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Cardiac ankyrins in health and disease

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Abstract

Ankyrins are critical components of ion channel and transporter signaling complexes in the cardiovascular system. Over the past five years, ankyrin dysfunction has been linked with abnormal ion channel and transporter membrane organization and fatal human arrhythmias. Loss-of-function variants in the ankyrin-B gene (*ANK2*) cause "ankyrin-B syndrome" (previously called type 4 long QT syndrome), manifested by a complex cardiac phenotype including ventricular arrhythmias and sudden cardiac death. More recently, dysfunction in the ankyrin-B-based targeting pathway has been linked with a highly penetrant and severe form of human sinus node disease. Ankyrin-G (a second ankyrin gene product) is required for normal expression, membrane localization, and biophysical function of the primary cardiac voltage-gated sodium channel, $Na_v1.5$. Loss of the ankyrin-G/$Na_v1.5$ interaction is associated with human cardiac arrhythmia (Brugada syndrome). Finally, in the past year ankyrin dysfunction has been associated with more common arrhythmia and cardiovascular disease phenotypes. Specifically, large animal studies reveal striking remodeling of ankyrin-B and associated proteins following myocardial infarction. Additionally, the *ANK2* locus has been linked with QT_c interval variability in the general human population. Together, these findings identify a host of unanticipated and exciting roles for ankyrin polypeptides in cardiac function. More broadly, these findings illustrate the importance of local membrane organization for normal cardiac physiology.

1. Introduction

Ankyrin polypeptides, once thought to solely serve structural roles in the erythrocyte, are now recognized as multifunctional proteins involved in targeting and stabilization of ion channels and transporters in diverse tissues and cell types including skeletal and cardiac myocytes, neurons, photoreceptors, and epithelial cells [1–4]. Ankyrin-R (*ANK1* gene) was originally identified in the late 1970s as a critical structural protein of the erythrocyte plasma membrane [5]. In the mid 1990s, human gene mutations in *ANK1* were discovered to cause erythrocyte spectrin-deficiency, resulting in hereditary spherocytosis and anemia [6]. Over the past decade, advances in ankyrin biology have generated new insights into the molecular mechanisms underlying a diverse range of human cardiovascular disorders. Ankyrin-B and ankyrin-G are now recognized to play essential roles in the targeting and membrane stabilization of ion channels and transporters in cardiomyocytes [7–16]. Inherited loss-of-function variants in the ankyrin-B gene (*ANK2)* cause "ankyrin-B syndrome" ventricular arrhythmias [10]. Moreover,

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ankyrin-B dysfunction was recently linked with human sinus node disease [17]. Ankyrin-G, a second ankyrin gene product, is required for targeting and membrane regulation of the primary voltage-gated sodium channel ($Na_V1.5$) in human heart [12,16]. A human mutation in the $Na_V1.5$ ankyrin-G-binding motif is associated with Brugada syndrome, an arrhythmia syndrome associated with risk of sudden death [12]. Thus, the identification of genetic variants in ankyrins and associated proteins (e.g. Na_V 1.5) has provided new information on the cellular and molecular basis of cardiac arrhythmias. This review will primarily focus on the emerging roles of ankyrin-B and ankyrin-G in cardiac function and cardiovascular disease.

2. Ankyrin genes

Ankyrin genes *ANK1* (human chromosome 8p11), *ANK2* (human chromosome 4q25–27), and *ANK3* (human chromosome 10q21) encode three canonical ankyrins: 210 kD ankyrin-R, 220 kD ankyrin-B, and 190 kD ankyrin-G, respectively [4,18]. Ankyrin-R expression is restricted to erythrocytes, neurons, and skeletal muscle, whereas ankyrin-B and ankyrin-G are ubiquitously expressed [4,18]. In addition to canonical ankyrins, alternative splicing of ankyrin genes produces a series of gene products with distinct subcellular distributions and functional properties [4,18,19]. For example, alternative splicing of *ANK2* results in differential distribution of 440 kD ankyrin-B (found at unmyelinated axons in neonatal brain) and 220 kD ankyrin-B (found in neural cell bodies, dendrites, and glial cells of adult brain) in the nervous system [20]. Likewise, alternative splicing of *ANK1* and *ANK2* genes in muscle results in differential subcellular location and function of ankyrin-R and ankyrin-B polypeptides [19, 21–27]. While all three ankyrin gene products have been identified in ventricular cardiomyocytes [7,8,12,28], the differential expression and subcellular distribution patterns of individual ankyrin gene products throughout the heart is currently unknown.

