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Review

Structure and function of the β subunit of voltage-gated Ca^{2+} channels ☆

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ABSTRACT

The voltage-gated Ca^{2+} channel β subunit ($Ca_v\beta$) is a cytosolic auxiliary subunit that plays an essential role in regulating the surface expression and gating properties of high-voltage activated (HVA) Ca^{2+} channels. It is also crucial for the modulation of HVA Ca^{2+} channels by G proteins, kinases, Ras-related RGK GTPases, and other proteins. There are indications that $Ca_v\beta$ may carry out Ca^{2+} channel-independent functions. $Ca_v\beta$ knockouts are either non-viable or result in a severe pathophysiology, and mutations in $Ca_v\beta$ have been implicated in disease. In this article, we review the structure and various biological functions of $Ca_v\beta$, as well as recent advances. This article is part of a Special Issue entitled: Calcium channels.

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Contents

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1. Introduction

Most voltage-gated ion channels are large protein complexes composed of a pore-forming subunit and one or more auxiliary subunits that regulate channel properties. Unlike the majority of voltage-gated channels, high-voltage-activated (HVA) Ca_v1 and Ca_v2 Ca^{2+} channels absolutely require an auxiliary β subunit ($Ca_v\beta$) for plasma membrane expression and proper gating [1]. $Ca_v\beta$ was first purified as part of the complex of skeletal muscle voltage-gated Ca^{2+} channels and was cloned in 1989 [2,3]. Subsequent cloning efforts revealed four subfamilies of $Ca_v\beta$s (β_1–β_4), encoded by four distinct genes and each with splice variants. In addition, early studies determined that $Ca_v\beta$ binds with high affinity to the pore-forming α_1 subunit ($Ca_v\alpha_1$) of voltage-gated calcium channels (VGCCs). The high-affinity site is located in the cytoplasmic linker connecting the first two of the four homologous repeats of $Ca_v\alpha_1$ (i.e., the I–II linker) and was named the α-interaction domain or AID (Fig. 1) [4–6]. Several crystal structures of $Ca_v\beta$ have been solved, providing great insights into the molecular mechanisms of $Ca_v\beta$ function. A comprehensive review on $Ca_v\beta$ is available [7]. Here, we highlight the most important features of $Ca_v\beta$ structure, function, and involvement in cell physiology and pathophysiology.

2. Structure of $CA_v\beta$

Based on amino acid sequence alignment, biochemical and functional studies, and molecular modeling, it became clear that $Ca_v\beta$ consists of a conserved core region flanked by non-conserved N- and C-termini (Fig. 1) [8–10]. The core region is composed of two highly conserved regions homologous to the Src homology 3 (SH3) and guanylate kinase (GK) domains, connected by a weakly conserved HOOK region. This SH3-HOOK-GK core can recapitulate many key functions of $Ca_v\beta$ [8,11–17]. In 2004, three independent research groups reported the crystal structures of the $Ca_v\beta$ core region of β_{2a}, β_3 and β_4, alone or in complex with the AID [11,18,19]. The structures confirmed the existence of an SH3-HOOK-GK module in the $Ca_v\beta$ core.

2.1. The N- and C-termini

The amino and carboxyl termini of $Ca_v\beta$ (abbreviated as $Ca_v\beta$-NT and $Ca_v\beta$-CT) are highly variable. They have not been crystallized, but an NMR structure has been obtained for the N-terminus of β_4. This structure reveals a fold consisting of two α-helices and two anti-parallel β-sheets [20]. There are currently no structures of $Ca_v\beta$-CT.

2.2. The GK domain

Guanylate kinases catalyze the formation of ADP and GDP from ATP and GMP. The general structural features of guanylate kinases [21,22] are preserved in the $Ca_v\beta$ GK domain ($Ca_v\beta$-GK), but structural variations exist in the catalytic site, and many key catalytic residues are absent in $Ca_v\beta$-GK [11,18,19]. Thus, $Ca_v\beta$-GK is catalytically inactive. Instead, $Ca_v\beta$-GK has evolved into a protein interaction module, binding

