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REVIEW ARTICLE

An update on axon initial segment structure and function

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Abstract

The axon initial segment (AIS) is a specialized subcellular region located at the proximal end of the axon and serves as the action potential initiation site due to the high density of ion channels. The AIS plays a critical role in maintaining neuronal polarity by regulating the trafficking and distribution of proteins that function in the dendritic or axonal compartment of the neuron. Due to the adaptive nature of AIS location and length, the excitability of neurons can be altered in response to activity. In this review, we briefly introduce the structure and function of AIS as well as discuss the recent progress in our understanding of AIS ion channel distribution and plasticity in different types of neurons. These would contribute to a better understanding of the AIS and give us a new perspective on AIS-related diseases.

Keywords: Axon initial segment; Ankyrin G; Ion channels; Plasticity

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1. Introduction

There are approximately 100 billion neurons in our brains. Neurons, as the most basic structural and functional units of the nervous system, are responsible for receiving and transmitting electrochemical signals in the nervous system and are involved in perception, calculation, learning, and memory. Signals exist in the form of action potentials (APs), which contain information encoded either in the firing time of the APs or in certain shape characteristics, such as the amplitude of APs[1]. Almost all neurons are composed of three parts: the soma, axon, and dendrite.

The axon initial segment (AIS) is a highly specialized region in the axon of neurons[1-5]. The AIS, which starts right after the axon hillock, is located approximately 20–60 μm from the proximal end of the axon and is distinguished from the axon by the dense framework of ion channels and other linker proteins. The most important function of the AIS is AP initiation, which could trigger the release of neurotransmitters in the synapses at the end of the axon. *In vivo* axons are usually tightly wrapped by lipid layers called the myelin sheath. The myelin sheath insulates axons from the outside environment, and as a result, there is no loss of current, thus ensuring the efficiency and fidelity of AP conduction.

2. Structure of AIS

The molecular structure of the AIS is relatively complex and is maintained by the accumulation of ankyrin G (AnkG) and βIV-spectrin[6,7]. AnkG, a product of the *ANK3* gene, is an anchoring protein at the AIS[8]. It forms a "scaffolding" structure with βIV-spectrin to anchor ion channels to the actin cytoskeleton. AnkG has three isoforms in mammals: 190-kDa AnkG, which is distributed in the cytoplasm; and 270-kDa and 480-kDa AnkG, which are the major isoforms that are specifically located at the AIS and nodes of Ranvier[8-10]. Following axon differentiation, AnkG begins to cluster at the proximal end of the axon and then recruits the other components of AIS[11,12]. The aminoterminal end of AnkG inserts into the actin/spectrin submembrane scaffold and anchors specific membrane proteins, including γ-aminobutyric acid type A ($GABA_A$) receptors, voltage-gated sodium (Na_v), and potassium (K_v) channels, as well as cellular adhesion molecules (CAMs), specifically the 186 kDa isoform of neurofascin 186 (NF-186) and neuronal (Nr)CAM, to the AIS[13] (Figure 1). Moreover, Wang *et al.* reported that neurons with αII spectrin deletion also showed the absence of AnkG, suggesting that αII spectrin and βIV-spectrin can interact to form spectrin tetramers that link adjacent actin rings and are required for AnkG clustering in the AIS[14].

In addition to actin, AnkG also interacts with microtubules through microtubule-associated proteins. For instance, a previous study has shown that the microtubule plus-end-binding (EB) proteins EB1 and EB3 are highly concentrated in mature AIS and can directly bind to AnkG, thus suggesting that EB1 and EB3 can stabilize the structure and microtubule network together[15]. Not only that, the dynein regulator nuclear distribution element-like 1 (NDEL1) localizes to the AIS through interaction with AnkG and controls cargo transport at the AIS[16].

In addition, there is a layer of specialized extracellular matrix anchored to the AIS that promotes and regulates the production of APs by various ion channels, such as Na_v, K_v, and calcium channels (Ca_v)[17-20], allowing neurons to generate a wide variety of AP waveforms[21]. Although the basic components of the AIS have been known for more than two decades, recent studies have indicated an increasing complexity of its types and the molecular composition and distribution of ion channels enriched within it.

