Ankyrin protein networks in membrane formation and stabilization

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Abstract

In eukaryotic cells, ankyrins serve as adaptor proteins that link membrane proteins to the underlying cytoskeleton. These adaptor proteins form protein complexes consisting of integral membrane proteins, signalling molecules and cytoskeletal components. With their modular architecture and ability to interact with many proteins, ankyrins organize and stabilize these protein networks, thereby establishing the infrastructure of membrane domains with specialized functions. To this end, ankyrin collaborates with a number of proteins including cytoskeletal proteins, cell adhesion molecules and large structural proteins. This review addresses the targeting and stabilization of protein networks related to ankyrin interactions with the cytoskeletal protein β-spectrin, L1-cell adhesion molecules and the large myofibrillar protein obscurin. The significance of these interactions for differential targeting of cardiac proteins and neuronal membrane formation is also presented. Finally, this review concludes with a discussion about ankyrin dysfunction in human diseases such as haemolytic anaemia, cardiac arrhythmia and neurological disorders.

Keywords: ankyrin • β-spectrin • obscurin • L1-CAM • cardiomyocyte • axon initial segment • node of Ranvier

Introduction

Unique cellular functions are the result of cell-type specific proteins and the distinctive arrangement of these proteins in the context of organelles, the cytoskeleton and the plasma membrane. In eukaryotic cells, there are a number of adaptor proteins that serve as the interface between the plasma membrane and cytoskeleton including 4.1 proteins, proteins from the ezrin–radixin–moesin family and ankyrins. These adaptor proteins organize protein networks with particular structural, signalling and electrogenic properties. Accordingly, these adaptor proteins are integral for the formation of subcellular domains with specialized functions such as ionic movement across the plasma membrane or providing adherence between cell membranes. The prevalence of human disease associated with dysfunction in these adaptor proteins and associated molecules attests to their significance for normal cellular physiology. This review focuses on the adaptor protein ankyrin and its role in forming protein networks that constitute specialized membrane domains in cardiomyocytes and neurons. Specifically, the first section provides a general overview of ankyrins covering topics such as functional domains, genes and alternative isoforms. The second section is focused on proteins that contribute to ankyrin targeting and stabilization at specialized cardiac and neuronal membrane domains. Such proteins include the cytoskeletal protein β-spectrin, the L1-family of cell adhesion molecules and the large structural protein obscurin. The final section describes ankyrin dysfunction in human diseases including haemolytic anaemia, cardiac arrhythmias and neurological disorders.
Ankyrins

Ankyrins are a family of adaptor proteins that link integral membrane proteins with the submembranous actin/β-spectrin cytoskeleton. The first ankyrin was characterized over 30 years ago as an adaptor protein that tethered the anion exchanger to β-spectrin in red blood cells [1]. Ankyrins are now regarded as pivotal choreographers in the formation of protein complexes consisting of ion channels and transporters, cell adhesion molecules, signalling proteins and cytoskeletal elements. These ankyrin-associated protein complexes comprise specialized membrane domains with distinct electrogenic and/or structure properties in eukaryotic cells. In addition, new functions now ascribed to ankyrin include membrane biogenesis and the formation of diffusion barriers that maintain the subcellular polarity of migrating proteins.

Ankyrin functional domains

The prototypical ankyrin consists of three functional domains (Fig. 1). The membrane-binding domain, which mediates ankyrin binding to integral membrane proteins, contains 24 ANK repeats assembled as a superhelical spiral. An ANK repeat consists of 33 amino acids arranged as two anti-parallel α-helices followed by a long loop [2]. Adjacent ANK repeats are connected by a β-hairpin loop and these solvent-exposed domains mediate protein interactions. The binding sites are often spread across adjacent β-hairpin loop tips. For example, ankyrin interacts with the sodium/calcium exchanger (NCX) via ANK repeats 16–18 [3], the inositol(1,4,5)-triphosphate receptor (IP₃R) via ANK repeats 22–24 [4] and the voltage-gated sodium channel via ANK repeats 14 and 15 [5]. Additional integral membrane proteins that interact with the ankyrin membrane-binding domain include the anion exchanger [6–8], sodium/potassium ATPase (NKA) [9, 10], voltage-gated potassium channel subunits (KCNQ2 and KCNQ3) [11–14] and the L1 family of cell adhesion molecules (Fig. 1) [15, 16].

The ankyrin-associated protein complex is tethered to the actin/spectrin cytoskeleton via its spectrin-binding domain. This domain is relatively large with a molecular weight of 62 kD, but the minimal spectrin-binding domain is contained within a 160 amino acid ZU-5 motif [17]. This motif has two conserved sites that are critical for spectrin-binding activity (ankyrin-B: DAR976, A1000 and ankyrin-G: DAR999, A1024) [17, 18]. For β-spectrin, the minimal ankyrin-binding domain is contained in spectrin repeats 14 and 15 [19–21]. The complementary electrostatic charges of ankyrin’s ZU-5 motif (positive) and β-spectrin’s repeat 14 (negative) suggest that the protein interactions are partially mediated by their oppositely charged domains [20]. With the exception of identifying the minimal ankyrin and spectrin binding sites, very little is known about the regulatory mechanisms of ankyrin/spectrin interactions. For example, it is not known whether different ankyrins preferentially associate with particular spectrins.

The C-terminal regulatory domain is comprised of a death domain and an unstructured stretch of 300 amino acids. This domain regulates protein interactions with ankyrin’s membrane-binding and spectrin-binding domains. For example, an alternative isoform of ankyrin-R lacking a portion of the C-terminal domain exhibits increased binding affinity for the anion exchanger and spectrin [22, 23]. This increase in binding affinity is reversed by co-expression of the C-terminal fragment, validating the autoinhibitory functions of the C-terminal regulatory domain [22, 23]. The regulatory activity appears to be mediated by an intra-molecular interaction between this domain and the first ANK repeat of the membrane-binding domain [24]. Additional evidence for an intra-molecular interaction comes from studies of ankyrin-B mutations in cardiac disorders. In particular, the ankyrin-B loss-of-function mutation E1425G impairs ankyrin-B binding to NCX, NKA and the IP₃ receptor [9]. Interestingly, this mutation is not found in the membrane-binding or the C-terminal regulatory domain, but at the junction of the spectrin-binding and C-terminal
Ankyrin-G

Ankyrin-R

160, 220 kD

107–130, 119 kD

210 kD

186, 215 kD

220, 440 kD

220 kD

190 kD

270, 480 kD

220 kD

190, 200–215 kD

Erythrocyte

186, 215 kD

Ankyrin genes, alternative splicing and the diversity of ankyrin polypeptides

The diversity of ankyrin polypeptides is the product of unexpectedly complex alternative splicing of three genes (see Table 1). Located on human chromosome 8p11, ANK1 contains 42 exons that are alternatively spliced to encode a variety of ankyrin-R isoforms expressed in erythrocytes, cardiac and skeletal muscle, and neurons [23, 25–33]. Ankyrin-B isoforms are encoded by ANK2, which is located on human chromosome 4q25–27 and contains 53 exons spanning approximately 560 kb [34, 35]. Ankyrin-B isoforms have been found in a variety of tissues including brain, heart, skeletal muscle and thymus [36–40]. Finally, the gene for ankyrin-G (ANK3) located on human chromosome 10q21 encodes numerous isoforms broadly expressed in epithelial tissue, kidney, skeletal and cardiac muscle, and brain [41–49].

