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REVIEW

The structure and function of TRIP8b, an auxiliary subunit of hyperpolarization-activated cyclic-nucleotide gated channels

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ABSTRACT

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are expressed throughout the mammalian central nervous system (CNS). These channels have been implicated in a wide range of diseases, including Major Depressive Disorder and multiple subtypes of epilepsy. The diversity of functions that HCN channels perform is in part attributable to differences in their subcellular localization. To facilitate a broad range of subcellular distributions, HCN channels are bound by auxiliary subunits that regulate surface trafficking and channel function. One of the best studied auxiliary subunits is tetratricopeptide-repeat containing, Rab8b-interacting protein (TRIP8b). TRIP8b is an extensively alternatively spliced protein whose only known function is to regulate HCN channels. TRIP8b binds to HCN pore-forming subunits at multiple interaction sites that differentially regulate HCN channel function and subcellular distribution. In this review, we summarize what is currently known about the structure and function of TRIP8b isoforms with an emphasis on the role of this auxiliary subunit in health and disease.

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Introduction

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are tetrameric voltage-gated channels that regulate cellular excitability [1,2]. In the central nervous system (CNS), these channels participate in a wide variety of physiological processes including dendritic integration [3,4], sleep and wake states [5,6], motor learning [7,8], taste [9], and fear learning [10]. HCN channels activate in response to hyperpolarization and are permeable to both Na^+ and K^+ such that their E_{rev} is depolarized relative to the resting membrane potential (E_{rev} often −25 to −40 mV) [1,2]. As a result, in many cell types (including cardiomyocytes, thalamocortical neurons, and CA1 pyramidal neurons) these channels are open at the resting membrane potential and contribute a depolarizing influence [1,2]. The channel's nonlinear properties make the precise function of the channel within a given cell difficult to predict. In thalamocortical neurons, I_h (the current mediated by HCN channels) interacts with T type calcium channels to generate rhythmic firing [5,6]. In contrast, HCN channels located in CA1 pyramidal cell dendrites limit temporal summation and reduce neuronal excitability [3,4].

In addition to activation by hyperpolarization, HCN channels are also directly regulated by cyclic nucleotide binding to a cytoplasmic cyclic nucleotide-binding domain (CNBD) of the channel [2,11]. Both cAMP and cGMP bind to the CNBD and speed channel activation at more depolarized potentials [1,12–15]. The magnitude of depolarization of the V_{50} in response to cAMP depends on which of the four HCN subunits (HCN1-4) is involved. HCN1 and HCN2 are the predominant subunits in the mammalian CNS [16] and while HCN1 exhibits a small shift in V_{50} in response to cyclic nucleotide binding, the V_{50} of HCN2 depolarizes to a larger degree [1,2]. *In vivo*, neuronal I_h is typically mediated by HCN1 and HCN2 homotetramers as well as heterotetramers which generate I_h with properties that are intermediate between the two subunits [12]. The incorporation of auxiliary subunits also modulates channel properties [12]. For additional background on the structure and function of HCN channels, the reader is advised

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to consult one of the several excellent reviews addressing these topics [1,2].

In many cell types, the function of HCN channels is intimately linked to their subcellular distribution. HCN channels within CA1 pyramidal neurons are located in a distal dendritic enrichment (DDE) pattern, in which the channels are highly expressed in the distal *stratum lacunosum moleculare* (SLM) of the apical dendrites relative to the more proximal *stratum radiatum* (SR) (Figure 1) [17]. This distribution facilitates the channel's role in regulating synaptic input, with its localization impacting entorhinal cortex inputs via the temporoammonic (TA) pathway (synapsing onto the SLM where HCN channels are expressed at high levels) to a greater degree than Schaffer collaterals arriving from CA3 (synapsing onto the SR where there are fewer HCN channels) [3,4,7,18,19]. Although somatic HCN channels contribute a predominantly depolarizing influence on the resting membrane potential, the net effect of CA1 dendritic channels is to dampen neuronal excitability [3,4,20]. A number of proteins bind HCN channels and are thought to influence their subcellular distribution *in vivo* including Filamin A [21,22], Mint2 [23], Tamalin, and tetratricopeptide-repeat containing, Rab8b-interacting protein (TRIP8b).

