Ankyrin-Based Trafficking and Scaffolding of Membrane Proteins: Implications for Plasma Membrane Stability, Formation, and Specialization

Gokay Yamankurt¹, Francis T Nguyen¹, Peter J Mohler²,⁴ and Shane R Cunha*⁴

¹Department of Integrative Biology and Pharmacology, University of Texas Health Science Center, USA
²Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, USA
³Department of Internal Medicine, Division of Cardiovascular Medicine, The Ohio State University, USA
⁴Department of Physiology and Cell Biology, The Ohio State University, USA

Abstract

Through the collaborative actions of β-spectrin and ankyrin, a cytoskeletal adaptor protein, integral and peripheral membrane proteins find order and stability in the relatively fluid environment of the plasma membrane. Not only is the ankyrin/β-spectrin complex responsible for the proper targeting and retention of membrane proteins but it facilitates the formation of multi-protein complexes to maximize local signaling between membrane and effector proteins. Dysfunction in ankyrin or β-spectrin causes deficiencies in fundamental cellular properties such as membrane stability, excitability, and adhesion. This review focuses on the direct effects of ankyrin function on membrane proteins in terms of binding and stability, intracellular transport, membrane targeting and retention, and altered biophysical properties. We propose that ankyrin and β-spectrin are important for the normal progression of many membrane proteins along their biosynthetic pathway from stabilizing the membrane protein to its proper trafficking and eventual targeting and retention at membrane domains. The second half of the review addresses how an ankyrin/membrane protein interaction influences the local membrane environment with particular emphasis on membrane stability, membrane domain formation, and membrane domain specialization. We propose that not only are ankyrins necessary for erythroid membrane stability but they are required in some cell types for membrane domain formation and integral for the formation of specialized membrane domains in myocytes.

Keywords: Ankyrin; Spectrin; Intracellular trafficking; Protein targeting; Membrane scaffolding; Ion channels; Cardiac arrhythmia

Introduction

Membrane proteins facilitate a variety of interactions between the external and internal cellular environments including the transport or exchange of ions and molecules, cellular adherence to a surrounding substrate or neighboring cell, and the translation of an extracellular signal into an altered cellular response. Given the importance of membrane proteins, many cellular processes are involved in the delivery, retention, and recycling of these proteins. Ankyrins are adaptor proteins that link membrane proteins to the underlying cytoskeleton. Both ankyrin and its cytoskeletal cohort β-spectrin have been linked to many steps in the biosynthetic pathway of membrane proteins from intracellular transport to membrane targeting and retention, in addition to clathrin-mediated endocytosis and endosomal recycling. This review is organized around two central themes: ankyrin function on membrane proteins and the cellular effects of ankyrin/membrane protein interactions. The first aim will address the direct effect of ankyrin function on membrane proteins in terms of protein binding, intracellular trafficking, membrane targeting and retention, and altered biophysical properties. In the later half, a discussion on the cellular effects of ankyrin/membrane protein interactions will include the mechanical stabilization of plasma membrane, membrane domain formation, and membrane-domain specialization.

Ankyrins

Ankyrins serve as an interface between membrane-bound proteins and the underlying cytoskeleton. This interaction contributes to the stability of the membrane protein’s location and expression within the plasma membrane. Ankyrins appear to be a metazoan invention as they have only been detected in worms, flies, rodents, and humans, but not in yeast or plants. In human, three genes ANK1, ANK2, and ANK3 encode isoforms of ankyrin-R, ankyrin-B, and ankyrin-G respectively. While very little is known about the regulation of ankyrin gene transcription, it will be complex because ankyrin genes are quite large with multiple first exons and numerous alternative transcripts have already been identified. Alternative splicing of ankyrin genes results in a diverse array of isoforms with unique functions and distinct expression patterns. Expression of ankyrin-R isoforms has been detected in erythrocytes, striated muscle, and some neurons. In contrast, isoforms of ankyrin-B and ankyrin-G have been detected in a greater variety of tissues. While some tissues such as the heart and cerebellum display all three ankyrin gene products, they are not functionally redundant, i.e. ankyrin-G cannot compensate for the loss of ankyrin-B in cardiomyocytes [1].

The prototypical ankyrin has three functional domains: a membrane-binding domain (MBD), spectrin-binding domain (SBD), and a C-terminal regulatory domain (CTD) (Figure 1). The membrane-binding domain is comprised of 24 consecutive ANK repeats with multiple first exons and numerous alternative transcripts have already been identified. Alternative splicing of ankyrin genes results in a diverse array of isoforms with unique functions and distinct expression patterns. Expression of ankyrin-R isoforms has been detected in erythrocytes, striated muscle, and some neurons. In contrast, isoforms of ankyrin-B and ankyrin-G have been detected in a greater variety of tissues. While some tissues such as the heart and cerebellum display all three ankyrin gene products, they are not functionally redundant, i.e. ankyrin-G cannot compensate for the loss of ankyrin-B in cardiomyocytes [1].

The prototypical ankyrin has three functional domains: a membrane-binding domain (MBD), spectrin-binding domain (SBD), and a C-terminal regulatory domain (CTD) (Figure 1). The membrane-binding domain is comprised of 24 consecutive ANK repeats that are arranged in a superhelical array forming a solenoid [2]. The ANK repeats have inherent spring-link qualities that confer resilience to the membrane-binding domain from mechanical perturbations that occur

*Corresponding author: Dr. Shane R. Cunha, University of Texas Health Science Center, Department of Integrative Biology and Pharmacology, 6431 Fannin St., MSE R356, Houston, TX 77030, USA, Tel: 713-500-7433; Fax: 713-500-0689; E-mail: Shane.R.Cunha@uth.tmc.edu

Received November 17, 2011; Accepted December 17, 2011; Published January 30, 2012

Citation: Yamankurt G, Nguyen FT, Mohler PJ, Cunha SR (2013) Ankyrin-Based Trafficking and Scaffolding of Membrane Proteins: Implications for Plasma Membrane Stability, Formation, and Specialization. J Proteomics Bioinform 6: 221-228. doi:10.4172/jpb.1000284

Copyright: © 2012 Yamankurt G, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

