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EDITORIAL

Elucidating sodium channel $Na_V1.5$ clustering in cardiac myocytes using super-resolution techniques

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This editorial refers to 'Super-resolution imaging reveals that loss of the C-terminus of connexin43 limits microtubule plus-end capture and $Na_V1.5$ localization at the intercalated disc' by E. Agullo-Pascual *et al.*, pp. 371–381, this issue.

The cardiac voltage-gated sodium channel, $Na_V1.5$, is responsible for conducting the inward sodium current (I_{Na}), which leads to the fast depolarization of the cardiac cell membrane. Mutations in SCN5A, the gene encoding $Na_V1.5$, that lead to alterations in I_{Na} are linked to many cardiac phenotypes including congenital long QT syndrome type 3, Brugada syndrome, atrial fibrillation, conduction slowing, and dilated cardiomyopathy. Several partner proteins have been described to associate with $Na_V1.5$, and the genes encoding some of these regulatory proteins have also been found to be mutated in patients with inherited forms of cardiac arrhythmias.[1]

Recent investigations have revealed that the expression level, cellular localization, and activity of $Na_V1.5$ are finely regulated by complex molecular and cellular mechanisms. Multiple pools of $Na_V1.5$ in cardiac cells have been identified,[2] depending on where they are targeted and with which partner proteins they interact (*Figure 1A*). Thus, proteins such as SAP97,[3] ankyrin-G, plakophilin 2 (PKP2), and connexin43 (Cx43)[4,5] have been described to interact with $Na_V1.5$. The importance of these interactions in targeting and stabilizing $Na_V1.5$ at the intercalated disc (ID), where cells are electrically and mechanically coupled, is only partially understood. $Na_V1.5$ is also expressed at the lateral membrane of cardiomyocytes, and its targeting to this compartment is regulated by the syntrophin/dystrophin complex.[6] Ankyrin-G has also been shown to associate with $Na_V1.5$ at the lateral membrane of cardiomyocytes as well as at the transverse tubules.[4]

Although several partners of $Na_V1.5$ have been identified in different membrane compartments of cardiomyocytes, the precise location of functional channels remained undefined. In a previous study, Gorelik and Delmar groups[7] demonstrated, using scanning ion conductance microscopy (a technique that allows 3D topography imaging of live cells with a resolution of $\leq$ 20 nm) and conventional cell-attached patch-clamp, that sodium channels not only segregate into ID vs. lateral membrane pools, but also cluster into highly confined functional nanodomains.

In this issue of *Cardiovascular Research* Agullo-Pascual *et al.*[8] report important new data regarding the organization of $Na_v1.5$ into macromolecular complexes at the ID of murine cardiomyocytes. Combining three sophisticated techniques, i.e. super-resolution fluorescence microscopy (SRFM), scanning patch-clamp (SPC), and macropatch current recordings, they characterized the relationship between Cx43, $Na_V1.5$, and the microtubule plus-end. This plus-end region of the microtubule contains tracking proteins which help to tether the end of the microtubule to the plasma membrane and facilitate the delivery of proteins to the cell surface at the ID of cardiomyocytes. Interestingly, among the different partners of $Na_V1.5$ at the ID, the cardiac gap junction protein Cx43 has been shown to regulate I_{Na}. A recent study[9] demonstrated a Cx43-dependent regulation of I_{Na} that led to ventricular arrhythmia while the gap junctional conductance was not impaired. Moreover, previous studies indicated that (i) capture of microtubules at the site of cell–cell contact involves association of cadherin-rich sites with the microtubule plus-end[10] and (ii) $Na_V1.5$ is delivered to the cell membrane via microtubules.[11] The authors of the present work have chosen to study the localization of $Na_V1.5$ and the microtubule plus-end tracking protein 'end-binding 1' (EB1) in relation to *N*-cadherin (a key protein of cell–cell junctions) at nanometric resolution and their dependence on Cx43 structures. Using SFRM, they identified *N*-cadherin signals at the cell end and used them as a reference point to define different clusters. After having identified EB1 clusters at the ID, the authors showed that these EB1 clusters were reduced in genetically modified mice where Cx43 was replaced by a truncated form lacking the last five amino acids (Cx43D378stop), while gap junction plaque formation was not altered. This reduction was accompanied by a reduction in $Na_V1.5$–ID clusters, but interestingly the localization of the $Na_V1.5$ scaffolding protein, ankyrin-G, was not changed. Macropatch recordings of isolated cells that were performed at the region previously occupied by the ID showed a reduced I_{Na} in Cx43D378stop cardiomyocytes compared with controls. SPC also revealed that unitary conductance of sodium channels was unchanged, thus concurring with the notion of a reduced proportion of functional $Na_V1.5$ channels in this ID region. Based on these observations, the authors proposed a model suggesting that Cx43 is part of a molecular complex that may capture the microtubule plus-end and allow for proper targeting of $Na_V1.5$ to the ID (*Figure 1B*).