Alternative splicing of the ankyrin genes enables ankyrin polypeptides to associate with many different proteins and display differential subcellular localization. For example, the targeting of ankyrin-G to the axon initial segments (AIS) of peripheral neurons is partly dependent on a serine/threonine-rich domain that is only present in neuronal isoforms of ankyrin-G [49]. In addition, alternative transcription of ANK1 results in small ankyrin-R isoforms that integrate into the sarcoplasmic reticulum (SR) of skeletal muscle via unique N-terminal transmembrane domains [25, 33, 50, 51]. With all ankyrin genes, the exons encoding the C-terminal regulatory domains are often extensively spliced. As mentioned previously, this domain regulates ankyrin’s association with integral membrane proteins and cytoskeletal elements. Altering the composition of this domain would partially explain both the functional diversity and differential localization of alternative ankyrin isoforms. Alternative splicing of exons encoding the membrane-binding domain would also contribute to ankyrin functional diversity. In heart, alternative splicing of the ANK2 gene removes key exons that encode known binding sites for ankyrin-B associated proteins NCX and IP3 receptor (reviewed in Van Oort, 2008) [34, 52]. Taken together, alternative splicing alters binding sites and targeting motifs in ankyrin, thereby enabling its selective association with particular proteins that ultimately culminate in the distinct subcellular localization of ankyrin-associated protein complexes. By some accounts, these mechanisms may also underlie a role for ankyrin in the formation of specialized membrane domains. The following section will discuss three proteins that contribute to the targeting and retention of ankyrin-associated protein complexes to specific membrane domains.

Mechanisms that target and stabilize ankyrin

β-spectrin

Spectrins are a family of filamentous proteins that assemble as heterotetramers of two α and two β subunits. In conjunction with actin filaments, these heterotetramers form the underlying cytoskeleton of plasma membranes in all metazoan cell types including erythrocytes, cardiomyocytes and neurons. While two genes encode the various isoforms of α-spectrin, five genes encode numerous isoforms of β-spectrin [53]. The prototypical β-spectrin contains an N-terminal actin-binding domain, 16 triple-helical spectrin repeats and a C-terminal pleckstrin homology domain. In addition to acting as molecular springs that ensure membrane resilience to mechanical stress, the spectrin repeats also mediate protein interactions. For example, ankyrin interacts with β-spectrin repeats 14 and 15 [19–21]. β-spectrin associates with the plasma membrane through adaptor proteins such as ankyrin and protein 4.1 [54], integral membrane proteins including the neuronal glutamate transporter [55] and phosphatidylinositol lipids [56]. In human, mutations to β-spectrin have been linked to hereditary elliptocytosis, a type of haemolytic anaemia, and spinocerebellar ataxia type 5, a progressive neurodegenerative disorder characterized by slurred speech and loss of coordination [57–59]. The association of spectrin mutations with these two seemingly disparate diseases underscores spectrin’s role as a multifunctional protein that not only insures membrane integrity in erythrocytes but also contributes to the stabilization of membrane proteins in the cerebellum.

Previous studies have focused on the relative significance of ankyrin and β-spectrin in the formation of specialized membrane
domains. This analysis includes examining the respective roles for ankyrin and β-spectrin in the targeting and stabilization of each other. Some studies have concluded that β-spectrin is targeted and stabilized by ankyrin. For example, in ventricular cardiomyocytes the M-line localization of β2-spectrin is dependent on ankyrin-B [17]. Likewise, ankyrin-B is necessary for β2-spectrin localization at the inner segments of rod photoreceptors [60]. In cerebellar-specific ankyrin-G knockout mice, the loss of ankyrin-G disrupts β2-spectrin localization at AIS in Purkinje cells [61]. Contrary to these findings, some studies have concluded that ankyrin targeting and stabilization is dependent on β-spectrin. In Drosophila melanogaster, ankyrin is localized to the basolateral domains of midgut copper cells through its interaction with β-spectrin [62].

Recently, an emerging theme is that ankyrin and β-spectrin are interdependent and are mutually involved in the formation and maintenance of membrane domains. For example, evidence from co-localization experiments demonstrate that expression of ankyrin-G or β4-spectrin at AIS of Purkinje neurons is dependent on each other [61, 63]. In bronchial epithelial cells, both ankyrin-G and β2-spectrin cooperate in the formation of lateral membrane domains. Specifically, loss of lateral membrane domains following AnkG-siRNA treatment is not rescued by exogenous expression of an ankyrin-G construct that lacks β-spectrin binding activity [64].

The relative prominence of ankyrin or spectrin in membrane biogenesis most likely depends on additional factors in the microdomain including cell adhesion molecules, other cytoskeletal elements and regional phospholipids. In support of this hypothesis, it was shown that different domains are necessary for β-spectrin targeting in various Drosophila tissues. For example, β-spectrin targeting in midgut copper cells is dependent on the C-terminal PH-domain, while β-spectrin targeting to neuronal plasma membranes requires both the PH and ankyrin-binding domains [65]. Interestingly, neither the PH nor the ankyrin-binding domains are required for β-spectrin targeting in salivary epithelial cells [65]. In fact, the novel targeting motif has yet to be identified. Ankyrin-independent β-spectrin targeting mechanisms may include phospholipids, integral membrane proteins (α-catenin) [66] and additional adaptor proteins (4.1 protein) [53]. Similar to β-spectrin, multiple ankyrin-G domains contribute to its retention at specific membrane domains. For example, axomelial targeting information is contained in the ankyrin-G spectrin-binding and C-terminal regulatory domains [49]. Furthermore, additional targeting motifs that restrict ankyrin-G expression to AIS are present in the serine/threonine rich and C-terminal regulatory domains [49]. These findings suggest that ankyrin-G targeting to AIS is dependent on multiple protein interactions with different ankyrin-G domains. Apparent discrepancies in the targeting and retention of ankyrin/spectrin complexes to different membrane domains may be explained by the involvement of multiple proteins. For example, as will be described in greater detail below, the cell adhesion molecule neurofascin recruits ankyrin-G to the nodes of Ranvier, but not to AIS of peripheral neurons [67–69].

L1-cell adhesion molecules

Ankyrins bind to a variety of cell adhesion molecules including CD44 [70], E-cadherin [71], β-dystroglycan [72] and members of the L1 family [15, 16]. This association is important for the proper subcellular targeting of these cell adhesion molecules. For example, ankyrin-G recruits E-cadherin to the lateral membrane domains of bronchial epithelial cells where in concert with β2-spectrin it facilitates membrane biogenesis [18, 64, 71]. Recently, it was shown that ankyrin-B and ankyrin-G are involved in the targeting and retention of β-dystroglycan to the sarcolemma, neuro-muscular junction and costameres of skeletal muscle [72].