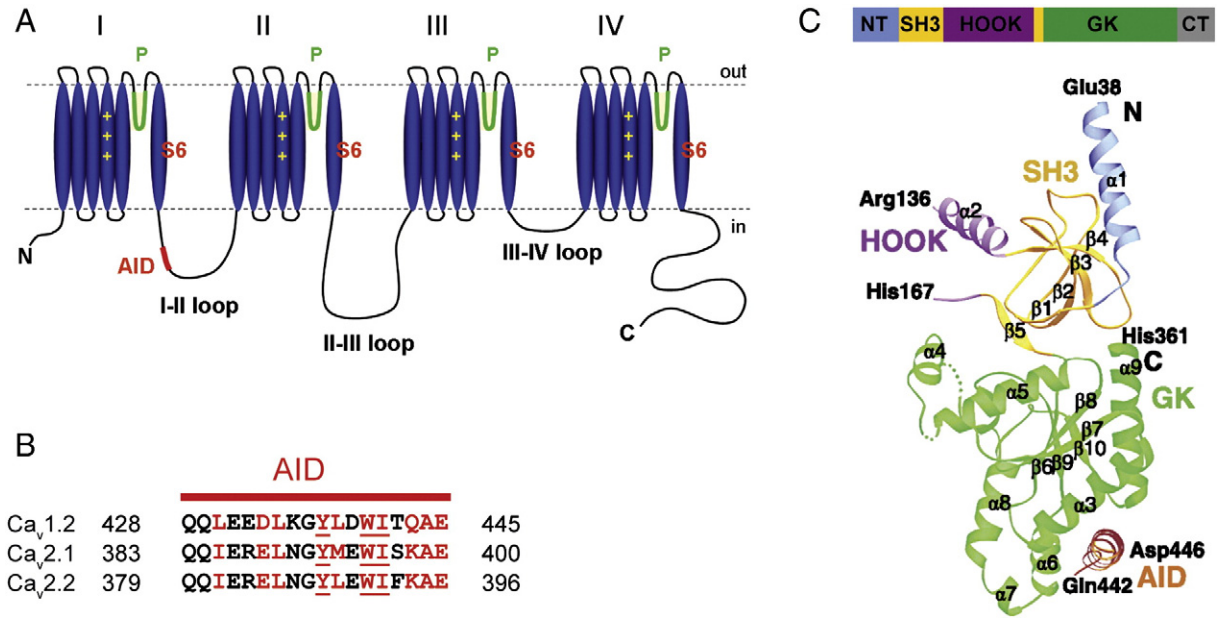

Fig. 1. VGCC topology and the structure of the $Ca_v\beta$ core in complex with the AID. (A) Schematic representation of the predicted transmembrane topology of the α_1 subunit of VGCC. The AID, marked in red, is located on the I–II linker. 'P' indicates the pore loops, located between transmembrane regions S5 and S6. '+' indicates the charged amino acids in S4—the voltage sensor. (B) Amino acid sequence alignment of the AID from the indicated calcium channel α_1 subunits. Residues involved in interactions with $Ca_v\beta$ are marked in red, with the most critical residues underlined. Residue numbers are indicated on both sides of the sequence. (C) $Ca_v\beta$ is organized into 5 regions represented schematically in the upper panel. The lower panel shows the crystal structure of the $Ca_v\beta_3$ core in complex with the AID (PDB accession code 1VYT) with the following regions: N-terminus (light blue), the SH3 domain (gold), part of the HOOK region (purple, residues 121–169), and the GK domain (green). Residues 137–166 of the HOOK region were disordered and are not included. The AID region of $Ca_v1.2$ (residues 422–446) is colored in orange.

tightly to $Ca_v\alpha_1$ through its high-affinity interaction with the AID (Fig. 1) [11,18,19,23]. Importantly, a large surface of the GK domain remains free to interact with other proteins, such as RGK GTPases (see review by Colecraft and colleagues in this issue) and BK channels [24].

2.3. The SH3 domain and the HOOK region

Classical SH3 domains mediate specific protein–protein interactions. They have a highly conserved hydrophobic surface, the PxxP-binding site, which binds to PxxP-sequences in target proteins. In general, SH3 domains have a well conserved and compact fold consisting of five sequential β-strands ($\beta_{strand\ 1-5}$) assembled into two orthogonally packed sheets [25]. However, in the $Ca_v\beta$ SH3 domain ($Ca_v\beta$-SH3) the last two β-strands are non-continuous, separated by the HOOK region [11,18,19] so that the SH3 domain has the following primary structure: $SH3_{\beta strand\ 1-4}$-HOOK-$SH3_{\beta strand\ 5}$ (Fig. 1). The crystal structures show that the PxxP-binding site in $Ca_v\beta$-SH3 is occluded by part of the HOOK region and a long loop connecting two of the four continuous β-sheets. It is conceivable that these two regions move and expose the PxxP-binding site, either when $Ca_v\beta$ is bound to full-length $Ca_v\alpha_1$ and/or when it interacts with other partners. Nevertheless, while binding between $Ca_v\beta$ and PxxP-containing proteins, such as dynamin, has been demonstrated [26], the putative PxxP-binding site itself has yet to be implicated.

The HOOK region is variable in length and amino acid sequence among the $Ca_v\beta$ subfamilies. In the crystal structures, a large portion of the HOOK is unresolved due to poor electron density, indicating that it has a high degree of flexibility [11,18,19]. As will be discussed later, the HOOK region plays an important role in regulating channel inactivation.

2.4. The SH3-GK intramolecular interaction

The crystal structures show that the SH3 and GK domains interact intramolecularly [11,18,19]. This interaction is strong enough that NT-$SH3_{\beta strand\ 1-4}$-HOOK module and the $SH3_{\beta strand\ 5}$-GK-CT module can associate biochemically in vitro and reconstitute the functionality of full-length $Ca_v\beta$s in situ [14,15,17,19,27–29].

It has recently been proposed that the intramolecular SH3-GK interaction can be disrupted by dynamin and replaced by an intermolecular interaction resulting in the dimerization of two $Ca_v\beta$s—a proposed mechanism for dynamin-mediated Ca^{2+} channel endocytosis (discussed later) [30].

2.5. The AID–$Ca_v\beta$ interaction

All $Ca_v\beta$s bind to the 18 amino acid AID in the I–II linker of Ca_v1 and Ca_v2 α_1 subunits (Fig. 1) [5]. The affinity of the AID–$Ca_v\beta$ interaction ranges from 2 to 54 nM in vitro [5,15,31–38]. Single mutations of several conserved residues in the AID, including Y10, W13 and I14, greatly weaken the AID–$Ca_v\beta$ interaction in vitro, and reduce or abolish $Ca_v\beta$-induced stimulation of Ca^{2+} channel current in heterologous expression systems [4,12,31,35,36,39–44], firmly establishing the role of the AID as the principal anchoring domain for $Ca_v\beta$.

The $Ca_v\beta$ crystal structures reveal a big surprise in regard to the region of $Ca_v\beta$ that interacts with the AID. A 31-amino acid segment of $Ca_v\beta$, referred to as the β-interacting domain or BID, had been described as the main binding site for the AID [8]. The BID was able to slightly enhance Ca^{2+} channel current and modulate gating [8], and several BID point mutations were able to weaken the $Ca_v\beta$/$Ca_v\alpha_1$ interaction and reduce BID-stimulated Ca^{2+} channel currents [8,31]. Thus, it had been generally accepted that $Ca_v\beta$ interacted with $Ca_v\alpha_1$ primarily through the BID. However, crystal structures of different AID–$Ca_v\beta$ core complexes reveal that the AID does not even contact the BID [11,18,19,23], which is buried inside $Ca_v\beta$. Thus, mutating the BID most likely alters the folding and/or structure of $Ca_v\beta$, which explains the disruptive effect of BID mutations [8,31]. The current enhancement by the BID peptide, on the other hand, is likely a non-specific effect, since a random peptide had a similar effect [11].

The crystal structures show that the AID binds to a hydrophobic groove in the GK domain termed the AID-binding pocket or ABP (Fig. 1, α-helices 3, 6 and 9 and some of their flanking loops) [11,19,35]. The interactions between the AID and the ABP are extensive and predominantly hydrophobic. These interactions account for the nM affinity of the AID–$Ca_v\beta$ binding. Functional studies show that mutating two or more key residues in the ABP severely weakens or completely abolishes the AID–$Ca_v\beta$ interaction [12,13].

