The axon initial segment and the maintenance of neuronal polarity

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Abstract | Ion channel clustering at the axon initial segment (AIS) and nodes of Ranvier has been suggested to be a key evolutionary innovation that enabled the development of the complex vertebrate nervous system. This innovation epitomizes a signature feature of neurons, namely polarity. The mechanisms that establish neuronal polarity, channel clustering and axon–dendrite identity during development are becoming clearer. However, much less is known about how polarity is maintained throughout life. Here, I review the role of the AIS in the development and maintenance of neuronal polarity and discuss how disrupted polarity may be a common component of many diseases and injuries that affect the nervous system.

Axon initial segment
The area of the axon near the soma that contains a high density of voltage-gated sodium channels, which are responsible for the initial depolarization that leads to the initiation of the action potential.

Nodes of Ranvier
Interruptions in the myelin sheath that covers axons. Nodes of Ranvier are enriched in Na^+ channels and facilitate the propagation of action potentials by saltatory conduction.

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Neurons are polarized in many different ways. For example, as originally articulated by Cajal[1], the law of dynamic polarization states that information flows along a neuron in one direction (for example, synapse → cell body → axon initial segment (AIS) → axon → terminal; FIG. 1a). Neurons receive excitatory and inhibitory synaptic inputs on their cell bodies and dendrites. In neurons that fire repeatedly, the summation of these synaptic inputs into bursts of action potentials is carried out by the AIS. The AIS is characterized by high densities of voltage-gated Na^+ and K^+ channels that initiate and modulate action potentials[2–5]. In the myelinated axons of vertebrates, action potentials then propagate rapidly along the axon through the activation of clustered Na^+ channels at the nodes of Ranvier. As the action potential reaches the axon terminal, neurotransmitters are released to propagate the signal across the synaptic cleft to the next cell. Thus, the function of the nervous system depends on the polarized, or directional, propagation of action potentials through a network of neurons and glia that together integrate, initiate and propagate action potentials.

The functional polarity of neurons depends on, and is reflected by, their anatomical polarity and the distinction between their somatodendritic and axonal domains (FIG. 1b). A large body of data suggests that during early development, axon–dendrite specification is established through a combination of extracellular cues and the activation of intracellular signalling pathways[6]. Neurons also exhibit a high degree of subcellular polarity, with ion channels, organelles and protein complexes restricted to distinct membrane domains or cellular compartments (FIG. 1c).

Each of these forms of neuronal polarity is regulated and exemplified by the AIS. As its name implies, the AIS is strategically located at the most proximal part of the axon, where it functions as both a physiological and a physical bridge between the somatodendritic and axonal domains (FIG. 2a). In addition to its location, the AIS can be identified by ultrastructural features, such as fasciculated microtubules[7], by its molecular constituents and by its electrophysiological properties[2]. Thus, the AIS functions as the nexus of neuronal polarity in the mature neuron.

Here, I review what is known about the role of the AIS in the establishment and maintenance of neuronal polarity. I discuss recent work that implicates altered AIS function and altered polarity in neuronal injury and disease. Finally, I provide — on the basis of recent results — some speculative ideas about how polarity and the properties of the AIS may be dynamic and contribute to non-synaptic neuronal plasticity.

Composition of the axon initial segment

The AIS has received much attention over the past several years as new AIS-specific or enriched proteins have been discovered and new insights into neuronal function have been gained from clearer descriptions of AIS physiology[8]. The nodes of Ranvier (FIG. 2b) have also been studied in detail because of their importance in demyelinating diseases and because node formation is the prototypical example of glial regulation of subcellular polarity[9,10]. The AIS and the nodes of Ranvier have a common molecular organization. Both structures consist mainly of ion channels, cell adhesion molecules,

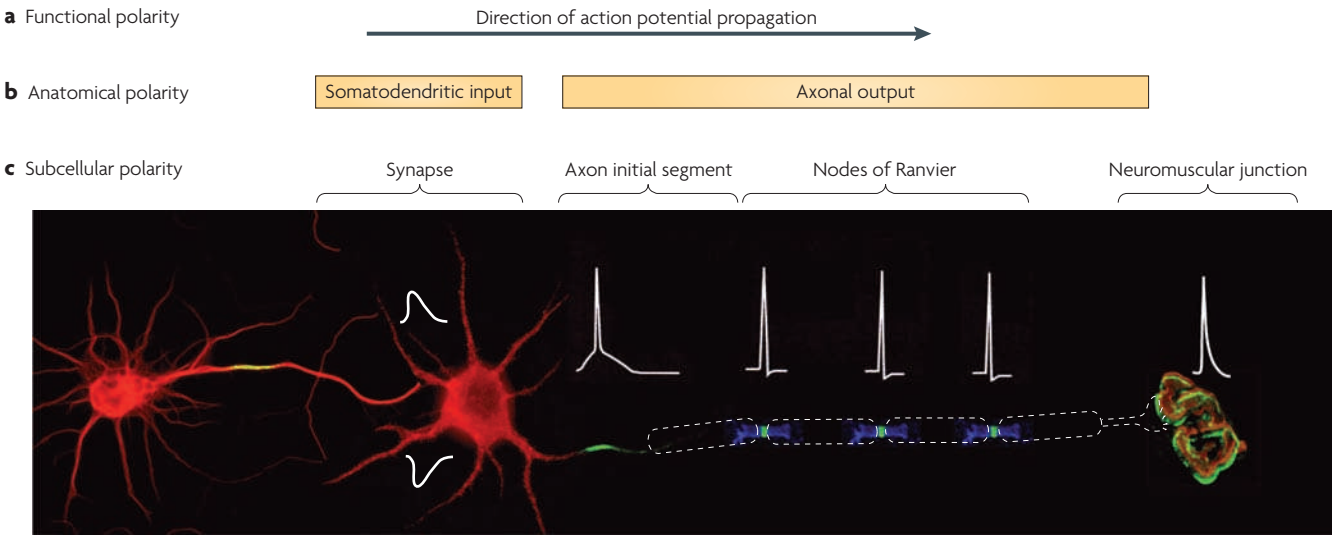


Figure 1 | **Neurons are highly polarized cells. a** | Neurons are functionally polarized because action potentials propagate in a single direction. Excitatory and inhibitory synaptic inputs are integrated at the axon initial segment (AIS). The resulting action potentials then propagate along the axon through the activity of ion channels clustered at nodes. Finally, neurotransmitter is released at the nerve terminal. **b** | Neurons are also anatomically polarized, as they can be subdivided into a somatodendritic input domain and an axonal output domain. The AIS separates these two domains. **c** | Neurons have a high degree of subcellular polarity, and synapses, the AIS, nodes of Ranvier and the neuromuscular junction are the main subcellular domains. Each of these domains is enriched in specific types of ion channels, receptors, adhesion molecules and molecular scaffolds that allow for the unidirectional propagation of action potentials. Each of these subcellular domains can also elicit unique electrophysiological responses (shown in white).

extracellular matrix molecules and cytoskeletal scaffolds[11] (FIG. 2c). Besides the main classes of proteins mentioned above, the AIS and nodes of Ranvier have other proteins in common, and these include kinases and accessory proteins with poorly understood functions[11]. Interestingly, genetic evidence suggests that this common molecular organization may be explained by the fact that the nodes evolved from the AIS[12] (BOX 1).

