Published in final edited form as:

Dev Neurobiol. 2011 June ; 71(6): 474–482. doi:10.1002/dneu.20843.

Maintenance of Neuronal Polarity

Tammy Szu-Yu Ho¹ and Matthew N. Rasband^{1,2}

¹ Program in Developmental Biology and Department of Neuroscience

² Baylor College of Medicine, Houston, TX 77030

Abstract

Neurons are highly polarized cells with distinct domains responsible for receiving, transmitting, and propagating electrical signals. Central to these functions is the axon initial segment (AIS), a short region of the axon adjacent to the cell body that is enriched in voltage-gated ion channels, cell adhesion molecules, and cytoskeletal scaffolding proteins. Traditionally, the function of the AIS has been limited to its role in action potential initiation and modulation. However, recent experiments indicate that it also plays essential roles in neuronal polarity. Here, we review how initial segments are assembled, and discuss proposed mechanisms for how the AIS contributes to maintenance of neuronal polarity.

Keywords

action potential; scaffold; cell adhesion molecule; ion channel

Introduction

After neurons receive information from presynaptic cells, the signals are transmitted through the dendrites and cell body, along the axon, and finally to the next cell in the circuit. This directional propagation of signals depends on the fact that neurons are polarized with two morphologically and functionally distinct domains: the somatodendritic and axonal domains. The somatodendritic domain receives and integrates input from presynaptic cells, whereas the axon sends output to target cells. Although disparate in both location and function, these two domains are connected by a single structure: the axon initial segment (AIS). The AIS functions as the site where the action potential is generated, and is roughly located at the beginning of the axon between the axon hillock and the start of the myelin sheath (Fig. 1; Ogawa and Rasband, 2008). Besides axon-dendrite polarity, neuronal membranes can be further subdivided into subcellular domains (which constitutes yet another form of polarity). These include the AIS, the nodes of Ranvier of myelinated axons, and both pre- and post-synaptic sites.

Dendrites, axons, and subcellular membrane domains consist of unique sets of proteins that perform essential functions in the integration of synaptic inputs and the initiation and propagation of action potentials. Given the importance of polarity, many studies have focused on uncovering the mechanisms that establish axon-dendrite polarity, and the subsequent assembly of membrane domains. Many factors involved in axon specification have been identified using *in vitro* and *in vivo* models. Together, these studies have revealed that both extracellular cues and intracellular signaling pathways are critical to establish

neuronal polarity *in vivo* (Barnes and Polleux, 2009). However, in contrast to the efforts to uncover developmental mechanisms of neuronal polarity, how polarity is maintained throughout the life of a neuron has received much less attention. Recently, several important studies demonstrated that besides its role in action potential initiation, the AIS functions to maintain axon-dendrite polarity (Hedstrom et al., 2008; Sobotzik et al., 2009; Song et al., 2009). In this review, we discuss the molecular organization and assembly of the AIS, and how this organization is necessary to maintain neuronal polarity both in health and disease.

AnkyrinG: master organizer of the AIS

Although the AIS has long been considered an essential subcellular domain for neuronal function, only recently has it begun to receive renewed attention. The resurgence in interest derives from the identification of several proteins that are found to cluster at the AIS. Subsequently, a number of studies have focused on the physiology of the AIS, plasticity of the AIS, AIS synapses, assembly of the AIS, and the importance of the AIS in neuronal polarity and injury (Ango et al., 2004; Khaliq and Raman, 2006; Goldberg et al., 2008; Kole et al., 2008; Schafer et al., 2009).

One characteristic feature is that many different kinds of proteins are clustered in very high densities at the AIS (Fig. 1). These AIS proteins include voltage-gated sodium (Nav) and potassium (Kv) channels (Fig. 1A), L1 family cell adhesion molecules (CAM; NF-186 and NrCAM), and the cytoskeletal proteins β IV spectrin and ankyrinG (ankG)(Fig. 1; Kordeli et al., 1995; Davis et al., 1996; Berghs et al., 2000; Devaux et al., 2004; Kole et al., 2008). Among the known AIS proteins, ankG is the master organizer for AIS assembly (Fig. 2A; Zhou et al., 1998; Jenkins and Bennett, 2001; Hedstrom et al., 2007). Two ankG isoforms, 480-kDa and 270-kDa ankG, have been found specifically localized at the AIS (Fig. 2Ai) and nodes of Ranvier (Kordeli et al., 1995). AnkG contains several domains including membrane-binding and spectrin-binding domains. The cytoplasmic domains of NF-186 and NrCAM have a highly conserved sequence (FIGQY) that binds to ankG's membrane-binding domain (Tuvia et al., 1997), whereas localization of β IV spectrin (Fig. 1B) to the AIS requires its 15th spectrin repeat which interacts with the spectrin-binding domain of ankG (Yang et al., 2007).

