Elucidating sodium channel $\text{Na}_v1.5$ clustering in cardiac myocytes using super-resolution techniques

Ludovic Gillet, Diana Shy, and Hugues Abriel*  
Department of Clinical Research, Ion Channels Research Group, University of Bern, Murtenstrasse 35, Bern 3010, Switzerland

This editorial refers to ‘Super-resolution imaging reveals that loss of the C-terminus of connexin43 limits microtubule plus-end capture and $\text{Na}_v1.5$ localization at the intercalated disc’ by E. Agullo-Pascual et al., pp. 371–381, this issue.

The cardiac voltage-gated sodium channel, $\text{Na}_v1.5$, is responsible for conducting the inward sodium current ($I_{\text{Na}}$), which leads to the fast depolarization of the cardiac cell membrane. Mutations in SCN5A, the gene encoding $\text{Na}_v1.5$, that lead to alterations in $I_{\text{Na}}$ 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 $\text{Na}_v1.5$, 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 $\text{Na}_v1.5$ are finely regulated by complex molecular and cellular mechanisms. Multiple pools of $\text{Na}_v1.5$ 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 $\text{Na}_v1.5$, and the genes encoding some of these regulatory proteins have also been found to be mutated in patients with inherited forms of cardiac arrhythmias.6

Although several partners of $\text{Na}_v1.5$ have been identified in different membrane compartments of cardiomyocytes, the precise location of functional channels remained undefined. In a previous study, Gorelik and Delmar groups7 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 $\text{Na}_v1.5$ 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, $\text{Na}_v1.5$, 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 $\text{Na}_v1.5$ at the ID, the cardiac gap junction protein Cx43 has been shown to regulate $I_{\text{Na}}$. A recent study9 demonstrated a Cx43-dependent regulation of $I_{\text{Na}}$ 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-end10 and (ii) $\text{Na}_v1.5$ is delivered to the cell membrane via microtubules.11 The authors of the present work have chosen to study the localization of $\text{Na}_v1.5$ 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 SRFM, 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 $\text{Na}_v1.5$–ID clusters, but interestingly the localization of the $\text{Na}_v1.5$ 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 $I_{\text{Na}}$ 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 $\text{Na}_v1.5$ 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 $\text{Na}_v1.5$ to the ID (Figure 1B).

This study contributes to the understanding of the mechanisms of $\text{Na}_v1.5$ cluster formation at the ID. Other partners of $\text{Na}_v1.5$ 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 $\text{Na}_v1.5$/EB1/Cx43 complex or acts independently is not defined yet. Further studies are needed to confirm whether $\text{Na}_v1.5$ associates with both Cx43 and EB1, and to identify the role of these proteins in the regulation of $\text{Na}_v1.5$ channel clustering.

The opinions expressed in this article are not necessarily those of the Editors of Cardiovascular Research or of the European Society of Cardiology.

* Corresponding author. Tel: +41 31 6320928; fax: +41 31 6320946. Email: hugues.abriel@difo.unibe.ch

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2014. For permissions please email: journals.permissions@oup.com.
ankyrin-G cluster, or to different NaV1.5 clusters, thus defining different NaV1.5 subpopulations at the ID, remains an open question. The organization of NaV1.5 clusters at the lateral membrane and t-tubules is also another question that remains to be addressed. Our group recently observed that the last three amino acids (SIV) of the C-terminus of NaV1.5 are essential for NaV1.5 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 NaV1.5 with a truncation of the SIV motif. This observation suggests that, either there is another pool of NaV1.5 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. Moreover, while interacting proteins may regulate NaV1.5 targeting, they may also influence the biophysical properties of sodium channel subpopulations. As an example, α-1-syntrophin (SNTA1) and NaV1.5 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, thus releasing inhibition of nNOS and leading to increased nitrosylation of NaV1.5 and causing late $I_{Na}$. Another NaV1.5 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 NaV1.5 when mutated, subsequently leading to an increase in late $I_{Na}$. Thus, identifying clusters of NaV1.5 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. 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.

**Conflict of interest:** none declared.

**Funding**

The group of H.A. is supported by a grant of the Swiss National Science Foundation 310030_14060 and from the European Community’s Seventh

---

**Figure 1** Multiple pools of NaV1.5 in cardiac myocyte. (A) Different NaV1.5 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 NaV1.5 cluster formation at the ID: Cx43 regulates EB1 capture, thus allowing for NaV1.5 delivery to N-cadherin-rich sites. The red circle defines the different partner proteins clustering at the ID.
References

1. Abriel H. Cardiac sodium channel Na(v)1.5 and interacting proteins: physiology and pathophysiology. J Mol Cell Cardiol 2010;48:2–11.