A main feature of the AIS is its enrichment for voltage-gated Na^+ channels. Na^+ channel immunostaining experiments have shown that the clustering of these channels is restricted to a stretch of the axon of about 35–45 μm in length[13,14], whereas electrophysiological and immunogold labelling measurements indicate that the Na^+ channel density in the AIS is about 40–50 times that in the soma or proximal dendrites[2,15]. This high degree of enrichment and subcellular polarity for Na^+ channels at the AIS facilitates a high Na^+ current density and a low action potential threshold[16].

Assembly of the axon initial segment

How does a neuron recruit Na^+ channels to the AIS during development and transition from an immature to a functionally mature and polarized state? AIS assembly is an intrinsic property of neurons, and no extracellular or glial-dependent cues are required[11]. By contrast, the formation and location of nodes of Ranvier are regulated by glial-derived signals[17–19].

The cytoskeletal scaffold protein ankyrin G (AnkG, also known as ANK3) is a master organizer of membrane domains and subcellular polarity in many cell types[20]. In neurons, AnkG is restricted to the AIS[21], and an AIS-targeting motif located in the cytoplasmic loop connecting domains II and III of Na^+ channels binds to AnkG[22,23]. The interaction between Na^+ channels and AnkG is facilitated by phosphorylation of the AIS-targeting motif by casein kinase II (CK2), which is enriched at the AIS and nodes of Ranvier[24]. Data from experiments in cultured neurons suggest that channels that are not anchored by AnkG (for example, those located in somatodendritic domains) are removed from the membrane by endocytosis[25]. Thus, the clustering of AIS membrane proteins depends on AnkG. Indeed, silencing AnkG expression by RNA interference or by genetic ablation in mice blocks the clustering of Na^+ channels at the AIS[26,27]. Loss of AnkG also blocks the subcellular polarization of the K^+ channels KCNQ2 and KCNQ3, the cell adhesion molecules neurofascin and neuronal cell adhesion molecule (NrCAM), the AIS extracellular matrix and the cytoskeletal protein βIV spectrin, which links AnkG to the actin cytoskeleton)[27–30]. Thus, AnkG functions as a scaffold to which all other AIS proteins are tethered directly or indirectly (FIG. 2c), and consequently AnkG establishes the subcellular polarity of these molecules.

Although all Na^+ channels harbour an AnkG-binding AIS localization motif, there are additional levels of subcellular polarity within the AIS. The voltage-gated Na^+ channel Nav1.6 is found in the distal AIS and the voltage-gated Na^+ channel Nav1.2 is found in the proximal AIS[13]. Similarly, members of the Kv1 family of voltage-gated K^+ channels are also found primarily in the distal AIS[31]. The regulation of these gradients of channels may have

an important role in nervous system function, as this differential distribution might contribute to the regulation of the initiation, modulation and backpropagation of action potentials.

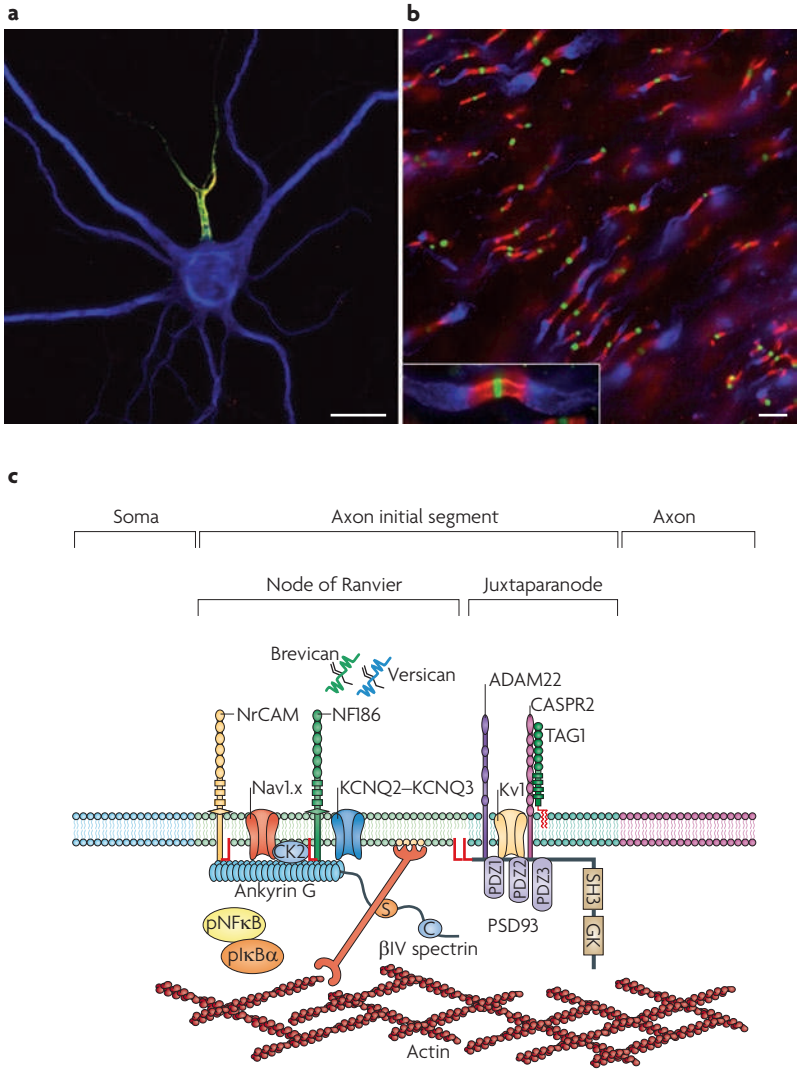


Figure 2 | **The axon initial segment and nodes of Ranvier are prototypical examples of subcellular polarity. a** | A cultured hippocampal neuron with high densities of ankyrin G (AnkG, also known as ANK3) (green) and neurofascin 186 (NF186) (the overlap between AnkG and NF186 is shown in yellow) at the axon initial segment (AIS). Microtubule-associated protein 2 (MAP2) (blue) is confined to the somatodendritic domain. The scale bar represents 20 μm. **b** | Nodes of Ranvier show a highly polarized organization that includes nodal Na^+ channels (green), paranodal contactin-associated protein (Caspr) (red) and juxtaparanodal K^+ channels (blue). The scale bar represents 5 μm. **c** | The molecular organization of the AIS, which has many features in common with nodes of Ranvier and juxtaparanodes. These domains are comprised of ion channels (Nav1.x, KCNQ2–KCNQ3 and Kv1.x), cell adhesion molecules (neuronal cell adhesion molecule (NrCAM), neurofascin 186 (NF186), a disintegrin and metalloproteinase domain-containing protein 22 (ADAM22), transient axonal glycoprotein 1 (TAG1, also known as contactin 2) and CASPR2), extracellular matrix molecules (brevican and versican), cytoskeletal scaffolds (AnkG, βIV spectrin and postsynaptic density protein 93 (PSD93)) and other signalling proteins, some with unknown roles (casein kinase II (CK2), phosphorylated nuclear factor-κB (pNFκB) and phosphorylated inhibitor of κBα (pIκBα)). Intriguingly, both nodal and juxtaparanodal proteins are located at the AIS, but they occupy mutually exclusive domains in myelinated axons. GK, guanylate kinase; PDZ, PSD95/discs large/zonula occludens; SH3, SRC homology 3.