Many studies have characterized the association of ankyrins with members of the L1 family of cell adhesion molecules that includes L1, close homologue of L1 (CHL1), neuron glia related CAM (NrCAM) and neurofascin. These proteins are predominantly expressed in the nervous system where they have been implicated in many aspects of neural differentiation including neurite outgrowth, axonal guidance and synaptogenesis [73]. The prototypical extracellular domain of L1 cell adhesion molecules has six immunoglobulin domains followed by four to five fibronectin type III domains. The cytoplasmic domain is relatively small ranging in size from 85 to 148 amino acids and contains the ankyrin-binding motif FIGQY that is highly conserved in L1 proteins [74–76]. Homophilic interactions between the extracellular domains of these proteins are stabilized by ankyrin binding to the FIGQY motif. Moreover, phosphorylation of the tyrosine residue in this motif disrupts ankyrin interaction with the C-terminal domain [74, 75]. For example, ankyrin/L1 interactions cause neuroblastoma cells to aggregate via homophilic interactions of the L1 extracellular domains [75]. These aggregates disperse following treatment with nerve growth factor because tyrosine phosphorylation of the FIGQY motif disrupts ankyrin binding to L1.

The phosphorylation-dependent regulation of ankyrin/L1 interaction allows L1 to function in two different capacities during neural development. In developmental events that favour static adhesion such as axon fasciculation or the formation of AIS, the tyrosine residue is not phosphorylated and ankyrin interacts with the L1 protein [73]. In contrast, for dynamic developmental processes such as neurite outgrowth and migration, L1 proteins are phosphorylated thereby inhibiting ankyrin interactions. Consistent with this hypothesis, neurite outgrowth is increased by 50% in cerebellar granular neurons following inhibition of L1/ankyrin interactions [77]. Interestingly, phosphorylated FIGQY preferentially interacts with the microtuble associated protein doublecortin that is linked to the neuronal migration disorder lissencephaly, which is characterized by thickening of the neocortex and reduced cortical gyraations [78, 79].

The relative contribution of ankyrin or L1 proteins to the targeting and retention of the other depends on the membrane domain. For example, ankyrin-G recruits neuronal isoforms of neurofascin (neurofascin-186) to AIS of cerebellar Purkinje neurons [80]. Similarly, in hippocampal neurons ankyrin-G depletion by RNA interference extinguishes AIS targeting of neurofascin-186, NaV1.6, and β4-spectrin [81]. As in the central nervous system,
ankyrin-G recruits neurofascin-186 to AIS of peripheral neurons, but interestingly their roles are reversed at the nodes of Ranvier (Fig. 2). Specifically, neurofascin-186 recruits ankyrin-G to the nodes of peripheral neurons [67–69]. Consistent with this finding, neurofascin-186 clusters at the nodes before ankyrin-G or voltage-gated sodium channels (KCNQ2/3) and β4-spectrin. In contrast, ankyrin-G localization to the AIS is not dependent on an extracellular cue or neurofascin-186. The AIS targeting of ankyrin-G appears to be mediated by an intrinsic mechanism that has yet to be discovered, but AIS targeting of ankyrin-G associated proteins (i.e. neurofascin-186, sodium and potassium channels, β4-spectrin) is dependent on ankyrin-G.

Fig. 2 Ankyrin-G targeting to membrane domains in the peripheral neuron. Ankyrin-G is recruited to the nodes of Ranvier by gliomedin, which is produced by Schwann cells and accumulates in the perinodal extracellular matrix. As a ligand for neurofascin-186, gliomedin causes the nodal clustering of this cell adhesion molecule, which in turn recruits to the nodal plasma membrane an ankyrin-G protein network consisting of voltage-gated sodium or potassium channels (KCNQ2/3) and β4-spectrin. In contrast, ankyrin-G localization to the AIS is not dependent on an extracellular cue or neurofascin-186. The AIS targeting of ankyrin-G appears to be mediated by an intrinsic mechanism that has yet to be discovered, but AIS targeting of ankyrin-G associated proteins (i.e. neurofascin-186, sodium and potassium channels, β4-spectrin) is dependent on ankyrin-G.

Decreasing gliomedin expression through RNA interference reduces the nodal expression of both neurofascin and NaV channels [84]. In summary, ankyrin interactions with L1 proteins demonstrate the relative contribution of intracellular and/or extracellular cues to the formation of specialized membrane domains. Namely, ankyrin-G clustering and the ensuing formation of nodes of Ranvier are dependent on extracellular cues from myelinating Schwann cells that are relayed via neurofascin-186.
Obscurin

In skeletal and cardiac tissue, large sarcomeric proteins coordinate the proper alignment of protein complexes to facilitate the integration of membrane structures including the SR and transverse tubules (T-tubules) with myofibrils. Obscurin is an 800 kD protein that regulates the formation and alignment of myofibrils. In skeletal muscle, obscurin is expressed early at the M-lines of developing sarcomeres in nascent myofibrils [85, 86]. Loss of obscurin activity causes the misalignment of M-lines in adjacent myofibrils and reduced myosin incorporation into maturing thick filaments [85, 87]. These observations are contradicted by the recent finding that there is no significant difference in skeletal muscle M-line architecture between obscurin knockout and wild-type mice [88]. It is possible that obscurin like 1 protein compensates for the loss of obscurin to preserve M-line architecture. Nevertheless, this knockout model demonstrates that obscurin plays a key role in establishing/maintaining the longitudinal SR that extends the length of a sarcomere and connects neighbouring junctional SR [88]. In zebrafish, obscurin deficiency due to antisense morpholino treatment results in ventricular hypoplasia and a reduced heart rate [89]. Obscurin mutations have been also associated with human cardiomyopathy [90].

While many models of obscurin deficiency demonstrate that this protein predominantly functions at the M-line, obscurin isoforms have also been detected at the M-line, Z-line, Z/I junctions and A/I junctions of skeletal muscle [91]. Multiple obscurin isoforms are produced by alternative splicing of the 117 exons that make up the obscurin gene OBSCN that is located on human chromosome 1q42.13 [92]. These isoforms contain variable combinations of modular domains including immunoglobulin (Ig) and fibronectin (Fn) repeats, a calcium/calmodulin binding domain, a Rho-GEF domain, and two serine/threonine kinase domains. Only the 800 kD obscurin isoform contains an ankyrin-binding site following the Rho-GEF domain in a unique C-terminal domain [92]. This isoform is predominantly localized to the M-line via the association of its N-terminal Ig domains with the M-line resident proteins myomesin and M-line titin (Fig. 3) [93]. Interestingly, titin mutations within the region of the obscurin-binding site are associated with tibial muscular dystrophy and limb girdle muscular dystrophy [94–96].

In ventricular cardiomyocytes, ankyrin-B is localized to two distinct domains: overlaying the structural and myofibrillar proteins of the M-line and at the SR/T-tubule junctions (Fig. 3). Recently, it was demonstrated that a subpopulation of ankyrin-B is targeted to the M-line via its interaction with the C-terminal domain of 800 kD obscurin [97]. This interaction is regulated by alternative splicing of an exon in the C-terminal regulatory domain that dramatically increases obscurin-binding activity of ankyrin. Moreover, it was demonstrated that a regulatory subunit of protein phosphatase 2A, a signalling molecule that controls the phosphorylation status of proteins such as myosin-binding protein C and troponin-I, is brought to the M-line via ankyrin-B/obscurin interactions [97].