Action potential initiation requires clustered Nav channels at the AIS (Kole et al., 2008). Nav channel subtypes clustered at the AIS include Nav1.1, Nav1.2 and Nav1.6, although different types of neurons can have different kinds of channels at the AIS (Boiko et al., 2003; Van Wart et al., 2007; Hu et al., 2009). A variety of Kv channels are also enriched at the AIS including KCNQ2/3 channels and Kv1 channel protein complexes (Devaux et al., 2004; Ogawa et al., 2008). The Nav channels and Kv KCNQ2/3 channels share a similar AIS-targeting motif that is essential for binding to ankG (Garrido et al., 2003; Lemaillat et al., 2003; Pan et al., 2006). During development of many neurons, Nav1.2 channels are expressed first and Nav1.6 channels appear later in the more mature AIS (Boiko et al., 2003). Furthermore, in cortical pyramidal neurons high-threshold Nav1.2 channels cluster at the proximal AIS and are responsible for action potential backpropagation, while action potentials are thought to originate from the distal part of the AIS, where low-threshold Nav1.6 channels accumulate (Hu et al., 2009). However, although these channels share a common ankG-targeting motif, the mechanisms regulating these additional levels of subcellular specialization within the AIS and the temporal expression of distinct Nav channel isoforms are unknown.

In ankG cerebellum-specific knockout mice, Nav channels are no longer enriched at the AIS of Purkinje neurons and granule cells (Zhou et al., 1998; Jenkins and Bennett, 2001). Functionally, loss of ankG causes Purkinje neurons to have impaired firing of action

potentials and a higher action potential threshold. Similarly, silencing of ankG expression in cultured hippocampal neurons blocks clustering of all other AIS proteins (Hedstrom et al., 2007). Recently, the protein kinase CK2 was shown to be enriched at the AIS and nodes where it phosphorylates Nav channels and greatly increases Nav channel affinity for ankG (Bréchet et al., 2008). Thus ankG acts as master scaffold and recruits many AIS proteins (Fig. 2Aii). However, despite its central role in AIS assembly, how ankG is recruited to the AIS, and when ankG is clustered at the AIS during axon specification *in vivo* remains unknown.

Besides Nav and KCNQ2/3 Kv channels, Kv1 channels are also enriched in the distal segment of the AIS (Inda et al., 2006; Lorincz and Nusser, 2008; Ogawa et al., 2008). These Kv1 channel protein complexes play important roles in regulating action potential threshold and frequency of firing (Kole et al., 2007; Goldberg et al., 2008). These Kv1 channels comprise a protein complex that has been most thoroughly studied at juxtaparanodal regions of myelinated axons (Rasband, 2004); Kv1 channels are excluded from nodes of Ranvier that, like the AIS, are enriched in ankG and Nav channels. Thus, it was a surprise when Kv1 channels and their associated proteins were found colocalized with ankG and Nav channels at the AIS. These protein complexes consist of the cell adhesion molecules Caspr2, TAG-1, and ADAM22, and the cytoskeletal scaffold PSD-93 (Ogawa et al., 2010). At juxtaparanodes, Caspr2 and TAG-1 are required for channel clustering. However, these cell adhesion molecules are dispensable for Kv1 channel clustering at the AIS. Intriguingly, mutations in Caspr2 have been linked to autism spectrum disorder, epilepsy, and mental retardation (Strauss et al., 2006; Bakkaloglu et al., 2008; Zweier et al., 2009). Based on the importance of the scaffolding protein ankG for clustering Nav channels at the AIS, Ogawa et al. (2008) examined the role of PSD-93 in Kv1 channel clustering at the AIS. They found that acute silencing of PSD-93 by shRNA knockdown blocked the clustering of Kv1 channels at the AIS. However, in a follow-up paper they also showed that PSD-93 null mice still had AIS Kv1 channels (Ogawa et al., 2010), indicating that other mechanisms may exist *in vivo* that can compensate for loss of PSD-93 and also cluster Kv1 channels. Together, these results emphasize the idea that scaffolding proteins are key determinants of the molecular assembly of the AIS, and that AIS are assembled from the “inside-out” rather than the “outside-in” (Fig. 2A). Future experiments will be needed to determine the dependence of PSD-93 and Kv1 channel clustering on ankG.

