

An update on transcriptional and post-translational regulation of brain voltage-gated sodium channels

Donatus O. Onwuli¹ · Pedro Beltran-Alvarez¹

Received: 15 October 2015 / Accepted: 16 October 2015 / Published online: 27 October 2015
© The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract Voltage-gated sodium channels are essential proteins in brain physiology, as they generate the sodium currents that initiate neuronal action potentials. Voltage-gated sodium channels expression, localisation and function are regulated by a range of transcriptional and post-translational mechanisms. Here, we review our understanding of regulation of brain voltage-gated sodium channels, in particular SCN1A (Na_v1.1), SCN2A (Na_v1.2), SCN3A (Na_v1.3) and SCN8A (Na_v1.6), by transcription factors, by alternative splicing, and by post-translational modifications. Our focus is strongly centred on recent research lines, and newly generated knowledge.

Keywords Voltage-gated sodium channel · Regulation · Transcription factor · Alternative splicing · Post-translational modification

Introduction

Voltage-gated sodium channels are essential proteins in brain physiology. Upon voltage-mediated activation, sodium channels produce sodium currents responsible for depolarisation of excitable cells, including neurons and cardiomyocytes. From the point of view of biomedical sciences and pathophysiology, brain disorders such as some forms of epilepsy have long been directly associated with voltage-gated sodium channel malfunction.

Sodium channels are thought to be macromolecular complexes composed of tens of different proteins (Abriel et al. 2015). The pore-forming protein is known as the α subunit, and is sufficient to generate sodium currents. All α subunits include a voltage sensor that promotes channel opening when the cell membrane is depolarized by a few millivolts. Sodium channels thus activate, generate the sodium currents that underlie the initial depolarisation phase of the action potential, and then inactivate within tens of milliseconds, critically shaping cell repolarisation (Zilberter et al. 1994).

There are nine isoforms of the voltage-gated sodium channel α subunit, and each form has distinct expression and electrophysiological patterns. In this review, we have considered the main sodium channel isoforms expressed in the central neuronal system (CNS), i.e., SCN1A (Na_v1.1), SCN2A (Na_v1.2), SCN3A (Na_v1.3) and SCN8A (Na_v1.6). Wherever relevant we have also included additional information regarding other isoforms, including SCN5A (generally known as the cardiac isoform, Na_v1.5) and SCN9A (mainly expressed in the peripheral nervous system, Na_v1.7), (Dib-Hajj et al. 2013).

Sodium channel α subunits are large (ca. 2000 residues), hydrophobic, integral membrane proteins that have been fascinating (and challenging) a range of scientific communities including biochemists, pharmacists, neuroscientists, and electrophysiologists for more than three decades (Catterall 2015). Although detailed mammalian voltage-gated sodium channel structures are not yet available, it is widely accepted that the topology of α subunits at the protein level consists of four homologous domains (termed DI to DIV), each consisting of six transmembrane helices, and joined by cytosolic interdomain linkers (Yu and Catterall 2003). The *N* and *C* termini of α subunits are also intracellular. Thus, cytosolic interdomain linkers, and *N*- and

✉ Pedro Beltran-Alvarez
p.beltran-alvarez@hull.ac.uk

¹ School of Biological, Biomedical and Environmental Sciences, University of Hull, Hardy Building Cottingham Road, Hull HU6 7RX, UK

C-terminal domains of α subunits are accessible to intracellular enzymes that catalyse post-translational modifications (PTM) of the channels.

In this review, we aim to integrate progress in our understanding of CNS voltage-gated sodium channel regulation at the transcriptional and post-translational level. The reader will find that much more is known on sodium channel PTMs than on the transcriptional mechanisms that regulate channel expression. Consequently, the weight of the review is balanced towards PTMs. Our focus is strongly centred on recent research lines, and newly generated knowledge. The goal is to facilitate dissemination of recent developments with a view on fostering further relevant research.

Regulation of brain sodium channel expression at the transcriptional level

In this section, we have considered the regulation of CNS voltage-gated sodium channels by transcription factors, and by alternative splicing. The regulation of sodium channels at the post-transcriptional level (e.g., by microRNAs) is out of the scope of the present review.

Regulation by transcription factors

Promoter regions of brain voltage-gated sodium channel genes have been described, including SCN1A (Dong et al. 2014; Long et al. 2008); SCN2A (Lu et al. 1998; Schade and Brown 2000), SCN3A (Martin et al. 2007), and SCN8A (Drews et al. 2005, 2007). Based on the sequence analyses and databases, several transcription factors have been proposed to control brain sodium channel expression (Long et al. 2008). Experimentally, a recent study has shown that SCN3A expression is regulated by promoter CpG methylation and Methyl-CpG-binding domain protein 2 (MBD2), (Li et al. 2015). MBD2 targets methylated CpG for demethylation, possibly leading to activated transcription. Consistently, knock-down of MBD2 decreased SCN3A mRNA levels in a neuroblastoma cell line. In seizure-induced mice, MBD2 expression was increased, which correlated with decreased CpG methylation, and enhanced SCN3A expression (Li et al. 2015).

Another recent development has been the identification of receptor for activated C kinase 1 (RACK1) as a repressor of SCN1A expression (Dong et al. 2014). The authors identified a transcriptional silencer in a region between +53 and +62 bp downstream of SCN1A promoter and used EMSA assays to uncover possible transcriptional regulators. RACK1 was found to bind to the silencer in NT2 cells (a pluripotent embryonal carcinoma cell line often used for differentiation into neurons). Knocking-down RACK1 in

NT2 cells markedly increased SCN1A mRNA levels (Dong et al. 2014).

Sodium channel macromolecular complexes may incorporate proteins classically known as voltage-gated sodium channel β subunits. These include five different proteins termed $\beta 1$, $\beta 1b$, $\beta 2$, $\beta 3$, and $\beta 4$. Many groups have studied the effect of β subunits on α subunit trafficking and electrophysiology, mainly from the point of view of protein–protein interactions (for a recent review, see Namadurai et al. 2015).

Additionally, sodium channel β subunits have been proposed to regulate α subunits at the transcriptional level. One of the first experimental observations was the increase in $Na_v1.1$ mRNA and protein levels in the presence of proteases targeting the $\beta 2$ subunit. The group of Kovacs, and others, has demonstrated the sequential mechanism by which, first, ADAM10 and BACE1 proteases cleave off the extracellular domain of the $\beta 2$ subunit. Second, γ -secretase releases the $\beta 2$ intracellular domain. And third, the $\beta 2$ intracellular domain induces an increase in $Na_v1.1$ mRNA and protein levels (Kim et al. 2005, 2007; Wong et al. 2005), although the precise pathways for $\beta 2$ internalisation into the cell nucleus remain unknown. BACE1-dependent sodium channel expression seems to be specific for $Na_v1.1$, and mRNA levels of other brain Na_v isoforms including $Na_v1.2$, $Na_v1.3$ and $Na_v1.6$ are relatively insensitive to BACE1 protease activity (Kim et al. 2007, 2011).

Likewise $\beta 2$, the $\beta 1$ subunit has been shown to regulate Na_v expression, and mouse models show changes in brain Na_v expression and localization upon $\beta 1$ deletion (Chen et al. 2004). In a recent development, $\beta 1$ subunit silencing has been shown to result in decreased $Na_v1.1$, $Na_v1.3$ and $Na_v1.6$ (but not $Na_v1.2$) mRNA and protein levels in cells models (Baroni et al. 2014), although the mechanism underlying this regulation was not investigated. Although $\beta 1$ subunit is a target for BACE1 *in vitro*, the question remains whether this is physiologically relevant (Wong et al. 2005).

Regulation by alternative splicing

The first evidences for alternative splicing of brain sodium channels were reported more than 20 years ago (Sarao et al. 1991; Gustafson et al. 1993), and splicing mechanisms are thought to be common to most brain Na_v isoforms (Copley 2004). In particular, SCN1A alternative splicing has been extensively studied due to its relevance in CNS disorders such as epilepsy (Lossin 2009; Schlachter et al. 2009; Le Gal et al. 2011; Thompson et al. 2011).