In addition to ankyrin-B, small ankyrin-R isoforms 1.5 and 1.9 interact with the carboxyl-terminus of 800 kD obscurin [50, 51, 98]. These small ankyrins are unique because they lack many of the domains found in a prototypical ankyrin. In fact, they only retain a small portion of the C-terminal regulatory domain of ankyrin-R. The amino terminal domain contains a novel stretch of amino acids that forms a transmembrane domain that integrates into the membrane of the SR. Based on their interactions with obscurin, these small isoforms are targeted to the M-line where it is thought that they position the developing SR in reference to myofibrils [50, 51, 98].

Ankyrins and disease

As the field of ankyrin biology continues to advance, the clinical manifestations of ankyrin dysfunction will be recognized as complex and multi-systemic for a number of reasons. First, ankyrins (R, G, B) and their alternative isoforms are expressed in many tissues (see Table 1). Often they display overlapping expression patterns in the same tissues. For example, isoforms of all three ankyrins are expressed in brain, heart and skeletal muscle. Findings from ankyrin knockout mouse models and cell-based structure/function analysis suggest that ankyrins are not functionally redundant. For example, ankyrin-G does not compensate for ankyrin-B loss in ventricular cardiomyocytes [99]. Nevertheless,
nothing is known about the relationship between ankyrins co-expressed in the same tissue, so the extent of functional redundancy between ankyrins has yet to be fully explored. Finally, owing to the structural and functional diversity of ankyrin isoforms across different tissues, an amino acid variation/mutation in ankyrin may be tolerated in a particular tissue background, all the while causing significant dysfunction in another tissue background. Moreover, the location of these variable residues may have a significant impact on ankyrin function. For example, 8 out of 9 loss-of-function mutations associated with ‘ankyrin-B syndrome’, which encompasses a wide range of cardiac disorders, reside within the C-terminal regulatory domain (see Table 2) [100–102]. The following sections will present diseases associated with ankyrin dysfunction in the erythrocyte, heart and brain.

Ankyrin-R

While ankyrin-R is expressed in red blood cells, muscle tissue and neurons, the most prominent phenotype associated with ankyrin-R mutations is hereditary spherocytosis. This haemolytic anaemia is characterized by increased haemolysis due to altered red blood cell morphology from the normal biconcave disk to a spherical conformation. Although it is rare, some neurological problems have been associated with hereditary spherocytosis [103]. As a model of ankyrin-R deficiency, normoblastosis (nb/nb) mouse display motor ataxia in addition to severe haemolytic anaemia [32, 104]. These deficiencies are the result of a hypomorphic mutation in ankyrin-R [105] and the movement disorder is consistent with the predominant expression of ankyrin-R in the cell bodies and dendrites of Purkinje and granule cells of the cerebellum [32, 104]. While multiple ankyrin-R isoforms have been characterized in skeletal muscle, no muscular dystrophies in human have been associated with ankyrin-R dysfunction.

Ankyrin-G

Recently, multiple genome wide association studies have linked single nucleotide polymorphisms in the ANK3 locus to bipolar disorder, a mental illness characterized by recurring episodes of mania and depression [106–108]. Considering neurotransmitters commonly associated with mood disorders (i.e. serotonergic, dopaminergic and cholinergic neurotransmitters) are not preferentially affected in bipolar disorder, the molecular basis for bipolar disorder may reflect wholesale changes to synaptic connectivity and neural circuitry. This view is consistent with the loss of synaptic connectivity between basket interneurons and Purkinje neurons in ankyrin-G deficient cerebellums [109]. To regulate neuronal excitability, basket interneurons form GABAergic synapses with AIS of Purkinje neurons, a developmental process guided by an ankyrin-G dependent subcellular gradient of neurofascin in Purkinje neurons [109]. Additional support for ankyrin function in synaptogenesis comes from genetic screens that identified the large isoform of Drosophila ankyrin 2 as a critical regulator of synaptic stability and maintenance at the Drosophila neuromuscular junction [110, 111]. Specifically, the loss of this ankyrin precipitates the disassembly and retraction of presynaptic boutons, caused by cytoskeletal destabilization following the loss of an interaction between synaptic microtubules and the extended C-terminal domain of this ankyrin [110, 111].

Another mechanism by which ankyrin-G impacts overall neural circuitry is through its targeting of voltage-gated sodium and potassium channels to AIS and nodes of Ranvier in neurons of the central nervous system. For example, ankyrin-G directs the targeting of voltage-gated sodium channels to AIS of granule cells and Purkinje neurons [80]. In the absence of ankyrin-G, Purkinje neurons display impaired ability to initiate action potentials and the mice demonstrate neuronal degeneration of the cerebellum and progressive motor ataxia [80].

Many disease-related mutations have been characterized in ankyrin-G associated proteins. For example, a mutation in cardiac voltage-gated sodium channel Nav1.5 was shown to disrupt the channel’s association with ankyrin-G and to cause Brugada syndrome, a cardiac disorder characterized by preordial ST segment elevation, right bundle branch block, and fatal arrhythmias [5, 45]. The Brugada syndrome mutation (E1053K) resides within a 9 amino acid ankyrin-binding motif that is highly conserved in the DII-DIII loops of voltage-gated sodium channels [45, 112, 113]. By disrupting the association of ankyrin-G and Nav1.5, this mutation impairs ankyrin-G dependent targeting of the channel to the intercalated disc resulting in decreased sodium channel current density ([I Na]) [5, 45]. Similar ankyrin-binding motifs are also present in the voltage-gated potassium channel subunits KCNQ2 and KCNQ3. These motifs are required for ankyrin-dependent targeting of these channel subunits to AIS where they control overall neuronal excitability [11, 13, 14].
Mutations in these subunits associated with benign familial neonatal convulsions (BFNC) cause epilepsy and myokymia [114–116]. Moreover, BFNC mutations in the KCNQ2 subunit that remove the ankyrin-binding site either through a frameshift or premature stop codon cause reduced axonal surface expression of potassium channels in hippocampal neurons [11]. Recently, the β1 subunit of cyclic nucleotide-gated channel (CNG-β1) was identified as a binding partner for ankyrin-G in the retina [117]. This interaction is necessary for ankyrin-dependent targeting of CNG-β1 to rod outer segments and domain formation [117]. Interestingly, the ankyrin-G binding site resides in the last twenty amino acids of CNG-β1 and a mutation that abolishes this domain is associated with retinitis pigmentosa, a progressive retinal dystrophy that causes gradual vision loss due to abnormalities in the photoreceptors or retinal pigment epithelium [117, 118]. Finally, both ankyrin-B and ankyrin-G were recently shown to interact with dystrophin, an essential component of the dystrophin glycoprotein complex that provides membrane stability by linking the actin-based cytoskeleton to integral membrane proteins connected to the extracellular matrix [72]. A dystrophin mutation linked to Becker muscular dystrophy was shown to reduce ankyrin binding thereby resulting in decreased sarcolemmal localization of dystrophin [72].