3. Structure and function of ankyrin polypeptides

Canonical ankyrins consist of four major domains: a membrane-binding domain (MBD), a spectrin-binding domain (SBD), a death domain (DD), and a C-terminal domain (CTD, Figure 1) [29]. The ankyrin membrane-binding domain (24 *ANK* repeats) interacts with a host of diverse membrane proteins, with each ankyrin polypeptide linked to a unique set of associated proteins (Table 1). Specifically, the ankyrin MBD interacts with ion channels, transporters, and pumps including the Na/K ATPase, voltage-gated Na^+ and K^+ channels, the Na/Ca exchanger, the ammonium transporter, the inositol 1,4,5 trisphosphate receptor (IP_3R), and the anion exchanger [7,10,12,30–34]. Additionally, ankyrin MBD interacts with cell adhesion molecules (CAMs) including CD44, E-cadherin, and L1 CAMs [4]. Interestingly, based on the ability of the MBD to interact simultaneously with multiple membrane proteins [13,35,36], a single ankyrin may orchestrate the formation of large homomeric or heteromeric protein complexes *in vivo*. This multivalent nature of ankyrin is clearly illustrated in the erythrocyte, where a single ankyrin-R polypeptide can organize homomeric protein complexes (coupling two dimers of the anion exchanger) as well as heteromeric complexes (coupling the anion exchanger with L1CAM) [35,36]. In cardiomyocytes, a single ankyrin-B polypeptide can form a ternary membrane protein complex of Na/Ca exchanger, Na^+/K^+ ATPase, and IP_3 receptor (Figure 1) [13].

The central spectrin-binding domain (SBD) links ankyrin to the actin-based cytoskeleton via β-spectrin isoforms [37]. This link between ankyrin and spectrin is critical for normal erythrocyte physiology, as mutations in ankyrin-R produce cell membrane instability and hemolytic anemia [38]. The mechanism by which ankyrin-R stabilizes the red blood cell membrane was initially thought to be solely through vertical association of the ankyrin/spectrin-associated cytoskeleton with the lipid bilayer [39]. However, ankyrins likely have additional key roles in formation and/or maintenance of spectrin/actin cytoskeleton as

evidenced by reduced spectrin levels in erythrocytes of humans and mice with ankyrin-R gene mutations [6,40]. Beyond the erythrocyte, coordinate expression of ankyrin and spectrin have been implicated in normal neuronal and epithelial cell membrane formation and function [41–44]. Importantly, the ankyrin SBD also interacts with downstream protein partners including B56α, the regulatory subunit of protein phosphatase 2A (Figure 1, Table 1, discussed below).

The ankyrin death and C-terminal domains comprise the ankyrin 'regulatory domain' that confers specificity of function for ankyrin gene products [10,45–47]. In cardiomyocytes, the ankyrin-B C-terminal domain interacts with the MBD and is important for driving ankyrin-B-specific functions [48]. Most importantly, the ankyrin-B C-terminal domain is the location of the majority of human *ANK2* arrhythmia loss-of-function variants [28,49]. Identified ankyrin regulatory domain interacting proteins include obscurin [21,23,27,50], Hdj1 [47], and Fas [51] (Table 1).

Finally, while specific binding sites on the ankyrin have yet to be mapped, recent work from Bennett and colleagues has identified new interactions of ankyrin polypeptides with cell adhesion molecules and ion channels. Specifically, in the past year ankyrin-G has been found to target cyclic nucleotide-gated channels in rod photoreceptors [2]. Moreover, ankyrin-G has been linked with the adherens junction protein E-cadherin in early embryos and epithelial cells [52]. Finally, ankyrin-B and ankyrin-G have been shown to associate with dystrophin and beta-dystroglycan in skeletal muscle [1]. Interestingly, a human dystrophin mutation associated with Becker muscular dystrophy blocks ankyrin-B/dystrophin interactions, resulting in mis-localization of dystrophin-Dp71 in skeletal muscle[1].

4. Ankyrin-B Syndrome

The importance of ankyrins for normal heart physiology is highlighted by the growing number of cardiac arrhythmia syndromes linked to ankyrin dysfunction. An early example may be found in the long QT (LQT) syndrome, a heterogenous group of inherited arrhythmogenic diseases characterized by prolonged corrected QT interval (QT_c) on the electrocardiogram (ECG) and susceptibility to life-threatening arrhythmias [53,54]. To date, nearly a dozen LQT syndromes have been identified. However, variants in three LQT genes account for the majority of clinical LQT syndrome cases. Specifically, LQT1-3 are associated with human variants in either potassium (LQT1,2) or sodium channel (LQT3) genes, leading to either decreased outward currents or increased inward currents, thereby delaying repolarization and prolonging the QT interval [54–57].