The AIS as a sorting station for protein trafficking

Once neurons are polarized during early development and axon specification has occurred, distinct sets of proteins are needed in axons and dendrites to carry out the unique functions of each domain. These proteins are selectively trafficked to, and accumulate within, unique cellular domains in ways that are just beginning to be described. One important mechanism contributing to polarized protein distribution utilizes the kinesin superfamily (KIF) motor proteins to selectively transport vesicular cargoes and proteins along microtubules. In neurons, microtubules are highly polarized with the ‘plus-end’ orientated away from the cell body, and the spacing between microtubules is larger in dendrites than in axons. The difference in spacing may reflect unique microtubule associated proteins found only in axonal or somatodendritic domains. For example, the microtubule associated protein 2 (MAP2) is restricted to somatodendritic domains and is often used as a very reliable marker of this domain. Fascicles of microtubules are the primary ultrastructural feature of the AIS, and until the advent of molecular markers for AIS proteins, was the only method to clearly distinguish AIS from other axonal regions (Palay et al., 1968). Motor proteins recognize distinct cargoes and transport them to their appropriate domains. For example, KIF17 and KIF5 transport NMDA and AMPA receptors, respectively, to dendrites, whereas KIF1 transports synaptic vesicle precursors to axons (for a detailed review on motor-dependent

directional transport in neurons see Hirokawa and Takemura, 2005). AIS microtubules may also be a preferred docking site for kinesin-mediated sorting. This idea finds experimental support in the observation that mutant forms of kinesin that can bind to microtubules, but not translocate along the microtubules, accumulate at the AIS (Nakata and Hirokawa, 2003). Furthermore, when cultured hippocampal neurons were transfected with a protein that binds to the tips of growing microtubules, it accumulated at the AIS (Nakata and Hirokawa, 2003). Taken together, these observations strongly support the notion that the AIS functions as a sorting station to either exclude, or permit, the directional, active transport of distinct cargoes into the axon.

The AIS as a barrier

An alternative mechanism to establish asymmetric, polarized distributions of proteins is through their selective elimination (or retention) via endocytosis (Sampo et al., 2003). For example, Na⁺ channels may be inserted into the plasma membrane of somatodendritic compartments, but they are retained in high density only at the AIS through selective retention by interaction with ankG. Without ankG binding, the channels are thought to be endocytosed and removed (Fache et al., 2004). However, even after proteins achieve a polarized distribution, without some mechanism to maintain their asymmetric locations, axonal and somatodendritic proteins would diffuse into inappropriate compartments where they would mix with one another. Thus, some mechanism should exist to compartmentalize these two domains and to prevent diffusional mixing.

In fact, previous studies showed that a barrier does exist, and that this barrier is located at the intersection between the somatodendritic and axonal domains, or the AIS (Fig. 2B). The AIS barrier restricts plasma membrane and cytoplasmic proteins, and it even regulates the lipid composition of each domain. Kobayashi et al. (1992) were the first to demonstrate the existence of a diffusion barrier in neurons. They fused liposomes containing fluorescent lipids with the membrane of axons in cultured hippocampal neurons and found that labeled lipids only distributed along the axon and were excluded from the membrane of dendrites and the cell body. Later, Nakada et al. (2003) followed the diffusion of individual phospholipids in the plasma membrane of cultured hippocampal neurons. They found the mobility of lipids is much less in AIS membranes, as compared to somatodendritic or axonal domains. In addition to lipids, AIS membrane proteins also have a reduced lateral mobility. Here, the CAM L1 was thought to be tethered to the cytoskeleton (L1 has an FIGQY ankG-interacting motif), thereby reducing its mobility in this domain. However, the GPI-anchored protein Thy1 was also shown to have a reduced lateral mobility. Its restricted movement in the membrane was proposed to be due to obstruction by the high density of AIS membrane proteins (Winckler et al., 1999). Although the mechanism responsible for this reduced mobility remains unknown, these examples demonstrate the presence of a barrier in the membrane of the AIS that acts on both lipids and membrane proteins.

In addition to lipids and membrane proteins, the diffusion barrier also extends to the AIS cytoplasm. Song et al. (2009) injected 10kD and 70kD fluorescence-labeled dextrans into the soma of cultured hippocampal neurons to examine their rates of diffusion into the axon. They found that in polarized neurons, 10kD dextrans spread throughout the axon and dendrites, but 70kD dextrans were excluded from the axon. In addition, they used fluorescence recovery after photobleaching (FRAP) to measure the diffusion rate of GFP at the AIS and more distal axon. They found that the recovery rate of GFP was significantly lower in the AIS than in the distal axon. Taken together, these results indicate that a barrier functions in both the plasma membrane and cytoplasm of the AIS to restrict proteins and lipids to somatodendritic or axonal domains (Fig. 2B).

How does the AIS function as a barrier to maintain neuronal polarity?

