FEBS Letters 587 (2013) 3159–3165

FEBS Letters

journal homepage: www.FEBSLetters.org

Protein arginine methyl transferases-3 and -5 increase cell surface expression of cardiac sodium channel

Pedro Beltran-Alvarez [a,b], Alexandsra Espejo [c], Ralf Schmauder [b], Carlos Beltran [d], Ralf Mrowka [e], Thomas Linke [b], Montserrat Batlle [f], Félix Pérez-Villa [f], Guillermo J. Pérez [a], Fabiana S. Scornik [a], Klaus Benndorf [b], Sara Pagans [a,1,*], Thomas Zimmer [b,1,*], Ramon Brugada [a,1,*]

[a] Cardiovascular Genetics Center, Institut d'Investigació Biomèdica de Girona, Department of Medical Sciences, School of Medicine, University of Girona, 17003 Girona, Spain
[b] Institute for Physiology II, University Hospital, 07743 Jena, Germany
[c] MD Anderson Cancer Center, Smithville, 78957 TX, United States
[d] Department of Mathematics, Statistics and Computation, University of Cantabria, 39005 Santander, Spain
[e] Experimental Nephrology KIMIII, University Hospital, 07743 Jena, Germany
[f] Thorax Institute, Cardiology Department, Hospital Clínic, University of Barcelona, Institute of Biomedical Research August Pi i Sunyer, 08036 Barcelona, Spain

ARTICLE INFO

Article history:
Received 21 June 2013
Revised 15 July 2013
Accepted 16 July 2013
Available online 31 July 2013

Edited by Robert B. Russell

Keywords:
Arginine methylation
Ion channel
Post-translational modification
Sodium channel

ABSTRACT

The α-subunit of the cardiac voltage-gated sodium channel ($Na_V1.5$) plays a central role in cardiomyocyte excitability. We have recently reported that $Na_V1.5$ is post-translationally modified by arginine methylation. Here, we aimed to identify the enzymes that methylate $Na_V1.5$, and to describe the role of arginine methylation on $Na_V1.5$ function. Our results show that protein arginine methyl transferase (PRMT)-3 and -5 methylate $Na_V1.5$ in vitro, interact with $Na_V1.5$ in human embryonic kidney (HEK) cells, and increase $Na_V1.5$ current density by enhancing $Na_V1.5$ cell surface expression. Our observations are the first evidence of regulation of a voltage-gated ion channel, including calcium, potassium, sodium and TRP channels, by arginine methylation.

Structured digital abstract:
PRMT5 physically interacts with **Nav1.5** by fluorescent resonance energy transfer (View interaction)
PRMT3 physically interacts with **Nav1.5** by fluorescent resonance energy transfer (View interaction)
Nav1.5 physically interacts with **PRMT3** by anti tag coimmunoprecipitation (View interaction)
PRMT1 physically interacts with **Nav1.5** by fluorescent resonance energy transfer (View interaction)
Nav1.5 physically interacts with **PRMT1** by anti tag coimmunoprecipitation (View interaction)

© 2013 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

Cardiac ion channels are essential for the generation of cardiomyocyte action potentials (AP). The cardiac voltage-gated sodium channel is responsible for the sodium inward current that initiates the AP, and consists of an α-subunit ($Na_V1.5$), which is necessary and sufficient for the generation of sodium currents, and accessory proteins [1]. The fine regulation of $Na_V1.5$ current is vital for proper cardiac function, and single mutations in $Na_V1.5$ can lead to cardiac arrhythmia conditions [2].

$Na_V1.5$ is predicted to be composed of four homologous domains, DI to DIV, joined by interdomain linkers. Interdomain linkers, N and C termini of the protein are cytosolic, and are accessible to post-translational modifications such as phosphorylation [3], and ubiquitination [4] which modulate $Na_V1.5$ function. Noteworthy, we have recently reported initial (40% sequence coverage) mass spectrometry evidence that identified arginine methylation (ArgMe) as a novel post-translational modification of $Na_V1.5$ [5]. ArgMe was observed in Arg residues within the interdomain linker between DI and DII of $Na_V1.5$ (L_{I-II}).

Abbreviations: AP, action potential; ArgMe, arginine methylation; bmp, beats per minute; CFP or YFP, cyan or yellow fluorescent protein; co-IP, co-immunoprecipitation; FRET, Förster resonance energy transfer; HEK, human embryonic kidney; IP, immunoprecipitation; L_{I-II}, linker between domains DI and DII of $Na_V1.5$; $Na_V1.5$, cardiac isoform of the voltage-gated sodium channel α subunit; PRMT, protein arginine methyltransferase; SAM, *S*-(5′-adenosyl)-L-methionine

* Corresponding authors: Cardiovascular Genetics Center, Institut d'Investigació Biomèdica de Girona, Department of Medical Sciences, School of Medicine, University of Girona, 17003 Girona, Spain.

E-mail addresses: pbeltran@idibgi.org, beltran@gencardio.com (P. Beltran-Alvarez), sara.pagans@udg.edu (S. Pagans), thomas.zimmer@mti.uni-jena.de (T. Zimmer), rbrugada@idibgi.org (R. Brugada).

[1] These authors contributed equally as senior authors.