J Proteomics Bioinform
ISSN: 0974-276X JPB, an open access journal

Membrane Protein Transporters

Volume 6(10) 221-228 (2013) - 221
in ankyrin-expressing tissues such as erythrocytes and striated muscle [3]. ANK repeats are a common motif for protein-protein interactions and most of ankyrin interactions with integral membrane proteins occur via the ANK repeats in the membrane-binding domain. The spectrin-binding domain contains a ZUS domain (from the mouse zona occludens 1 (ZO-1) and the C. elegans uncoordinated protein 5 (unc5)) that comprises the minimal binding domain for spectrin [4]. In contrast, spectrin repeats 14 and 15 comprise the minimal binding domain for ankyrin [5-7]. In addition to spectrin, the spectrin-binding domain has been shown to interact with B56alpha, a regulatory subunit of protein phosphatase 2A, and dynactin-4, an adaptor protein that links the dynein motor to membrane cargo [8,9]. As different ankyrin gene products are relatively homologous within the MBD and SBD, the C-terminal regulatory domain, which is the least homologous, governs the specificity of ankyrin function and its subcellular localization. The CTD confers ankyrin specificity by regulating ankyrin interactions with itself, the cytoskeleton, and integral membrane proteins [10-12]. Not surprisingly, many of the missense mutations associated with ankyrin dysfunction have been localized to the C-terminal regulatory domain [13]. The prevalence of ankyrin dysfunction in a variety of human disorders including hemolytic anemia, cardiac arrhythmias, and neonatal diabetes highlights the significance of ankyrin function for normal cellular physiology [14-18].

Ankyrin functions

Binding and stabilization of membrane proteins: Ankyrin interacts with a variety of integral membrane proteins including ion channels, transporters, and cell adhesion molecules (see Table 1). Ankyrin-associated ion channels include voltage-gated sodium channels (Na_A1.1, Na_A1.2, Na_A1.3, Na_A1.4, Na_A1.5, and Na_A1.6) and potassium channels (K_C3, K_C7, the L-type voltage-gated calcium channel Ca_A1.3, inositol trisphosphate receptors (IP_R), and the potassium inward rectifying channel subunit Kir6.2 [17,19-26]. Ankyrin-associated ion transporters include the sodium/calcium exchanger (NCX), sodium/potassium ATPase (NKA), anion exchangers (AE1, AE2, AE3), hydrogen/potassium ATPase, and the RhBG ammonium transporter [27-34]. Ankyrin-associated cell adhesion molecules include the family of L1-CAMs, E-cadherin, CD44, and β-dystroglycan [35-40]. While the structural requirements underlying many of these interactions have yet to be elucidated, previous studies have demonstrated that MBD ANK repeats mediate ankyrin interactions with integral membrane proteins. Some interactions involve one ANK repeat, while other interactions require multiple consecutive ANK repeats. For example, ankyrin-G ANK repeat 14 or 15 is sufficient to bind Na_A1.5, while the NCX binding site is spread across ankyrin-B ANK repeats 16, 17, and 18 [41,42]. One ankyrin molecule can interact with multiple membrane proteins simultaneously, thereby allowing for multi-protein complex formation. Another important aspect of ankyrin binding is that this interaction stabilizes the membrane protein. In ankyrin haploinsufficiency, ankyrin-associated membrane proteins including NCX, NKA, IP_R, Na_A1.5, and Ca_A1.3 display reduced protein expression and membrane localization [21,42-44]. More detailed studies have demonstrated that ankyrin-B binding to the IP_R lengthens the receptor’s half-life from 3.7 hours to 11.7 hours [45]. Likewise, NCX binding to ankyrin-B extends the exchanger’s half-life from 17.8 hours to 27.2 hours [41]. In summary, multiple membrane proteins can simultaneously bind to an ankyrin membrane-binding domain and this interaction stabilizes the membrane proteins.

Intracellular trafficking of membrane proteins: While a lot of ankyrin biology research has focused on the targeting and retention of integral membrane proteins at the plasma membrane, there is evidence that ankyrin and β-spectrin interact with membrane proteins at many steps along the membrane protein’s biosynthetic pathway. First, isoforms of ankyrin (Ank195 and AnkG119) and β-spectrin (β3-

---

**Table 1:** Proteins that interact with ankyrins (R, B, or G) are grouped according to their general function. Sites of interaction on ankyrin are listed (MBD: membrane-binding domain, SBD: spectrin-binding domain, DD: death domain, CTD: C-terminal domain).

| Protein | Ankyrin | Domain |
|---------|---------|--------|
| Rh antigen | R | MBD |
| IP_R | B | MBD |
| Ca_A1.3 | B | MBD |
| Kir6.2 | B | MBD |
| Na_A1.5 | G | MBD |
| Na_A1.7 | G | MBD |
| K_C3 | G | SBD |
| CNG-β | G | SBD |
| Anion exchanger (AE1, 2, 3) | R | MBD |
| NCX | R, B | MBD |
| NKA | R, B, G | MBD |
| Ammonium transporter | R, G | CTD |
| Cell adhesion molecules: | | |
| CD44 | R | MBD |
| L1-CAM family | R, B, G | MBD |
| E-cadherin | G | SBD |
| β-dystroglycan | G | SBD |
| Cytoskeleton/structure: | | |
| β-spectrin | R, B, G | SBD |
| Obscurin | R, B, G | CTD |
| Dystrophin | B, G | CTD |
| Filamin C | G | CTD |
| Plakophilin-2 | G | CTD |
| Plecin | G | CTD |
| Intracellular transport: | | |
| Claudin | R | MBD |
| Tubulin | R, B | MBD |
| EHD1-4 | B | MBD |
| Dynactin-4 | B | SBD |
| EB1/3 | G | SBD |
| Other: | | |
| PP2A | B | SBD |
| Hdg1/Hsp40 | B | CTD |
| Fas | G | DD |
| Tiam-1 | R, G | MBD |
| Sigma receptor | B | SBD |

