and 40 µm from the soma. For instance, in layer 5 pyramidal neurons, the AP initiation zone is located in the distal part of the AIS, that is at 35–40 µm from the axon hillock (Palmer and Stuart, 2006; Yu *et al*., 2008). **See also**: **Action Potential: Ionic Mechanisms**; **Action Potentials: Generation and Propagation**

Action potential threshold

The threshold represents a critical feature of the all-or-none property of the AP initiation. Classically, the threshold is defined

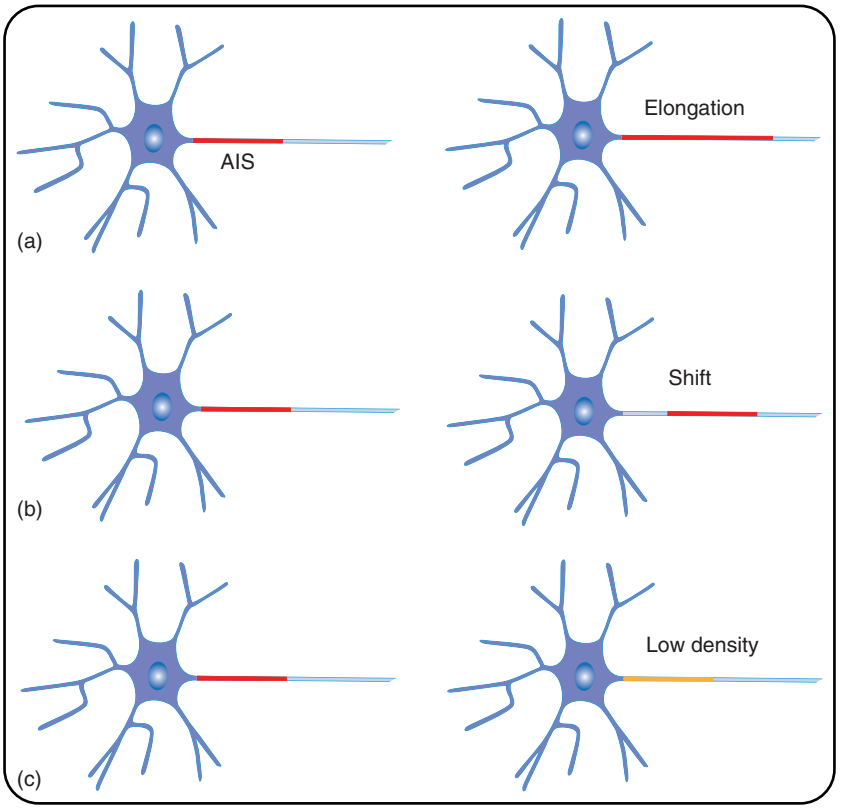


Figure 3 Plasticity of the AIS. (a) Elongation of the AIS. Following activity deprivation, the length of the AIS is increased. Reproduced with permission from Kuba *et al.* (2010). © Springer Nature. (b) Shift in the AIS position. Following activity enhancement, the AIS position is shifted away from the soma. Reproduced with permission from Grubb and Burrone (2010). © Springer Nature. (c) Change in composition of the AIS. Following stimulation of P2X7 receptor, the density of ankyrin G is reduced. Reproduced with permission from Del Puerto *et al.* (2015). © Oxford University Press.

as the voltage where the summed inward membrane current exceeds the outward current. It means that the voltage threshold of the AP is lower when the contribution of voltage-gated potassium channels is blocked pharmacologically (Higgs and Spain, 2011). Because of the high concentration of Na_v channels at the AIS, one could conclude that the spike threshold is lower in the AIS compared to the soma. Yet, although the minimal current required to elicit a single spike is indeed lower in the AIS compared to the cell body, the threshold of the AP is much higher in the AIS compared to the soma (Kole and Stuart, 2008) (**Figure 2b**). This counterintuitive observation results in fact from geometrical factors. Because of the compartmentalisation of spike initiation, the AP is sharper and its threshold is more hyperpolarised in the somatic compartment than in the AIS (Brette, 2013).

The spike threshold measured at the soma display high variability. In fact, an AP induced by highly synchronous inputs (or preferred stimulus) has a much lower threshold than an AP induced by weakly synchronous inputs (or not preferred stimulus) (Azouz and Gray, 2000). The mechanism underlying spike-threshold adaptation is thought to imply inactivation of sodium channel in the AIS (Wester and Contreras, 2013).