0014-5793/$36.00 © 2013 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.febslet.2013.07.043

ArgMe is catalysed by protein arginine methyltransferases (PRMT), which transfer methyl groups from the cofactor *S*-adenosyl-L-methionine (SAM) to the protein, leading to mono- or dimethylated arginines [6]. In the latter case, methylation can be asymmetric or symmetric [7]. To date, 9 PRMTs have been identified in the human genome [8], namely PRMT1 to -9. PRMT1, -2, -3, -4, -6 and -8 are Type I PRMT that produce monomethyl and asymmetric dimethyl arginines [9]. PRMT5 is a Type II PRMT that produces monomethyl and symmetric dimethyl arginines [10], while PRMT7 leads to monomethyl arginines only [11]. PRMT9 has not been biochemically characterised to date. The addition of methyl groups modifies the shape of the arginine residue, turning it more bulky and hydrophobic, and may block a potential hydrogen bond donor.

Evidence indicating that $Na_V1.5$ is modified by ArgMe has opened new questions in the sodium channel field. In this context, we set ourselves to identify the PRMTs that mediate $Na_V1.5$ ArgMe, and to describe their effect on $Na_V1.5$ function.

2. Materials and methods

2.1. Plasmids

A pcDNA3 plasmid that encodes $Na_V1.5$ as a FLAG fusion, which has previously been described [12–14], was used in electrophysiological, co-IP, and biotin pull-down experiments. For Förster resonance energy transfer (FRET) measurements, a $Na_V1.5$-CFP construct, which localises to the endoplasmic reticulum in HEK cells, was used [15].

PRMTs for in vitro methylation assays were cloned into pGEX or Myc vectors. GST-PRMT1, -2, -3, -4, -6, and -7, as well as Myc-PRMT5, have been described [11,16]. To perform methylation studies in cells, FRET, co-IP, electrophysiological, and biotin pull-down experiments, PRMT-YFP fusion proteins were produced. PRMT1, -2, -3, -5, -6 and -7 were cloned from human cardiac cDNA (the study was approved by the institutional ethical committee of the Dr. Josep Trueta Hospital, Girona, Spain) into pcDNA3.1 (Invitrogen) as C-terminal YFP fusion proteins. PRMT4 could not be amplified from cardiac cDNA and a plasmid template was used.

L_{I-II} ($Na_V1.5$ residues 416–711) was cloned into pcDNA3.1 as L_{I-II}-FLAG-YFP fusion. Plasmids for expression in *Escherichia coli* were pETM15b (EMBL) in which L_{I-II} or the C-terminal domain ($Na_V1.5$ residues 1773–2016) were cloned as N-terminal His-tagged proteins.

For electrophysiological and FRET assays, HEK cells were transfected using calcium phosphate precipitation. For all other experiments, cells were transfected using GeneCellin (BioCellChallenge).

2.2. Detection of methylated L_{I-II} in cells

HEK cells were transiently transfected with the L_{I-II}-FLAG-YFP plasmid (2 μg), and harvested 30 h later. Soluble lysates were incubated with α-GFP antibody (Abcam) coupled to protein A beads (GE Healthcare). Immunoprecipitated proteins were detected with α-FLAG (M2, Sigma), α-ASYM24, or α-SYM11 (Merck).

2.3. In vitro methylation assays

L_{I-II} and the C-terminal domain of $Na_V1.5$ were expressed in *E. coli* as 6×His fusions, and purified using Ni-NTA chromatography. In vitro methylation assays were conducted as described [17]. Recombinant GST-PRMT1, -2, -3, -4, -6 and -7 were expressed and purified from *E. coli*. Myc-tagged human PRMT5 was expressed and purified from HeLa cells. Reactions were done in the presence of 0.5 μCi *S*-adenosyl-L-[methyl-^{3}H]methionine (^{3}H-SAM, GE Healthcare) for 1 h at 30 °C in a final volume of 30 μl.

2.4. FRET between $Na_V1.5$ and PRMTs

HEK cells expressing $Na_V1.5$-CFP (0.5 μg plasmid) together with the corresponding PRMT–YFP (1.5 μg plasmid), or YFP as a negative control (0.5 μg plasmid), were grown for 24–48 h after transfection. For every $Na_V1.5$/PRMT pair, 4 independent transfections were done. In every measuring session, 5–10 negative controls were measured in parallel. Cells were examined by confocal microscopy on a LSM 710 (Zeiss, Jena, Germany) using a C-Apochromat 40×/1.2 NA water-immersion objective lens at room temperature. FRET was calculated using the sensitised emission method. Reported apparent FRET efficiencies ($FRET_{app}$) are the mean ± S.E.M. of individual cell $FRET_{app}$ values for a given $Na_V1.5$-CFP/PRMT–YFP pair.

2.5. Co-IP of PRMT1 and -3, with $Na_V1.5$

HEK cells were transiently transfected with FLAG $Na_V1.5$ (1 μg plasmid), together with GFP (0.5 μg plasmid), or PRMT1-, or -3–YFP (1.5 μg plasmid). Cells were lysed 48 h after transfection, and $Na_V1.5$ was immunoprecipitated ($n = 3$) using FLAG chromatography (Sigma). PRMTs were detected using an α-GFP antibody (Abcam), and $Na_V1.5$ was detected using an α-$Na_V1.5$ antibody (Alomone).