Although by definition a neuron must have an axon to assemble an AIS, the relationship between AIS assembly and axon specification *in vivo* has not been determined yet. However, in cultured hippocampal neurons, AnkG can be first detected after the breaking of symmetry and specification of the axon has already taken place[32].

Little is known about the mechanisms that recruit AnkG to the AIS. Efforts to define individual domains of AnkG that might be involved in its targeting to the AIS have proven unsuccessful[33], and none of the binding partners (such as βIV spectrin and neurofascin 186 (NF186)) for AnkG seem to be required for its clustering at the AIS[27]. A recent study showed that phosphorylated inhibitor of κBα (pIκBα), an inhibitor of nuclear factor-κB (NFκB), is enriched at the AIS[34]. IκBα is phosphorylated by IκB kinases (IKKs) and pharmacological inhibition of IKKs impaired the clustering of AnkG at the AIS, causing retention of AnkG in the cell soma[35]. These observations suggest that pIκBα is an important upstream regulator or cofactor for AnkG polarization and trafficking to the AIS. However, the molecular details of how pIκBα performs this function are not yet understood.

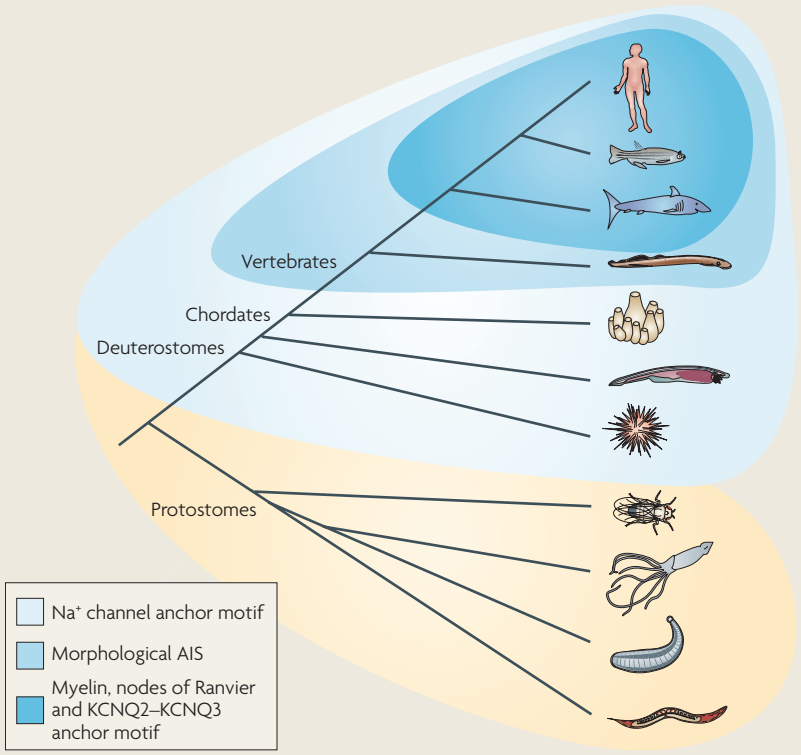
Maintenance of neuronal polarity

The existence of a barrier. During brain development, the emergence of neuronal polarity is a rapid event. In the mouse cortex this occurs between embryonic days 14 and 18 (E14–E18)[6]. In contrast to this brief developmental window, polarity must be maintained for much longer periods of time, over many decades in the case of humans. How is this accomplished? Neurons could sustain the positive and negative intracellular signalling pathways that were used to establish axon–dendrite polarity[36], but this would burden neurons with an additional metabolic demand on top of the continuous energetic requirements of electrical signalling. A simple, alternative solution would be for neurons to build a physical barrier, or fence, that prevents the mixing of somatodendritic and axonal molecules, thereby maintaining anatomical polarity. After such a barrier is in place, the energy-dependent signalling pathways that regulate axon specification can be shut down.

Early support for the barrier model in vertebrates was provided in a study in which fluorescent phospholipids were fused to axonal membranes[37]. Instead of diffusing throughout the cell membrane, the phospholipids were trapped in the axonal domain, suggesting that a diffusion barrier between the somatodendritic and axonal regions restricted their mobility[37]. A more recent study[38] measured the diffusion of individual fluorescent phospholipid molecules in the axonal membrane and showed that their diffusion was also blocked at the AIS. The distribution of membrane proteins is also restricted by the AIS diffusion barrier. For example, the lateral mobility of the membrane proteins NCAM-L1 (an AnkG binding protein found in the AIS and the distal segment of the axon) and THY1 (a glycosyl-phosphatidyl inositol (GPI)-anchored protein) have been shown to be reduced in the AIS relative to other axonal locations[39].

Box 1 | **Evolutionary origin of ion channel clustering**

The clustering of Na^+ and KCNQ2–KCNQ3 K^+ channels at the axon initial segment (AIS) depends on a common cytoplasmic anchor motif that mediates the interaction of these channels with ankyrin G (AnkG, also known as ANK3)[22,23,28]. Phylogenetic analyses of these ion channels show that the anchor motif arose first in basal chordates and that all orthologous Na^+ channels in jawed vertebrates contain the anchor motif[12] (see the figure). Although a morphologically and functionally identifiable AIS with high densities of Na^+ channels can be found in lampreys — jawless vertebrates that lack myelin and nodes of Ranvier — the AIS anchor motif did not evolve in KCNQ2–KCNQ3 K^+ channels until much later in evolution, at about the same time as myelin. This observation suggests that the unique advantages of myelination (that is, reduced membrane capacitance, increased transmembrane resistance and clustered ion channels permitting saltatory conduction) drove the subsequent evolution of the KCNQ2–KCNQ3 K^+ channel, so that the K^+ channels independently evolved the capacity to localize to nodes and the AIS. The independent development of the AIS anchor motif in KCNQ2–KCNQ3 and Na^+ channels provides a remarkable example of convergent molecular evolution and suggests that stringent structural restrictions for the assembly of protein complexes exist at the AIS. Furthermore, the data support the conclusion that the nodes of Ranvier evolved from the AIS. Thus, the AIS is not only at the centre of functional and anatomic polarity of vertebrate neurons but is also located at the centre of the evolution of neuronal polarity. Figure is modified from REF. 12.