Ankyrin-B

While ankyrin-B is expressed in a variety of tissues, human mutations to ankyrin-B have almost exclusively been associated with cardiac disorders (see Table 2). The uniform expression of ankyrin-B polypeptides in different cardiac regions (i.e. ventricles, atria, sinoatrial node) accounts for the diversity of ankyrin-B associated cardiac disorders including bradycardia, sinus arrhythmia, delayed conduction/conduction block, idiopathic ventricular fibrillation and catecholaminergic polymorphic ventricular tachycardia [100–102]. In the ventricles, ankyrin-B is responsible for the proper targeting and retention of ion channels and transporters that regulate local calcium dynamics at the SR/T-tubule junction (Fig. 3). In ankyrin-B haploinsufficient mice, ventricular cardiomyocytes display reduced expression and proper SR/T-tubule localization of NCX, NKA and the IP3 receptor [9, 101, 102]. Furthermore, when given a catecholamine bolus during exercise, these mice exhibit ventricular tachycardia and often sudden death, thereby mimicking human cases of catecholaminergic ventricular tachycardia [100–102]. In the ventricles, ankyrin-B is necessary for the proper targeting of ion channels and transporters associated with calcium influx and efflux in the cardiac pacemaker, or sinoatrial node [119]. In addition to reduced expression of NCX, NKA and IP3 receptor, sinoatrial cells from ankyrin-B heterozygous mice display inappropriate targeting of Cav1.3, an important regulator of extracellular calcium entry that is necessary for normal cardiac pacemaker function [119]. Future research efforts to assess ankyrin-B activity in other cardiac regions (i.e. the atrium and other components of the conduction system) will advance our understanding of ankyrin-B function in normal cardiac physiology.

Studies of the ankyrin-B knockout mouse provide insight into potential disorders linked to human mutations in ankyrin-B. For example, ankyrin-B deficiency in mouse has been reported to be associated with mild skeletal muscular phenotypes including elevated creatine kinase levels and sporadically disorganized sarcomeres [40]. As mentioned above, ankyrin-B was recently found to interact with dystrophin consistent with its role in skeletal muscle organization and integrity. To date, no human skeletal muscle disorder has been associated with an ankyrin-B mutation, although co-expression of ankyrin-G and ankyrin-R isoforms may compensate for ankyrin-B dysfunction.

Ankyrin-B deficient mice also exhibit significant neurological pathology including hypoplasia of the corpus callosum and pyramidal tracts, dilated ventricles and severe postnatal degeneration of the optic nerve [39]. At the cellular level, many of these abnormalities are the result of ankyrin-B functions in neurite outgrowth, axonal guidance and axon fasciculation. At the molecular level, these functions are fulfilled by ankyrin-B interactions with the cell adhesion molecule L1. In fact, many of the neurological defects manifested in ankyrin-B deficient mice are mimicked in L1-deficient mice including axon guidance errors in pyramidal tracts and corpus callosum, degeneration of sensory axons and enlarged lateral ventricles [120–123]. The neuropathology manifested in ankyrin-B deficient mice may also arise in part from a loss of ankyrin-B dependent targeting of β-dystroglycan to astrocyte endfeet, which ensures the compact formation of the glia limitans that serves as an interface between cerebral spinal fluid and the brain [72].

In many animal models, both ankyrin and L1 are involved in axonal guidance and fasciculation. For example, mutations to the ankyrin-related gene unc-44 in C. elegans result in abnormal axonal guidance and fasciculation [124, 125]. Likewise, mutations to the Drosophila homologue of L1 neuroglin cause abnormal pathfinding of motorneuron projections and impaired contralateral projections of olfactory receptor neurons [126, 127]. Two different mechanisms have been shown to regulate L1-mediated axonal guidance and neurite outgrowth. First, L1 interacts with neuroligin-1, which is a receptor for the semaphorin family of chemoeattracants and repellents [128–130]. Second, the homophilic interactions between L1 proteins activate receptor tyrosine kinases (i.e. the epidermal and fibroblast growth factor receptors) and second messenger pathways (i.e. MAP kinase and phospholipase C) [131]. Tyrosine phosphorylation of the FIGQY motif abolishes ankyrin interaction with L1, and neurite outgrowth is positively regulated by untethered L1 molecules. Interestingly, mutations in the L1 FIGQY motif have been linked to human cases of CRASH syndrome that is characterized by corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraplegia and hydrocephalus [132, 133].
Conclusions

While ankyrins were initially characterized as anchors tethering membrane proteins to the underlying cytoskeleton, they are now thought to play a crucial role in the formation of specialized membrane domains. With their modular architecture and ability to interact with many proteins, ankyrins organize and stabilize protein networks in collaboration with other proteins including cytoskeletal proteins, cell adhesion molecules and large structural proteins. Cytoskeletal proteins such as β-spectrin contribute stability to the ankyrin protein network. This stability underlies the structural integrity of the plasma membrane such that cells remain resilient to mechanical stress associated with development and normal cellular functions. Furthermore, incorporation of β-spectrin allows the ankyrin protein network to remain static in the fluid lipid bilayer thereby accommodating the initiation, development and maintenance of membrane domains. Cell adhesion molecules enable extracellular signals from neighbouring cells or the extracellular matrix to interact with the ankyrin protein complex and to guide the formation of membrane domains such as the nodes of Ranvier in peripheral neurons. Ankyrin interactions with cell adhesion molecules are important for connecting adjacent cells and anchoring cellular projections that migrate through the extracellular matrix. Finally, ankyrin interactions with large structural proteins such as obscurin allow for the proper integration of ankyrin protein complexes into the larger cellular scheme, ensuring its correct placement in the context of other protein networks and neighbouring cellular structures. While the targeting and stabilization of ankyrin protein networks are facilitated by each of the aforementioned proteins, it is certain that many additional proteins will contribute to ankyrin-dependent membrane formation. The tailoring of ankyrin polypeptides through alternative splicing enables modified ankyrins to adapt to the variable conditions presented during the formation of different specialized domains. This versatility accounts for ankyrin's multifunctional capabilities in diverse cellular backgrounds (i.e. erythrocytes versus cardiomyocytes) and their apt intercellular functioning within different organelles (i.e. lysosome, trans Golgi network and SR) [41, 47, 134, 135]. As the field of ankyrin biology continues to develop, so too will our appreciation of ankyrin's pivotal role in normal and diseased cellular conditions.