2.6. Electrophysiological recordings

Sodium currents in HEK cells transiently expressing FLAG-$Na_V1.5$ (1.5 μg plasmid) together with the corresponding PRMT–YFP (0.5 μg plasmid), were measured between 25 and 35 h after transfection. The total number of independent transfections was 5 (PRMT3 and -5), 3 (PRMT1), or 2 (PRMT2, -4, and -7). The day after transfection, SAM (0.15 mM, final concentration) was added to the medium, and sodium currents were measured at room temperature. In every session, 3–10 negative controls (i.e. cells co-expressing $Na_V1.5$ and YFP) were measured in parallel.

Currents were obtained by patch clamp whole-cell recording using an Axopatch 200B amplifier (Molecular Devices). Activation data at test potentials of −80 to +50 mV (in 5 or 10 mV steps) were fitted to a Boltzmann equation, of the form $g = g_{max}/(1 + \exp[(V_{1/2} - V_m)/s])$, where g is the conductance, g_{max} the maximum conductance, V_m the membrane potential, $V_{1/2}$ the voltage at which half of the channels are activated, and s the slope factor. Inactivation data at test potentials of −140 to −30 mV (in 5 or 10 mV steps, for 250 ms) were fitted to a Boltzmann equation of the form $I = I_{max}/(1 + \exp[(V_m - V_{1/2})/s])$, where I is the peak current amplitude, I_{max} the maximum peak current amplitude, V_m the membrane potential, $V_{1/2}$ the voltage at which half of the channels are inactivated, and s the slope factor. Kinetics of $Na_V1.5$ inactivation were fitted to a monoexponential function, from which time constant τ (inactivation) between −40 and 30 mV was estimated. $Na_V1.5$ recovery from inactivation data (at recovery times of 250 μs, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 100, 250, 500, 1000 and 1900 ms at −120 mV) were fitted to monoexponential functions to determine recovery time constant τ. All reported errors are S.E.M.

2.7. Biotin pull-down of cell surface proteins

Biotin pull-down of FLAG-$Na_V1.5$ was performed from transiently transfected HEK cells ($n = 2$) and from a previously developed [5] cell line stably expressing FLAG-$Na_V1.5$ ($n = 2$). Cells were transfected with $Na_V1.5$ (1 μg plasmid) together with YFP

(0.5 μg plasmid), PRMT1, -3 or -5 (1.5 μg plasmid). After 24 h, cells were treated with reactive biotin (Pierce) for 60 min at 4 °C, and lysed. Soluble fractions were incubated with neutravidin (Pierce) overnight at 4 °C. Pull-downs were probed with α-Na_V1.5 antibody. Protein bands were quantified using ImageJ (NIH).

2.8. Cardiac AP simulations

We used the ten Tusscher–Panfilov 2006 model [18]. The model was modified to reproduce the observed changes in sodium current density in cells overexpressing PRMT3 or -5. We multiplied the fast sodium current by a factor of $(1 + p)$, where p was 0.46 or 0.35 (PRMT3-, or -5-cardiomyocytes, respectively). Used protocols were an adaptation of recently reported protocols to simulate cardiac arrhythmias [19].

3. Results

3.1. $L_{I–II}$ is a substrate for Type I and Type II PRMTs

The linker between DI and DII ($L_{I–II}$) of Na_V1.5 is a *hot-spot* for ArgMe within Na_V1.5 [5]. To test an isolated $L_{I–II}$ protein as substrate for PRMTs, we overexpressed $L_{I–II}$ in HEK cells. Antibodies specific to asymmetric, and symmetric, dimethyl arginines recognised $L_{I–II}$ (Fig. 1A), indicating that $L_{I–II}$ was a target for Type I and Type II PRMTs.

To identify the PRMTs that methylate $L_{I–II}$, we performed in vitro methylation assays using recombinant PRMTs and ^{3}H-SAM. We purified untagged $L_{I–II}$, from *E. coli* (Fig. 1B), as substrate. The C-terminal domain of Na_V1.5 was included as negative control. We screened all known PRMTs excluding PRMT8, which is specifically expressed in the central nervous system [20], and PRMT9, which has not been characterised [9]. Consistent with our findings in cells, we observed methylation of $L_{I–II}$ by Type I (PRMT1, -3, -4 and -6), and Type II (PRMT5) PRMTs (Fig. 1C). PRMT5 showed weak activity towards $L_{I–II}$ in vitro, but the fact that $L_{I–II}$ was symmetrically dimethylated in cells strongly indicates that PRMT5, the only Type II PRMT [9,10,21], methylates Na_V1.5. The observation that PRMT1 methylates the C-terminal domain was unexpected. Methylated protein bands at lower molecular weight most likely corresponded to degradation products, which might serve as preferred recognition motifs.

3.2. Na_V1.5 interacts with PRMT1, -3 and -5

Next, we screened for interactions between full length Na_V1.5 and PRMTs. We observed significant FRET efficiency ($FRET_{app}$) between Na_V1.5–CFP and PRMT1–, -3–, or -5–YFP, compared to control (YFP) cells, and cells co-expressing PRMT2, -4, and -7 (Fig. 2A and B, and Supplementary Fig. S1). PRMT6 was discarded for later experiments, because it localised exclusively to the cell nucleus (not shown), in agreement with previous reports [16,22–24].