Fujiwara et al. (2002) first proposed a general model to explain how phospholipids can have a much lower diffusion rate in cells than expected based on measurements in pure lipid membranes. They proposed an anchored membrane-protein picket fence model where membrane phospholipids are temporarily restricted or confined to small membrane domains or compartments. They later applied this model to the AIS (Nakada et al., 2003), and proposed that the high density of membrane proteins at the AIS blocks the diffusion of lipids and membrane proteins through steric hindrance and hydrodynamic friction. Furthermore, they demonstrated that these compartments depend on polymerized actin, strongly implicating the underlying cytoskeleton in regulating phospholipid diffusion rates, even in the outer leaflet of the membrane bilayer (Fujiwara et al., 2002; Nakada et al., 2003). Consistent with this idea, Nakada et al. (2003) also showed that like ankG and β IV spectrin, actin is highly enriched at the AIS, and the establishment of the lipid barrier coincided with the clustering of AIS proteins.

The idea that actin plays a key role in the barrier function of the AIS is supported by several lines of evidence. First, partial depolymerization of actin with latrunculin increased the mobility of phospholipids in the AIS membrane, whereas stabilization of actin filaments with jasplakinolide decreased the mobility of phospholipids in the AIS membrane (Nakada et al., 2003). Second, Winckler et al. (1999) showed that by disrupting actin, the distribution of the L1 CAM homologue NgCAM, which is normally restricted to axons of control neurons, became randomized to both axons and dendrites. Third, disruption of actin allowed 70kD dextrans to enter the axon after somatic loading, while these same dextrans are excluded from the axon in control neurons (Song et al., 2009). And fourth, the GFP diffusion rate at the AIS increased with latrunculin treatment (Song et al., 2009). Taken together, these data strongly support the notion that actin plays a key role in regulating the asymmetric distribution of both membrane proteins and lipids, as well as cytoplasmic proteins and cargoes (Fig. 2Bi).

AnkyrinG maintains neuronal polarity

As described above, ankG is essential for clustering of AIS membrane proteins. If the anchored membrane-protein picket fence model is correct, then ankG should be necessary for the asymmetric distribution of phospholipids and membrane proteins, and may even be an important regulator of neuronal polarity, since ankG recruits β IV spectrin to the AIS (Yang et al., 2007), and β IV spectrin binds to actin via its N-terminal actin-binding domain. Thus, ankG is ideally positioned to link the membrane barrier to the cytoplasmic barrier. Indeed, β IV spectrin-deficient mice show impaired neuronal polarity, with the L1 cell adhesion molecule no longer being restricted to axonal domains (Nishimura et al., 2007). To first test the role of ankG in *maintaining* AIS structure, Hedstrom et al. (2008) silenced ankG expression in fully polarized cultured hippocampal neurons using virus to transduce ankG shRNA. Loss of ankG in hippocampal neurons caused disassembly of the AIS, including loss of clustered Nav channels, NF186, NrCAM and β IV spectrin. Thus ankG is necessary for both AIS assembly and maintenance. Hedstrom et al. (2008) also determined if loss of ankG affected *maintenance* of neuronal polarity by examining the asymmetric distribution of somatodendritic proteins. They found that upon dismantling the AIS, the former axon acquired many of the molecular and structural features of dendrites, including the proteins MAP2 and the K⁺/Cl⁻ cotransporter KCC2 (normally restricted to somatodendritic domains), and the former axon even developed spines enriched in PSD-95 (Fig. 2Bii). Consistent with these *in vitro* results, Sobotzik et al. (2009) showed that ankG is also necessary to maintain neuronal polarity *in vivo*. Using mice with ankG-deficient Purkinje neurons, they were able to identify glutamatergic, axonal spines enriched with postsynaptic

density protein ProSAP1/Shank2. Ultrastructural analyses of these ankG-deficient Purkinje neurons showed many of the characteristic features of the AIS were absent, including the dense undercoating and fascicles of microtubules. Importantly, in these ankG-deficient Purkinje neurons the axon was still identifiable as a single process opposite the large dendritic tree. This suggests that ankG is not required to break symmetry and specify an axon. Taken together, these results show that ankG is necessary for 1. assembly and maintenance of the unique ion channel protein complex at the AIS, and 2. maintenance of axon-dendrite polarity.