Binding to the AID does not significantly change the $Ca_v\beta$ structure, except for some small and localized changes near the ABP. On the other hand, the AID undergoes a dramatic change in secondary structure when it is engulfed by the ABP. When alone, the AID forms a random coil in solution, as shown by circular dichroism spectrum measurements [18]; when bound to $Ca_v\beta$, the AID forms a continuous α-helix, as shown in the crystal structures. Importantly, the 22 amino acid region that links the first S6 segment of $Ca_v\alpha_1$ (i.e., IS6) to the AID seems to form an α-helix [45]. Thus, a picture emerges that in the presence of $Ca_v\beta$ the entire region from IS6 to the AID adopts a continuous α-helical structure. Indeed, two recent crystal structures of a β_2 core in complex with large parts of the I–II linker of Ca_v1 or Ca_v2 channels show a continuous α-helical structure upstream of the AID (towards IS6), albeit with some differences between Ca_v1 and Ca_v2 channels [23]. This rigid structure is crucial for $Ca_v\beta$ regulation of Ca^{2+} channel gating, as will be discussed later.

3. The functions of $Ca_v\beta$

$Ca_v\beta$ regulates multiple aspects of HVA channel physiology including surface expression, degradation, and gating (Fig. 2). $Ca_v\beta$ is also critical for the regulation of VGCC by lipids, G proteins, RGK GTPases (Rem, Rem2, Rad and Gem/Kir, reviewed in this issue by Colecraft and colleagues), kinases, phosphatases, and other signaling proteins (Fig. 2) [for a comprehensive review see 7]. We highlight here the most important functions of $Ca_v\beta$ as well as recent advances.

3.1. Membrane targeting of $Ca_v\beta$

$Ca_v\beta$s are cytosolic proteins. This is based both on primary sequence analyses [3,46] and subcellular localization when $Ca_v\beta$ is expressed alone, in the absence a $Ca_v\alpha_1$ [16,42] (a few important exceptions are discussed below). In the presence of $Ca_v\alpha_1$, $Ca_v\beta$ switches its localization from cytosolic to membrane-bound. This translocation depends on the AID–$Ca_v\beta$ interaction. Single point mutations in the AID of $Ca_v1.2$ that disrupt binding with $Ca_v\beta$ abolish both membrane localization and dendritic clustering of $Ca_v\beta$ in hippocampal neurons [47].

Some $Ca_v\beta$s can independently be localized to the plasma membrane [48,49], most notably β_{2a} [50–53]. β_{2a} is tethered to the plasma membrane through dynamic palmitoylation of two cysteines (Cys 3, 4) in its N-terminus [50–53]. However, palmitoylation alone may not be sufficient for membrane localization because implanting the β_{2a} N-terminus into other $Ca_v\beta$s imparts palmitoylation but not membrane localization [51]. Thus, β_{2a} probably possesses some additional determinants that help target it to the plasma membrane. Importantly, membrane tethering of $Ca_v\beta$ coincides with many functional effects, especially slowed inactivation, as we discuss later.

3.2. $Ca_v\beta$ is required for normal channel expression

Ca_v1 and Ca_v2 α_1 subunits show little or no surface expression and produce very small or no currents when expressed without auxiliary subunits. Upon the coexpression of $Ca_v\beta$, Ca^{+2} currents are increased

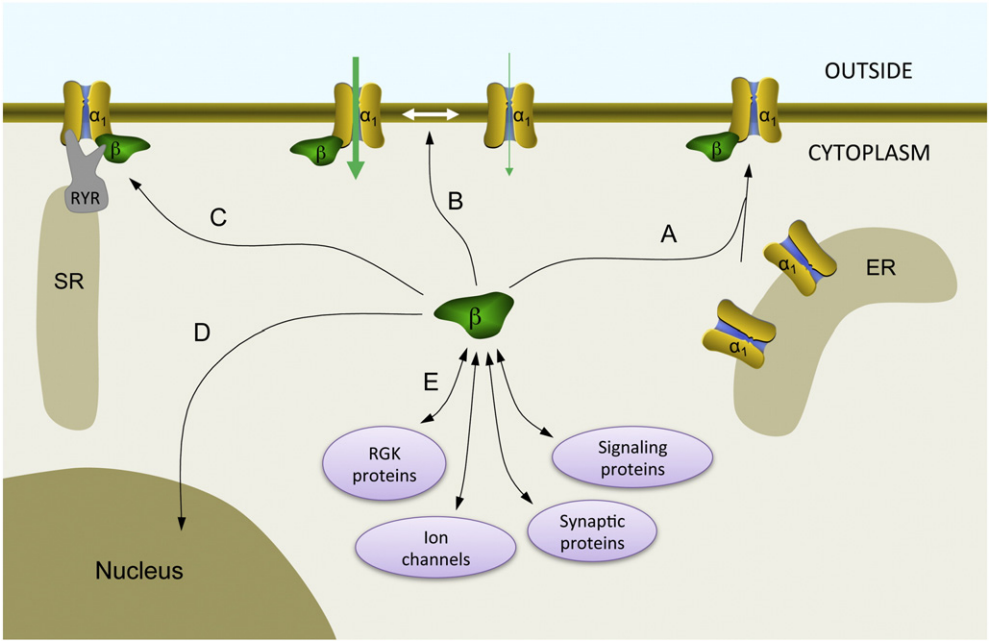

Fig. 2. Major functions of $Ca_v\beta$. (A) $Ca_v\beta$ enhances $Ca_v\alpha_1$ localization to the plasma membrane by preventing $Ca_v\alpha_1$ degradation and exposing ER export signals on $Ca_v\alpha_1$. (B) $Ca_v\beta$ promotes VGCC gating, resulting in an overall enhancement of current. (C) $Ca_v\beta$ interacts with the ryanodine receptor (RYR) in the sarcoplasmic reticulum (SR) of muscle cells and is critical for excitation–contraction coupling. (D) $Ca_v\beta$ can be translocated into the nucleus where it may participate in transcriptional regulation. (E) $Ca_v\beta$ interacts directly with many intracellular proteins that regulate VGCC function. The strongest of those regulators are RGK proteins, which potently inhibit VGCCs (reviewed in this issue by Colecraft and colleagues, also see [7]). Other partners include ion channels (e.g., BK_{Ca} and bestrophin), synaptic proteins (e.g., synaptotagmin I and RIMI), and signaling proteins (such as kinases, phosphatases, dynamin, and Ahnak) [for extensive review see 7].

