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INVITED REVIEW

Microtubule plus-end tracking proteins: novel modulators of cardiac sodium channels and arrhythmogenesis

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Abstract

The cardiac sodium channel $Na_V1.5$ is an essential modulator of cardiac excitability, with decreased $Na_V1.5$ levels at the plasma membrane and consequent reduction in sodium current (I_{Na}) leading to potentially lethal cardiac arrhythmias. $Na_V1.5$ is distributed in a specific pattern at the plasma membrane of cardiomyocytes, with localization at the crests, grooves, and T-tubules of the lateral membrane and particularly high levels at the intercalated disc region. $Na_V1.5$ forms a large macromolecular complex with and is regulated by interacting proteins, some of which are specifically localized at either the lateral membrane or intercalated disc. One of the $Na_V1.5$ trafficking routes is via microtubules (MTs), which are regulated by MT plus-end tracking proteins (+TIPs). In our search for mechanisms involved in targeted delivery of $Na_V1.5$, we here provide an overview of previously demonstrated interactions between $Na_V1.5$ interacting proteins and +TIPs, which potentially (in)directly impact on $Na_V1.5$ trafficking. Strikingly, +TIPs interact extensively with several intercalated disc- and lateral membrane-specific $Na_V1.5$ interacting proteins. Recent work indicates that this interplay of +TIPs and $Na_V1.5$ interacting proteins mediates the targeted delivery of $Na_V1.5$ at specific cardiomyocyte subcellular domains, while also being potentially relevant for the trafficking of other ion channels. These observations are especially relevant for diseases associated with loss of $Na_V1.5$ specifically at the lateral membrane (such as Duchenne muscular dystrophy), or at the intercalated disc (for example, arrhythmogenic cardiomyopathy), and open up potential avenues for development of new anti-arrhythmic therapies.

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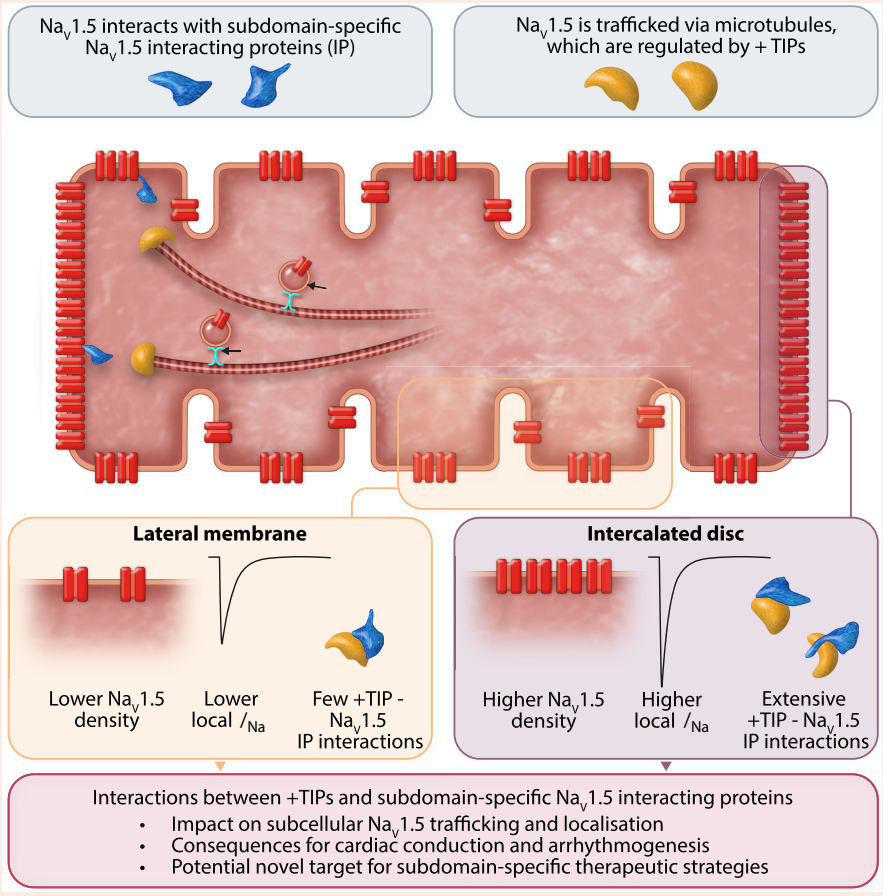
Graphical Abstract



Keywords Cardiac sodium channel • Regulation • Subcellular • Microtubules • Plus-end tracking proteins

1. Introduction

The cardiac isoform of the voltage-dependent sodium channel, Na$_V$1.5 (encoded by the *SCN5A* gene), mediates influx of sodium ions into the myocyte (sodium current, I_{Na}) and is consequently crucial for cardiac excitability and electrical propagation. Dysfunction of Na$_V$1.5, either by inherited disease or acquired secondary to cardiac disease, is linked to a high risk for arrhythmias and sudden cardiac death. Inherited disorders caused by *SCN5A* mutations include long QT syndrome type 3, Brugada syndrome, and cardiac conduction disease.[1] Moreover, *SCN5A* mutations have been identified in patients with arrhythmogenic cardiomyopathy, a disease associated with reduced I_{Na} as well as structural abnormalities, which are driven by disruptions in the adhesion complex at the intercalated discs in cardiomyocytes.[2,3] Research from the last decade has demonstrated that Na$_V$1.5 is heterogeneously distributed within different subcellular domains of cardiomyocytes and is enriched at the intercalated discs. Na$_V$1.5 forms macromolecular complexes with interacting proteins,[4] some of which are specifically present in certain subcellular compartments, conferring subcellular domain-specific modulatory effects on I_{Na}.

One of the modes of Na$_V$1.5 trafficking to the plasma membrane is by microtubules (MTs), which are in turn modulated by MT plus-end tracking proteins (+TIPs). +TIPs have been studied extensively in various cell types including neurones, where they are crucial in the formation of the axon initial segment and Ranvier nodes, which are both highly enriched in ion channels, and play a central role in AP initiation and saltatory conduction, respectively.[5–7] In cardiomyocytes, certain +TIPs have been shown to mediate trafficking of gap junction components and ion channels including Na$_V$1.5.[8–10] We have furthermore demonstrated a modulatory effect of the +TIP end binding (EB) protein EB1 on I_{Na} and cardiac conduction in addition to its involvement in intercalated disc-specific targeting of Na$_V$1.5.[11] More recently, the gene *MAPRE2* encoding end binding protein 2 (EB2) was identified in a genome-wide association study for Brugada syndrome in addition to a functional impact of EB2 on I_{Na} and conduction,[12] further emphasizing the relevance of +TIPs for cardiac electrical (dys)function. Based on these recent observations, we explored existing literature and found that a number of other +TIPs are known to interact with Na$_V$1.5 interacting proteins and hence may also be of potential relevance for targeted Na$_V$1.5 trafficking. While a direct regulatory impact on Na$_V$1.5 has not been experimentally proven for many of these +TIPs, available literature indicates a role for +TIPs in modulating cardiac (electrical) function and ion channel biology. We therefore here review current insight into the interplay between Na$_V$1.5, interacting proteins, and +TIPs, their putative role in trafficking and distinct subcellular localization of Na$_V$1.5 in cardiomyocytes, and the potential arrhythmogenic and therapeutic implications of this 'exciting' interplay of proteins.

2. Na$_V$1.5 structure and function

Na$_V$1.5 consists of a cytoplasmic N-terminus, four transmembrane domains (DI—DIV), which are connected by cytoplasmic linkers, and a

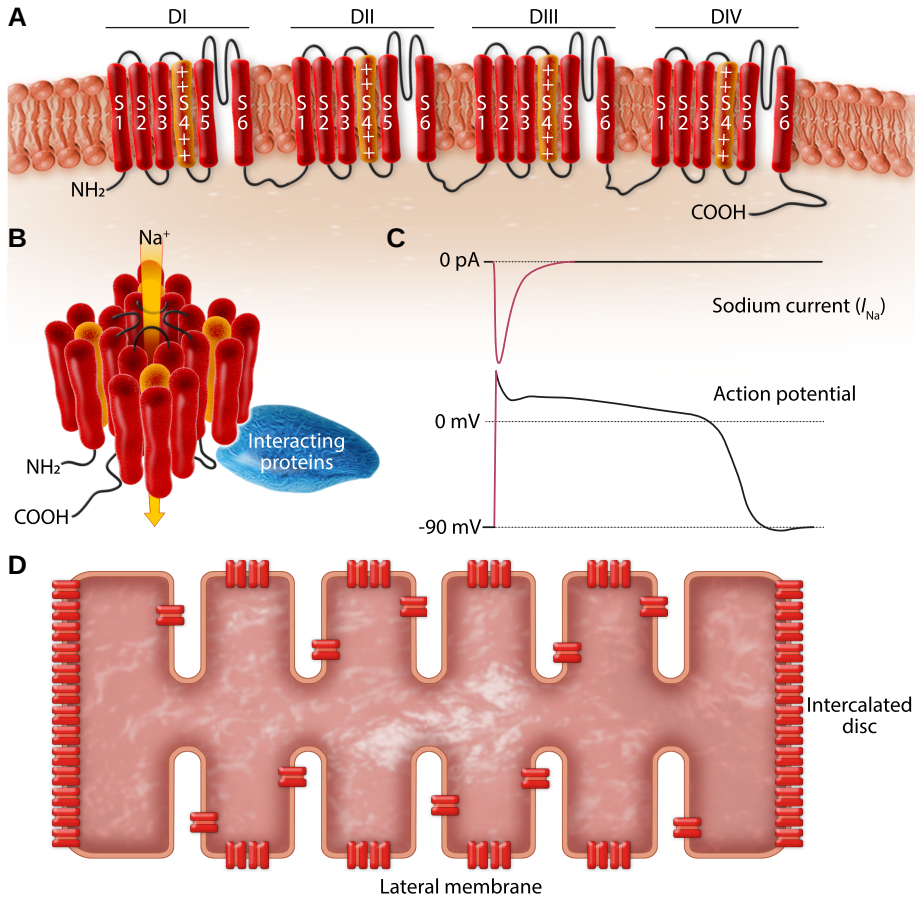


Figure 1 $Na_V1.5$ structure, function, and cellular distribution. (*A*) Protein structure of the α-subunit of the cardiac sodium channel, $Na_V1.5$, encoded by *SCN5A*, and (*B*) visualization of the 3D structure and binding of interacting proteins. (*C*) I_{Na} modulated by $Na_V1.5$ (top panel) and its effect on the action potential in ventricular cardiomyocytes (upstroke phase, bottom panel). (*D*) Schematic visualization of $Na_V1.5$ localization in adult cardiomyocytes, with localization at the lateral membrane and enrichment at the intercalated discs.