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References

1. Bennett V. Immunoactive forms of human erythrocyte ankyrin are present in diverse cells and tissues. Nature. 1979; 281: 597–9.
2. Michaeley P, Tomchick DR, Machius M, et al. Crystal structure of a 12 ANK repeat stack from human ankyrinR. EMBO J. 2002; 21: 6387–96.
3. Cunha SR, Bhasin N, Mohler PJ. Targeting and stability of Na/Ca exchanger 1 in cardiomyocytes requires direct interaction with the membrane adaptor ankyrin-B. J Biol Chem. 2007; 282: 4875–83.
4. Mohler PJ, Davis JQ, Davis LH, et al. Inositol 1,4,5-trisphosphate receptor localization and stability in neonatal cardiomyocytes requires interaction with ankyrin-B. J Biol Chem. 2004; 279: 12980–7.
5. Lowe JS, Palygin O, Bhasin N, et al. Voltage-gated Nav channel targeting in the heart requires an ankyrin-G dependent cellular pathway. J Cell Biol. 2008; 180: 173–86.
6. Davis JQ, Bennett V. The anion exchanger and Na+/K+-ATPase interact with distinct sites on ankyrin in vitro assays. J Biol Chem. 1990; 265: 17252–6.
7. Davis LH, Otto E, Bennett V. Specific 33-residue repeat(s) of erythrocyte ankyrin associate with the anion exchanger. J Biol Chem. 1991; 266: 11163–9.
8. Michaeley P, Bennett V. The ANK repeats of erythrocyte ankyrin form two distinct but cooperative binding sites for the erythrocyte anion exchanger. J Biol Chem. 1995; 270: 22050–7.
9. Mohler PJ, Davis JQ, Bennett V. Ankyrin-B coordinates the Na/K ATPase, Na/Ca exchanger, and InsP(3) receptor in a cardiac T-tubule/SR microdomain. PLoS Biol. 2005; 3: 2158–67.
10. Nelson WJ, Veshnock PJ. Ankyrin binding to (Na+ + K+)ATPase and implications for the organization of membrane domains in polarized cells. Nature. 1987; 328: 533–6.
11. Chung HJ, Jan YN, Jan LY. Polarized axonal surface expression of neuronal KCNQ channels is mediated by multiple signals in the KCNQ2 and KCNQ3 C-terminal domains. Proc Natl Acad Sci USA. 2006; 103: 1–15.
12. Hill AS, Nishino A, Nakajo K, et al. Ion channel clustering at the axon initial segment and node of Ranvier evolved sequentially in early chordates. PLoS Genetics. 2008; 4; e1000317.
13. Pan Z, Kao T, Horvath Z, et al. A common ankyrin-G-based mechanism retains KCNQ and NaV channels at electrically active domains of the axon. J Neurosci. 2006; 26: 2599–613.
14. Rasmussen HB, Frokjaer-Jensen C, Jensen CS, et al. Requirement of subunit co-assembly and ankyrin-G for M-channel localization at the axon initial segment. J Cell Sci. 2007; 120: 953–63.
15. Davis JQ, Bennett V. Ankyrin binding activity shared by the neurofascin/L1/NrCAM family of nervous system cell adhesion molecules. J Biol Chem. 1994; 269: 27163–6.
16. Davis JQ, McLaughlin T, Bennett V. Ankyrin-binding proteins related to nervous system cell adhesion molecules: candidates to provide transmembrane and intercellular connections in adult brain. J Cell Biol. 1993; 121: 321–33.
17. Mohler PJ, Yoon W, Bennett V. Ankyrin-B targets beta2-spectrin to an intracellular compartment in neonatal cardiomyocytes. J Biol Chem. 2004; 279: 40185–93.
18. Kizhatil K, Yoon W, Mohler PJ, et al. Ankyrin-G and beta2-spectrin collaborate in biogenesis of lateral membrane of...
human bronchial epithelial cells. J Biol Chem. 2007; 282: 2029–37.

19. Davis L, Abdi K, Machius M, et al. Localization and structure of the ankyrin-binding site on beta2-spectrin. J Biol Chem. 2009; 284: 6982–7.

20. Ipsaro JJ, Huang L, Mondragon A. Structures of the spectrin-ankyrin interaction binding domains. Blood. 2009; 113: 5385–93.

21. Kennedy SP, Warren SL, Forget BG, et al. Ankyrin binds to the 15th repetitive unit of erythroid and nonerythroid beta-spectrin. J Cell Biol. 1991; 115: 267–77.

22. Davis LH, Davis JQ, Bennett V. Ankyrin regulation: an alternatively spliced segment of the regulatory domain functions as an intramodular modulator. J Biol Chem. 1992; 267: 18966–72.

23. Hall TG, Bennett V. Regulatory domains of erythrocyte ankyrin. J Biol Chem. 1987; 262: 10537–45.

24. Abdi KM, Mohler PJ, Davis JQ, et al. Isoform specificity of ankyrin-B: a site in the divergent C-terminal domain is required for intramodular association. J Biol Chem. 2006; 281: 5741–9.

25. Birkenmeier CS, Sharp JJ, Gifford EJ, et al. An alternative first exon in the distal end of the erythroid ankyrin gene leads to production of a small isoform containing an NH2-terminal membrane anchor. Genomics. 1998; 50: 79–88.

26. Birkenmeier CS, White RA, Peters LL, et al. Complex patterns of sequence variation and multiple 5’ and 3’ ends are found among transcripts of the erythroid ankyrin gene. J Biol Chem. 1993; 268: 9533–40.

27. Gallagher PG, Forget BG. An alternate promoter directs expression of a truncated, muscle-specific isoform of the human ankyrin 1 gene. J Biol Chem. 1998; 273: 1339–48.

28. Gallagher PG, Tse WT, Scarpa AL, et al. Structure and organization of the human ankyrin-1 gene. Basis for complexity of pre-mRNA processing. J Biol Chem. 1997; 272: 19229–8.

29. Lambert S, Yu H, Prchal JT, et al. cDNA sequence for human erythrocyte ankyrin. Proc Natl Acad Sci USA. 1990; 87: 1730–4.

30. Lux SE, Tse WT, Menninger JC, et al. Hereditary spherocytosis associated with deletion of human erythrocyte ankyrin gene on chromosome 8. Nature. 1990; 345: 736–9.

31. Peters LL, Birkenmeier CS, Bronson RT, et al. Purkinje cell degeneration associated with erythroid ankyrin deficiency in nb/nb mice. J Cell Biol. 1991; 114: 1233–41.

32. Zhou D, Birkenmeier CS, Williams MW, et al. Small, membrane-bound, alternatively spliced forms of ankyrin 1 associated with the sarcoplasmic reticulum of mammalian skeletal muscle. J Cell Biol. 1997; 136: 621–31.

33. Cunha SR, Le Scouarnec S, Schott JJ, et al. Exon organization and novel alternative splicing of the human ANK2 gene: implications for cardiac function and human cardiac disease. J Mol Cell Cardiol. 2008; 45: 724–34.

34. Tse WT, Menninger JC, Yang-Feng TL, et al. Isolation and chromosomal localization of a novel nonerythroid ankyrin gene. Genomics. 1998; 50: 105–66.

35. Kunimoto M, Otto E, Bennett V. A new 440-kD isoform is the major ankyrin in neonatal rat brain. J Cell Biol. 1991; 115: 1319–31.

36. Otto E, Kunimoto M, McLaughlin T, et al. Identification of a novel ankyrin isoform (AnkG190) in kidney and lung that associates with the plasma membrane and binds alpha-Na, K-ATPase. J Biol Chem. 1998; 273: 23952–8.

37. Zhang X, Bennett V. Restriction of 440/270-kD ankyrin G to axon proximal segments requires multiple ankyrin G-specific domains. J Cell Biol. 1998; 142: 1571–81.