The best studied SCN1A splicing variants are often referred to as the adult and neonatal forms, although both forms are expressed in adults. They result from the mutually exclusive expression of either exon 5A (adult) or 5N

(neonatal). Common SCN1A polymorphisms can have a massive effect on the expression of the 5N variant in normal adults (Tate et al. 2005; Heinzen et al. 2007). The 5A/5N splicing event can also be modulated by splice-modifier proteins, including sodium channel modifier 1 (SCNM1). Very recently, a mutation in SCNM1 has been linked to epilepsies possibly via regulation of SCN1A splicing leading to reduction of the variant containing exon 5N (Kasteleijn-Nolst Trenité et al. 2015). SCNM1, as well as other splicing regulators such as Rbfox2, can also modulate SCN8A splicing (Buchner et al. 2003; Gehman et al. 2012).

Regulation of brain sodium channels at the post-translational level

From biochemical assays in vitro to targeted purification of proteins from tissues, research in sodium channel PTMs has recently expanded from (immuno) chemical methods to embrace mass spectrometry and proteomics. Here, we review our current understanding of some of the best known sodium channel PTMs. As before, we have included Na_v1.1, Na_v1.2, Na_v1.3, and Na_v1.6. Where relevant, Na_v1.5 has also been considered because of the wealth of available Na_v1.5 PTM data. In particular, Na_v1.5 phosphorylation, ubiquitylation, and arginine methylation have been studied in detail (“Phosphorylation”, “Ubiquitylation” and “Arginine methylation”, respectively).

Previously in “Regulation by transcription factors”, we have reviewed our knowledge of sodium channel β subunit processing by proteases, leading to transcriptional regulation of α subunits. In “Regulation of brain sodium channels by proteases”, we have included available data on direct proteolysis of α subunits. Although proteolysis is in most cases associated with degradation, it can also be regarded as PTM if it is limited and specific (Rogers and Overall 2013).

Phosphorylation

Phosphorylation is the most experimentally observed PTM at the proteome-wide level, and it is certainly thought to be the most abundant PTM along with *N*-glycosylation (Khoury et al. 2011). Sodium channels are no exception to the rule and phosphorylation is the most studied and observed sodium channel PTM.

Identified phosphorylation sites

The aim of this subsection is to comprehensively collect and update the repertoire of sodium channel ‘phosphorylatable’ sites (Table 1). These include phosphosites identified

by the use of in vitro assays and heterologous expression experiments, as well as those identified in sodium channels isolated from native sources. Data in Table 1 are taken from classical papers (Berendt et al. 2010), and previous reviews (Cerda et al. 2011; Baek et al. 2011), and updated to include recent original articles that described novel phosphosites (Marionneau et al. 2012; Baek et al. 2014; Herren et al. 2015). Functional consequences of Na_v phosphorylation are discussed below.

Is it safe to assume that if a Na_v isoform is phosphorylated at a certain residue, then our favourite isoform will also be, provided the site is conserved? The general answer is No. Visual analysis of phosphosite conservation in Table 1 leaves little room for hope, at least according to the data currently available. The exception is the interdomain linker between domains I and II, which is considered the PTM hot-spot (Cantrell and Catterall 2001), and where one can find phosphosites conserved among 3, sometimes 4, of the considered Na_v isoforms. Nevertheless, due to the substoichiometric and labile nature of phosphorylation, the failure to detect a protein modification does not imply that a residue is not phosphorylated. Perhaps future comprehensive proteomic studies will demonstrate higher degree in phosphosite conservation among Na_v isoforms.

Specificity of the functional effect of phosphorylation among Na_v isoforms

Most of the phosphosites included in Table 1 were identified by proteomics and mass spectrometry methods, and we currently lack information on which protein kinase may catalyse phosphorylation of many of the Na_v phosphosites. Nevertheless, it has long been known that protein kinase C (PKC) and cAMP-dependent kinase (PKA) can phosphorylate brain Na_v channels (West et al. 1991; Numann et al. 1991; Li et al. 1992, 1993). Other kinases involved in regulating brain Na_v phosphorylation are glycogen synthase kinase 3 (GSK3) (James et al. 2015), protein kinase CK2 (Hien et al. 2014), A kinase-anchoring protein 15 (Few et al. 2007), Fyn tyrosine kinase (Beacham et al. 2007), and p38 mitogen-kinase activated protein kinase (Wittmack et al. 2005).

The functional effects of channel phosphorylation on Na_v electrophysiology often depend on the specific isoform of interest. For instance, phosphorylation by PKA and PKC results in attenuation of Na_v1.2 currents due to defective channel trafficking to the cell surface (Li et al. 1992). But Na_v1.6 channels are relatively insensitive to PKA/PKC regulation (Chen et al. 2008), and Na_v1.5 currents are enhanced by PKA activation due to increased Na_v1.5 expression at the cell surface (Hallaq et al. 2006). Subtle variations in the primary sequence of Na_v isoforms must underlie such differences. For instance, it is thought that

