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The β Subunit of Voltage-Gated Ca^{2+} Channels

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Abstract

Calcium regulates a wide spectrum of physiological processes such as heartbeat, muscle contraction, neuronal communication, hormone release, cell division, and gene transcription. Major entry-ways for Ca^{2+} in excitable cells are high-voltage activated (HVA) Ca^{2+}channels. These are plasma membrane proteins composed of several subunits, including α_1, $\alpha_2\delta$, β, and γ. Although the principal α_1 subunit ($Ca_v\alpha_1$) contains the channel pore, gating machinery and most drug binding sites, the cytosolic auxiliary β subunit ($Ca_v\beta$) plays an essential role in regulating the surface expression and gating properties of HVA Ca^{2+} channels. $Ca_v\beta$ is also crucial for the modulation of HVA Ca^{2+} channels by G proteins, kinases, and the Ras-related RGK GTPases. New proteins have emerged in recent years that modulate HVA Ca^{2+} channels by binding to $Ca_v\beta$. There are also indications that $Ca_v\beta$ may carry out Ca^{2+} channel-independent functions, including directly regulating gene transcription. All four subtypes of $Ca_v\beta$, encoded by different genes, have a modular organization, consisting of three variable regions, a conserved guanylate kinase (GK) domain, and a conserved Src-homology 3 (SH3) domain, placing them into the membrane-associated guanylate kinase (MAGUK) protein family. Crystal structures of $Ca_v\beta$s reveal how they interact with $Ca_v\alpha_1$, open new research avenues, and prompt new inquiries. In this article, we review the structure and various biological functions of $Ca_v\beta$, with both a historical perspective as well as an emphasis on recent advances.

I. INTRODUCTION

Calcium is arguably one of life's most important elements. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is kept at very low levels (~100 nM) under resting conditions, but it rises sharply (to tens or hundreds of μM) upon stimulation. This allows Ca^{2+} to play a crucial role in numerous biological processes, including neurotransmitter and hormone release, muscle excitation-contraction coupling, cell division, tumorigenesis, differentiation, migration, and cell death. In addition, Ca^{2+} influx across the plasma membrane causes changes in cellular excitability. Mechanisms that rigorously control intracellular Ca^{2+} levels are therefore essential for eukaryotic cell function. $[Ca^{2+}]_i$ is maintained at low levels by Ca^{2+}-ATPases through active extrusion of cytosolic Ca^{2+} to the extracellular milieu or into intracellular

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DISCLOSURES
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organelles. On the other hand, Ca^{2+} entry into cells is mediated primarily by passive flow through voltage-, ligand-, temperature-, and mechanical stretch-gated ion channels.

The principal Ca^{2+} entryways of nerve, muscle, and some endocrine cells are voltage-gated Ca^{2+} channels (VGCCs). They were discovered in 1953 with the unexpected observation that crab muscle action potentials (APs) persist in the absence of external Na^+, unlike squid nerve APs (145). Muscle APs were then found to increase with increasing extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$), consistent with a Ca^{2+} conductance (200). Similar currents were later found in nerve, endocrine, and other tissues in diverse organisms (12, 221,225, 253, 291, 304). Based on the membrane voltage required for activation, VGCCs were subsequently classified into high-voltage activated (HVA) and low-voltage activated (LVA) channels (65, 66,146, 293). Later studies further classified Ca^{2+} currents into L-, N-, P/Q-, R-, and T-type currents, which exhibit distinct biophysical and pharmacological properties (127, 137,292, 335, 359, 408, 444, 446, 500).

Molecular characterization of VGCCs began with the purification and cloning of the skeletal muscle Ca^{2+} channel (also called dihydropyridine receptor or DHPR) (107, 430, 434). The purified channel complex is composed of five subunits, termed α_1 (175 kDa), α_2 (143 kDa), β (54 kDa), δ (24–27 kDa), and γ (30 kDa). α_2 and δ are linked posttranslationally by disulfide bonds into a single subunit referred to as $\alpha_2\delta$ (430). Subsequent research showed that L-, N-, P/Q- and R-type channels are made up of α_1, $\alpha_2\delta$, β, and, in some tissues, γ subunits (Fig. 1*A*). T-type channels, on the other hand, appear to require only an α_1 subunit (351, 352).

The α_1 subunit ($Ca_v\alpha_1$) is the principal component of VGCCs and is responsible for their unique biophysical and pharmacological properties. However, proper trafficking and functioning of L-, N-, P/Q- and R-type channels require the auxiliary subunits. In particular, the β subunit ($Ca_v\beta$) plays a crucial role in trafficking the channels to the plasma membrane, fine-tuning channel gating, and regulating channel modulation by other proteins and signaling molecules. Crystal structures of the core region of three distinct $Ca_v\beta$s have opened up new avenues for investigating the molecular basis of $Ca_v\beta$'s actions. There is also emerging evidence that $Ca_v\beta$ may possess functions unrelated to VGCCs. This review focuses on the molecular biology, structure, function, and channelopathy of $Ca_v\beta$, beginning with a brief overview of all VGCC subunits. Summaries of classical and recent work on VGCC electrophysiology, pharmacology, biochemistry, molecular biology, modulation, cell biology, and pathophysiology can be found in numerous excellent reviews (20, 22,72–74, 108, 126, 138, 189, 216, 220, 234, 237, 246, 318, 351, 389, 423, 440, 445, 495).

A. The α_1 Subunit

$Ca_v\alpha_1$ is the principal subunit of VGCCs. It is a 190- to 250-kDa protein containing four homologous repeats (I–IV) connected through cytoplasmic loops (Fig. 1*B*). Each repeat has six predicted transmembrane segments (S1–S6) and a reentrant pore-forming loop (P-loop) between S5 and S6. The four P-loops form the ion-selectivity filter, where four highly conserved negatively charged amino acids (glutamate or aspartate), one from each P-loop, form a signature locus that is essential for selecting and conducting Ca^{2+} (256, 266,389, 482). Similar to K^+ channels (128, 243, 290), the S6 segments form the inner pore (505), and the S4 segments' positively charged amino acids form part of the voltage sensor. The voltage-dependent movement of this sensor results in channel opening and closing. Furthermore, the majority of drug and toxin binding sites are located on $Ca_v\alpha_1$ (72). Thus $Ca_v\alpha_1$ possesses all the key features that define a VGCC, including pharmacological and biophysical properties such as gating, ion selectivity, and permeation.

Mammalian $Ca_v\alpha_1$ are encoded by 10 distinct genes. Based on amino acid sequence similarity, $Ca_v\alpha_1$ are divided into three subfamilies: Ca_v1, Ca_v2, and Ca_v3 (reviewed in Refs. 10, 72, 141, 486). The Ca_v1 subfamily includes channels that conduct L-type Ca^{2+} currents; the Ca_v2 subfamily includes channels that conduct N-, P/Q-, and R-type Ca^{2+} currents; and the Ca_v3 subfamily includes channels that conduct T-type Ca^{2+} currents (Fig. 1*C*).

B. The $\alpha_2\delta$ Subunit

The Ca_v1 and Ca_v2 subfamilies contain an auxiliary $\alpha_2\delta$ subunit (reviewed in Ref. 112). To date, there are four known $\alpha_2\delta$ subunits, each encoded by a unique gene and all possessing splice variants (Fig. 1*D*). Each $\alpha_2\delta$ protein is encoded by a single messenger RNA and is posttranslationally cleaved and then linked by disulfide bonds (259, 367). The δ peptide, originally presumed to be transmembrane but recently shown to be attached to the membrane through a glycosylphosphatidylinositol linker (113), anchors the larger extracellular α_2 peptide in place (Fig. 1*A*). $\alpha_2\delta$ subunits can modify channel biophysical properties (63, 406, 459), but their main role is to increase Ca^{2+} channel current (63, 111,174, 259, 260, 322, 406, 459) by promoting trafficking of $Ca_v\alpha_1$ to the plasma membrane and/or by increasing its retention there (32, 64,194, 385). More recently, it was reported that $\alpha_2\delta$ functioned as a thrombospondin receptor to regulate excitatory synpatogenesis, independently from its regulation of VGCC activity (140, 267).

In two different mouse strains, naturally occurring mutations that lead to the loss of the full-length $\alpha_2\delta_2$ protein cause the *ducky* phenotype. This is characterized by shortened life spans, absence epilepsy, spike wave seizures, cerebellar ataxia, and decreased Purkinje cell dendritic arborization and firing rates (112, 260). $\alpha_2\delta_2$ knockouts also have abnormalities in the cardiovascular, immune, respiratory, and nervous systems. Irregularities in the cardiovascular system are also found in $\alpha_2\delta_1$ knockouts (169). $\alpha_2\delta_3$-null *Drosophila* are not viable, and the mutants have significantly impaired synaptic transmission (123, 267). Upregulation of $\alpha_2\delta_1$, on the other hand, is associated with neuropathic pain (283, 284). Importantly, $\alpha_2\delta_1$ is the main target of the antiepilepsy and antineuropathic pain drugs gabapentin and pregabalin, respectively (150, 169, 254).

C. The γ Subunit

There are eight different γ subunit genes, all yielding proteins with four transmembrane segments and intracellular amino (NH_2) and carboxy (COOH) termini. γ_1 was the first cloned γ subunit (182, 238, 430) and was copurified with muscle VGCCs, consistent with its primary role as a VGCC subunit. γ_1 knockout mice are viable, morphologically indistinguishable from wild-type (WT) mice, but have larger Ca^{2+} currents with altered inactivation kinetics (168). γ_2, γ_3, and γ_4 also associate with VGCCs (250, 399). γ_{1-4}

subunits have been shown to produce varying effects on VGCC activity, depending on the partnered $Ca_v\alpha_1$ and $Ca_v\beta$ (134, 168,2017, 250, 258, 277, 379, 406, 467). The most consistent effect is a small reduction of current, caused mainly by a hyperpolarizing shift of the voltage dependence of inactivation and/or a positive shift of the voltage dependence of activation. However, unlike $\alpha_2\delta$ subunits, whose primary role is regulating VGCCs, γ subunits have more diverse biological functions. Since the discovery that mutations in γ_2 underlie the *stargazer* mouse phenotype (277), which includes absence epilepsy and defects in the cerebellum and inner ear, it has become clear that γ_2 and three other closely related γ subunits (γ_3, γ_4, and γ_8) regulate the trafficking, localization, and biophysical properties of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (41, 82,249, 343, 442). They are therefore referred to as transmembrane AMPA receptor regulatory proteins (TARPs). Indeed, acting as TARPs seems to be the primary role of γ_2, γ_3, γ_4, γ_8, and probably γ_7 (252). While the function of γ_5 remains unknown, γ_6 is suggested to inhibit $Ca_v3.1$ channels (288), and γ_7 is involved in the turnover of the mRNA of $Ca_v2.2$ and other proteins (149, 323). For recent reviews on γ subunits, see References 41, 82, 249, 320, 343, 350, 382.

D. The β Subunit

Purified Ca_v1 and Ca_v2 channels contain a tightly bound cytosolic $Ca_v\beta$ protein. There are four subfamilies of $Ca_v\beta$s (β_1– (β_4), each with splice variants, encoded by four distinct genes. All four $Ca_v\beta$s can dramatically enhance Ca^{2+} channel currents when they are coexpressed in heterologous expression systems along with a Ca_v1 or Ca_v2 (α_1 subunit (268, 319,322, 361, 405, 450, 467, 470). $Ca_v\beta$s also change the voltage dependence and kinetics of activation and inactivation (247, 268,322, 332, 406, 412, 418, 450, 495); however, they do not affect ion permeation (183, 405,458; but see Ref. 390). Furthermore, $Ca_v\beta$ either regulates or is indispensable for the modulation of Ca_v1 and Ca_v2 channels by protein kinases, G proteins, and small RGK (Rem, Rem2, Rad, Gem/Kir) proteins. Not surprisingly, $Ca_v\beta$ knockouts are either nonviable (in the case of (β_1 and (β_2) or result in a severe pathophysiology (in the case of (β_3 and (β_4).

The rest of this review is devoted to $Ca_v\beta$.

II. CLONING OF $Ca_v\beta$

Molecular studies on $Ca_v\beta$ can be traced back to the first purification and identification of the components of the skeletal muscle DHPR (107). With the use of a combination of chromatography, sucrose gradient sedimentation, and labeling with a high-affinity DHPR-specific ligand, three noncovalently attached subunits were purified: the largest 160-kDa subunit was named (α, a 53-kDa subunit was named (β, and a 32-kDa subunit was named (γ (107). Subsequent purification studies of skeletal and neuronal Ca^{2+} channel complexes showed the presence of similar protein bands (4, 59,114, 162, 278, 322, 381, 430, 434) and established that the DHPR actually consisted of five subunits, including (α_1 (175 kDa), (α_2 (143 kDa), (β (54 kDa), (δ (24–27 kDa), and (γ (30 kDa) (430, 434).

Cloning of the first $Ca_v\beta$ was accomplished by Ruth et al. (381) using a classical approach based on peptide sequences derived from a purified skeletal muscle (β subunit. This (β subunit is now referred to as (β_{1a}. This cloning paved the way for the identification of other (β subunits, their genes, and splice variants. Using a labeled skeletal muscle (β_{1a} cDNA, Pragnell et al. (362) screened a rat brain cDNA library and cloned a new (β subunit, which later turned out to be a splice variant of (β_1 named (β_{1b} (360) (see sect. IV). Perez-Reyes et al. (353) also screened a rat brain cDNA library with (β_{1a} and, using low-stringency hybridization, uncovered another new (β subunit, which was encoded by a different gene and named (β_2 (now named (β_{2a}). Screening a cardiac cDNA library, Hullin et al. (230) found (β_{2a} and two other (β_2 splice variants ((β_{2b} and (β_{2c}); in addition, they isolated the cDNA for (β_3. Meanwhile, using degenerate primers corresponding to the conserved domains of (β_1 and (β_2 to perform reverse-transcription PCR, Castellano et al. (67, 68) cloned (β_3 and (β_4 from a rat brain cDNA library.

The cloning of $\text{Ca}_v\beta$s subsequently led to the mapping of the four $\text{Ca}_v\beta$ genes (55, 94,143, 347, 438) to chromosomes 17, 10, 12, and 2 for (β_1, (β_2, (β_3, and (β_4, respectively, and to the discovery of many other splice variants (see sect. IV).

III. STRUCTURE OF $\text{Ca}_v\beta$

Prior to the determination of the crystal structure of $\text{Ca}_v\beta$, it was already well recognized, based on amino acid sequence alignment, biochemical and functional studies, and molecular modeling, that $\text{Ca}_v\beta$ has a modular structure consisting of five distinct regions (40, 93,119, 203, 342, 361). The first, third, and fifth regions are variable in length and amino acid sequence, whereas the second and fourth regions are highly conserved and are homologous to the Src homology 3 (SH3) and guanylate kinase (GK) domains, respectively. The SH3 domain is a common protein interaction module present in diverse groups of proteins (reviewed in Ref. 307). The GK domain, originally found in guanylate kinase from baker's yeast (416), is also engaged in protein-protein interactions (136, 170, 431). The middle three regions of $\text{Ca}_v\beta$ constitute the so-called $\text{Ca}_v\beta$ core, which is able to reconstitute many key functions of $\text{Ca}_v\beta$ (83, 84,119, 176, 206, 313, 342, 502). In addition, early studies determined that $\text{Ca}_v\beta$ binds with high affinity to $\text{Ca}_v\alpha_1$. This high-affinity site is located in the cytoplasmic loop connecting the first two homologous repeats (i.e., the I–II loop) of $\text{Ca}_v\alpha_1$ and was named the (α-interaction domain or AID (121, 361, 472) (Fig. 1*E*).

In 2004, three groups simultaneously and independently reported the crystal structure of the core of (β_{2a}, (β_3 and (β_4, either alone or in complex with the AID (84, 341, 447). The structures show that the $\text{Ca}_v\beta$ core indeed contains an SH3 domain and a GK domain, which are connected by a so-called HOOK region (Fig. 2*A*).