38. Kontogianni-Konstantopoulo A, Jones EM, Van Rossom DB, et al. Obscurin is a ligand for small ankyrin 1 in skeletal muscle. Mol Biol Cell. 2003; 14: 1138–48.

39. van Oort RJ, Altamirano J, Lederer WJ, et al. Alternative splicing: a key mechanism for ankyrin-B functional diversity? J Mol Cell Cardiol. 2008; 45: 709–11.

40. Bennett V, Baines AJ. Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. Physiol Rev. 2001; 81: 1353–92.

41. An X, Debnath G, Guo X, et al. Identification and functional characterization of protein 4.1R and actin-binding sites in erythrocyte beta spectrin: regulation of the interactions by phosphatidylinositol-4,
5-bisphosphate. Biochemistry. 2005; 44: 10681–8.

55. Jackson M, Song W, Liu MY, et al. Modulation of the neuronal glutamate transporter EAAT4 by two interacting proteins. Nature. 2001; 410: 89–93.

56. Wang DS, Shaw G. The association of the C-terminal region of beta I sigma II spectrin to brain membranes is mediated by a PH domain, does not require membrane proteins, and coincides with an inositol-1,4,5 triphosphate binding site. Biochem Biophys Res Commun. 1995; 217: 608–15.

57. Deauaney J, Alloisio N, Morle L, et al. Molecular genetics of hereditary elliptocytosis and hereditary spherocytosis. Ann Genet. 1996; 39: 209–21.

58. Tse WT, Lecomte MC, Costa FF, et al. Point mutation in the beta-spectrin gene associated with alpha U74 hereditary elliptocytosis. Implications for the mechanism of spectrin dimer self-association. J Clin Invest. 1990; 86: 909–16.

59. Soong BW, Paulson HL. Spincerebellar ataxias: an update. Curr Opin Neurol. 2007; 20: 438–46.

60. Kizhatil K, Sandhu NK, Peachey NS, et al. Neuron. 2005; 48: 737–42.

61. Ayalon G, Davis JQ, Scotand PB, et al. An ankyrin-based mechanism for functional organization of dystrophin and dystroglycan. J Biol Chem. 2008; 135: 1189–200.

62. Hortsch M, Nagaraj K, Godenschwege TA. The interaction between L1-type proteins and ankyrins-a master switch for L1-type CAM function. Cell Mol Biol Lett. 2009; 125: 739–46.

63. Jagadeesh P, Sastry G, Prasad A, et al. Obscurin determines the architecture of the nodes of Ranvier. Neuron. 2005; 47: 703–14.

64. Garver TD, Ren Q, Tuvia S, et al. Tyrosine phosphorylation at a site highly conserved in the L1 family of cell adhesion molecules abolishes ankyrin binding and increases lateral mobility of neurofascin. J Biol Chem. 1997; 137: 703–14.

65. Tuvia S, Garver TD, Bennett V. The phospho-FIGQY tyrosine in the cytoplasmic domain of neurofascin is required for the expression of doublecortin in recognition of the phospho-FIGQY tyrosine in the cytoplasmic domain of neurofascin. J Neurosci. 2002; 22: 7948–58.

66. Zhou D, Lambert S, Malen PL, et al. 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: 1295–304.

67. Hemstrom KL, Xu X, Ogawa Y, et al. Neurofascin assembles a specialized extracellular matrix at the axon initial segment. J Cell Biol. 2007; 178: 875–86.

68. Custer AW, Kazarina-Novyes K, Sakurui T, et al. The role of the ankyrin-binding protein NrCAM in node of Ranvier formation. J Neurosci. 2003; 23: 10032–9.

69. Lambert S, Davis JQ, Bennett V. Morphogenesis of the node of Ranvier: co-clusters of ankyrin and ankyrin-binding integral proteins define early developmental intermediates. J Neurosci. 1997; 17: 7025–36.

70. Eshed Y, Feinberg K, Poliak S, et al. Giomerin mediates Schwann-cell-axon interaction and the molecular assembly of the nodes of Ranvier. Neuron. 2005; 47: 215–29.

71. Kontrogianni-Konstantopoulos A, Catino DH, Strong JC, et al. Obscurin modulates the assembly and organization of sarcosomes and the sarcoplasmic reticulum. FASEB J 2006; 20: 2102–11.

72. Young P, Ehler E, Gautel M, Obscurin, a giant sarcromeric Rho guanine nucleotide exchange factor protein involved in sarcomere assembly. J Cell Biol. 2001; 154: 123–36.

73. Borisov AB, Sutter SB, Kontrogianni-Konstantopoulos A, et al. Essential role of obscurin in cardiac myofibrillogenesis and hypertrophic response: evidence from small interfering RNA-mediated gene silencing. Histochim Cell Biol. 2006; 125: 227–38.

74. Lange S, Ouyang K, Meyer G, et al. Obscurin determines the architecture of the longitudinal sarcoplasmic reticulum. J Cell Sci. 2009; 122: 2640–50.

75. Raaeker MO, Su F, Geisler SB, et al. Obscurin is required for the lateral alignment of striated myofibrils in zebrafish. Dev Dyn. 2006; 235: 2018–29.

76. Arimura T, Matsumoto Y, Okazaki O, et al. Structural analysis of obscurin gene in hypertrophic cardiomyopathy. Biochem Biophy Res Commun. 2007; 362: 281–7.
91. Bowman AL, Kontrogianni-Konstantopoulos A, Hirsch SS, et al. Different obscure isoforms localize to distinct sites at sarcosomes. FEBS Lett. 2007; 581: 1549–54.

92. Fukuzawa A, Idowu S, Gautel M. Complete human gene structure of obscure: implications for isoform generation by differential splicing. J Muscle Res Cell Motil. 2005; 26: 427–34.

93. Fukuzawa A, Lange S, Holt M, et al. Interactions with titin and myomesin target obscure and obscure-like 1 to the M-band: implications for hereditary myopathies. J Cell Sci. 2008; 121: 1841–51.

94. Hackman P, Vihola A, Haravuori H, et al. Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. Am J Hum Genet. 2002; 71: 492–500.

95. Udd B, Vihola A, Sarparanta J, et al. Titinopathies and extension of the M-line mutation phenotype beyond distal myopathy and LGMD2J. Neurology. 2005; 64: 636–42.

96. Van den Bergh PY, Bouquiaux O, Verellen C, et al. Tibial muscular dystrophy in a Belgian family. Ann Neurol. 2003; 54: 248–51.

97. Cunha SR, Mohler PJ. Obscurin targets ankyrin-B and protein phosphatase 2A to the cardiac M-line. J Biol Chem. 2008; 283: 31968–80.

98. Armani A, Galli S, Giacomello E, et al. Molecular interactions with obscurin are involved in the localization of muscle-specific small ankyrin1 isoforms to subcompartments of the sarcoplasmic reticulum. Exp Cell Res. 2006; 321: 3546–58.

99. Mohler PJ, Hoffman JA, Davis JQ, et al. Isoform specificity among ankyrins: an amphipathic alpha-helix in the divergent regulatory domain of ankyrin-B interacts with the molecular co-chaperone Hsp40. J Biol Chem. 2004; 279: 25798–804.