cytoplasmic C-terminal domain (*Figure 1A*).[13] Each transmembrane domain contains six segments (S1–S6), which are linked by extracellular and cytoplasmic loops. Of these segments, S1–S4 contain the voltage sensing domain. $Na_V1.5$ folds into a 3D structure creating a transmembrane pore, which forms a macromolecular complex with interacting proteins (*Figure 1B*). Upon small depolarization of the plasma membrane, the highly-charged S4 segment moves towards the extracellular space, opening the pore and allowing the passage of ions through the channel.[14] Segments S5 and S6 form the channel's pore and are connected by the extracellular P-loops, which act as an ion selectivity filter allowing only sodium ions to pass through the channel.[15] Upon depolarization, the channel activates (opens) quickly and typically inactivates (closes) within 1 ms allowing a brief, but large influx of sodium (peak I_{Na}) and thereby mediating the upstroke of the action potential (*Figure 1C*). Reduction of peak I_{Na} decreases upstroke velocity of the action potential and can lead to conduction slowing, which may result in cardiac arrhythmias and sudden cardiac death.[16,17] After activation, $Na_V1.5$ inactivation is mediated by the intracellular loop connecting DIII and DIV, the extracellular S5–S6 loop, and the C-terminal domain of the channel. While peak I_{Na} is typically brief due to its fast activation and inactivation, incomplete channel inactivation may allow for a 'persistent' or 'late' sodium current ($I_{Na,L}$). This $I_{Na,L}$ is small in physiological conditions but is enhanced in pathophysiological conditions, leading to persistent entry of sodium into the cell and consequently a prolonged action potential duration.[18,19] These electrogenic alterations in $Na_V1.5$ function can occur secondary to acquired disorders or due to mutations in either *SCN5A* or in genes modulating *SCN5A* expression or $Na_V1.5$ function, such as genes encoding $Na_V1.5$ interacting proteins, as previously reviewed.[20,21] Recent advances in cryogenic electron microscopy have allowed for investigation of $Na_V1.5$ structure in miniscule detail, providing novel insights into ion selectivity filter and inactivation gate structure, voltage-dependent activation, antiarrhythmic drug action, mutation sites, and arrhythmia mechanisms.[22]

3. Subcellular distribution of $Na_V1.5$ in cardiomyocytes

Individual cardiomyocytes are coupled tightly to neighbouring cardiomyocytes in both a structural and electrical fashion, which is mainly mediated by the short ends of cardiomyocytes, also known as the intercalated discs. Classically, intercalated discs were considered to consist of three major structures: desmosomes and adherens junctions achieving mechanical coupling, and gap junctions allowing electrical coupling. However, as reviewed in detail elsewhere,[23] these segments are highly interlinked and cooperate rather than being isolated components serving distinct purposes. Meanwhile, the long ends of cardiomyocytes, or lateral membrane, are also tightly mechanically coupled, creating a complex 3D structure.[24]

Moreover, similar to the intercalated discs, the lateral membrane also plays a role in the propagation of electrical potential.[25] The lateral membrane is structured by a repeated pattern of crests which align with contractile elements, and grooves which align with Z-discs and contain T-tubules.[26]

At the plasma membrane of cardiomyocytes, $Na_V1.5$ displays a specific distribution, with localization at the crests, grooves, and T-tubules of the lateral membrane, as well as at the intercalated discs (*Figure 1D*).[26,27] Intriguingly, differential I_{Na} has been described at the intercalated discs and lateral membrane, with larger I_{Na} at the intercalated discs.[28] Moreover, biophysical properties of I_{Na}, as well as $Na_V1.5$ cluster organization differ between the intercalated disc and lateral membrane.[10] These differences in $Na_V1.5$ properties have been suggested to be driven, at least in part, by interactions with subcellular domain-specific interacting proteins, regulating both $Na_V1.5$ function and subcellular domain-specific localization (*Table 1*).[10,29,30] Indeed, interacting proteins can affect $Na_V1.5$ functional properties (i.e. current amplitude, gating properties) through interactions and/or by inducing $Na_V1.5$ post-translational modifications, as described in detail in previous reviews.[72,73] An additional explanation for this specific localization of $Na_V1.5$ and enrichment at the intercalated discs lies in the possibility that $Na_V1.5$ interacting proteins regulate $Na_V1.5$ trafficking towards specific locations. Indeed, dysfunction of lateral membrane- or intercalated disc-specific $Na_V1.5$ interacting proteins leads to loss of $Na_V1.5$ localization at the site of the dysfunctional protein and are associated with cardiac arrhythmias. For instance, loss of $Na_V1.5$ at the intercalated discs is observed in arrhythmogenic cardiomyopathy secondary to intercalated disc-specific PKP2 dysfunction, while loss of dystrophin at the lateral membrane in Duchenne muscular dystrophy leads to loss of $Na_V1.5$ specifically at this microdomain.[26,37,52] As discussed in more detail below, trafficking of $Na_V1.5$ to distinct subcellular domains may furthermore be regulated by proteins that impact on the MT network, the predominant trafficking pathway by which ion channels reach the cell membrane.

4. MT-dependent $Na_V1.5$ trafficking in cardiomyocytes

The functional channel protein turnover time (half-life) of $Na_V1.5$ is around 35 h,[74] necessitating constant trafficking of newly synthesized $Na_V1.5$ towards the membrane (anterograde trafficking) as well as internalization of $Na_V1.5$ from the membrane (retrograde trafficking). As reviewed previously,[75,76] like most ion channels, $Na_V1.5$ is synthesized and assembled in the endoplasmic reticulum (ER), after which it is transported to the Golgi apparatus. There, $Na_V1.5$ is further processed, and ultimately targeted to the plasma membrane, predominantly via MT-dependent mechanisms.[77]

MTs, which are part of the cytoskeleton, are long, hollow tubes with a diameter of approximately 25 nm composed of α-tubulin and β-tubulin heterodimers. MTs mainly originate from MT-organizing centres, structures rich in γ-tubulin ring complexes, which embed the base of the MT, the MT minus-end.[78] The major MT-organizing centre in cardiomyocytes is located around the nuclear envelope and co-localizes with the Golgi apparatus.[79,80] Apart from the main Golgi apparatus surrounding the nucleus, additional small Golgi elements are scattered around the cell, which also act as MT-organizing centres.[81] Hence, the MT-organizing centres at the Golgi apparatus and Golgi elements allow distribution of proteins across the cell via MTs. The MT minus-end is relatively static, and MT dynamic behaviour is therefore largely restricted to the other end of the MT: the MT plus-end. This highly dynamic structure constantly switches between states of growth and shrinkage, which is known as dynamic instability. The switch of shrinkage to growth is known as rescue, while the switch from growth to shrinkage has been termed catastrophe. Dynamic instability is regulated by MT associated proteins, and in particular by +TIPs, which localize at the MT plus-end.[78] Changes in MT dynamics may have significant impact on the trafficking of proteins to and from the plasma membrane.[82]

Long-range intracellular transport across MTs is mediated by motor proteins, which carry cargo vesicles containing cellular components including various ion channels.[83] Two superfamilies of MT motor proteins exist, which generally move in opposite directions, with kinesins primarily going from the MT minus-end to the plus-end,[84] and dyneins moving from the plus-end to the minus-end.[85] Since trafficking across MTs occurs in both the plus-end and minus-end direction, MTs are vital in both delivery of newly formed cellular components as well as internalization of the latter prior to degradation. Hence, as visualized in *Figure 2A*, trafficking of proteins over MTs is regulated by various modulators, including (i) motor proteins carrying cargo in different directions and at various speeds, (ii) proteins connecting to the MT minus-end, stabilizing the MT and acting as a base, and (iii) +TIPs which regulate MT dynamics and interact with proteins from certain structures, allowing plus-end directed vesicles to be delivered at the right cellular domain. For targeted delivery of proteins such as $Na_V1.5$ to specific subcellular domains in cardiomyocytes, the third described manner of trafficking regulation by +TIPs is likely most relevant.

5. +TIPs: regulators of MT-dependent trafficking

+TIPs regulate MT dynamics as well as MT interactions, thereby representing crucial modulators of MT-dependent trafficking. Binding of specific +TIPs promotes MT stabilization, reducing MT catastrophe and promoting MT growth.[78,86] Moreover, +TIPs can act as a bridge between MTs and other proteins or cellular structures, thereby facilitating MT anchoring at specific targets and allowing delivery of cargo carried across the MT.[78] Indeed, interactions between +TIPs and proteins at the plasma membrane have been shown to be essential in the localized delivery of transmembrane proteins,[87] and these interactions are therefore of potential relevance for $Na_V1.5$ trafficking to the plasma membrane. Still, while the impact of +TIPs and interactions with other proteins on MT dynamics, cell development, control of cell polarity, signalling, and targeted delivery of ion channels has been explored extensively in neurones,[88] the exact role of these +TIPs in trafficking of cardiac ion channels is largely unknown. As shown in *Table 2*, various +TIPs are expressed in cardiac tissue. Drawing from knowledge from the neurology field and the association of certain +TIPs with cardiac disease, we can speculate about the impact of +TIPs on cardiac ion channel trafficking, and $Na_V1.5$ in particular. Specifically, we here propose a mechanism in which interactions between +TIPs and subcellular domain-specific $Na_V1.5$ interacting proteins regulate MT dynamics and anchoring, allowing for targeted delivery of $Na_V1.5$ (*Figure 2B*). As discussed below, a number of different +TIPs exist, some of which have been shown to impact on $Na_V1.5$ and/or cardiac electrical (dys)function, while for others such a modulatory role may be hypothesized based on available information.