The AIS and maintenance of polarity in human diseases

As described above, disruption of the AIS cytoskeleton by genetic or pharmacological treatments causes loss of neuronal polarity and loss of the highly concentrated Nav channel clusters necessary for action potential initiation. Therefore, diseases or injuries that affect the structure or function of the AIS cytoskeleton would be expected to have profound consequences for nervous system function. For example, when ankG is not present in Purkinje neurons, mice exhibit tremors, progressive ataxia, and loss of polarity (Zhou et al., 1998; Sobotzik et al., 2009). Furthermore, genome wide association studies have linked ankG to bipolar disorder (Ferreira et al., 2008), emphasizing the importance of these cytoskeletal proteins for normal nervous system function. Recently, Schafer et al. (2009) demonstrated that disruption of the ankG/ β IV spectrin-based AIS cytoskeleton is a novel mechanism for nervous system injury. Specifically, they showed that after ischemic neuronal injury, both ankG and β IV spectrin are proteolyzed by calpain, a calcium-dependent cysteine protease (Fig. 2Ci). Proteolysis of the AIS cytoskeleton caused AIS disassembly including loss of Nav channel clusters, followed by loss of axon-dendrite polarity. The effect of injury on AIS integrity was both rapid and irreversible, occurred before cell death, and was not due to axon degeneration. These data suggest that additional, previously unappreciated causes of nervous system dysfunction after injury may include loss of the ion channel complexes necessary to integrate and initiate action potentials, as well as loss of normal neuronal polarity. Thus, therapeutic strategies aimed at nervous system repair may also require preservation of the AIS cytoskeleton as an important component of any treatment.

Conclusion

The AIS is a fascinating example of a cellular structure that regulates a myriad of neuronal properties including excitability and maintenance of polarity. Recent work also strongly suggests that the AIS may be much more malleable than originally thought, and that its rearrangement may even underlie previously unappreciated forms of non-synaptic neuronal plasticity. For example, Grubb and Burrone (2010) demonstrated that membrane depolarization or patterned activity can cause the physical translocation of the AIS to more distal regions of the axon (Fig. 2Di). This translocation caused neurons to have much higher current thresholds for action potential initiation. Similarly, Kuba et al. (2010) showed that deprivation of auditory inputs caused neurons in the avian brainstem to increase the size of their AIS, resulting in a more excitable cell (Fig. 2Dii). Finally, Fried et al. (2009) found that the size and location of the AIS varied among functional classes of retinal ganglion cell types. Together, these studies suggest that the properties of the AIS are plastic, and can be tuned to the unique requirements of the neuron. Furthermore, these results emphasize that the AIS can dramatically shape the input-output function of a neuron. Although the molecular mechanisms underlying changes in the location and size of the AIS are not known, the changes are reversible and Ca^{2+} dependent (Grubb and Burrone, 2010). Given ankG's central role in regulating the distribution of ion channels, we propose that these plastic changes converge on the stability and location of ankG. It will be interesting to

determine if altered activity results in regulated dismantling and re-assembly of the AIS cytoskeleton (reminiscent of the Ca²⁺-dependent dismantling after nervous system injury), or if changes converge on the actin cytoskeleton. It will also be interesting to determine if under these conditions where plastic changes occur, whether neuronal polarity is also affected.

In summary, the AIS is a specialized subdomain of neurons at the center of both functional and morphological polarity. It is designed to integrate and initiate electrical signals, and to regulate the asymmetric, polarized distribution of ion channels, membrane proteins, lipids, cytoplasmic proteins, and trafficking of organelles and vesicles. Central to these functions is the AIS cytoskeleton, whose assembly depends most critically on the large scaffolding protein ankG. Much remains unknown about how the AIS cytoskeleton regulates these various neuronal properties, but future studies will no doubt reveal exciting and unexpected mechanisms.

Acknowledgments

Supported by NIH grant NS044916. MNR is a Harry Weaver Neuroscience Scholar of the National Multiple Sclerosis Society.