The existence of an SH3-HOOK-GK module places $\text{Ca}_v\beta$ in a family of proteins called the membrane-associated guanylate kinases (MAGUKs). MAGUKs, which include proteins such as PSD95, SAP97, CASK, Shank, and Homer, function as scaffold molecules that play a key role in organizing multiprotein complexes at functionally specialized regions such as synapses and other cellular junctions (136, 170, 431). MAGUKs contain an SH3-HOOK-GK module; in addition, they also contain one or more PDZ domains in the NH_2 terminus, which serve protein-protein interaction and oligomerization functions. $\text{Ca}_v\beta$ is only partially related to MAGUKs structurally, however, because it does not contain a well-defined PDZ domain. Not surprisingly, the functions of $Ca_v\beta$ are markedly different from those of MAGUKs.

A. The GK Domain

Guanylate kinases are members of the nucleotide monophosphate kinase family that exists in organisms ranging from bacteria to humans. They catalyze the reversible phosphoryl transfer from ATP to GMP to produce ADP and GDP. Crystal structures of yeast guanylate kinases show that these enzymes have a compact structure with well-defined domains and folds and a catalytic site harboring the GMP- and ATP-binding pockets (43, 415, 416). The general structural features of yeast guanylate kinases are preserved in the $Ca_v\beta$ GK domain ($Ca_v\beta$-GK), but large structural variations exist in the catalytic site, and many key catalytic residues are absent in $Ca_v\beta$-GK (84, 341, 447). Thus $Ca_v\beta$-GK is catalytically inactive. Similarly, the GK domain of MAGUKs does not possess catalytic activity, as indicated by the structural changes in the catalytic site and the lack of critical catalytic residues (285, 312, 437). Instead, the GK domains in these proteins have evolved into a protein interaction module. The $Ca_v\beta$ structures show that $Ca_v\beta$-GK binds tightly to the AID in $Ca_v\alpha_1$ (84, 341, 447) (Fig. 2*A*), an interaction that will be further discussed in detail.

B. The SH3 Domain and the HOOK Region

Classical SH3 domains have a well-conserved and compact fold consisting of five sequential β-strands (βstrand 1–5) assembled into two orthogonally packed sheets (271). They mediate specific protein-protein interactions by binding to PxxP-containing motifs in target proteins, through a surface formed by a cluster of highly conserved hydrophobic residues. The $Ca_v\beta$ SH3 domain ($Ca_v\beta$-SH3) has a similar fold as canonical SH3 domains do, but its last two β sheets are noncontinuous, separated by the HOOK region (84, 341, 447) (Fig. 2*A*). This split configuration is also shared by the SH3 domain of PSD-95, a MAGUK (312, 437). $Ca_v\beta$-SH3 contains a well-preserved PxxP motif-binding site and therefore has the potential to bind PxxP motif-containing proteins. However, in the crystal structures, this binding site is partly shielded by the HOOK region and a long loop connecting two of the four continuous β sheets. Thus access to this site requires movement of these two regions. Such conformational changes are conceivable when $Ca_v\beta$ is bound to full-length $Ca_v\alpha_1$ and/or when it interacts with other partners, but are yet to be demonstrated. In contrast, the PxxP motif-binding site of the SH3 domain of PSD-95 is unobstructed (312, 437), consistent with the observation that the SH3 domain of MAGUKs can associate directly with PxxP motif-containing proteins (177, 306).

The HOOK region is variable in length and amino acid sequence among the $Ca_v\beta$ subfamilies (Fig. 3). 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 (84, 341, 447). As will be discussed below, the HOOK region plays an important role in regulating channel inactivation.

C. The NH_2 Terminus

The NH_2 and COOH termini of $Ca_v\beta$ (abbreviated as $Ca_v\beta$-NT and $Ca_v\beta$-CT) are highly variable in length and amino acid composition (Fig. 3). There is yet no structure available for $Ca_v\beta$-CT. However, an NMR structure of the NH_2 terminus of β_4 was solved recently, revealing a fold consisting of two α-helices and two antiparallel β sheets (451). This structure also shows that, unlike previously thought (203), $Ca_v\beta$-NT does not have a PDZ fold, which consists of five β sheets (380). Incidentally, one of the two α-helices in the NMR structure is equivalent to the very first α-helix in the $Ca_v\beta$ core structures. Superposition of this helix in the two structures reveals that the NH_2 terminus is oriented away from the core (Fig. 4).

D. The SH3-GK Intramolecular Interaction

The crystal structures of the $Ca_v\beta$ core show that the SH3 and GK domains interact intramolecularly (84, 341, 447). The affinity of this interaction is unknown, but the interaction is strong enough such that hemi-$Ca_v\beta$ fragments containing the 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 when they are coexpressed in cells (298, 313,342, 431, 432, 447). In fact, one of the β_{2a} structures was obtained from cocrystals of two β_{2a} hemifragments truncated at the HOOK region (447).

The last β sheet of $Ca_v\beta$-SH3 ($SH3_{\beta strand\ 5}$), which is separated from the rest of the SH3 domain by the HOOK region, is critical for the strong intramolecular SH3-GK interaction (83, 298,313, 342, 431, 432). This β sheet is directly connected to the GK domain, and it interacts extensively with both the GK domain and the rest of the SH3 domain (83). As a result, $SH3_{\beta strand5}$ glues the NT-$SH3_{\beta strand1–4}$-HOOK module and the $SH3_{\beta strand5}$-GK-CT module together and strengthens the otherwise weak interactions at the SH3-GK interface (83).

As in MAGUKs, the SH3-GK intramolecular interaction is important for the function of $Ca_v\beta$ (83, 298,313, 431, 432). Weakening this interaction by mutating the SH3-GK interface or by inserting flexible linkers between the SH3 domain and the GK domain severely compromises the gating effects of $Ca_v\beta$ (83). Thus mutations, modifications, or protein-protein interactions that alter the SH3-GK intramolecular interaction may produce significant functional consequences.

E. The AID-$Ca_v\beta$ Interaction

Which regions anchor $Ca_v\beta$ to $Ca_v\alpha_1$? By screening an epitope library of 20,000 Ca_v1.1 fragments, Pragnell et al. (361) identified a region in the I–II loop that binds β_{1b}. This region, known as the AID, is comprised of 18 residues, with a conserved consensus motif (QQxExxLxGYxxWIxxxE) in all Ca_v1 and Ca_v2 α_1 subunits (Fig. 1*E*). The AID binds to all four $Ca_v\beta$s (121). The affinity of the AID-$Ca_v\beta$ interaction ranges from 2 to 54 nM, depending on the AID/$Ca_v\beta$ or $Ca_v\alpha_1$/$Ca_v\beta$ pair and the method of affinity measurement (30, 56,62, 120, 121, 179, 342, 371, 395, 448). Single mutations of several conserved residues in the AID, including Y10, W13, and I14, greatly weaken the AID-$Ca_v\beta$ interaction, as indicated by in vitro binding experiments and by the reduction or abolishment of $Ca_v\beta$-induced stimulation of Ca^{2+} channel current in heterologous expression systems (33, 34,56, 120, 181, 185, 206, 218, 276, 361, 448). Thus the role of the AID as the principal interacting domain with $Ca_v\beta$ is firmly established.

Which region(s) of $Ca_v\beta$ interact with the AID? In an influential study, De Waard et al. (119) described a 31-amino acid segment of $Ca_v\beta$, referred to as the β-interacting domain or BID, as the main binding site for the AID. The BID was able to slightly enhance Ca^{2+} channel current and modulate gating (119), 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 (119, 120).

For the next decade, it had been generally accepted that $Ca_v\beta$ interacted with $Ca_v\alpha_1$ primarily through the BID. Surprisingly, however, the crystal structures of two different AID-$Ca_v\beta$ core complexes reveal that the AID does not bind the BID (84, 341, 447). Indeed, the AID and the BID do not come into direct contact (Fig. 2*B*). Instead, the AID binds to a hydrophobic groove in the GK domain termed the AID-binding pocket (ABP; Fig. 2*C*) (84, 447, 448). The AID occupies only a tiny fraction of the $Ca_v\beta$ surface area, raising the possibility that other domains of $Ca_v\beta$ are involved in interactions with other regions of $Ca_v\alpha_1$ or with other proteins. As will be discussed later, both are indeed the case.

The AID-GK domain interactions are extensive and predominantly hydrophobic (Fig. 2*C*). These interactions account for the 2–54 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 (206, 502).

The binding of the AID with $Ca_v\beta$ does not significantly change the $Ca_v\beta$ structure, except for some small and localized changes near the ABP. Importantly, however, the AID undergoes a dramatic change in secondary structure when it is engulfed by the ABP. When alone, the AID exists as a random coil in solution, as determined by circular dichroism spectrum measurements (341). When bound to $Ca_v\beta$, the AID forms a continuous α-helix, as shown in the crystal structures. Together with the observation that the 22-amino acid linker between the AID and the first S6 segment of $Ca_v\alpha_1$ (i.e., IS6) also forms an α-helix (9), a picture emerges that the entire region encompassing IS6 and the AID adopts a continuous α-helical structure in the presence of $Ca_v\beta$ (Fig. 4). This structural hallmark is crucial for the regulation of Ca^{2+} channel gating by $Ca_v\beta$, as will be discussed later.

Since the publication of the $Ca_v\beta$ structures, some investigators have been continuing to perform or interpret experiments based on the notion that the BID interacts with $Ca_v\alpha_1$ (85, 281,302, 388, 441, 506), so before leaving this section, we briefly revisit the BID. The crystal structures show that the BID spans three different regions of $Ca_v\beta$ (SH3, HOOK and GK) and that most of it is completely buried (Fig. 2*B*). Thus the BID does not directly interact with $Ca_v\alpha_1$; rather, it is crucial for maintaining the SH3-GK intramolecular interaction and the structural integrity of $Ca_v\beta$. Of the four residues in the BID whose mutations weakened the $Ca_v\beta/Ca_v\alpha_1$ interaction, three were proline and one was tyrosine (119, 120). Mutating these residues most likely alters the folding and/or structure of $Ca_v\beta$, which explains its inability to bind $Ca_v\alpha_1$.

But how could the BID enhance Ca^{2+} channel current (119)? While the mechanism of this action remains unclear, it reminds us of an experiment of our own in which a random 43-amino acid peptide (which has no sequence similarity in GenBank) was coexpressed with

Cav2.1 and $\alpha_2\delta$ in *Xenopus* oocytes. This random peptide significantly increased Ca^{2+} channel currents (compared with no $Ca_v\beta$), to ~50% of β_3-induced current. This obvious nonspecific effect, reported in 2004 (84), suggests that the BID-induced current increase may also be a nonspecific effect. Given these structural and functional information, it is prudent to exercise caution when interpreting experimental data concerning the BID.

IV. $Ca_v\beta$ SPLICE VARIANTS AND THEIR TISSUE DISTRIBUTION

Mammalian $Ca_v\beta$s are encoded by four distinct genes, *Cacnb 1–4*. They all have 14 exons except *Cacnb3*, which has 13, and each $Ca_v\beta$ has 2 or more splice variants. Figures 5 and 6 show most of the human $Ca_v\beta$ splice variants found thus far. The five distinct domains and regions of $Ca_v\beta$ are mapped onto their corresponding exons and protein sequences. Alternative splicing occurs in those exons that encode the variable domains or regions, namely, the NH_2 and COOH termini and the HOOK region. The four different *Cacnb* genes utilize different alternative splicing sites. *Cacnb1* and *Cacnb2*, which produce β_1 and β_2, respectively, exhibit alternative splicing in exon 7, giving rise to divergent HOOK regions. *Cacnb1* is also alternatively spliced in the COOH terminus, with exon 14 either included or excluded. On the other hand, *Cacnb2* is alternatively spliced extensively in the NH2 terminus, yielding highly diversified NH_2 termini. *Cacnb4* has no alternative splicing in the HOOK region but has NH_2- and possibly COOH-terminal alternative splicing. *Cacnb3* has no alternatively spliced exons, but like all other *Cacnb* genes, produces a truncated isoform.

Table 1 shows the tissue distribution of some $Ca_v\beta$ splice variants. As expected, $Ca_v\beta$s are abundantly expressed in excitable tissues such as the brain, heart, and muscles. While some splice variants (e.g., β_{1b} and β_{2b}) are widely expressed, others (e.g., β_{1a}, β_{2d}, and β_{2e}) have a more restricted expression. The expression of some splice variants is developmentally regulated. For example, β_{1b} and β_4 expression increases with development, whereas β_{2c}, β_{2d}, and β_{2e} expression decreases with development. It is important to note that, in most cases, protein but not mRNA expression was listed. Immunolocalization experiments should be interpreted cautiously since an antibody may recognize several splice variants, for example, an antibody against the β_2 COOH terminus will recognize all β_2 splice variants.

Since the association between $Ca_v\beta$ and $Ca_v\alpha_1$ is promiscuous (i.e., any full-length $Ca_v\beta$ can associate with any Ca_v1 or Ca_v2 α_1 subunit), alternative splicing greatly increases the molecular diversity and functionality of HVA Ca^{2+} channels. Furthermore, some splice variants may take on functions other than regulating HVA Ca^{2+} channels (see sect. XII). Thus a major future challenge (and a fruitful area of research) is to determine how alternative splicing is regulated in various tissues and at different developmental stages.

V. $Ca_v\beta$ REGULATES THE SURFACE EXPRESSION OF HIGH-VOLTAGE ACTIVATED Ca^{2+} CHANNELS

The α_1 subunit of Ca_v1 and Ca_v2 channels cannot reach the membrane by itself; it shows no surface expression and produces very small or no currents when expressed without auxiliary subunits. Coexpression of $Ca_v\beta$ with $Ca_v\alpha_1$ increases currents by orders of magnitude, depending on factors such as the expression system, DNA or RNA concentration, VGCC turnover rate, inhibitory factors present, the α_1/β combination, etc. (reviewed in Refs. 10, 40, 125, 237, 495). The current increase reflects enhanced channel expression on the plasma membrane and also an increase in channel open probability. In this section we discuss the evidence and mechanisms of increased channel surface expression.

A. $Ca_v\beta$ Is Required for Normal Channel Expression

It has been well established that $Ca_v\beta$ can function as a chaperone to dramatically increase the surface expression of Ca_v1 and Ca_v2 channels. This is observed in various heterologous expression systems with all four subfamilies of $Ca_v\beta$ and all Ca_v1 and Ca_v2 subunits (14, 50,88, 93, 191, 245, 247, 248, 276, 322, 353, 458, 481, 495). The increased surface expression can be detected by $Ca_v\alpha_1$ epitope tag staining, surface biotinylation, gating charge measurements, or increased Ca^{2+} channel current. An important point to mention is that *Xenopus* oocytes, a widely used expression system for studies of VGCCs, have two endogenous β subunits that share 98% homology with β_3 (436). These endogenous subunits are expressed at sufficient levels to transport a small number of exogenously expressed $Ca_v\alpha_1$ to the plasma membrane and hence lead to small Ca^{2+} channel currents in the absence of an exogenous $Ca_v\beta$. Antisense oligonucleotides against endogenous β_3 are able to suppress these currents (62, 436). Little or no endogenous $Ca_v\beta$ was detected in widely used mammalian cell lines such as HEK 293 cells, COS cells, and CHO cells (276, 315). Nevertheless, expression of $Ca_v\alpha_1$ alone in these cells can produce measureable, albeit miniscule, Ca^{2+} channel currents (245, 247,248, 303, 407, 418, 436), suggesting that either a very small fraction of $Ca_v\alpha_1$ can be trafficked to the plasma membrane in the absence of $Ca_v\beta$ or these cells contain low levels of endogenous $Ca_v\beta$s.

$Ca_v\beta$ also enhances Ca^{2+} channel surface expression in vivo. For example, β_1 and β_2 knockout mice have severely reduced Ca^{2+} currents in muscle and heart (see sect. XIII). Knockdown of $Ca_v\beta$ also decreases endogenous Ca^{2+} currents in neuronal cells (35, 279). Conversely, overexpression of $Ca_v\beta$ using adenoviruses increases Ca^{2+} channel current density in native cardiac cells, suggesting that Ca^{2+} channel surface expression may be limited by the availability of $Ca_v\beta$ (336, 465).