5.1 EB proteins

In mammals, the EB protein family consists of three proteins: EB1, EB2, and EB3,[119] which bind directly to the plus-end of MTs via a calponin homology (CH) domain.[94,120] In neurones, EB1 is essential for the targeted delivery of potassium and sodium channels at the axon initial segment and Ranvier nodes.[5,121,122] Similarly, EB1 has been shown to modulate the delivery of Cx43 to the adherens junctions in the intercalated discs of cardiomyocytes,[87] thereby impacting on cell adhesion.[8] EB proteins are considered autonomous +TIPs in that they accumulate at the ends of growing MTs independently of other proteins. However, by doing that they generate a platform at MT ends to which other +TIPs can bind and exert their function. As such, EB1 plays a central role in the interaction between MT plus-end and +TIPs, with many +TIPs binding to MT ends via a serine-x-isoleucine-proline (SxIP) motif in the C-terminal domain of EB1 (the EB-homology domain).[123] We recently identified EB1 as a modulator of $Na_V1.5$, with EB1 overexpression promoting $Na_V1.5$ forward trafficking in HEK293 cells.[11] Furthermore, as shown in *Figure 3*, in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) EB1

Table 1 **$Na_V1.5$ interacting proteins and their localization in adult cardiomyocytes as described in previous research**

Gene	Protein	Cellular domain	References
SCN1B	β1 (non-phosphorylated); pYβ1 (tyrosine-phosphorylated)	Lateral membrane; Intercalated discs	31–33
SCN2B	β2	Intercalated discs	33–36
SCN3B	β3	Lateral membrane	35,36
SCN4B	β4	atrium: lateral membrane, ventricle: intercalated discs	35,36
SNTA1	α1-syntrophin	Lateral membrane	37
SNTB1	β1-syntrophin	Lateral membrane	37
CASK	Calcium/calmodulin-dependent serine protein kinase (CASK)	Lateral membrane	38
ANK2	Ankyrin-B	Lateral membrane	31
ACTN2	α-actinin-2	Z-discs, intercalated discs	39,40
TCAP	Telethonin	Z-discs, intercalated discs	41
LDB3	Z-band alternatively spliced PDZ motif protein (ZASP)	Z-discs, intercalated discs	42
CAV3	Caveolin-3	Lateral membrane, intercalated discs	43,44
FGF13	Fibroblast growth factor 13 (FGF13)	Lateral membrane, intercalated discs	45,46
ANK3	Ankyrin-G	Lateral membrane, intercalated discs	47–50
SPTBN4	β_{IV}-spectrin	Intercalated discs	51
GJA1	Connexin 43	Intercalated discs	10,31
PKP2	Plakophilin-2	Intercalated discs	30,52
DSG2	Desmoglein-2	Intercalated discs	53
CDH2	N-cadherin	Intercalated discs	31,54
DCTN2	Dynactin subunit 2 (p50/dynamitin)	Intercalated discs	55,56
SAP97	SAP97; DLG1	Intercalated discs	29,57
CXADR	Coxsackie and adenovirus receptor (CAR)	Intercalated discs	58
YWHAH	14-3-3η	Intercalated discs	59,60
MOG1	MOG1	Intercalated disc-enriched	61,62
CAMK2D	CaMKIIδc	Intercalated disc-enriched	51,63
NEDD4L	Nedd4-2/Nedd4-like	Cytoplasm	64,65
CALM	Calmodulin	Cytoplasm	66,67
FGF12	Fibroblast growth factor 12 (FGF12); fibroblast homologous factor 1B (FHF1B)	Unknown	68,69
GPD1L	Glycerol-3-phosphate dehydrogenase 1-like	Unknown	70
PTPH1	Protein tyrosine phosphatase H1	Unknown	71

overexpression increased I_{Na} and enhanced $Na_V1.5$ localization at the plasma membrane, while knockout of *MAPRE1* (encoding EB1) decreased I_{Na}.[11] In addition, acute knockout of the *MAPRE1* homologue (*mapre1b*) in zebrafish resulted in decreased ventricular conduction velocity (*Figure 3*),[11] underlining the functional importance of EB1. EB1 and EB3 are structurally and functionally similar, and both promote MT dynamicity.[124,125] Together with EB1, EB3 is involved in maintaining the axon initial segment in neurones and has interacting proteins in common with EB1, demonstrating functional overlap between EB1 and EB3.[122,124] However, evidence for a functional impact of EB3 in cardiomyocytes is (as yet) lacking.

While EB1 and EB3 are functionally similar, EB2 is considered both structurally and functionally different,[125] playing an essential role in the regulation of mitosis and MT reorganization upon cell differentiation.[126,127] However, recent studies have also highlighted a functional overlap between EB1 and EB2, revealing that anterograde trafficking of the Ca^{2+}-activated non-selective cationic channel transient receptor potential melastatin 4 (TRPM4) is regulated by both EB1 and EB2.[128] *TRPM4* mutations have been associated with cardiac conduction disorders and Brugada syndrome, and *Trpm4*-deficient mice display ventricular conduction slowing and reduced I_{Na}.[129] More recently, a genome wide association study (GWAS) performed by our group identified an association between *MAPRE2* (encoding EB2) and Brugada syndrome, and further exploration established that the Brugada syndrome risk allele was associated with lower *MAPRE2* expression in ventricular tissue.[12] Moreover, ventricular conduction slowing in zebrafish and reduced I_{Na} in hiPSC-CMs was observed upon *MAPRE2*/EB2 knock-out (*Figure 3*). These findings not only indicate that similar to EB1, EB2 impacts on $Na_V1.5$ trafficking in cardiomyocytes but also identify MT-dependent trafficking as a potential new mechanism underlying Brugada syndrome with a particular role for EB proteins and possibly also other +TIPs.

5.2 Cytoplasmic linker associated proteins and interacting proteins

Cytoplasmic linker associated protein 1 and −2 (CLASP1/2) are +TIPs that promote MT growth and stabilization and are required for cell polarization, division, and migration.[91] While CLASPs can bind to MTs directly via tumor overexpressed gene-like (TOGL)-domains, their binding to MT ends is significantly enhanced via interaction with EB1.[92] Crucially, CLASPs suppress MT catastrophe and promote stabilization independently, but this function is enhanced when CLASP interacts with EB1.[93] Both CLASP1 and CLASP2 are regulated by glycogen synthase kinase 3β (GSK3β), a constitutively active kinase which phosphorylates CLASPs, thereby decreasing interactions between CLASPs and their interacting partners EB1 and MTs.[130] Hence, in areas where GSK3β is locally inactivated, CLASPs are dephosphorylated and become potent MT end stabilizing proteins.[131] In neurones, CLASP2 regulates cell polarity and is a key regulator of axon and dendrite outgrowth, as well as synapse formation.[132] We recently demonstrated that modulation of interactions between CLASP2, EB1, and MTs by pharmacological GSK3β inhibition impacts on $Na_V1.5$ and I_{Na} in mouse

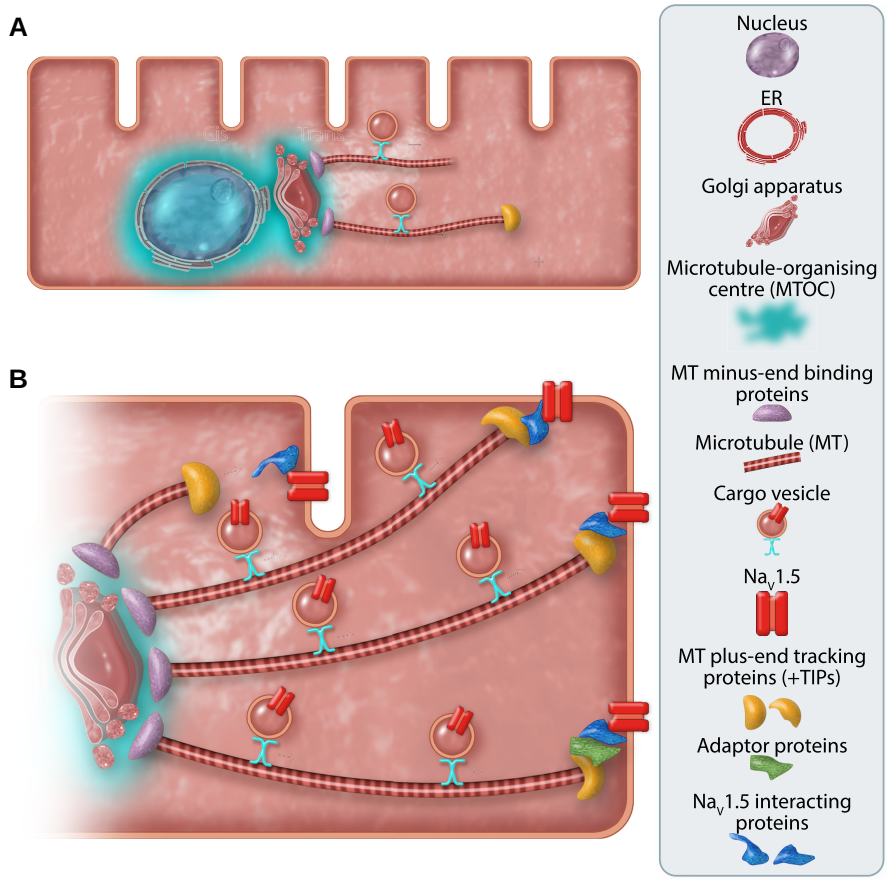


Figure 2 MT-dependent trafficking and proposed mechanism of targeted delivery of $Na_V1.5$. (*A*) Schematic overview of MT-dependent anterograde trafficking and regulators of MTs. From left to right: The nucleus is surrounded by the ER, and the Golgi apparatus. The MT-organizing centre co-localizes with Golgi apparatus and contains the MT minus-EB proteins, which stabilize the minus-end (−) of MTs. Vesicles containing cargo originating from the Golgi apparatus can be loaded on MTs and transported to the MT plus-end (+) by motor proteins. MTs are regulated by +TIPs, which bind the MT plus-end, controlling MT dynamics and stability, as well as allowing for interactions to mediate cargo delivery. (*B*) Proposed mechanism regulating $Na_V1.5$ targeted delivery by associations between +TIPs and $Na_V1.5$ interacting proteins. $Na_V1.5$ interacting proteins bait growing MTs through attracting +TIPs, and allow MT stabilization upon interacting with +TIPs, promoting $Na_V1.5$ delivery. These $Na_V1.5$ interacting proteins differ between cardiomyocyte subcellular domains (i.e. lateral membrane, intercalated discs), attracting specific +TIPs to these distinct locations. Associations between $Na_V1.5$ interacting proteins and +TIPs can arise from direct binding, or indirect interactions via adaptor proteins.

cardiomyocytes (*Figure 4*).[11] Moreover, this effect of GSK3β inhibition was absent in cardiomyocytes from *Clasp2*-deficient mice, demonstrating that CLASP2 or CLASP2-EB1 interactions modulate $Na_V1.5$ trafficking to the intercalated discs.[11] Conversely, although an interplay of CLASP1 and −2 has been shown to be crucial for polarized trafficking in motile epithelial cells,[133] the role of CLASP1 in ion channel trafficking remains to be explored.