To validate our FRET results, we performed co-IP experiments in HEK cells. After IP of Na_V1.5 by FLAG-affinity, we detected co-IP of PRMT1– and -3–YFP (Fig. 2C). PRMT5 was excluded from FLAG co-IP experiments, as it cross-reacts with α-FLAG antibodies [9].

3.3. Na_V1.5 cell surface expression is increased by PRMT3 and -5

To address the effect of PRMTs on Na_V1.5 electrophysiology, we co-transfected HEK cells with Na_V1.5 and PRMTs. We observed increased Na_V1.5 current density (in pA/pF) in cells co-expressing PRMT3 (102 ± 10), or PRMT5 (95 ± 9), compared to control cells (70 ± 6), (Fig. 3A–C). Na_V1.5 current density in cells co-expressing PRMT1 (77 ± 7) was not significantly higher than in control cells. Na_V1.5 voltage-dependent activation and inactivation, recovery from inactivation (Supplementary Table S1), and kinetics of inactivation (Supplementary Fig. S2) were unchanged in all cases.

We hypothesised that the observed increase in Na_V1.5 current density was due to higher Na_V1.5 cell surface expression. Biotin pull-down experiments revealed ca. 30% increased Na_V1.5 cell surface expression in cells co-expressing PRMT3 or -5, compared to control, and PRMT1 cells (Fig. 3, D–F), in reasonable agreement with electrophysiological findings (46% and 35% higher current density in the presence of PRMT3 and -5, respectively).

3.4. Increased Na_V1.5 ArgMe changes cardiac AP dynamics in silico

To extend our findings to a cardiac context, we generated computer cardiomyocyte AP simulations. We considered control

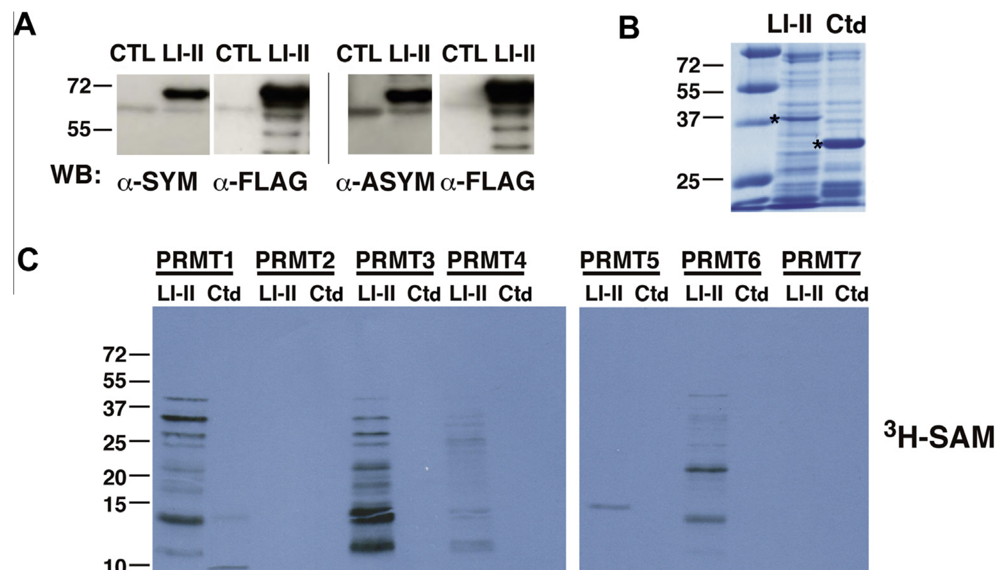

Fig. 1. $L_{I–II}$ is a substrate for PRMTs. (A) $L_{I–II}$-FLAG-YFP recognised by antibodies against symmetric (α-SYM), and asymmetric (α-ASYM) dimethyl arginine. Molecular weights are in kDa. Expected size: 61 kDa. (B) Expression of $L_{I–II}$ and the C-terminal domain of Na_V1.5 (Ctd) in *E. coli* (marked with stars). (C) In vitro methylation assays. Molecular weights are in kDa.