Binding of $Ca_v\beta$ to the AID of Ca_v1 and Ca_v2 is essential for its chaperone effect. Point mutations in the AID that weaken or abolish the AID-$Ca_v\beta$ interaction severely reduce or abolish $Ca_v\beta$-stimulated Ca^{2+} channel current (33, 34,56, 120, 181, 185, 206, 218, 276, 336, 361, 448). Deleting the AID altogether, not surprisingly, abolishes $Ca_v\beta$-induced current enhancement (185, 298). Likewise, mutations in the ABP that weaken or abolish the AID-$Ca_v\beta$ interaction also reduce or abolish $Ca_v\beta$-stimulated Ca^{2+} channel expression and current (206, 502). Recent studies show that 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 (129, 206).

How does $Ca_v\beta$ enhance Ca^{2+} channel surface expression? One hypothesis is that $Ca_v\beta$ shields or disrupts one or more ER retention signals on the I–II loop of $Ca_v\alpha_1$ (39), and several lines of evidence support this hypothesis. The I–II loop of Cav1.2 and Ca_v2 can trap α_1 subunits in the ER (except $Ca_v1.1$), but the I–II loop of Cav3.1 (a T channel) fails to do so. Also, tagging a *Shaker* K^+ channel with the I–II loop of $Ca_v1.2$ or $Ca_v2.1$ decreases its expression by approximately sevenfold, while coexpression of $Ca_v\beta$ prevents this downregulation (39). Moreover, deleting the I–II loop from $Ca_v1.2$ (Δ389–423) increases its surface expression in the absence of $Ca_v\beta$ (39).

However, some results are inconsistent with this hypothesis. *1*) The I–II loop of $Ca_v1.1$ does not cause ER retention of a CD8 peptide (99). *2*) CD4 fusion constructs of the I–II loop of $Ca_v1.2$ and $Ca_v2.2$ are trafficked efficiently to the plasma membrane, rather than being retained in the ER (5). *3*) Transplanting the I–II loop of $Ca_v2.2$ into $Ca_v3.1$ causes $Ca_v\beta$-independent current upregulation instead of downregulation (9).

An alternative possibility is that additional trafficking signals exist in the NH_2 and COOH termini of $Ca_v\alpha_1$ (99, 163,175, 262, 466). However, the NH_2 and COOH termini of Ca_v1 and Ca_v2 are not conserved, and yet, the chaperone function of $Ca_v\beta$ is universal, suggesting that any ER retention signals in the NH_2 and COOH termini may only be modulatory.

Recently, a new study suggested that $Ca_v\beta$ increases $Ca_v\alpha_1$ expression on the plasma membrane by preventing its ubiquitination and proteasomal degradation (5). Thus $Ca_v\beta$ may simply be required to help $Ca_v\alpha_1$ escape the degradation pathway.

B. Membrane Association and Subcellular Targeting of $Ca_v\beta$

$Ca_v\beta$s are expected to have a cytosolic localization based on analyses of their amino acid sequence (353, 381). This is true for the majority of $Ca_v\beta$ splice variants when they are expressed alone, without a $Ca_v\alpha_1$ (with a few exceptions discussed below; Refs. 176, 181). However, some $Ca_v\beta$s, most notably β_{2a}, can be localized to the plasma membrane on their own. β_{2a} is linked to the plasma membrane through palmitoyl groups that are covalently attached to two cysteines (Cys 3, 4) in the NH_2 terminus (86, 87). When palmitoylation is abolished, in a double Cys→Ser mutant, membrane localization disappears (87). Importantly, β_{2a} palmitoylation can be dynamically regulated in vivo, adding a layer of physiological control (232, 464). However, palmitoylation alone may not be sufficient for membrane localization because implanting the β_{2a} NH_2 terminus into other $Ca_v\beta$s does not yield membrane localization (87, but see Ref. 369). Thus β_{2a} probably possesses additional determinants that help target it to the plasma membrane. Another β_2 subunit, β_{2e}, is not palmitoylated but is found at the plasma membrane (433). The underlying mechanism is yet unknown. Finally, β_{1b} is localized to the plasma membrane in COS-7 cells (44, 50), but this is not observed in tsA201 cells (87) or primary cardiomyocytes (93). The reason for the discrepancy is unclear, but in COS-7 cells, the membrane localization is attributed to a COOH-terminal acidic motif (WEEEEDYEEE) whose deletion diminishes membrane localization. When this motif is fused to β_3, which is normally cytosolic, it migrates to the plasma membrane (44, 50). As will be discussed in section VI, membrane localization of $Ca_v\beta$ coincides with many functional effects, especially slowed inactivation.

In the presence of $Ca_v\alpha_1$, all $Ca_v\beta$s localize to the plasma membrane through their association with $Ca_v\alpha_1$; however, they may be targeted to different subcellular locations depending on which $Ca_v\alpha_1$ they associate with. For example, β_3 and β_4, which predominantly associate with presynaptic Ca_v2 channels, can be found in axons, whereas β_1 is scarce in this compartment (336); instead, β_1 is found in postsynaptic compartments (soma and dendrites). In skeletal muscle, β_{1a} is targeted to the triads through its association with $Ca_v1.1$ (333). When exogenously expressed in epithelial cells, β_{1b} is localized on the apical membrane with $Ca_v2.1$ but on the basolateral membrane with $Ca_v1.2$ (44). Conversely, $Ca_v\beta$ may affect the subcellular localization of $Ca_v\alpha_1$. For example, β_{1a} helps arrange L-type Ca^{2+} channels as tetrads in the t tubules of skeletal muscles (see sect. XI*A*; Refs. 164, 191, 394, 507), and β_4 is implicated in the synaptic localization of P/Q-type channels in cultured hippocampal neurons (474). Furthermore, through interactions with different proteins, $Ca_v\beta$ helps attach Ca^{2+} channels to synaptic vesicles (257), the cytoskeleton (223), or the surface of sarcoplasmic reticulum (333). These examples illustrate the role of $Ca_v\beta$ as a scaffold protein.

VI. $Ca_v\beta$ REGULATION OF Ca^{2+} CHANNEL GATING

Once the Ca^{2+} channel complex reaches the plasma membrane, $Ca_v\beta$ powerfully modulates its gating. The main features of gating modulation are the enhancement of voltage-dependent activation (VDA) and voltage-dependent inactivation (VDI). β_{2a} is unique in that it inhibits VDI. This section describes these $Ca_v\beta$ effects and their mechanisms.

A. $Ca_v\beta$ Enhances Voltage-Dependent Activation

All $Ca_v\beta$s shift the voltage dependence of activation to more hyperpolarized voltages (by ~10–15 mV, Table 2 and Fig. 7). This was shown for both Ca_v1 and Ca_v2 channels in various expression systems (61, 116,229, 245, 268, 322, 406, 412, 414, 443, 495). The shift can also be observed in vivo in some knockout mice (191, 325, 468), while in some other cases, it is probably obscured by the compensatory effects of other $Ca_v\beta$ genes (31, 330, 331). In addition, the speed of activation is increased in general (268, 412), but it could appear slower depending on the stimulus voltage (353) and the particular α_1/β pair (184, 245,443, 459).

These effects are also visible at the single-channel level. Thus channels without a $Ca_v\beta$ tend to open less frequently, open for a shorter duration, and require more positive activation voltages. $Ca_v\beta$ coexpression greatly increases channel open probability (P_o) and shortens the latency to first opening (93, 125,215, 229, 295, 457). Notably, β_{2a} produces the most dramatic increase in P_o (76, 93, 132).

Normal VDA is largely reconstituted by the core region of $Ca_v\beta$ (206). Deleting the entire $Ca_v\beta$ COOH terminus has no effect on VDA, at least for $Ca_v2.1$ channels expressed in *Xenopus* oocytes (206). The NH_2 terminus, however, appears to have a small role in modulating VDA. For example, β_{4b}, which has a longer NH_2 terminus compared with β_{4a}, induces a larger hyperpolarizing shift in the activation of some $Ca_v\alpha_1$ (208).

B. $Ca_v\beta$ Promotes Voltage-Dependent Inactivation, Except β_{2a}

VDI reduces the amount of Ca^{2+} entering the cell following depolarization and decreases the number of channels responsive to subsequent depolarizations. $Ca_v\beta$ is a key modulator of VDI, as first demonstrated in 1991 (268, 406, 450) and subsequently confirmed for various α_1/β combinations in different expression systems (116, 137,245, 340, 348, 414, 417, 458). Several aspects of VDI are affected by $Ca_v\beta$. *1*) β_1, most β_2 splice variants, β_3, and β_4 shift the voltage dependence of inactivation to more hyperpolarized voltages (by ~10–20 mV; Table 2 and Fig. 7), whereby weaker depolarizations are able to inactivate the channels. β_{2a}, however, causes a shift to more depolarized voltages (by ~10 mV) (40, 93,119, 132, 206, 218, 245, 276, 314, 340, 369). *2*) $Ca_v\beta$s (except β_{2a}) promote the process of "closed state" inactivation exhibited by Ca_v2 channels when they rapidly transition between closed and open states, such as during a train of action potentials ($\beta3 > \beta1b = \beta_4 >> \beta2a$; Refs. 348, 491). Similarly, a large hyperpolarization of steady-state inactivation (approximately ~40 mV) is observed when β_3 is overexpressed with N- and R-type channels, dramatically increasing the population of inactivated channels at resting conditions (491). *3*) β_1, most β_2 splice variants, β_3, and β_4 speed up the inactivation kinetics, whereas β_{2a} and β_{2e} slow down inactivation (Table 2 and Fig. 7).

The unique effects of β_{2a} on VDI are largely abolished when palmitoylation of β_{2a} is disrupted by mutating its two NH_2-terminal cysteine residues to serine (β_{2a} C3,4S) (365, 369). WT β_{2a}-like properties can be restored when a transmembrane segment of an unrelated membrane protein is fused to this mutant, suggesting that membrane anchorage rather than palmitoylation per se is critical for β_{2a}'s unique functions (369). Supporting this idea, the nonpalmitoylated but membrane-attached β_{2e} has properties similar to β_{2a} (433).

Multiple domains and regions of $Ca_v\beta$ are involved in the regulation of VDI. The GK domain alone, when expressed together with $Ca_v2.1$ and $\alpha_2\delta$ in *Xenopus* oocytes, has been shown to speed up VDI and hyperpolarize the voltage dependence of VDI (206). The GK domain of all four subfamilies of $Ca_v\beta$ produces the same effects (Fig. 7, *D* and *E*; Ref. 206), as expected from its high degree of amino acid conservation. Similarly, the GK domain of β_{2a} greatly accelerates VDI and hyperpolarizes the voltage dependence of VDI of $Ca_v2.2$ channels expressed in oocytes and tsA-201 cells (129, 372). On the other hand, it has been reported that refolded and purified proteins of β_{2a} and β_{1b} GK domains slow down VDI and depolarize the voltage dependence of $Ca_v2.3$ channels expressed in oocytes (187). The discrepancy between these studies may result from the use of different $Ca_v\alpha_1$ or from RNA versus protein injection, but it should be noted that the refolded and purified GK domains appear to be dimerized proteins (187), and it is unknown whether and how dimerization changes the function of the GK domain.

The HOOK plays an important role in regulating VDI, as first suggested by chimeric studies between different $Ca_v\beta$s (364, 420). Two recent studies based on structurally defined $Ca_v\beta$ domains provide more definitive evidence. *1*) Swapping the HOOK between the core regions (SH3-HOOK-GK) of β_{1b} and β_{2a}, which have opposite effects on VDI, also swaps their effects on VDI (206). *2*) Deleting the HOOK in either β_{2a} core or full-length β_{2a} results in increased VDI (372). These studies, in conjunction with those discussed earlier, indicate that both membrane attachment through palmitoylation and a long HOOK region contribute to the unique effects of β_{2a} on VDI.

The role of the NH_2 terminus of $Ca_v\beta$ in regulating VDI has long been established. Deleting or shortening the NH_2 terminus, or swapping the NH_2 terminus of different $Ca_v\beta$s markedly alters VDI (236, 340,364, 420). β_2 or β_4 splice variants differing in the NH_2 terminus exhibit markedly different VDI (208, 209,215, 433). As discussed above, the palmitoylation site of β_{2a} is in the NH_2 terminus.

Surprisingly, the COOH terminus of $Ca_v\beta$ seems to play a very limited or no role in regulating VDI, even though it is highly variable among the four $Ca_v\beta$ subfamilies. Thus, although a very small change in the inactivation kinetics of $Ca_v2.1$ channels is observed when the COOH terminus of β_4 is deleted (460), exchanging the COOH terminus between β_3 and β_4 or deleting the entire COOH terminus of any of the four $Ca_v\beta$s has little effect on VDI of Cav2.2 channels or $Ca_v2.1$ channels (206, 420). It remains to be determined whether the COOH terminus exerts a more prominent effect on VDI under other conditions and for certain combinations of $Ca_v\alpha_1$ and $Ca_v\beta$. Intriguingly, a β_4 COOH-terminal truncation mutant missing the last 38 amino acids, which causes slightly faster inactivation of $Ca_v2.1$ channels at moderate depolarizations, was identified in a juvenile myoclonic epilepsy patient (142). Whether the very subtle change in Ca^{2+} channel inactivation underlies the disease is unclear.

C. A Unified Model for $Ca_v\beta$ Regulation of Ca^{2+} Channel Gating

How does $Ca_v\beta$ regulate VDA and VDI of Ca_v1 and Ca_v2 channels? Before addressing this question, we first briefly discuss the pore structure, the location of the activation gate, and the mechanism of VDI of VGCCs.

The external pore, including the ion selectivity filter, of VGCCs is formed by the pore loop between the S5 and S6 transmembrane segments of each of the four homologous repeats of $Ca_v\alpha_1$; point mutations in this region, especially of the four conserved glutamate or aspartate residues, drastically alter ion selectivity, permeation, and pore blockage (256, 266,389, 482). The inner pore is formed by all four S6 segments of $Ca_v\alpha_1$, as demonstrated by the substituted cystine accessibility method (505). Cystine accessibility studies also indicate that the activation gate is located at the cytoplasmic end of the S6 segments (476). The S6 segments, together with the I–II loop and the NH2 and COOH termini of $Ca_v\alpha_1$, are involved in controlling or regulating VDI (for review, see Refs. 212, 422). Although the precise molecular mechanism of VDI is unknown, a prevalent model is that the I–II loop of $Ca_v\alpha_1$ functions as a “hinged lid” to physically occlude the pore by binding to the cytoplasmic ends of the S6 segments (421, 422), reminiscent of VDI of voltage-gated Na^+ channels (71). Which amino acids form the inactivation gate and its receptor site remain unknown. An alternative model is that VDI is produced by a constriction of the pore (151). Either way, the S6 segments constitute a converging point through which both VDA and VDI are controlled and regulated.

The biochemical, functional, and structural studies presented above support a unified model for $Ca_v\beta$ regulation of VDA and VDI of VGCCs (9, 125,151, 206, 298, 341, 448, 455, 461). This model has two central components.

First, the high-affinity AID-GK domain interaction and a rigid IS6-AID linker are essential for $Ca_v\beta$ regulation of VGCC gating. As mentioned in section III*E*, in the presence of $Ca_v\beta$, through the AID-GK domain interaction, the entire region encompassing the IS6 segment and the end of the AID becomes a continuous α-helix (9, 151, 341). Via this rigid structure,

$Ca_v\beta$ gains a lever with which to regulate both activation and inactivation (Fig. 4). Thus $Ca_v\beta$ binding adds mass and tension to IS6 and the I–II loop, which most likely affects the energetics of voltage-dependent movement of both IS6 and the inactivation gate, thereby directly changing the voltage dependence and kinetics of activation and inactivation. This explains why the GK domain alone is capable of affecting both activation and inactivation (129, 206, 372). Equally important, the AID-GK domain interaction anchors $Ca_v\beta$ to $Ca_v\alpha_1$, thereby enabling interactions between $Ca_v\beta$ and other parts of $Ca_v\alpha_1$ that are of intrinsic low affinity but are important for $Ca_v\beta$'s gating effects (see below). Supporting an essential role of the AID-GK domain interaction, many studies show that $Ca_v\beta$ regulation of gating is abolished by mutations in the AID (33, 34,56, 120, 181, 185, 206, 218, 276, 361, 448) or in the ABP (206, 502). However, one difficulty in interpreting these and similar experiments is that those mutations dramatically reduce or abolish $Ca_v\beta$-stimulated Ca^{2+} channel surface expression, leaving minuscule currents to be scrutinized. This problem is circumvented in several recent studies where the rigid α-helical structure of the IS6-AID linker was disrupted by substituting linker residues with glycines, or inserting multiple glycines in the linker, while leaving the AID-GK domain interaction intact. These substitutions or insertions do not affect $Ca_v\beta$-enhanced Ca^{2+} channel surface expression, but they severely compromise or eliminate the ability of $Ca_v\beta$ to regulate Ca_v1 and Ca_v2 channel activation and inactivation (151, 455, 502). These results underscore the essential role of a rigid IS6-AID linker in $Ca_v\beta$ regulation of VGCC gating.