100. Mohler PJ, Le Scouarnec S, Denjoy I, et al. Defining the cellular phenotype of “ankyrin-B” variants: human ANK2 variants associated with clinical phenotypes display a spectrum of activities in cardiomyocytes. Circulation. 2007; 115: 432–41.

101. Mohler PJ, Schott JJ, Gramolini AO, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature. 2003; 421: 634–9.

102. Mohler PJ, Sliwa K, Napolitano C, et al. A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. Proc Natl Acad Sci USA. 2004; 101: 9317–42.

103. Birkenmeier CS, Barker JE. Hereditary haemolytic anaemias: unexpected sequelae of mutations in the genes for erythroid membrane skeletal proteins. J Path. 2004; 204: 450–9.

104. Kordeli E, Bennett V. Distinct ankyrin isoforms at neuron cell body and nodes of Ranvier resolved using erythrocyte ankyrin-deficient mice. J Cell Biol. 1991; 114: 1243–59.

105. Birkenmeier CS, Gifford EJ, Barker JE. Normoblastosis, a murine model for ankyrin-deficient hemolytic anemia, is caused by a hypomorphic mutation in the erythroid ankyrin gene Ank1. Hematol J. 2003; 4: 445–9.

106. Ferreira MA, D’Onovano MC, Meng YA, et al. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet. 2008; 40: 1056–66.

107. Schulze TG, Detera-Wadleigh SD, Akula N, et al. Two variants in ankyrin 3 (ANK3) are independent genetic risk factors for bipolar disorder. Mol Psychiatry. 2009; 14: 487–91.

108. Scott LJ, Muglia P, Kong XQ, et al. Genome-wide association and meta-analysis of bipolar disorder. Mol Psychiatry. 2009; 14: 487–91.

109. Koch I, Schwarz H, Beuchle D, et al. Drosophila ankyrin 2 is required for synaptic stability. Neuron. 2008; 58: 210–22.

110. Pielage J, Cheng L, Fetter RD, et al. A presynaptic giant ankyrin stabilizes the NMJ through regulation of presynaptic microtubules and transsynaptic cell adhesion. Neuron. 2008; 58: 195–209.

111. Garrido JJ, Giraud P, Carlier E, et al. A targeting motif involved in sodium channel clustering at the axonal initial segment. Science. 2003; 300: 2091–4.

112. 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: 27333–9.

113. Biervert C, Schroeder BC, Kubisch C, et al. A potassium channel mutation in neonatal human epilepsy. Science. 1998; 279: 403–6.

114. Dedeck K, Kunath B, Kananura C, et al. Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K⁺ channel. Proc Natl Acad Sci USA. 2001; 98: 12272–7.

115. Singh NA, Charlier C, Stauffer D, et al. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nat Genet. 1998; 18: 25–9.

116. Kizhatil K, Baker SA, Arshavsky VY, et al. Ankyrin-G promotes cyclic nucleotide-gated channel transport to rod photoreceptor sensory cilia. Science. 2009; 323: 1614–7.

117. Kondo H, Qin M, Mizota A, et al. A homozygosity-based search for mutations in patients with autosomal recessive retinitis pigmentosa, using microsatellite markers. Invest Ophthalmol Vis Sci. 2004; 45: 4433–9.

118. Le Scouarnec S, Bhasin N, Vieyres C, et al. Dysfunction in ankyrin-B-dependent ion channel and transporter targeting causes human sinus node disease. Proc Natl Acad Sci USA. 2008; 105: 15617–22.

119. Cohen NR, Taylor JS, Scott LB, et al. Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. Curr Biol. 1998; 8: 26–33.

120. Dahme M, Bartsch U, Martini R, et al. Disruption of the mouse L1 gene leads to malformations of the nervous system. Nat Genet. 1997; 17: 346–9.

121. Demyanyenko GP, Tsai AY, Maness PF. Abnormalities in neuronal process extension, hippocampal development, and the ventricular system of L1 knockout mice. J Neurosci. 1999; 19: 4907–20.

122. Haney CA, Sahenk Z, Li C, et al. Heterophilic binding of L1 on unmethylated sensory axons mediates Schwann cell adhesion and is required for axonal survival. J Cell Biol. 1999; 146: 1173–84.

123. Otsuka AJ, Boontrakulpoontawee P, Rebeiz N, et al. Novel UNC-44 A013 ankyrin is required for axonal guidance in C. elegans, contains six highly repetitive STEP blocks separated by seven potential transmembrane domains, and is localized to neuronal processes and the periphery of neuronal cell bodies. J Neurobiol. 2002; 50: 333–49.

124. Otsuka AJ, Franco R, Yang B, et al. An ankyrin-related gene (unc-44) is necessary for proper axonal guidance in Caenorhabditis elegans. J Cell Biol. 1995; 129: 1081–92.

125. Hall SG, Bieber AJ. Mutations in the Drosophila neuroglian cell adhesion molecule affect motor neuron pathfinding and cell adhesion. J Cell. Mol. Med. Vol 13, No 11-12, 2009
peripheral nervous system patterning. J Neurobiol. 1997; 32: 325–40.

127. Chen W, Hing H. The L1-CAM, Neuroglian, functions in glial cells for Drosophila antennal lobe development. Dev Neurobiol. 2008; 68: 1029–45.

128. Castellani V, Falk J, Rougon G. Semaphorin3A-induced receptor endocytosis during axon guidance responses is mediated by L1 CAM. Mol Cell Neurosci. 2004; 26: 89–100.

129. Castellani V, De Angelis E, Kenwrick S, et al. Cis and trans interactions of L1 with neuropilin-1 control axonal responses to semaphorin 3A. EMBO J. 2002; 21: 6348–57.

130. Castellani V, Chedotal A, Schachner M, et al. Analysis of the L1-deficient mouse phenotype reveals cross-talk between Sema3A and L1 signaling pathways in axonal guidance. Neuron. 2000; 27: 237–49.

131. Chen L, Ong B, Bennett V. LAD-1, the Caenorhabditis elegans L1CAM homologue, participates in embryonic and gonadal morphogenesis and is a substrate for fibroblast growth factor pathway-dependent phosphotyrosine-based signaling. J Cell Biol. 2001; 154: 841–56.

132. Needham LK, Thelen K, Maness PF. Cytoplasmic domain mutations of the L1 cell adhesion molecule reduce L1-ankyrin interactions. J Neurosci. 2001; 21: 1490–500.

133. Fransen E, Van Camp G, D’Hooge R, et al. Genotype-phenotype correlation in L1 associated diseases. J Med Genet. 1998; 35: 399–404.

134. Hoock TC, Peters LL, Lux SE. Isoforms of ankyrin-3 that lack the NH2-terminal repeats associate with mouse macrophage lysosomes. J Cell Biol. 1997; 136: 1059–70.

135. Beck KA, Buchanan JA, Nelson WJ. Golgi membrane skeleton: identification, localization and oligomerization of a 195 kDa ankyrin isoform associated with the Golgi complex. J Cell Sci. 1997; 110: 1239–49.