Structure of the TRIP8b-HCN Interaction

TRIP8b binds to HCN pore-forming subunits in a 1:1 ratio at two locations and has been shown to facilitate the DDE of HCN channels in CA1 pyramidal neurons (Figure 1) [24–26]. The first binding site involves the CNBD of HCN channels and a 40 amino acid stretch of TRIP8b known as the upstream site (Figure 2) [27,28]. The second interaction site occurs between the C terminal tail of HCN channels and the TPR domains of TRIP8b, termed the downstream interaction site [25]. Both interaction sites occur within regions of TRIP8b that are common to all known CNS isoforms of TRIP8b. As a result, the effect of TRIP8b on HCN channel properties (such as activation kinetics and voltage dependence) are consistent across TRIP8b isoforms, although the different isoforms of TRIP8b have varying effects on the surface trafficking and subcellular distribution of HCN channels.

The upstream interaction is primarily responsible for TRIP8b-dependent regulation of HCN channel gating [29,30]. In the presence of TRIP8b, HCN channels open at more hyperpolarized potentials as a result of TRIP8b antagonizing cAMP binding to the CNBD region of HCN

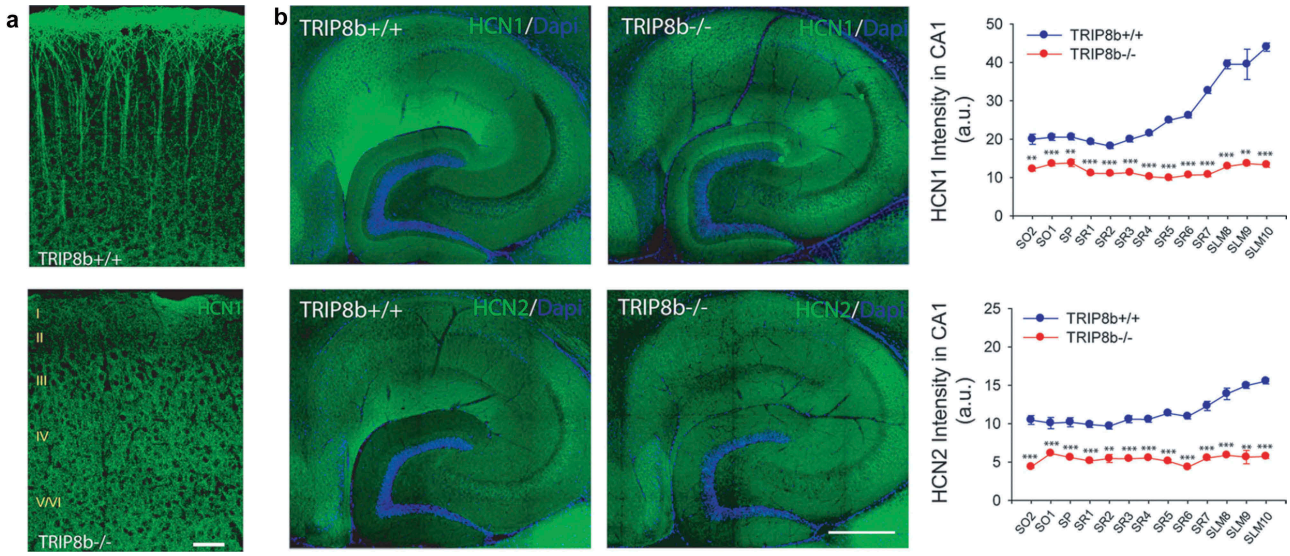

Figure 1. Image reproduced from Lewis et al 2011 [24]. Copyright 2011 Society for Neuroscience. (a) Layer V neocortical pyramidal neuron dendrites of wild type (above) and *Trip8b*$^{-/-}$ mice stained for HCN1 (in green). Note the reduced staining in the superficial layers of the *Trip8b*$^{-/-}$ mice. Scale bar 50 microns. (b) Sagittal images of wild type (left two panels) and *Trip8b*$^{-/-}$ mice (right two panels) stained for HCN1 (top two panels) and HCN2 (bottom two panels) with quantification of HCN1 (far right, top) and HCN2 (far right, bottom). Note the absence of HCN1 and HCN2 staining in the distal dendrites of the CA1 region of the *Trip8b*$^{-/-}$ mice. Scale bar 400 microns. **p < 0.01, ***p < 0.001.