Many cytoplasmic proteins are also excluded from the axoplasm, indicating that the AIS barrier is not confined to the plasma membrane. For example, microtubule-associated protein 2 (MAP2) is found exclusively in somatodendritic regions of the cell and begins to be excluded from the axon when the AIS is being assembled. The existence of a cytoplasmic barrier was confirmed in an experiment in which fluorescently labelled dextrans of different sizes were loaded into the soma of hippocampal neurons, after which their diffusion rates into the axon were measured[40]. Although small dextrans readily diffused into the axon, large ones did not. The degree of exclusion depended on the age of the neuron and the assembly of the AIS[40]. Furthermore, examining the movement of transport vesicles showed that vesicles containing dendritic cargoes were excluded from the axon[40]. Considered together, these experiments provide support for the existence of a barrier that restricts lipids, cytoplasmic and membrane proteins and transport vesicles to distinct compartments of the polarized neuron. For a discussion of the evolutionary origins of the AIS barrier, see BOX 2.

The nature of the barrier. What molecular mechanisms could underlie such a barrier? It has been suggested that the high density of membrane proteins found at the AIS might create a 'fence' that physically prevents the free diffusion of lipids and other membrane proteins[38] (FIG. 3a). This hypothesis is based on the observation that the rate of diffusion of phospholipids decreases during development as Na^+ channels and other membrane proteins accumulate at the AIS. Alternatively (or in addition), the lipid composition of the AIS itself may be regulated by proteins that are present in the AIS (FIG. 3b). For example, βIV spectrin has a carboxy-terminal pleckstrin homology (PH) domain that has been proposed to contribute to cytoskeleton–plasma membrane adhesion and membrane polarization through binding to phosphoinositides[41]. Although the mobility of lipids has not been measured in mutant mice lacking the PH domain of βIV spectrin, the nodes of Ranvier in these mice have impaired structural and molecular polarity[42,43], indicating that the PH domain has crucial structural and functional roles at polarized domains of myelinated axons. In addition, the AIS is enriched in proteins with lipid modifications. The cell adhesion molecules NF186 and

Box 2 | **Evolutionary origin of the axonal barrier**

Drosophila neurons have an intrinsic axonal barrier that restricts axon guidance receptors to distinct axonal domains. The receptor tyrosine kinase Derailed (DRL) is located in proximal domains of axons, whereas Roundabout 2 (ROBO2) and ROBO3 are restricted to distal segments, with a sharp transition in protein distribution between the two domains[96]. Measurement of the rate of protein diffusion in these two domains revealed that the site of transition between DRL and ROBO2 and ROBO3 corresponds to a region in which the lateral mobility of proteins in the membrane is highly restricted, which suggests the existence of a diffusion barrier in the axon. In *Drosophila* the barrier exists at some distance from the cell body rather than at the transition from the somatodendritic to the axonal domain as in vertebrates. The axonal barrier in *Drosophila* might have first arisen during evolution as a patterning mechanism, although the molecular mechanisms underlying this compartmentalization between proximal and distal axonal domains are unknown. Future studies on polarity in this model organism may provide clues about the origins of the AIS barrier in vertebrates.

NrCAM and the scaffolding protein postsynaptic density protein 93 (PSD93) are all palmitoylated, whereas transient axonal glycoprotein 1 (TAG1, also known as contactin 2) is tethered to the plasma membrane by GPI[44–46]. These lipid modifications often have key roles in regulating protein trafficking, localization and function in specialized lipid microdomains[47]. However, as the dextrans used in the study mentioned above[40] were cytoplasmic, their exclusion from the axon cannot be exclusively attributed to a high density of AIS membrane proteins or to a unique composition of lipids at the AIS.

Mechanistic insights into the nature of the barrier were provided by a study that showed that differences in the mobility of membrane proteins and lipids could be eliminated by disruption of the actin cytoskeleton[38,39]. Moreover, actin depolymerization allowed large cytoplasmic dextrans to bypass the AIS and enter the axon[40]. This suggests that the high density of proteins at the AIS is unlikely to be solely responsible for the diffusion barrier. The actin cytoskeleton is also a crucial regulator of Na^+ channel stability in the AIS membrane, and this finding reconciled conflicting results from immunostaining and patch-clamp measurements of AIS membrane, which suggested high and low densities of Na^+ channels at the AIS, respectively[48,49]. Electrophysiological measurements showed high densities of Na^+ channels after the channels had been uncoupled from the actin cytoskeleton by cytochalisin treatment[2]. Thus, in previous patch-clamp experiments[49] the channels had remained firmly anchored to the actin–spectrin–AnkG-based cytoskeleton instead of being incorporated into the pulled patch, which led to an underestimation of their density. Taken together, these results indicate that actin is actively involved in the maintenance of the AIS barrier and neuronal polarity (FIG. 3c).

Actin is enriched at the AIS[38], possibly through binding to the amino-terminal domain of βIV spectrin[20] (FIG. 2c). Thus, βIV spectrin may contribute to neuronal polarity by assembling and stabilizing the actin cytoskeleton at the AIS. Consistent with this idea, mutant mice lacking βIV spectrin have impaired axonal polarization[50]. However, as βIV spectrin is recruited to the AIS by AnkG[14], actin assembly at the AIS may ultimately depend on AnkG clustering.

Ankyrin G maintains neuronal polarity. As described above, AnkG is necessary to establish polarized membrane domains in the axon. Is AnkG also essential for maintaining a polarized subcellular organization? To directly test the role of AnkG in mature, polarized neurons, AnkG expression was suppressed in hippocampal neurons using short hairpin RNA[51]. When AnkG was lost from the axon, Na^+ channels, βIV spectrin and the AIS cell adhesion molecules NF186 and NrCAM could no longer be detected at the site at which the AIS was formerly located. This result shows that in addition to its role in the assembly of the AIS during development, AnkG maintains the molecular organization of the AIS in mature neurons. Thus, the unique extracellular matrix found at the AIS[52] is not sufficient to retain AIS-specific membrane proteins. Studies based on cell culture suggest that AnkG is also required for the assembly of nodes of Ranvier in the peripheral nervous system[53], but it is not known whether it is necessary to maintain these same nodal protein complexes *in vivo* or in the CNS.

If the AIS barrier depends on the high density of actin and membrane proteins found at the AIS (FIG. 3), AnkG is directly responsible for the assembly and maintenance of the barrier. To test this possibility, the polarized distribution of axonal and somatodendritic proteins was evaluated in mature hippocampal neurons in which AnkG expression was silenced[51] (FIG. 4a). Remarkably, in cells that lost AnkG, one process could be identified that had the molecular characteristics of both axons and dendrites; axons were identified by NF186 immunostaining and MAP2 was used to distinguish dendrites. In the 'axons' of the infected cells lacking AnkG, NF186 was only found far from the cell body, and MAP2 immunoeactivity could be detected several hundred micrometres along the proximal part of the former axon (FIG. 4c). This result suggests that somatodendritic proteins entered into the axon following the loss of AnkG from the AIS, causing a wave of dedifferentiation and a switch from axonal to dendritic properties. For example, the somatodendritic K^+/Cl^- co-transporter (KCC2, also known as SLC12A5), a membrane protein, was detected in the axonal domain[51]. Notably, these former axons developed spine-like protrusions (FIG. 4b) that were enriched in PSD95, a postsynaptic marker of excitatory synapses[51].