This study contributes to the understanding of the mechanisms of $Na_V1.5$ cluster formation at the ID. Other partners of $Na_V1.5$ have been proposed to control its expression at the ID, especially PKP2 and SAP97. While PKP2 has been proposed to be part of a Cx43/ankyrin-G complex, whether SAP97 belongs to the same $Na_V1.5$/EB1/

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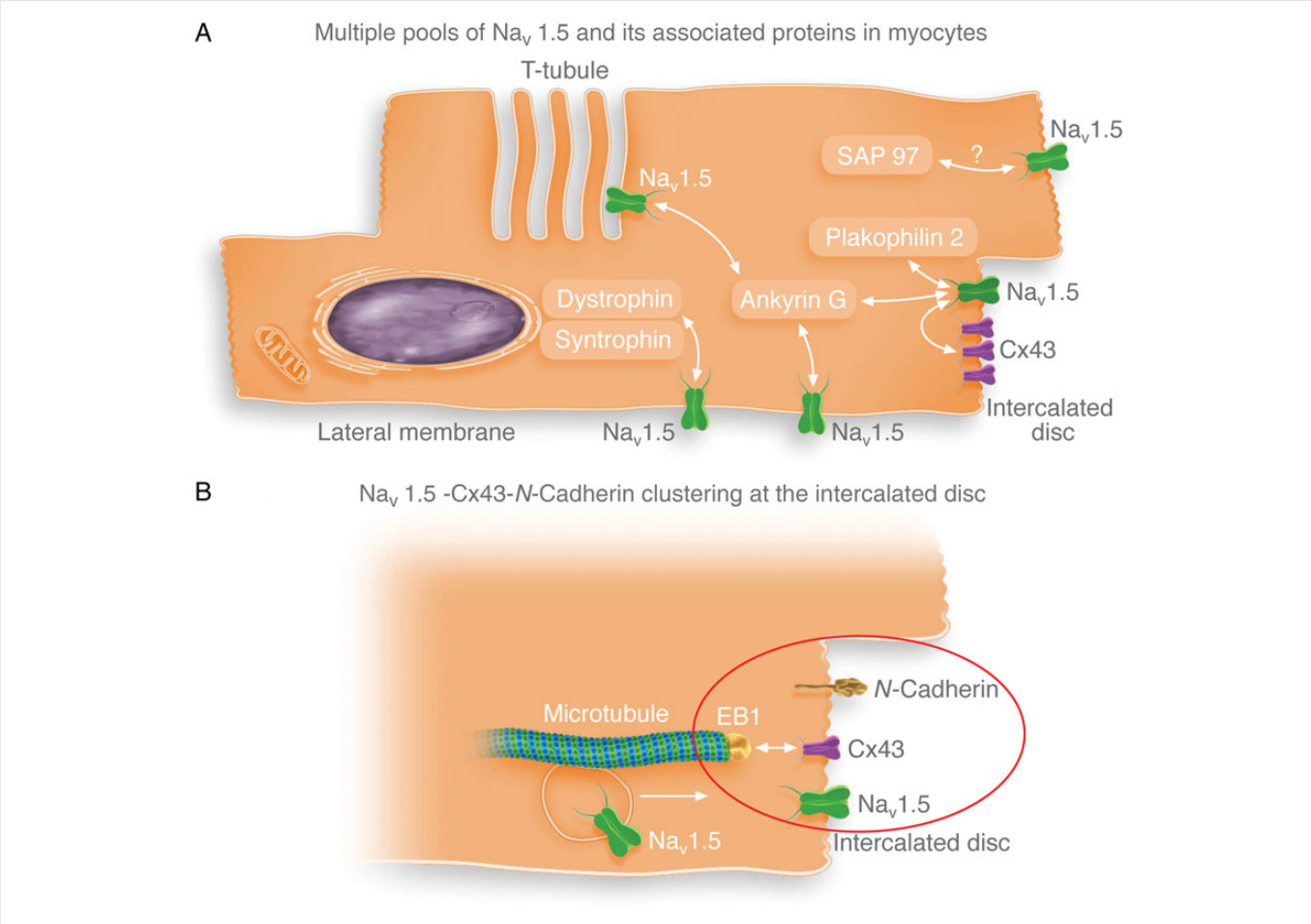