CLASPs were named CLIP-associated proteins because of their ability to interact with CAP-Gly domain container linker protein (CLIP)-1 and −2 (also known as CLIP-170 and −115, respectively). Although both CLIPs contain MT-binding glycine-rich CAP-Gly domains, and therefore interact with MTs directly,[134] CLIP1 was found to track the MT plus-end largely in an EB1-dependent manner.[94,135] Similar to CLASPs, CLIPs appear to promote MT rescue, albeit via a different mechanism.[134] Importantly, CLIP1 binding to the MT plus-end is regulated by AMP-activated protein kinase (AMPK), which phosphorylates CLIP1 reducing its affinity for the MT plus-end.[136] In cardiomyocytes, phosphorylated (active) AMPK is mainly present at the intercalated discs, and controls cell size and shape; AMPK is furthermore known to modulate ion channels and transporters.[137] Moreover, inhibition of CLIP1 phosphorylation by AMPK resulted in cardiac dysfunction, myocardial fibrosis, dilated cardiomyopathy, and cardiomyocyte elongation,[138] emphasizing the importance of this interplay between CLIP1 and AMPK for normal cardiac function. Still, the role of CLIP1 in regulation of protein trafficking remains largely unclear. Interestingly though, BIN1, a modulator of tubule formation and positioning of the L-type calcium channel (LTCC) at the T-tubules of cardiomyocytes,[139] interacts with CLIP1.[95] As such, CLIP1 may prove to be a modulator of LTCC trafficking to the T-tubules at the lateral membrane of cardiomyocytes. Hence, CLIP1 interacts with various proteins at both the lateral membrane and intercalated discs of cardiomyocytes, and as such may potentially regulate ion channel trafficking; however, a connection to $Na_V1.5$ can as yet not be made.

5.3 Adenomatous polyposis coli and associated proteins

Adenomatous polyposis coli (APC) is a tumour suppressor gene which is a negative regulator of Wnt/β-catenin signalling, and is involved in embryonic cardiac development.[97] APC can form a multiprotein complex with Axin, GSK3β, and β-catenin; a complex known as the β-catenin destruction

Table 2 Microtubule plus-end tracking proteins (+TIPs) present in cardiac tissue, and their localization in adult cardiomyocytes as described in previous research. ER: endoplasmic reticulum, EB1: co-localization with end binding protein 1.

Gene	Protein	Cellular domain	Reference
MAPRE1	End binding 1 protein (EB1)	Golgi, intercalated discs, +TIP	9,89
MAPRE2	End binding 2 protein (EB2)	+TIP	89
MAPRE3	End binding 3 protein (EB3)	Golgi, T-tubules/Z-discs, +TIP	89,90
CLASP1	Cytoplasmic linker associated protein 1	+TIP	91,92
CLASP2	Cytoplasmic linker associated protein 2	+TIP	92,93
CLIP1	CAP-Gly domain containing linker protein 1; CLIP-170	T-tubules, +TIP	94,95
CLIP2	CAP-Gly domain containing linker protein 2; CLIP-115	+TIP	96
APC	Adenomatous polyposis coli	+TIP	97
DCTN1	Dynactin subunit 1; $p150^{Glued}$	ER, +TIP	98
CDK5RAP2	CDK5 regulatory subunit associated protein 2	Centrosome, Golgi, +TIP	99,100
PDE4DIP	Phosphodiesterase 4D interacting protein; myomegalin	Centrosome, ER, Golgi, +TIP	101
CKAP5	Cytoskeleton associated protein 5; Ch-TOG	+TIP	102
SLAIN2	SLAIN motif family member 2	+TIP	103
CENPF	Centromere protein F (CENP-F)	+TIP	104
PPP1R13L	Protein phosphatase 1 regulatory subunit 13 like (iASPP)	+TIP, intercalated discs	105–107
MACF1	Microtubule-actin crosslinking factor 1 (ACF7)	Golgi, +TIP	108,109
DST	Dystonin (MACF2, BPAG1)	+TIP, Z-discs, intercalated discs	110
TRIO	Rho guanine nucleotide exchange factor TRIO	+TIP	111
NAV1	Neurone navigator 1	+TIP	111,112
NAV2	Neurone navigator 2	+TIP	111
STIM1	Stromal interaction molecule 1	ER, Z-discs, +TIP	113,114
TTBK2	Tau tubulin kinase 2	+TIP	115
MTUS2	Microtubule associated scaffold protein 2 (Tip150, KIAA0774)	+TIP	116
KIF5B	Kinesin-1	EB1, intercalated discs, +TIP	9
KIF17	Kinesin-2	EB1, +TIP	117
KIF3A/B	Kinesin family member 3A/B	+TIP	118

complex, which localizes at the adherens junctions in epithelial cells and is regulated by E-cadherin.[140] While the localization of the β-catenin destruction complex in cardiomyocytes remains to be discovered, disruption of the Wnt/β-catenin pathway has been linked to loss of I_{Na} and arrhythmogenic cardiomyopathy, a disease which mainly affects the intercalated discs of cardiomyocytes.[3,141] In addition, human and hamster hypertrophic hearts, β-catenin was increased at the intercalated discs of cardiomyocytes, likely mediated by concomitant changes in APC and/or GSK3β.[142] This accumulation of β-catenin at the intercalated discs is thought to impact on cell-cell adhesion and affect transcription.[142] Indeed, cytosolic β-catenin is able to translocate to the nucleus and restrict activation of target genes, and hence alterations in β-catenin during pathophysiological conditions may increase transcription of pro-hypertrophic and pro-adipogenic genes.[143] In addition to its effects on β-catenin, APC interacts with MTs and the other +TIPs EB1 and CLIP1, promoting MT stability and potentially affecting trafficking.[144,145] While no studies have investigated the effects of APC on ion channel trafficking, it is conceivable that alterations in APC may affect (the interaction between) GSK3β and β-catenin, and consequently impact on GSK3β accumulation at the intercalated discs which in turn may regulate $Na_V1.5/I_{Na}$ within this subcellular domain, as has been demonstrated in the setting of arrhythmogenic cardiomyopathy.[11,146]

5.4 Auxiliary EB1-dependent +TIPs

As mentioned previously, most +TIPs are dependent on EB1 for their accumulation at the end of growing MTs. One of these is MT-associated scaffold protein 2 (MTUS2), which regulates MT dynamics by facilitating plus-EB of the MT depolymerase mitotic centromere-associated kinesin (MCAK; also known as KIF2C).[116] Interestingly, MTUS2 interacts with the actin-binding and cytoskeletal protein cortactin (CTTN),[147] which in turn interacts with the potassium channel $K_V1.5$ (conducting the ultra-rapidly activating delayed rectifier K^+ current, I_{Kur}) at the sarcolemma of cardiomyocytes, and is required for regulation of $K_V1.5$ by N-cadherin.[148] Since N-cadherin is specifically localized at the intercalated discs of cardiomyocytes, where it interacts with $Na_V1.5$,[54] it is conceivable that MTUS2 also modulates intercalated disc-specific $Na_V1.5$ trafficking. However, the impact of altered MTUS2 or its interacting partner CTTN on $Na_V1.5$ has as yet not been explored.

Other potential regulators of $Na_V1.5$ trafficking include CDK5 regulatory subunit-associated protein 2 (CDK5RAP2), which is mainly localized at Golgi-based MT-organizing centres,[99] and additionally tracks MT plus-ends in a EB1-dependent manner, thereby promoting MT growth, stability, and bundling.[100] A paralogue of CDK5RAP2, phosphodiesterase 4D interacting protein (PDE4DIP; also known as myomegalin), has been shown to control MT organization as well as being essential for MT growth.[149] PDE4DIP forms a complex with myopodin, α-actinin, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and A-kinase anchoring proteins (AKAPs).[150] Specifically, isoform 8 of PDE4DIP is required for proper Golgi organization, and interaction between this isoform and EB1 was necessary to facilitate ER-to-Golgi trafficking.[101] On the other hand, another isoform named 'SMYLE' forms a complex with EB1, CDK5RAP2 and A-kinase anchoring protein 9 (AKAP9), and promotes MT assembly and stabilization at the cell periphery in an EB1-dependent manner.[151] Therefore, CDK5RAP2 together with the 'SMYLE' isoform of PDE4DIP appears potentially relevant for modulation of trafficking of proteins to the plasma membrane.

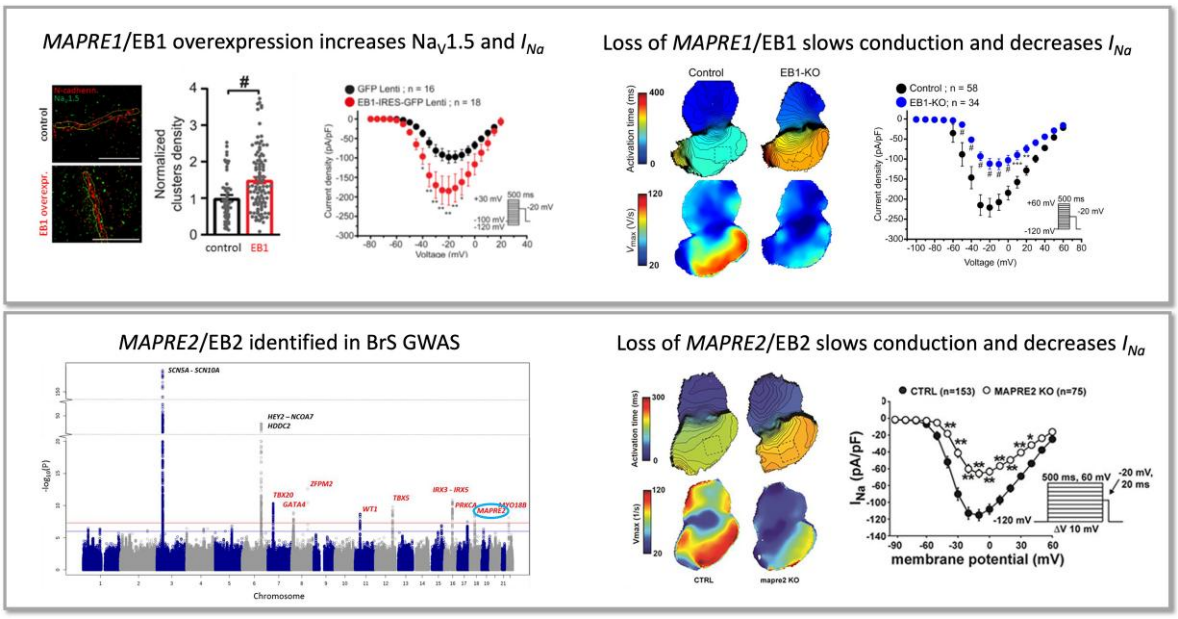


Figure 3 Impact of the MT plus EB proteins EB1 and EB2 on $Na_V1.5$, I_{Na}, and cardiac conduction. Top panel: Lentiviral transduction of EB1 in hiPSC-derived cardiomyocytes (hiPSC-CM) increased membrane $Na_V1.5$ cluster density and I_{Na} (left), whereas knockdown of *Mapre1*/EB1 in zebrafish and hiPSC-CM induced cardiac conduction slowing and reduced I_{Na}, respectively (right). Reproduced from,[11] with permission. Lower panel: *MAPRE2*/EB2 was identified in a GWAS for Brugada syndrome (BrS) (left), and knockdown of *Mapre2*/EB2 in zebrafish and hiPSC-CM-induced cardiac conduction slowing and reduced I_{Na}, respectively (right). Reproduced from,[12] with permission.