Independent and complementary experiments used mice that specifically lacked AnkG in cerebellar Purkinje neurons to determine the role of AnkG in neuronal polarity[54]. The unique anatomy of Purkinje neurons (which have an elaborate dendritic tree and a single axon) allowed the unequivocal identification of the axon opposite to the dendritic arbour, even in the absence of AnkG[54] (FIG. 4d). This suggests that AnkG is not required

for the developmental and anatomical specification of an axon. However, as in *in vitro* experiments, loss of AnkG caused axons to acquire dendritic spines with postsynaptic proteins and receptors (FIG. 4e,f). Finally, knockdown of AnkG in cultured hippocampal neurons also resulted in the loss of the cytoplasmic diffusion barrier, allowing large dextrans to enter the axon[40]. Taken together, these experiments support the conclusion that AnkG is required for the maintenance of distinct axonal and somatodendritic domains.

To summarize, the initial specification of the axon (that is, the establishment of axon–dendrite polarity) does not seem to depend on AnkG. However, after polarity has been established, AnkG is required not only to maintain subcellular polarity (that is, the clustering of AIS proteins) but also to maintain the distinction between axonal and somatodendritic domains. Finally, in the absence of AnkG axons acquire the molecular and structural features of a dendrite, and this finding supports the notion that the default identity for neural processes is dendritic.

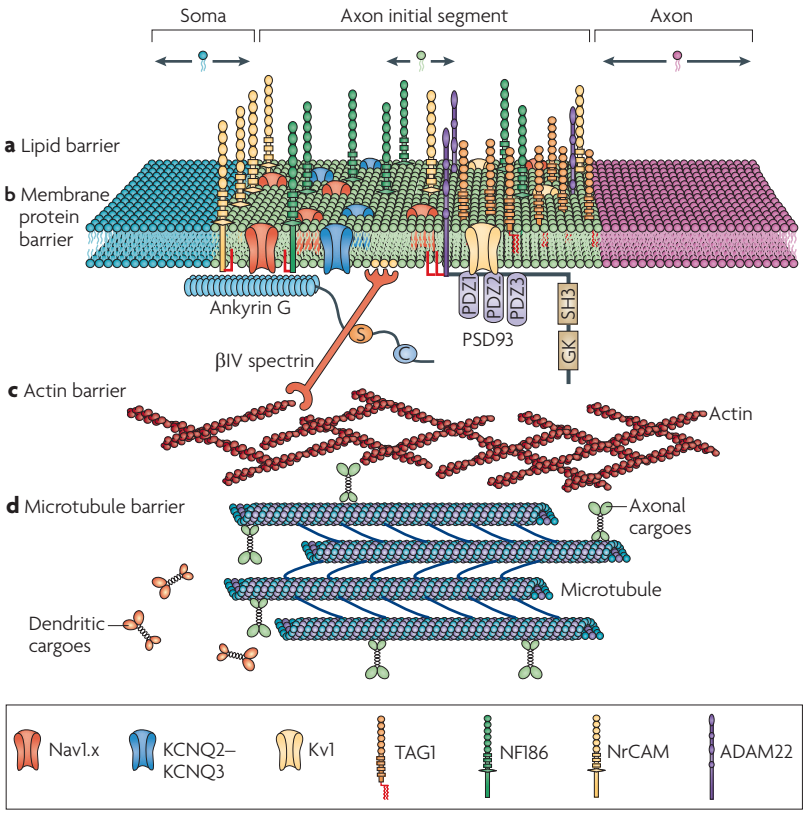


Figure 3 | **Molecular substrates of the axon initial segment barrier.** Four potential mechanisms have been proposed to contribute to the axon initial segment (AIS) barrier. **a** | The lipid composition of the AIS plasma membrane may directly influence mobility and diffusion rates in the AIS. The lipid composition can be directly regulated by post-translational modifications of membrane and cytoplasmic proteins or by phospholipid–cytoskeleton interactions. **b** | The high density of membrane proteins creates a membrane diffusion barrier that limits the lateral mobility of other transmembrane proteins and lipids at the AIS. The high density of these proteins is established through binding to ankyrin G. **c** | Actin filaments can contribute to the maintenance of neuronal polarity and the AIS barrier by limiting the entry of cytoplasmic proteins into the axon and by contributing to the maintenance of protein density at the AIS membrane through interactions with βIV spectrin. **d** | Microtubule fascicles with unique cross-bridges at the AIS allow axonal cargoes but not dendritic cargoes to enter the axon, thus contributing to the maintenance of neuronal polarity. ADAM22, a disintegrin and metalloproteinase domain-containing protein 22; GK, guanylate kinase; NF186, neurofascin 186; NrCAM, neuronal cell adhesion molecule; PDZ, PSD95/discs large/zonula occludens; PSD93, postsynaptic density protein 93; SH3, SRC homology 3; TAG1, transient axonal glycoprotein 1 (also known as contactin 2).

Polarized trafficking. Besides actin, βIV spectrin and AnkG, what other cytoskeletal constituents influence polarity? High-resolution electron microscopy shows that a cardinal feature of the AIS is its uniquely fasciculated microtubule network[55]. Consistent with the notion that microtubules may have important roles in polarity[56], their stabilization promotes the formation of multiple axons *in vitro*[57]. At present the molecular basis of microtubule fascicles at the AIS is unknown, and it is also unknown whether these fascicles are permissive or inhibitory to axonal trafficking of distinct cytoplasmic vesicles. However, electron microscopy analysis of the AIS of AnkG-deficient Purkinje neurons shows that proximal regions of the axon lack microtubule fascicles, suggesting that fascicles depend on AnkG and do not direct AnkG recruitment to the AIS[54].

Within the AIS, microtubules are highly polarized, and the fast-growing 'plus-end' is oriented away from the cell body. This orientation is thought to contribute to the polarized trafficking mechanisms that depend on specific kinesins, their distinct cargoes and the post-translational modifications of the microtubules along which they travel[40,58–60] (FIG. 3d). Microtubules at the AIS function as a preferential sorting site for kinesin-mediated polarized trafficking. This notion is supported by results from *in vitro* experiments using mutant forms of kinesin that cannot translocate or dissociate from the microtubules and that thereby reveal the site of their initial recruitment and interaction with microtubules[61]. For example, when a mutant form of the kinesin KIF5 was transfected into hippocampal neurons in culture, it accumulated at the AIS[61], indicating that the microtubule network at the AIS functions as a preferential docking site for KIF5. After binding to microtubules, motor proteins are thought to recognize cargoes that are destined for axons or dendrites through adaptor or scaffold proteins[60]. Thus, AIS microtubules also play an important part in the maintenance of neuronal polarity through polarized trafficking.

