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Review

Cardiac sodium channel $Na_V1.5$ distribution in myocytes via interacting proteins: The multiple pool model ☆

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ABSTRACT

The cardiac sodium current (I_{Na}) is responsible for the rapid depolarization of cardiac cells, thus allowing for their contraction. It is also involved in regulating the duration of the cardiac action potential (AP) and propagation of the impulse throughout the myocardium. Cardiac I_{Na} is generated by the voltage-gated Na^+ channel, $Na_V1.5$, a 2016-residue protein which forms the pore of the channel. Over the past years, hundreds of mutations in *SCN5A*, the human gene coding for $Na_V1.5$, have been linked to many cardiac electrical disorders, including the congenital and acquired long QT syndrome, Brugada syndrome, conduction slowing, sick sinus syndrome, atrial fibrillation, and dilated cardiomyopathy. Similar to many membrane proteins, $Na_V1.5$ has been found to be regulated by several interacting proteins. In some cases, these different proteins, which reside in distinct membrane compartments (i.e. lateral membrane vs. intercalated disks), have been shown to interact with the same regulatory domain of $Na_V1.5$, thus suggesting that several pools of $Na_V1.5$ channels may co-exist in cardiac cells. The aim of this review article is to summarize the recent works that demonstrate its interaction with regulatory proteins and illustrate the model that the sodium channel $Na_V1.5$ resides in distinct and different pools in cardiac cells. This article is part of a Special Issue entitled: Cardiomyocyte Biology: Cardiac Pathways of Differentiation, Metabolism and Contraction.

1. Introduction

The contraction of cardiac cells is triggered by the fast and long-lasting electrical depolarization of the membrane, called the action potential (AP). Among the many different ionic currents that are involved in generating the cardiac AP, the cardiac sodium current (I_{Na}) plays a central role in the early depolarization of the AP, the duration of the AP, and the propagation of the electrical impulse from one cell to the other [23]. The main voltage-gated sodium channel expressed in cardiac cells is $Na_V1.5$ [26] (Fig. 1A), which is the pore-forming protein of about 220 kDa, also called α-subunit. It has been shown to assemble with small (about 30–40 kDa), single trans-membrane segment proteins called β-subunits [8]. Four of these β-subunits have been found to be encoded in the human genome [8].

Since 1995, hundreds of mutations in *SCN5A*, the human gene coding for $Na_V1.5$, have been linked to many cardiac electrical disorders, including the congenital and acquired long QT syndrome, Brugada syndrome, conduction slowing, sick sinus syndrome, atrial fibrillation, and dilated cardiomyopathy [75]. This long list of allelic diseases clearly underlines the central role of $Na_V1.5$ in normal physiology and diseases, but also raises the question as to whether or not this channel may have, as of yet, unrecognized functions. Similar to many membrane proteins, $Na_V1.5$ has been found to be regulated by several interacting proteins [1]. For some of these cases, these different proteins, which reside in specific subcellular regions of the cardiomyocyte (i.e. lateral membrane vs. intercalated disks), have been shown to interact with the same regulatory domain of $Na_V1.5$, thus suggesting that several pools of $Na_V1.5$ channels may co-exist in cardiac cells. Mutations in the genes coding for several of these partner proteins were found in patients with inherited arrhythmias, such as congenital long QT syndrome (LQTS) [11] and Brugada syndrome (BrS) [46]. The proteins interacting with $Na_V1.5$ can act as: anchoring/adaptor proteins involved in trafficking, targeting, and anchoring of the channel protein to specific membrane compartments; as enzymes interacting with and modifying the channel structure *via* post-translational modifications (e.g. protein kinases or ubiquitin ligases); and as proteins modulating the biophysical properties of $Na_V1.5$ upon binding (Table 1).

In this review article, we first describe the proteins that interact with $Na_V1.5$. These interacting proteins were discovered either by performing protein–protein interaction screens, such as yeast two-hybrid assays, or by using proteomic-based protein identification assays. The sites of interaction, often protein–protein interaction modules, were mapped on the sequence of $Na_V1.5$ as described in Fig. 1A. Second, we will review the experimental evidence obtained recently that suggest that $Na_V1.5$ is part of distinct pools in cardiomyocytes.

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2. Interacting partners/proteins of $Na_V1.5$

$Na_V1.5$ interacts with several partner proteins that regulate the channel's biology and function [1]. Specific regions of $Na_V1.5$ have been identified as essential for these interactions.

2.1. Associated proteins that interact with the C-terminal domain of $Na_V1.5$

The 243-residue, intracellular C-terminal part of $Na_V1.5$ contains several protein–protein interaction motifs [2]. The most well-characterized interaction sites are a calmodulin-binding IQ motif, a PY motif, and a PDZ domain-binding motif.

2.1.1. Proteins interacting with the PDZ domain-binding motif

PDZ domains are conserved domains that interact with the carboxy-terminal ends of target proteins. PDZ is the acronym for PSD-95 (post-synaptic density protein), DlgA (the *Drosophila* disks-large tumor suppressor protein), and ZO-1 (the tight junction protein, zonula occludens-1). This domain has been identified in a variety of proteins that are often found in structures at the plasma membrane and are involved in signal transduction pathways [60]. The last three residues of the $Na_V1.5$ C-terminus (Ser–Ile–Val, SIV) constitute the SIV-motif that interacts with PDZ domain-bearing proteins that contain a class I PDZ-domain (i.e. a domain that recognizes the C-terminal –Ser/Thr-X-ψ-Val sequence, where X is any amino acid and ψ is any hydrophobic amino acid) [20]. Several proteins such as PTPH1, SAP97, and syntrophins have been described to interact with this PDZ domain-binding motif.