---

**Figure 1:** Ankyrin functional domains. The prototypical ankyrin has three functional domains. The membrane-binding domain (MBD) is comprised of 24 ANK repeats that bind many membrane proteins. The spectrin-binding domain (SBD) includes the ZUS motif, which is the minimal binding domain for spectrin. The death domain (DD) and C-terminal domain (CTD) regulate ankyrin’s association with itself, membrane proteins, and the cytoskeleton.
spectrin) have been associated with the trans Golgi network [46-52].
Furthermore, the association of β3-spectrin with the Golgi is partially
regulated by an ADP ribosylation factor-dependent increase in the
level of phosphatidylinositol 4,5-bisphosphate in the Golgi membrane
[53]. β3-spectrin is connected to the molecular motor dynein through
its interaction with actin-related protein (Arp1) that is a component of
the dynactin complex [54]. Considering its interactions with the
dynein/dynactin complex and its notable similarities to other coat-like
proteins such as clathrin, β3-spectrin is thought to be involved in
vesicular trafficking. Using a dominant negative construct to disrupt
Golgi-targeting of the endogenous β-spectrin, it was demonstrated that
the endoplasmic reticulum (ER) to Golgi transport of both the α- and
β-subunits of NKA is dependent upon β3-spectrin [49]. In support of
these findings, β3-spectrin null mice display a large number of vesicles
around the Golgi [55]. Similar to β3-spectrin, ankyrin is involved in
ER to Golgi transport of the NKA α-subunit. Ankyrin interacts with
a specific binding domain in the α-subunit that is necessary for the
subunit’s ER to Golgi transport [56]. Furthermore, normal ER to
Golgi trafficking of the α-subunit is disrupted by over-expression of
this domain. In contrast, ER to Golgi trafficking is rescued by inclusion
of this ankyrin-binding domain in a fusion protein that would have
otherwise remained in the ER [56]. Other converging evidence in
support of ankyrin’s role in intracellular trafficking includes a direct
interaction of ankyrin with tubulin [57-59] and dynactin-4 [8,35].
In addition, a new study demonstrated a direct interaction between
ankylin-G and the microtubule plus-end binding proteins EB1 and
EB3 [60]. This interaction partially regulates ankyrin-G’s subcellular
localization at the axon initial segments of neurons, suggesting that
ankylin-G may play an important role at the dynamic interface of the
plus-end microtubule and the actin cytoskeleton.

While both ankyrin and β-spectrin have been associated with
microtubule-based transport, ankyrin is also connected to clathrin-
mediated endocytosis and endosomal-based trafficking. Ankyrin-R
MBD binds to clathrin [61]. In addition, ankyrin-B MBD interacts
with all four of the Eps15-homology domain containing (EHD)
proteins, which are involved in endosomal-based anterograde and
retrograde trafficking of membrane proteins [62]. Even though all
four EHD proteins are expressed in the heart, there appears to be a
preferential interaction between ankyrin-B and EHD3 given that
ankylin-B hapolinsufficiency caused the most significant up-regulation
of EHD3 protein expression. In support of the hypothesis that EHD3
is involved in anterograde transport of ankyrin-B associated proteins,
NCX membrane expression was increased by EHD3 over-expression
and conversely its expression was decreased by EHD3 down-regulation
[62]. Finally, a truncated isoform of ankyrin-G has been detected in
late endosomal compartments that are immunopositive for the
lysosomal-associated membrane glycoprotein, suggesting that there
is a lysosomal-specific ankyrin isoform in addition to a Golgi-specific
isoform [63].

Targeting and scaffolding of membrane proteins: One of the
more obvious deficiencies associated with ankyrin dysfunction is the
loss of membrane protein targeting and scaffolding. For example,
ankylin-B hapolinsufficiency causes a decrease in the membrane
expression of the L-type voltage-gated calcium channel Ca1.3 in both
sinusoidal cells and atrial myocytes [16,21]. Interestingly, ankyrin-B
is required for full membrane expression of Ca1.3 and decreased channel
expression is associated with sinus node disease and atrial fibrillation
[16,21]. In the nervous system, the intrinsic self-assembly of axon
initial segments is predominantly mediated by ankyrin-G-dependent
retention of voltage-gate sodium and potassium channels, as well as the
cell adhesion molecule neurofascin-186.

The axon initial segment (AIS) is a highly specialized region of the
nerve that initiates the action potential thereby facilitating electrical
and chemical communication between neurons. Action potential
initiation is achieved through the coordinated activities of a variety of
voltage-gated ion channels clustered at the AIS. Ankyrin-G is critical
for AIS-enrichment of voltage-gated sodium channels Na1.2 and
Na1.6, in addition to the enrichment of potassium channel subunits
KCNO2 and KCNO3 [20,22-24,64]. In the absence of ankyrin-G in
cerebellar Purkinje neurons, voltage-gated sodium channels are no
longer properly localized to AIS and the neurons display reduced action
potential generation [64]. Ankyrin-G interaction with voltage-gated
sodium channels is positively regulated by channel phosphorylation
by the AIS-enriched CK2 kinase [65,66]. Ankyrin-G also targets cell
adhesion molecules including neurofascin-186 and neuron glia-related
Cell adhesion molecule neurofascin-186 (NF-186) is the target of synapse formation from GABAergic basket interneurons [67]. In hippocampal neurons, neurofascin-186 recruits the chondroitin sulfate proteoglycan brevican to the AIS, which has an inhibitory effect on interactions between presynaptic and postsynaptic membranes [68,69]. Ankyrin-G retention of voltage-gated ion channels and cell adhesion molecules is absolutely essential for AIS intracellular and extracellular formation.

Modulation of membrane protein biophysical properties: While the effect of ankyrin-binding on the biophysical properties of membrane proteins has not been studied in great detail, ankyrin has been shown to alter intrinsic biophysical properties of voltage-gated sodium channels and the potassium inward rectifying channel subunit Kir6.2. Mohler et al. [15] described a missense mutation in the ankyrin-binding domain of Na1.5 that disrupted channel association with ankyrin-G and was linked to Brugada syndrome, a cardiac disorder caused by decreased sodium current density. The missense mutation causes changes in activation and inactivation states of Na1.5 in a heterologous expression system. Similarly, Shirahata et al. [70] has demonstrated that ankyrin-G accelerates the rate of Na1.6 inactivation in a heterologous expression system. On the contrary, Lowe et al. [42] found no change in the inactivation state of Na1.5 following ankyrin-G knockdown in cardiomyocytes. These conflicting results warrant additional studies to clarify the effect of ankyrin-G on intrinsic properties of the voltage-gated sodium channel.