Stromal interaction molecule-1 (STIM1) is a calcium-sensing transmembrane protein localized on the ER membrane and binds the MT plus-end in an EB1-dependent manner.[113] In cardiomyocytes, STIM1 is involved in Ca^{2+} homeostasis, thereby also impacting on cardiac function and focal adhesion turnover.[114] Moreover, STIM1 is an activator of the Ca^{2+}-dependent calcineurin-NFAT pathway, thereby regulating cardiomyocyte hypertrophy.[152] Recently, it was demonstrated that inducible cardiomyocyte-specific STIM1 knockdown in adult mice resulted in conduction slowing and increased cardiac arrhythmias, while $Na_V1.5$ expression was in fact increased.[153] Phospho-CaMKII upregulation was also observed in these hearts; although $I_{Na}/I_{Na,L}$ was not investigated, it is possible that the observed conduction slowing despite $Na_V1.5$ upregulation may be explained by enhanced $I_{Na,L}$ and dysfunctional Ca^{2+} handling leading to reduced $Na_V1.5$ availability and impaired connexin conductance.

Another EB1-dependent +TIP of interest is inhibitor of apoptosis stimulating protein of P53 (iASPP), encoded by protein phosphatase 1 regulatory subunit 13 like (*PPP1R13L*), whose deficiency leads to an autosomal recessive cardio-cutaneous syndrome associated with lethal dilated cardiomyopathy in various animal models,[154] as well as in humans.[155] iASPP is highly present at the intercalated discs of cardiomyocytes, where it interacts with the desmosomal proteins desmoplakin (DSP) and desmin, and regulates their localization at the intercalated discs.[105] Strikingly, iASPP dysfunction resulted in right-ventricular dilatation and an arrhythmogenic cardiomyopathy phenotype in a murine model, while iASPP levels were reduced at the intercalated discs of cardiomyocytes from arrhythmogenic cardiomyopathy patients.[105] Therefore, iASPP appears to be a major regulator of the cardiac desmosome, regulating desmosomal protein localization as well as potentially other intercalated disc-specific proteins, including for instance $Na_V1.5$ (which is reduced at the intercalated discs in arrhythmogenic cardiomyopathy). In fact, iASPP interacts with EB1 and EB3 via a SxIP motif,[106] and a recent paper identified a complex of iASPP, Myosin-Ic (Myo1c), and EB1 which contributes to MT capture at the cell cortex.[107] Therefore, it can be speculated that iASPP contributes to intercalated disc-specific targeting via the MT network, but data on the impact of iASPP on MT-dependent trafficking are currently lacking.

5.5 +TIPs containing spectrin (like) repeats

Several MT-associated proteins are part of the spectrin superfamily, proteins that serve as a link between cytoskeletal elements, the nucleus, and the cell membrane which are characterized by the presence of spectrin repeats; domains composed of three α-helices.[156] As these proteins are generally very large (100–600 kD) and under great mechanical strain, spectrin repeats are considered to function as flexible modules that allow maintenance of structural integrity. Additionally, spectrin repeats allow interactions with the cytoskeleton, signalling proteins, and potentially transmembrane proteins.[157] The spectrin superfamily includes proteins such as α-actinin and dystrophin, which are known to modulate $Na_V1.5$ (as further discussed below). In particular, syntrophin binds directly to the spectrin-like domain of dystrophin, forming a large macromolecular complex which also contains $Na_V1.5$.[158,159] Another subclass is formed by the spectraplakins, which are able to interact with intermediate filaments, F-actin, and MTs, and function as cytoskeletal crosslinkers. Examples of spectraplakins expressed in heart include DSP, MT-actin cross-linking factor 1 (MACF1), and dystonin (DST), of which MACF1 and DST have been found to function as +TIP. In mice, an important role for MACF1 in cardiac adaptation to pressure overload has been described, with maladaptive MT redistribution in the setting of dysfunctional MACF1.[160] Additionally, MACF1 was shown to accumulate at the MT plus-end, guiding MTs along actin bundles to the cell cortex, as well as promoting MT stability in endodermal cells.[108] Moreover, in skin cells, MACF1 co-immunoprecipitated with the +TIPs EB1, CLASP1, and CLASP2 and appeared to regulate MTs targeting to focal adhesion complexes,[109] which are mainly located at the intercalated discs of cardiomyocytes. However, information about subcellular localization of MACF1 in cardiomyocytes is currently lacking. In contrast, the subcellular localization of DST has been studied in detail in hearts of various species,

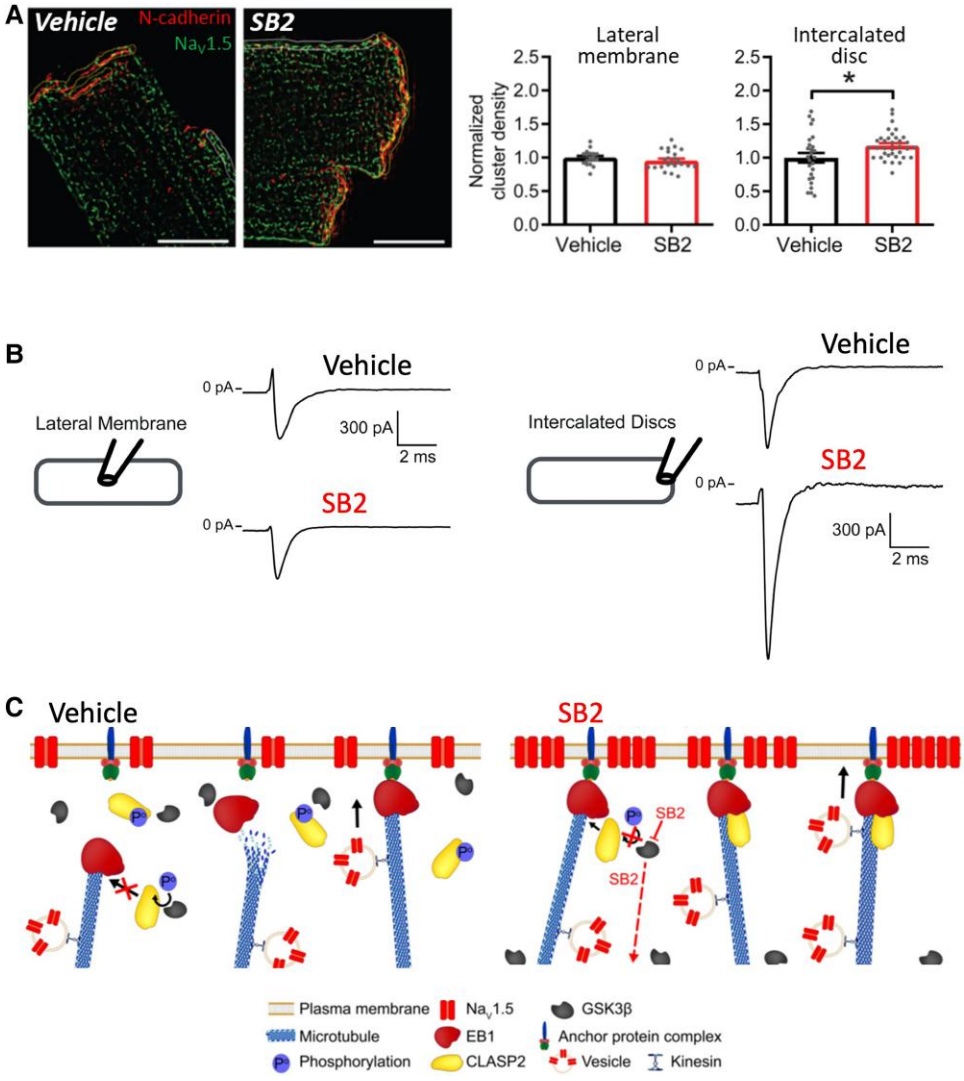


Figure 4 Subdomain-specific modulation of I_{Na} and $Na_V1.5$. In mouse cardiomyocytes, the compound SB216763 (SB2) increased $Na_V1.5$ cluster density (*A*) and I_{Na} (*B*) at the intercalated discs but not at the lateral membrane. (*C*) SB2 prevents GSK3β-mediated CLASP2 phosphorylation, thereby enhancing EB1-CLASP2-MT interactions, resulting in increased $Na_V1.5$ delivery at the intercalated discs. Redrawn from,[11] with permission.

where DST localized in a cross-striated pattern and at the intercalated discs.[110] Similar to MACF1, DST interacts with EB1, but also with EB3, and thereby modulates vesicular transport across MTs in myoblasts.[158] Deficiency of DSTin mice has been shown to increase *Anf* and decrease *Serca2a* expression, indicative of cardiac remodelling.[159] As both MACF1 and DST interact with MTs and intercalated disc-enhanced proteins, they represent a potential target for the modulation of MT-dependent ion channel trafficking in cardiomyocytes.