An additional factor that is important for $Ca_v\beta$ regulation of gating is the orientation of $Ca_v\beta$ relative to $Ca_v\alpha_1$ (455, 502). Inserting five alanine residues in the IS6-AID linker, which is expected to maintain the α-helical structure of the linker but induce a 180° rotation of $Ca_v\beta$ with respect to $Ca_v\alpha_1$, markedly diminishes $Ca_v\beta$ regulation of activation, while insertion of seven alanines, which produces two full turns, has no significant detrimental effect (502). Similarly, deleting one or three residues in the IS6-AID linker totally abolishes $Ca_v\beta$ regulation of both activation and inactivation (455). 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 $Ca_v\beta$ regulation of VGCC gating.

Second, intrinsically low-affinity interactions between $Ca_v\beta$ and $Ca_v\alpha_1$ are crucial for $Ca_v\beta$ regulation of VGCC gating (especially VDI), and these interactions confer each $Ca_v\beta$ its distinct modulatory effect and α_1/β pair-specific gating properties. Besides the AID-GK domain interaction, other direct contacts between $Ca_v\beta$ and $Ca_v\alpha_1$ have been observed in vitro. For example, the $Ca_v\beta$ SH3 domain interacts with the I–II loop, but at a region different from the AID (298), and a COOH-terminal region conserved only in β_2 binds to a COOH-terminal region of $Ca_v1.2$ where calmodulin (CaM) also binds (270). The same Cav1.2 COOH-terminal region also binds to a β_{2a} construct containing the N-$SH3_{\beta strand1–4}$-HOOK module (501). Other regions of $Ca_v\alpha_1$, including the NH_2 and COOH termini and the III–IV loop, have also been shown to interact directly with $Ca_v\beta$ (366, 436,460, 461). It remains to be determined which regions of $Ca_v\beta$ they associate with, but the $Ca_v\beta$ NH_2 terminus and HOOK are prime candidates since they are critically involved in regulating VDI. These additional α_1/β interactions have intrinsic low affinity, and on their own, do not produce significant gating effects. However, the strength of these interactions increases dramatically when $Ca_v\beta$ is anchored to $Ca_v\alpha_1$ by the AID-GK domain interaction. These notions are supported by the aforementioned mutagenesis/insertion studies in the AID, the ABP, and the IS6-AID linker. Further supporting these ideas, Chen et al. (83) reported that, without changing the AID-GK domain interaction, splitting β_{2a} into two connected modules (N-SH3-HOOK and GK-C) through the insertion of increasingly longer flexible linkers between the SH3 and GK domains leads to a gradual diminishment of the effect of the N-SH3-HOOK module on VDI (83). This result indicates that keeping the N-SH3-HOOK module near $Ca_v\alpha_1$ is essential for its modulatory effect. A future challenge is to develop ways to precisely map the interface of intrinsically low-affinity $Ca_v\alpha_1/Ca_v\beta$ interactions, which might be too weak to be identified biochemically and might require more than one $Ca_v\alpha_1$ region.

How low-affinity α_1/β interactions regulate gating is unclear. These interactions could pull on $Ca_v\beta$ and thereby modulate the movement of IS6 and the presumed inactivation gate in the I–II loop. They may also interfere with intramolecular interactions between the I–II loop and other parts of $Ca_v\alpha_1$, such as the NH_2 and COOH termini and the III–IV loop, where point mutations and deletions cause marked changes of VDI (for review, see Refs. 212, 422). These intramolecular interactions, as well as the low-affinity α_1/β interactions, are α_1 or α_1/β pair specific (2, 99,178, 262, 369, 386, 404, 436, 460, 501). Thus, to fully appreciate the physiological importance of $Ca_v\beta$ regulation of VGCC gating, it is crucial to examine the pairing of $Ca_v\alpha_1$ and $Ca_v\beta$ in different tissues and cell types, in different subcellular locations, and at different developmental stages.

A final point that should be mentioned here is that many proteins that interact directly with $Ca_v\beta$ have been shown to regulate VGCC gating, such as RGK proteins (see sect. IX), Best1 (493), and RIM1 (257) (see sect. XI).

D. Can $Ca_v\beta$ Produce AID-Independent Gating Effects?

Several reports, which at first seemed to contradict the model presented above, are in fact in accord with the model upon closer examination. It has been shown that β_{2a} is able to modulate VDA and VDI of Ca^{2+} channels formed by a mutant $Ca_v2.1$ subunit ($Ca_v2.1\_\Delta AID$) whose AID is deleted (298). This result led the authors to conclude that essential $Ca_v\beta$ modulatory properties are AID independent. This result, however, has an alternative explanation: β_{2a} can be anchored to the plasma membrane through palmitoylation, and this membrane tethering might mimic, at least partially, the anchoring role of the AID-GK domain interaction, bringing β_{2a} near Cav2.1_ΔAID subunits and promoting the functionally important low-affinity α_1/β interactions alluded to above. Indeed, this result lends strong support to the second part of the model discussed above, i.e., there are low-affinity interactions between $Ca_v\beta$ and $Ca_v\alpha_1$ that are crucial for $Ca_v\beta$ regulation of VGCC gating.

Several studies reported that a 41-amino acid β_2 COOH-terminal fragment and some $Ca_v\beta$ splice variants, including β_{2f}, $\beta_{2\Delta g}$, β_{1d}, and chicken β_{4c}, all of which lack most or the entire GK domain (and hence cannot bind the AID), are all able to enhance Ca^{2+} channel currents and/or regulate their gating (92, 204, 270). However, these effects are much weaker than those produced by full-length $Ca_v\beta$s. Moreover, the specificity of these effects is called into question by the clear nonspecific effects of two short peptides that do not exist in nature: a

35-amino acid peptide containing the BID and a 43-amino acid peptide with a random sequence, both of which are able to stimulate Ca^{2+} channel expression and weakly modulate gating (84, 119, 120). Nevertheless, given that β_{2f} and $\beta_{2\Delta g}$ are found in native cells (204, 270), they could affect Ca^{2+} channel gating through low-affinity α_1/β interactions if they are expressed at very high levels. At present, the physiological role of β_{2f} and $\beta_{2\Delta g}$ remains unknown.

E. $Ca_v\beta$ Regulation of Ca^{2+}-Dependent Inactivation and Facilitation

HVA Ca^{2+} channels are strongly regulated by another type of inactivation that depends on Ca^{2+} influx, namely, Ca^{2+}-dependent inactivation (CDI) (for reviews, see Refs. 53, 78, 201, 358), which serves as a negative-feedback mechanism. CDI is mediated by the ubiquitous Ca^{2+}-sensing protein CaM, which is constitutively bound to the $Ca_v\alpha_1$ COOH terminus (494, 509). The exact molecular mechanism of CDI is unclear, as is the relationship between CDI and VDI, but a recent study shows that two of the elements critical for VDI, $Ca_v\beta$ and a rigid IS6-AID linker, are also essential for CDI (151). Glycine (but not alanine) substitutions that disrupt the α-helix of the IS6-AID linker dramatically slow CDI. The absence of $Ca_v\beta$ binding to $Ca_v\alpha_1$, ensured by mutating the AID, produces similar results (151). Thus CDI and VDI appear to share a common mechanism by which conformational changes caused by CaM-$Ca_v\alpha_1$ interactions or $Ca_v\beta$-$Ca_v\alpha_1$ interactions are transmitted to the pore through the rigid IS6-AID linker.

HVA Ca^{2+} channels also undergo Ca^{2+}-dependent facilitation (CDF), which occurs during repetitive channel activation, such as during a train of action potentials (for reviews, see Refs. 53, 201, 358). This process, which is dependent on CaM binding to the $Ca_v\alpha_1$ COOH terminus, also requires $Ca_v\beta$ binding to the AID and an intact IS6-AID α-helix (151). Interestingly, CDF is readily observed with β_{2a} but not with β_{1b} or β_4 (80, 272). The main reason for this difference is probably that channels with β_{2a} inactivate much slower; slow inactivation not only allows the unmasking of CDF but also further stimulates CDF by permitting a larger Ca^{2+} influx.

F. $Ca_v\beta$ Regulation of Voltage-Dependent Facilitation

L-type Ca^{2+} channels exhibit voltage-dependent facilitation (VDF) (47). VDF is manifested as a gradual increase in L-type current during high-frequency action potentials, and it partly explains activity-dependent enhancement of L-type currents in skeletal muscle, brain, and heart. VDF can be differentiated from CDF by using Ba^{2+} as the charge carrier; it is accompanied by an increase in high P_o gating (357) and may be dependent on phosphorylation (273). Like CDF, VDF depends on the presence of $Ca_v\beta$ (47, 75; but see Ref. 274); it is supported by β_1 and β_3 but not β_{2a} (47, 75,365; but see Ref. 109). Some of the discrepancies in the literature may result from the following reasons. *1*) Differences in L-type channel splice variants and the $\alpha_2\delta$ subunits used affect the results. For example, $\alpha_2\delta_1$ and $\alpha_2\delta_3$ seem to mask VDF by increasing inactivation (109). *2*) The β_{2a}-containing channels already have a high P_o, so VDF is harder to observe in these channels. *3*) Nonpalmitoylated β_{2a} mutants can restore VDF (365), suggesting that different levels of palmitoylation may contribute some variations in the results.

The GK domain alone appears to be necessary and sufficient to confer VDF; deleting other domains, including the SH3 domain, separately or in combination, spares VDF (77). Hence, it is possible that VDF, just like CDF, CDI, VDI, and VDA, relies on the rigid IS6-AID linker and $Ca_v\beta$ to affect gating. It would be of interest to investigate whether glycine substitution or insertion in the IS6-AID linker also affects VDF.

VII. STOICHIOMETRY AND REVERSIBILITY OF THE $Ca_v\alpha_1$-$Ca_v\beta$ INTERACTION

How many β subunits need to bind to each $Ca_v\alpha_1$ to bring about the aforementioned trafficking and gating effects? Is the $Ca_v\alpha_1$-$Ca_v\beta$ interaction reversible? This section discusses these two important issues.

A. $Ca_v\alpha_1$ 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 $Ca_v\beta$ (430, 473). This remains the prevalent view today, but it comes after a brief competition with the idea of a 1 $Ca_v\alpha_1$:2 or more $Ca_v\beta$ stoichiometry (62, 436).

As mentioned in section V*A*, *Xenopus* oocytes express two endogenous β_3-like subunits, called β_{3xo} (436). When $Ca_v\alpha_1$ cRNA is injected into *Xenopus* oocytes alone, a small fraction of the $Ca_v\alpha_1$ is transported to the plasma membrane by β_{3xo} (436). Coinjection of a mammalian $Ca_v\beta$ or either of the two *Xenopus* β subunits greatly increases Ca^{2+} channel current and changes its gating properties. These results led to the proposal that the "$Ca_v\alpha_1$-alone" channels in fact contained a β_{3xo} and that one or more exogenous $Ca_v\beta$ bind the $Ca_v\alpha_1/\beta_{3xo}$ complex to form a higher order complex with modulated gating (436). Subsequently, by varying the concentration of coexpressed β_3, it was found that β_3 produced the trafficking effect with a sevenfold higher apparent affinity than it did gating modulation (17 vs. 120 nM) (62). This result was initially explained by one of two hypotheses: either two $Ca_v\beta$s bind a single $Ca_v\alpha_1$ or the mature $Ca_v\alpha_1$ on the plasma membrane has a lower affinity for $Ca_v\beta$ than the nascent $Ca_v\alpha_1$ does (62).

Subsequent extensive studies indicate that $Ca_v\beta$ associates with $Ca_v\alpha_1$ in a 1:1 stoichiometry and that this stoichiometry is determined by the AID-GK domain inter- action. *1*) Channels coexpressed with a mixture of β_{2a} and β_3 form two biophysically distinct channel populations, rather than a single population of "mixed"-channel type (245). *2*) Colecraft and colleagues (110) covalently linked a single β_{2b} to the COOH terminus of $Ca_v1.2$ (creating $Ca_v1.2$-β_{2b}) and found that the channels formed by Cav1.2-β_{2b} exhibited the same gating properties as channels formed by the coexpression of $Ca_v1.2$ and β_{2b} did. Moreover, coexpression of β_{2a} and Cav1.2-β_{2b} did not further change channel gating. *3*) The crystal structures of the AID-$Ca_v\beta$ core complexes clearly show that each $Ca_v\beta$ binds a single AID (84, 341, 447). *4*) Mutations of key residues in the AID or the ABP abolish both $Ca_v\beta$-mediated Ca^{2+} channel surface expression and gating modulation (33, 34,56, 120, 181, 185, 206, 218, 276, 361, 448, 502).

B. The $Ca_v\alpha_1$-$Ca_v\beta$ Interaction Is Reversible

The affinity of the AID-$Ca_v\beta$ interaction measured in vitro is very high, with a K_d ranging from 2 to 54 nM (30, 56,62, 120, 121, 179, 342, 371, 395, 448). The affinity of $Ca_v\alpha_1$-$Ca_v\beta$ interactions in cells is less certain but seems to be lower (218), probably partly due to competition for $Ca_v\alpha_1$ and $Ca_v\beta$ binding by other proteins. The lower affinity likely permits a more dynamic $Ca_v\alpha_1$-$Ca_v\beta$ interaction. Indeed, several studies support the notion that the $Ca_v\alpha_1$-$Ca_v\beta$ interaction is reversible in intact cells. *1*) Injection of β_3 protein into oocytes expressing L-type $Ca_v\alpha_1$ alone quickly alters Ca^{2+} channel gating properties, suggesting that some channels on the plasma membrane are devoid of $Ca_v\beta$ (480). *2*) A synthetic AID peptide can significantly reduce the P_o of channels formed by L-type $Ca_v\alpha_1$ and β_{2a} in HEK 293 cells when it is applied to the cytoplasmic side of inside-out membrane patches, but it has no effect on channels containing no β_{2a}, suggesting that the AID peptide can compete off bound β_{2a} (224). *3*) Injection of β_{2a} protein into oocytes expressing Cav2.3 and β_{1b} results in a dramatic inhibition and slowing down of inactivation, consistent with β_{2a} replacing previously bound β_{1b} and overtaking the channel (218).

That *Xenopus* oocytes have endogenous $Ca_v\beta$s and that the $Ca_v\alpha_1$-$Ca_v\beta$ interaction has a 1:1 stoichiometry and is reversible provide a straightforward explanation for why Ca^{2+} currents can be recorded in oocytes expressing $Ca_v\alpha_1$ alone, and why the gating properties of these currents can be modulated by exogenous $Ca_v\beta$: the endogenous β_{3XO} subunits are expressed at high enough levels to interact with a small fraction of the nascent $Ca_v\alpha_1$ in the ER and transport them to the plasma membrane; there, β_{3XO} eventually dissociates from $Ca_v\alpha_1$, leaving most of the channels devoid of a β subunit because the cytoplasmic concentration of β_{3XO} is too low to rebind these β-less channels. The β-less channels, however, can associate with exogenously overexpressed $Ca_v\beta$ to form a stable $Ca_v\alpha_1/Ca_v\beta$ complex, as long as the cytoplasmic concentration of $Ca_v\beta$ is a few fold higher than the K_d of the $Ca_v\alpha_1$-$Ca_v\beta$ interaction. It is likely that this is also the scenario in mammalian expression systems.