Table 1 Phosphosites of different Na_v isoforms

MEQTVLVPPG	PDSFNFFTRE	SLAAIERRIA	E EKAKNPKPD	----KKDDDE	46	Na _v 1.1	mouse	(N terminus)
MARSVLVPPG	PDSFRFFTRE	SLAAIEQRIA	E EKAKRPKQE	---RKDEDDE	47	Na _v 1.2	rat	(N terminus)
MAQALLVPPG	PESFRLFTRE	SLAAIEKRAA	E EKAKPKKE	----QDNDDE	46	Na _v 1.3	human	(N terminus)
M-ANFLLPRG	TSSFRFRFTRE	SLAAIEKRMA	E KQARGSTTL	QESREGLPEE	49	Na _v 1.5	human	(N terminus)
MAARVLAPPG	PDSFKPFTPE	SLANIERRIA	E SKLKKPKKA	DGSHREDDDE	50	Na _v 1.6	mouse	(N terminus)
NGPKPNSDLE	AGKNLPFIYG	DIPPEMVSEP	LEDLDPYYIN	KKTFIVLNKG	96	Na _v 1.1	mouse	(N terminus)
NGPKPNSDLE	AGKSLPFIYG	DIPPEMVSEP	LEDLDPYYIN	KKTFIVLNKG	97	Na _v 1.2	rat	(N terminus)
NKPKPNSDLE	AGKNLPFIYG	DIPPEMVSEP	LEDLDPYYIN	KKTFIVMNKG	96	Na _v 1.3	human	(N terminus)
EAPRPQLDLQ	ASKKLPDLYG	NPPQELIGEP	LEDLDPFYST	QKTFIVLNKG	99	Na _v 1.5	human	(N terminus)
SKPKPNSDLE	AGKSLPFIYG	DIPQGLVAVP	LEDFDPYYLT	QKTFVVLNRG	100	Na _v 1.6	mouse	(N terminus)
KAIRFRSATS	ALYILTPFNP	LRKIAIKILV	HSLFSLMLIC	TILTNCVFMT	146	Na _v 1.1	mouse	(N terminus)
KAIRFRSATS	ALYILTPFNP	IRKLAIKILV	HSLFVNLIC	TILTNCVFMT	147	Na _v 1.2	rat	(N terminus)
KAIRFRSATS	ALYILTPFNP	VRKIAIKILV	HSLFSLMLIC	TILTNCVFMT	146	Na _v 1.3	human	(N terminus)
KTIFRFSATN	ALYVLSPFHP	IRRAAVKILV	HSLFNLMLIC	TILTNCVFMA	149	Na _v 1.5	human	(N terminus)
KTLFRFSATP	ALYILSPFNL	IRRIAIIKILI	HSVFSMIIC	TILTNCVFMT	150	Na _v 1.6	mouse	(N terminus)
EFQQMLEQLK	KQEEAAQQA	ATTASE----	-HSRE--PS	AAGRLSDSSS	484	Na _v 1.1	mouse	(Linker DI-DII)
EFQQMLEQLK	KQEEAQ-AA	AAAAA----	-ESRDFSGAG	GIGVFSSESS	488	Na _v 1.2	rat	(Linker DI-DII)
EFQQMLEQLK	KQEEAQ--A	VAAASA----	-ASRDFSGIG	GLGELLESSS	486	Na _v 1.3	human	(Linker DI-DII)
RFQEAEMMLK	KEHEALT---	-----	-----IR	GVDTVSRSSL	461	Na _v 1.5	human	(Linker DI-DII)
EFKAMLEQLK	KQEEAQ-AA	AMATSAGTVS	EDAIEEEDG	GVGS-PRSSS	478	Na _v 1.6	mouse	(Linker DI-DII)
EASKLSSKSA	KERRNRRKKR	KQKEQSGGEE	K-DDDEFHKS	ESEDSIRRKG	533	Na _v 1.1	mouse	(Linker DI-DII)
VASKLSSKSE	KELKNRRKKK	KQKEQAGEEE	K--EAVRKS	ASEDSIRKKG	536	Na _v 1.2	rat	(Linker DI-DII)
EASKLSSKSA	KEWRNRRKKR	RQREHLEGN	KGERDSFPKS	ESEDSVKKRS	536	Na _v 1.3	human	(Linker DI-DII)
EMSPPLAVPNS	HERRSKRRKR	---MSSGTE	ECGEDRLPKS	DSEDPGR---	504	Na _v 1.5	human	(Linker DI-DII)
ELSKLSSKSA	KERRNRRKKR	KQKELSEGEE	KGDPEKVFKS	ESEDMRRKA	528	Na _v 1.6	mouse	(Linker DI-DII)
FRFSIEGNRL	TYEKRYSSPH	QSLLSIRGSL	FSPRRNSRTS	LF SFR--GRA	581	Na _v 1.1	mouse	(Linker DI-DII)
FQFSLEGSRL	TYEKRFSSPH	QSLLSIRGSL	FSPRRNSRAS	LFNFK--GRV	584	Na _v 1.2	rat	(Linker DI-DII)
FLFSMDGNRL	TSDKKFCSPH	QSLLSIRGSL	FSPRRNSKTS	LF SFR--GRA	584	Na _v 1.3	human	(Linker DI-DII)
-----	-----AMNH	LSLTRGLSRT	SMKPRSSRGS	IFTFR--R--	534	Na _v 1.5	human	(Linker DI-DII)
FR--LPDNRI	G--RKFSIMN	QSLLSIPGSP	FLSRHNSKSS	IFSFRGPGRF	574	Na _v 1.6	mouse	(Linker DI-DII)
KDVGSENDFA	DDEHSTFEDN	ESRRDSLFPV	RRHGERRN--	---SNLSQTS	626	Na _v 1.1	mouse	(Linker DI-DII)
KDIGSENDFA	DDEHSTFEDN	DSRRDSLFPV	HRHGERRP--	---SNVQAS	629	Na _v 1.2	rat	(Linker DI-DII)
KDVGSENDFA	DDEHSTFEDS	ESRRDSLFPV	HRHGERRN--	---SNVQAS	629	Na _v 1.3	human	(Linker DI-DII)
RDLGSEADFA	DDENSTAGES	ESHHTSLLVP	--WPLRR--	---SAQGQPS	577	Na _v 1.5	human	(Linker DI-DII)
RDPGSENEFA	DDEHSTVEES	EGRRDSLFPV	IRARERRSSY	SGYSGYSQCS	624	Na _v 1.6	mouse	(Linker DI-DII)
RSSRMLAVFP	ANGKMHSTVD	CNGVVSLVG-	GSPVPTSPVG	QLLPEVIIDK	675	Na _v 1.1	mouse	(Linker DI-DII)
RASRGITPLP	MNGKMHSAVD	CNGVVSLVG-	GPSALTSVPV	QLLPE-----	673	Na _v 1.2	rat	(Linker DI-DII)
MSSRMVPLP	ANGKMHSTVD	CNGVVSLVG-	GPSALTSPTG	QLPPE-----	673	Na _v 1.3	human	(Linker DI-DII)
PGTSA-PGHA	LHGKKNSTVD	CNGVVSLLGA	GDPEATSPGS	HLLRPVMLEH	626	Na _v 1.5	human	(Linker DI-DII)
RSSRIFPPLR	RSVKNRSTVD	CNGVVSLIG-	----PGSHIG	RLLPE-----	664	Na _v 1.6	mouse	(Linker DI-DII)
PATDDNGTTT	EEMRKRSS	SFHVSMDFLE	DPSQRQRAMS	IASILNTV-	724	Na _v 1.1	mouse	(Linker DI-DII)
-----GTTT	EETIRKRSS	SYHVSMDLLE	DP-SRQRAMS	MASILNTM-	715	Na _v 1.2	rat	(Linker DI-DII)
-----GTTT	EETVRKRRLS	SYQISMEMLE	DSSGRQRAVS	IASILNTM-	716	Na _v 1.3	human	(Linker DI-DII)
PP--DTTTPS	EEPGGPQMLT	SQAPCVDFGE	EPGARQRALS	AVSVLTSAL-	673	Na _v 1.5	human	(Linker DI-DII)
-----ATT	EVEIKKKGPG	SLLVSMEQLA	SYGRKDRINS	IMSVVTNTLV	707	Na _v 1.6	mouse	(Linker DI-DII)
EELEESRQKC	PWCYKFSNI	FLIWDCSPYW	LKVKHIVNLV	VMPDFVDLAI	774	Na _v 1.1	mouse	(Linker DI-DII)
EELEESRQKC	PWCYKFSNI	FLIWDCSPYW	LKVKHIVNLV	VMPDFVDLAI	765	Na _v 1.2	rat	(Linker DI-DII)
EELEESRQKC	PWCYKFSNI	FLIWDCSPYW	LKVKHIVNLV	VMPDFVDLAI	766	Na _v 1.3	human	(Linker DI-DII)
EELEESRQKC	PWCYKFSNI	FLIWDCSPYW	LKVKHIVNLV	VMPDFVDLAI	723	Na _v 1.5	human	(Linker DI-DII)
EELEESRQKC	PWCYKFSNI	FLIWDCSPYW	LKVKHIVNLV	VMPDFVDLAI	757	Na _v 1.6	mouse	(Linker DI-DII)