by orders of magnitude [9,54–57] due to enhanced channel surface expression and open probability. The dramatic increase in surface expression of all Ca_v1 and Ca_v2 α_1 subunits can be observed both in native cells [58–60] and in various heterologous expression systems with any of the four subfamilies of $Ca_v\beta$. Thus, $Ca_v\beta$ is required for HVA channel surface expression. The increased surface expression is dependent on $Ca_v\beta$ binding to the AID, as point mutations in the AID or the ABP that weaken or abolish the AID–$Ca_v\beta$ interaction severely reduce or abolish $Ca_v\beta$-stimulated Ca^{2+} channel current [4,12,13]. The GK domain itself can largely recapitulate the chaperone function of full-length $Ca_v\beta$s, greatly increasing Ca^{2+} channel surface expression and current in *Xenopus* oocytes and mammalian cells [12,62].

It should be noted that some expression systems, such as *Xenopus* oocytes, have endogenous $Ca_v\beta$s that can transport a small number of exogenously expressed $Ca_v\alpha_1$ to the plasma membrane [62]. This yields small Ca^{2+} channel currents that can be suppressed by antisense oligonucleotides against endogenous $Ca_v\beta$ [34,62].

How does $Ca_v\beta$ enhance Ca^{2+} channel surface expression? An early hypothesis was that $Ca_v\beta$ shields or disrupts one or more ER retention signals on the I–II linker of $Ca_v\alpha_1$ [63]. However, several results are inconsistent with this hypothesis: (i) I–II linkers from several different $Ca_v\alpha_1$ subunits do not cause ER retention of CD8 or CD4 peptides [64,65]; (ii) In the absence of $Ca_v\beta$, transplanting the I–II linker of different HVA $Ca_v\alpha_1$ subunits ($Ca_v2.2$, $Ca_v2.1$ and $Ca_v1.2$) into a T-type channel ($Ca_v3.1$), which does not require $Ca_v\beta$ for its function, causes current upregulation instead of downregulation [45,66]; (iii) several labs implicated regions other than the I–II linker in $Ca_v\alpha_1$ trafficking [64,67–71].

These inconsistencies prompted a re-evaluation of the mechanism of $Ca_v\beta$-mediated upregulation of HVA channel expression. In a recent study [66], all of the L-type $Ca_v1.2$ channel intracellular linkers were systematically transplanted into the T-type channel, individually or in combination. This was followed by careful examination of the linkers' ER export and ER retention properties, in the presence or absence of a $Ca_v\beta$, by monitoring channel surface expression. The results suggest that the I–II linker of $Ca_v1.2$ has an ER export signal composed of 9 acidic residues downstream of the AID. All other intracellular linkers, including the N- and C-termini, were found to contain overall ER retention signals. Thus, it was proposed that the intracellular regions of $Ca_v\alpha_1$ form a complex that yields a prevailing ER retention signal, and when $Ca_v\beta$ binds to the I–II linker, it orchestrates a switch in the complex such that the ER export signal becomes dominant, enhancing $Ca_v\alpha_1$ surface expression. In this process, the $Ca_v\alpha_1$ C-terminus plays an essential role since it is absolutely required (but not sufficient) for $Ca_v\beta$-dependent channel upregulation [66].

A few recent studies have proposed that $Ca_v\beta$ increases channel surface expression by preventing $Ca_v\alpha_1$ ubiquitination and proteasomal degradation [71–73]. In the absence of $Ca_v\beta$, a proteasome inhibitor (MG132) can rescue $Ca_v\alpha_1$ surface expression. $Ca_v\beta$ coexpression, on the other hand, decreased $Ca_v1.2$ ubiquitination and association with proteins involved in proteasomal degradation, suggesting that $Ca_v\beta$ could be rerouting channels away from predestined proteasomal degradation. This mechanism was proposed for $Ca_v2.2$ channels [72]. However, Cav2.1 channels do not appear to be subject to this type of regulation [71]. For an extensive review on VGCC trafficking see [74].

3.3. $Ca_v\beta$ regulates Ca^{2+} channel gating

Besides enhancing channel surface expression, $Ca_v\beta$ regulates channel gating. Here we describe the effects of $Ca_v\beta$ on channel activation and voltage- and Ca^{2+}-dependent inactivation. We also discuss a unifying model for the mechanism of $Ca_v\beta$-mediated gating regulation. The effects of $Ca_v\beta$ on Ca^{2+} channel facilitation have been reviewed previously [7].

3.3.1. $Ca_v\beta$ enhances channel activation

All $Ca_v\beta$s facilitate channel opening by shifting the voltage dependence of channel activation by ~10–15 mV to more hyperpolarized voltages [75–77]. This is reflected as an increase in the open probability at the single channel level [78,79], with β_{2a} producing the most dramatic increase in channel open probability [79–81]. $Ca_v\beta$ also often speeds channel activation [1,82], which is observed as a shortened

latency to first channel opening in single channel recordings [83,84]. Many of these effects can be reconstituted by the core region of $Ca_v\beta$ [12] and, in some cases, the GK domain alone [85].

3.3.2. $Ca_v\beta$ enhances inactivation, except β_{2a}

Calcium channels inactivate in a voltage- and Ca^{2+}-dependent manner (VDI and CDI respectively). This process is modulated by $Ca_v\beta$ in at least 3 ways: (1) $Ca_v\beta$ generally shifts the voltage dependence of inactivation to more hyperpolarized voltages by ~10–20 mV (Fig. 3), enhancing VDI. Similarly, $Ca_v\beta$ increases CDI [86]. β_{2a}, however, shifts the voltage dependence of inactivation to more depolarized voltages by ~10 mV, reducing VDI [12,87]. (2) $Ca_v\beta$ (except β_{2a}) promotes Ca_v2 channels' 'closed state' inactivation [88,89]. (3) $Ca_v\beta$ generally accelerates inactivation kinetics, but β_{2a} and β_{2e} decelerate inactivation kinetics (Fig. 3).