3. Ion channels in the AIS

The entire AIS is enriched with many sodium channels of different subtypes[22]. AnkG binds to the interaction motif within the II-III intracellular loop of Na_v channels[23-26]. Experiments have shown that the concentration of sodium ion (Na^+) channels in neuronal AIS is approximately 19-fold of that in the cell body[27], 50-fold of that in the proximal dendrite[28], and 30-fold of that in the distal axon[29]. The main subtypes of Na^+ channels at the AIS are $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$[30,31]. During different developmental stages, the expression and subcellular distribution of various subtypes of Na^+ channels vary. For example, during early neuronal development, $Na_v1.2$ channels are initially found in the AIS, while $Na_v1.6$ is the dominant subtype in mature neurons[20,29,30,32,33]. Moreover, in cortical neurons, high-threshold $Na_v1.2$ channels are preferentially located at the proximal end of the AIS, while low-threshold $Na_v1.6$ channels are found at the distal end of the AIS[27]. Na_v channels are primarily responsible for the initiation of

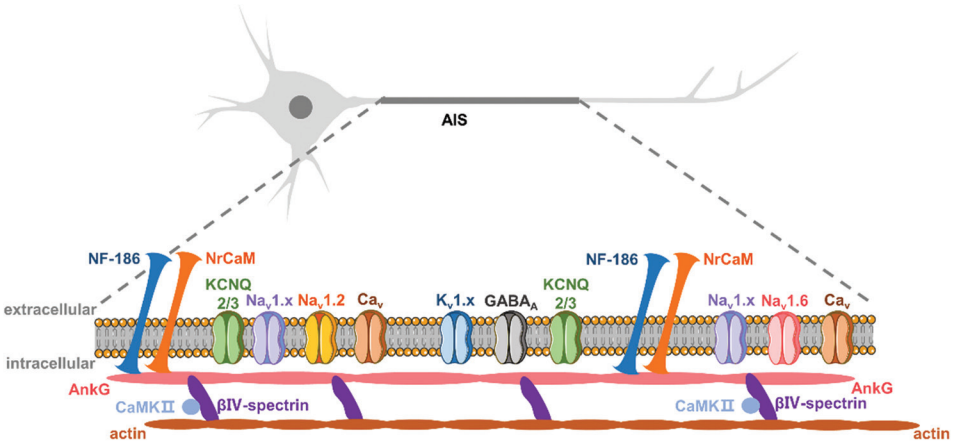

Figure 1. A simplified diagram of the molecular structure of the axon initial segment (AIS) in a neuron. AIS aggregates many anchoring and cytoskeletal proteins, such as ankyrin G (AnkG) and βIV spectrin. Skeleton proteins are anchored by a variety of ion channels, such as voltage-gated sodium channels ($Na_v1.x$), voltage-gated potassium channels ($K_v1.x$), voltage-gated calcium channels (Ca_v), and γ-aminobutyric acid type A ($GABA_A$) receptors. The AIS comprises many other component molecules and ion channels, and its complexity is constantly growing with advancements in research.

APs in the AIS[2,5]. This polarized distribution may reflect discrete functions of Na^+ channel subtypes in AP initiation and backpropagation.

Potassium ion (K^+) channels in the AIS are important for repolarization of AP. The density of K^+ channels in the distal AIS is also 10-fold of that in the cell body[34]. $K_v7.2$ and $K_v7.3$ (KCNQ2/3) channels form homomeric or heteromeric complexes, which interact with AnkG and gather in the AIS[35]. Although $K_v1.1$ and $K_v1.2$ channels do not bind to AnkG, they still cluster at the distal end of the AIS by binding to the scaffold protein postsynaptic density-93 (PSD93)[20,36].

Calcium ion (Ca^{2+}) channels have also been reported in the AIS[17]. Previous study has shown that Ca^{2+} channels may be modulated by dopaminergic signaling[37]. Unlike Na^+ and K^+ channels in the AIS, very little is known about the molecular mechanisms of Ca^{2+} channels.