Table 1 continued

YVKRKIYEFI	QQSFVKKQKI	LDEIKPLDDL	NNRKDNCSN	HT----TEIG	1070	Na _v 1.1	mouse	(Linker DII-DIII)
FVKRKIREFI	QKAFVRKQKA	LDEIKPLEDL	NNKKD SCI SN	HTT---IEIG	1062	Na _v 1.2	rat	(Linker DII-DIII)
YVKNKMRFCF	QKAFFRKPKV	LEIHE-----	GNKIDSCMSN	NTG---IEIS	1058	Na _v 1.3	human	(Linker DII-DIII)
FVKRTTWDFC	CGLLRQRFPQK	PAALAAQQQL	----P SCI AT	PYSPPPPETE	1017	Na _v 1.5	human	(Linker DII-DIII)
WAKVKVHAFM	QAHF--KQRE	ADEVKPLDEL	YEKKANCIAN	HTG---VDIH	1052	Na _v 1.6	mouse	(Linker DII-DIII)
KDLCLKDV-	--NGTTS G IG	TGSSVEKYII	DESDYMSFIN	NPSLTVTVPI	1117	Na _v 1.1	mouse	(Linker DII-DIII)
KDLNLYKDG-	--NGTTS G I-	-GSSVEKYVV	DESDYMSFIN	NPSLTVTVPI	1107	Na _v 1.2	rat	(Linker DII-DIII)
KELNLYLRDG-	--NGTTS G VG	TGSSVEKYVI	DENDYMSFIN	NPSLTVTVPI	1105	Na _v 1.3	human	(Linker DII-DIII)
KVPPTRKETR	FEEGEQPGQG	TPGDPE----	-----	-----PVCVPI	1049	Na _v 1.5	human	(Linker DII-DIII)
RNGDFQKNG-	--NGTTS G I-	-GSSVEKYII	DE-DH M SFIN	NPNTLTVRPI	1096	Na _v 1.6	mouse	(Linker DII-DIII)
AVGE S DFENL	NTEDFS S ESD	LEESKEKLE	-----	-----	1147	Na _v 1.1	mouse	(Linker DII-DIII)
ALGESDFENL	NTEEFSS S ESD	MEESKEKLN-	-----	-----	1136	Na _v 1.2	rat	(Linker DII-DIII)
AVGESDFENL	NTEEFSS S ESE	LEESKEKLN-	-----	-----	1134	Na _v 1.3	human	(Linker DII-DIII)
AVAESD T DDQ	EEDENS L GT	EEESSK Q QES	QPVSGG P EAP	PDSRT W SQVS	1099	Na _v 1.5	human	(Linker DII-DIII)
AVGESDFENL	NTEDV S ESD	PEGSKDKLD-	-----	-----	1125	Na _v 1.6	mouse	(Linker DII-DIII)
LYFVIFIIIFG	SFFTLN L FIG	VIIDN F NQOK	KKFGG Q DIFM	TEE Q KKY N A	1510	Na _v 1.1	mouse	(Linker DIII-DIV)
LYFVIFIIIFG	SFFTLN L FIG	VIIDN F NQOK	KKFGG Q DIFM	TEE Q KK Y NA	1500	Na _v 1.2	rat	(Linker DIII-DIV)
LYFVIFIIIFG	SFFTLN L FIG	VIIDN F NQOK	KKFGG Q DIFM	TEE Q KKY N A	1495	Na _v 1.3	human	(Linker DIII-DIV)
IYFVIFIIIFG	SFFTLN L FIG	VIIDN F NQOK	KKLGG Q DIFM	TEE Q KKY N A	1497	Na _v 1.5	human	(Linker DIII-DIV)
IYFVIFIIIFG	SFFTLN L FIG	VIIDN F NQOK	KKFGG Q DIFM	TEE Q KKY N A	1489	Na _v 1.6	mouse	(Linker DIII-DIV)
MKKLG S KKPQ	KPIPR P GNKF	QGMV F DFVTR	QVFDIS I MIL	ICLN M V T MMV	1560	Na _v 1.1	mouse	(Linker DIII-DIV)
MKKLG S KKPQ	KPIPR P ANKF	QGMV F DFVTK	QVFDIS I MIL	ICLN M V T MMV	1550	Na _v 1.2	rat	(Linker DIII-DIV)
MKKLG S KKPQ	KPIPR P ANKF	QGMV F DFVTR	QVFDIS I MIL	ICLN M V T MMV	1545	Na _v 1.3	human	(Linker DIII-DIV)
MKKLG S KKPQ	KPIPR P L N KY	QGFI F DI V TK	QAFD V T I MFL	ICLN M V T MMV	1547	Na _v 1.5	human	(Linker DIII-DIV)
MKKLG S KKPQ	KPIPR P L N KI	QGIV F DF V TQ	QAFD I V I MML	ICLN M V T MMV	1539	Na _v 1.6	mouse	(Linker DIII-DIV)
RIHCLDILFA	F T K R VL G ESG	EMD A L R I Q ME	ER F MA S NP S K	V S Y Q P I T T T L	1910	Na _v 1.1	mouse	(C terminus)
RIHCLDILFA	F T K R VL G ESG	EMD A L R I Q ME	ER F MA S NP S K	V S Y E P I T T T L	1900	Na _v 1.2	rat	(C terminus)
RIHCLDILFA	F T K R VL G ESG	EMD A L R I Q ME	DR F MA S NP S K	V S Y E P I T T T L	1895	Na _v 1.3	human	(C terminus)
RIHCMDILFA	F T K R VL G ES G	EMD A L K I Q ME	E K F M A A NP S K	I S Y E P I T T T L	1896	Na _v 1.5	human	(C terminus)
RIHCLDILFA	F T K R VL G DSG	ELD I L R Q Q ME	ER F V A SNP S K	V S Y E P I T T T L	1888	Na _v 1.6	mouse	(C terminus)
KRKQEEVSAV	I I Q R A Y RRHL	L K R T V K Q A S F	T Y N K N K L-KG	--G A N L L V KE	1957	Na _v 1.1	mouse	(C terminus)
KRKQEEVSAI	V I Q R A Y RRYL	L K Q K V K K V S S	I Y K K D K G-KE	--D E G T PIKE	1947	Na _v 1.2	rat	(C terminus)
KRKQEEVSAI	V I Q R N F R C YL	L K Q R L K N I S S	N Y N K E A I-KG	--R I D L PIKQ	1942	Na _v 1.3	human	(C terminus)
RRKHEEVSAV	V I Q R A F RRHL	L Q R S L K H A S F	L F R Q Q A G- S G	L S E E D A P E R E	1945	Na _v 1.5	human	(C terminus)
RRKQEEVSAV	V L Q R A Y R G H L	A R R G F-----	-----	-----I C R	1916	Na _v 1.6	mouse	(C terminus)
DMLIDRI-NE	N----S I TEK	T D L T M S T A AC	P S S Y D R V T K P	I V E K H E ---Q	1999	Na _v 1.1	mouse	(C terminus)
DIITDKL-NE	N----S T PEK	T D V T P S T T S-	P S S Y D S V T K P	E E K E F E ---K	1988	Na _v 1.2	rat	(C terminus)
DMIIDKL-NG	N----S T PEK	T D G S S T S-	P S S Y D S V T K P	D E K E F E ---K	1983	Na _v 1.3	human	(C terminus)
GLIAYVM-SE	N F S R P L G P P S	S S S I S T S F -	P S S Y D S V T R A	T S D N L Q V R G S	1993	Na _v 1.5	human	(C terminus)
KITSNKLENG	G----T H R E K	K E S T P S T A S-	L P S Y D S V T K P	D E K E Q Q R A E E	1961	Na _v 1.6	mouse	(C terminus)
EGKDEKAKGK	-----	---			2009	Na _v 1.1	mouse	(C terminus)
DKSEKEDKGG	-----	D I R E SKK			2005	Na _v 1.2	rat	(C terminus)
DKPEKESKGG	-----	E V R E N Q K			2000	Na _v 1.3	human	(C terminus)
DYSH S EDLAD	F P P S PD R D R E	S I V			2016	Na _v 1.5	human	(C terminus)
GRRERAKRQK	-----	E V R E SKK			1978	Na _v 1.6	mouse	(C terminus)

Phosphorylated residues are shown bold and shadowed

Na_v1.5 phosphorylation by PKA at S528 masks an endoplasmic reticulum retention signal (RRR₅₃₅), thereby promoting Na_v1.5 trafficking to the membrane (Zhou et al. 2002). This endoplasmic reticulum signal is absent in Na_v1.2 (RVK₅₈₅) and modified in Na_v1.6 (RFR₅₇₅).

Opposite functional effects of post-translational modifications on distinct Na_v isoforms have also been observed after phosphorylation by Fyn kinase. Fyn kinase phosphorylates essential tyrosine residues within the inactivation gate of sodium channels, including the equivalent Y1498

(Na_v1.2) and Y1495 (Na_v1.5). Yet, the functional effect of phosphorylation by Fyn on channel inactivation is a negative (Na_v1.2) or positive (Na_v1.5) shift in the voltage dependence of inactivation (Beacham et al. 2007; Ahern et al. 2005). The simplest explanation is that Fyn phosphorylates other Tyr residues within Na_v1.2 and Na_v1.5 sequences, and this has indeed been demonstrated for Na_v1.2 (including Y66, Y1497, and Y1893), (Beacham et al. 2007). Nevertheless, recent work has reported that distinct splicing variants of the same Na_v isoform show different electrophysiological behaviour upon phosphorylation by Fyn, which introduces another level of complexity (Iqbal et al. 2015).

Ubiquitylation

Protein ubiquitylation (or ubiquitination) is a post-translational modification that involves the orchestrated function of three types of enzymes. First, ubiquitin activating enzyme (E1) catalyses thioester formation between the C terminus of ubiquitin and an internal cysteine. Second, activated ubiquitin is transferred to the ubiquitin conjugating enzyme (E2). Third, ubiquitylation of the substrate protein is catalysed by ubiquitin ligases (E3), which covalently attach ubiquitin molecules to lysine residues within the target sequence. Ubiquitylation is often associated with protein degradation.

There are hundreds of E3 ubiquitin ligases, usually classified into two groups: HECT (homologous to E6-AP C terminus) ligases, and RING (really interesting new gene) ligases (Goel et al. 2015). Until 2015, it was thought that only HECT ligases could catalyse sodium channel ubiquitylation (see below).

The most studied molecular mechanism for sodium channel ubiquitylation involves channel recognition by Nedd4-2 ubiquitin ligases (HECT-type ligases) via protein–protein interaction between the WW4 domain of Nedd4-2, and the PY motif of neuronal and cardiac sodium channels (Fotia et al. 2004; van Bemmelen et al. 2004). Ubiquitylation by Nedd4-2 has been shown to tag sodium channels for internalisation from the cell surface, including Na_v1.2 (Fotia et al. 2004), Na_v1.6 (Gasser et al. 2010), and Na_v1.5 (Rougier et al. 2005). However, in most cases, the precise modification site(s), i.e., the Lys residues that are ubiquitylated, remain to be confirmed.