References

- Ango F, di Cristo G, Higashiyama H, Bennett V, Wu P, Huang ZJ. Ankyrin-based subcellular gradient of neurofascin, an immunoglobulin family protein, directs GABAergic innervation at purkinje axon initial segment. *Cell*. 2004; 119:257–272. [PubMed: 15479642]
- Bakkaloglu B, O'Roak BJ, Louvi A, Gupta AR, Abelson JF, Morgan TM, Chawarska K, Klin A, Ercan-Sencicek AG, Stillman AA, Tanriver G, Abrahams BS, Duvall JA, Robbins EM, Geschwind DH, Biederer T, Gunel M, Lifton RP, State MW. Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. *Am J Hum Genet*. 2008; 82:165–173. [PubMed: 18179895]
- Barnes AP, Polleux F. Establishment of axon-dendrite polarity in developing neurons. *Annu Rev Neurosci*. 2009; 32:347–381. [PubMed: 19400726]
- Berghs S, Aggujaro D, Dirkx R, Maksimova E, Stabach P, Hermel JM, Zhang JP, Philbrick W, Slepnev V, Ort T, Solimena M. betaIV spectrin, a new spectrin localized at axon initial segments and nodes of ranvier in the central and peripheral nervous system. *J Cell Biol*. 2000; 151:985–1002. [PubMed: 11086001]
- Boiko T, Van Wart A, Caldwell JH, Levinson SR, Trimmer JS, Matthews G. Functional specialization of the axon initial segment by isoform-specific sodium channel targeting. *J Neurosci*. 2003; 23:2306–2313. [PubMed: 12657689]
- Bréchet A, Fache MP, Brachet A, Ferracci G, Baude A, Irondelle M, Pereira S, Leterrier C, Dargent B. Protein kinase CK2 contributes to the organization of sodium channels in axonal membranes by regulating their interaction with ankyrin G. *Journal of Cell Biology*. 2008 in press.
- Davis JQ, Lambert S, Bennett V. Molecular composition of the node of Ranvier: identification of ankyrin-binding cell adhesion molecules neurofascin (mucin+/third FNIII domain-) and NrCAM at nodal axon segments. *Journal of Cell Biology*. 1996; 135:1355–1367. [PubMed: 8947556]
- Devaux JJ, Kleopa KA, Cooper EC, Scherer SS. KCNQ2 Is a Nodal K⁺ Channel. *J Neurosci*. 2004; 24:1236–1244. [PubMed: 14762142]
- Fache MP, Moussif A, Fernandes F, Giraud P, Garrido JJ, Dargent B. Endocytotic elimination and domain-selective tethering constitute a potential mechanism of protein segregation at the axonal initial segment. *J Cell Biol*. 2004; 166:571–578. [PubMed: 15302857]
- Ferreira MA, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L, Fan J, Kirov G, Perlis RH, Green EK, Smoller JW, Grozeva D, Stone J, Nikolov I, Chambert K, Hamshere ML, Nimgaonkar VL, Moskvina V, Thase ME, Caesar S, Sachs GS, Franklin J, Gordon-Smith K, Ardlie KG, Gabriel SB, Fraser C, Blumenstiel B, Defelice M, Breen G, Gill M, Morris DW, Elkin A, Muir WJ, McGhee KA, Williamson R, Macintyre DJ, Maclean AW, St Clair D, Robinson M, Van Beck

- M, Pereira AC, Kandaswamy R, McQuillin A, Collier DA, Bass NJ, Young AH, Lawrence J, Nicol Ferrier I, Anjorin A, Farmer A, Curtis D, Scolnick EM, McGuffin P, Daly MJ, Corvin AP, Holmans PA, Blackwood DH, Gurling HM, Owen MJ, Purcell SM, Sklar P, Craddock N. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet.* 2008
- Fried SI, Lasker AC, Desai NJ, Eddington DK, Rizzo JF 3rd. Axonal sodium-channel bands shape the response to electric stimulation in retinal ganglion cells. *J Neurophysiol.* 2009; 101:1972–1987. [PubMed: 19193771]
- Fujiwara T, Ritchie K, Murakoshi H, Jacobson K, Kusumi A. Phospholipids undergo hop diffusion in compartmentalized cell membrane. *J Cell Biol.* 2002; 157:1071–1081. [PubMed: 12058021]
- Garrido JJ, Giraud P, Carlier E, Fernandes F, Moussif A, Fache MP, Debanne D, Dargent B. A targeting motif involved in sodium channel clustering at the axonal initial segment. *Science.* 2003; 300:2091–2094. [PubMed: 12829783]
- Goldberg EM, Clark BD, Zagha E, Nahmani M, Erisir A, Rudy B. K⁺ channels at the axon initial segment dampen near-threshold excitability of neocortical fast-spiking GABAergic interneurons. *Neuron.* 2008; 58:387–400. [PubMed: 18466749]
- Grubb MS, Burrone J. Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability. *Nature.* 2010; 465:1070–1074. [PubMed: 20543823]
- Hedstrom KL, Ogawa Y, Rasband MN. AnkyrinG is required for maintenance of the axon initial segment and neuronal polarity. *J Cell Biol.* 2008; 183:635–640. [PubMed: 19001126]
- Hedstrom KL, Xu X, Ogawa Y, Frischknecht R, Seidenbecher CI, Shrager P, Rasband MN. Neurofascin assembles a specialized extracellular matrix at the axon initial segment. *J Cell Biol.* 2007; 178:875–886. [PubMed: 17709431]
- Hirokawa N, Takemura R. Molecular motors and mechanisms of directional transport in neurons. *Nat Rev Neurosci.* 2005; 6:201–214. [PubMed: 15711600]
- Hu W, Tian C, Li T, Yang M, Hou H, Shu Y. Distinct contributions of Na(v)1.6 and Na(v)1.2 in action potential initiation and backpropagation. *Nat Neurosci.* 2009; 12:996–1002. [PubMed: 19633666]
- Inda MC, Defelipe J, Munoz A. Voltage-gated ion channels in the axon initial segment of human cortical pyramidal cells and their relationship with chandelier cells. *Proc Natl Acad Sci U S A.* 2006
- Jenkins SM, Bennett V. Ankyrin-G coordinates assembly of the spectrin-based membrane skeleton, voltage-gated sodium channels, and L1 CAMs at Purkinje neuron initial segments. *J Cell Biol.* 2001; 155:739–746. [PubMed: 11724816]
- Khaliq ZM, Raman IM. Relative contributions of axonal and somatic Na channels to action potential initiation in cerebellar Purkinje neurons. *J Neurosci.* 2006; 26:1935–1944. [PubMed: 16481425]
- Kobayashi T, Storrie B, Simons K, Dotti CG. A functional barrier to movement of lipids in polarized neurons. *Nature.* 1992; 359:647–650. [PubMed: 1406997]
- Kole MH, Ilschner SU, Kampa BM, Williams SR, Ruben PC, Stuart GJ. Action potential generation requires a high sodium channel density in the axon initial segment. *Nat Neurosci.* 2008; 11:178–186. [PubMed: 18204443]
- Kole MH, Letzkus JJ, Stuart GJ. Axon initial segment Kv1 channels control axonal action potential waveform and synaptic efficacy. *Neuron.* 2007; 55:633–647. [PubMed: 17698015]
- Kordeli E, Lambert S, Bennett V. AnkyrinG. A new ankyrin gene with neural-specific isoforms localized at the axonal initial segment and node of Ranvier. *J Biol Chem.* 1995; 270:2352–2359. [PubMed: 7836469]
- Kuba H, Oichi Y, Ohmori H. Presynaptic activity regulates Na(+) channel distribution at the axon initial segment. *Nature.* 2010; 465:1075–1078. [PubMed: 20543825]
- Lemaitre G, Walker B, Lambert S. Identification of a conserved ankyrin-binding motif in the family of sodium channel alpha subunits. *J Biol Chem.* 2003; 278:27333–27339. [PubMed: 12716895]
- Lorincz A, Nusser Z. Cell-type-dependent molecular composition of the axon initial segment. *J Neurosci.* 2008; 28:14329–14340. [PubMed: 19118165]
- Nakada C, Ritchie K, Oba Y, Nakamura M, Hotta Y, Iino R, Kasai RS, Yamaguchi K, Fujiwara T, Kusumi A. Accumulation of anchored proteins forms membrane diffusion barriers during neuronal polarization. *Nat Cell Biol.* 2003; 5:626–632. [PubMed: 12819789]