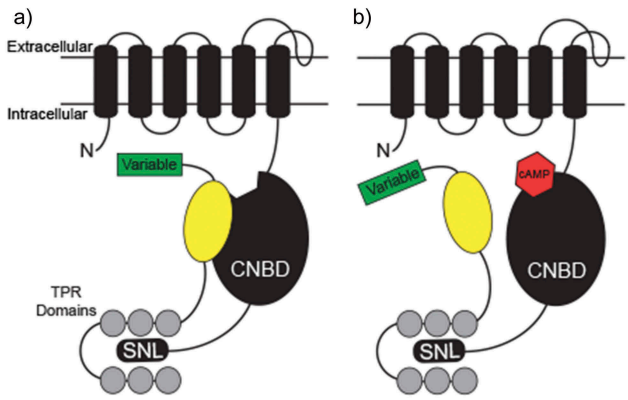

Figure 2. Schematic of the TRIP8b-HCN interaction. Adapted from Foote et al 2019 [54]. A single TRIP8b protein is shown bound to a single HCN monomer, although *in vivo* HCN channels exist as heterotetramers bound to TRIP8b in a 1:1 ratio. Note that the CNBD is bound either by TRIP8b (a) or by cAMP (b). The TPR domains of TRIP8b (shown as gray circles) bind to the C terminus of the HCN monomer. This figure was originally published in the Journal of Biological Chemistry. Foote KM, Lyman KA, Han Y, Michailidis IE, Heuermann RJ, Mandikian D, Trimmer JS, Swanson GT, Chetkovich DM. Phosphorylation of the HCN channel auxiliary subunit TRIP8b is altered in an animal model of temporal lobe epilepsy and modulates channel function. J. Biol. Chem. 2019; 294: 15743–58. © the Author(s). The American Society for Biochemistry and Molecular Biology.

channels. Although this observation was made electrophysiologically soon after the discovery of TRIP8b, it required significant experimentation by multiple groups in order to accurately describe the biochemical interaction [30]. Recent NMR studies have confirmed that cAMP and TRIP8b directly compete for binding the CNBD of HCN [27]. This is principally due to a small domain of TRIP8b (residues 235–275 in mouse isoform 1a-4) [27], although nearby TRIP8b domains allosterically influence this interaction as well [27,31]. Consistent with this interpretation, mutations such as HCN1(R538E) and HCN2(R591E) that disrupt cAMP binding to the CNBD also block upstream TRIP8b binding [30].

The downstream interaction site occurs between the TPR domains of TRIP8b and the C terminus of HCN channels. The crystal structure of the downstream interaction site has been solved using the conserved TRIP8b TPR domains (mouse isoform 1a-4 residues 241–602) in complex with a short peptide fragment corresponding to the C terminus of HCN2 (-RLSSNL) [25]. TRIP8b contains 6 TPR domains, organized into 2 groups of 3 domains separated by a 45 amino acid hinge region. The 2 groups of TPR domains clamp onto the C terminal peptide of HCN in an interaction that is nearly identical to that of Peroxin5 (PEX5) binding to peroxisomal targeting signal 1 (PTS1) motifs [25,32,33]. PEX5 is a cytosolic protein involved in trafficking proteins into peroxisomes by binding to a wide variety of C terminal PTS1 motifs with the consensus sequence (S/A/C/K/N)(K/R/H/Q/N)(S/L/I) [32,34]. The significant conservation between the structure of TRIP8b and PEX5, as well as their similarities in substrate binding, raises the question of what accounts for their differing functions *in vivo* [33]. The TPR domains of TRIP8b have been observed to bind certain PTS1 motifs with greater affinity than HCN C terminal peptides in biophysical assays, yet the majority of TRIP8b is bound to HCN *in vivo* [35,36]. Conversely, human peroxisomal proteins that terminate in – SNL (identical to the C terminus of HCN1, 2, and 4) have been discovered [37], but HCN channels have never been identified in peroxisomes despite multiple electron microscopy characterization studies [16,17,24,38–45]. Although TRIP8b and PEX5 have structurally similar binding at their TPR domains, there appear to be crucial differences in stabilizing their respective interactions which may ultimately explain their distinct *in vivo* substrate specificities [25,32]. For example, post-translational modifications play an important role in regulating PEX5 binding and the two TRIP8b-HCN interaction sites are thought to allosterically promote binding of TRIP8b to HCN channels [36].