Another class of cytoskeletal proteins that could potentially influence neuronal polarity are neurofilaments, as these are highly expressed in axons but not in dendrites and can influence axonal transport[62]. However, transgenic mice in which neurofilaments are restricted to the cell soma develop axons with normal subcellular polarity (for example, intact nodes of Ranvier)[63], which suggests that neurofilaments are not crucial regulators of polarity or of AnkG localization.

Neuronal polarity and disease

The loss of neuronal polarity owing to structural and/or functional changes in AIS properties would be expected to have dramatic consequences for the overall function

of the nervous system. For example, even small changes in the density of Na^+ or K^+ channels could change the excitability of a neuron, and reduced integrity of the AIS barrier would be expected to affect the normal distribution of axonal and somatodendritic proteins in the cell. Although the maintenance of polarity is clearly essential for neurons, its disruption following disease or injury has not been considered to be a major contributor to dysfunctions in the nervous system. However, it was recently shown that both *in vitro* and *in vivo*, ischaemic injury causes rapid and specific proteolysis of AnkG and βIV spectrin[64] (FIG. 5a). Proteolysis of AnkG at the AIS was due to activation of the Ca^{2+}-dependent cysteine protease calpain and was accompanied by loss of Na^+ channels and neuronal polarity. After proteolysis of AnkG and βIV spectrin, MAP2 (which is normally restricted to the somatodendritic domain) was distributed throughout the axon[64]. Importantly, AnkG and βIV spectrin proteolysis preceded and occurred independently of cell death, suggesting that injured yet viable neurons can become non-functional owing to the loss of neuronal polarity and AIS Na^+ channels (FIG. 5b).

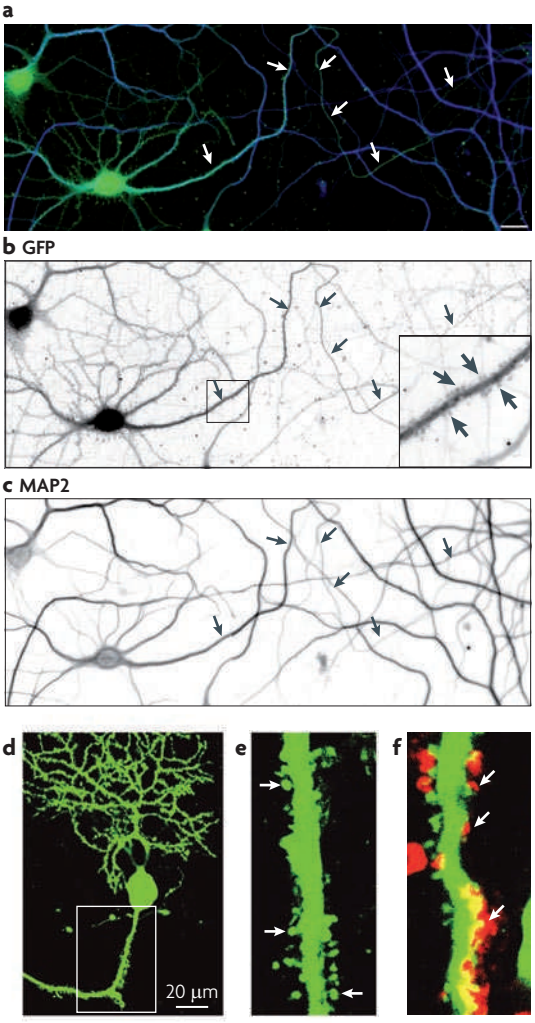


Figure 4 | **Ankyrin G is required to maintain neuronal polarity.** Micrograph (**a**) showing cultured hippocampal neurons infected with an adenoviral vector expressing green fluorescent protein (GFP) and a short hairpin RNA (shRNA) sequence for ankyrin G (AnkG, also known as ANK3) (the scale bar represents 20 µm). Ten days after infection, the axon (arrows) acquires dendritic features, such as spines (**b**, inset), and expresses the dendritic marker microtubule associated protein 2 (MAP2) (**c**). Purkinje neurons lacking AnkG develop an axon (**d**) that exhibits spine-like protrusions (indicated by arrows in **e**). These protrusions are contacted by neighbouring vesicular glutamate transporter 1 (VGLUT1)-labelled axonal terminals (arrows in **f**), which suggests that the protrusions form functional excitatory synapses. Parts **a**–**c** are modified, with permission, from REF. 51 © (2008) Rockefeller University Press. Parts **d**–**f** are modified, with permission, from REF. 54 © (2009) United States National Academy of Sciences.

However, it is well established that polarity can show plasticity in response to axonal injury, resulting in the conversion of a dendrite into an axon or the development of supernumerary axons[65–67] (FIG. 5c). In particular, a study using cultured hippocampal neurons[68] showed that the conversion of a dendrite into an axon was determined by the location of the injury. When the axon was cut far from the cell body, it regenerated and maintained its identity, but if the cut was located close to the cell body (35 µm or less) the stump of the axon degenerated and a random dendrite was converted into an axon. Although it was originally proposed that this switch depended on differences between the stability and post-translational modifications of microtubules[68], an equally plausible explanation for the switch is that the injuries located close to the cell body disrupt the AIS and, therefore, neuronal polarity. Thus, treatments for various forms of injury to the nervous system might benefit from incorporating polarity preserving or restoring strategies in addition to neuroprotective strategies. However, the possibility that loss of polarity, and loss of the AIS in particular, may be beneficial in some situations should also be considered. For example, loss of AnkG may promote neuronal survival by diminishing excitotoxicity through the loss of Nav channels[64]. Alternatively, loss of polarity may actually drive the development of a new axon that can regenerate through or around the site of the injury. For example, after spinal cord injury in cats, spinal interneurons develop an axon from a distal dendrite, and these new axons can traverse through the site of the injury[66,67].

The examples given above suggest that disruption of the proteins that form the AIS may be an important contributor to nervous system dysfunction after injury. Loss of neuronal polarity may also have a role in the pathophysiology of mental or cognitive disorders. For example, recent genome-wide association analyses identified the genes that encode AnkG and contactin-associated protein-like 2 (CASPR2, which is also enriched at the AIS[69]) as major susceptibility loci for bipolar disorder, autism spectrum disorders and mental retardation[70,71]. Whether the manifestation of these diseases is in any way related to altered polarity, to altered density of ion channels or to AIS function is currently unknown.