There is usually only one axon in a neuron. The so-called somatic axon grows from the cell body through a short axon hillock. However, in cultured neurons, approximately 20% of neuronal axons grow from the dendrite, which we refer to as dendritic axons. This type of axon has been observed in rat pyramidal cells in previous studies[29,38,39]. According to a recent study, axons emanate from the basal dendritic arbor in more than half of all CA1 pyramidal neurons and 30% of those in rat pups[38]. Correspondingly, the AIS also has different origins. In this review, "dendritic AIS" refers to AIS with a dendritic origin, whereas "somatic AIS" refers to AIS with a somatic origin. Our previous study has shown that although somatic AIS has a similar length as dendritic AIS, the distribution of $Na_v1.2$ was significantly narrower in dendritic AIS[40].

4. Physiological functions of the AIS

APs are initiated at the AIS. In the 1950s when the structure and molecular composition of the AIS were mostly unknown, people speculated that the AIS or axon hillock was the AP initiation site based on intracellular recording observations[41-43]. The AIS has a lower AP threshold than the somatodendritic domain[42]. In an autoradiography study using Na^+ channel-binding ^{125}I-scorpion toxin, it was first revealed that Na^+ channel density was higher at the AIS[44]. In recent years, more and more studies have shown that the AIS is the AP initiation site in different types of CNS neurons[28,40,45,46]. Using quantitative freeze-fracture electron microscopy immunogold labeling, Lorincz *et al.* have confirmed that the concentration of $Na_v1.6$ channels at the AIS and nodes of Ranvier is approximately 40-fold higher than that at the soma in hippocampal neurons[29]. Our previous work has also confirmed that AP is initiated at the distal end of the AIS through voltage imaging technology[40]. As a result of its ability to determine the onset of AP, kinetic dysfunction in AIS can lead to neurological disorders, such as epilepsy[47-50].

Second, the AIS can "filter" molecules to separate the cell body from the axon, limiting the diffusion and exchange of molecules in different parts, and maintaining cell polarity[6,22,51]. Since it is located between the axon and the soma, it serves as a critical regulatory center that controls the proteins entering into the axon, and thus plays a crucial role in maintaining the neuron's polarity[52]. Either for cytoplasmic proteins or for vesicles, the AIS has been reported to have filtering functions. There is an actin-based cytoplasmic filter that could prevent the diffusion of soluble macromolecules larger than 13 nm. This has been proposed as a mechanism for regulating cytoplasmic protein movement[53,54]. Within the AIS, studies have shown different trafficking pathways in vesicles carrying axonal or dendritic protein. For instance, vesicles containing axonal proteins are preferentially trafficked into the axon[55]. On the other hand, the trafficking of vesicles carrying dendritic proteins is stopped in the AIS and redirected back to the soma[55-59].

5. Plasticity of the AIS

AIS plasticity refers to the adaptive responses in the nervous system to change its structure and function in response to neuronal activity[60,61]. Ion channel compositions at the AIS vary across different types of neurons. The differences in AIS length and location may be the basis for the different neuronal firing properties. For instance, retinal ganglion cells with different visual properties have different AIS lengths and locations in the axons[62]. Despite the importance of these differences for neuronal function, the mechanisms controlling AIS length and location remain largely unknown. Recent studies have revealed that AIS length or location is dynamically regulated in response to normal developmental and pathological conditions. For example, in rat cortical neurons, the length of AIS undergoes dynamic changes during their visual system development[63]. Previous studies have investigated the long-term activity-dependent plasticity regulation of the AIS in neurons *in vivo* and *in vitro* and found that the position and length of the AIS would make corresponding changes and adapt to changes in external factors[60,61,64,65]. This phenomenon, also known as AIS plasticity, is usually accompanied by alterations in neuronal excitability.

Several studies have suggested that neurons may alter excitability by changing their AIS length or location in response to changes in developmental and pathological conditions. For example, when avian nucleus magnocellularis neurons are deprived of auditory inputs,

their AIS will be elongated and proximally shifted closer to the soma[61]. Moreover, electrophysiology recordings have shown that input-deprived neurons tend to be more excitable, indicating that to regulate neuronal excitability and homeostasis as well as maintain neuronal circuit output, neurons might need to adapt to presynaptic activity by altering their AIS properties.

In *in vivo* experiments using chicken embryos, Hiroshi Kuba *et al*. found that depriving chickens of auditory input (destroying the cochlea) resulted in the elongation of the AIS of chicken brainstem auditory neurons (Chick NM). In the experiment, 7 days after auditory input deprivation, the AIS length increased to 1.7 times the original length. The distribution of Na^+ channels and the excitability of neurons increased, but the density and distribution of Na^+ channels barely changed[61]. In addition, different decibels of auditory stimulation may cause the AIS to shorten or lengthen, suggesting that the AIS can change its length according to the activity level of presynaptic neurons.