The potassium inward rectifying channel subunit (Kir6.2) is
another membrane protein that has altered biophysical properties upon
ankylin-binding. Kir6.2 is an ATP-sensitive channel that links cellular
metabolism with cellular excitability. Increased metabolism elevates
intracellular ATP that binds to Kir6.2 and closes the channel, leading to
membrane depolarization and cellular excitability. It has been shown
that ankylin-B selectively binds to the pore-forming channel subunit
Kir6.2, but not to Kir6.1 [17,71]. Moreover, the ankyrin-B/Kir6.2
protein complex includes the regulatory sulfonylurea receptor subunits
SUR1 and SUR2, although ankyrin-B does not directly bind to these
regulatory subunits [17,71]. Ankyrin-B/Kir6.2 interaction enhances
channel membrane expression and decreases the channel’s ATP
sensitivity [17,71]. The molecular mechanisms underlying the ankyrin-
dependent decrease in ATP sensitivity have yet to be discovered, but
the ankyrin-B interaction may cause steric hindrance between the ATP
molecule and Kir6.2.
Cellular effects of ankyrin/membrane protein interactions

Maintenance of plasma membrane mechanical stability: The bicarbonate/chloride exchanger band 3 (or anion exchanger 1, AE1) is the most abundant membrane protein in erythrocytes. In addition to playing a key role in carbon dioxide transport in blood, the anion exchanger also serves as a point of attachment for the erythroid cytoskeleton. Membrane-bound AE1 predominantly exists as a dimer and tetramer. The dimer is attached to the cytoskeleton through the junctional complex with its principal components AE1, protein 4.1, p55, and glycophorin. In contrast, the tetramer is linked to the cytoskeleton through the ankyrin complex that contains the core subunits AE1, ankyrin-R, and protein 4.2. Mutations to proteins in the ankyrin complex are generally associated with hereditary spherocytosis, a type of hemolytic anemia that is quite common, but renders the erythrocytes vulnerable to mechanical and osmotic disruption [72]. Of the subunits in the ankyrin complex, ankyrin-R mutations are the most predominant cause of hereditary spherocytosis (HS). At the molecular level, disrupting any component of the ankyrin complex compromises the attachment between the erythroid membrane and its underlying cytoskeleton; therefore, ankyrin-R interactions with AE1 are critical for the normal conformation and stability of erythroid membranes.

Membrane domain formation: In some cell types, the interaction of ankyrin with cell adhesion molecules and cytoskeletal proteins is important for the formation and/or maintenance of membrane domains. Specifically, it has been demonstrated that ankyrin-G is necessary for lateral membrane biogenesis in bronchial epithelial cells [36,73]. Ankyrin-G directly interacts with the cytoplasmic domain of the cell adhesion molecule E-cadherin leading to the retention of E-cadherin and β-catenin at nascent adherens junctions in growing lateral membrane domains [73,74]. Ankyrin-G recruitment of β2-spectrin stabilizes the developing adherens junctions and allows for the accumulation of lateral membrane. When ankyrin-G or β2-spectrin is reduced by siRNA treatment, there is a complete loss of lateral membrane biogenesis and a compensatory increase in the apical and basal membranes [73,74]. Interestingly, the apical to basal polarity in these epithelial cells is maintained despite the loss of lateral membrane [73,74]. Not only does ankyrin-G and β2-spectrin stabilize the lateral membrane domain expression of E-cadherin, but they also play important roles in the transport of E-cadherin from the trans-Golgi network. Disruption of ankyrin-G/E-cadherin interactions significantly increases the mislocalization of E-cadherin to the trans-Golgi network [36]. Lateral membrane biogenesis in bronchial epithelial cells is dependent on the post-Golgi transport and membrane stabilization of E-cadherin by an ankyrin-G/β2-spectrin protein complex.

In the mammalian retina, ankyrin-G is also important for the biogenesis of outer segments of rod photoreceptors. Specifically, ankyrin-G-treated retinas displayed significantly shortened rod outer segments compared to the control-treated retinas [75]. While the molecular mechanisms underlying this ankyrin-G-dependent membrane biogenesis has yet to be characterized, future studies should focus on ankyrin-associated cell adhesion molecules.

Membrane domain specialization

Transverse-tubules: In cardiac ventricular myocytes, transverse-tubules (T-tubules) are invaginations of the plasma membrane that maximize the interface between the sarcolemma and extracellular milieu. They facilitate the rapid and efficient propagation of membrane depolarization to the myocyte interior thereby ensuring the rapid and synchronized release of intracellular calcium from the sarcoplasmic reticulum (SR). T-tubules are enriched with ion channels and transporters that mediate the transmembrane flux of calcium ions. Calcium-induced calcium release from the SR is predominantly regulated by the coordinated activities of the L-type voltage-gated calcium channel (or dihydropyridine receptor, DHPR) and the ryanodine receptor (RyR) (Figure 2). As an integral membrane protein in the T-tubule, DHPR is aligned opposite the RyR, a SR integral membrane protein, through actions of the pore-forming channel subunit [76] or the β1 auxiliary subunit [77,78]. The T-tubule is also enriched with NCX that acts in conjunction with the sarcoplasmic reticulum calcium ATPase (SERCA) to reduce cytosolic calcium levels during the myocyte relaxation phase. NCX is functionally coupled to NKA and the proper targeting/retention of this protein complex at the T-tubules is dependent upon interactions with ankyrin-B (Figure 2). Ankyrin-B directly binds NCX and NKA [43], an interaction that stabilizes NCX protein [41]. Ankyrin-B haploinsufficiency results in reduced NCX and NKA protein expression and membrane localization at T-tubules [41,43,44]. Reduced T-tubular NCX function increases post-systolic calcium levels in the cytosol, thereby enhancing SERCA’s contribution to cytosolic calcium removal and resulting in elevated SR calcium stores [44]. Therefore, ankyrin-B-dependent targeting and retention of NCX and NKA at T-tubules contributes to the functional specialization of this domain, i.e. the normal efflux of calcium ions during the myocyte relaxation phase.