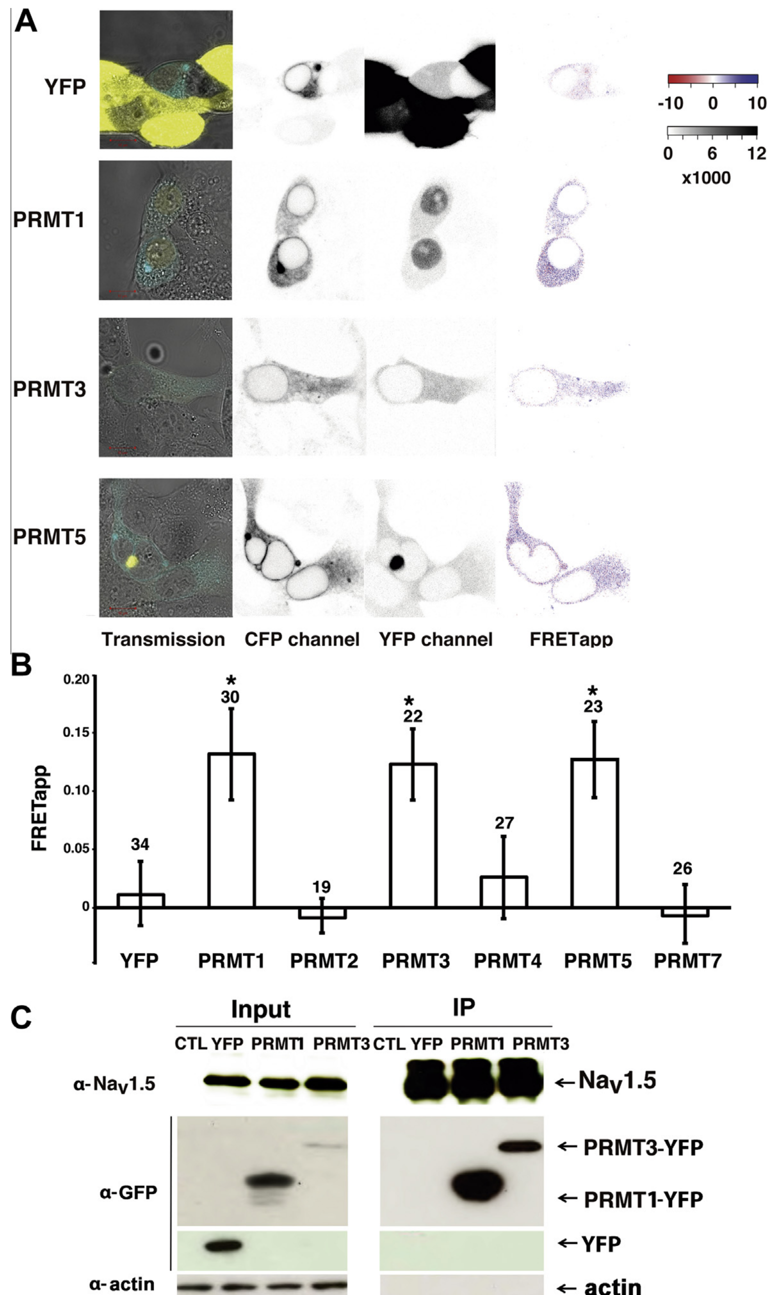

Fig. 2. $Na_V1.5$ interacts with PRMT1, -3 and -5. (A) Representative cells co-expressing $Na_V1.5$-CFP and YFP (control), PRMT1–, -3–, or -5–YFP. Shown: transmission image including CFP and YFP fluorescence; CFP and YFP channels at respective excitation and emission; and corrected $FRET_{app}$ image. Scale bars (of 40 µm length) represent low (red) to high (blue) $FRET_{app}$; and low (white) to high (black) CFP or YFP intensity. (B) $FRET_{app}$ between $Na_V1.5$–CFP and PRMT–YFP. Over each bar the number of evaluated cells is indicated. *Significant ($P < 0.002$ ANOVA test). (C) Representative gel picture ($n = 3$) before (input), and after IP of $Na_V1.5$. PRMT1–YFP (67 kDa) and -3–YFP (86 kDa), but not YFP neither actin, co-IP with $Na_V1.5$.

cardiomyocytes, as well as PRMT3- or -5-cardiomyocytes with 46% or 35% (respectively), higher sodium current density. We performed 6000 cycles at 60 beats per minute (bpm), and then increased pacing abruptly to ca. 150 bpm. Under these conditions, control cardiomyocytes led to alternans (Fig. 4), while PRMT3- and -5-cardiomyocytes generated APs at pacing rate, the duration of which was stabilised after 10 stimuli.

4. Discussion

Recently, we have revealed ArgMe as a novel $Na_V1.5$ post-translational modification [5]. Now, we present the first systematic analysis of the effect of individual PRMTs on the function of a voltage-gated ion channel, and demonstrate that PRMT3 and -5 increase $Na_V1.5$ cell surface expression. Future work will elucidate whether this is due to facilitated trafficking to the plasma membrane (a proposed effect of $Na_V1.5$ phosphorylation [3]), or extended residence time at the cell surface (ArgMe by PRMT3 may inhibit ubiquitination and thereby proteolysis of target proteins [25]).

PRMT1 methylated L_{I-II}, and interacted with $Na_V1.5$ in cells, but did not regulate $Na_V1.5$ cell surface expression under our experimental conditions. This may be related to the observation that PRMT1 was the only PRMT that methylated the C-terminal domain