Figure 1 Multiple pools of $Na_V1.5$ in cardiac myocyte. (*A*) Different $Na_V1.5$ pools have been identified at the ID, lateral membrane, or t-tubules of cardiac cells, depending on which partner proteins they interact. It has to be noted that, in each compartment, sodium channels also cluster into highly confined functional nanodomains. (*B*) A model proposed by Agullo-Pascual *et al.* for $Na_V1.5$ cluster formation at the ID: Cx43 regulates EB1 capture, thus allowing for $Na_V1.5$ delivery to *N*-cadherin-rich sites. The red circle defines the different partner proteins clustering at the ID.

ankyrin-G cluster, or to different $Na_V1.5$ clusters, thus defining different $Na_V1.5$ subpopulations at the ID, remains an open question. The organization of $Na_V1.5$ clusters at the lateral membrane and t-tubules is also another question that remains to be addressed. Our group recently observed[6] that the last three amino acids (SIV) of the C-terminus of $Na_V1.5$ are essential for $Na_V1.5$ expression at the lateral membrane of cardiomyocytes and also for cardiac conduction. Macropatch recordings showed a 60% decrease in I_{Na}, recorded at the lateral membrane of cardiomyocytes from mice expressing $Na_V1.5$ with a truncation of the SIV motif. This observation suggests that, either there is another pool of $Na_V1.5$ at the lateral membrane that is responsible for the 40% remaining I_{Na} and that the targeting of these channels is independent of the C-terminal SIV motif or, that other sodium channel isoforms are present at the lateral membrane, as already proposed.[12,13] Moreover, while interacting proteins may regulate $Na_v1.5$ targeting, they may also influence the biophysical properties of sodium channel subpopulations. As an example, α-1-syntrophin (SNTA1) and $Na_V1.5$ interact at the lateral membrane of ventricular cardiomyocytes, and the congenital long QT syndrome mutation A390V in SNTA1 was shown to disrupt the association of plasma membrane Ca2+-ATPase 4b (PMCA4b) from the neuronal nitric oxide synthase (nNOS)–SNTA1–PMCA4b complex,[14] thus releasing inhibition of nNOS and leading to increased nitrosylation of $Na_V1.5$ and causing late I_{Na}. Another $Na_V1.5$ partner, caveolin-3, that localizes at the lateral membrane and t-tubules, has also been described to interact with the channel and to increase nNOS-dependent nitrosylation of $Na_V1.5$ when mutated, subsequently leading to an increase in late I_{Na}.[15] Thus, identifying clusters of $Na_V1.5$ channels which present different biophysical properties would be of great interest, especially in the selective targeting of a population of channels with novel pharmacological agents.

In conclusion, the study from Agullo-Pascual *et al.*[8] represents an important step in the ambitious endeavour that aims at providing a complete understanding of the diversity of the cardiac sodium channel landscape. The combination of cutting-edge techniques, such as SRFM and SPC, and the use of different available animal models will greatly contribute towards reaching this aim in the future. To develop new therapeutic interventions that are aimed at restoring normal sodium channel function, it is crucial to fully understand the cellular mechanisms that lead to the formation of sodium channel macromolecular complexes within cardiac cells.

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