Seminars in Cell & Developmental Biology 22 (2011) 166–170

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Ankyrin-based cellular pathways for cardiac ion channel and transporter targeting and regulation

Shane R. Cunha[a,b,1], Peter J. Mohler[a,b,*]

[a] Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, IA 52242, United States
[b] Department of Molecular Physiology and Biophysics, University of Iowa Carver College of Medicine, Iowa City, IA 52242, United States

ARTICLE INFO

Article history:
Available online 8 October 2010

Keywords:
Ankyrin
Nav1.5
Intercalated disc
Transverse-tubule
Arrhythmia
Sodium channel
Targeting

ABSTRACT

The coordinate activities of ion channels and transporters regulate myocyte membrane excitability and normal cardiac function. Dysfunction in cardiac ion channel and transporter function may result in cardiac arrhythmias and sudden cardiac death. While the past fifteen years have linked defects in ion channel biophysical properties with human disease, more recent findings illustrate that ion channel and transporter localization within cardiomyocytes is equally critical for normal membrane excitability and tissue function. Ankyrins are a family of multifunctional adapter proteins required for the expression, membrane localization, and regulation of select cardiac ion channels and transporters. Notably, loss of ankyrin expression in mice, and ankyrin loss-of-function in humans is now associated with defects in myocyte excitability and cardiac physiology. Here, we provide an overview of the roles of ankyrin polypeptides in cardiac physiology, as well as review other recently identified pathways required for the membrane expression and regulation of key cardiac ion channels and transporters.

Contents

1. Introduction

The excitability of neurons, cardiomyocytes, and endocrine cells is regulated by the expression and function of voltage-gated ion channels in the plasma membrane. In the heart, voltage-gated sodium, potassium, and calcium channels regulate cardiomyocyte excitability including the generation and propagation of action potentials. For example, voltage-gated sodium channels determine the amplitude and slope of action potentials along with controlling conduction velocity throughout the cardiac tissue. Dysfunction of the cardiac sodium channel results in increased risk for ventricular arrhythmias such as Brugada syndrome and long QT syndrome type 3 (reviewed in [1]). These life-threatening cardiac arrhythmias may arise from mutations in the sodium channel that alter gating properties, decrease protein expression, or cause the mis-localization of sodium channel subunits. Notably, while many properties of cardiac voltage-gated sodium channels have been well established over the past twenty years (i.e. biophysical properties, critical structural domains, and signaling motifs), the specific mechanisms underlying the membrane targeting, stabilization, and local regulation of these essential membrane proteins have received less attention. Over the past decade, findings from mice and humans have implicated ankyrin polypeptides in ion channel and transporter targeting in cardiomyocytes. In fact, ankyrin dysfunction has been linked with defective targeting of voltage-gated sodium channels, as well as Na/Ca exchanger, Na/K ATPase, and Kir6.2 in heart.

* Corresponding author at: Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, IA 52242, United States. Tel.: +1 319 335 9691.
E-mail address: peter-mohler@uiowa.edu (P.J. Mohler).
[1] Current address: Department of Integrative Biology and Pharmacology, University of Texas Health Science Center, Houston, TX 77030, United States.

1084-9521/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2010.09.013

Moreover, ankyrin dysfunction for cardiac membrane protein targeting has been linked with potentially fatal human arrhythmias. This review will focus on the role of ankyrin polypeptides in cardiac ion channel and transporter targeting, with an emphasis on the role of ankyrin-G for voltage-gated sodium channel localization. Additionally, we will detail known mechanisms for regulating ankyrin function and specificity in excitable cells. Finally, we will describe current knowledge on the mechanisms underlying ankyrin function in membrane protein delivery, as well as post-transcriptional mechanisms that modulate ankyrin interactions in excitable cells.

2. Ankyrins

Ankyrins are a family of adaptor proteins that link integral membrane proteins including ion channels, transporters, and cell adhesion molecules to the actin/spectrin cytoskeleton. The ankyrin family consists of numerous ankyrin polypeptides resulting from tissue-specific alternative splicing (reviewed in [2]) of three ankyrin genes (*ANK1*: ankyrin-R, *ANK2*: ankyrin-B, *ANK3*: ankyrin-G). The prototypical ankyrin consists of three functional domains: the membrane-binding domain, spectrin-binding domain, and C-terminal regulatory domain (Fig. 1). The membrane-binding domain is made up of 24 *ANK* repeats that assemble as a suprahelical spiral [3]. The *ANK* repeat is a common protein motif (33 amino acid motif, comprised of two alpha-helices) that mediates protein–protein interactions. *ANK* repeats of the membrane-binding domain mediate ankyrin interactions with integral membrane proteins such as the voltage-gated sodium channels, sodium/calcium exchanger (NCX), inositol$_{(1,4,5)}$-trisphosphate receptor (IP_3 receptor), ATP-sensitive potassium channel subunit Kir6.2, and the L1 family of cell adhesion molecules [4–11]. The spectrin-binding domain interacts with β-spectrin thereby tethering ankyrin-associated integral membrane proteins to the cytoskeleton [12]. The spectrin-binding domain also interacts with the regulatory subunit of protein phosphatase 2A (PP2A) suggesting that another function of ankyrin is to organize local signaling networks [13]. Ankyrin interactions with β-spectrin and integral membrane proteins are partially regulated by the C-terminal regulatory domain. This domain most likely regulates ankyrin specificity for particular interacting proteins and directs ankyrin subcellular targeting [14–16]. The functional significance of this domain is highlighted by the prevalence of human disease-associated variants within this domain of ankyrin-B [17].