Very recently, compelling evidence has been presented that shows ubiquitylation of sodium channels in zebra fish CNS by RNF121, a member of the RING family of E3 ubiquitin ligases (Ogino et al. 2015). From the initial observation that zebra fish bearing mutations in RNF121 present defective Na_v trafficking in neurons and skeletal muscle, the investigators moved on to perform heterologous expression of Na_v1.6 and RNF121 in HEK 293T cells. Results

showed increased Na_v1.6 degradation upon co-expression of RNF121 but, intriguingly, enhanced Na_v1.6 membrane localization when co-expressed with RNF121 *and* auxiliary Na_v β subunits (Ogino et al. 2015).

Arginine methylation

Arginine methylation consists on the addition of methyl groups to arginine residues of proteins. Arginine methylation is catalysed by protein arginine methyl transferases (PRMTs) that transfer a methyl group from *S*-adenosyl-*L*-methionine (SAM) to the target arginine. Arginine methylation has recently been reported as a novel post-translational modification of the voltage-gated sodium channel family using Na_v1.5 as a model system (Beltran-Alvarez et al. 2011).

The groups of Comb and Trimmer have described arginine methylation of brain sodium channels. Using a proteomic approach and bespoke antibodies that recognise peptides bearing methylated arginine, the group of Comb reported arginine methylation of Na_v1.1, Na_v1.2 and Na_v1.5 in the mouse brain (Guo et al. 2014). In parallel, the group of Trimmer described arginine methylation of Na_v1.2 purified from rat brain (Baek et al. 2014). We analysed the methylation sites reported by the three referenced articles (Beltran-Alvarez et al. 2011; Guo et al. 2014; Baek et al. 2014), and found that three sites have been observed by at least two independent studies (Table 2).

The functional consequences of sodium channel modification by arginine methylation have been documented. Available electrophysiological data are consistent with an increase in sodium current density, most likely due to enhanced Na_v membrane expression (Beltran-Alvarez et al. 2013; Baek et al. 2014). Additionally, the group of Trimmer reported considerable acceleration in Na_v1.2 recovery from inactivation when arginine methylation was enhanced (Baek et al. 2014). Remarkably, arginine methylation is an example of PTM conservation among Na_v isoforms, even if catalysed by different enzymes: Na_v1.2 is methylated by PRMT8 (mostly expressed in the CNS), while Na_v1.5 methylation is catalysed by PRMT3 and -5 (ubiquitously expressed).

Other known post-translational modifications

We would like to mention that sodium channels have long been known to undergo cysteine modifications including *S*-palmitoylation (Schmidt and Catterall 1987; Bosmans et al. 2011), and *S*-nitrosylation (Renganathan et al. 2002). Methionine oxidation of sodium channels has previously been reviewed (Cui et al. 2012). SUMOylation of the Na_v1.7 isoform has been described, but available data suggest that SUMOylation may not be conserved in CNS Na_v isoforms (Dustrude et al. 2013).

Table 2 Hot spots of arginine methylation sites

Methylated residue (rat $\text{Na}_v1.2$ numbering)	Isoform where methylation was observed (isoform numbering)	Species and tissue	References	Notes
R563	$\text{Na}_v1.1$ (560) $\text{Na}_v1.2$ (563) $\text{Na}_v1.2$ (563)	Mouse, brain Mouse, brain Rat, brain	Guo et al. 2014 Guo et al. 2014 Baek et al. 2014	Observed in human $\text{Na}_v1.5$ (R513) expressed in HEK 293 cells (Beltran-Alvarez et al. 2011). $\text{Na}_v1.5$ peptide containing R513 is methylated in vitro by PRMT3 (Beltran-Alvarez et al. 2015).
R570	$\text{Na}_v1.2$ (570) $\text{Na}_v1.2$ (570)	Mouse, brain Rat, brain	Guo et al. 2014 Baek et al. 2014	
R574	$\text{Na}_v1.5$ (526) $\text{Na}_v1.5$ (526)	Mouse, brain Heart, human	Guo et al. 2014 Beltran-Alvarez et al. 2014	Observed in human $\text{Na}_v1.5$ (R526) expressed in HEK 293T cells (Beltran-Alvarez et al. 2011).

Methylation sites reported by at least two independent studies

Another well-known PTM, *N*-glycosylation, has been mostly studied in the cardiac isoform of the sodium channel, and several excellent reviews have recently been published (Baycin-Hizal et al. 2014; Marionneau and Abriel 2015). Perhaps the latest studies are those from the Chatelier and the Decosterd–Abriel groups, which have proposed alternative trafficking pathways for differentially glycosylated Na_v , using $\text{Na}_v1.5$ and $\text{Na}_v1.7$ as study models (Mercier et al. 2015; Laedermann et al. 2013, respectively).

Other possible post-translational modifications?

The advent of large-scale proteomics including the publication of human proteome maps is revolutionising life sciences. The ion channel field can also benefit from the analysis of big data to anticipate and identify challenges and opportunities, particularly in the field of PTMs. With this in mind, we searched Phosphositeplus (Hornbeck et al. 2015) for PTMs of Na_v isoforms. The database contains potentially novel sodium channel modifications including Lys acetylation, which is reported for $\text{Na}_v1.1$, $\text{Na}_v1.2$, $\text{Na}_v1.3$, $\text{Na}_v1.5$ and $\text{Na}_v1.6$, and Lys methylation, which is included for $\text{Na}_v1.2$ and $\text{Na}_v1.6$.

Although promising at first sight, available data must be regarded with care. Conservation of the reported post-translationally acetylated or methylated Lys site among Na_v isoforms was very low. The finding worth mentioning was interspecies conservation of $\text{Na}_v1.1$ acetylation at K1948 in human and mouse samples. Although K1948 acetylation was observed in unrelated experiments, it must be noted that the source of tissue was not brain but colon cancer.

Cross-talk between sodium channel PTMs

Cross-talk, or interplay, between PTMs includes the regulatory mechanisms by which PTMs work together to determine protein function. Cross-talk between sodium channel phosphorylation, and arginine methylation, has been reported. The group of Trimmer reported cross-talk between $\text{Na}_v1.2$ arginine methylation and phosphorylation (Baek et al. 2014). In this study, the authors studied $\text{Na}_v1.2$ PTMs in the rat brain. $\text{Na}_v1.2$ was immunopurified, digested and subjected to mass spectrometry analysis. An initial observation was that detected $\text{Na}_v1.2$ peptides harboured either arginine methylation or phosphorylation, but not both PTMs on the same peptide. Convincingly, these two PTMs were reciprocally regulated in response to acute seizure: e.g., R563 methylation (see also Table 2) increased but S554 and S568 phosphorylation decreased after induction of seizure in rats (Baek et al. 2014). The most likely mechanism for this interplay between sodium channel arginine methylation and phosphorylation is the modification of kinetic specificity constants of serine phosphorylation

upon methylation of a neighbouring arginine, and viceversa (Beltran-Alvarez et al. 2015). Nevertheless, the functional consequences of phosphorylation—arginine methylation cross-talk remain to be elucidated.

Additionally, cross-regulation between $\text{Na}_v1.6$ phosphorylation, and ubiquitylation, has been observed. On the one hand, $\text{Na}_v1.6$ is phosphorylated by p38 MAPK at position S553. On the other, $\text{Na}_v1.6$ is ubiquitylated by Nedd4-2 after recognition of the PY motif (Pro-Ser-Tyr) at the C terminus of the channel. Results from the group of Dib-Hajj suggested that S553 phosphorylation enables further $\text{Na}_v1.6$ ubiquitylation and internalisation of the channel (Gasser et al. 2010). A similar mechanism has recently been proposed for $\text{Na}_v1.2$ whereby phosphorylation of T1966 by GSK3 primes recognition by Nedd4-2 via the $\text{Na}_v1.2$ PY motif (PPSY₁₉₇₅), (James et al. 2015).

Regulation of brain sodium channels by proteases

Voltage-gated sodium channel density has long been known to be regulated by proteases under normal (Paillart et al. 1996) and stress conditions (Iwata et al. 2004). Among the most important proteases in mammalian cells stand the calpains, which target hundreds of proteins (Grimm et al. 2012). The group of Meany has revealed the bases of calpain-dependent proteolysis of $\text{Na}_v1.2$.