Type 4 long QT syndrome (LQTS4) was first described in 1995, and further defined in 2003 [10,58]. Linkage analysis studies on a four-generation French family with two consecutive cases of sudden death revealed a new LQTS locus on chromosome 4q25–27 [58]. Electrocardiographic screening of 56 members in the family revealed prolonged QT_c in 21 individuals. Further clinical evaluation of the 21 affected family members identified a unique clinical phenotype characterized by severe sinus node dysfunction (all 21 affected patients), atrial fibrillation (12 of 21 adult patients) and notched biphasic T wave morphology (19 of 21 patients), in addition to the more common LQT features of prolonged QT_c, syncope (4 of 21 patients) and sudden death (2 cases) [58].

In 2003, the human *ANK2* gene encoding ankyrin-B was identified as the affected gene in the large French kindred [10], serving as the first example of an LQT syndrome gene that encoded a protein other than an ion channel or channel subunit. Sequencing of the *ANK2* gene identified an A-to-G missense mutation at position 4274 in exon 36, resulting in the substitution of glycine for a glutamic acid at amino acid residue 1425 (E1425G) in the ankyrin-B spectrin-binding domain [10]. Twenty-two of 24 affected members who carried the E1425G variant displayed

prolonged QT_c. This variant was not found in unaffected family members or control subjects with normal ECGs [10].

Ankyrin-$B^{+/-}$ mice and human *ANK2* E1425G carriers display a number of common cardiac phenotypes including severe bradycardia, prolonged QT interval, heart rate variability, and episodes of isorhythmic atrio-ventricular dissociation [10,17]. Furthermore, similar to "ankyrin-B syndrome" patients, ankyrin-$B^{+/-}$ mice display polymorphic ventricular tachycardia, syncope, and sudden cardiac death in response to exercise and catecholamine injection [10]. Ankyrin-B localizes to both the M-line and Z-line in adult ventricular myocytes, and at M-lines in primary neonatal cardiomyocytes (Z-line/T-tubule domains not developed) [8,10,13,28]. Interestingly, ankyrin-$B^{+/-}$ cardiomyocytes show selective reduction of ankyrin-B levels at transverse-tubule/sarcoplasmic reticulum sites [10]. This reduction is associated with aberrant expression and membrane localization of ankyrin-B-associated proteins Na/Ca exchanger, Na/K ATPase, and IP_3R [10]. Importantly, these phenotypes are rescued by exogenous expression of ankyrin-B in cardiomyocytes, but not by an ankyrin-B variant containing the E1425G mutation [10].

At the level of the single cell, ankyrin-$B^{+/-}$ ventricular cardiomyocytes display elevated sarcoplasmic reticulum Ca^{2+} load and catecholamine-induced afterdepolarizations [10]. Cellular afterdepolarizations are likely caused by coordinate loss of membrane Na/Ca exchanger and Na/K ATPase due to disruption of ankyrin-based targeting. Similar to the activity of digitalis, decreased expression of membrane Na/K ATPase and Na/Ca exchanger in ankyrin-$B^{+/-}$ myocytes results in elevated cytosolic Na+ concentrations, that in turn decreases Ca^{2+} extrusion by the Na/Ca exchanger [10,13,15]. Ultimately, ankyrin-$B^{+/-}$ myocytes develop sarcoplasmic reticulum Ca^{2+} overload and increased likelihood for catecholamine-induced afterdepolarizations [10]. These events may result in triggered arrhythmias *in vivo* [59–61].

Since the discovery of the first *ANK2* loss-of-function variant (E1425G), analysis of human populations has identified a select number of additional *ANK2* human loss-of-function variants [28,49,62,63], as well as a host of benign polymorphisms [49]. Loss-of-function gene variants are associated with a wide spectrum of phenotypes including sinus node dysfunction, atrial fibrillation, conduction defects, and ventricular arrhythmias [10,28,49]. Interestingly, despite its presence in the original French kindred [10], prolonged QT interval is not a consistent phenotype of many carriers with specific *ANK2* variants [28,49].