A dynamic and reversible $Ca_v\alpha_1$-$Ca_v\beta$ association might play an important role in regulating Ca^{2+} channel activity, especially during development when changes in the expression level of different $Ca_v\beta$ isoforms occur (311, 435, 449). It has been shown that the $Ca_v\beta$ component of N-type Ca^{2+} channels changes during postnatal development, from $\beta_{1b} > \beta_3 >> \beta_2$ at P2 to $\beta_3 > \beta_{1b} = \beta_4$ at P14 and adult age (449). This study further shows that although no N-type channels associate with β_4 at P2, 14 and 25% of N-type channels contain β_4 at P14 and adult age, respectively.

VIII. ROLE OF $Ca_v\beta$ IN G PROTEIN INHIBITION OF Ca_v2 CHANNELS

VGCCs are susceptible to negative-feedback inhibition by hormones and neurotransmitters through the activation of G protein-coupled receptors (GPCRs). An extensively studied form of inhibition is the G protein-mediated, membrane-delimited, and voltage-dependent inhibition of members of the Ca_v2 channel family (i.e., N-, PQ-, and R-type channels). It is believed that this inhibition contributes to presynaptic inhibition and short-term synaptic plasticity (36, 52, 126, 219, 440, 471). This inhibition is mediated by the direct binding of G protein $G_{\beta\gamma}$ subunits to the channel (213, 233), and it demonstrates three hallmarks: *1*) it shifts channel activation to more depolarized potentials (23); *2*) it is accompanied by a slowing of channel activation (23), resulting from latent $G_{\beta\gamma}$ unbinding from the channel (139, 244, 349); and *3*) it can be reversed by a strong conditioning depolarizing prepulse, which accelerates $G_{\beta\gamma}$ dissociation from the channel in a phenomenon known as prepulse facilitation, or PPF (23, 139, 251). Below we discuss the role of $Ca_v\beta$ in the $G_{\beta\gamma}$-mediated, voltage-dependent inhibition. For in-depth reviews on other aspects of this inhibition, see References 117, 126, 138, 424, 440, and 497.

A. $Ca_v\beta$ Is Required for Voltage-Dependent $G_{\beta\gamma}$ Inhibition

It has long been observed that some effects of $G_{\beta\gamma}$ on VGCCs, such as the slowing of activation and the depolarizing shift of the voltage dependence of activation, are opposite to those of $Ca_v\beta$, raising the possibility that $G_{\beta\gamma}$ and $Ca_v\beta$ compete with each other (48, 60). Supporting this idea, early studies found that knockdown of endogenous $Ca_v\beta$ in neurons increased GPCR-induced inhibition of Ca^{2+} currents (60), and coexpression of $Ca_v\beta$ with $Ca_v\alpha_1$ in oocytes decreased G protein-mediated inhibition (48, 366). However, later studies showed that in COS-7 cells G protein inhibition of N-type Ca^{2+} channels was markedly enhanced by coexpressed $Ca_v\beta$s (315), and that in tsA-201 cells, a mutant $Ca_v2.2$ that contained a point mutation in the AID (W391A) and was unable to associate with $Ca_v\beta$ could no longer display voltage-dependent G protein inhibition (276). The latter studies indicate that $Ca_v\beta$ is essential for voltage-dependent G protein inhibition of N-type Ca^{2+} channels.

The discrepancy among these studies could arise from many factors. In particular, in the early studies (48, 60, 366), G protein inhibition was examined at a single voltage, which could complicate the interpretation because $G_{\beta\gamma}$ and $Ca_v\beta$ both shift the voltage dependence of channel activation, but in opposite directions. Another factor could be the difficulty of *1*) characterizing inhibition of tiny Ca^{2+} channel currents typically recorded in the absence of coexpressed $Ca_v\beta$, and *2*) excluding the contribution of endogenous $Ca_v\beta$s. To overcome these difficulties, a mutant β_{2a} subunit (named β_{2a}_Mut2) was created by mutating two key AID-binding residues (M245 and L249) to alanine (502). When coexpressed with $Ca_v2.1$ in *Xenopus* oocytes, β_{2a}_Mut2 is still capable of promoting channel trafficking, but owing to its reduced affinity for the AID, it can be washed off from the surface of Ca^{2+} channels in excised membrane patches (502). With the use of this approach, large populations of Ca^{2+} channels devoid of $Ca_v\beta$ can be generated on the plasma membrane. Such β-less channels are still inhibited by purified $G_{\beta\gamma}$ protein applied to the cytoplasmic side of the channels; however, all the hallmarks of voltage-dependent inhibition are absent (502). This finding strongly supports the notion that $Ca_v\beta$ is indispensible for voltage-dependent $G_{\beta\gamma}$ inhibition.

Although β-less channels do not display voltage-dependent G protein inhibition, they can still be inhibited by G proteins in a voltage-independent manner. For example, the mutant $Ca_v2.2$ harboring the W391A mutation is still susceptible to voltage-independent G protein inhibition (276). Likewise, the β-less $Ca_v2.1$ channels (produced by washing off the bound β_{2a}_Mut2 in inside-out membrane patches) are also still inhibited by $G_{\beta\gamma}$, but without any voltage-dependent features (502). These findings indicate that $G_{\beta\gamma}$ can bind $Ca_v\alpha_1$ in the absence of $Ca_v\beta$. Thus the essential role of $Ca_v\beta$ is to enable voltage-dependent dissociation of $G_{\beta\gamma}$ from the inhibited channels, a process that gives rise to the voltage dependence of $G_{\beta\gamma}$ inhibition (45).

B. $G_{\beta\gamma}$ Does Not Displace $Ca_v\beta$

Another important question is whether $Ca_v\beta$ and $G_{\beta\gamma}$ coexist on $Ca_v\alpha_1$ during voltage-dependent inhibition. The apparent opposing actions of $G_{\beta\gamma}$ and $Ca_v\beta$ prompted the hypothesis that $G_{\beta\gamma}$ displaces $Ca_v\beta$ from $Ca_v\alpha_1$ (48, 60, 366, 375). This conclusion was also reached in a study showing that Förster resonance energy transfer (FRET) signals between $Ca_v\beta$ and $Ca_v\alpha_1$ change during $G_{\beta\gamma}$ inhibition (387). However, functional antagonism does not necessarily indicate direct competition, and, while FRET signal changes are indicative of protein conformational changes, they are inadequate in demonstrating protein dissociation (3, 231, 490).

On the contrary, several lines of evidence indicate that $Ca_v\beta$ remains associated with $Ca_v\alpha_1$ during $G_{\beta\gamma}$ modulation. *1*) Different subfamilies of $Ca_v\beta$ have different effects on the magnitude and properties of voltage-dependent $G_{\beta\gamma}$ inhibition, with β_{2a} being the least effective in promoting this inhibition (61, 129, 147, 316, 376). *2*) $Ca_v\beta$ increases the rate of $G_{\beta\gamma}$ dissociation (as determined by the time constant of PPF) from the inhibited channels, but the efficacy of the four $Ca_v\beta$s is different (with a rank order of $\beta_3 > \beta_4 > \beta_{1b} > \beta_{2a}$; Refs. 61, 147). These observations (*1* and *2*) are most easily explained if $Ca_v\alpha_1$, $Ca_v\beta$, and $G_{\beta\gamma}$ form a tripartite complex during $G_{\beta\gamma}$ modulation. *3*) Since $Ca_v\beta$ critically affects VDA and VDI, these properties are expected to change if $Ca_v\beta$ were dislodged from the channel. However, the voltage dependence and kinetics of VDI remain unchanged before, during, and after $G_{\beta\gamma}$ modulation (23, 316, 502). Similarly, the voltage dependence of activation is unchanged before and after $G_{\beta\gamma}$ modulation (502). These results support the notion that $Ca_v\beta$ is not dislodged by $G_{\beta\gamma}$ from the inhibited channels.

C. The Rigid IS6-AID α-Helix Is Necessary for Voltage-Dependent $G_{\beta\gamma}$ Inhibition

The antagonistic effects of $Ca_v\beta$ and $G_{\beta\gamma}$ on channel activation suggest that their actions are related structurally and mechanistically. As mentioned in section VI*C*, disruption of the α-helical structure of the IS6-AID linker (by inserting 3–7 glycine residues in the linker or by substituting 3 linker residues with glycine) abolishes $Ca_v\beta$ modulation of VGCC gating (151, 455, 502). The same maneuver also completely eliminates voltage-dependent $G_{\beta\gamma}$ inhibition but spares voltage-independent $G_{\beta\gamma}$ inhibition (502), indicating that a rigid IS6-AID helix is not necessary for $G_{\beta\gamma}$ binding to $Ca_v\alpha_1$ but is essential for voltage-dependent dissociation of $G_{\beta\gamma}$. Strikingly, both voltage-dependent and -independent $G_{\beta\gamma}$ inhibition are abolished when five alanine residues are inserted into the IS6-AID linker of $Ca_v2.1$, which is likely to maintain the β-helical structure of the linker but produce a ~180° rotation of $Ca_v\beta$ with respect to $Ca_v\alpha_1$ (502). It is possible that $G_{\beta\gamma}$ can no longer bind to this mutant $Ca_v2.1$, but further investigation is necessary to confirm this speculation.

Consistent with the requirement of a rigid IS6-AID linker, two recent studies show that the GK domain alone is sufficient to support voltage-dependent G protein inhibition in both N- and P/Q-type channels (129, 502). On the other hand, the observation that different isoforms of $Ca_v\beta$ differentially modulate voltage-dependent G protein inhibition indicates that other

$Ca_v\beta$ domains and regions can fine-tune this process. Indeed, the HOOK region has been suggested to play a role in enhancing the voltage-dependent dissociation of $G_{\beta\gamma}$ (129).

On another note, the differential effect of different $Ca_v\beta$s on $G_{\beta\gamma}$-mediated inhibition can be further exposed by the expression of other proteins. For example, RGS2, which is a member of the regulators of G protein signaling that catalyze GTP hydrolysis and terminate G protein signaling (498), can unmask differences in G-protein modulation of P/Q-type channels containing different types of $Ca_v\beta$ (300).

D. Model for the Voltage Dependence of $G_{\beta\gamma}$ Inhibition

Before presenting a model for voltage-dependent $G_{\beta\gamma}$ inhibition, we first consider the molecular components involved in this process. *1*) Several distinct regions in $Ca_v\alpha_1$, all of which bind $G_{\beta\gamma}$ in vitro, play a role in voltage-dependent $G_{\beta\gamma}$ inhibition, including the NH_2 terminus (3, 345), the I–II loop (118, 346, 439, 496), and to a lesser extent, the COOH terminus (3, 189, 231, 282, 366, 499). The NH_2 terminus of $Ca_v\alpha_1$ binds directly to the I–II loop, and together they form a $G_{\beta\gamma}$-gated inhibitory module (3). *2*) The I–II loop has two $G_{\beta\gamma}$ binding sites: one extends from the COOH-terminal end of IS6 to the NH_2-terminal end of the AID and contains a signature $G_{\beta\gamma}$-interacting QxxER motif (QQIER in $Ca_v2.1$), and the other is located further downstream of the AID (118, 439, 496). The downstream site, termed the G protein interaction domain or GID, is likely to be an anchoring site for $G_{\beta\gamma}$. It has a ~20 nM affinity for $G_{\beta\gamma}$ (118, 496), and when applied as a 21-amino acid peptide, it can prevent PPF. The upstream site containing the QxxER motif has a ~60 nM affinity for $G_{\beta\gamma}$, and its mutations attenuate $G_{\beta\gamma}$ modulation (214). However, this site may serve only as a secondary $G_{\beta\gamma}$-binding site in the holo-channel for three reasons. *1*) It is partially buried by $Ca_v\beta$, as shown by the AID-$Ca_v\beta$ core crystal structures (84, 341, 447). Hence, $G_{\beta\gamma}$ binding to the upstream site is significantly weaker in the presence of $Ca_v\beta$ than in the absence of $Ca_v\beta$ (502). *2*) The QxxER motif is unlikely to become completely available, since $Ca_v\beta$ does not vacate from the $G_{\beta\gamma}$-bound channels (61, 231, 315, 502), as discussed above. When seven alanine residues are inserted in the upstream site, which is expected to prevent $G_{\beta\gamma}$ binding to this site, voltage-dependent $G_{\beta\gamma}$ inhibition remains intact (502). *3*) As discussed in section III*E*, $Ca_v\beta$ binding to the AID results in the formation of an α-helix extending continuously from IS6 to the AID. The integrity of this α-helix is critical for voltage-dependent $G_{\beta\gamma}$ inhibition, as mentioned above.

Figure 8 depicts an allosteric model proposed recently for the origin of the voltage dependence of $G_{\beta\gamma}$ inhibition of Ca_v2 channels (502). This model links voltage-dependent dissociation of $G_{\beta\gamma}$ to the voltage-dependent movement of IS6 and to the obligatory role of $Ca_v\beta$. The pocket where $G_{\beta\gamma}$ binds in the holo-channel to produce the voltage-dependent inhibition is still unknown, but it is postulated to be downstream of the COOH-terminal end of the AID and is formed collectively by portions in the NH_2 terminus, the I–II loop, and the COOH terminus (and possibly yet unknown regions). Under the resting condition and in the presence of $G_{\beta\gamma}$, the channel is inhibited (Fig. 8*A*, *left*). Upon depolarization, the S6 segments of $Ca_v\alpha_1$ move, and owing to the continuous rigid α-helical structure of the IS6-AID linker, this movement is transmitted to and beyond the AID, resulting in a movement of the distal I–II loop and, consequently, a conformational change of the $G_{\beta\gamma}$-binding pocket.

Such a chain of events ultimately leads to the disassembly of the NH_2 terminus-I–II loop inhibitory module and the dissociation of $G_{\beta\gamma}$ from the channel (Fig. 8*A*, *right*), which account for the slowing of the activation kinetics and prepulse facilitation. In the absence of $Ca_v\beta$, $G_{\beta\gamma}$ can still bind to the holo-channel but cannot be discharged by the depolarizing potential, because the $G_{\beta\gamma}$-binding pocket is uncoupled from IS6 as a result of the unwinding of the AID into a random coil (Fig. 8*B*). Such uncoupling can also be produced by glycine insertions in the IS6-AID linker (Fig. 8*C*). In summary, this model postulates that the voltage dependence of $G_{\beta\gamma}$ inhibition of Ca_v2 channels arises from the voltage-dependent movement of IS6 and that $Ca_v\beta$ and a rigid IS6-AID linker play a pivotal role in translating this movement to $G_{\beta\gamma}$ dissociation.

IX. ROLE OF $Ca_v\beta$ IN RGK INHIBITION OF HIGH-VOLTAGE ACTIVATED Ca^{2+} CHANNELS

The RGK (Rad, Rem, Rem2, Gem/Kir) family of Ras-related monomeric small GTP-binding proteins has emerged as potent inhibitors of HVA Ca^{2+} channels (27, 155). There are four members in this family: Rad (Ras associated with diabetes; Ref. 370), Rem (or Ges = human ortholog; Ref. 153), Rem2 (157), and Gem/Kir (91, 296). They share a conserved Ras-like core but differ from other Ras members in that their GTP/GDP-binding domains have nonconserved mutations that alter or abolish the GTP/GDP cycle (410, 489). They also contain extended NH_2 and COOH termini. The COOH terminus has a motif that can anchor them to the membrane (reviewed in Refs. 81, 104, 211, 296) and is critical for their function (81, 104, 105, 488). RGK proteins have two known functions: shaping cytoskeletal dynamics and inhibiting HVA Ca^{2+} channels (24, 27, 104, 255, 324). These two functions can be differentially regulated; for example, RGK modification of cytoskeletal reorganization, but not inhibition of HVA Ca^{2+} channels, is attenuated by dephosphorylation of certain RGK residues (152, 463). The physiological importance of RGK inhibition of HVA Ca^{2+} channels is illustrated by recent in vivo studies that manipulate endogenous Rad levels with consequences for the heart (79, 462, 478). For example, dominant negative suppression of endogenous Rad in the heart increases L-type Ca^{2+} channel currents and action potential duration in cardiac cells and causes longer QT intervals and arrhythmias (478). Here, we only discuss RGK inhibition of HVA Ca^{2+} channels and the role of $Ca_v\beta$ in this process. For more comprehensive reviews of RGK proteins and their functions, see References 104 and 255.