The unique effects of β_{2a} on inactivation are largely due to its palmitoylation [90,91], but it seems that membrane anchorage rather than palmitoylation per se is critical [91,92]. Indeed, the non-palmitoylated but membrane-attached β_{2e} has properties similar to β_{2a} [49].

The molecular determinants on $Ca_v\beta$ that regulate channel inactivation are many. The N-terminus is clearly important, as indicated by the observation that natural β_2 and β_4 splice variants differing in their N-termini exhibit markedly different effects on VDI [49,93–95]. The C-terminus, on the other hand, seems to play a limited or no role in regulating VDI [12,96]. All $Ca_v\beta$-GK domains, including that of β_{2a}, speed VDI and hyperpolarize the voltage dependence of VDI [12,61,97]. The HOOK domain is also important as swapping the HOOK between the core regions (SH3-HOOK-GK) of β_{1b} and β_{2a} also swaps their effects on VDI [12].

3.3.3. How does $Ca_v\beta$ regulate voltage-dependent activation and inactivation of HVA channels?

To answer this question, it is necessary to mention the molecular determinants of voltage-dependent activation (VDA) and inactivation (VDI) of VGCCs. For VDA, they include (i) the external pore and the ion selectivity filter formed by the pore loop between the S5 and S6 transmembrane segments of $Ca_v\alpha_1$ [98–101], (ii) the inner pore formed by all four S6 segments of $Ca_v\alpha_1$ [102], and (iii) the activation gate, located at the cytoplasmic end of the S6 segments [103].

The molecular determinants of VDI include the cytosolic ends of the S6 segments, the I–II linker, considered to be the inactivation gate, and the N- and C-termini of $Ca_v\alpha_1$ [for review, see 86,104,105].

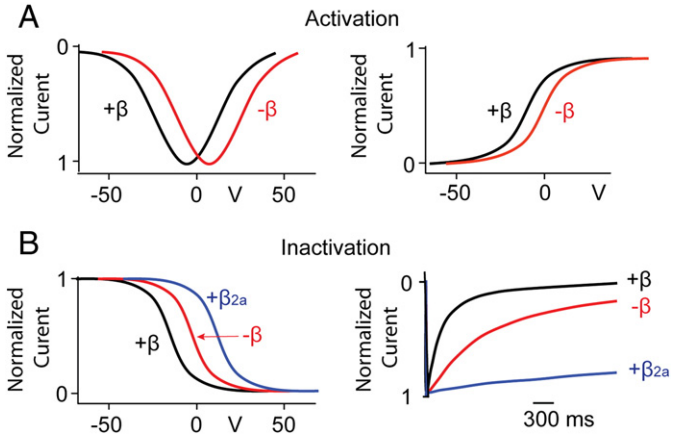

Fig. 3. Modulation of HVA Ca^{2+} channel gating by $Ca_v\beta$. (A) Activation: $Ca_v\beta$ shifts the current–voltage curve (left panel) and the activation curve (right panel) to more hyperpolarized voltages. (B) Inactivation: $Ca_v\beta$ shifts the voltage dependence of inactivation to more hyperpolarized voltages, except β_{2a}, which shifts it to more depolarized voltages (left panel). All $Ca_v\beta$ subunits speed the kinetics of inactivation, except β_{2a}, which slows the kinetics of channel inactivation (right panel). All traces are schematic representations.

Thus, the S6 segments are critical for both VDA and VDI. Notably, the AID, to which $Ca_v\beta$ binds, is connected to the IS6 segment through a short linker. Based on extensive studies [12,18,29,35,45,55,86,106,107], a unified model for $Ca_v\beta$ regulation of VDA and VDI of VGCCs has emerged. First, when $Ca_v\beta$ is bound to the AID, the entire region starting with IS6 to the end of the AID becomes a continuous α-helix [18,23,45,86]. This rigid structure allows $Ca_v\beta$ to regulate both activation and inactivation, most likely by changing the energetics of voltage-dependent movement of both IS6 and the inactivation gate. When the integrity of this rigid α-helix is disrupted by the insertion of glycine residues, the ability of $Ca_v\beta$ to regulate VDA, VDI and CDI is severely compromised, while Ca^{2+} channel surface expression remains unaffected [13,86,107]. These results underscore the essential role of a rigid IS6-AID linker in $Ca_v\beta$ regulation of VGCC gating. Further supporting this notion, the GK domain, which is the minimal part of $Ca_v\beta$ that can bind to the AID and presumably induce the formation of the rigid α-helix, can significantly impact (but not entirely normalize) activation and inactivation [12,61,97].

Second, and equally important for the model, the anchoring of $Ca_v\beta$ to $Ca_v\alpha_1$ through the AID-GK interaction enables the formation of intrinsically low-affinity interactions between $Ca_v\beta$ and other parts of $Ca_v\alpha_1$ that fully normalize channel gating [reviewed in 7].

An additional factor important for $Ca_v\beta$ regulation of gating is the orientation of $Ca_v\beta$ relative to $Ca_v\alpha_1$ [13,107,108]. Insertions or deletions in the IS6-AID linker, which are expected to maintain the α-helical structure of the linker but induce a 180° rotation of $Ca_v\beta$ with respect to $Ca_v\alpha_1$, diminish $Ca_v\beta$ regulation of activation and inactivation [13,107]. These studies are consistent with the notion that additional contacts between $Ca_v\beta$ and $Ca_v\alpha_1$ besides the AID-GK domain interaction are critical for VGCC gating.

4. $Ca_v\beta$ stoichiometry with $Ca_v\alpha_1$

4.1. $Ca_v\alpha_1$ and $Ca_v\beta$ are paired with a 1:1 stoichiometry

Early biochemical studies suggest that skeletal and neuronal VGCCs contain a single $Ca_v\alpha_1$ and a single $Ca_v\beta$ [109,110]. Extensive recent studies indicate that this is indeed the case and that the 1:1 stoichiometry is determined by the AID-GK domain interaction. (i) Covalently linked $Ca_v1.2$ and $Ca_v\beta_{2b}$ ($Ca_v1.2$-$Ca_v\beta_{2b}$) have the same gating properties as channels formed by the coexpression of $Ca_v1.2$ and β_{2b}. Moreover, when β_{2a}, which slows inactivation, is coexpressed with $Ca_v1.2$-β_{2b}, gating properties remain unchanged [111]. (ii) The simultaneous coexpression of β_{2a} and β_3 with $Ca_v\alpha_1$ yields two biophysically distinct channel populations, rather than a single population with intermediate biophysical properties [92,112]. (iii) The crystal structures of the AID–$Ca_v\beta$ core complexes clearly show that each $Ca_v\beta$ binds a single AID [11,18,19,23], and mutations of key residues in the AID or the ABP abolish both $Ca_v\beta$-mediated Ca^{2+} channel surface expression and gating modulation [4,12,13,39,44].