ANK2 variants expressed in primary cardiomyocytes demonstrate a range of functional perturbations [49]. In fact, based on both clinical and *in vitro* phenotypes, *ANK2* loss-of-function variants are categorized into three distinct functional classes. Variants in the first class (G1406C, R1450W, and L1503V) are primarily associated with less severe clinical phenotypes (or asymptomatic individuals) and display only mild *in vitro* cellular phenotypes compared with wild-type neonatal cardiomyocytes [28,49]. Variants in the second class (T1404I, T1552N, and V1777M) are generally associated with more severe human ventricular phenotypes and behave as loss-of-function alleles in the context of a primary myocyte [28, 49]. The most severe ankyrin-B loss-of-function (dominant-negative) activity *in vitro* is associated with the third class of variants (E1425G, V1516D, and R1788W) [28,49], which are also linked with the most severe clinical phenotypes [28,49]. Together, these findings demonstrate that *ANK2* variants produce a spectrum of *in vitro* defects corresponding to a wide range of clinical phenotypes. However, additional cellular studies are necessary to determine the molecular mechanism underlying phenotypic variability of each human *ANK2* variant.

5. Ankyrin-B is essential for sinoatrial node automaticity

While early studies focused on the role of ankyrin-B in ventricular cardiomyocytes and arrhythmias, it is now clear that ankyrin-B is expressed throughout the heart. In fact, recent studies have identified an important role for ankyrin-B in sinoatrial node pacemaking. Sinus node dysfunction (SND), or "sick sinus syndrome" is considered a disease of the elderly with an exponential increase in its frequency with age [64,65]. In fact, SND affects one in every 600 individuals over age 65 years [66]. Furthermore, SND is a common cause of bradycardia and syncope and accounts for 50% of pacemaker implantations in the United States [66]. Despite its impact, the etiology of SND is heterogenous and not fully understood at this time. At the cellular level, replacement of nodal tissue by "fibrous tissue" has remained a prevailing theory [65,67]. However, in recent years, discovery of single gene defects associated with ion channels has expanded our understanding of sinus node dysfunction to include "channelopathies" and "familial" forms of the disease that can manifest at significantly younger ages [68–70].

Our group recently mapped two families with a severe and highly penetrant sinus node dysfunction to *ANK2* [17]. In the first family, 25 of 74 members were affected. Thirteen members had atrial fibrillation and 14 patients underwent permanent pacemaker implantation. Gene sequencing of the available family members revealed all 25 affected members as carriers of *ANK2* mutation (E1425G), whereas none of the unaffected subjects were carriers. In the second family, 13 of 44 screened individuals had SND. Three members manifested atrial fibrillation and six patients required pacemaker implantation for SND. Twelve members presented with an abnormal sinusoidal T and prominent U waves, and QT_c prolongation, similar to the first family. Echocardiographic screening revealed atrial septal defect in five cases. Out of 36 members of this family who underwent genotype testing, 20 were carriers of a common haplotype at the *ANK2* locus, while the other 16 were non-carriers [17]. All thirteen affected members were carriers of the *ANK2* disease haplotype. The maximum LOD score for marker D4S1616 showed evidence for strong linkage ($Z_{max} = 5.9$). Immunoblot analysis of a muscle biopsy revealed a striking decrease in ankyrin-B expression in one of the affected patients compared with samples from two unaffected individuals [17]. Collectively, these data demonstrate that the *ANK2* variants with decreased ankyrin-B expression are strongly associated with a familial form of SND that may occur at much younger age than typical SND.

Similar to patients with ankyrin-B-associated SND, mice lacking ankyrin-B display severe SND. Moreover, immunoblots of sinoatrial node (SAN) cells from ankyrin-$B^{+/-}$ mice show reduced expression of Na/K ATPase, Na/Ca exchanger, and IP_3 receptor [17]. Interestingly, the distribution of one L-type calcium channel isoform, $Ca_v1.3$, was also affected in ankyrin-$B^{+/-}$ SAN cells [17]. Moreover, L-type Ca^{2+} current was significantly reduced in isolated ankyrin-$B^{+/-}$ SAN cells. Finally, ankyrin-$B^{+/-}$ SAN cells display abnormal calcium handling and automaticity [17]. Collectively, these data indicate that a full complement of ankyrin-B is required for proper expression and membrane localization of $Ca_v1.3$, Na/K ATPase, Na/Ca exchanger, and IP_3 receptor in SAN cells. These findings provide a new cellular mechanism underlying human sinus node dysfunction and further highlight the importance of ankyrin-based targeting pathways in ion channel physiology. Finally, as noted above, individuals harboring *ANK2* variants may display atrial fibrillation in addition to SND [10,17]. To date, the role of ankyrin-B in the vertebrate atria has not been defined. However, based on findings in other excitable cardiac cell types, it is likely that ankyrin-B dysfunction will result in defects in normal atrial excitability due to abnormal ion channel and transporter targeting.