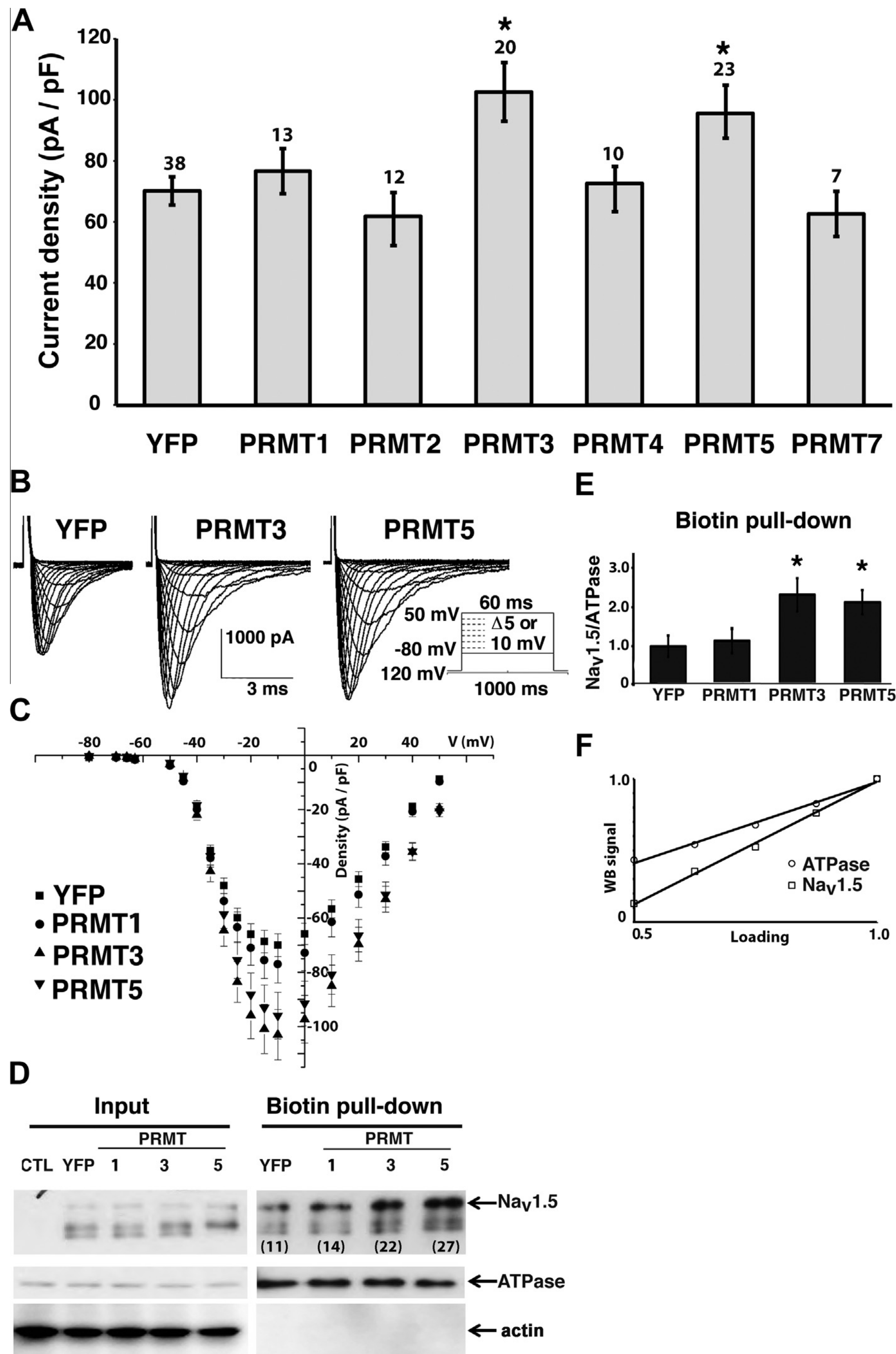

Fig. 3. $Na_V1.5$ cell surface expression is increased by PRMT3 and -5. (A) $Na_V1.5$ peak current density at −10 mV in cells co-expressing PRMTs. Over each bar the number of evaluated cells is indicated. *Significant ($P < 0.01$ ANOVA test). (B) Representative $Na_V1.5$ currents in control (YFP), PRMT3 or -5 cells. (C) I–V curves in control (YFP) cells, PRMT1, -3 or -5 cells. (D) Representative gel picture ($n = 4$) after biotin pull-down from non-transfected (CTL), control (YFP), PRMT1, -3 or -5 cells. The Na^+/K^+ ATPase α subunit was probed as a loading control for pull-downed samples. Actin was included as marker for cytosolic proteins contamination in biotin pull-downs (not observed). Shown between brackets, quantification of signal intensity of $Na_V1.5$ bands. (E) Bar plot showing ratio of $Na_V1.5$/ATPase intensities in cells co-expressing PRMT1, -3 or -5, normalised to control (YFP). Note that $Na_V1.5$ signal intensity in PRMT3 and -5 was 2.3 and 2.1 times higher, respectively, than control experiments. *Significant ($P < 0.05$ unpaired *t*-Student test). (F) Calibration of Western blot signal for $Na_V1.5$ and ATPase under our experimental conditions. $Na_V1.5$ detection was not in the linear range: doubling the loading doubled ATPase signal, but increased $Na_V1.5$ signal by a factor of 7.7. We estimate that the observed increase in $Na_V1.5$ signal by a factor of 2.3 and 2.1 corresponds to ca. 30% and 27% (PRMT3 and -5, respectively) higher $Na_V1.5$ cell surface expression.

in vitro, however, the individual effect of ArgMe of specific Arg residues on $Na_V1.5$ cell surface expression, needs be described by further work. PRMT2, -4, -6 and -7 are most likely not relevant to full length $Na_V1.5$, and we relate the observed in vitro methylation of L_{I-II} by PRMT4 and -6 to the suitability of an isolated L_{I-II} protein as substrate for ArgMe.

The observation of an effect on $Na_V1.5$ cell surface expression upon co-expression of PRMT3 and -5 suggests that PRMT concentration is an important factor to regulate levels of $Na_V1.5$ ArgMe. In the human heart, where $Na_V1.5$ is modified by ArgMe [5], expression of $Na_V1.5$, PRMT3 and -5 may be dysregulated e.g. in cardiac disease [26,27], which in turn may affect $Na_V1.5$ ArgMe.