Gee and co-workers first investigated the possibility that syntrophins bind to voltage-gated sodium channels via their PDZ domains [25]. They were able to co-purify a complex formed by sodium channels, syntrophins, and dystrophin from extracts of skeletal and cardiac muscle. Peptides corresponding to the last 10 amino acids of the $Na_V1.4$ and $Na_V1.5$ C-terminus were sufficient to inhibit the binding of native sodium channels to syntrophin PDZ domain fusion proteins and to bind specifically to PDZ domains from α1, β1, and β2-syntrophin. Using yeast two-hybrid and glutathione S-transferase (GST) pull-down experiments, Ou and co-workers showed that the PDZ domain of syntrophin γ2 directly interacts with the C-terminus of $Na_V1.5$. They investigated the functional consequences of this interaction [51] and found that co-expression of syntrophin γ2 with $Na_V1.5$ shifted the steady-state activation of the sodium current (I_{Na}). In a later study, our group demonstrated that $Na_V1.5$ interacts with dystrophin *via* syntrophins. In dystrophin-deficient mouse hearts, the protein level of $Na_V1.5$ was decreased, resulting in a reduced cellular I_{Na} and conduction defects that suggest that dystrophin regulates the expression level of $Na_V1.5$ [24].

The second protein reported to interact with the PDZ domain-binding motif of $Na_V1.5$ is protein tyrosine phosphatase (PTPH1), a protein expressed in many different tissues, including the heart [30]. In this work, our group showed that co-expression of PTPH1 with $Na_V1.5$ shifted the availability of sodium channels towards hyperpolarized potentials and that this effect was abrogated when the C-terminal SIV motif of $Na_V1.5$ was mutated. With this observation that more than one protein can interact with the same motif (SIV), it was proposed that $Na_V1.5$ may be part of different multi-protein complexes, depending upon the membrane compartment to which it is located.

More recently, we demonstrated in human atria and mouse ventricles, a specific interaction between the PDZ domain-binding motif of $Na_V1.5$ and a major membrane associated guanylate kinase (MAGUK) scaffolding protein, SAP97 [53]. Immunostainings performed on rat heart sections showed that $Na_V1.5$ and SAP97 are both co-localized at intercalated disks, and electrophysiological studies demonstrated that sodium currents were reduced in rat atrial myocytes, infected with shRNA-containing lentiviruses. Silencing of endogenous SAP97 expression in HEK293 cells, which were transiently transfected with $Na_V1.5$, resulted in reduced sodium current. The current recorded in SAP97-silenced cells was comparable to the ones recorded in cells that were transfected with $Na_V1.5$ channels lacking the last three amino acids (the SIV motif) of the C-terminus. Surface expression of $Na_V1.5$ was decreased in silenced cells which may underlie the reduced current

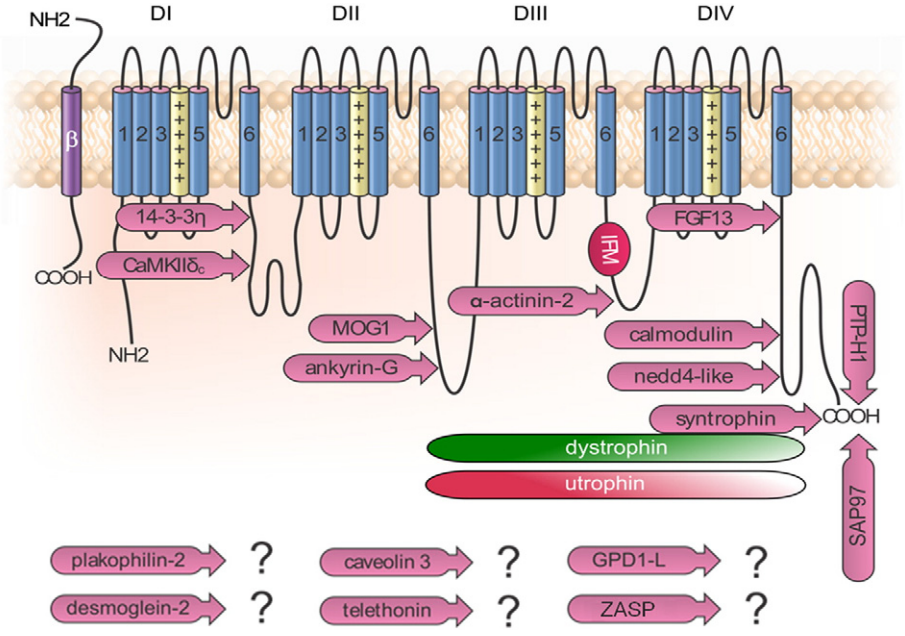

Fig. 1. Topology of $Na_V1.5$ and its interaction with various regulatory proteins. The pore-forming alpha subunit of $Na_V1.5$ consists of four homologous domains (DI-DIV), connected together by intracellular and extracellular loops. Each of these domains contains segments that contribute to the lining of the channel pore (segments S5 and S6) as well as a voltage sensor segment (S4). Several regulatory proteins for $Na_V1.5$ were identified and their sites of interaction have been mapped on the alpha subunit of the $Na_V1.5$ channel. Many of these interactions are shown to take place at the intracellular loops or the C-terminus of $Na_V1.5$. For a few proteins that have been shown (through co-immunoprecipitation) to associate with $Na_V1.5$, the sites of interaction on the $Na_V1.5$ channel are still unknown.