6. Ankyrin-B and obscurin target cardiac protein phosphatase 2A

Ankyrin-B plays a critical role in cardiomyocyte signaling pathways. Specifically, ankyrin-B was recently identified as a binding partner in ventricular myocytes for B56α, the regulatory subunit of protein phosphatase 2A (PP2A) [14]. PP2A is a multifunctional serine/threonine phosphatase linked with cardiac β-adrenergic signaling [71–73]. Furthermore, PP2A regulates a number of ion channels and transporters including L-type Ca^{2+}-channels [71,74], ryanodine receptor [75], IP_3 receptor [76], and Na/K ATPase [77]. Ankyrin-B and B56α are both localized over the M-line in neonatal cardiomyocytes, as well as at M-lines and Z-lines in adult cardiomyocytes [14]. Work from Bhasin *et al.* recently demonstrated that cardiomyocytes lacking ankyrin-B expression display a striking loss of B56α [14]. In a related study, Cunha *et al*. determined that the localization of ankyrin-B and PP2A is dependent on interaction of ankyrin-B with the large Rho-GEF obscurin [27] (previously identified as binding partner of small ankyrin-R isoforms in skeletal muscle [21,23]). While these data demonstrate that ankyrin-B, obscurin and PP2A are physiological binding partners in cardiomyocytes, the targets of ankyrin-B-associated PP2A are currently unknown. Additionally, future studies are needed to define the role for ankyrin-B/obscurin interactions. Elegant work from Bloch and colleagues in skeletal muscle, suggest ankyrin-B may play a role in obscurin-dependent regulation of myocyte myofibrillogenesis [50,78,79].

More recently, Terentyev and colleagues showed that inhibition of B56α in myocytes (by overexpression of *miR-1*) resulted in RyR2 hyperphosphorylation (by CaMKII) and myocyte electrical instability [80]. Interestingly, B56α loss was associated with spontaneous SR calcium release and afterdepolarizations [80], similar to phenotypes observed in ankyrin-$B^{+/-}$ myocytes lacking normal B56α targeting [10,14]. It is therefore intriguing to speculate that defects in CaMKII-based local signaling pathways may contribute to human ankyrin-B-linked catecholamine-induced arrhythmias.

7. Ankyrin-B in acquired arrhythmias

Recent large animal studies suggest that ankyrin-B dysfunction is associated with common acquired forms of heart disease [81]. Electrical remodeling in the peri-infarct zone creates a substrate favorable to the initiation and maintenance of reentrant arrhythmias following myocardial infarction [82,83]. An important component of this remodeling process involves the redistribution of ion channels in surviving myocytes near the infarct [84,85]. Recently, ankyrin-B levels were shown to be significantly affected following myocardial infarction in a large animal model [81]. Moreover, abnormal expression of ankyrin-B at both mRNA and protein levels was associated with reduced expression, and/or abnormal distribution of ankyrin-B associated membrane proteins (IP_3R, NCX1, Na^+/K^+ ATPase, and PP2A) [81]. These findings provide the first data on the role of ankyrin-B in acquired arrhythmias, and suggest that ankyrin-B may represent a central player in the genesis of electrical remodeling and arrhythmias following myocardial infarction.

8. Common genetic variants in ANK2 are associated with QT interval variability

Recent data from large human population studies have raised the exciting prospect that variability in ankyrin-B function may influence arrhythmia susceptibility in the general human population. Prolongation of QT interval causes susceptibility to polymorphic ventricular tachycardia and sudden death [86,87]. Conversely, shortening of QT interval may facilitate life-threatening reentrant arrhythmias in vulnerable patients [88]. In the general population, the QT interval is distributed normally, and may be influenced by many parameters including congenital factors, heart rate, age, sex, electrolyte levels, and many medications [89]. The rare

genetic variants affecting ion channel subunits or ionic currents have long been recognized to cause repolarization abnormalities and long QT syndrome [10,53,55,56,90]. Recently, through linkage disequilibrium (LD) mapping analysis, investigators of the large human KORA study explored the association between common genetic variants and QT interval variability in the general population [91,92]. Among other common genetic variants affecting repolarization [91,93,94], the study identified new common genetic variants in the 5′ genomic region of *ANK2* associated with variations in QT interval [92]. In particular, one single nucleotide polymorphism (SNP), rs6850768, was found to be associated with slightly shorter QT intervals [92]. These findings suggest a link between common genetic variants in *ANK2* and QT interval duration irrespective of confounding factors such as heart rate, age, sex and medication.