The isoforms of TRIP8b

TRIP8b is an extensively alternatively spliced protein (Figure 3) and while there are three known promoters for TRIP8b (termed a, b, and c), only transcripts from a and b have been detected in the brain [28]. Transcripts arising from the third promoter (c) have been detected in the testes but contain only the 1c exon followed by exons 10–16 [28]. As this transcript has not been shown to be translated *in vivo* we will not consider it further here [28]. From the a and b promoters, 11 known mRNA transcripts have been detected and 9 different proteins are predicted to be translated [28]. The properties of the distinct TRIP8b

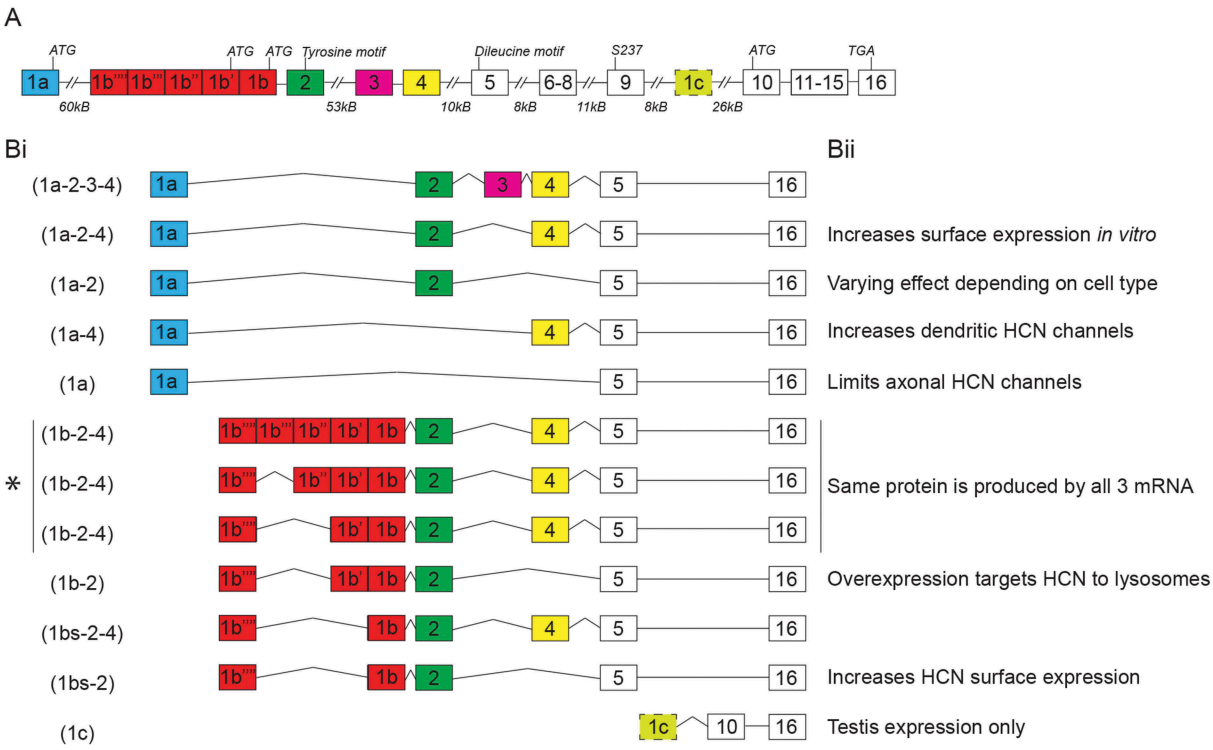

Figure 3. Schematic of the isoforms of TRIP8b. Adapted from Lewis et al 2009 [28]. Copyright 2009 Society for Neuroscience. (a) Schematic for *Pex5 l*, the gene encoding TRIP8b. Note that throughout this manuscript we have referred to the gene encoding TRIP8b as *Trip8b* for simplicity. As described in the text, there are three promoters (a,b,c) with multiple N terminal exons that are variably included.

isoforms have been analyzed both *in vitro* and *in vivo*, although differences in heterologous expression systems have occasionally produced conflicting results. We have adopted the convention of naming each TRIP8b isoform according to the variably spliced exons that are included before the conserved exons 5–16. For example, TRIP8b (1a-4) refers to the isoform generated from the a promoter and contains exons 1a, 4, and 5–16 while TRIP8b(1b-2) refers to the isoform generated from the b promoter and contains exons 1b, 2, and 5–16 (Figure 3Bi).