- Nakata T, Hirokawa N. Microtubules provide directional cues for polarized axonal transport through interaction with kinesin motor head. *J Cell Biol.* 2003; 162:1045–1055. [PubMed: 12975348]
- Nishimura K, Akiyama H, Komada M, Kamiguchi H. betaIV-spectrin forms a diffusion barrier against L1CAM at the axon initial segment. *Mol Cell Neurosci.* 2007; 34:422–430. [PubMed: 17223356]
- Ogawa Y, Horresh I, Trimmer JS, Brecht DS, Peles E, Rasband MN. Postsynaptic density-93 clusters Kv1 channels at axon initial segments independently of Caspr2. *J Neurosci.* 2008; 28:5731–5739. [PubMed: 18509034]
- Ogawa Y, Oses-Prieto J, Kim MY, Horresh I, Peles E, Burlingame AL, Trimmer JS, Meijer D, Rasband MN. ADAM22, a Kv1 channel-interacting protein, recruits membrane-associated guanylate kinases to juxtaparanodes of myelinated axons. *J Neurosci.* 2010; 30:1038–1048. [PubMed: 20089912]
- Ogawa Y, Rasband MN. The functional organization and assembly of the axon initial segment. *Curr Opin Neurobiol.* 2008; 18:307–313. [PubMed: 18801432]
- Palay SL, Sotelo C, Peters A, Orkand PM. The axon hillock and the initial segment. *J Cell Biol.* 1968; 38:193–201. [PubMed: 5691973]
- Pan Z, Kao T, Horvath Z, Lemos J, Sul J-Y, Cranstoun SD, Bennett MV, Scherer SS, Cooper EC. A common ankyrin-G-based mechanism retains KCNQ and Nav channels at electrically active domains of the axon. *J Neurosci.* 2006; 26:2599–2613. [PubMed: 16525039]
- Rasband MN. It's 'juxta' potassium channel. *J Neurosci Res.* 2004; 76:749–757. [PubMed: 15160387]
- Sampo B, Kaech S, Kunz S, Banker G. Two distinct mechanisms target membrane proteins to the axonal surface. *Neuron.* 2003; 37:611–624. [PubMed: 12597859]
- Schafer DP, Jha S, Liu F, Akella T, McCullough LD, Rasband MN. Disruption of the axon initial segment cytoskeleton is a new mechanism for neuronal injury. *J Neurosci.* 2009; 29:13242–13254. [PubMed: 19846712]
- Sobotzik JM, Sie JM, Politi C, Del Turco D, Bennett V, Deller T, Schultz C. AnkyrinG is required to maintain axo-dendritic polarity in vivo. *Proc Natl Acad Sci U S A.* 2009; 106:17564–17569. [PubMed: 19805144]
- Song AH, Wang D, Chen G, Li Y, Luo J, Duan S, Poo MM. A selective filter for cytoplasmic transport at the axon initial segment. *Cell.* 2009; 136:1148–1160. [PubMed: 19268344]
- Strauss KA, Puffenberger EG, Huentelman MJ, Gottlieb S, Dobrin SE, Parod JM, Stephan DA, Morton DH. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. *N Engl J Med.* 2006; 354:1370–1377. [PubMed: 16571880]
- Tuvia S, Garver TD, Bennett V. The phosphorylation state of the FIGQY tyrosine of neurofascin determines ankyrin-binding activity and patterns of cell segregation. *Proc Natl Acad Sci U S A.* 1997; 94:12957–12962. [PubMed: 9371782]
- Van Wart A, Trimmer JS, Matthews G. Polarized distribution of ion channels within microdomains of the axon initial segment. *J Comp Neurol.* 2007; 500:339–352. [PubMed: 17111377]
- Winckler B, Forscher P, Mellman I. A diffusion barrier maintains distribution of membrane proteins in polarized neurons. *Nature.* 1999; 397:698–701. [PubMed: 10067893]
- Yang Y, Ogawa Y, Hedstrom KL, Rasband MN. {beta}IV spectrin is recruited to axon initial segments and nodes of Ranvier by ankyrinG. *J Cell Biol.* 2007; 176:509–519. [PubMed: 17283186]
- Zhou D, Lambert S, Malen PL, Carpenter S, Boland LM, Bennett V. AnkyrinG is required for clustering of voltage-gated Na channels at axon initial segments and for normal action potential firing. *J Cell Biol.* 1998; 143:1295–1304. [PubMed: 9832557]
- Zweier C, de Jong EK, Zweier M, Orrico A, Ousager LB, Collins AL, Bijlsma EK, Oortveld MA, Ekici AB, Reis A, Schenck A, Rauch A. CNTNAP2 and NRXN1 are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in *Drosophila*. *Am J Hum Genet.* 2009; 85:655–666. [PubMed: 19896112]