Intercalated disc: In ventricular cardiomyocytes, intercalated discs (ICD) are specialized domains that mediate the end-end contact between adjoining myocytes and allow for electrical and mechanical continuity between these cells. In the intercalated discs, desmosomes and adherens junctions function in the mechanical adhesion between neighboring myocytes, while connexons (or gap junctions) facilitate the electrical coupling between these cells. Each ICD junctional complex has distinct protein components with specialized functions; nevertheless, these complexes are interconnected and functionally dependent on each other. Ankyrin-G interacts with the desmosomal protein plakophilin-2 and the gap junction protein connexin43 (Figure 2). Decreasing ankyrin-G expression results in reduced ICD localization of plakophilin-2 and diminished intercellular adhesion [79]. Furthermore, reduced ankyrin-G expression causes a decrease in protein expression and ICD localization of connexin43 resulting in decreased junctional conductance [79]. Interestingly, ankyrin-G and plakophilin-2 appear to mutually facilitate their retention at the ICD because siRNA-mediated plakophilin-2 knockdown decreases the ICD localization of both ankyrin-G and the voltage-gated sodium channel Na+,1.5 [79]. Ankyrin-G scaffolding of plakophilin-2 and connexin43 contributes to the electromechanical coupling between adjoining cardiomyocytes.

The voltage-gated sodium channel Na+,1.5 initiates the rapid upstroke of the cardiac action potential. This channel displays differential subcellular localization in ventricular cardiomyocytes. While a small population of Na+,1.5 has been localized to lateral membranes, the most abundant population is localized at the ICD (Figure 2). Na+,1.5 differential localization arises from the channel’s association with different protein complexes. Lateral membrane localization is the result of channel association with the syntrophin-dystrophin complex, while ICD localization of the channel is regulated by ICD-resident proteins synapse associated protein 97 (SAP97) and ankyrin-G. Syntrophin interacts with the PDZ domain encoded by the last three amino acids in Na+,1.5 (Ser-Ile-Val). Disrupting this interaction leads to decreased
Na<sub>1.5</sub> localization in lateral membranes, reduced sodium current density, and attenuated impulse propagation [80,81]. Interestingly, the ICD-resident protein SAP97 also interacts with Na<sub>1.5</sub> via its last three amino acids. Inhibiting this interaction reduces Na<sub>1.5</sub> protein expression and localization at pseudo-ICDs, in addition to reducing total sodium current density [80]. An unresolved question about the differential localization of Na<sub>1.5</sub> is what regulates channel interaction with syntrophin or SAP97 given that they both share the same binding site on Na<sub>1.5</sub>.

In ventricular myocytes, ankyrin-G is required for the targeting and retention of Na<sub>1.5</sub> at intercalated discs. Ankyrin-G directly binds to Na<sub>1.5</sub> via a conserved ankyrin-binding domain present in the cytoplasmic loop between the DII and DIII homologous domains [15]. Disruption of this interaction causes the loss of Na<sub>1.5</sub> membrane expression at ICDs and reduced sodium current density [15,42]. Ankyrin-G-dependent enrichment of Na<sub>1.5</sub> at the ICD is important for action potential propagation between adjoining myocytes [82]. Interestingly, a Na<sub>1.5</sub> missense mutation that disrupted ankyrin-G binding and reduced channel membrane localization was linked to a case of Brugada syndrome, which is a cardiac arrhythmia characterized by ventricular conduction abnormalities and reduced Na<sub>1.5</sub> function [15]. Phosphorylation also regulates Na<sub>1.5</sub> channel activity and the costamere β4-spectrin/calmodulin kinase II (CaMKII) binding protein [83]. A direct interaction between ankyrin-G and β4-spectrin retains CaMKII in close proximity to Na<sub>1.5</sub> [83]. Channel phosphorylation by CaMKII enhances the peak sodium current and changes the channel’s inactivation gating
Costameres: Costameres are submembranous protein complexes that facilitate the lateral transmission of contractile force to the sarcomere, surrounding extracellular matrix, and neighboring myocytes. They overlie the Z-lines, which define the boundaries of an individual sarcomere, and facilitate mechanotransduction through the actions of focal adhesion proteins such as vinculin, α-actinin, and β1 integrin. Also residing in the costamere, the dystrophin-glycoprotein complex (DGC) connects the myocyte cytoskeleton through the sarcomere to the surrounding extracellular matrix thereby providing structural integrity for the sarcomemal membrane. While several transmembrane and peripheral proteins contribute to DGC stability and sarcomemal integrity, both dystrophin and dystroglycan play central roles in this complex. Dystroglycan is proteolytically processed into an extracellular α-subunit and transmembrane-spanning β-subunit. The link between the extracellular matrix (ECM) and the cytoskeleton is mediated by β-dystroglycan by binding to the ECM-associated α-dystroglycan and dystrophin, which binds to actin and intermediate filaments.

Both ankyrin-B and ankyrin-G have been associated with the recruitment and retention of DGC components to the costamere. Specifically, ankyrin-B and ankyrin-G bind to dystrophin, while ankyrin-G binds to β-dystroglycan [35] (Figure 2). The sarcomemal recruitment/retention of dystrophin by ankyrin-B is dependent on ankyrin-B interaction with dynactin-4, a component of the dynactin complex that links membrane cargo to the submembranous actin filaments. In skeletal muscle, the loss of ankyrin-B or its intermediary dynactin-4 results in decreased sarcomemal localization of dystrophin, β-dystroglycan, and costamere-associated microtubules [8]. Not surprisingly, ankyrin-B haploinsufficient mice display greater muscle damage following exercise compared to their wild-type littermates [8].

While ankyrin-B regulates the sarcomemal localization of dystrophin and β-dystroglycan, ankyrin-G is important for the retention of these proteins at the costamere [35]. In skeletal muscle, the loss of ankyrin-G reduces the costameric localization of both dystrophin and β-dystroglycan, while their sarcomemal localization remains intact [35]. Based on these findings, it has been suggested that dystrophin, β-dystroglycan and a subset of microtubules are initially recruited/retained at the sarcomemra by an ankyrin-B/dynactin-4 protein complex and the further refinement of their localization to costameres is facilitated by an ankyrin-G/β2-spectrin protein complex. While this is a very tentative model, unresolved issues include the relationship between ankyrin-G and ankyrin-B at the costameres, the function of DGC adaptor proteins such as syntrophins in this protein complex, and the characterization of different ankyrin isoforms at the costameres.