9. Ankyrin-G is required for targeting of cardiac $Na_v1.5$

Voltage-gated sodium channels (Na_v) are required for normal vertebrate cardiac function. Dysfunction in cardiac $Na_v1.5$ may result in type 3 long QT syndrome, conduction defects, sick sinus syndrome, and Brugada syndrome [90,95–97]. While the mechanisms underlying $Na_v1.5$ channel biophysics are well described, little is known regarding the pathways required for targeting and regulation of $Na_v1.5$ at cardiac membrane domains.

In the nervous system, ankyrin-G is required for Na_v channel targeting to excitable membrane domains [41,98,99]. Na_v channels co-localize with ankyrin-G throughout the brain (axon initial segments, nodes of Ranvier) and at the neuromuscular junction [100–102]. Moreover, Na_v channels and ankyrin-G co-purify from brain [103,104]. Mice lacking ankyrin-G (cerebellar-specific knock-out) display striking reduction in Na_v channel targeting to axon initial segments and nodes of Ranvier [98]. Additionally, mice with cerebellar-knockout of ankyrin-G display aberrant distribution of β_4-spectrin, and neurofascin [41]. Ankyrin-G cerebellar-specific knockout mice display abnormal action potentials and ataxia, likely due to abnormal inter-neuronal circuitry [98]. Finally, recent work from Cooper and colleagues demonstrates a role of ankyrin-G in targeting potassium channels (*KCNQ2/KCNQ3*, encode subthreshold M-currents required for stabilizing neuronal resting potential and preventing repetitive action potential firing) to specific membrane domains [105,106]. Together, these studies conclusively link ankyrin-G function with the targeting of critical ion channels in excitable cells.

In 2003, two independent groups identified the structural requirements on brain Na_v channels ($Na_v1.2$) for ankyrin-G association as a highly conserved motif in the Na_v channel DII-DIII cytoplasmic domain [107,108]. Based on the conservation of the ankyrin-G-binding motif across Na_v channel gene products (including $Na_v1.5$), our group hypothesized that ankyrin-G regulates $Na_v1.5$ channel targeting in excitable myocytes. Ankyrin-G co-immunoprecipitates with $Na_v1.5$ from detergent-soluble lysates of heart, and directly interacts with $Na_v1.5$ using purified proteins [12]. Consistent with an *in vivo* interaction, ankyrin-G and $Na_v1.5$ are co-expressed at cardiac excitable membrane domains including the intercalated disc and transverse-tubule [12,16]. Finally, as predicted from previous studies, $Na_v1.5$ lacking the putative ankyrin-G-binding motif (nine residues) fails to associate with ankyrin-G [12].

More recently, our group utilized viral expression of ankyrin-G shRNAs to assess the requirement of ankyrin-G expression for $Na_v1.5$ targeting and function in myocytes [16]. Reduction of ankyrin-G at the level of the single myocyte results in a marked reduction in $Na_v1.5$ channel expression, targeting, and I_{Na} [16]. These phenotypes were specific for the ankyrin-G/$Na_v1.5$ pathway as Na/Ca exchanger and $Ca_v1.2$ targeting were unaffected by the ankyrin-G shRNA [16]. Finally abnormal $Na_v1.5$ phenotypes were rescued by exogenous expression of ankyrin-G, but not an ankyrin-G mutant lacking $Na_v1.5$-binding activity [16]. Together, these data support the role of a direct $Na_v1.5$/ankyrin-G interaction for normal expression, targeting, and function of membrane $Na_v1.5$ in cardiomyocytes.

10. Dysfunction in the ankyrin-G-based Nav1.5 channel targeting pathway is associated with human Brugada syndrome arrhythmia

The Brugada syndrome is an autosomal-dominant, potentially-fatal cardiac arrhythmia syndrome characterized by ST segment elevation in the precordial leads associated with right bundle branch block and T-wave inversion [109]. In affected individuals, sudden death is most prevalent at night, and is the result of ventricular fibrillation [110,111]. Gene variants in *SCN5A* (encodes $Na_v1.5$) account for up to ~30% of Brugada syndrome cases [112], and are associated with $Na_v1.5$ biophysical defects that disrupt inward (depolarizing) sodium current [113]. The vast majority of human $Na_v1.5$ gene mutations affect channel biophysical properties.