Using rat brain homogenates, they showed that calpain cleaves $\text{Na}_v1.2$ (but not $\text{Na}_v1.1$) at two sites, i.e., the inter-domain linkers between domains I and II, and between domains II and III (von Reyn et al. 2009). Intriguingly, most of the calpain sodium channel fragment products localise at the plasma membrane 6 h after calpain activation, and possibly interact (von Reyn et al. 2009). Perhaps the simplest explanation is that distinct sodium fragments still retain the protein–protein interactions that hold the sodium channel macromolecular complex together, and thus control the break-down of the complex. A more thought-provoking alternative is that sodium channel post-translational proteolysis creates new proteins with modified biological activities.

The group of Meany has dissected the mechanisms of $\text{Na}_v1.2$ proteolysis in cellular and mouse models of neuronal injury (von Reyn et al. 2012; Schoch et al. 2013), opening opportunities for treatment and therapy of traumatic brain injury. In this line, other researchers have recently described the beneficial effect of calpain inhibitors on brain sodium channel expression and electrophysiology in a model of diabetic neuropathy (Kharatmal et al. 2015).

The other example of sodium channel processing by proteases is the excision of the initiation methionine by aminopeptidases. This has been shown for $\text{Na}_v1.5$ (followed by N-terminal acetylation of the resulting initiation alanine) in cardiac disease (Beltran-Alvarez et al. 2014).

Whether $\text{Na}_v1.5$ or other Na_v isoforms are devoid of Met residues (or post-translationally acetylated) in normal tissue is unknown.

Conclusions and perspective

Research in the voltage-gated sodium channel field has grown linearly for the last 20 years. While the interest in transcriptional mechanisms regulating sodium channel expression has also grown steadily, we have observed an exponential trend in the number of publications related to sodium channel post-translational regulation. We predict that this growth will keep pace over the coming years. The aim of this review was to provide the current state of the art of the transcriptional and post-translational regulation of sodium channels, and thus set the ground for further research opportunities and discoveries.

Our understanding of transcriptional mechanisms governing brain sodium channel expression is far from comprehensive, and the ongoing research efforts of the ENCODE Consortium will surely encourage groups around the globe to dissect the molecular mechanism controlling Na_v transcription. Analogously, there are new questions in the field of PTM of sodium channels, in particular related to cross-talk among co-occurring types of PTM. As an example, the functional consequences of the interplay between phosphorylation and arginine methylation are intriguing, because the latter is thought to be a rather stable PTM (Bedford and Clarke 2009). The dynamic sequence of PTM events, thus, acquires vital relevance. Our incomplete understanding of proteolysis and degradation pathways of sodium channels also warrants further research in the area.

From the point of view of cell biology, biochemistry and electrophysiology, we predict that major advances in our understanding of Na_v regulation will be made in two main directions. First, systems biology approaches will integrate knowledge on Na_v biology, including transcriptional and post-translational regulation. This may be done using mathematical models and simulations of protein expression, function and degradation at the single molecule level, or, e.g., at the level of action potentials. Second, structural insights into whole sodium channel proteins, or isolated domains, will provide the framework to rationalise possible interactions between PTMs.

Additionally, research on Na_v is intrinsically associated to biomedical sciences, given the prominent relevance of these channels in a range of neurological and cardiac disorders. In this respect, in the following years we expect reports on quantitative experiments identifying changes in PTM patterns in disease (some recent examples include Baek et al. 2014; and Herren et al. 2015). The effect of sodium channel proteolysis in major neurological diseases

is also an emerging field of research (Corbett et al. 2013), which includes the identification of genetic mutations in proteases affecting sodium channel levels (Kim et al. 2014).

Acknowledgments DOO acknowledges Rivers State University of Science & Technology Port Harcourt Nigeria, and TETFund Nigeria (Academic Staff Training and Development Unit) for funding. We are grateful to Sandra Jones and John Greenman (School of Biological, Biomedical and Environmental Sciences, University of Hull) for guidance.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. This review contains data published previously only. This article does not contain any studies with human participants or animals performed by any of the authors.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Abriel H, Rougier JS, Jalife J (2015) Ion channel macromolecular complexes in cardiomyocytes: roles in sudden cardiac death. *Circ Res* 116(12):1971–1988
- Ahern CA, Zhang JF, Wookalis MJ, Horn R (2005) Modulation of the cardiac sodium channel NaV1.5 by Fyn, a Src family tyrosine kinase. *Circ Res* 96(9):991–998
- Baek JH, Cerda O, Trimmer JS (2011) Mass spectrometry-based phosphoproteomics reveals multisite phosphorylation on mammalian brain voltage-gated sodium and potassium channels. *Semin Cell Dev Biol* 22(2):153–159
- Baek JH, Rubinstein M, Scheuer T, Trimmer JS (2014) Reciprocal changes in phosphorylation and methylation of mammalian brain sodium channels in response to seizures. *J Biol Chem* 289(22):15363–15373
- Baroni D, Picco C, Barbieri R, Moran O (2014) Antisense-mediated post-transcriptional silencing of SCN1B gene modulates sodium channel functional expression. *Biol Cell* 106(1):13–29
- Baycin-Hizal D, Gottschalk A, Jacobson E, Mai S, Wolozny D, Zhang H, Krag SS, Betenbaugh MJ (2014) Physiologic and pathophysiologic consequences of altered sialylation and glycosylation on ion channel function. *Biochem Biophys Res* 453(2):243–253
- Beacham D, Ahn M, Catterall WA, Scheuer T (2007) Sites and molecular mechanisms of modulation of Na(v)1.2 channels by Fyn tyrosine kinase. *J Neurosci* 27(43):11543–11551
- Bedford MT, Clarke SG (2009) Protein arginine methylation in mammals: who, what, and why. *Mol Cell* 33(1):1–13
- Beltran-Alvarez P, Pagans S, Brugada R (2011) The cardiac sodium channel is post-translationally modified by arginine methylation. *J Proteome Res* 10(8):3712–3719
- Beltran-Alvarez P, Espejo A, Schmauder R, Beltran C, Mrowka R, Linke T, Batlle M, Pérez-Villa F, Pérez GJ, Scornik FS, Benndorf K, Pagans S, Zimmer T, Brugada R (2013) Protein arginine methyl transferases-3 and -5 increase cell surface expression of cardiac sodium channel. *FEBS Lett* 587(19):3159–3165
- Beltran-Alvarez P, Tarradas A, Chiva C, Pérez-Serra A, Batlle M, Pérez-Villa F, Schulte U, Sabidó E, Brugada R, Pagans S (2014) Identification of *N*-terminal protein acetylation and arginine methylation of the voltage-gated sodium channel in end-stage heart failure human heart. *J Mol Cell Cardiol* 76:126–129
- Beltran-Alvarez P, Feixas F, Osuna S, Díaz-Hernández R, Brugada R, Pagans S (2015) Interplay between R513 methylation and S516 phosphorylation of the cardiac voltage-gated sodium channel. *Amino Acids* 47(2):429–434
- Berendt FJ, Park KS, Trimmer JS (2010) Multisite phosphorylation of voltage-gated sodium channel alpha subunits from rat brain. *J Proteome Res* 9(4):1976–1984
- Bosmans F, Milescu M, Swartz KJ (2011) Palmitoylation influences the function and pharmacology of sodium channels. *Proc Natl Acad Sci USA* 108(50):20213–20218
- Buchner DA, Trudeau M, Meisler MH (2003) SCN1M1, a putative RNA splicing factor that modifies disease severity in mice. *Science* 301(5635):967–969
- Cantrell AR, Catterall WA (2001) Neuromodulation of Na⁺ channels: an unexpected form of cellular plasticity. *Nat Rev Neurosci* 2(6):397–407
- Catterall WA (2015) Finding channels. *J Biol Chem*. doi:10.1074/jbc.X115.683383
- Cerda O, Baek JH, Trimmer JS (2011) Mining recent brain proteomic databases for ion channel phosphosite nuggets. *J Gen Physiol* 137(1):3–16
- Chen C, Westenbroek RE, Xu X, Edwards CA, Sorenson DR, Chen Y, McEwen DP, O'Malley HA, Bharucha V, Meadows LS, Knudsen GA, Vilaythong A, Noebels JL, Saunders TL, Scheuer T, Shrager P, Catterall WA, Isom LL (2004) Mice lacking sodium channel beta1 subunits display defects in neuronal excitability, sodium channel expression, and nodal architecture. *J Neurosci* 24(16):4030–4042
- Chen Y, Yu FH, Sharp EM, Beacham D, Scheuer T, Catterall WA (2008) Functional properties and differential neuromodulation of Na(v)1.6 channels. *Mol Cell Neurosci* 38(4):607–615
- Copley RR (2004) Evolutionary convergence of alternative splicing in ion channels. *Trends Genet* 20(4):171–176
- Corbett BF, Leiser SC, Ling HP, Nagy R, Breyse N, Zhang X, Hazra A, Brown JT, Randall AD, Wood A, Pangalos MN, Reinhart PH, Chin J (2013) Sodium channel cleavage is associated with aberrant neuronal activity and cognitive deficits in a mouse model of Alzheimer's disease. *J Neurosci* 33(16):7020–7026
- Cui ZJ, Han ZQ, Li ZY (2012) Modulating protein activity and cellular function by methionine residue oxidation. *Amino Acids* 43(2):505–517
- Dib-Hajj SD, Yang Y, Black JA, Waxman SG (2013) The Na(V)1.7 sodium channel: from molecule to man. *Nat Rev Neurosci* 14(1):49–62
- Dong ZF, Tang LJ, Deng GF, Zeng T, Liu SJ, Wan RP, Liu T, Zhao QH, Yi YH, Liao WP, Long YS (2014) Transcription of the human sodium channel SCN1A gene is repressed by a scaffolding protein RACK1. *Mol Neurobiol* 50(2):438–448
- Drews VL, Lieberman AP, Meisler MH (2005) Multiple transcripts of sodium channel SCN8A (Na(V)1.6) with alternative 5'- and 3'-untranslated regions and initial characterization of the SCN8A promoter. *Genomics* 85(2):245–257
- Drews VL, Shi K, de Haan G, Meisler MH (2007) Identification of evolutionarily conserved, functional noncoding elements in the promoter region of the sodium channel gene SCN8A. *Mamm Genome* 18(10):723–731
- Dustrude ET, Wilson SM, Ju W, Xiao Y, Khanna R (2013) CRMP2 protein SUMOylation modulates NaV1.7 channel trafficking. *J Biol Chem* 288(34):24316–24331
- Few WP, Scheuer T, Catterall WA (2007) Dopamine modulation of neuronal Na(+) channels requires binding of A kinase-anchoring