Plasticity of the Axon Initial Segment

Structural plasticity

Far away from being a static domain, the AIS shows a high degree of structural and functional plasticity. The AIS can adapt its length, position and composition in response to changes in activity in a homeostatic manner (**Figure 3**). For instance, elongation of the AIS has been shown to occur in auditory neurons after sensory deprivation (Kuba *et al.*, 2010) (**Figure 3a**). In contrast, in hippocampal neurons, the AIS has been shown to move away from the soma following chronic activity increase induced *in vitro* by elevating external K^+ or by optogenetic stimulation (Grubb and Burrone, 2010) (**Figure 3b**). Along the same line, shift in AIS position has been reported following persistent blockade of Kv7 channels (Lezmy *et al.*, 2017). Recent studies have shown that input number and frequency can modulate position and/or length of the AIS. This kind of plasticity happens in visual cortex during development, where the AIS shortens in layer II/III cortical neurons from postnatal day 10 to postnatal day 35 at the same time that the number of synaptopodin clusters increases.

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However, sensory deprivation during this period elongates the AIS and reduces the number of AIS clusters (Schluter *et al*., 2017). It has been proposed that changes in cisternal organelle organisation may be responsible for this plasticity. Furthermore, recent studies show that dendrites calibre affect AIS structural plasticity.

Besides structural plasticity induced by activity, the AIS can adapt its composition of voltage-gated ion channels following stimulation of ligand-gated receptors (**Figure 3c**). Activation of the purinergic P2X7 receptor decreases ankyrinG and voltage-gated sodium channels concentration at the AIS, without affecting the length or position of the AIS (Del Puerto *et al*., 2015). In contrast, P2X7 inactivation was able to recover control levels of sodium channels. Cannabinoid receptors are also related to changes in AIS composition. During early initial development of hippocampal neurons, suppression of cannabinoid receptor 1 (CB1) decreases dendritic arbour size which is correlated to a decrease on ankyrinG expression (Tapia *et al*., 2017).

Functional plasticity

While elongation of the AIS corresponds to an increase in Na_v channel number and subsequently to an increase in excitability (Kuba *et al*., 2010), the functional effect of a shift in AIS position is still matter of debate. In fact, the shift in AIS position away from the soma has been classically interpreted as a decrease in excitability (Grubb and Burrone, 2010). But because of compartmentalisation of spike initiation and its effect on spike threshold, the distal AIS shift may be seen as an increased excitability (Brette, 2013). Voltage-gated potassium channels in the AIS play a critical role in setting global excitability. These channels are also subject of activity-dependent regulation. For instance, chronic activity deprivation induced by pharmacological blockade of glutamate receptors downregulates Kv1 channels in hippocampal neurons (Cudmore *et al*., 2010). In auditory neurons, the elongation of the AIS is associated with the switch from Kv1 channels to Kv7 channels (Kuba *et al*., 2015). **See also**: **Memory (Mechanisms Other than LTP)**

Conclusion

In conclusion, the AIS is a complex assembly of ion channels, scaffold proteins and cytoskeleton. Its role in the AP initiation makes it a major player in neuron physiology. As most of neuronal elements, the structure and function of the AIS is subject to activity-dependent plasticity. Although many aspects of AIS biology and physiology have been characterised in detail, further work will certainly reveal its last secrets.

Inputs originated from the dendrites are integrated at the AIS where the AP is triggered. The propagation of the spikes to the presynaptic terminal is made possible by nodes of Ranvier, which composition is similar to AIS. The membrane of AIS is characterised by a high concentration of sodium channels (Na_v), 50 times higher than in soma. Other potassium and calcium voltage-gated ion channels (K_v and Ca_v) contribute to modulate excitability. These channels are anchored and concentrated through scaffold proteins like AnkyrinG and PSD-93. AnkyrinG is anchored to the AIS actin microfilaments by βIV-spectrin. Both actin microfilaments and microtubules have distinctive characteristics at the AIS and its contribution to AIS structure and function is still mostly unknown. All this complex structure is modulated through recently identified kinases (such as CK2 or GSK3) and phosphatases (such as calcineurin).

Glossary

Action potential Electrical impulsion resulting from the opening of voltage-gated sodium channels.

Axon Neuronal process that conducts electrical impulses away from the cell body of the neuron towards presynaptic terminals.

Axon initial segment Axonal domain responsible for the initiation of the action potential and the maintenance of neuronal polarity.

Neuron An electrically excitable cell that receives, integrates and transmits information to other cells (generally neurons) through electrical and chemical signals.

Plasticity The ability of neurons including synapses and ion channels to adapt their function from past experience.

Potassium channels A family of transmembrane proteins that are permeable to potassium ions. In contrast with sodium channels, potassium channels are formed by the assembly of two or four subunits.

Sodium channels A pore-forming transmembrane protein that is permeable to sodium ions.

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