A. $Ca_v\beta$ Is Essential for RGK Inhibition of HVA Ca^{2+} Channels

All members of the RGK family are able to inhibit, in a voltage-independent manner, HVA Ca^{2+} channels when expressed in various heterologous expression systems (15, 24–27, 81, 101, 102, 154–156, 165, 281, 397, 478, 487, 488). This inhibition depends on $Ca_v\beta$, as RGK proteins do not affect Ca^{2+} channel currents recorded in cells expressing only $Ca_v\alpha_1$ (27, 155, 397 but see Ref. 106). Consistent with this notion, RGK proteins do not affect the activity of T-type Ca^{2+} channels, which do not associate with $Ca_v\beta$ nor require $Ca_v\beta$ for their activity (81, 155). Furthermore, RGK proteins interact directly with $Ca_v\beta$, both in vitro and in cells (24–28, 101, 102, 154–156, 165, 281, 487), and this interaction seems to be promiscuous whereby any RGK protein can interact with any full-length $Ca_v\beta$. A structural model of Gem-β_3 interaction has been recently developed (28) based on systematic mutagenesis analysis and homology modeling based on the crystal structure of the AID-β_3 core complex (84) and a crystal structure of GDP-bound Gem (PDB 2G3Y). This model shows that Gem binds to the β_3 GK domain at a site distinct from the AID-binding pocket and that residues D194, D270, and D272 in β_3 and R196, V223, and H225 in Gem are critical for this interaction. Supporting this model, mutating these residues individually or in combination severely weakens or abolishes in vitro binding of Gem and β_3 (28, 144).

Three mechanisms of RGK inhibition have been reported. The first mechanism, reported in the first study on RGK regulation of HVA Ca^{2+} channels (27) and advanced in subsequent studies mostly from the same groups (24–26, 28, 388, 478), is that all RGK proteins disrupt the trafficking of HVA Ca^{2+} channels to the plasma membrane and hence reduce the number of surface Ca^{2+} channels, as determined primarily by imaging the subcellular localization of epitope-tagged $Ca_v\alpha_1$. It was hypothesized that RGK proteins compete with $Ca_v\alpha_1$ by sequestering $Ca_v\beta$ in the cytoplasm and/or the nucleus, thus leaving $Ca_v\alpha_1$ trapped in the ER. Several observations are consistent with this hypothesis. *1*) Nuclear targeting of Rem and Rad causes nuclear sequestration of $Ca_v\beta$ (24). *2*) In addition to $Ca_v\beta$, all RGK proteins also interact with CaM and 14 –3-3 (24 –26, 158, 297). Abolishing CaM and 14 –3-3 binding or CaM binding alone results in nuclear accumulation of RGK proteins (24 –26), suggesting that these interactions regulate the subcellular localization of RGK proteins. Moreover, Rem2 and Gem (but not Rad and Rem) mutants deficient in CaM binding are unable to inhibit HVA Ca^{2+} channel currents (24 –27, 463).

On the other hand, other findings are inconsistent with the aforementioned mechanism (i.e., sequestration of $Ca_v\beta$ and disruption of channel surface expression). *1*) $Ca_v\beta$-$Ca_v\alpha_1$ binding through the AID-GK domain interaction is much stronger than RGK-$Ca_v\beta$ binding (154), making it unlikely for RGK proteins to compete off $Ca_v\beta$ from $Ca_v\alpha_1$. *2*) Several studies show that $Ca_v\alpha_1$, $Ca_v\beta$, and RGK proteins form a trimeric complex in vitro and in cells (28, 101, 144, 154, 487). *3*) Work from several groups show that RGK proteins can inhibit HVA Ca^{2+} channels without affecting their surface expression, with the latter determined by surface binding of a radioactive toxin (81), surface biotinylation (144, 154, 156), or gating charge measurement (487). These observations suggest that cytoplasmic and/or nuclear sequestration of $Ca_v\beta$ by RGK proteins occurs via a mechanism other than competition with the AID and may, if at all, only partially account for the observed RGK inhibition of HVA Ca^{2+} channels.

The second mechanism, closely related to the first one, is that RGK proteins decrease the number of surface Ca^{2+} channels by increasing their internalization. A recent study shows that Rem enhances dynamin-mediated endocytosis of L-type channels expressed in HEK 293 cells (488).

The third mechanism of RKG inhibition is the suppression of the activity of channels already on the plasma membrane. Such direct inhibition has been observed for different RGK proteins and different HVA Ca^{2+} channels in a variety of expression systems. For example, Rem2 inhibits endogenous surface N-type channels in native neurons (81), Rem inhibits surface L-type channels expressed in pancreatic β-cells (154, 156) and HEK 293 cells (488), and rapid translocation of a recombinant Rem derivative acutely inhibits L- and N-type channels expressed in tsA201 cells (487). Recently, Gem inhibition of P/Q-type channels was reconstituted in inside-out membrane patches by direct application of a purified Gem protein domain (144). Furthermore, it was found that this acute inhibition was completely abolished when $Ca_v\beta$ was washed away from the patch (using the strategy mentioned in sect. VIII*A*), leaving P/Q channels β-less, but it was fully restored after application of a purified $Ca_v\beta$ protein, demonstrating that $Ca_v\beta$ is indispensable for Gem inhibition of surface P/Q channels. Finally, in agreement with a recent study (281), it was found that the $Ca_v\beta$ GK domain alone was sufficient to support Gem inhibition of P/Q channels expressed in *Xenopus* oocytes (144).

The absolute requirement of $Ca_v\beta$ for RGK-mediated inhibition of HVA Ca^{2+} channels is reminiscent of such a requirement for voltage-dependent $G_{\beta\gamma}$ inhibition of N- and P/Q-type channels. The latter process further requires a rigid α-helical structure of the IS6-AID linker. It was shown, however, that disrupting the IS6-AID α-helix by glycine insertions did not affect Gem inhibition of P/Q channels (144). This result suggests that Gem inhibition uses a fundamentally different mechanism from voltage-dependent $G_{\beta\gamma}$ inhibition and may not involve IS6-transmitted conformational changes in the pore.

The three mechanisms of RGK inhibition discussed above operate on different time scales and are not mutually exclusive. For example, in the case of Rem inhibition of L-type channels expressed in HEK 293 cells, both reduction of surface channel density and direct inhibition of surface channels occur (488). Which mechanism dominates or is utilized in native cells remains to be determined, but it likely depends on the type and expression level of RGK proteins as well as Ca^{2+} channel subunits.

B. A New Paradigm for RGK Inhibition of HVA Ca^{2+} Channels

As mentioned above, all members of the RGK family can interact with all four subfamilies of $Ca_v\beta$. The RGK-$Ca_v\beta$ interaction has been widely presumed to be essential for RGK inhibition of HVA Ca^{2+} channels (15, 24–27, 81, 101, 102, 104, 154–156, 165, 206, 281, 397, 478). However, it was recently reported that while $Ca_v\beta$ is required for Gem inhibition of surface P/Q-type channels, the interaction between $Ca_v\beta$ and Gem is not (144). In this study, residues D194, D270, and D272 in β_3 and R196, V223, and H225 in Gem were simultaneously mutated to alanine; these residues are predicted and shown to be critical for the Gem-β_3 interaction (28, 144). This combination of mutations completely abolished binding of Gem and β_3, and yet, the mutant Gem (named Gem_Mut3) was still fully capable of inhibiting P/Q channels containing either the mutant or WT β_3 (144). Mean-while, it was found that $Ca_v2.1$ could be coimmunoprecipitated by WT Gem or Gem_Mut3, either in the presence or absence of β_3, suggesting that Gem directly interacts with $Ca_v2.1$. Chimeric studies with PQ- and the RGK-insensitive T-type channels indicate that the IIS1–IIS3 region of $Ca_v\alpha_1$ is essential for gem inhibition (144).

Based on these results and those discussed above, we propose a "$Ca_v\beta$-priming" model for Gem inhibition of P/Q-type Ca^{2+} channels on the plasma membrane (Fig. 9; Ref 144). (This model may be applicable to Rem and Rem2 since they also inhibit surface Ca^{2+} channels.) A distinct feature of this model is that the interaction between Gem and $Ca_v\beta$ is not necessary for Gem's inhibitory effect, but a direct association between Gem and $Ca_v2.1$ is essential. In this model, Gem interacts directly with $Ca_v2.1$ through an anchoring site, with or without $Ca_v\beta$ being present. In the presence of $Ca_v\beta$ and Gem, $Ca_v2.1$ forms a multimeric complex with both proteins on the plasma membrane (Fig. 9*A*). Binding of $Ca_v\beta$ to $Ca_v2.1$ produces a conformational change, resulting in the formation of an inhibitory site in $Ca_v2.1$ where Gem binds to produce inhibition (Fig. 9*A*). When $Ca_v\beta$ dissociates or is washed off from surface $Ca_v2.1$, the inhibitory site disappears, rendering Gem unable to inhibit $Ca_v2.1$, even though it can remain attached to $Ca_v2.1$ via the anchoring site (Fig. 9*B*). When $Ca_v\beta$ and Gem mutants that cannot bind to each other are used (Fig. 9*C*), inhibition can nevertheless proceed since the ability of $Ca_v\beta$ and Gem to bind $Ca_v2.1$ is not compromised. Thus the essential role of $Ca_v\beta$ is to convert $Ca_v2.1$ into a state permissive for Gem inhibition.

At present, this model remains speculative, and many questions remain unanswered. For example, where is the anchoring site for Gem on $Ca_v2.1$? Where is the inhibitory site? How does $Ca_v\beta$ binding to $Ca_v2.1$ create the inhibitory site? How does Gem binding to the inhibitory site lead to channel inhibition? Does the Gem-$Ca_v\beta$ interaction play any role at all? With regard to the last question, we speculate that in native cells, with physiological levels of Gem protein, the Gem-$Ca_v\beta$ interaction may increase the effective concentration of Gem near surface Ca^{2+} channels and thereby facilitate Gem inhibition.

X. ROLE OF $Ca_v\beta$ IN PHOSPHO- AND LIPID REGULATION OF HIGH-VOLTAGE ACTIVATED Ca^{2+} CHANNELS

Phosphorylation allows dynamic regulation of protein functions, including those of HVA Ca^{2+} channels. During the "fight-or-flight" response, for example, β-adrenergic stimulation leads to PKA-dependent upregulation of L-type Ca^{2+} channel currents, which results in a faster and stronger heartbeat. The activity of HVA Ca^{2+} channels is regulated by a variety of protein kinases and phosphatases. While $Ca_v\alpha_1$ is often the target of phosphorylation, $Ca_v\beta$ can nevertheless modulate the effect of such phosphorylation, and in some cases, $Ca_v\beta$ itself is the target. HVA Ca^{2+} channels can also be regulated by membrane lipids and their metabolic products. This section discusses the role of $Ca_v\beta$ in phospho- and lipid regulation of HVA Ca^{2+} channels.

A. Ca^{2+}/Calmodulin-Dependent Kinase II

Ca^{2+}/calmodulin-dependent kinase II (CaMKII) is among the most abundant enzymes in many cell types. Recent studies show that CaMKII can interact directly with $Ca_v\alpha_1$ of HVA Ca^{2+} channels and regulate their activities (242, 273, 358). In cardiac and smooth muscle cells, CaMKII plays a role in the facilitation of L-type Ca^{2+} channels (133, 192, 308). In cardiomyocytes, the molecular mechanism partly involves CaMKII-mediated phosphorylation of the β_{2a} subunit (192). CaMKII binds to the COOH terminus of β_{2a} and phosphorylates it at T498, which leads to an upregulation of L-type currents (192). This upregulation is not observed in the absence of β_{2a} or when a nonphosphorylatable β_{2a} mutant, β_{2a} (T498A), is coexpressed in tsA201 cells. This mutant can also act as a dominant negative, preventing CaMKII-mediated facilitation of endogenous Ca^{2+} currents. Moreover, T498 phosphorylation promotes the dissociation of CaMKII from β_{2a}, which may serve as a negative-feedback mechanism (193). Since most β_2 splice variants have a common COOH terminus identical to that of β_{2a} (Fig. 6), it would be interesting to examine whether CaMKII also interacts with and phosphorylates these β_2 variants.

CaMKII has also been shown to associate with β_{1b} in vitro, but not with β_3 and β_4 (193). A recent study further shows that CaMKII coimmunoprecipitates with forebrain L-type Ca^{2+} channel complexes containing β_1 or β_2 but not β_4 (1). β_{1b}, β_3, and β_4 have also been shown to be phosphorylated by CaMKII (193), but the physiological consequences remain to be determined.

B. Mitogen-Activated Protein Kinase

Mitogen-activated protein kinase (MAPK) is a member of a signaling network that responds to extracellular stimuli and induces diverse physiological and pathological processes. The small monomeric G protein, Ras, can upregulate Ca^{2+} currents in dorsal root ganglion neurons through the activation of the MAPK signaling pathway (160). In COS-7 cells, this upregulation is shown to require $Ca_v\beta$, because in the absence of $Ca_v\beta$, MAPK-dependent upregulation is abolished (159). Furthermore, different $Ca_v\beta$s support different degrees of upregulation. For example, in the presence of β_{2a}, but not other $Ca_v\beta$s, Ca^{2+} channels are partially resistant to inhibition by an antagonist of MAPKK, the exclusive activator of MAPK (159). It is speculated that MAPK directly phosphorylates the channel complex (159). While both $Ca_v\alpha_1$ and $Ca_v\beta$ have consensus MAPK phosphorylation sites, it is unclear which, if any, are phosphorylated in cells.

C. Phosphoinositide 3-Kinase and Protein Kinase B (or Akt)

Some external stimuli (e.g., insulin-like growth factor) activate receptors that are associated with tyrosine kinases and upregulate L- and N-type Ca^{2+} channel currents (42). The mechanism likely involves phosphoinositide 3-kinase (PI3K) activation and subsequent production of phosphatidylinositol 4,5-bisphosphate (PIP_2) and phosphatidylinositol 3,4,5-trisphosphate (PIP_3), known regulators of HVA Ca^{2+} channels (for reviews, see Refs. 122, 318). Increased PIP_3 levels recruit protein kinase B (PKB) to the membrane, which can phosphorylate β_{2a} at S574 (454). This results in an upregulation of currents conducted by channels containing β_{2a} and $Ca_v1.2$ or $Ca_v2.2$, mainly by increasing surface expression (454). Ca^{2+} channels containing an unphosphorylatable β_{2a} (bearing the S574A mutation) are resistant to upregulation by PI3K/PKB, whereas those containing a phosphorylation-mimic β_{2a} (bearing the S574E mutation) exhibit tonically increased current and are insensitive to upregulation by PI3K. The permissive role of β_{2a} is not shared by other $Ca_v\beta$s and does not depend on palmitoylation (454).

A similar PI3K/PKB signaling pathway may play a role in maintaining normal cardiac function. Thus, in the absence of active PKB (created in a cardiac-specific conditional knockout), L-type Ca^{2+} channel surface expression is greatly reduced, leading to severe cardiomyopathy (70). This deficit results from lysosomal degradation of L-type channels, initiated by conserved PEST sequences (signals for rapid protein degradation) in $Ca_v1.2$. When PKB is active, it binds and phosphorylates β_2, which in turn masks the degradation signals and leads to an increased channel surface expression (70).

D. cAMP-Dependent Protein Kinase

cAMP-dependent protein kinase (PKA)-mediated upregulation of cardiac L-type Ca^{2+} channel currents was one of the first examples of ion channel modulation. During the "fight-or-flight" response, β-adrenergic stimulation, through G proteins and adenylate cyclase, results in increased levels of cAMP and subsequent activation of PKA, eventually leading to dramatic increases of cardiac L-type Ca^{2+} currents and the consequent faster and stronger heartbeat (for reviews, see Refs. 72, 309). In spite of intense research, the target of PKA phosphorylation that underlies L-type current upregulation still remains obscure, partly owing to the difficulty of reconstituting this regulation in heterologous expression systems. In vivo, this upregulation is accompanied by increased phosphorylation of both $Ca_v1.2$ and β_2 (54, 72, 114, 115, 180, 197). A PKA phosphorylation site on $Ca_v1.2$ (S1928) has been proposed to mediate the β-adrenergic response (115). However, a recent study using the $Ca_v1.2$ (S1928A) knock-in mice shows that basal L-type currents and the upregulation of L-type currents by PKA and β-adrenergic receptor stimulation are unchanged (275), indicating that PKA phosphorylation of S1928 is not the underlying cause for L-type current upregulation.