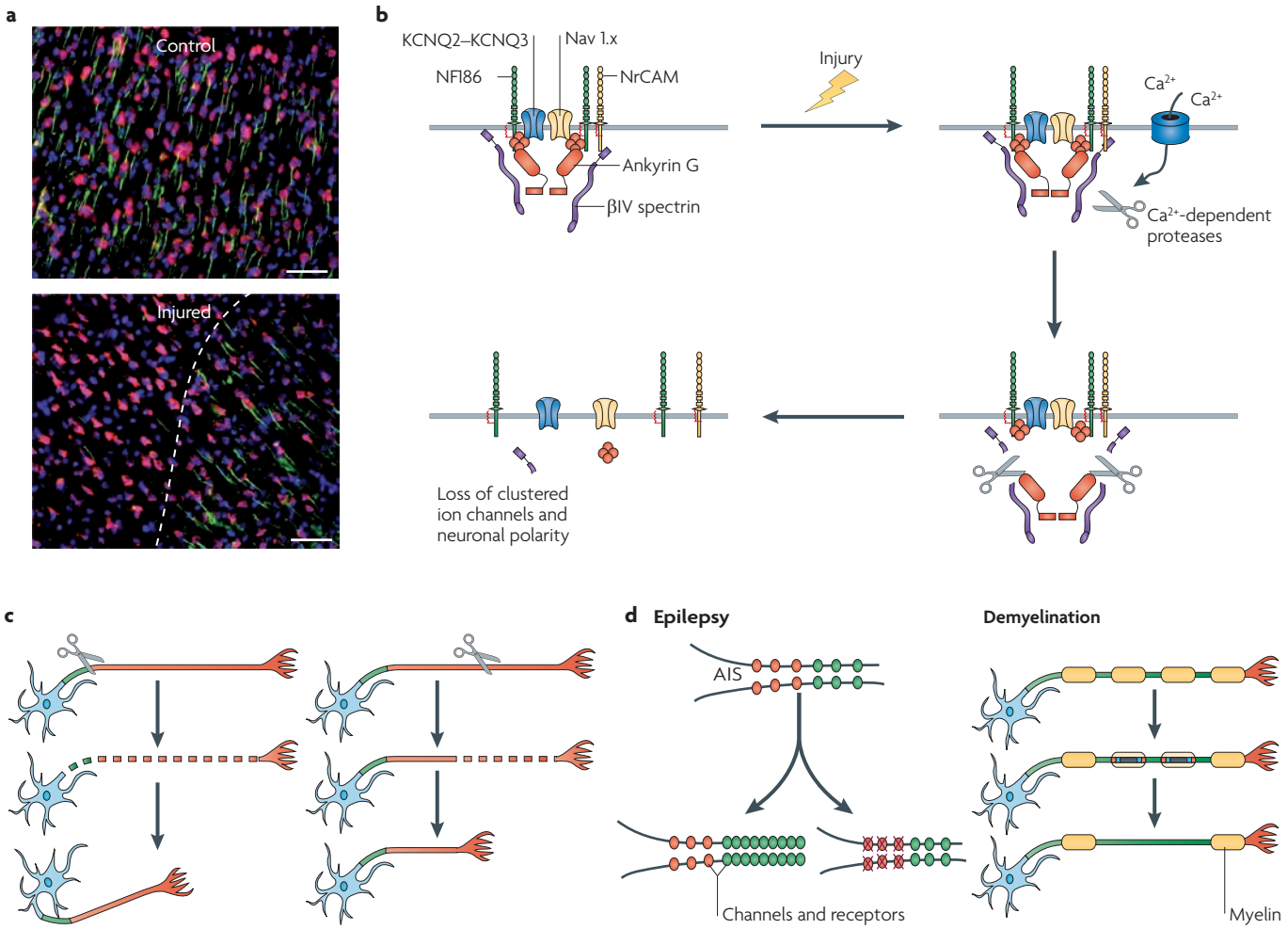


Figure 5 | **Nervous system injury and disease alters neuronal polarity. a** | Ischaemic injury (that is, stroke) disrupts the axon initial segment (AIS) cytoskeleton. The micrograph shows AIS stained for the cytoskeletal protein βIV spectrin (green), neurons labelled with NeuroTrace (red) and nuclei labelled with Hoechst stain (blue). The dashed line indicates the transition from the injured brain, in which the AISs are missing, to the area not exposed to ischaemia, where the AISs remain intact. The scale bars represent 50 μm. **b** | The cascade of events leading to loss of neuronal polarity after injury. Injury increases cytoplasmic Ca^{2+}, which activates the Ca^{2+}-dependent cysteine-protease calpain. Calpain (indicated by the scissors) cleaves ankyrin G (AnkG, also known as ANK3) and βIV spectrin, leading to the declustering of ion channels and loss of polarity. **c** | The type and location of axonal injury determines the consequence for neuronal polarity. Axonal transection near the cell body causes a dendrite-to-axon identity switch (left panel), whereas transection far from the cell body does not (right panel). **d** | Disruption of channel and receptor density, function or location can cause or result from altered nervous system function. For example, epilepsy can increase channel densities (left) or result from altered AIS channel function (right). Demyelination leads to altered subcellular polarity of axons. Parts **a** and **b** are modified, with permission, from REF. 64 © (2009) The Society for Neuroscience.

A link between schizophrenia and alterations in the AIS has also been proposed on the basis of the observation that inhibitory chandelier neurons, which form unique GABA (γ-aminobutyric acid)-ergic synapses on the AIS of cortical pyramidal neurons, showed reduced GABA release in patients with schizophrenia[72]. As a result, these patients have dramatically increased levels of α2 subunits of $GABA_A$ receptors at the AIS. Intriguingly, one study showed decreased AnkG levels in superficial cortical layers of individuals with schizophrenia[73], and loss of AnkG (leading to mislocalized NF186) has been shown to impair the formation of GABAergic synapses at the AIS of Purkinje neurons in mice[74]. However, the observed increase in $GABA_A$ receptor α2 subunits at the AIS seems to be paradoxical, as loss of NF186 at the AIS prevents the clustering of gephyrin, the postsynaptic scaffolding protein that is required for clustering GABA receptors[75]. In any case, an appropriate level of GABAergic signalling at the AIS seems to depend on the polarized localization of NF186 through its interactions with AnkG, and disruptions in these interactions may contribute to schizophrenia.

Altered function of Na^+ and K^+ channels at the AIS can also lead to severe CNS impairments. For example, loss-of-function mutations in the gene encoding the Na^+ channel Nav1.1 cause severe myoclonic

epilepsy in infancy owing to defects at the level of the AIS in parvalbumin-positive inhibitory interneurons[76]. Moreover, mutations in *Nav1.2* (also known as *SCN2A*), *Navβ1* (also known as *SCN1B*) and the K^+ channel genes *KCNQ2* and *KCNQ3* lead to generalized epilepsies[77]. Intriguingly, expression levels of the Na^+ channel Nav1.6 and AnkG were reported to be increased at the AIS in an animal model for epilepsy[78]. These results suggest that altered CNS activity by itself can induce changes in the molecular composition of the AIS that might contribute to ongoing pathophysiology. The dysregulation of ion channel expression has previously been proposed as a new form of channelopathy[79]. A subset of these 'acquired channelopathies' may be a consequence of disrupted polarity due to altered channel and/or receptor density or localization (FIG. 5d).