4.2. Dimerization of $Ca_v\beta$

Several recent studies suggested that $Ca_v\beta$ fragments can associate to form GK–GK [83,113] or SH3–SH3 domain dimers [30]. While the molecular mechanism of GK–GK domain dimerization is unclear [113], SH3 domain dimerization seems to depend on a cysteine residue that participates in forming an SH3–SH3 domain disulfide bond [30].

In addition to fragment dimerization, full-length $Ca_v\beta$ dimerization and oligomerization have been proposed, including homodimerization for β_3 and β_{2a}, and heterodimerization between β_3 and other $Ca_v\beta$s [30,113]. Higher order $Ca_v\beta$ oligomers (3 or more $Ca_v\beta$s) have also been reported, based on limited data from co-immunoprecipitation and native gel analyses under reducing and non-reducing conditions [113]. The molecular mechanisms of full-length $Ca_v\beta$ oligomerization are

unknown. Mutating the cysteine that holds together the SH3 dimer disrupts SH3–SH3 dimerization but fails to prevent full-length $Ca_v\beta_{2a}$ from dimerizing [30]. Similarly, mutations that can individually disrupt GK fragment dimerization fail to prevent full-length $Ca_v\beta_3$ dimerization [113]. It is possible that both the GK and SH3 domains lend residues for $Ca_v\beta$ dimerization.

It was recently reported that $Ca_v\beta$ dimerization is critical for dynamin-mediated channel internalization [26,30]. However, this was shown for Shaker K^+ channels and Ca^{2+} channels with a deleted AID, while WT Cav1.2 channels prevent both $Ca_v\beta$ dimerization and dynamin-mediated internalization. The steps in dynamin-mediated internalization are unclear but may involve the formation of a quaternary complex between two $Ca_v\beta$s and two dynamin molecules [30]. Finally, the fact that WT $Ca_v\alpha_1$ prevents $Ca_v\beta$ dimerization is consistent with a 1:1 stoichimetry of the $Ca_v\alpha_1/Ca_v\beta$ complex.

5. The role of $Ca_v\beta$ in $G_{\beta\gamma}$ inhibition of Ca_v2 channels

Ca_v2 channels are susceptible to several kinds of inhibition by hormones and neurotransmitters through the activation of G protein coupled receptors (see Currie and Zamponi review in this issue). The most prominent type of inhibition is the membrane-delimited, voltage-dependent inhibition mediated by the direct binding of G protein $G_{\beta\gamma}$ subunits to the channel's α_1 subunit [114,115].

5.1. $Ca_v\beta$ is required for voltage-dependent $G_{\beta\gamma}$ inhibition

Many studies indicate that $Ca_v\beta$ is essential for $G_{\beta\gamma}$-dependent channel inhibition. In COS-7 cells, G protein inhibition of N-type Ca^{2+} channels was markedly enhanced by coexpressed $Ca_v\beta$s [116]; in tsA-201 cells, a point mutation in the AID of $Ca_v2.2$ (W391A) that disrupted $Ca_v\beta$ binding abolished voltage-dependent G protein inhibition [43]. In a recent study that directly tested whether $Ca_v\beta$ is required for $G_{\beta\gamma}$ inhibition [13], large populations of Ca^{2+} channels devoid of $Ca_v\beta$ were obtained by washing away a mutant $Ca_v\beta$ that was loosely bound to the AID but was still able to chaperone channels to the membrane. Such β-less channels were still inhibited by purified $G_{\beta\gamma}$ protein applied to the cytoplasmic side of the macropatch; however, all the hallmarks of voltage-dependent inhibition were absent [13,117,118]. When $Ca_v\beta$ was supplied, $G_{\beta\gamma}$ inhibition became voltage-dependent [13]. These results suggest that in the absence of $Ca_v\beta$, $G_{\beta\gamma}$ can bind the channel and inhibit it in a voltage-independent manner [13,43]. They also suggest that under physiological conditions, $Ca_v\beta$ remains bound to the channel during $G_{\beta\gamma}$ inhibition, enhancing the dissociation of $G_{\beta\gamma}$ from the channels and giving rise to the voltage dependence of inhibition [119]. There is further evidence supporting the notion that $Ca_v\beta$ remains associated with $Ca_v\alpha_1$ during $G_{\beta\gamma}$ modulation. (1) Different $Ca_v\beta$s have different effects on voltage-dependent $G_{\beta\gamma}$ inhibition, with β_{2a} being the least effective in promoting this inhibition [61,120–123]. In addition, the efficacy of the four $Ca_v\beta$s to increase the rate of $G_{\beta\gamma}$ dissociation from the channel is different [121,122]. (2) VDA and VDI, which are significantly modulated by $Ca_v\beta$, remain unchanged before, during, and after $G_{\beta\gamma}$ modulation [13,117,123].

5.2. An allosteric model for the voltage-dependent G protein inhibition of VGCC

An allosteric model was recently proposed for the origin of the voltage dependence of $G_{\beta\gamma}$ inhibition of Ca_v2 channels [13]. There are several components in this model. First, although the $G_{\beta\gamma}$-binding pocket in the holo-channel is still unknown, it is likely formed by several regions including the I–II linker, the N-terminus, and the C-terminus of $Ca_v\alpha_1$ [124]. Second, binding of $Ca_v\beta$ transforms IS6 and a large portion of the I–II linker, including the AID, into an α-helix. This allows movements in IS6, following a depolarization, to be efficiently transduced to dismantle the $G_{\beta\gamma}$ binding pocket, causing $G_{\beta\gamma}$ dissociation. In the absence of $Ca_v\beta$, the AID is a random coil [18] and IS6 movements cannot be efficiently transmitted to the I–II linker. Thus, $G_{\beta\gamma}$ stays on the channel, inhibiting it with no voltage dependence. Corroborating this model, mutations that disrupt the rigid α-helix encompassing IS6 and the AID abolish the voltage dependence of $G_{\beta\gamma}$ inhibition in the presence of $Ca_v\beta$ [13,61].