On the other hand, it has been shown that, in vitro, PKA phosphorylates three sites on β_{2a}: S459, S478, and S479 (180). It was initially proposed that β_{2a} phosphorylation at S478 and S479 is critical for L-type channel upregulation (54, 173). This was based on the lack of or reduced upregulation of currents conducted by L-type channels containing β_{2a} (S478A/S479A) (54, 173). A caveat is that these studies were done either with a truncated $Ca_v1.2$ (54) or without comparative experiments with WT β_{2a} (173). The role of β_{2a} phosphorylation in L-type current upregulation has been strongly challenged by a recent study, which shows that in cardiac muscle cells L-type channels containing β_{2a}(S459A/S478A/S479A) exhibit the same degree of PKA-mediated upregulation as channels containing WT β_{2a} (321). This study further demonstrates that the extent of PKA modulation is influenced by the associated $Ca_v\beta$. Thus channels containing β_{1b} show the strongest upregulation, followed by those containing β_3 and β_4, whereas channels containing β_{2a} show the least modulation (probably because β_{2a} dramatically increases channel P_o in the first place, as mentioned in sect. VI*A*). Since β_{1b}, β_3, and β_4 do not share the aforementioned PKA phosphorylation sites in β_{2a}, the latter observation further strengthens the notion that PKA phosphorylation of β_{2a} does not play an essential role in the upregulation of cardiac L-type currents. Thus identifying the site(s) of PKA phosphorylation that give rise to this event remains a stubborn challenge.

Lastly, $Ca_v1.3$ channels can also be potentiated by PKA phosphorylation (287). The extent and duration of this potentiation also depend on the identity of the associated $Ca_v\beta$ (287).

E. Protein Kinase C

Protein kinase C (PKC) can enhance some neuronal L- and N-type Ca^{2+} channel currents (483). In Ca_v2 channels, the enhancement partly results from the disruption of $G_{\beta\gamma}$ inhibition (19, 21, 96, 202, 426, 496). This is achieved by phosphorylating the I–II loop of $Ca_v\alpha_1$, which may block $G_{\beta\gamma}$ binding, considering that the I–II loop contributes to form the $G_{\beta\gamma}$-binding pocket (see sect. VIII*D*). Since $Ca_v\beta$ also binds to the I–II loop, it is conceivable that its binding is sensitive to PKC phosphorylation, or vice versa. In fact, pharmacological activation of PKC can increase $Ca_v2.2$ and $Ca_v2.3$ channel currents, but only in the presence of $Ca_v\beta$, and transferring the I–II loop from these channels to the unresponsive $Ca_v2.1$ and $Ca_v1.2$ channels can transfer PKC sensitivity (49, 413). Thus it seems that $Ca_v\beta$ is permissive for PKC modulation of certain $Ca_v\alpha_1$. However, the effects of PKC phosphorylation of $Ca_v\beta$ itself (e.g., β2a; Ref. 180) are unknown.

F. cGMP-Dependent Protein Kinase

cGMP-dependent protein kinase (PKG) is activated by cGMP and can phosphorylate both $Ca_v1.2$ and β_{2a}-$Ca_v1.2$ at S1928 (the same residue that can also be phsophorylated by PKA) and β_{2a} at S496 (484, 485). Activation of PKG results in inhibition of channels containing $Ca_v1.2$ and β_{2a} in HEK cells (484). This inhibition is abolished by the β_{2a} S496A mutation, suggesting that PKG phosphorylation of β_{2a} is critical for this process. The physiological consequences of this inhibition remain to be determined.

G. Arachidonic Acid

Like many other types of ion channels, the activity of HVA Ca^{2+} channels can be regulated by membrane lipids and their metabolic products (for reviews, see Refs. 46, 122, 172, 318, 373). One example is the voltage-independent inhibition of HVA Ca^{2+} channels upon PIP_2 depletion from the plasma membrane (171, 475). Another example is channel inhibition by arachidonic acid (AA), an unsaturated fatty acid released from phospholipids (including PIP_2) by the action of some phospholipases (18, 289, 374, 403). The magnitude of AA inhibition depends on the partnered $Ca_v\beta$ in the channel (374). In particular, β_{2a} seems to dampen AA inhibition of $Ca_v1.3$ channels. This unique effect of β_{2a} is abolished when palmitoylation is eliminated in a mutant β_{2a} (β_{2a} C3,4S). Attaching a transmembrane segment of an unrelated membrane protein to the NH_2 terminus of this mutant β_{2a} does not restore the dampening effect, suggesting that palmitoylation rather than membrane anchorage per se is responsible for the antagonizing effect of β_{2a} on AA-mediated inhibition. It is proposed that the palmitoyl groups of β_{2a} compete with AA for a common binding site on $Ca_v1.3$ (374). This unique ability of β_{2a} has also been proposed to underlie the enhancement of $Ca_v2.2$ channels containing β_{2a} by the stimulation of G_q-coupled receptors, which, in contrast, causes inhibition of channels containing β_{1b}, β_3, or β_4 (210).

XI. INTERACTION OF $Ca_v\beta$ WITH OTHER PROTEINS

For many years, $Ca_v\alpha_1$ was the only known interacting partner for $Ca_v\beta$. In recent years, however, a growing number of proteins have been found to interact with $Ca_v\beta$, in some cases with significant functional impact. This section reviews some of these interactions and their functional consequences. Two more examples are discussed in section XII, in the context of a potential role of $Ca_v\beta$ in transcriptional regulation.

A. Ryanodine Receptors

In skeletal muscle, Ca^{2+} channel complexes (DHPRs), which are made up of $Ca_v1.1$, β_{1a}, $\alpha_2\delta_1$, and γ_1 subunits, are arranged on the plasma membrane of t tubules in tetrads, which are in contact with ordered RyRs in the membrane of adjoining sarcoplasmic reticulum (SR) (for reviews, see Refs. 167, 363). This physical arrangement is required for efficient excitation-contraction (EC) coupling. It has been shown that β_{1a}, which is expressed exclusively in skeletal muscle, is indispensible for EC coupling (for reviews, see Refs. 100, 164). This is because β_{1a} is essential not only for the surface expression of DHPRs (37, 38, 191, 393, 394, 402) but also for the tetrad formation (393, 394). It seems that β_{1a} allosterically primes $Ca_v1.1$ to properly interact with RyRs to form the tetrads (393). Expression of exogenous $Ca_v\beta$ in skeletal muscle cells isolated from β_{1a}-null mice or zebrafish can fully rescue L-type Ca^{2+} channel current, but only β_{1a} (and β_{1c}) can normalize EC coupling (38, 393, 402). This unique ability is due to the presence of a heptad repeat (L478-V485-L492) in the distal COOH terminus of β_{1a} (393, 401, 402). When this region is deleted from β_{1a}, EC coupling is lost, and when it is transferred to β_{2a}, EC coupling is observed. It is unclear, however, where the heptad repeat binds (402). What is certain is that β_{1a} can bind directly to the RyR (85, 164). On the RyR, β_{1a} binds to a highly charged region (KKKRRxxR), whose mutation attenuates EC coupling (85). Interestingly, two pathogenic mutations, R3348H and P3527S, occur in this region of the RyR and cause malignant hyperthermia susceptibility (384) and multi-minicore congenital myopathy (148), respectively. It remains to be determined whether the loss of interaction with β_{1a} is the underlying cause.

B. Ahnak

Ahnak, a ubiquitous large (700 kDa) signaling and scaffolding protein involved in diverse aspects of cell physiology and pathophysiology (reviewed in Refs. 7, 195), has been shown to interact with $Ca_v\beta$ in several distinct cell types, including cardiac cells, osteoblasts, and T lymphocytes (6, 199, 223, 305, 398). This interaction provides a potential link between Ca^{2+} channels, the cytoskeleton, and cellular organelles. Multiple regions in the COOH terminus of Ahnak can bind β_{2a} in vitro, with apparent affinities ranging from 50 to ~300 nM (196, 199, 223). The Ahnak-interacting region on β_{2a} is unknown, but since Ahnak coimmunoprecipitates with β_{1b}, β_3, and β_{2a} (6, 199, 398), it is likely to be in the conserved GK or SH3 domains.

It has been proposed that the Ahnak-β_{2a} interaction plays a role in PKA-mediated upregulation of cardiac L-type currents (195, 196). Both β_{2a} and Ahnak can be phosphorylated by PKA, and this phosphorylation weakens the binding between the two proteins by ~50% (196). This effect is accompanied by L-type current upregulation, a hyperpolarizing shift in the voltage dependence of activation, and an occlusion of subsequent PKA effects (196, 199). Thus it was suggested that under basal conditions, L-type channel activity is suppressed by the Ahnak-β_{2a} interaction and that PKA phosphorylation of both proteins disengages β_{2a} from Ahnak and hence relieves this tonic inhibition (195). However, a challenge to this proposal is that PKA phosphorylation of β_{2a} does not appear to play a role in the β-adrenergic receptor stimulation-induced upregulation of cardiac L-type currents, as discussed in section X*D*.

In the immune system, T cells are central in cell-mediated immunity. T cells are nonexcitable cells, yet they express all four Ca_v1 channels and all four $Ca_v\beta$s (241, 419). Activation of T-cell antigen receptors (TCR) causes Ca^{2+} influx, which is key for T-cell activation. This Ca^{2+} influx is thought to be mediated in part by Ca_v1 channels, although the mechanism of channel activation remains unclear (11, 241,305, 419). $CD4^+$ T lymphocytes isolated from β_3- and β_4-null mice and $CD8^+$ T cells isolated from β_3-null mice display impaired TCR-triggered Ca^{2+} response (11, 241), presumably because of deficient surface expression of Ca_v1 channels. T cells from Ahnak1 knockout mice also respond poorly to TRC stimulation and have impaired Ca^{2+} influx (305). It is proposed that this deficit results from decreased plasma membrane expression of Ca_v1 channels, owing to the lack of the Ahnak1-$Ca_v\beta$ interaction (305); however, this hypothesis needs further testing.

C. BK_{Ca} Potassium Channels

Large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) are synergistically activated by membrane depolarization and intracellular Ca^{2+} and play important roles in various physiological processes (383). Even though BK_{Ca} channels have their own auxiliary subunits, it has been shown recently that BK_{Ca} channels bind directly to the Ca^{2+} channel β_1 subunit (508). In HEK 293T cells, this interaction dampens the Ca^{2+} sensitivity of BK_{Ca} channels and slows their activation and deactivation. The GK domain of β_1 is necessary and sufficient for these effects, suggesting that other $Ca_v\beta$s may share this β_1 function, but this remains to be determined. It is also unknown whether the $Ca_v\beta$-BK_{Ca} channel interaction occurs in native cells, and whether and how this interaction affects HVA Ca^{2+} channels.

D. Bestrophin

Bestrophin (Best1) is a 585– 604 amino acid chloride channel expressed in the retinal pigment epithelium (RPE) whose mutations cause Best's and other retinopathies (see review in Ref. 205). It modulates L-type Ca^{2+} channel gating and blocks L-type channel-mediated rises in $[Ca^{2+}]_i$ in RPE (301, 378). Recently, it was shown that Best1 binds the Ca^{2+} channel β_4 subunit and that its effects disappear in the absence of β_4, suggesting that Best1 may be acting through β_4 on $Ca_v1.3$ channels (493). Befittingly, β_4 knockout mice also have rethinopathies (301). The COOH terminus of Best1, which on its own does not generate Cl^- currents, can also inhibit L-type channels. It contains a predicted proline-rich domain (PRD) whose mutation abolishes the effects of Best1. It is proposed that this PRD binds to the SH3 domain of β_4, disrupts the GK-SH3 interaction, and causes L-type channel inhibition (493). However, direct evidence for such a mechanism is still lacking. It would be interesting to examine whether the PxxP-binding region of β_4 binds Best1. As discussed in section III*B*, although this region is occluded in the $Ca_v\beta$ crystal structures (84, 341, 447), it could conceivably become accessible when $Ca_v\beta$ is bound to $Ca_v\alpha_1$ and other proteins.

E. Dynamin

A recent study reported that full-length β_{2a} interacts in vitro with dynamin, a multi-partner GTPase involved in endocytosis (186). This interaction was presumed to involve a PRD of dynamin and the SH3 domain of β_{2a}, since a purified β_{2a} fragment (amino acids 24–136) containing the four contiguous β sheets of the SH3 domain was found to interact with dynamin and this interaction was partially blocked by the dynamin PRD. This β_{2a} fragment was able to markedly suppress the surface expression of $Ca_v1.2$ channels, and this suppression depended on dynamin. It was proposed that the dynamin-SH3 domain interaction links HVA Ca^{2+} channels to the endocytotic machinery (186). It should be noted, however, that the β_{2a} fragment used in this study lacks the fifth (i.e., the last) β sheet of the SH3 domain and the HOOK region, which hinders access to the PxxP-binding region of $Ca_v\beta$ (84, 341, 447). To determine whether the PxxP-binding region of β_{2a} is involved in the interaction between dynamin and full-length β_{2a}, it would be useful to examine whether this interaction is abolished by selective mutations of β_{2a} residues that are presumably directly involved in binding PRDs.

F. Synaptic Proteins: Synaptotagmin I and RIM1

The α_1 subunit of presynaptic HVA Ca^{2+} channels physically interacts with presynaptic proteins, including syntaxin, SNAP-25, and synaptotagmin I; these interactions are important for synaptic vesicle docking and fusion (reviewed in Ref. 400). Recent studies show that $Ca_v\beta$ can also interact with synaptic proteins (257, 452). For example, the NH_2 terminus of β_3 and β_{4a} (but not β_{4b}) binds to synaptotagmin I, and this interaction is abolished by a high concentration (10 mM) of Ca^{2+} (452). However, the physiological importance of this interaction is yet unknown. Another study shows that RIM1, a presynaptic protein critical for synaptic transmission and plasticity (69, 392), binds directly and with a high affinity (35 nM) to β_{4b} and β_{2a}. This interaction is mediated by the COOH terminus of RIM1, and the SH3-HOOK-GK module of $Ca_v\beta$ is sufficient for binding to occur. The most prominent effect of the RIM1-$Ca_v\beta$ interaction on HVA Ca^{2+} channels is the slowing of VDI and a hyperpolarizing shift of the voltage dependence of inactivation. This effect is observed on recombinant L-, N-, P/Q-, and R-type channels containing β_{4b} and on recombinant P/Q-type channels containing β_{1a}, β_{2a}, β_3, or β_{4b}. RIM1 may also play a role in anchoring synaptic vesicles to presynaptic VGCCs through binding to the synaptic vesicle protein Rab3. Consequently, overexpression of a mutant $Ca_v\beta$ that is unable to bind $Ca_v\alpha_1$ can attenuate vesicle docking at the presynaptic membrane in PC12 cells, presumably by competing with the WT $Ca_v\alpha_1/\beta$ complex for RIM1 binding. Furthermore, overexpression of RIM1 in PC12 cells and cultured cerebellar neurons enhances neurotransmitter release. This study establishes a direct role of $Ca_v\beta$ in the physical organization of the synaptic vesicle release machinery (257).

G. Zinc Transporter 1

Zinc transporter 1 (ZnT-1), a ubiquitous transmembrane protein involved in zinc transport and metabolism, binds directly to β_{2a} (280) and has been shown to inhibit L-type Ca^{2+} channels in heterologous expression systems and native cells (29, 280,337, 396). When coexpressed with $Ca_v1.2$ and β_{2a} in *Xenopus* oocytes, ZnT-1 reduced $Ca_v1.2$ channel currents (280). This inhibitory effect disappeared in the absence of β_{2a} or in the presence of an excess amount of β_{2a}. ZnT-1 reduced the surface expression of $Ca_v1.2$ without changing its total expression level when they were coexpressed in HEK 293T cells (280). The authors proposed that ZnT-1, through direct binding to $Ca_v\beta$, inhibits L-type channels by reducing their trafficking to the plasma membrane (280). It remains to be examined whether ZnT-1 physically interacts with other types of $Ca_v\beta$s and whether it inhibits Ca_v2 channels.