Figure 1.

The axon initial segment (AIS) is highly enriched in ion channels and cytoskeletal and scaffolding proteins. *A*, Cultured hippocampal neurons immunostained using antibodies against ankyrinG (green), Na⁺ channels (Pan Nav, red), and the somatodendritic marker MAP2 (microtubule associated protein 2; blue). *B*, Cortex from rat brain immunostained for the cytoskeletal scaffolding protein βIV spectrin (green), the Na⁺ channel accessory protein FGF14 (red), and Hoechst to indicate nuclei (blue). Image was kindly provided by Ms. Kelli Baalman. Scale bars, *A*, 20 μm; *B*, 25 μm.

Figure 2.

Assembly, maintenance, and plasticity of the AIS. **A**, During early development neurons become polarized with an axon and somatodendritic domain. This is accompanied by the redistribution of proteins into distinct cellular compartments. After axon specification, ankG becomes restricted to the AIS (i), where it functions as a scaffold to which many other AIS proteins become attached (ii). Upon assembly of the AIS, MAP2 (blue) becomes excluded from the distal axon. **B**, Actin and ankyrinG play key roles in maintaining neuronal polarity and restricting membrane proteins to either axonal or somatodendritic domains. (i) Loss of actin results in mixing of membrane proteins and loss of the cytoplasmic filter, permitting somatodendritic cargoes (yellow) to enter the axon. (ii) Loss of ankG causes the former axon to dedifferentiate and acquire many of the structural and molecular characteristics of a dendrite, including MAP2 and spines with post-synaptic densities (orange). **C**, Disease or injury causes influx of Ca^{2+} which activates the protease calpain (scissors). (i) Calpain cleaves the AIS cytoskeletal proteins ankG and β IV spectrin, (ii) leading to loss of neuronal polarity. **D**, The AIS can be plastic under different conditions of activity. (i) Under conditions of chronic activity, the AIS can translocate to more distal regions of the axon, thereby increasing threshold for action potential initiation. (ii) Under conditions where

activity is lost, the AIS can expand and become larger, making it easier for the neuron to reach threshold and fire an action potential.