In addition, Grubb and Burrone have demonstrated a distal shift in AIS location, up to 17 μm away from the cell body, after 48 h of *in vitro* culture of rat hippocampal neurons under high-concentration K^+ treatment (15 mM potassium chloride [KCl]) to the extracellular fluid. In the AIS, AnkG and other associated proteins, such as Na^+ channels and βIV-spectrin, moved accordingly, but there was no observable change in AIS length. After washing, the AIS returned to its original position in relation to the cell body, and after 48 h of treatment with 15 mM KCl, neuronal excitability reduced, and a stronger current stimulation was needed for the neurons to fire an AP[60].

Our previous study has shown that after treating cultured hippocampal neurons with 20 mM glucose for 3 h, the neurons from both rats and mice showed changes in AIS length or location but in different ways[40]: in rat neurons, the AIS length became shorter, and the distance from the cell body increased because the proximal end of the AIS was pushed away from the cell body, while the distal end did not change; in mouse neurons, the AIS was longer, but there was no change in distance from the cell body as the distal end of the AIS moved away from the cell body, while the proximal end of the AIS showed almost no change. However, in both mouse and rat neurons, the dendritic AIS barely changed after the 20 mM glucose treatment, indicating that even in the presence of the same external stimulus, AIS plasticity varies between different types of neurons.

AIS plasticity can also occur under certain pathophysiological conditions. A previous study has shown AIS plasticity in barrel cortex L5 pyramidal neurons in a mild traumatic brain injury model, where the AIS was shortened and AP intensity was attenuated[66]. Similarly, a previous study has reported a molecular remodeling of the AIS, which was observed in the surviving peri-infarct region after focal cortical stroke[67]. Moreover, *de novo* AIS formation has been observed within the peri-infarct cortex, suggesting that stroke-induced axonal sprouting may contribute to the formation of new functional axons[67].

6. Conclusions

The AIS is a remarkable neuronal structure comprising a complex assembly of membrane proteins, scaffold proteins, cytoskeletal adaptors, and voltage-gated ion channels. The precise molecular distribution of the AIS ensures proper neuronal excitability by regulating the generation of APs. The dynamic properties of the AIS allow the regulation of neuronal excitability in response to neuronal activities both inside and outside the cell, thereby maintaining homeostasis and the output of neural circuits. Dendritic AIS may play an important role in the homeostatic regulation of physiological functions in the nervous system due to its insensitivity to external stimulus. The AIS also demarcates the boundary between the dendritic-somatic and axonal compartments while allowing axonal proteins to pass through. Moreover, certain structural properties of the AIS, such as length and/or location, can change through activity-dependent manners. The structural plasticity of the AIS is known as an important factor for the establishment and maintenance of nervous system functions. During AIS plasticity, the proteins in the AIS may undergo a demolition and reconstruction process. However, we still have very limited knowledge of the AIS.

From research on the brain of Alzheimer's disease (AD) patients, we are aware that axonal pathology is associated with neuroaxonal dystrophy and amyloid plaque formation[68]. Our previous study has also shown that AIS pathology may occur even before axonal pathology[54]. As a result of the key role AIS plays in AP bursts, the dysfunction of AIS may lead to certain neurological diseases. For example, mutations in ion channel subtypes could cause epilepsy[48]. In addition, *ANK3* (gene encoding AnkG) mutation has been found to be associated with some neuronal diseases, such as bipolar disorder[69], intellectual disability[70], schizophrenia, and autism spectrum disorder[71,72]. In mouse models, the loss of βIV-spectrin function resulted in ataxia and central auditory deafness[73,74]. Moreover, patient with the pathogenic variant of *SPNB4* (gene encoding human βIV-spectrin) has also been associated with myopathies and auditory neuropathies[75]. Our previous study has shown that AnkG deficiency in the AIS could play a significant role in AD pathology[54]. Therefore, restoring or preserving the structure and function of the AIS will be an important strategy for the treatment of these diseases.

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Conflict of interest

The authors declare no potential conflicts of interest.

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Ethics approval and consent to participate

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