Cardiovascular Research (2016) **110**, 163–164
doi:10.1093/cvr/cvw055

EDITORIAL

Complexity of cardiac ion channel macromolecular complexes

Peter J. Mohler[1] and Hugues Abriel[2]*

[1]Departments of Physiology & Cell Biology and Internal Medicine, The Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus, OH, USA; and [2]Department of Clinical Research, Ion Channel Research Group, University of Bern, Bern, Switzerland

Online publish-ahead-of-print 16 March 2016

This editorial refers to 'Nav1.5 N-terminal domain binding to α1-syntrophin increases membrane density of human Kir2.1, Kir2.2 and Nav1.5 channels' by M. Matamoros *et al.*, pp. 279–290.

Proper cardiac function depends on a myriad of fine-tuned molecular interactions within myocytes.[1] The critical role of these interactions is clearly illustrated by a growing list of human cardiovascular diseases due to defects in the biogenesis and maintenance of large macromolecular complexes.[2] This paradigm is particularly relevant in the field of human cardiac arrhythmias where 'gain-of-function' or 'loss-of-function' mutations leading to seemingly small changes in the protein structure of ion channel proteins may lead to potential fatal events.

These findings are particularly surprising due to the fact that there exist many regulatory and counter-regulatory mechanisms that compensate to preserve cardiac electrical and structural function. For nearly two decades, cardiac ion channels have been shown to exist not simply as a pore-forming subunit, but as constituents of large, diverse, and fluid multiprotein complexes.[2] In fact, over the past few years, the emergence of new and highly sophisticated cell and molecular technologies has propelled our understanding into the 'life', i.e. cell biology, of these ion channels in highly compartmentalized cardiac cells.[1]

While all cardiac ion channels appear to be regulated through multiple protein interactions and post-translational modifications, the regulation of the primary cardiac voltage-gated Nav1.5 channel (*SCN5A*) has gained significant attention because of its critical role in myocardial excitability and direct links with human disease.[3] To date, hundreds of *SCN5A* mutations are linked to a long list of pathological phenotypes, congenital long-QT and Brugada syndromes being the most prevalent.[4] More than a dozen proteins interact with Nav1.5 and regulate either its function or its cellular fate.[3] In several cases, the genes coding for these Nav1.5-regulatory proteins are also mutated in patients with cardiac channelopathies. It has been observed that Nav1.5 is sequestered in distinct pools at the surface of cardiac cells.[5] Somewhat simplified, the current model is that the lateral membrane pool of Nav1.5 channels is complexed with the dystrophin–syntrophin multiprotein complex, while the intercalated disk pool interacts with ankyrin-G.[5] Recent studies using genetically modified mouse models demonstrated the *in vivo* and functional relevance of these distinct Nav1.5 pools. Makara *et al.*[6] showed that Nav1.5 expression at the intercalated disk was dependent on the expression of ankyrin-G in this compartment. Furthermore, this interaction with ankyrin-G and protein partner β-4 spectrin recruits CaMKIId to Nav1.5 for regulation of late sodium current.[7 8 9] Studies from the Abriel group demonstrated that the C-terminal PDZ-binding domain of Nav1.5 consisting of Ser-Ile-Val was key for the expression of a specific Nav1.5 channel population at the lateral sarcolemma.[10] The current model is that Nav1.5 PDZ-binding motif permits the interaction with α1-syntrophin and consequently dystrophin[5] (*Figure 1*). These multiprotein complexes are specific for the lateral membrane because neither syntrophin protein nor dystrophin expression was observed at the intercalated disks.[12] In dystrophin-deficient mice[12] and mice expressing a PDZ-binding motif truncated version of Nav1.5,[10] a strong, but not complete, reduction of Nav1.5 expression was observed. The remaining population at the lateral sarcolemma is represented as a punctate signal consistent with a T-tubular pool of Nav1.5 channels.[10] As indicated above, mutations in *SNTA1* gene, coding for alpha1-syntrophin, cause congenital long-QT syndrome by increasing the late sodium current.[13]