Disruption of subcellular polarity at nodes of Ranvier can be another important contributor to dysfunctions in the nervous system. For example, in multiple sclerosis, demyelination leads to the dissolution and loss of Na^+ channel clusters at the nodes, the dismantling of paranodal junctions and the loss of the clustered K^+ channels that reside beneath the myelin sheath and flank the nodes in a region known as the juxtaparanode[80,81] (FIG. 2b). The abnormal localization and density of Na^+ channels in demyelinated axons has been proposed to be a major contributor to the axonal degeneration seen in individuals with multiple sclerosis[82]. However, some patients with multiple sclerosis generate antibodies against NF186 (REF. 83), and animal models suggest that the binding of antibodies against NF186 to the nodes of Ranvier (and possibly the AIS) can cause axon degeneration. Therefore, this may represent an alternative mechanism to demyelination for the disruption of cellular polarity at nodes of Ranvier, which in turn might contribute to the progression of the disease. In autoimmune disorders of the peripheral nervous system, such as acute motor axonal neuropathy (AMAN), a variant of Guillain–Barré syndrome, and experimental allergic neuritis, the nodes of Ranvier are specifically disrupted, which leads to blocked action potential conduction[84,85]. Taken together, these examples show how disruption and/or altered functioning of the AIS or other polarized axonal membrane domains can contribute to the pathophysiology of many diseases of the nervous system.

Plasticity of axon initial segment properties

As maintenance of neuronal polarity is important for the functioning of the nervous system, it might be assumed that neuronal polarity and the subcellular organization of axons are static and unchanging. However, recent data show that the structural and functional organization of the AIS and the nodes of Ranvier are much more dynamic than was originally thought and are a previously unrecognized substrate for neuronal plasticity. Some ways in which AIS properties are modulated include the differential expression of ion channels, gradients of ion channels within the AIS, expression of accessory subunits, ion channel phosphorylation, altered length and position (relative to the cell body) of the AIS and GABAergic innervation of the AIS[8,13,24,78,86–90]. However, the mechanisms that regulate these dynamic and plastic properties of the AIS are mostly unknown.

AIS plasticity has been clearly demonstrated in the avian auditory system. For example, in neurons found in the nucleus laminaris, which have important roles in sound localization, both the length and the position of the AIS are regulated to achieve the highest sensitivity to interaural time difference. Specifically, the position of the AIS changes depending on the characteristic sound frequency to which each neuron responds[86]. Subsequent studies have shown that deprivation of presynaptic activity can directly regulate the length and position of the AIS[90]. Similarly, simple chronic depolarization, or chronic stimulation, of cultured mammalian neurons can cause a substantial proximal–distal shift in the locations of AnkG, βIV spectrin and Na^+ channels[89]. This change in AIS position is accompanied by substantial increases in the current thresholds required to induce spiking behaviour. Thus, the position of the AIS can be 'tuned' for optimal function in an activity-dependent manner, therefore providing a powerful feedback mechanism to efficiently regulate neural activity.

It is interesting to speculate about the mechanisms underlying such plasticity of AIS function and the conditions under which it might occur. Although synaptic plasticity can rapidly fine-tune neuronal properties and circuits[91], the extremely stable nature of AIS proteins[51] suggests that changes occurring at the AIS are likely to take much longer than that, and probably occur over hours or days. Consistent with this idea, a 17 μm shift in AIS location requires two days of chronic membrane depolarization[89]. Furthermore, as the stability and half-life of AIS membrane proteins depend on AnkG, changes in AIS properties are likely to reflect changes in AnkG properties rather than dynamic changes in expression levels of other AIS proteins, such as Na^+ or K^+ channels. Intriguingly, *in vitro* studies of AIS plasticity indicate that T- and/or L-type voltage-gated Ca^{2+} channels are required for the translocation of the AIS to more distal axonal locations[89]. This is reminiscent of the Ca^{2+}-dependent dismantling of the AIS under injury conditions[64]. It will be interesting to determine whether the physiological conditions that modulate the properties of the AIS impinge directly on the stability, turnover rate and/or expression level of AnkG. Exceptions to this could include rapid changes in the phosphorylation state of channels or other proteins that could have an immediate impact on a channel's biophysical properties, on a channel's ability to interact with AnkG and on neuronal function.

Another remarkable example of how function can influence the subcellular polarity of axons is seen in the myelinated CNS axons that conduct action potentials encoding interaural time differences. Previous models used to explain sound localization suggested that coincidence detection relied solely on axon length to provide temporal offset[92]. However, careful anatomical measurements of axon length indicated that axon length could not account for the high fidelity of coincidence detection[93]. Instead, both the spacing between nodes and the diameter of axons were regulated to provide the

Paranodal junctions
Major sites of physical interaction between myelin-forming glial cells and the axon. They are located on either side of the nodes of Ranvier and are characterized by septate-like junctions.

Juxtaparanodes
Regions beneath the myelin sheath and flanking each node of Ranvier where voltage-gated K^+ channels are clustered.

Interaural time difference
The time difference for the arrival of a sound between two ears.

Coincidence detection
A mechanism whereby neurons encode information by detecting two simultaneous inputs from distinct sources.

temporal offset. As node assembly, node spacing and axon diameter are controlled by myelinating glia[9,94,95], these data suggest that activity-dependent neuron–glia interactions shape the polarized organization and structure of the axon. It is currently unknown how this occurs, how neurons measure distances between nodes and whether other circuits in the CNS also actively regulate nodal spacing.

The future of polarity

As described in this Review, the AIS is a key regulator of neuronal polarity. Therefore, future experiments should focus on how the AIS performs this function. However, the molecular mechanisms that regulate AIS assembly (upstream of AnkG) and plasticity in both health and disease remain poorly understood. Important unanswered questions include: what mechanisms recruit AnkG to the AIS? How does actin create a barrier at the AIS? What molecular 'tags' permit vesicular cargo to bypass the AIS barrier and enter the axon, and what prevents other cargoes from entering? What physiological properties and molecular mechanisms control the density, composition and gradients of channels within the AIS? How can neuronal polarity be preserved or restored after injury?

And what neural circuits are influenced by plasticity of polarity, plasticity of the AIS and nodal properties? To help resolve these questions, further research could be based on our knowledge of another highly polarized structure in the nervous system: the synapse. Rapid advances in our understanding of synaptic structure, function and plasticity have been made possible by the advent of electrophysiological, proteomic and molecular methods that have defined hundreds of synaptic proteins, their interactions and their functions in neuronal physiology. However, the scarcity of proteins described at the AIS and the limited methods for studying AIS physiology have been major impediments to determining the mechanisms underlying AIS structure, function and plasticity. Therefore, a transformation in our understanding of neuronal polarity and the mechanisms underlying its plasticity will begin with the identification and functional characterization of the proteins found at the AIS under normal and pathological conditions. In addition, we need to develop model systems that enable the electrophysiological characterization and manipulation of AIS properties. These efforts will no doubt reveal fascinating AIS-based mechanisms that maintain and regulate neuronal polarity.

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Competing interests statement

The author declares no competing financial interests.

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FURTHER INFORMATION

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