Another spectrin repeat-containing protein present in cardiac tissue is the Trio Rho guanine nucleotide exchange factor (TRIO), which is a +TIP through interaction with EB1 and is required for neurite outgrowth.[111] Importantly, this function of TRIO was regulated by neurone navigators (NAVs); SxIP motif-containing +TIPs which can interact with MTs directly and via EB1, and which interact with TRIO at the MT plus-end.[111] However, despite the fact that TRIO, NAV1, and NAV2 are expressed in the human heart, there are no reports on their impact of cardiac physiology or protein trafficking. In contrast, centromere-binding protein F (CENP-F), a large protein containing various protein-binding domains including a spectrin repeat,[161] has been shown to be relevant for cardiac pathology. Apart from its involvement in mitosis, CENP-F binds both polymerizing and depolymerizing MTs, is a +TIP, and couples cargo to MTs.[104] Additionally, CENP-F modulates MT-based trafficking through interactions with syntaxin 4 and SNAP25, promoting cell coupling in fibroblasts, indicating increased connexin localization at the cell membrane.[162] In mice, loss of CENP-F is associated with dilated cardiomyopathy, disruption of Z-lines, MT structure, and mitochondrial localization, as well as loss of intercalated disc structures, and myocardial fibrosis.[163,164] In human cardiac tissue of end-stage dilated cardiomyopathy, a downregulation of CENP-F has been observed, and common variants in *CENPF* have been linked to increased risk of heart failure.[163]

5.6 Motor proteins and associated proteins acting as +TIP

As described above, the motor proteins kinesin and dynein mediate intracellular transport across MTs from the MT minus-end to the plus-end and vice versa, respectively. Dynactin is a co-factor of dynein-1 and in neurones is necessary for the initiation of retrograde transport from distal axons.[165]

In addition to this, the p150^{Glued} subunit of the dynactin protein complex has been shown to be an EB1- and CLIP1-dependent +TIP.[98,166] Moreover, p150^{Glued} promotes MT stabilization by preventing MT catastrophe, although it was suggested that this function is specific for neuronal isoforms.[167] Interestingly, p150^{Glued} also impacts on Cx43 trafficking, as it is required for targeted delivery of Cx43 at the adherens junction in cardiomyocytes.[87] Therefore, the interplay between p150^{Glued} and EB1 may also be relevant for $Na_V1.5$ delivery at the intercalated discs of cardiomyocytes.

Among the kinesin motor proteins, the KIF5B subunit of kinesin-1 is mainly present at the intercalated discs of cardiomyocytes, and is involved in the trafficking of several ion channels as well as Cx43.[9,168,169] Importantly, KIF5B co-localizes with EB1 and promotes MT elongation and stability via c-Jun N-terminal kinase (JNK),[9,170] and can therefore be considered a +TIP. Similarly, kinesin family member 3A (KIF3A) and −3B (KIF3B), which together with kinesin-associated protein 3 (KAP3) forms a plus-end directed motor complex, is involved in the trafficking of ion channels, as well as MT regulation.[118,121] Specifically, this complex associates with APC and β-catenin, and has been suggested to mediate the transport of these two MT regulators to the MT plus-end, thereby regulating MT dynamics; as discussed above, alterations in APC/GSK3β/β-catenin may conceivably affect $Na_V1.5$ localization.[171] Moreover, the KIF3A/B-KAP3 complex mediates the trafficking of N-cadherin,[172] which is specifically located at the intercalated discs in cardiomyocytes and interacts with $Na_V1.5$. In addition, kinesin family member 17 (KIF17), a kinesin-2 family motor protein involved in ion channel trafficking in neurones,[173] is enriched at the MT plus-end, where it interacts with APC and EB1, and promotes MT stabilization.[117,174] Hence, motor proteins may exert their modulatory effect on ion channel trafficking through various mechanisms and targets.

6. Interactions between +TIPs and $Na_V1.5$ interacting proteins to determine specific localization

As illustrated above, several +TIPs represent potentially promising targets for the regulation of $Na_V1.5$ trafficking, as evidenced by our recent demonstration of a modulatory effect for EB1 and CLASP2 on $Na_V1.5$.[11] Further exploration of the interplay between +TIPs and $Na_V1.5$ interacting proteins may uncover potential avenues for regulating cardiomyocyte subcellular domain-specific trafficking of $Na_V1.5$. Indeed, as visualized in *Figure 5*, +TIPs are predicted to interact extensively with $Na_V1.5$ interacting proteins, either directly, or indirectly via intermediate 'adaptor' proteins (see *Table 3*). Hence, we propose that interactions between $Na_V1.5$ interacting proteins and +TIPs modulate MTs, allowing targeted delivery of $Na_V1.5$ in specific subcellular domains. This modulation can occur via two mechanisms, which are not mutually exclusive. First, $Na_V1.5$ interacting proteins potentially attract +TIP-capped MTs, mediating an enrichment of MTs at the site of the $Na_V1.5$ interacting protein, enhancing $Na_V1.5$ trafficking to this site. Second, the interaction between +TIPs and $Na_V1.5$ interacting proteins could 'anchor' the MT, causing MTs to stay for a prolonged period and allowing vesicles carrying $Na_V1.5$ to be delivered at this site. Since several $Na_V1.5$ interacting proteins are specifically localized in distinct subcellular domains of cardiomyocytes (*Table 1*), the interactions between +TIPs and proteins in the $Na_V1.5$ macromolecular complex are clear potential facilitators of the targeted delivery of $Na_V1.5$. This concept has already been demonstrated in neurones, where Ankyrin-G (AnkG) mediates formation of the Ranvier nodes and axon initial segment,[215] and interactions between AnkG and EB1 mediate specific localization of ion channels (including sodium channels) at these neuronal subcellular locations.[5,122] Apart from EB1, AnkG interacts with the +TIPs EB2 and EB3,[122] as well as KIF5B,[205] confirming the central role of AnkG in the regulation of MT-dependent trafficking. As discussed in the next sections, evidence is emerging that $Na_V1.5$ interacting proteins and +TIPs play a similar role in targeting of $Na_V1.5$ to distinct microdomains within cardiomyocytes.

6.1 Lateral membrane-directed trafficking of $Na_V1.5$—role of interacting proteins and +TIPs

$Na_V1.5$ is located at the crests, grooves, and t-tubular structures of the lateral membrane, and its localization at the lateral membrane is considered highly dependent on dystrophin.[26,37] As mentioned above, the +TIP dystrophin attracts growing MTs and interacts directly with MTs via a spectrin-like repeat,[216] and loss of dystrophin in Duchenne mouse models leads to a dysfunctional MT network as well as altered expression and function of various ion channels with $Na_V1.5$ specifically reduced at the lateral membrane.[217,218] Although dystrophin may not interact with $Na_V1.5$ directly, it serves as a scaffolding protein, forming a complex with a wide range of proteins including the $Na_V1.5$ interacting proteins α1- and β1-syntrophin,[37] AnkG, Ankyrin-B (AnkB),[31,208] and calcium/calmodulin-dependent serine protein kinase (CASK),[38] thereby linking $Na_V1.5$ to the dystrophin-glycoprotein complex. Hence, other $Na_V1.5$ interacting proteins within the dystrophin-glycoprotein complex may prove be more relevant for the regulation of $Na_V1.5$ trafficking to the lateral membrane than dystrophin itself. Indeed, we previously demonstrated that $Na_V1.5$ levels and I_{Na} were specifically decreased at the lateral membrane of cardiomyocytes upon disruption of the syntrophin interaction site of $Na_V1.5$.[25] In addition, dystrophin and syntrophin also interact with α- and β-dystrobrevin, which in turn interact with the +TIP KIF5B.[207,209] CASK localizes at the lateral membrane and silencing of CASK in cardiomyocytes increased I_{Na} and $Na_V1.5$ specifically at the lateral membrane,[38] suggesting that the presence of CASK under normal conditions may limit trafficking of $Na_V1.5$ to this microdomain and hence may be (at least partially) responsible for the relatively low levels of $Na_V1.5$ at this subcellular domain.

In addition to the proteins mentioned above, α-actinin-2, a protein which links transmembrane proteins to the actin cytoskeleton is connected to the dystrophin-glycoprotein complex by interacting with dystrophin.[219] Of note, α-actinin-2 is markedly present at the Z-discs, which overlaps with the groove of the lateral membrane, where it co-localizes with $Na_V1.5$.[26] Moreover, α-actinin-2 interacts with $Na_V1.5$, and enhances its positioning at the plasma membrane.[39] Therefore, although a strong connection with +TIPs has not yet been established, α-actinin-2 is likely involved in the trafficking of $Na_V1.5$ to the lateral membrane. However, as α-actinin-2 is also present at the intercalated discs,[26,39] it might not exclusively modulate trafficking to the lateral membrane, but also to the intercalated discs of cardiomyocytes. Taken together, we found a number of lateral membrane-specific $Na_V1.5$ interacting proteins (listed in *Table 2*) to interact with +TIPs, either directly or via adaptor proteins, as summarized in *Figure 4*. Although relatively few interactions were found, these interactions potentially modulate trafficking of $Na_V1.5$ to the lateral membrane.

6.2 Regulation of $Na_V1.5$ trafficking to the intercalated discs—role of interacting proteins and +TIPs

As previously mentioned, the intercalated discs of cardiomyocytes show similarities with the axon initial segment and Ranvier nodes of neurones. Indeed, AnkG interacts with various intercalated disc-enriched $Na_V1.5$ interacting proteins including CaMKIIδ, βIV-spectrin, plakophilin-2 (PKP2), and Cx43, and mediates $Na_V1.5$ delivery at the intercalated discs of cardiomyocytes.[47–49] In addition, AnkG is also present at the lateral membrane, where it has been reported to interact with dystrophin,[208] a MT-associated protein which complexes with $Na_V1.5$ within this subcellular domain. Disruption of $Na_V1.5$-AnkG interactions leads to altered I_{Na} characteristics, as well as removal of $Na_V1.5$ from both the intercalated discs and lateral membrane.[220] Since some papers report similar intracellular distributions of $Na_V1.5$ and AnkG, with enrichment at the intercalated discs of cardiomyocytes and relatively low levels at the lateral membrane,[47] $Na_V1.5$ levels may simply correlate with AnkG levels; however, no consensus about the distribution of AnkG has been reached, with some reports