The heart expresses protein products of all three ankyrin genes including the 190 kDa isoform of ankyrin-G, the 160 and 220 kDa isoforms of ankyrin-B, and the 210 kDa isoform of ankyrin-R. While the molecular basis for ankyrin-R function in heart has yet to be fully elucidated, there is some understanding as to how ankyrin-B and ankyrin-G function in heart. Specifically, ankyrin-B is important for the proper targeting and stability of NCX, IP_3 receptor, and sodium/potassium ATPase (NKA) at membrane junctions of the transverse-tubules (T-tubules) with sarcoplasmic reticulum (SR) [5,18]. Ankyrin-B also regulates the protein expression and membrane targeting of K_{ATP} channel subunit Kir6.2 to T-tubules in addition to modulating K_{ATP} channel ATP sensitivity [8,9,19]. In contrast, as addressed in greater detail below, ankyrin-G is important for the protein expression and proper targeting of the voltage-gated sodium channel Nav1.5 to intercalated disc membranes [4,7].

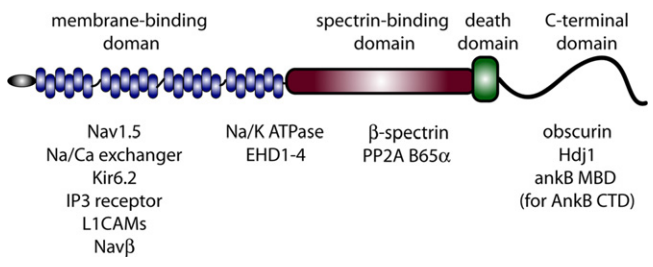

Fig. 1. Ankyrin domain organization and associated proteins. Canonical ankyrins display an amino-terminal membrane-binding domain comprised of 24 consecutive *ANK* repeats (blue), a spectrin-binding domain (red), a death domain (green), and C-terminal domain (black). Validated binding partners for cardiac ankyrin-B and ankyrin-G are noted below the domain of interaction. Note that both Na/K ATPase and EHD1-4 may require interaction sites on both membrane- and spectrin-binding domains.

3. Ankyrin-dependent targeting of cardiac voltage-gated sodium channels

The voltage-gated sodium channel (Nav) consists of a pore-forming α-subunit and one or more auxiliary β-subunits [20]. In addition to alternative splice variants, there are 10 different α-subunits encoded by different genes that individually produce a ~260 kDa membrane protein. The α-subunits display differential tissue, cellular, and subcellular expression patterns. A prototypical α-subunit has four domains (DI-DIV) that contain six α-helical transmembrane segments (S1–S6) (Fig. 2). The S4 segment is the voltage sensor and the membrane-embedded loop between segments S5 and S6 confers ion selectivity. The transmembrane and extracellular domains of the α-subunits share a significant degree of homology. In contrast, the intracellular domains are more divergent and account for the α-subunit's unique biophysical properties and expression patterns. By itself, the α-subunit harbors the fundamental properties of a sodium channel (pore formation, ion selectivity, and rapid inactivation), while the β-subunits modulate the channel's biophysical properties in addition to regulating channel expression and localization in the plasma membrane [21]. Four genes encode the β-subunits that are single-pass transmembrane proteins with an extracellular immunoglobulin domain that mediates homophilic interactions between adjacent β-subunits.

In heart, the most prevalent sodium channel alpha-subunit is TTX-resistant Nav1.5. This subunit is predominantly expressed at the intercalated disc membrane [4,22,23] where gap junctions, adherens junctions, and desmosomes link neighboring cardiomyocytes both electrically and mechanically. Expression of Nav1.5 at the intercalated disc facilitates action potential propagation throughout the working myocardium. Nav1.5 channels have also been detected on T-tubules and the peripheral sarcolemma. Additional α-subunits expressed on T-tubules include the TTX-sensitive Nav1.1, Nav1.2, Nav1.3, Nav1.4, and Nav1.6 [23,24]. These subunits are notably less abundant than TTX-resistant Nav1.5, but still estimated to account for 10–20% of the total sodium current in adult cardiomyocytes.

There are four Nav channel β-subunits: β1, β2, β3, and β4. All four subunits including an alternative β1A isoform are expressed in the heart [25]. An individual β-subunit has a single-pass transmembrane domain, an extracellular domain with an Ig domain, and an intracellular domain. The rates of activation, inactivation, and recovery from inactivation for α-subunits can be altered by β-subunits depending on the α-subunit and the cell-type background. In addition, β-subunits can increase the membrane expression of α-subunits. For example, the β1-subunit increases membrane expression of a brain sodium channel by 2–4-fold in a heterologous cell line [26]. Finally, the significance of β-subunits for proper sodium channel activity and normal cardiac physiology is highlighted by multiple studies that link cardiac β-subunits mutations to cardiac arrhythmia disorders [27,28]. While Nav β-subunits are clearly important for cardiac I_{Na}, it will be important in the future to define how and where α- and β-subunits co-assemble in the biosynthetic pathway. Moreover, it will be interesting to determine how β-subunits may recruit signaling molecules to the larger