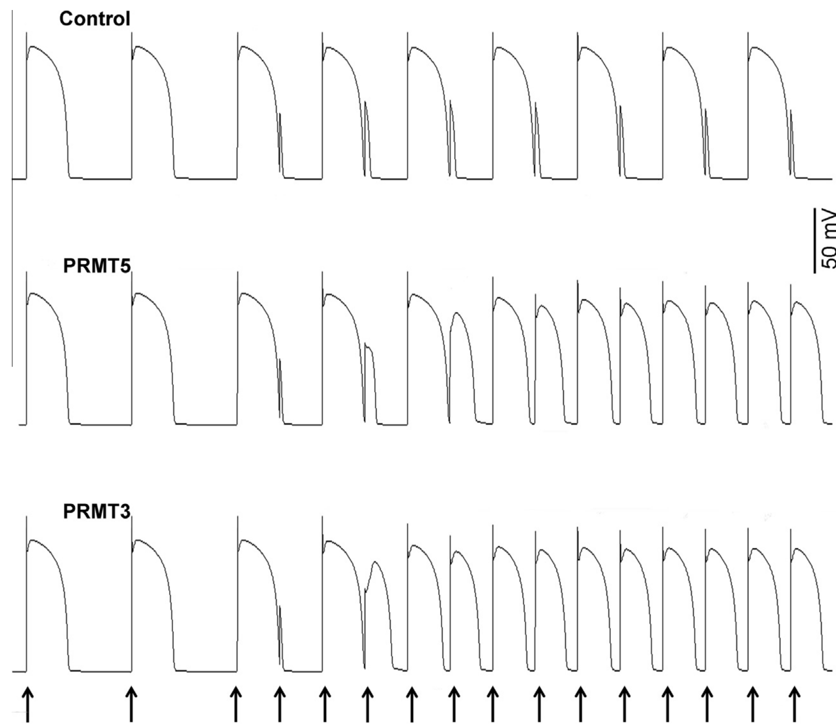

Fig. 4. Increased $Na_V1.5$ ArgMe changes AP dynamics in silico. APs of control, PRMT5- and -3-cardiomyocytes paced at ca. 150 beats per minute (bpm) after 6000 conditioning pulses at 60 bpm, the last two of which are shown. Arrows indicate pacing.

Our simulations suggested that cardiomyocytes with increased $Na_V1.5$ ArgMe sustained action potentials at high pacing when control cardiomyocytes did not. Validation of our data, which have been obtained in accepted (but heterologous) systems, in the cardiac pathophysiology, will further the relevance of $Na_V1.5$ ArgMe.

In conclusion, the observation of $Na_V1.5$ regulation by PRMT3 and -5 contributes to growing evidence that $Na_V1.5$ is modulated by multiple processes [28], continues to expand ArgMe to non-histone proteins [21], and should trigger studies of ArgMe in other members of the voltage-gated ion channel superfamily, which consists of 143 proteins [29].

Acknowledgments

The authors gratefully thank Dr. Mark Bedford (MD Anderson Cancer Center) for his supervision of radioactive assays; and Karin Schoknecht (University Hospital Jena) for her outstanding technical support. This work was supported by the Fundació La Caixa (Barcelona, Spain), and the Spanish Government (Grants Nos. SAF2011-27627 and MTM2010-16051). P.B.-A. acknowledges a Sara Borrell postdoctoral fellowship (CD10/00275). Funding sources had no involvement in study design; collection, analysis and interpretation of data; writing of the report; or decision to submit the article for publication.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.07.043.