TRIP8b(1a-4)

TRIP8b isoform (1a-4) is the most abundant TRIP8b isoform in the mammalian brain and reliably increases the surface expression of HCN channels [28,46]. Two mouse lines have been used to study TRIP8b. One is a global TRIP8b knockout in which all TRIP8b isoforms are eliminated (*Trip8*$^{-/-}$) (Figure 1) [24]. The second line is missing exons 1b and 2 (*Trip8b(1b-2)*$^{-/-}$) [47], which has made it an ideal tool to study the function of the two most common TRIP8b isoforms (1a and 1a-4). Although this mouse continues to express TRIP8b(1a-3-4), this isoform comprises less than 5% of total TRIP8b [46]. *Trip8b(1b-2)*$^{-/-}$ animals have a normal distribution of HCN1 in CA1 dendrites and normal somatic I_h, indicating that *in vivo*, either isoform 1a or 1a-4 is sufficient for this effect [47,48]. Using an antibody that specifically detects exon 4, Piskorowski and colleagues confirmed that TRIP8b(1a-4) is colocalized with HCN channels in CA1 dendrites [47]. Finally, AAV mediated expression of TRIP8b(1a-4) in the dorsal CA1 of *Trip8b*$^{-/-}$ animals restores the DDE of HCN channels and rescues somatic I_h [49]. Combined, these results strongly suggest that TRIP8b(1a-4) is sufficient for the dendritic enrichment of HCN channels in CA1 pyramidal neurons.

Although expression of TRIP8b(1a-4) increases dendritic HCN channels, it remains unclear how precisely this pattern of HCN channel expression comes about. One possibility is that TRIP8b functions principally to prevent HCN channel degradation by lysosomes [24]. A second possibility

(which is not mutually exclusive) is that the variably spliced region plays a role in actively transporting HCN channels into the distal dendrites. In favor of this point, differentially spliced isoforms of TRIP8b have been shown to perform distinct functions as described below. There is also an apparent requirement for both TRIP8b-HCN binding sites to be functioning simultaneously. Several groups have demonstrated that both the upstream and downstream TRIP8b-HCN interactions are required for dendritic enrichment of HCN channels by TRIP8b in CA1 [47,49]. In contrast to the dual binding site requirement *in vivo*, only a single binding site is required for TRIP8b to facilitate HCN channel surface trafficking *in vitro* in heterologous expression systems [30,32]. These results raise the possibility that a specific conformation of TRIP8b-HCN must be achieved or be sufficiently stabilized in order for trafficking by other proteins to occur.

While the precise role of TRIP8b in facilitating HCN DDE remains unclear, there is evidence that it is a regulated process dependent on extracellular signals. Using a slice culture model, Shin et al observed a dependence on TA signaling from the entorhinal cortex in order to establish and maintain the DDE of HCN1 channels [50]. These experiments suggested that glutamatergic signaling from the entorhinal cortex led to activation of calcium/calmodulin-dependent protein kinase II (CaMKII), and ultimately to the DDE of HCN channels, consistent with prior lesion experiments of the TA pathway finding reduced HCN expression [38]. This hypothesis also mirrors findings from physiology experiments, where CaMKII activation by Ca^{2+} influx through NMDA receptors leads to increased I_h [51]. However, another group did not observe a dependence on TA signaling but instead noted a role for Reelin signaling in the establishment of DDE [52]. The discrepancy between these two studies was suggested to be the result of differences in calretinin positive interneuron activity between the two preparations, given that synaptic activity has been shown to drive the release of Reelin from these cell types in other brain regions [53]. This hypothesis would produce an elegant reconciliation and suggest a model where TA inputs ultimately drive calretinin positive interneurons to release Reelin, which in turn leads to DDE of HCN channels [52].

Post-translational modifications of TRIP8b provide an additional layer of regulation that is beginning to be explored. Foote et al identified a serine residue within the CNBD-binding domain of TRIP8b that is phosphorylated by CaMKII and PKA (Figure 3(a)) [54]. Phosphorylation of this site has been observed for isoform 1a-4 and increases the strength of HCN channel binding leading to differences in HCN channel voltage dependence. This phosphorylation occurs within the distal dendrites of CA1 pyramidal and layer 5 neocortical cells raising the possibility that it may be involved in regulating trafficking as well [54].

Similar to the hippocampus, neocortical HCN channels are also enriched in the distal dendrites of layer 5 pyramidal neurons in a TRIP8b-dependent manner (Figure 1) [24,55]. However, while corticospinal neurons express high levels of HCN1 and TRIP8b, neighboring corticostriatal and corticocortical neurons express much lower levels [56]. These differences suggest an additional layer of complexity in specifying TRIP8b and HCN channel expression beyond local Reelin signaling [52].