We hypothesized that defects in normal $Na_v1.5$ membrane expression may result in an alternative mechanism for the Brugada syndrome. In 2002, Priori and colleagues identified a G3157A *SCN5A* variant in a proband with Brugada syndrome [113]. Subsequent analysis revealed that this variant (resulting in Nav1.5 E1053K) is located in the ankyrin-binding motif of Nav1.5 DII-DIII[12]. Interestingly, $Na_v1.5$ E1053K lacks *in vitro* ankyrin–binding activity [12]. Moreover, $Na_v1.5$ E1053K displays abnormal intercalated disc and transverse-tubule targeting when introduced into adult rat cardiomyocytes [12]. Thus, the human Brugada syndrome is associated with an *SCN5A* mutation that affects normal $Na_v1.5$ membrane targeting (consistent with $Na_v1.5$ loss-of-function). Ankyrin-G may play an unexpected secondary role in $Na_v1.5$ channel biophysical properties. Specifically, $Na_v1.5$ E1053K lacking ankyrin-binding displays a more negative threshold for activation, faster inactivation, and slower recovery than wild-type channel when analyzed in HEK293 cells [12]. Clearly, additional experiments in primary myocytes will be necessary to further define a potential role of ankyrin-G for Na_v channel biophysical regulation.

Thus, ankyrin-G is critical for the cellular machinery required for Na_v channel targeting in cardiomyocytes. As Na_v channel function is essential for excitable cell function, future experiments to define additional components of the ankyrin-dependent targeting pathway will be critical for our understanding of myocyte cell biology, as well as for defining potential future targets for the treatment of excitable cell disease.

11. Summary

Exciting discoveries over the past decade have established the importance of local signaling domains for excitable cell function. While key myocyte membrane receptors, transporters, and ion channels remain the primary mediators of cardiomyocyte signaling, the proteins responsible for local organization of ion channels and transporters with signaling and effector proteins are now recognized as critical determinants of normal myocyte function. Over the past five years, ankyrin-B and ankyrin-G have been recognized as essential cellular components for targeting ion channels and transporters to specialized membrane micro-domains in the heart. Dysfunction in cardiac ankyrin polypeptides results in several congenital arrhythmia syndromes including ankyrin-B syndrome, congenital sinus node dysfunction, and Brugada syndrome. Furthermore, recent large animal studies have linked ankyrin-B dysfunction to more common acquired arrhythmias (e.g. after myocardial infarction). Moreover, ankyrin-B expression has been linked to QT interval variability in the general population suggesting a broader role for ankyrins in regulating heart function. In conclusion, recent developments in ankyrin biology have expanded our understanding of the molecular bases of human arrhythmias. At the same time, many questions remain and further studies are needed to unravel the cellular pathways underlying ankyrin function in diverse excitable cardiac tissue.