- protein 15 and PKA by a modified leucine zipper motif. *Proc Natl Acad Sci USA* 104(12):5187–5192
- Fotia AB, Ekberg J, Adams DJ, Cook DI, Poronnik P, Kumar S (2004) Regulation of neuronal voltage-gated sodium channels by the ubiquitin-protein ligases Nedd4 and Nedd4-2. *J Biol Chem* 279(28):28930–28935
- Gasser A, Cheng X, Gilmore ES, Tyrrell L, Waxman SG, Dib-Hajj SD (2010) Two Nedd4-binding motifs underlie modulation of sodium channel Nav1.6 by p38 MAPK. *J Biol Chem* 285(34):26149–26161
- Gehman LT, Meera P, Stoilov P, Shiue L, O'Brien JE, Meisler MH, Ares M Jr, Otis TS, Black DL (2012) The splicing regulator Rbfox2 is required for both cerebellar development and mature motor function. *Genes Dev* 26(5):445–460
- Goel P, Manning JA, Kumar S (2015) NEDD4-2 (NEDD4L): the ubiquitin ligase for multiple membrane proteins. *Gene* 557(1):1–10
- Grimm S, Höhn A, Grune T (2012) Oxidative protein damage and the proteasome. *Amino Acids* 42(1):23–38
- Guo A, Gu H, Zhou J, Mulhern D, Wang Y, Lee KA, Yang V, Aguiar M, Kornhauser J, Jia X, Ren J, Beausoleil SA, Silva JC, Vemulapalli V, Bedford MT, Comb MJ (2014) Immunoaffinity enrichment and mass spectrometry analysis of protein methylation. *Mol Cell Proteomics* 13(1):372–387
- Gustafson TA, Clevinger EC, O'Neill TJ, Yarowsky PJ, Krueger BK (1993) Mutually exclusive exon splicing of type III brain sodium channel alpha subunit RNA generates developmentally regulated isoforms in rat brain. *J Biol Chem* 268(25):18648–18653
- Hallaq H, Yang Z, Viswanathan PC, Fukuda K, Shen W, Wang DW, Wells KS, Zhou J, Yi J, Murray KT (2006) Quantitation of protein kinase A-mediated trafficking of cardiac sodium channels in living cells. *Cardiovasc Res* 72(2):250–261
- Heinzen EL, Yoon W, Tate SK, Sen A, Wood NW, Sisodiya SM, Goldstein DB (2007) Nova2 interacts with a *cis*-acting polymorphism to influence the proportions of drug-responsive splice variants of SCN1A. *Am J Hum Genet* 80(5):876–883
- Herren AW, Weber DM, Rigor RR, Margulies KB, Phinney BS, Bers DM (2015) CaMKII phosphorylation of Na(V)1.5: novel *in vitro* sites identified by mass spectrometry and reduced S516 phosphorylation in human heart failure. *J Proteome Res* 14(5):2298–2311
- Hien YE, Montersino A, Castets F, Leterrier C, Filhol O, Vacher H, Dargent B (2014) CK2 accumulation at the axon initial segment depends on sodium channel Nav1. *FEBS Lett* 588(18):3403–3408
- Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E (2015) PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res* 43(1):D512–D520
- Iqbal SM, Andavan GS, Lemmens-Gruber R (2015) Differential modulation of fast inactivation in cardiac sodium channel splice variants by fyn tyrosine kinase. *Cell Physiol Biochem* 37(3):825–837
- Iwata A, Stys PK, Wolf JA, Chen XH, Taylor AG, Meaney DF, Smith DH (2004) Traumatic axonal injury induces proteolytic cleavage of the voltage-gated sodium channels modulated by tetrodotoxin and protease inhibitors. *J Neurosci* 24(19):4605–4613
- James TF, Nenov MN, Wildburger NC, Lichti CF, Luisi J, Vergara F, Panova-Electronova NI, Nilsson CL, Rudra JS, Green TA, Labate D, Laezza F (2015) The Nav1.2 channel is regulated by GSK3. *Biochim Biophys Acta* 1850(4):832–844
- Kasteleijn-Nolst Trenité DG, Volkers L, Strengman E, Schippers HM, Perquin W, de Haan GJ, Gkoutidi AO, van't Slot R, de Graaf SF, Jovic-Jakubi B, Capovilla G, Covanis A, Parisi P, Veggiotti P, Brinciotti M, Incorpora G, Piccioli M, Cantonetti L, Berkovic SF, Scheffer IE, Brilstra EH, Sonsma AC, Bader AJ, de Kovel CG, Koeleman BP (2015) Clinical and genetic analysis of a family with two rare reflex epilepsies. *Seizure* 29:90–96
- Kharatmal SB, Singh JN, Sharma SS (2015) Calpain inhibitor, MDL 28170 confer electrophysiological, nociceptive and biochemical improvement in diabetic neuropathy. *Neuropharmacology* 97:113–121
- Khoury GA, Baliban RC, Floudas CA. (2011) Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. *Sci Rep*, 1 pii: srep00090
- Kim DY, Ingano LA, Carey BW, Pettingell WH, Kovacs DM (2005) Presenilin/gamma-secretase-mediated cleavage of the voltage-gated sodium channel beta2-subunit regulates cell adhesion and migration. *J Biol Chem* 280(24):23251–23261
- Kim DY, Carey BW, Wang H, Ingano LA, Binshtok AM, Wertz MH, Pettingell WH, He P, Lee VM, Woolf CJ, Kovacs DM (2007) BACE1 regulates voltage-gated sodium channels and neuronal activity. *Nat Cell Biol* 9(7):755–764
- Kim DY, Gersbacher MT, Inquimbert P, Kovacs DM (2011) Reduced sodium channel Na(v)1.1 levels in BACE1-null mice. *J Biol Chem* 286(10):8106–8116
- Kim DY, Wertz MH, Gautam V, D'Avanzo C, Bhattacharyya R, Kovacs DM (2014) The E280A presenilin mutation reduces voltage-gated sodium channel levels in neuronal cells. *Neurodegener Dis* 13(2–3):64–68
- Laedermann CJ, Syam N, Pertin M, Decosterd I, Abriel H (2013) β 1- and β 3-voltage-gated sodium channel subunits modulate cell surface expression and glycosylation of Nav1.7 in HEK293 cells. *Front Cell Neurosci* 7:137
- Le Gal F, Salzmann A, Crespel A, Malafosse A (2011) Replication of association between a SCN1A splice variant and febrile seizures. *Epilepsia* 52(10):e135–e138
- Li M, West JW, Lai Y, Scheuer T, Catterall WA (1992) Functional modulation of brain sodium channels by cAMP-dependent phosphorylation. *Neuron* 8(6):1151–1159
- Li M, West JW, Numann R, Murphy BJ, Scheuer T, Catterall WA (1993) Convergent regulation of sodium channels by protein kinase C and cAMP-dependent protein kinase. *Science* 261(5127):1439–1442
- Li HJ, Wan RP, Tang LJ, Liu SJ, Zhao QH, Gao MM, Yi YH, Liao WP, Sun XF, Long YS (2015) Alteration of Scn3a expression is mediated via CpG methylation and MBD2 in mouse hippocampus during postnatal development and seizure condition. *Biochim Biophys Acta* 1849(1):1–9
- Long YS, Zhao QH, Su T, Cai YL, Zeng Y, Shi YW, Yi YH, Chang HH, Liao WP (2008) Identification of the promoter region and the 5'-untranslated exons of the human voltage-gated sodium channel Nav1.1 gene (SCN1A) and enhancement of gene expression by the 5'-untranslated exons. *J Neurosci Res* 86(15):3375–3381
- Lossin C (2009) A catalog of SCN1A variants. *Brain Dev* 31(2):114–130
- Lu CM, Eichelberger JS, Beckman ML, Schade SD, Brown GB (1998) Isolation of the 5'-flanking region for human brain sodium channel subtype II alpha-subunit. *J Mol Neurosci* 11(3):179–182
- Marionneau C, Abriel H (2015) Regulation of the cardiac Na⁺ channel Nav1.5 by post-translational modifications. *J Mol Cell Cardiol* 82:36–47
- Marionneau C, Lichti CF, Lindenbaum P, Charpentier F, Nerbonne JM, Townsend RR, Mérot J (2012) Mass spectrometry-based identification of native cardiac Nav1.5 channel α subunit phosphorylation sites. *J Proteome Res* 11(12):5994–6007
- Martin MS, Tang B, Ta N, Escayg A (2007) Characterization of 5' untranslated regions of the voltage-gated sodium channels SCN1A, SCN2A, and SCN3A and identification of cis-conserved noncoding sequences. *Genomics* 90(2):225–235
- Mercier A, Clément R, Harnois T, Bourmeyster N, Bois P, Chatelier A (2015) Nav1.5 channels can reach the plasma membrane through distinct N-glycosylation states. *Biochim Biophys Acta* 1850(6):1215–1223