TRIP8b(1a)

TRIP8b(1a) is the second most abundant isoform in the adult brain after TRIP8b(1a-4) and its function appears to depend heavily on cellular context [46]. When overexpressed in HEK293 cells, TRIP8b(1a) increases HCN surface expression [28]. However, when overexpressed in oocytes, the same isoform reduces HCN surface expression through a mechanism that requires a dileucine motif in exon 5 [46]. It is notable that this dileucine motif is conserved in every TRIP8b isoform, yet most isoforms still have a net effect of increasing HCN surface expression [28]. This could signify that the motif is inactive or that its function is overwhelmed by some other motif's effect in other isoforms. *In vivo* experiments have yielded results that are consistent with those from oocytes. In a viral rescue experiment in *Hcn1*$^{-/-}$ animals, overexpression of TRIP8b(1a-4) along with HCN1 led to expression of HCN1 in the axons of CA1 pyramidal neurons [47]. However, rescue with TRIP8b(1a) prevented the expression of HCN1 in

the CA1 axons in a manner that was again dependent on the exon 5 dileucine motif [47]. Beyond the CA1 region, other groups have also examined the function of the TRIP8b(1a) isoform. In the dentate gyrus (DG), Wilkars et al. noted that there was more HCN1 expression in the molecular layer (ML) of the DG in *Trip8b*$^{-/-}$ animals compared to *Trip8b(1b-2)*$^{-/-}$ animals [48,50]. This raises the possibility that either TRIP8b(1a) or TRIP8b(1a-4) acts to keep HCN1 out of the axons terminating in the ML [48,50]. In the context of data showing that TRIP8b(1a) keeps HCN1 out of CA1 pyramidal neuron axons *in vivo* [47], it is likely the case that TRIP8b(1a) plays a general role in preventing axonal localization of HCN channels, although it remains to be seen what the precise function of this isoform is in other cellular contexts.

The remaining 1a isoforms

Exon 2 contains a tyrosine based trafficking motif, discussed in detail below in the context of TRIP8b (1b-2) [46]. Expression of TRIP8b(1a-2) in oocytes has virtually no effect on HCN surface expression, but mutating this tyrosine motif causes TRIP8b(1a-2) to increase surface expression, suggesting an equilibrium between competing processes [46]. The remaining 1a isoforms are collectively thought to represent at most 16% of total TRIP8b in the brain, but likely much less [46]. These isoforms have been challenging to study because neither specific antibodies nor genetic models to isolate their function have been developed.

TRIP8b(1b-2)

The 1b-2 isoform downregulates HCN channels from the surface of oocytes, HEK293 cells, and neurons [28,46,57]. Exon 2 of TRIP8b(1b-2) contains a tyrosine based trafficking motif that binds adaptor proteins in order to facilitate clathrin mediated endocytosis [46,58] and mutations of the motif prevent TRIP8b(1b-2)-mediated internalization of HCN channels in oocytes [46]. Interestingly, this suggests a mechanism that is distinct from the dileucine motif in exon 5 that is required for TRIP8b(1a)-mediated internalization and further broadens TRIP8b's functions [46,59].

Trip8b$^{-/-}$ animals have fewer HCN channels, an effect that has been attributed in part to increased lysosomal trafficking of the channels in the absence of TRIP8b [24]. TRIP8b(1b-2) appears to be unique amongst TRIP8b isoforms in *facilitating* trafficking of HCN channels to lysosomes [46,59]. Although the effect of TRIP8b(1b-2) on HCN channel trafficking has been strikingly consistent, it remains unclear what role the isoform plays physiologically and which cells it is expressed in. One hypothesis is that TRIP8b(1b-2) is expressed in mature oligodendrocytes [47,60]. HCN2 is expressed in these cells [16], and an RNA-seq database examining oligodendrocyte RNA during differentiation shows a substantial upregulation of both *Hcn2* and *Pex5l*, the gene encoding TRIP8b [61]. However, it remains an open question whether HCN2 channels are active in oligodendrocytes and what role they might play in this electrically silent cell type [62–64].

In the rat hippocampus, TRIP8b transcripts produced from the a promoter are expressed at constant levels throughout development while those from the b promoter increase during the first 3 weeks of life [28]. The b promoter contains an additional level of complexity, as the transcripts produced from this promoter are predicted to variably include a 25 amino acid segment into the first (1b) exon (the variant lacking the 25 amino acid segment is designated 1bs for "1b short"). Unlike TRIP8b(1b-2), TRIP8b(1bs-2) *increases* surface expression of HCN1 in HEK293 cells and indicates that the presence of the 25 amino acid segment of 1b in combination with exon 2 leads to the internalization of HCN1 channels [28]. This suggests that perhaps the 25 amino acid sequence is necessary for binding partner recruitment because TRIP8b(1b-2)-mediated internalization still requires a tyrosine motif in exon 2 [46].