Recent work also demonstrated that Nav1.5 protein may not only interact with regulatory proteins, but also with other ion channel proteins, among them other Nav1.5 subunits.[14] In addition, Milstein *et al.* showed in a seminal paper[15] that the expression of Nav1.5 was reciprocally regulated with the Kir2.1 channel in cardiac cells. This potassium channel plays a key role in cardiac excitability through control of the resting membrane potential and is involved in the repolarization of the cardiac action potential.[16] Mutations in the gene encoding Kir2.1 (*KCNJ2*) are linked to Andersen's syndrome (multiple cardiac phenotypes including delayed cardiac repolarization). On the basis of these results with multiple experimental approaches, Nav1.5 and Kir2.1 interact via the MAGUK protein SAP97.[15] Co-stabilization, i.e. regulation of the internalization process, was observed to be one of the mechanisms of this reciprocal regulation. New work[11] from the group of Delpón in Madrid in collaboration with the Jalife group provide new insights into the role of accessory proteins for Nav1.5 and potassium channel regulation. Matamoros *et al.*,[11] using multiple cellular models and biochemistry approaches, identified an unexpected second

* Corresponding author. Tel: +41 31 6320928; fax: +41 31 6320946; E-mail: hugues.abriel@dkf.unibe.ch

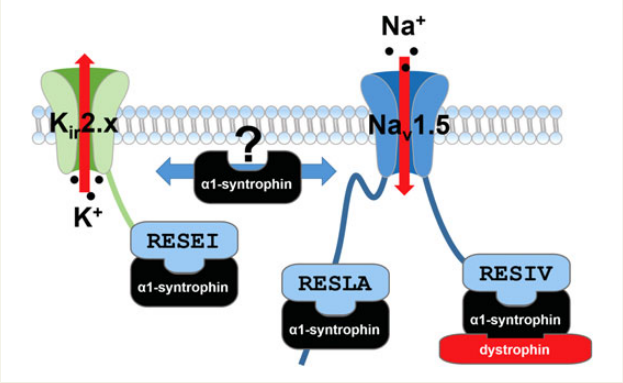

Figure 1 Matamoros *et al.*[11] demonstrated the key role of α1-syntrophin protein in mediating the interaction and reciprocal regulation between Kir2.1–2.2 (but not Kir2.3) and Nav1.5. Alpha-1 syntrophin, via its PDZ domain, binds to Kir2.x and Nav1.5 channels via cannonical C-terminal sequences, i.e. RESEI and RESIV, respectively. The authors also revealed a non-cannonical sequence (RESLA) in the N-terminal domain of Nav1.5. The molecular details about how α-1 syntrophin binding to these different ion channel domains may permit their interaction and regulation are, however, still unknown (question mark).

α1-syntrophin-binding site in the N-terminus domain of Nav1.5 (*Figure 1*). This finding is notable, as to the best of our knowledge, it represents the first evidence that a regulatory protein interacts with the Nav1.5 N-terminus. The authors provide evidence for a non-canonical PDZ-like-binding site with sequence resemblances to the C-terminal SIV binding motif. Thus, the cardiac sodium channel may interact with two α1-syntrophin molecules: one binding at the N-terminus with the second at the C-terminus (*Figure 1*). In their study, the authors show that syntrophin interactions are important for regulating the membrane density of not only Nav1.5, but also Kir2.1 and Kir2.2. Finally, the authors demonstrate that the Nav1.5 N-terminal domain displays 'chaperone-like' activity and that this activity is important for modulation of both Na^+ and K^+ channel function. While these new findings provide important new insight into the mechanisms underlying the post-transcriptional regulation of Nav1.5, this study raises a number of important new questions for the field. For example, can a single Nav1.5 channel simultaneously associate with two syntrophin molecules? Alternatively, does the N-terminal syntrophin interaction regulate anterograde trafficking to the lateral membrane while the C-terminal syntrophin interaction alters Nav1.5 lateral membrane stability? What are the molecular details of the interaction between Nav1.5 and Kir2.x channels? Finally, can N-terminal Nav1.5 N-terminal peptides be introduced *in vivo* to modulate Na^+ and K^+ channels and tune cardiac excitability in disease? In summary, the work from Matamoros[11] *et al.* further adds to our knowledge of the incredible complexity underlying ion channel regulation in the vertebrate heart. It also raises many questions that will have to be answered in future studies.

Conflict of interest: none declared.

Funding

The group of HA is supported by a grant of the Swiss National Science Foundation (310030_14060). P.J.M. is supported by NIH (HL084583, HL083422, and HL114383), and the American Heart Association.