References

[1] Abriel, H. (2010) Cardiac sodium channel Na(v)1.5 and interacting proteins: physiology and pathophysiology. J. Mol. Cell. Cardiol. 48, 2–11.
[2] Zimmer, T. and Surber, R. (2008) Scn5a channelopathies–an update on mutations and mechanisms. Prog. Biophys. Mol. Biol. 98, 120–136.
[3] Rook, M.B., Evers, M.M., Vos, M.A. and Bierhuizen, M.F. (2012) Biology of cardiac sodium channel Nav1.5 expression. Cardiovasc. Res. 93, 12–23.
[4] van Bemmelen, M.X., Rougier, J.S., Gavillet, B., Apotheloz, F., Daidie, D., Tateyama, M., Rivolta, I., Thomas, M.A., Kass, R.S., Staub, O. and Abriel, H. (2004) Cardiac voltage-gated sodium channel Nav1.5 is regulated by Nedd4-2 mediated ubiquitination. Circ. Res. 95, 284–291.
[5] Beltran-Alvarez, P., Pagans, S. and Brugada, R. (2011) The cardiac sodium channel is post-translationally modified by arginine methylation. J. Proteome Res. 10, 3712–3719.
[6] Pang, C.N., Gasteiger, E. and Wilkins, M.R. (2010) Identification of arginine- and lysine-methylation in the proteome of *Saccharomyces cerevisiae* and its functional implications. BMC Genomics 11, 92.
[7] Bannister, A.J., Schneider, R. and Kouzarides, T. (2002) Histone methylation: dynamic or static? Cell 109, 801–806.
[8] Petrossian, T.C. and Clarke, S.G. (2011) Uncovering the human methyltransferasome. Mol. Cell. Proteomics 10. M110.000976.
[9] Bedford, M.T. and Clarke, S.G. (2009) Protein arginine methylation in mammals: who, what, and why. Mol. Cell 33, 1–13.
[10] Branscombe, T.L., Frankel, A., Lee, J.H., Cook, J.R., Yang, Z., Pestka, S. and Clarke, S. (2001) Prmt5 (janus kinase-binding protein 1) catalyzes the formation of symmetric dimethylarginine residues in proteins. J. Biol. Chem. 276, 32971–32976.
[11] Zurita-Lopez, C.I., Sandberg, T., Kelly, R. and Clarke, S.G. (2012) Human protein arginine methyltransferase 7 (PRMT7) is a type iii enzyme forming omega-ng-monomethylated arginine residues. J. Biol. Chem. 287, 7859–7870.
[12] Tan, B.H., Valdivia, C.R., Song, C. and Makielski, J.C. (2006) Partial expression defect for the SCN5A missense mutation G1406R depends on splice variant background Q1077 and rescue by mexiletine. Am. J. Physiol. Heart Circ. Physiol. 291, H1822–1828.
[13] Wu, G., Ai, T., Kim, J.J., Mohapatra, B., Xi, Y., Li, Z., Abbasi, S., Purevjav, E., Samani, K., Ackerman, M.J., Qi, M., et al. (2008) Alpha-1-syntrophin mutation and the long-QT syndrome: a disease of sodium channel disruption. Circ. Arrhythm. Electrophysiol. 1, 193–201.
[14] Makita, N., Behr, E., Shimizu, W., Horie, M., Sunami, A., Crotti, L., Schulze-Bahr, E., Fukuhara, S., Mochizuki, N., Makiyama, T., et al. (2008) The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. J. Clin. Invest. 118, 2219–2229.
[15] Zimmer, T., Biskup, C., Dugarmaa, S., Vogel, F., Steinbis, M., Bohle, T., Wu, Y.S., Dumaine, R. and Benndorf, K. (2002) Functional expression of gfp-linked human heart sodium channel (hh1) and subcellular localization of the a subunit in HEK cells and dog cardiac myocytes. J. Membr. Biol. 186, 1–12.
[16] Frankel, A., Yadav, N., Lee, J., Branscombe, T.L., Clarke, S. and Bedford, M.T. (2002) The novel human protein arginine *n*-methyltransferase prmt6 is a nuclear enzyme displaying unique substrate specificity. J. Biol. Chem. 277, 3537–3543.
[17] Cheng, D., Vemulapalli, V. and Bedford, M.T. (2012) Methods applied to the study of protein arginine methylation. Methods Enzymol. 512, 71–92.

[18] ten Tusscher, K.H. and Panfilov, A.V. (2006) Alternans and spiral breakup in a human ventricular tissue model. Am. J. Physiol. Heart Circ. Physiol. 291, H1088–1100.
[19] Castro, J., Rodriguez, J.F., Laguna, P. and Pueyo, E. (2011) A human ventricular cell model for investigation of cardiac arrhythmias under hyperkalaemic conditions. Phil. Trans. R. Soc. A 369, 4205–4232.
[20] Kousaka, A., Mori, Y., Koyama, Y., Taneda, T., Miyata, S. and Tohyama, M. (2009) The distribution and characterization of endogenous protein arginine *n*-methyltransferase 8 in mouse CNS. Neuroscience 163, 1146–1157.
[21] Clarke, S.G. (2013) Protein methylation at the surface and buried deep: thinking outside the histone box. Trends Biochem. Sci. 38, 243–252.
[22] Herrmann, F., Pably, P., Eckerich, C., Bedford, M.T. and Fackelmayer, F.O. (2009) Human protein arginine methyltransferases in vivo–distinct properties of eight canonical members of the prmt family. J. Cell Sci. 122, 667–677.
[23] Herrmann, F., Lee, J., Bedford, M.T. and Fackelmayer, F.O. (2005) Dynamics of human protein arginine methyltransferase 1 (PRMT1) in vivo. J. Biol. Chem. 280, 38005–38010.
[24] Waldmann, T., Izzo, A., Kamieniarz, K., Richter, F., Vogler, C., Sarg, B., Lindner, H., Young, N.L., Mittler, G., et al. (2011) Methylation of H2AR29 is a novel repressive PRMT6 target. Epigenetics Chromatin 4, 11.
[25] Choi, S., Jung, C.R., Kim, J.Y. and Im, D.S. (2008) PRMT3 inhibits ubiquitination of ribosomal protein S2 and together forms an active enzyme complex. Biochim. Biophys. Acta 1780, 1062–1069.
[26] Kupershmidt, I., Su, Q.J., Grewal, A., Sundaresh, S., Halperin, I., Flynn, J., Shekar, M., Wang, H., Park, J., Cui, W., et al. (2010) Ontology-based meta-analysis of global collections of high-throughput public data. PLoS ONE 5, 9.
[27] Borlak, J. and Thum, T. (2003) Hallmarks of ion channel gene expression in end-stage heart failure. FASEB J. 17, 1592–1608.
[28] Priori, S.G. (2010) The fifteen years of discoveries that shaped molecular electrophysiology: time for appraisal. Circ. Res. 107, 451–456.
[29] Yu, F.H., Yarov-Yarovoy, V., Gutman, G.A. and Catterall, W.A. (2005) Overview of molecular relationships in the voltage-gated ion channel superfamily. Pharmacol. Rev. 57, 387–395.