The physiological role of TRIP8b

Learning and memory

Regulation of I_h is a common feature of several forms of long-term potentiation (LTP) and long-term depression (LTD). While a thorough explanation of

this topic is beyond the scope of this review (but see [65]), several observations suggest a role for TRIP8b in homeostatic alterations of I_h. Loss of TRIP8b leads to broader EPSPs in CA1 pyramidal neurons in response to inputs synapsing onto the SLM but does not affect Schaffer collateral (SC) inputs to the more proximal SR [66]. Despite the lack of a difference in SC EPSPs, *Trip8b*$^{-/-}$ cells still exhibited greater short-term potentiation in response to theta burst pairing of the SC EPSPs, although the two genotypes converged over a period of 30 minutes. I_h-dependent membrane properties (ie resonance frequency) that ordinarily increase after SC LTP also failed to do so in *Trip8b*$^{-/-}$ animals [66]. Overall, these results suggest that TRIP8b is likely involved in homeostatic I_h regulation following SC LTP, but it remains unclear how exactly TRIP8b is involved. Likely possibilities are that TRIP8b either ensures an adequate subcellular HCN channel pool is available for transport or plays an active role in trafficking the channels to the cell surface in response to post-translational regulation [66].

Major Depressive Disorder (MDD)

Recent work in several brain regions has implicated HCN channels in MDD (reviewed elsewhere [67]). In many regions outside the hippocampus, animal models pertinent to MDD have observed that loss of I_h is associated with more depression-like behavior [68–70]. However, in CA1 neurons of the hippocampus the opposite trend has been observed with more HCN channel expression in the dorsal hippocampi of rodents after a behavioral paradigm meant to induce depression-like behavior [71]. The dorsal CA1 region has not classically been linked to MDD [72,73] although several studies have shown a reduction in hippocampal excitability in MDD patients and animal models [74,75]. This suggests a model where increasing HCN channel expression limits the excitability of CA1 pyramidal neurons and ultimately dampens hippocampal output. Although TRIP8b is likely involved in mediating changes in HCN channel expression because of its strong association with CA1 HCN channels, TRIP8b has only specifically been investigated in one study [76]. In that report, TRIP8b(1a-4) was upregulated following chronic social defeat [76].

The data linking dorsal hippocampal HCN1 channels to antidepressant-like behavior is extensive. *Trip8b*$^{-/-}$ animals were initially found to spend less time immobile in the tail suspension test (TST) and forced swim test (FST), two key antidepressant screening assays [24]. Similar observations were also made for *Hcn1*$^{-/-}$ mice and animals lacking HCN2. Knockdown of HCN1 only in the dorsal CA1 also produces antidepressant-like changes in TST and FST performance as well as anxiolysis [71,77]. Rescue experiments in which TRIP8b(1a-4) is expressed in the dorsal hippocampi of *Trip8b*$^{-/-}$ animals rescues TST and FST performance back to that of wild type animals [49]. Additionally, expression of a dominant negative TRIP8b isoform (TRIP8b(1b-2)) in the dorsal hippocampus of wild type animals also produces antidepressant-like changes in behavior [57]. These studies all point to a mechanism where changes in dorsal CA1 excitability translate into an antidepressant-like phenotype [57,77]. Interestingly, a second mechanism has also recently been proposed for the antidepressant-like phenotype of *Trip8b*$^{-/-}$ mice. Yun and colleagues observed that *Trip8b*$^{-/-}$ mice have increased adult neurogenesis in the DG and suggested that this change in neurogenesis is important for the antidepressant-like phenotype [76].

Despite these intriguing animal studies, virtually no data exists linking human pathology directly to TRIP8b. However, loss-of-function mutations in TRIP8b would be expected to confer *protection* from MDD given that more antidepressant-like behavior is seen in *Trip8b*$^{-/-}$ mice. As nearly all genome wide association studies of psychiatric disease identify genes that may increase the risk of disease, it is unlikely that mutations of TRIP8b would be identified. The TRIP8b-HCN interaction has become the focus of efforts to develop novel antidepressants because of the robust findings linking disruption of TRIP8b-mediated HCN channel trafficking to antidepressant-like behavior [78–80]. The brain-specific expression of TRIP8b makes the interaction between TRIP8b and HCN subunits an attractive drug target that could circumvent the prominent role that HCN channels play in cardiac rhythmicity [81,82].