6. Role of $Ca_v\beta$ in the regulation of Ca^{2+} channels by PIP_2 and arachidonic acid

6.1. β_{2a} dampens the inhibitory effect of PIP_2 depletion

Phosphatidylinositol-4, 5-biphosphate (PIP_2), a membrane phospholipid composed of two long fatty acid chains attached to a phosphoinositol head group, is necessary for the maintenance of HVA currents [125–128], and PIP_2 depletion following Gq-coupled receptor stimulation results in voltage-independent inhibition of HVA channels [92,125,129,130]. Recently, it was demonstrated that the coexpression of β_{2a} with $Ca_v2.1$, $Ca_v2.2$ and $Ca_v1.3$ channels can largely prevent channel inhibition upon PIP_2 depletion [92]. This effect was the direct result of β_{2a} palmitoylation since preventing palmitoylation abolished channel protection from PIP_2 depletion. Moreover, imparting palmitoylation to β_3, by fusion to an unrelated palmitoylated peptide, enabled the modified β_3 to protect the channels from PIP_2 depletion [92]. The proposed molecular mechanism for β_{2a}'s action is that the two β_{2a} palmitoyl groups, which are long fatty acid chains, can stabilize Ca^{2+} channels by substituting for PIP_2. Although the PIP_2 binding site on VGCCs is unknown, it was proposed to be 'bidentate'—one region binds the PIP_2 fatty acid chains, and the other binds to the PIP_2 head group. When both sites are occupied, the channel is 'stretched' in a more active conformation. It was further suggested that β_{2a} can engage both sites to maintain channel activity even in the absence of PIP_2 [92].

6.2. β_{2a} suppresses channel inhibition by arachidonic acid

Many phospholipids, including PIP_2, can be metabolized by lipases to arachidonic acid (AA), an unsaturated fatty acid without a head group. The accumulation of AA inhibits HVA Ca^{2+} channels [131–133]. It was recently suggested that this inhibition was the result of occupying only a single site within the bidentate lipid binding site on the channels [92]. The inhibitory action of AA on $Ca_v1.3$ is attenuated in the presence of β_{2a}, but not other $Ca_v\beta$s [134,135]. This dampening effect critically depends on β_{2a} palmitoylation per se, rather than membrane anchorage. Thus, the palmitoyl groups of β_{2a} can both compete with AA to prevent VGCC inhibition, and also substitute for PIP_2 and protect channels from PIP_2 depletion, as discussed above. Both actions likely occur via the same bidentate lipid binding site on VGCCs [92,134,136]. Finally, the competition of the β_{2a} palmitoyl groups with AA can be prevented by manipulating the IS6-AID linker to change the orientation of $Ca_v\beta$ in relation to $Ca_v\alpha_1$ [108], suggesting that β_{2a} palmitoyl groups have a precise binding site on the channel, likely the same site where PIP_2 and AA bind.

7. $Ca_v\beta$ may have transcriptional activity

Several short $Ca_v\beta$ isoforms have been cloned that do not contain a GK domain [83,137–140]. In the first such report, a short $Ca_v\beta$ that lacks 90% of the GK domain and the entire C-terminus was cloned form chicken brain and named β_{4c} [140]. This cβ_{4c} has almost no effect on $Ca_v2.1$ channels expressed in *Xenopus* oocytes but it can dose-dependently attenuate the repressor function of heterochromatin protein 1 (HP1), a chromatin organizer. These findings suggest that cβ_{4c} may function as a transcription regulator. Similar results have been recently reported for a human β_{4c} isoform found in the nuclei

of vestibular neurons [141]. Full-length $Ca_v\beta$ has also been implicated in transcriptional regulation. β_3, for example, can directly interact with and suppress the transcriptional activity of Pax6(S), in vitro [142]. In HEK 293T cells, coexpression of β_3 and Pax6(S) results in the translocation of β_3 from the cytoplasm to the nucleus. Several other studies have shown nuclear targeting of $Ca_v\beta$ in native cells [79,143,144]. It remains to be determined which specific genes are the targets of $Ca_v\beta$ transcriptional regulation. It is also unclear whether nuclear targeting of $Ca_v\beta$ is correlated with VGCC activity; one recent study suggests that L-type Ca^{2+} channel activity diminishes the nuclear targeting of β_{4b} in the cerebellum [143].

8. $Ca_v\beta$ knockouts and pathophysiology

Because of the essential role of $Ca_v\beta$ to enable the surface expression and functional modulation of HVA Ca^{2+} channels, $Ca_v\beta$ mutations have been implicated in human disease. $Ca_v\beta$ knockout animals and mutants have severe phenotypes that are in some cases lethal. Ultimately, what determines the phenotypical outcome of a knockout mouse is the ability of the remaining $Ca_v\beta$s to compensate for the lost functions.

8.1. β_1

$Ca_v\beta_1$ is expressed in brain, heart, skeletal muscle, spleen, T cells and other tissues [reviewed in 7]. The β_{1a} isoform, however, is exclusively expressed in skeletal muscle where it partners with skeletal muscle $Ca_v1.1$, and is irreplaceable for excitation–contraction coupling. Thus, β_1 knockout mice, similar to $Ca_v1.1$ knockouts, are born motionless and die immediately from asphyxiation [75]. A similar phenotype is observed in zebrafish [145].

Paradoxically, the increased expression of β_{1a} in aging mice was recently proposed to cause skeletal muscle weakness due to decreased levels of $Ca_v1.1$ channel expression [146]. Knockdown of β_1 in aging mice could increase muscle force and Cav1.1 expression levels to those observed in young mice. This is a surprising effect considering $Ca_v\beta$ normally enhances VGCC expression, as is the case in young mice [146]. It remains to be determined what age-related factors turn $Ca_v\beta$ from a positive to a negative regulator of $Ca_v1.1$ expression.