References

1. Balse E, Steele DF, Abriel H, Coulombe A, Fedida D, Hatem SN. Dynamic of ion channel expression at the plasma membrane of cardiomyocytes. *Physiol Rev* 2012;**92**:1317–1358.
2. Mohler PJ, Wehrens XHT. Mechanisms of human arrhythmia syndromes: abnormal cardiac macromolecular interactions. *Physiology (Bethesda)* 2007;**22**:342–350.
3. Abriel H. Cardiac sodium channel Nav1.5 and interacting proteins: physiology and pathophysiology. *J Mol Cell Cardiol* 2010;**48**:2–11.
4. Veerman CC, Wilde AAM, Lodder EM. The cardiac sodium channel gene SCN5A and its gene product NaV1.5: role in physiology and pathophysiology. *Gene* 2015;**573**:177–187.
5. Shy D, Gillet L, Abriel H. Cardiac sodium channel Nav1.5 distribution in myocytes via interacting proteins: the multiple pool model. *Biochim Biophys Acta* 2013;**1833**:886–894.
6. Makara MA, Curran J, Little SC, Musa H, Polina I, Smith SA, Wright PJ, Unudurthi SD, Snyder J, Bennett V, Hund TJ, Mohler PJ. Ankyrin-G coordinates intercalated disc signaling platform to regulate cardiac excitability in vivo. *Circ Res* 2014;**115**:929–938.
7. Hund TJ, Koval OM, Li J, Wright PJ, Qian L, Snyder JS, Gudmundsson H, Kline CF, Davidson NP, Cardona N, Rasband MN, Anderson ME, Mohler PJ. A bIV-spectrin/CaMKII signaling complex is essential for membrane excitability in mice. *J Clin Invest* 2010;**120**:3508–3519.
8. Koval OM, Snyder JS, Wolf RM, Pavlovicz RE, Glynn P, Curran J, Leymaster ND, Dun W, Wright PJ, Cardona N, Qian L, Mitchell CC, Boyden PA, Binkley PF, Li C, Anderson ME, Mohler PJ, Hund TJ. Ca^{2+}/calmodulin-dependent protein kinase II-based regulation of voltage-gated Na+ channel in cardiac disease. *Circulation* 2012;**126**:2084–2094.
9. Glynn P, Musa H, Wu X, Unudurthi SD, Little S, Qian L, Wright PJ, Radwanski PB, Gyorke S, Mohler PJ, Hund TJ. Voltage-gated sodium channel phosphorylation at Ser571 regulates late current, arrhythmia, and cardiac function in vivo. *Circulation* 2015;**132**:567–577.
10. Shy D, Gillet L, Ogrodnik J, Albesa M, Verkerk AO, Wolswinkel R, Rougier J-S, Barc J, Essers MC, Syam N, Marsman RF, van Mil AM, Rotman S, Redon R, Bezzina CR, Remme CA, Abriel H. PDZ-domain-binding motif regulates cardiomyocyte compartment-specific $Na_v1.5$ channel expression and function. *Circulation* 2014;**130**:147–160.
11. Matamoros M, Pérez-Hernández M, Guerrero-Serna G, Amorós I, Barana A, Núñez M, Ponce-Balbuena D, Sacristan S, Gomez R, Tamargo J, Caballero R, Jalife J, Delpon E. Nav1.5 N-terminal domain binding to α1-syntrophin increases membrane density of human Kir2.1, Kir2.2 and Nav1.5 channels. *Cardiovasc Res* 2016;**110**:279–290.
12. Petitprez S, Zmoos AF, Ogrodnik J, Balse E, Raad N, El-Haou S, Albesa M, Bittihn P, Luther S, Lehnart SE, Hatem SN, Coulombe A, Abriel H. SAP97 and dystrophin macromolecular complexes determine two pools of cardiac sodium channels Nav1.5 in cardiomyocytes. *Circ Res* 2011;**108**:294–304.
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. Clatot J, Ziyadeh-Isleem A, Maugenre S, Denjoy I, Liu H, Dilanian G, Hatem SN, Deschenes I, Coulombe A, Guicheney P, Neyroud N. Dominant-negative effect of SCN5A N-terminal mutations through the interaction of Nav1.5 alpha-subunits. *Cardiovasc Res* 2012;**96**:53–63.
15. Milstein ML, Musa H, Balbuena DP, Anumonwo JM, Auerbach DS, Furspan PB, Hou L, Hu B, Schumacher SM, Vaidyanathan R, Martens JR, Jalife J. Dynamic reciprocity of sodium and potassium channel expression in a macromolecular complex controls cardiac excitability and arrhythmia. *Proc Natl Acad Sci USA* 2012;**109**:E2134–E2143.
16. Anumonwo JMB, Lopatin AN. Cardiac strong inward rectifier potassium channels. *J Mol Cell Cardiol* 2010;**48**:45–54.