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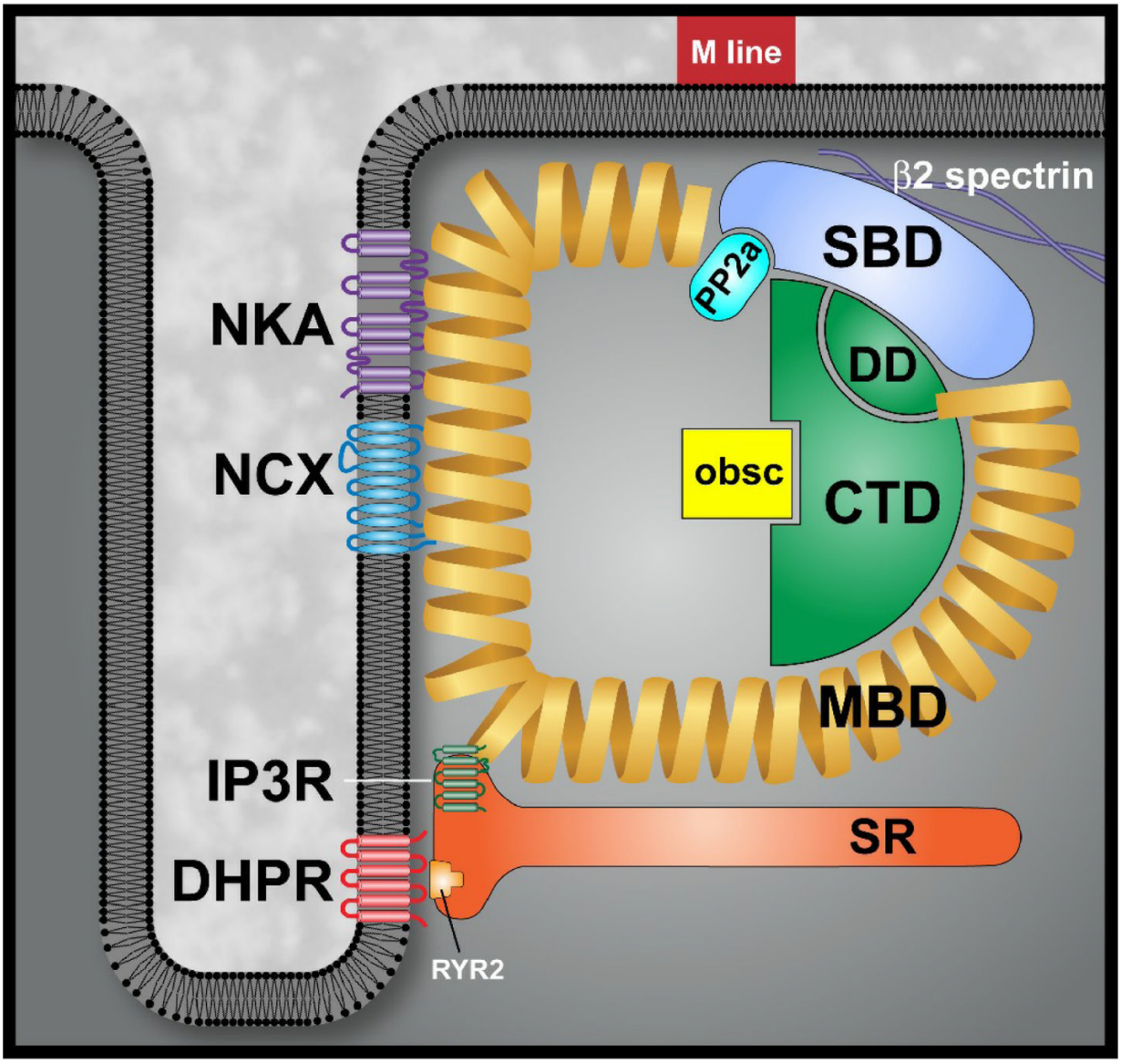

Figure 1. Cardiac ankyrin-B membrane-associated protein complex

Ankyrins are comprised of four distinct folding domains including a membrane-binding domain (MBD), spectrin-binding domain (SBD), C-terminal domain (CTD) and death domain (DD). Together, the CTD and DD comprise the regulatory domain. In heart, ankyrin-B targets ion channels and transporters including Na/K ATPase (NKA), Na/Ca exchanger (NCX) and IP3 receptor (IP3R) in cardiomyocytes. Thorugh interactions with obscurin (obs), ankyrin-B also targets protein phosphatase 2A (PP2A) via the PP2A B56α subunit.

Table 1

Ankyrin-interacting proteins

Protein Class	Protein	Ankyrin(s)	Domain
Channel/Transporter/Pumps	Anion Exchanger	R,G,B	MBD
	Na/K ATPase	R,B,G	MBD/SBD
	Na/Ca exchanger	R, B	MBD
	Nav channels	G	MBD
	Ammonium	G	MBD
	Transporter		
	InsP3 receptor	B	MBD
	Kv3.1	G	?
	Kv7.2, Kv7.3	G	MBD
	Cyclic nucleotide- gated channel	G	?
Cell adhesion Molecules	L1CAMs	R,B,G	MBD
	E-cadherin	G	?
	dystrophin	B,G	?
	Beta-dystroglycan	G	?
	CD44	R,B	MBD
	Nav channel β subunit	R,B,G	MBD
Cell Transport Proteins	Clathrin	R	MBD
	Dynactin-4	B	?
Signaling Proteins	B56α subunit of	B	SBD
	PP2A		
	Hdj-1	B	CTD
	Fas	G	DD
Cytoskeletal Proteins	Obscurin	R,B	CTD
	Spectrin	R,B,G	SBD
	Tubulin	B,R	?

Abbreviations: R, ankyrin-R; B, ankyrin-B, G, ankyrin-G; MBD, membrane-binding domain, SBD, spectrin-binding domain; DD, death domain; CTD, C-terminal domain.