- Namadurai S, Yereddi NR, Cusdin FS, Huang CL, Chirgadze DY, Jackson AP (2015) A new look at sodium channel β subunits. *Open Biol* 5(1):140192
- Numann R, Catterall WA, Scheuer T (1991) Functional modulation of brain sodium channels by protein kinase C phosphorylation. *Science* 254(5028):115–118
- Ogino K, Low SE, Yamada K, Saint-Amant L, Zhou W, Muto A, Asakawa K, Nakai J, Kawakami K, Kuwada JY, Hirata H (2015) RING finger protein 121 facilitates the degradation and membrane localization of voltage-gated sodium channels. *Proc Natl Acad Sci USA* 112(9):2859–2864
- Paillart C, Boudier JL, Boudier JA, Rochat H, Couraud F, Dargent B (1996) Activity-induced internalization and rapid degradation of sodium channels in cultured fetal neurons. *J Cell Biol* 134(2):499–509
- Renganathan M, Cummins TR, Waxman SG (2002) Nitric oxide blocks fast, slow, and persistent Na⁺ channels in C-type DRG neurons by S-nitrosylation. *J Neurophysiol* 87(2):761–775
- Rogers LD, Overall CM (2013) Proteolytic post-translational modification of proteins: proteomic tools and methodology. *Mol Cell Proteomics* 12(12):3532–3542
- Rougier JS, van Bemmelen MX, Bruce MC, Jespersen T, Gavillet B, Apothéoz F, Cordonier S, Staub O, Rotin D, Abriel H (2005) Molecular determinants of voltage-gated sodium channel regulation by the Nedd4/Nedd4-like proteins. *Am J Physiol Cell Physiol* 288(3):C692–C701
- Sarao R, Gupta SK, Auld VJ, Dunn RJ (1991) Developmentally regulated alternative RNA splicing of rat brain sodium channel mRNAs. *Nucleic Acids Res* 19(20):5673–5679
- Schade SD, Brown GB (2000) Identifying the promoter region of the human brain sodium channel subtype II gene (SCN2A). *Brain Res Mol Brain Res* 81(1–2):187–190
- Schlachter K, Gruber-Sedlmayr U, Stogmann E, Lausecker M, Hotzy C, Balzar J, Schuh E, Baumgartner C, Mueller JC, Illig T, Wichmann HE, Lichtner P, Meitinger T, Strom TM, Zimprich A, Zimprich F (2009) A splice site variant in the sodium channel gene SCN1A confers risk of febrile seizures. *Neurology* 72(11):974–978
- Schmidt JW, Catterall WA (1987) Palmitoylation, sulfation, and glycosylation of the alpha subunit of the sodium channel. Role of post-translational modifications in channel assembly. *J Biol Chem* 262(28):13713–13723
- Schoch KM, von Reyn CR, Bian J, Telling GC, Meaney DF, Saatman KE (2013) Brain injury-induced proteolysis is reduced in a novel calpastatin-overexpressing transgenic mouse. *J Neurochem* 125(6):909–920
- Tate SK, Depondt C, Sisodiya SM, Cavalleri GL, Schorge S, Soranzo N, Thom M, Sen A, Shorvon SD, Sander JW, Wood NW, Goldstein DB (2005) Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. *Proc Natl Acad Sci USA* 102:5507–5512
- Thompson CH, Kahlig KM, George AL Jr (2011) SCN1A splice variants exhibit divergent sensitivity to commonly used antiepileptic drugs. *Epilepsia* 52(5):1000–1009
- van Bemmelen MX, Rougier JS, Gavillet B, Apothéoz F, Daidié D, Tateyama M, Rivolta I, Thomas MA, Kass RS, Staub O, Abriel H (2004) Cardiac voltage-gated sodium channel Nav1.5 is regulated by Nedd4-2 mediated ubiquitination. *Circ Res* 95(3):284–291
- von Reyn CR, Spaethling JM, Mesfin MN, Ma M, Neumar RW, Smith DH, Siman R, Meaney DF (2009) Calpain mediates proteolysis of the voltage-gated sodium channel alpha-subunit. *J Neurosci* 29(33):10350–10356
- von Reyn CR, Mott RE, Siman R, Smith DH, Meaney DF (2012) Mechanisms of calpain mediated proteolysis of voltage gated sodium channel α -subunits following in vitro dynamic stretch injury. *J Neurochem* 121(5):793–805
- West JW, Numann R, Murphy BJ, Scheuer T, Catterall WA (1991) A phosphorylation site in the Na⁺ channel required for modulation by protein kinase C. *Science* 254(5033):866–868
- Wittmack EK, Rush AM, Hudmon A, Waxman SG, Dib-Hajj SD (2005) Voltage-gated sodium channel Nav1.6 is modulated by p38 mitogen-activated protein kinase. *J Neurosci* 25(28):6621–6630
- Wong HK, Sakurai T, Oyama F, Kaneko K, Wada K, Miyazaki H, Kurosawa M, De Strooper B, Saftig P, Nukina N (2005) beta Subunits of voltage-gated sodium channels are novel substrates of beta-site amyloid precursor protein-cleaving enzyme (BACE1) and gamma-secretase. *J Biol Chem* 280(24):23009–23017
- Yu FH, Catterall WA (2003) Overview of the voltage-gated sodium channel family. *Genome Biol* 4(3):207
- Zhou J, Shin HG, Yi J, Shen W, Williams CP, Murray KT (2002) Phosphorylation and putative ER retention signals are required for protein kinase A-mediated potentiation of cardiac sodium current. *Circ Res* 91(6):540–546
- Zilberter YuI, Starmer CF, Starobin J, Grant AO (1994) Late Na channels in cardiac cells: the physiological role of background Na channels. *Biophys J* 67(1):153–160