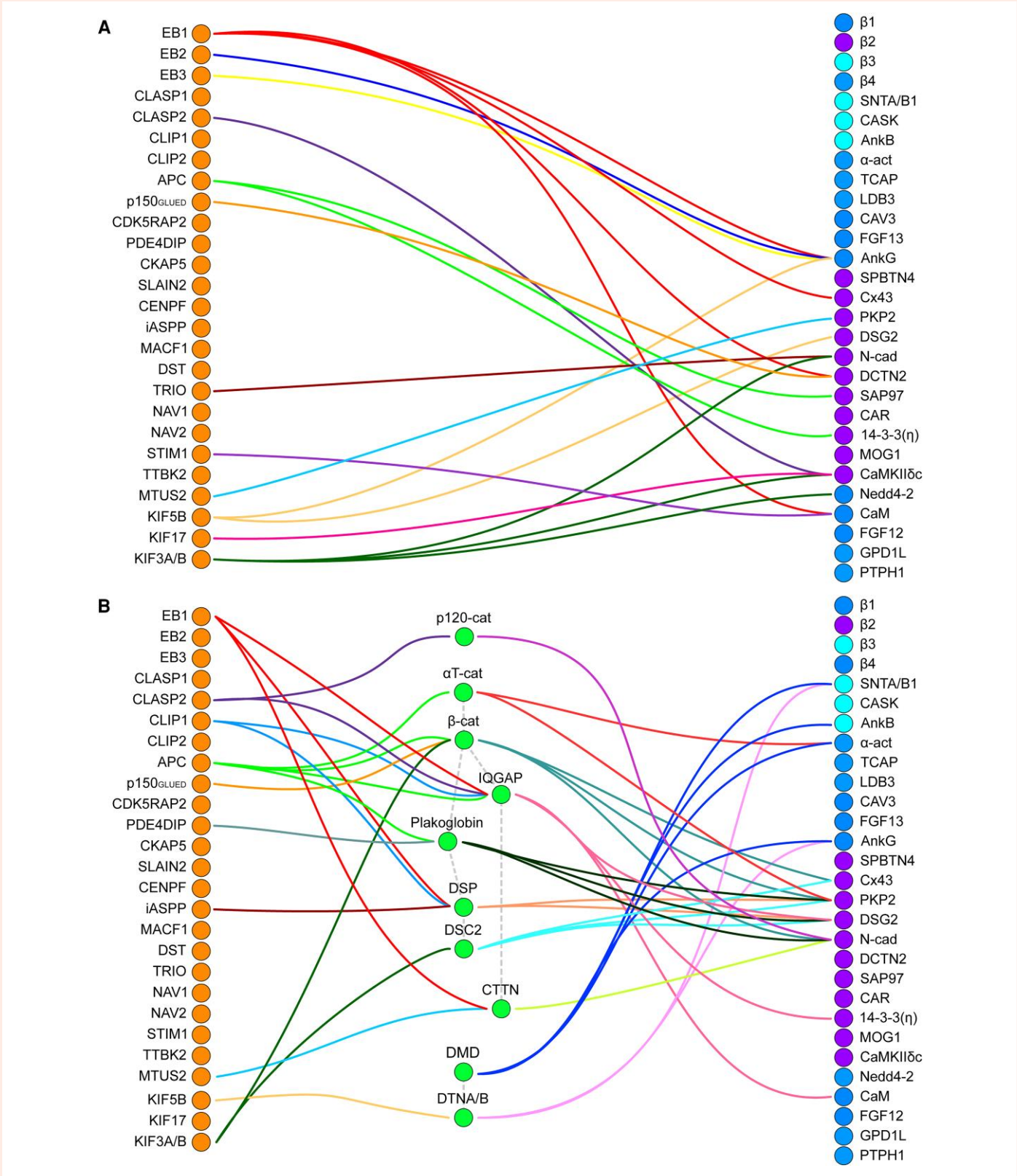


Figure 5 Interactions between +TIPs and $Na_V1.5$ interacting proteins. Overview of described interactions between +TIPs (left, orange) and $Na_V1.5$ interacting proteins (right) specifically located at the lateral membrane (cyan), at the intercalated discs (purple) or which are not specifically located at the lateral membrane or intercalated discs (blue). (*A*) Direct associations between +TIPs and $Na_V1.5$ interacting proteins. (*B*) Indirect associations via adaptor proteins (centre, green). Interactions between +TIPs and $Na_V1.5$ interacting proteins or adaptor proteins are marked by a solid, coloured line. Interactions between individual adaptor proteins are illustrated by a dashed grey line.

Table 3 Overview of interactions between plus-end tracking proteins (+TIPs) and $Na_V1.5$ interacting proteins, either through direct interaction or indirect via 'anchor' proteins, as described in referenced literature. Interactions between 'anchor' proteins as displayed in *Figure 5* are not shown in this table. "-" indicates absence of interactions.

+TIP	Associated $Na_V1.5$ interacting protein(s)	Associated 'anchor' protein(s)	Indirectly associated $Na_V1.5$ interacting protein(s)
EB1	Ankyrin-G,[122] connexin 43,[87] dynamitin,[175] calmodulin[176]	IQGAP,[177] desmoplakin,[178] cortactin[177]	Plakophilin-2,[179] desmoglein-2,[180] N-cadherin,[148] 14-3-3,[181] calmodulin[182]
EB2	Ankyrin-G[122]	—	—
EB3	Ankyrin-G[122]	—	—
CLASP1	—	—	—
CLASP2	CaMKII[183]	p120-catenin,[184] IQGAP[145]	Desmoglein-2,[180] N-cadherin,[185] 14-3-3,[181] calmodulin[182]
CLIP1	—	IQGAP,[186] desmoplakin[187]	Plakophilin-2,[179] desmoglein-2,[180] N-cadherin, 14-3-3,[181] calmodulin[182]
CLIP2	—	—	—
APC	SAP97,[188] 14-3-3[181]	αT-catenin,[189] β-catenin,[190] IQGAP,[145] plakoglobin[191]	α-actinin,[192] connexin 43,[193] plakophilin-2,[179,194,195] desmoglein-2, N-cadherin[196,197,198],14-3-3,[181] calmodulin[182]
$p150^{Glued}$	Dynamitin[199]	β-catenin[87]	Connexin 43,[193] plakophilin-2,[179] N-cadherin[196]
CDK5RAP2	—	—	—
PDE4DIP	—	Plakoglobin[200]	Plakophilin-2,[194] desmoglein-2,[201] N-cadherin[197,198]
CKAP5	—	—	—
SLAIN2	—	—	—
CENP-F		—	—
iASPP	—	Desmoplakin[105]	Connexin 43, plakophilin-2,[179] desmoglein-2[180]
MACF1	—	—	—
DST	—	—	—
TRIO	N-cadherin[202]	—	—
NAV1	—	—	—
NAV2	—	—	—
STIM1	Calmodulin[203]	—	—
TTBK2	—	—	—
MTUS2	Plakophilin-2[204]	Cortactin[147]	N-cadherin[148]
KIF5B	Ankyrin-G,[205] desmoglein-2[206]	α/β-dystrobrevin,[207] (dystrophin[208,209])	α/β-syntrophin-1,[209] Ankyrin-G,[208] (ankyrin-B,[208] α-actinin)
KIF17	CaMKII[210]	—	—
KIF3A/B	N-cadherin,[172] CaMKII,[211] Nedd4-2[212]	β-catenin,[171] desmocollin-2[206]	Connexin 43,[193,213] plakophilin-2,[179,206] desmoglein-2,[214] N-cadherin[196]

showing no enrichment at the intercalated discs.[48] As in neurones, AnkG and βIV-spectrin attract CaMKIIδ to a specific site of the cardiomyocyte (i.e. intercalated discs), a process which is essential for the formation of the intercalated discs, cardiac excitability, and $Na_V1.5$ levels at the intercalated discs.[47,51] Although no +TIPs were found to associate with βIV-spectrin, CaMKIIδ has been shown to interact with, and phosphorylate KIF17.[210] CaMKIIδ has also been suggested to interact with CLASP2 and KIF3A,[183,211] therefore likely regulating the MT-dependent trafficking of intercalated disc-specific proteins including $Na_V1.5$.

Apart from the AnkG complex, other proteins have also been proposed to impact $Na_V1.5$ localization at the intercalated discs, including SAP97,[29,57] which interacts with the +TIP APC.[188] SAP97 forms part of an interaction complex together with $Na_V1.5$ and $K_{ir}2.1$,[57] and cardiac-specific deletion of *Sap97* in mice resulted in altered sodium and potassium currents in addition to pro-arrhythmia; a putative mutation in the *DLG1* gene encoding SAP97 was furthermore identified in a Brugada syndrome patient.[221] Furthermore, as mentioned above, dysfunction of the gap junctional protein Cx43 has been shown to result in loss of $Na_V1.5$ at the intercalated discs, demonstrating that $Na_V1.5$ trafficking to the intercalated discs is modulated by Cx43;[10] these two proteins likely share a common trafficking mechanism. This is emphasized by observations regarding the effect of the protein 14-3-3, which modulates Cx43 trafficking and gap junction size,[59] while also regulating I_{Na} and $Na_V1.5$ at the intercalated discs.[60] Of note, 14–3–3 interacts with the +TIPs APC,[181] EB1, CLASP2, and CLIP1 via IQGAP1,[130,177] thereby potentially regulating Cx43 and $Na_V1.5$ trafficking. Moreover, another study exploring the role of +TIPs in the trafficking mechanism of Cx43 unravelled a vital role of +TIPs in the trafficking of Cx43 to gap junctions. Here, it was found that this targeted trafficking of Cx43 is mediated by interactions between the +TIPs EB1 and $p150^{Glued}$, and the intercalated disc-enriched proteins β-catenin and N-cadherin.[87] As described above and illustrated in *Figure 3*, our recent observations also established the relevance of EB1 for $Na_V1.5$ trafficking.[11] We furthermore demonstrated that $Na_V1.5$ localization and I_{Na} are enhanced specifically at the intercalated discs of adult murine cardiomyocytes by the GSK3β inhibitor SB216763 (*Figure 4*).[11] Importantly, GSK3β phosphorylates CLASP2 and thereby reduces its binding to EB1, IQGAP1 and MTs, while GSK3β inhibition by SB216763 enhances these interactions.[130] The crucial involvement of CLASP2 in this process was confirmed by the observation that SB216763 did not increase I_{Na} in *Clasp2*-deficient mice. These findings confirm that +TIPs impact on $Na_V1.5$ localization, and that modulation of +TIP interactions can alter $Na_V1.5$ distribution and I_{Na} at specific subcellular domains.