Epilepsy

HCN channels and TRIP8b have been implicated in several epilepsy subtypes including temporal lobe epilepsy (TLE) and absence epilepsy [83–87]. Animal models of TLE have consistently

demonstrated reduced I_h, hyperpolarization of channel activation, and mislocalization of HCN channels within CA1 pyramidal cells [88–91]. These changes likely increase the propensity of the network to spontaneous seizures as loss of HCN channel function within the CA1 has been linked to increased neuronal excitability, seizure susceptibility, and seizure-related death [7,77,92,93,94]. The exact mechanisms by which HCN1 channel function is lost in the distal dendrites of CA1 pyramidal cells in TLE are unknown. Studies have either reported a global loss of HCN1 expression [90,91,95] or a loss specifically in the distal dendrites that correlates with reduced TRIP8b interaction [89]. Some authors have attributed the loss of HCN1 protein to transcriptional repression [90,96] while others cite post-translational modifications of HCN channels and TRIP8b [54,97]. CaMKII activity is necessary for the DDE of HCN1 channels in CA1 and CaMKII activity is reduced in a rat KA model of TLE [54]. KA-mediated status epilepticus leads to a reduction in phosphorylation of a key serine residue of TRIP8b in a CaMKII consensus sequence, suggesting that this alteration may be mechanistically involved in mislocalization of HCN channels in TLE [54]. Overall, there is evidence suggesting that impaired TRIP8b function explains some of the HCN channel deficits observed in TLE.

Absence seizures are defined by transient staring spells lasting for a few seconds without loss of muscle tone [98,99]. These seizures are accompanied by a characteristic 3 Hz spike and wave discharge on EEG that is thought to represent the cortical correlate of bursting activity in thalamocortical (TC) neurons [99]. I_h is intimately involved in regulating TC activity and the production of absence epilepsy [6,60,98,100,101]. TRIP8b plays a role in scaffolding HCN channels in thalamic neurons, and *Trip8b*$^{-/-}$ animals also have an absence epilepsy phenotype [102]. Both TC neurons and layer 5b corticothalamic neurons have less I_h in *Trip8b*$^{-/-}$ animals and exhibit a corresponding hyperpolarization of the membrane potential [60,102]. In contrast to these two glutamatergic neuron types, loss of TRIP8b did not affect I_h in GABAergic neurons of the reticular thalamic nucleus (RTN) [60,102]. Compared with the severe absence epilepsy phenotype of animals lacking HCN2, the milder absence phenotype of the *Trip8b*$^{-/-}$ animals suggested that TRIP8b-independent HCN2 function in the RTN is sufficient to stabilize the network and substantially reduce absence seizure frequency in these mice [60].

Conclusion

HCN channels have been implicated in a number of other pathophysiological states, including fragile X syndrome [103], neurofibromatosis [104], autism [105], Parkinson's disease [106], Alzheimer's disease [45], and neuropathic pain [107]. As such, a thorough understanding of HCN channel function may have therapeutic implications for a broad number of disorders. To date, pharmacological efforts targeting HCN channels in the CNS have been limited by their expression in cardiac tissue. Therapeutics aimed at altering TRIP8b function may be an attractive strategy by improving specificity and avoiding cardiac effects. By targeting individual TRIP8b isoforms, it may be possible to regulate HCN function in a given cell type with a precision that is not possible by directly targeting the pore-forming subunits themselves.

What remains to be determined are the rules for predicting TRIP8b function from its exons. It is unknown whether the *only* function of TRIP8b(1a-4) is in dendritic enrichment of HCN channels in CA1, or if the same isoform could behave differently in other cell types. Similarly, although TRIP8b(1b-2) has been used as a tool to manipulate CA1 HCN channel trafficking [57], its function and *in vivo* expression pattern remain undefined. Prior work has demonstrated specific roles for functional domains within TRIP8b exons, however, extrapolating these observations may be overly simplistic. AP2-mediated internalization of HCN channels is accomplished by a functional domain in exon 2 [46], but this only reliably occurs in the context of the 1b-2 isoform. These results suggest that the net effect of a given TRIP8b isoform is an emergent property of the entire isoform in a given cellular context.

Alterations in HCN channel function have been implicated in a number of biological processes and it is likely the case that TRIP8b mediates

many of them in the hippocampus. Post-translational modifications of TRIP8b have only recently begun to be investigated although these mechanisms could mediate the association (or dissociation) of the TRIP8b-HCN interaction and explain rapid changes in HCN channel surface trafficking. This could also provide an explanation for the changes in HCN surface expression seen in response to certain pharmacologic agents, including lamotrigine [108] and gabapentin [109]. Although there is much work to be done, understanding the precise role played by TRIP8b in regulating HCN channel function could reveal new therapeutic targets for a range of psychiatric and neurologic disorders.

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