2. Shy D, Gillet L, Abriel H. Cardiac sodium channel Na,1.5 distribution in myocytes via interacting proteins: the multiple pool model. Biochim Biophys Acta 2013;1833:886–894.

3. Petitprez S, Zmoos AF, Ogrodnik J, Balse E, Raad N, El-Haou S, Albesa M, Bitbin P, Luther S, Lehnt SE, Hatem SN, Coulombre A, Abriel H. Sap97 and dystrophin macromolecular complexes determine two pools of cardiac sodium channels Na,1.5 in cardiomyocytes. Circ Res 2011;108:294–304.

4. Hund TJ, Koval OM, Li J, Wright PJ, Qian L, Snyder JS, Gudmundsson H, Kline CF, Davidson NP, Cardona N, Rasband MN, Cardona N, Anderson ME, Mohler PJ. A beta(IV)-spectrin/CaMKII signaling complex is essential for membrane excitability in mice. J Clin Invest 2010;120:3508–3519.

5. Sato PY, Coombs W, Lin X, Nekrasova O, Green KJ, Isom LL, Taffet SM, Delmar M. Interactions between ankyrin-g, plakophilin-2, and connexin43 at the cardiac intercalated disc. Circ Res 2011;109:193–201.

6. Bhargava A, Lin X, Novak P, Mehta K, Korchev Y, Delmar M, Gorelik J. Super-resolution scanning patch clamp reveals clustering of functional ion channels in adult ventricular myocyte. Circ Res 2013;112:1112–1120.

7. Agullo-Pascual E, Lin X, Leo-Macias A, Zhang M, Liang FX, Li Z, Pfenniger A, Lukkemeier I, Keegan S, Fenyo D, Willecke K, Rothenberg E, Delmar M. Super-resolution imaging reveals that loss of the C-terminus of connexin43 limits microtubule plus-end capture and Na,1.5 localization at the intercalated disc. Cardiovasc Res 2014;104:371–381.

8. Lukkemeier I, Requardt RP, Lin X, Sasse P, Andrie R, Schricket JW, Chkourko H, Bukauskas FF, Kim JS, Frank M, Malan D, Zhang J, Wirth A, Dobrowolski R, Mohler PJ, Offermanns S, Fleischmann BK, Delmar M, Willecke K. Deletion of the last five C-terminal amino acid residues of connexin43 leads to lethal ventricular arrhythmias in mice without affecting coupling via gap junction channels. Basic Res Cardiol 2013;108:348.

9. Shaw RM, Fay Aj, Puthenveedu MA, von Zastrow M, Jan YN, Jan LY. Microtubule plus-end-tracking proteins target gap junctions directly from the cell interior to adherens junctions. Cell 2007;128:547–560.

10. Casini S, Tan HL, Demirayak I, Remme CA, Amin AS, Scicluna BP, Chaytan H, Ruitjer JM, Bezerra CR, van Ginneken AC, Veldkamp MW. Tubulin polymerization modifies cardiac sodium channel expression and gating. Cardiovasc Res 2010;85:691–700.

11. Verkerk AO, van Ginneken AC, van Veen TA, Tan HL. Effects of heart failure on brain-type Na⁺ channels in rabbit ventricular myocytes. Eurocerc 2007;9:571–577.

12. Lin X, Liu N, Lu J, Zhang J, Anumonwo JM, Isom LL, Fishman GI, Delmar M. Subcellular heterogeneity of sodium current properties in adult cardiac ventricular myocytes. Heart Rhythm 2011;8:1923–1930.

13. Ueda K, Valdivia C, Medeiros-Domingo A, Tester DJ, Vatta M, Farrugia G, Ackerman MJ, Makielski JC. Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5a macromolecular complex. Proc Natl Acad Sci USA 2008;105:9355–9360.

14. Cheng J, Valdivia CR, Vaidyanathan R, Balijepalli RC, Ackerman MJ, Makielski JC, Cavedolin-3 suppresses late sodium current by inhibiting nNOS-dependent S-nitrosylation of SCN5a. J Mol Cell Cardiol 2013;51:1102–1110.