As mentioned before, N-cadherin and β-catenin are required for targeted delivery of Cx43. These proteins, together with αT-catenin, p120-catenin, plakoglobin,[23] and the dynamitin component of the dynactin complex (DCTN2),[222] are present in the adherens junction component of the intercalated disc. Of these, N-cadherin and DCTN2 interact with $Na_V1.5$,[31,54,55] while N-cadherin also interacts with the actin cytoskeleton regulator CTTN,[148] which in turn interacts with IQGAP.[177] Of note, while DCTN2 interacts with the +TIPs EB1 and $p150^{Glued}$,[199] it inhibits motor protein-mediated MT transport by disrupting the dynactin complex, and overexpression of DCTN2 resulted in decreased $Na_V1.5$ at the plasma membrane and reduced I_{Na}.[55] Aforementioned adherens junctional proteins and their interactors are connected extensively to +TIPs, interacting with EB1,[177] CLASP2,[145] CLIP1,[186] APC,[144] $p150^{Glued}$,[87,199] PDE4DIP,[200] TRIO,[202] MTUS2,[147] and KIF3A/B,[172] thereby facilitating interaction with MTs. The adherens junction and desmosome structures at the intercalated disc are extensively linked, with adherens junctional N-cadherin interacting with the desmosome, while desmosomal PKP2 interacts with the adherens junction. Indeed, several proteins are present in both structures, with also αT- and β-catenin, plakoglobin, and AnkG overlapping between structures and interacting with PKP2.[23,49,194,206] Accordingly, Cx43 delivery to the intercalated discs is also heavily dependent on several 'desmosomal' proteins, with PKP2 and DSP modulating Cx43 levels at the sarcolemma the latter through interactions with EB1.[178,223] Indeed, both Cx43 and PKP2 interact with β-catenin[179,193] and desmocollin-2 (DSC2).[179,206,214] Moreover, PKP2 interacts with desmoglein-2 (DSG2),[179] and both interact with $Na_V1.5$,[30,52,53] as well as plakoglobin.[194,201] In addition, DSG2 interacts with IQGAP1,[176] forming a complex containing Cx43, PKP2, DSG2, N-cadherin, αT-, β-, and p120-catenin, plakoglobin, IQGAP1, CTTN, DSP, and DSC2. This complex is highly connected to +TIPs, interacting with EB1,[87,177,178] CLASP2,[145] CLIP1,[186] APC,[144] $p150^{Glued}$,[87] PDE4DIP,[200] iASPP,[105] TRIO,[172] MTUS2,[147,204] KIF5B[206] and KIF3A/B.[171,172,206] Hence, gap junctional Cx43 and desmosomal proteins are widely connected to +TIPs, and Cx43, PKP2, and DSG2 interact with $Na_V1.5$ and modulate I_{Na},[10,30,52,53,224] Crucially, dysfunction of PKP2, DSG2, DSC2, and DSP is associated with arrhythmogenic cardiomyopathy,[225–227] a disease characterized by loss of Cx43 and $Na_V1.5$ at the intercalated disc. It remains to be investigated if and to what extent (alterations in) +TIPs play a modulatory role in this setting potentially contributing to the I_{Na} reduction, conduction slowing, and pro-arrhythmia observed in arrhythmogenic cardiomyopathy.

7. Potential implications of +TIPs for arrhythmogenesis and therapy

Disruption of $Na_V1.5$ function and availability and consequent cardiac conduction slowing are well-defined risk factors for the development of potentially life-threatening cardiac arrhythmias.[1] As discussed in this review, several +TIPs modulate $Na_V1.5$ localization and function; in particular, previous work by us and others highlighted the impact of EB1 and EB2 on I_{Na}, $Na_V1.5$ and cardiac conduction and identified a role for *MAPRE2*/EB2 in Brugada syndrome (*Figure 3*).[11,12] In addition, a myriad of +TIPs have been found to associate with $Na_V1.5$ interacting proteins and are therefore potentially involved in the trafficking of $Na_V1.5$. Importantly, alterations in the +TIPs EB1,[11] EB2,[12] CLASP2,[228] APC,[142] CLIP1,[138] CENP-F,[163,164] MACF1,[160] and iASPP[105] are associated with cardiac conduction slowing, contractile dysfunction and/or myocardial structural abnormalities, emphasizing the impact of +TIP function on cardiac (electro)physiology. Of these, EB1, CLASP2, APC, CLIP1, and iASPP interact preferentially or exclusively with $Na_V1.5$ interacting proteins located at the intercalated discs. Hence, disruption of interactions between +TIPs and $Na_V1.5$ interacting proteins at the intercalated discs may be especially pathogenic. In this respect, the impact of +TIPs on Cx43, either directly or through co-regulation or co-trafficking with $Na_V1.5$, is also of potential importance for arrhythmogenesis, since changes in Cx43 may contribute to conduction abnormalities. As detailed above, alterations in $Na_V1.5$ and/or Cx43 are of particular relevance in the setting of arrhythmogenic cardiomyopathy. While we here focus on the impact of +TIPs on $Na_V1.5$ trafficking, it is important to emphasize that +TIPs also modulate trafficking of other cardiac ion channels. This may occur as a consequence of a direct effect of +TIPs on these ion channels; alternatively, the effects of +TIPs may occur indirectly, either by impacting on interacting proteins regulating ion channel targeting and/or function, or by modulating multiple ion channels which traffic together. For instance, $K_V1.5$ which generates the ultrarapid-delayed potassium current (I_{Kur}) is regulated by CTTN, which interacts with the +TIP MTUS2.[148] Additionally, $Na_V1.5$ co-traffics with $K_{ir}2.1$ (generating inward rectifier current I_{K1})[229] and potentially impacts trafficking of $K_V4.3$ (generating transient outward potassium current I_{to})[230] Hence, +TIPs also regulate other ion channels, either directly or via $Na_V1.5$. Importantly, this impact on other ion channels is not necessarily detrimental and can be important to maintain the balance between ion currents required for proper AP generation and propagation. For instance, enhanced I_{K1} would hyperpolarize the resting membrane potential and enhance $Na_V1.5$ availability. Clearly, the potential impact of +TIPs on ion channel trafficking are complex and require further detailed exploration.

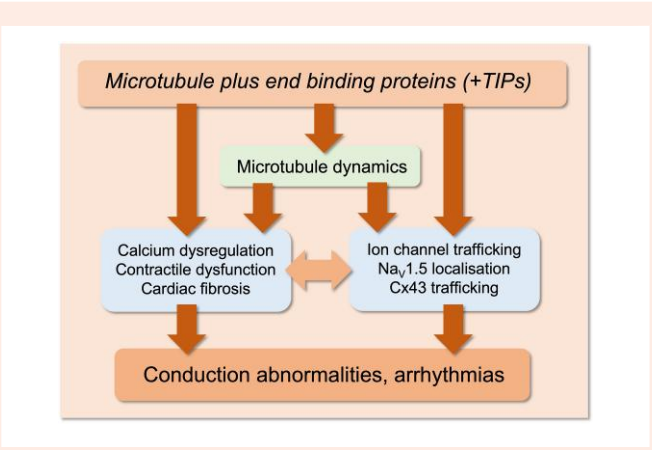


Figure 6 +TIPs may impact on cardiac conduction and arrhythmogenesis through various mechanisms.

In addition to their potential effects of +TIPs on ion channel trafficking and function, their impact on cardiac contractile function and structural derangements may also have clear pro-arrhythmic consequences. For example, deficiency of iASPP has been shown to induce right-ventricular dilatation and an arrhythmogenic cardiomyopathy phenotype in mice, likely through its impact on desmosomal proteins.[105] Similarly, loss of CENP-F has been associated with dilated cardiomyopathy, loss of intercalated disc structures, and fibrosis in murine hearts.[163,164] Additionally, a core function of +TIPs is to regulate MT dynamics, thereby also impacting cardiomyocyte structural and functional integrity, contractility and stiffness, as well as calcium homeostasis, which are key determinants of cardiac function.[231] Apart from +TIPs, MT dynamics are also regulated by a multitude of regulatory mechanisms, including interactions with other cytoskeletal components, focal adhesions, and mechanical stress, underlining the complexity of MT-dependent processes.[231,232] Hence, +TIPs may modulate cardiac arrhythmogenesis through various direct and indirect mechanisms, as summarized in *Figure 6*.

From a therapeutic perspective, there are only very limited options for pharmacological targeting of $Na_V1.5$-based channels. Development of efficacious sodium channel modulators may in fact be limited by the subcellular diversity in $Na_V1.5$ within cardiomyocyte microdomains. Hence, targeting either lateral membrane- or intercalated disc-specific interacting proteins may ultimately prove to be a more specific approach to prevent conduction abnormalities and arrhythmias. In addition, the current review also highlights the potential for +TIPs as novel therapeutic targets aimed at modulating $Na_V1.5$ in a microdomain-specific manner. Indeed, we recently

demonstrated that the compound SB216763, annotated as a GSK3β inhibitor, enhanced I_{Na} and $Na_V1.5$ at the intercalated discs but not at the lateral membrane of mouse cardiomyocytes (*Figure 4*), opening up new avenues for development of novel therapeutics.[11] While modulating GSK3β activity may not be a potential useful therapeutic approach given its widespread involvement in cellular processes, defining the exact mechanism involved in the observed effect of SB216763 on intercalated disc-specific modulation of I_{Na}, such as for instance the displacement of GSK3β from the intercalated discs,[11] may facilitate the design of more refined compounds. Similarly, increased mechanistic insight into other +TIPs and their regulation may uncover additional strategies for subdomain-specific targeting of ion channels, including $Na_V1.5$. Moreover, gene therapy may ultimately allow cardiac-specific targeting of +TIPs and MTs, by-passing potential therapeutic difficulties associated with the ubiquitous expression of this protein network.

8. Conclusions

Cardiomyocytes express a large number of +TIPs which not only modulate MT function but also regulate trafficking and delivery of proteins including ion channels to the cell membrane. In particular, we here focused on their newly identified role in $Na_V1.5$ trafficking, cardiac (electro)physiology, and arrhythmogenesis. Based on our previous work and the available literature, we propose that the interplay between +TIPs and $Na_V1.5$ interacting proteins is an essential modulator of $Na_V1.5$ delivery to specific subcellular domains at the plasma membrane of cardiomyocytes. While this novel concept is partly of hypothetical nature, our recent findings combined with available knowledge are consistent with a more general role for +TIPs in modulating cardiac (electrical) function and regulating trafficking of ion channels, including Nav1.5. In line with the specific $Na_V1.5$ distribution observed in cardiomyocytes, it appears that +TIPs interact with intercalated disc-specific $Na_V1.5$ interacting proteins to a wide extent, while fewer interactions are observed in the lateral membrane. While it is possible that not all interactions described here are relevant for $Na_V1.5$ trafficking, it is tempting to speculate that the high number of interactions with +TIPs explains the relative abundance of $Na_V1.5$ at the intercalated discs. Studies have so far focused mostly on ventricular cardiomyocytes, and it will be interesting to explore whether different expression levels or distribution of +TIPs contribute to variation in ion channel function in other regions of the heart, including atrial and conduction system cells. Moreover, given the fact that certain ion channels are known to be jointly trafficked and/or regulated in cardiomyocytes, it is possible that they are modulated by shared MT/+TIP-related pathways. Although as yet few mutations in +TIPs have been firmly linked to electrical disorders, future genetic studies may focus on +TIPs as potential candidate genes or modifiers in the setting of inherited arrhythmias. In addition, further research is required to explore the potential of modulating +TIPs and their interacting partners with the aim of developing more refined, microdomain-specific therapeutic approaches to target ion channels and ultimately prevent cardiac arrhythmias.

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Data availability

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