8.2. β_2

$Ca_v\beta_2$ and its various splice variants are expressed in brain, heart, lung, nerve endings at the neuro-muscular junction, T cells, osteoblasts and other tissues [7]. It is also the predominant $Ca_v\beta$ in the heart, especially $Ca_v\beta_{2b}$ [79]. β_2 knockouts die prenatally at embryonic day 10.5 due to lack of cardiac contractions [147,148]. Interestingly, when β_2 expression is restored to the heart of β_2-knockout animals using a cardiac muscle-specific promoter, the animals survive but are deaf due to several deficiencies in the inner ear, including a dramatic reduction in the expression of $Ca_v1.3$ channels [147,149]. These 'rescued' mice also have defects in vision with a phenotype similar to human patients with congenital stationary night blindness [150].

It is not clear whether β_2 is essential only during certain stages of development or throughout life. A recent study in which the β_2 gene was conditionally knocked out in adult mouse cardiomyocytes gave unanticipated results [151]. Peak calcium currents were reduced by only ~30% and the mice had no obvious impairment, suggesting that β_2 may be more critical for the developing than the adult heart [151].

Two point mutations in β_{2b} have been implicated in cardiovascular human diseases. The S481L mutation, which occurs in the C-terminus of β_{2b}, contributes to a type of sudden death syndrome characterized by a short QT interval and an elevated ST-segment [152]. The other mutation, in the β_{2b} N-terminus (T11I), causes accelerated inactivation of cardiac L-type channels and is linked to the Brugada syndrome [153].

8.3. β_3

$Ca_v\beta_3$ knockouts are viable [154,155], with reduced perception of inflammatory pain but unaltered mechanically or thermally induced pain. This is likely the result of reduced N-type calcium channel expression in dorsal root ganglia [77]. $Ca_v\beta_3$ knockouts also have abnormally high insulin secretion at high blood glucose concentrations [156]. A high salt diet causes abnormally elevated blood pressure, a reduction in plasma catecholamine levels, and a hypertrophy of heart and aortic smooth muscle [155,157]. These results point to a compromised sympathetic control in β_3 knockout mice, likely due to reduced N- and L-type channel activity [154]. Behaviorally, β_3-null mice exhibit impaired working memory, but some forms of hippocampus-dependent learning are enhanced [158,159]. Knockout mice also have lower anxiety, increased aggression, and increased nighttime activity [159]. Finally, both β_3 and β_4 knockout mice have abnormal T-cell signaling, revealing an unanticipated function of $Ca_v\beta$s in the immune system [160].

A recent study comparing epileptic patients with non-epileptic individuals identified 3 mutations in β_3 that were present only in patients; however, it is difficult to conclude whether these mutations are the cause of epilepsy [161].

8.4. β_4

Lethargic mice are naturally occurring β_4 knockouts [162–164]. They have an insertion that causes exon skipping and a premature stop codon in the gene for β_4. These mice exhibit ataxia, seizures, absence epilepsy, and paroxysmal dyskinesia [162,165,166]. Some of this phenotype is contributed by a 50% upregulation of T-type Ca^{2+} channels in thalamic neurons [167]. Other characteristics of lethargic mice include reduced excitatory neurotransmission in the thalamus [168] and a modified electro-oculogram [169]. Interestingly, and perhaps indicative of $Ca_v\beta$ functions that are independent of VGCC, β_4 knockouts have aberrant splenic and thymic involution [163,164] and renal cysts [161]. Similar to β_3 knockouts, $CD4^+$ T cells have attenuated receptor-mediated Ca^{2+} responses [160].

In humans, an R468Q mutation in the gene encoding β_4 is associated with a history of febrile seizures, presumably due to the enhancement of $Ca_v2.1$ currents [170]. In addition, a juvenile myoclonic epilepsy patient was found to have a truncated β_4 (R482x) [171]. Interestingly, two families with different disease histories—one with episodic ataxia and the other with generalized epilepsy and praxis-induced seizures—share the same mutation in β_4 (C104F) [171], highlighting the importance of genetic background in disease penetrance.

9. Future directions

Past studies have provided great insights into the structure, function, and physiology of $Ca_v\beta$. They have also opened new avenues for future research and prompted many intriguing questions that remain to be answered, some of which we highlight below.

1) Knockout mice provide a useful tool in the study of $Ca_v\beta$ physiology. Conditional and tissue-specific knockouts are particularly useful as they circumvent compensatory mechanisms that are triggered in conventional knockout mice. This advantage was highlighted in several recent studies that found unexpected mouse phenotypes when $Ca_v\beta$ was conditionally knocked out [147,150,151]. We anticipate that future conditional knockouts would similarly provide great insights into the physiological functions of $Ca_v\beta$ and the significance of its diversity.

2) $Ca_v\beta$ has functions that are independent of VGCC [reviewed in 7]. Most notable is a possible role in transcriptional regulation, which has been demonstrated for both short splice variants and full-length $Ca_v\beta$. It is not clear, however, how and which tissues benefit from such regulation under physiological conditions. It would also be interesting to know which signaling events trigger the nuclear translocation of $Ca_v\beta$.
3) There are currently two prevalent hypothesis that explain the role of $Ca_v\beta$ in VGCC trafficking: (1) $Ca_v\beta$ alters the balance of ER retention and ER export signals on $Ca_v\alpha_1$ in favor of the latter, and (2) $Ca_v\beta$ protects channels from predestined proteasomal degradation. These two hypotheses are not mutually exclusive but their relationship remains unclear. It also remains to be determined whether the proposed mechanisms of $Ca_v\beta$-mediated channel trafficking apply to all Ca_v1 and Ca_v2 channels.
4) The crystal structures of the $Ca_v\beta$ core have provided great insights into the molecular mechanisms of $Ca_v\beta$ function. It would be of great interest to obtain high-resolution structures of full-length $Ca_v\beta$ and of $Ca_v\beta$ in complex with its various interacting partners, such as RGK proteins, dynamin or the ryanodine receptor. Such structures would shed significant light on how different cellular signals converge on $Ca_v\beta$ to regulate VGCC function.
5) $Ca_v\beta$s have many interacting partners (Fig. 2, also reviewed in [7]), some of which significantly impact the function of VGCCs. The recent findings that $Ca_v\beta$ interacts with synaptic proteins [172,173] uncover new roles for $Ca_v\beta$ in the organization of the synaptic vesicle release machinery and reveal a new facet of VGCC physiology. We anticipate that the search and study of new $Ca_v\beta$ partners will continue to be an interesting and productive area of research in the future.

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