Many different ankyrin-G isoforms are expressed in striated muscle due to alternative splicing. In addition to the full-length ankyrin-G isoform with all three functional domains, truncated isoforms lacking the membrane-binding domain have been detected in skeletal and cardiac tissue. Interestingly, these truncated isoforms include a novel stretch of 76 amino acids in the C-terminal domain that have been previously shown to mediate interactions with obscurin, a large structural protein implicated in myofibrillogenesis and predominantly localized to the sarcomere M-line [84]. In addition to obscurin, two actin-binding scaffolding proteins plectin and filamin that localize to the costamere interact with this 76 amino acid domain [85]. In the costamere, plectin and filamin act as adaptor/scaffolding proteins that interact with components of the DGC and the β-integrin complex (Figure 2). While the interactions with plectin and filamin most likely contribute to the stability of these truncated ankyrin-G isoforms at costameres, the costameric functions of these isoforms remain to be determined. In addition, the relationship between these truncated isoforms and the full-length version is another unresolved issue.

Conclusions

Since the initial discovery of the ankyrin/β-spectrin cytoskeletal complex some 35 years ago, there has been a tremendous growth in our understanding of how this complex functions in both normal and diseased states. Historically, ankyrin dysfunction was only associated with haemolytic anaemia, but now dysfunction of ankyrin and associated proteins has been connected to numerous cardiac arrhythmias, epilepsy, bipolar disorder, and a type of neonatal diabetes. The vast majority of our knowledge about ankyrin biology has come from analysis and interpretation of ankyrin and β-spectrin function at the plasma membrane in a static situation. While this analysis has been tremendously productive, it doesn’t provide a complete view of the entirety of ankyrin/β-spectrin functions. For example, fundamental unresolved questions include what regulates ankyrin specificity for membrane proteins, where along the biosynthetic pathway does ankyrin interact with membrane proteins, and how does the ankyrin/β-spectrin complex orchestrate differential targeting of membrane proteins. Given that both ankyrin and β-spectrin are involved with elemental biological processes such as establishing subcellular polarity, maintaining membrane excitability, and reinforcing adhesive junctions, it will come as no surprise if they are implicated in the molecular pathogenesis of many more diseases.

Sources of Funding

We acknowledge support from the National Institutes of Health (HL084583, HL083422 to PJM; HL092232 to SRC), Pew Scholars Trust (PJM), and Fondation Leducq award to the Alliance for Calmodulin Kinase Signaling in Heart Disease (PJM).

References

1. Mohler PJ, Gramolini AO, Bennett V (2002) The ankyrin-B C-terminal domain determines activity of ankyrin-B/G chimeras in rescue of abnormal inositol 1,4,5-trisphosphate and ryanodine receptor distribution in ankyrin-B (-/-) neonatal cardiomyocytes. J Biol Chem 277: 10599-10607.
2. Michaelis P, Tomchick DR, Machius M, Anderson RG (2002) Crystal structure of a 12 ANK repeat stack from human ankyrinR. EMBO J 21: 6387-6396.
3. Lee G, Abdi K, Jiang Y, Michaelis P, Bennett V, et al. (2006) Nanospring behaviour of ankyrin repeats. Nature 440: 246-249.
4. Mohler PJ, Yoon W, Bennett V (2004) Ankyrin-B targets beta2-spectrin to an intracellular compartment in neonatal cardiomyocytes. J Biol Chem 279: 40185-40193.
5. Davis L, Abdi K, Machius M, Brautigam C, Tomchick DR, et al. (2009) Localization and structure of the ankyrin-binding site on beta2-spectrin. J Biol Chem 284: 6962-6967.
6. Ipsaro JJ, Huang L, Mondragón A (2009) Structures of the spectrin-ankyrin interaction binding domains. Blood 113: 5385-5393.
7. Kennedy SD, Warren SL, Forget BG, Morrow JS (1991) Ankyrin binds to the 15th repetitive unit of erythroid and nonerythroid beta-spectrin. J Cell Biol 115: 267-277.
8. Ayalon G, Hostettler JD, Hoffman J, Kizhatil K, Davis JO, et al. (2011) Ankyrin-B interactions with spectrin and dynactin-4 are required for dystrophin-based protection of skeletal muscle from exercise injury. J Biol Chem 286: 7370-7378.
9. Bhasin N, Cunha SR, Mudannayake M, Gigena MS, Rogers TB, et al. (2007) Molecular basis for PP2A regulatory subunit B56alpha targeting in cardiomyocytes. Am J Physiol Heart Circ Physiol 293: H109-119.

Citation: Yaman Kurt G, Nguyen FT, Mohler PJ, Cunha SR (2013) Ankyrin-Based Trafficking and Scaffolding of Membrane Proteins: Implications for Plasma Membrane Stability, Formation, and Specialization. J Proteomics Bioinform 6: 221-228. doi:10.4172/jpb.1000284
Citation: Yamanikurt G, Nguyen FT, Mohler PJ, Cunha SR (2013) Ankyrin-Based Trafficking and Scaffolding of Membrane Proteins: Implications for Plasma Membrane Stability, Formation, and Specialization. J Proteomics Bioinform 6: 221-228. doi:10.4172/jpb.1000284

30. Koob R, Zimmermann M, Schoner W, Drenckhahn D (1988) Colocalization and copericitation of ankyrin and Na,K-ATPase in kidney epithelial cells. Eur J Cell Biol 45: 230-237.

31. Morrow JS, Cicic CI, Ardito T, Mann AS, Kashgarian M (1989) Ankyrin links fodrin to the alpha subunit of Na,K-ATPase in Madin-Darby canine kidney cells and in intact renal tubule cells. J Cell Biol 108: 455-465.

32. Nelson WJ, Yeshnock PJ (1987) Ankyrin binding to (Na+ + K+)ATPase and implications for the organization of membrane domains in polarized cells. Nature 328: 533-536.

33. Festy F, Robert JC, Brasseur R, Thomas A (2001) Interaction between the N-terminal domain of gastric H,K-ATPase and the spectrin binding domain of ankyrin III. J Biol Chem 276: 7721-7726.

34. Nicolas V, Mourou-Chanteloup I, Lopez C, Gane P, Gimm A, et al. (2006) Functional interaction between Rh proteins and the spectrin-based skeleton in erythroid and epithelial cells. Transfus Clin Biol 13: 23-28.

35. Ayalon G, Davis JQ, Scotland PB, Bennett V (2008) An ankyrin-based mechanism for functional organization of dystrophin and dystroglycan. Cell 135: 1189-1200.

36. Kizhatil K, Davis JQ, Davis L, Hoffman J, Hogan BL, et al. (2007) Ankyrin-G is a molecular partner of E-cadherin in epithelial cells and early embryos. J Biol Chem 282: 26552-26561.

37. Davis LH, Bennett V (1994) Identification of two regions of beta G spectrin that bind to distinct sites in brain membranes. J Biol Chem 269: 4409-4416.

38. Davis JQ, Bennett V (1993) Ankyrin-binding activity of nervous system cell adhesion molecules expressed in adult brain. J Cell Sci Suppl 17: 109-117.

39. Lokeshwar VB, Fregien N, Bourguignon LY (1994) Ankyrin-binding domain of CD44(GP85) is required for the expression of hyaluronic acid-mediated adhesion function. J Cell Biol 126: 1099-1109.

40. Hortsch M, O'Shea KS, Zhao G, Kim F, Vallejo Y, et al. (1998) A conserved role for L1 as a transmembrane link between neuronal adhesion and membrane cytoskeleton assembly. Cell Adhes Commun 5: 61-73.

41. Cunha SR, Bhasin N, Mohler PJ (2007) Targeting and stability of Na/Ca exchanger 1 in cardiomyocytes requires direct interaction with the membrane adaptors ankyrin-B and ankyrin-G. J Biol Chem 282: 4857-4863.

42. Lowe JS, Palygin G, Bhasin N, Hund TJ, Boyden PA, et al. (2008) Voltage-gated Na channel targeting in the heart requires an ankyrin-G-dependent cellular pathway. J Cell Biol 180: 173-186.

43. Mohler PJ, Davis JQ, Bennett V (2005) Ankyrin-B coordinates the Na/K ATPase, Na/Ca exchanger, and InsP3 receptor in a cardiac T-tubule/SR microdomain. PLoS Biol 3: e423.

44. Mohler PJ, Schott JJ, Gramolini AD, Dilly KW, Guatimosim S, et al. (2003) Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature 421: 634-639.

45. Mohler PJ, Davis JQ, Davis LH, Hoffman JA, Michaely P, et al. (2004) Inositol 1,4,5-trisphosphate receptor localization and stability in neonatal cardiomyocytes requires interaction with ankyrin-B. J Biol Chem 279: 12980-12987.

46. Beck KA, Buchanan JA, Malhotra V, Nelson WJ (1994) Golgi spectrin: identification of an erythroid beta-spectrin homolog associated with the Golgi complex. J Cell Biol 127: 707-723.

47. Beck KA, Buchanan JA, Nelson WJ (1997) Golgi membrane skeleton: identification, localization and oligomerization of a 195 kDa ankyrin isoform associated with the Golgi complex. J Cell Sci 110: 1239-1249.

48. Devarajan P, Stabach PR, Mann AS, Ardito T, Kashgarian M, et al. (1996) Identification of a small cytoplasmic ankyrin (AnkG119) in the kidney and muscle that binds beta I sigma spectrin and associates with the Golgi apparatus. J Cell Biol 133: 819-830.

49. Devarajan P, Stabach PR, De Matteis MA, Morrow JS (1997) Na,K-ATPase transport from endoplasmic reticulum to Golgi requires the spectrin-ankyrin G119 skeleton in Madin Darby canine kidney cells. Proc Natl Acad Sci U S A 94: 10711-10716.

50. Fath KR, Trimbur GM, Burgess DR (1997) Molecular motors and a spectrin-complex. Membrane Protein Transporters

ISSN: 0974-276X JPB, an open access journal
Volume 6(10) 221-228 (2013) - 227
expressed betall spectrin associated with Golgi and cytoplasmic vesicles. Proc Natl Acad Sci U S A 95: 14158-14163.

53. Gudi A, Santone I, Peritel P, Devarajan P, Babarach PR, et al. (1998) ADP ribosylation factor regulates spectrin binding to the Golgi complex. Proc Natl Acad Sci U S A 95: 8607-8612.

54. Holleran EA, Ligun LA, Tokito M, Stankewich MC, Morrow JS, et al. (2001) beta III spectrin binds to the AFG1 subunit of dynamin. J Biol Chem 276: 36598-36605.

55. Perkins EM, Clarkson YL, Sabatier N, Longhurst DM, Millward CP, et al. (2010) Loss of beta-III spectrin leads to Purkinje cell dysfunction recapitulating the behavior and neuropathology of spinocerebellar ataxia type 5 in humans. J Neurosci 30: 4857-4867.

56. Stabach PR, Devarajan P, Stankewich MC, Bannyaik S, Morrow JS (2008) Ankyrin facilitates intracellular trafficking of alpha1-Na-K+ ATPase in polarized cells. Am J Physiol Cell Physiol 295: C1202-1214.

57. Bennett V, Davis J (1981) Erythrocyte ankyrin: immunoreactive analogues are associated with mitotic structures in cultured cells and with microtubules in brain. Proc Natl Acad Sci U S A 78: 7550-7554.

58. Davis JQ, Bennett V (1984) Brain ankyrin. A membrane-associated protein with binding sites for spectrin, tubulin, and the cytoplasmic domain of the erythrocyte anion channel. J Biol Chem 259: 13550-13559.

59. Davis LH, Otto E, Bennett V (1991) Specific 33-residue repeat(s) of erythrocyte ankyrin associate with the anion exchanger. J Biol Chem 266: 11163-11169.

60. Leterrier C, Vacher H, Fache MP, d’Ortoli SA, Castets F, et al. (2011) End-binding proteins EB3 and EB1 link microtubules to ankyrin G in the axon initial segment. Proc Natl Acad Sci U S A 108: 8926-8931.

61. Michaela P, Kannal A, Anderson RG, Bennett V (1999) A requirement for ankyrin binding to clathrin during coated pit budding. J Biol Chem 274: 35908-35913.

62. Gudmundsson H, Hund JT, Wright PJ, Kline CF, Snyder JS, et al. (2010) EH domain proteins regulate cardiac membrane protein targeting. Circ Res 107: 84-95.

63. Hoock TC, Peters LL, Lux SE (1997) Isomers of ankyrin-3 that lack the NH2-terminal repeats associate with mouse macrophage lysosomes. J Cell Biol 136: 1059-1070.

64. Zhou D, Lambert S, Malen PL, Carpenter S, Boland LM, et al. (1998) AnkyrinG is required for clustering of voltage-gated Na channels at axon initial segments and for normal action potential firing. J Cell Biol 143: 1295-1304.

65. Bréchet A, Fache MP, Brachet A, Ferracci G, Baude A, et al. (2008) Protein kinase CK2 contributes to the organization of sodium channels in axonal membranes by regulating their interactions with ankyrin G. J Cell Biol 183: 1101-1114.

66. Sanchez-Ponce D, Munoz A, Garrido JJ (2011) Casein kinase 2 and microtubules control axon initial segment formation. Mol Cell Neurosci 46: 222-234.

67. Ango F, di Cristo G, Higashiyama H, Bennett V, Wu P, et al. (2004) Ankyrin-based subcellular gradient of neurofascin, an immunoglobulin family protein, directs GABAergic innervation to purkinje axon initial segment. Cell 119: 257-272.

68. Pyka M, Wetzl C, Aguado A, Geissler M, Hatt H, et al. (2011) Chondroitin sulphate proteoglycans regulate astrocyte-dependent synaptogenesis and modulate synaptic activity in primary embryonic hippocampal neurons. Eur J Neurosci 33: 2187-2202.

69. Hedstrom KL, Xu X, Ogawa Y, Frischknecht R, Seidenbecher CI, et al. (2007) Neurofascin assembles a specialized extracellular matrix at the axon initial segment. J Cell Biol 178: 875-886.

70. Shirahata E, Iwasaki H, Takagi M, Lin C, Bennett V, et al. (2006) Ankyrin-G regulates inactivation gating of the neuronal sodium channel, Nav1.6. J Neurophysiol 96: 1347-1357.

71. Li J, Kline CF, Hund TJ, Anderson ME, Mohler PJ (2010) Ankyrin-G regulates Kir2.1 membrane expression and function in heart. J Biol Chem 285: 28723-28730.

72. van den Akker H, Satchwell TJ, Williamson RC, Toye AM (2010) Band 3 multiprotein complexes in the red cell membrane; of mice and men. Blood Cells Mol Dis 45: 1-8.

73. Kuzhati K, Bennett V (2004) Lateral membrane biogenesis in human bronchial epithelial cells requires 190-kDa ankyrin-G. J Biol Chem 279: 16706-16714.

74. Kuzhati K, Yoon W, Mohler PJ, Davis LH, Hoffman JA, et al. (2007) Ankyrin-G and beta2-spectrin collaborate in biogenesis of lateral membrane of human bronchial epithelial cells. J Biol Chem 282: 2029-2037.

75. Kuzhati K, Baker SA, Arshavsky VY, Bennett V (2009) Ankyrin-G promotes cyclic nucleotide-gated channel transport to rod photoreceptor sensory cilia. Science 323: 1614-1617.

76. Casarotto MG, Cui Y, Karunasekara Y, Harvey PJ, Norris N, et al. (2006) Structural and functional characterization of interactions between the dihydropyridine receptor II-III loop and the rymodine receptor. Clin Exp Pharmacol Physiol 33: 1114-1117.

77. Cheng W, Altajf X, Ronij M, Coronado R (2005) Interaction between the dihydropyridine receptor Ca2+ channel-beta-subunit and rymodine receptor type 1 strengthens excitation-contraction coupling. Proc Natl Acad Sci U S A 102: 19225-19230.

78. Rebbbeck RT, Karunasekara Y, Gallant EM, Board PG, Beard NA, et al. (2011) The beta(1a) subunit of the skeletal DHPR binds to skeletal RyR1 and activates the channel via its 35-residue C-terminal tail. Biophy J 100: 922-930.

79. Sato PY, Coombs W, Lin X, Nekrasova O, Green KJ, et al. (2011) Interactions between ankyrin-G, Plakophilin-2, and Connexin43 at the cardiac intercalated disc. Circ Res 109: 193-201.

80. Petitprez S, Zmook AF, Ogrodnik J, Balse E, Raad N, et al. (2011) SAP97 and dystrophiin macromolecular complexes determine two pools of cardiac sodium channels Nav1.5 in cardiomycocytes. Circ Res 108: 294-304.

81. Gavillet B, Rougier JS, Domenighetti AA, Behar R, Boixel C, et al. (2006) Cardiac sodium channel Nav1.5 is regulated by a multiprotein complex composed of syntrophins and dystrophin. Circ Res 99: 407-414.

82. Kucera JP, Rohr S, Rudy Y (2002) Localization of sodium channels in intercalated disks modulates cardiac conduction. Circ Res 91: 1176-1182.

83. Hund TJ, Koval OM, Li J, Wright PJ, Qian L, et al. (2010) A beta(1a)-spectrin/chiral domain proteins regulate cardiac membrane protein targeting. Cell Res 107: 154: 123-136.

84. Maiewiland Y, Klauza I, Kordeli E (2011) Novel interactions of ankyrins-G at the costameres: the muscle-specific Obscurin/Titin-Binding-related Domain (OTBD) binds plectin and filamin C. Exp Cell Res 317: 724-736.

Submit your next manuscript and get advantages of OMICS Group Services

Unique features:
- User friendly/feasible website-transition of your paper to 50 world’s leading languages
- Audio Version of published paper
- Digital articles to share and explore

Special features:
- 250 Open Access Journals
- 20,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at PubMed (portal), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at http://www.editorialmanager.com/proteomics

Citation: Yamankurt G, Nguyen FT, Mohler PJ, Cunha SR (2013) Ankyrin-Based Trafficking and Scaffolding of Membrane Proteins: Implications for Plasma Membrane Stability, Formation, and Specialization. J Proteomics Bioinform 6: 221-228. doi:10.4172/jpb.1000284

This article was originally published in a special issue, Membrane Protein Transporters handled by Editor(s), Dr. Mobeen Raja, University of Alberta, Canada

Citation: Yamankurt G, Nguyen FT, Mohler PJ, Cunha SR (2013) Ankyrin-Based Trafficking and Scaffolding of Membrane Proteins: Implications for Plasma Membrane Stability, Formation, and Specialization. J Proteomics Bioinform 6: 221-228. doi:10.4172/jpb.1000284

This article was originally published in a special issue, Membrane Protein Transporters handled by Editor(s), Dr. Mobeen Raja, University of Alberta, Canada