XII. Ca^{2+} CHANNEL-INDEPENDENT FUNCTIONS OF $Ca_v\beta$

Until recently, the functions of $Ca_v\beta$ have been exclusively linked to VGCCs. However, a stream of recent studies suggests that $Ca_v\beta$ may possess functions independent of their association with VGCCs. This line of inquiry began with the cloning of various short isoforms of $Ca_v\beta$, some of which lacked the GK domain. This inability to engage in the high-affinity AID-GK domain interaction with $Ca_v\alpha_1$ raised questions about their functions (92, 166,204, 217, 229). The first study examining possible alternative functions of truncated splice variants of $Ca_v\beta$ centered on a β_4 splice variant expressed in chicken cochlea and brain, termed β_{4c} (217); to avoid confusion, we refer to it as $c\beta_{4c}$. $c\beta_{4c}$ is truncated after exon 8 and thus lacks 90% of the GK domain and the entire COOH terminus (Fig. 6). As expected, $c\beta_{4c}$ barely affects $Ca_v2.1$ channels coexpressed with $\alpha_2\delta$ in *Xenopus* oocytes. However, $c\beta_{4c}$ interacts directly with the scaffolding domain of heterochromatin protein 1 (HP1), a nuclear protein involved in gene silencing and transcriptional regulation. Both proteins are colocalized in the nuclei of cochlear hair cells, and their coexpression in tsA201 cells causes translocation of $c\beta_{4c}$ from the cytoplasm to the nucleus. Moreover, $c\beta_{4c}$ attenuates the repressor function of HP1 in a dose-response manner. The effects on HP1 are specific since a longer isoform, β_{4a}, has no effect. These findings suggest that $c\beta_{4c}$ may function as a transcription regulator.

In a recent study, Zhang et al. (504) reported that full-length β_3 could directly interact with a new splicing isoform of Pax6, a transcription factor critical for the development of the eye and nervous system. The new isoform, named Pax6(S), has a truncated COOH terminus with a unique serine-rich tail. The interaction between Pax6(S) and β_3 is conferred mainly by the S tail and the SH3-HOOK-GK module of β_3. Since the other three subtypes of $Ca_v\beta$ can also interact with Pax6(S), the binding site for the S tail likely resides in the conserved SH3 or GK domain. Coexpression of Pax6(S) with $Ca_v2.1$ channels containing β_3 in *Xenopus* oocytes does not alter channel properties; however, the in vitro transcriptional activity of Pax6(S) is markedly suppressed by β_3. Furthermore, co-expression of β_3 and Pax6(S) in HEK 293T cells results in the translocation of β_3 from the cytoplasm to the nucleus. These results suggest that full-length $Ca_v\beta$s may function as transcription regulators (504).

This notion is further supported by other recent studies (16, 427), which show that, upon neuronal differentiation, full-length β_{4a} physically interacts with B56δ, a nuclear regulatory subunit of phosphatase 2A (PP2A). The β_{4a}/B56δ complex relocates to the nucleus, where it associates with nucleosomes and regulates the dephosphorylation of histones, a key mechanism in transcriptional regulation. A mutant β_4 lacking the last 38 COOH-terminal residues and associated with a case of juvenile myoclonic epilepsy (142), can neither associate with B56δ nor translocate to the nucleus, and its in vitro transcriptional regulation activity is different from that of WT β_4 (16, 427). Formation of the β_{4a}/B56δ complex requires an intact intramolecular SH3-GK interaction.

Consistent with the notion that full-length $Ca_v\beta$s may function as transcription regulators, it has been shown that full-length $Ca_v\beta$s can be targeted to the nucleus in native cells. For example, β_4, and to a lesser extent, β_{1b} and β_3, are translocated into the nucleus when they are exogenously expressed in cardiac cells (93). A recent study reports that endogenous β_4 is present in the nuclei of cerebellar granule cells and Purkinje cells (425). When heterologously expressed in skeletal myotubes or cultured hippocampal neurons, β_{4b} is robustly targeted to the nucleus, whereas other $Ca_v\beta$s are not. Nuclear localization of β_{4b} is dependent on an Arg-Arg-Ser motif in the NH_2 terminus, which is necessary since deleting this motif decreases nuclear targeting of β_{4b}. This motif is also sufficient since fusing it to β_{4a} increases nuclear targeting of the resulting chimera. Importantly, nuclear targeting of β_{4b} is diminished upon increased electrical activity and Ca^{2+} influx through L-type Ca^{2+} channels, suggesting a potential physiological function (425).

Another possible VGCC-independent function for β_4 is demonstrated by a recent study in zebrafish (135), which express all four subtypes of $Ca_v\beta$s (506). Morpholino knockdown of zebrafish β_4 abolishes or retards epiboly, an early development process, due to disturbances in mitotic and postmitotic cytoskeletal rearrangements. Epiboly can be rescued by coinjecting full-length human β_{4a} or β_{4b} cRNA. Interestingly, epiboly can also be rescued upon coinjection of a mutant β_{4a}, which contains a triple mutation (M204A/L208A/L350A) in its AID-binding pocket and cannot bind $Ca_v\alpha_1$ or enhance Ca^{2+} channel currents in *Xenopus* oocytes. These results suggest an involvement of β_4 in zebrafish early development, probably through VGCC-independent actions. It remains to be determined how β_4 is involved and whether this function is shared by other $Ca_v\beta$s.

In a study using β_3 knockout mice, high glucose conditions caused pancreatic β cells to produce twofold more insulin than their WT counterparts (31). No change in VGCC currents was detected, but the β_3-deficient cells exhibited a higher frequency of glucose-induced intracellular $[Ca^{2+}]$ oscillations, accompanied by increased IP_3 production and increased Ca^{2+} release from intracellular stores. On the basis of the colocalization of these proteins in pancreatic β cells, it was hypothesized that β_3 may directly interact with IP_3 receptors to cause some of these effects (31).

In snails (*Lymnaea*), the expression of the sole $Ca_v\beta$ ($LCa_v\beta$) is temporally and spatially uncoupled from the expression of LCa_v2, a *Lymnaea* homolog of the mammalian Ca_v2 family of VGCCs (409). Functionally, $LCa_v\beta$ does not modulate the surface expression or gating of LCa_v2 when they are coexpressed in tsA201 cells, even though they show current upregulation and gating modulation when they are partnered with rat $Ca_v\alpha_1$ and $Ca_v\beta$, respectively (409). Furthermore, knockdown of LCa_v2, but not $LCa_v\beta$, alters neurite morphology. These results suggest that $LCa_v\beta$ may have VGCC-independent functions, which remain to be elucidated. This work illustrates that studies in simple model organisms might be beneficial to our understanding of the full spectrum of $Ca_v\beta$ functions.

XIII. $Ca_v\beta$ KNOCKOUTS AND PATHOPHYSIOLOGY

As expected, because of the essential role of $Ca_v\beta$ in the surface expression and functional modulation of HVA Ca^{2+} channels, $Ca_v\beta$ knockouts or mutations can produce severe functional deficits and, in some cases, are lethal. The phenotype of $Ca_v\beta$ knockout mice depends on the ability of the remaining three $Ca_v\beta$ genes to compensate. This section discusses the phenotypes and pathophysiology of $Ca_v\beta$ knockouts and mutations.

A. Gene Knockouts and Mutants

1. β*1*—As mentioned in section XI*A*, β_{1a} is irreplaceable in partnering with $Ca_v1.1$ channels to enable skeletal muscle EC coupling. Thus β_1 knockout mice, similar to $Ca_v1.1$ knockouts, are born motionless and die immediately from asphyxiation (191). Skeletal muscles isolated from β_1-null mice are twitchless upon electrical stimulation, and action potentials do not elicit Ca^{2+} transients. L-type Ca^{2+} channel currents and the surface expression of $Ca_v1.1$ subunits are much reduced in these muscles, but caffeine can still cause contractions, indicating that internal Ca^{2+} stores are intact (191). Transgenic expression of β_{1a} exclusively in the skeletal muscle of β_1-null mice rescues the mice, which exhibit no obvious phenotype, suggesting that the remaining $Ca_v\beta$ genes can compensate for the functions of β_1 in other tissues (14).

Zebrafish β_1 knockouts also exist (393, 394). They have the *Relaxed* phenotype and die paralyzed days after hatching, with completely deficient EC coupling. Skeletal muscles from β_1-null zebrafish have no tetrads and show reduced depolarization-induced Ca^{2+} transients, but exhibit normal caffeine-induced Ca^{2+} transients (394). Unlike in β_1-null mice, targeting of $Ca_v1.1$ subunits to t tubules and the formation of *triads* are preserved (394), suggesting a nonessential role of β_1 in these processes in zebrafish. It remains to be determined whether other $Ca_v\beta$s are expressed in β_1-null zebrafish skeletal muscles or whether zebrafish $Ca_v1.1$ is able to traffic to the plasma membrane on its own.

2. β*2*—Several β_2 splice variants are the predominant $Ca_v\beta$s expressed in the heart (Table 1). Thus it is no surprise that β_2 knockouts have no cardiac contractions and are nonviable beyond embryonic day 10.5 (14, 468). This is due to diminished L-type Ca^{2+} channel currents in cardiomyocytes and cardiac failure-associated defective remodeling of blood vessels. The β_2-null phenotype can be rescued by the expression of β_2 under a cardiac muscle-specific promoter (14). These partial knockouts revealed an essential role of β_2 in tissues besides the heart: such mice (lacking β_2 in all but cardiac tissues) are deaf due to a dramatic reduction in the membrane expression of $Ca_v1.3$ channels in inner hair cells, coupled with decreased exocytosis, improper hair cell development, and defective cochlear amplification (331). These "rescued" mice also have defects in vision with a phenotype similar to human patients with congenital stationary night blindness (13).

Given the knockout results, genetic mutations in β_1 and β_2 are expected to affect mainly skeletal and cardiac muscles, respectively. While no β_1 mutations have been associated with genetic diseases thus far, β_2 mutations have. Thus a mutation in the COOH terminus of β_{2b} (CACNB2b), S481L, contributes to a type of sudden death syndrome characterized by a short QT interval and an elevated ST segment (8), which are categorized into a group of genetic heart diseases called the Brugada syndrome. This mutation decreases $Ca_v1.2$ currents by ~75% in an expression system (CHO-K1 cells). Another mutation, in the β_{2b} NH_2 terminus (T11I), causes accelerated inactivation of cardiac L-type channels and is also linked to the Brugada syndrome (97). This mutation occurs in exon 2C of the CACNB2 gene and only affects β_{2b}, the most abundant $Ca_v\beta$ isoform in the heart (93). A recent study suggests that variations in CACNB2 may be also associated with a heightened risk for Alzheimer's disease (286).

3. β_3—β_3 Knockouts are viable and were initially found to be normal (328, 330). Later studies, however, uncovered a wide spectrum of abnormalities, especially under stress conditions. For example, at high glucose concentrations, the frequency of $[Ca^{2+}]_i$ oscillations and the resulting insulin secretion from pancreatic β cells are potentiated (31). This is likely due to the attenuation of β_3-mediated inhibition of IP_3 production (31, 339). Also, a high-salt diet causes elevation of blood pressure, reduction in plasma catecholamine levels, and a hypertrophy of heart and aortic smooth muscle (328,). The latter effects, as well as observations from mice overexpressing β_3 (327), are consistent with a function of β_3 in sympathetic control. In this regard, β_3 knockouts resemble N-type channel ($Ca_v2.2$) knockouts (428,), which is not surprising since N-type channels preferably partner with β_3 (294, 325–327, 395). Indeed, sympathetic neurons from β_3-null mice have reduced N- and L-type channels activity (330). N-type current is also decreased in dorsal root neurons, which dampens inflammatory pain, but not mechanical or thermal pain (325). In the brain, N-type channel expression is reduced by ~40% (326); in the hippocampus, expression of NR2B (an NMDA receptor subunit), NMDA receptor currents, and long-term potentiation are all increased (239). Some forms of hippocampus-dependent learning and memory appear to be enhanced, but working memory is impaired (239,). Furthermore, pruning of visual retinocollicular pathways is developmentally reduced and delayed (98). Behaviorally, β_3-null mice have lower anxiety, increased aggression, and increased night-time activity (326). Finally, β_3-null mice show abnormal signaling in CD4 T-cells, where receptor-mediated Ca^{2+} responses, nuclear translocation of NFAT, and cytokine production are all attenuated (11).

4. β_4—Mutated β_4 was first reported in *lethargic* mice (55, 130, 131). The naturally occurring null mutation is a four nucleotide insertion in *Cacnb4*, causing a translational frame shift and a premature stop codon. *Lethargic* mice have ataxia, seizures, absence epilepsy, and paroxysmal dyskinesia (17, 55, 227). The abnormal phenotype appears after postnatal day 15, a time when WT animals have an increase in β_4 expression in the brain (311). The upregulation of β_4 in WT mice is particularly robust in cerebellar granule and Purkinje neurons, which likely explains the ataxia in null mice (55). T-type Ca^{2+} channel upregulation (by ~50%) in thalamic neurons of *lethargic* mice likely contributes to the seizures (503). It is not clear why the remaining $Ca_v\beta$s fail to compensate for the lack of β_4, but this could be partly because of the unique interactions between the NH_2 and COOH termini of β_4 with other proteins (for examples, see Refs. 51, 121, 420, 460, 461, 474). Nevertheless, in *lethargic* mice, there is increased pairing of $Ca_v2.2$ and $Ca_v2.1$ with other $Ca_v\beta$s; in particular, both β_{1b} and $Ca_v2.2/\beta_{1b}$ complexes are upregulated, similar to what is found in the developing brain (310, 311). Some other characteristics of *lethargic* mice include lower N-type channel expression in the forebrain and cerebellum (310), reduced excitatory neurotransmission in the thalamus (57), a modified electro-oculogram (301), splenic and thymic involution (130, 131), and renal cysts (130). Similar to β_3 knockouts, $CD4^+$ T-cells have attenuated receptor-mediated Ca^{2+} responses, nuclear translocation of NFAT, and cytokine production (11).

Since β_4 is the predominant partner for P/Q-type ($Ca_v2.1$) channels in brain (311), it is no surprise that *tottering* mice (161), with mutations in $Ca_v2.1$, have a phenotype very similar to *lethargic* mice (356). Both *tottering* and *lethargic* mice are also models for epilepsy (17, 226). Indeed, there are examples where mutations in β_4 precipitate epilepsy and ataxia in humans. In one case, an R468Q mutation in CACNB4, which enhances $Ca_v2.1$ current, was associated with a history of febrile seizures (338). In another, truncated β_4 (R482x) that only has a very minor effect on HVA Ca^{2+} channel properties was found in a juvenile myoclonic epilepsy patient (142). In yet another case, a mutation in the SH3 domain of β_4 (C104F) causes different symptoms in two different families: episodic ataxia in one and generalized epilepsy and praxis-induced seizures in the other, presumably as a result of different genetic backgrounds (142).

B. $Ca_v\beta$ in Pathophysiology

Changes in the expression level of various $Ca_v\beta$s have been reported in certain pathological conditions. For example, in hypertrophic obstructive cardiomyopathy, β_2 is upregulated, which likely drives the observed increase in Ca^{2+} channels (198, 465). Downregulation of $Ca_v\beta$ is observed in allografts from diastolically failing hearts (228), in pancreatic islets from type 2 diabetic rats (235), and during atrial fibrillation (188). However, these observations are only correlative, and it remains unclear whether these changes are causative or incidental to the disease.

In the Lambert-Eaton myasthenic syndrome (LEMS), an autoimmune disease, autoantibodies against the extracellular loops of presynaptic Ca^{2+} channels disrupt channel arrays at the neuromuscular junction and impair synaptic transmission (269, 299). However, antibodies against β_3 and β_4 are also common in sera from LEMS patients (55% of the time), including in five of five LEMS patients who also had small-cell lung carcinoma (368). In some instances, the $Ca_v\beta$ autoantibodies can prevent $Ca_v\alpha_1$-$Ca_v\beta$ binding (368, 377). It is unclear, however, how $Ca_v\beta$ autoantibodies contribute to the disease, since $Ca_v\beta$ is an intracellular protein and is unlikely to be a target of the $Ca_v\beta$ autoantibodies in the intact muscle. Indeed, immunization of rats with a purified $Ca_v\beta$ protein causes no neuropathy in spite of the induction of high antibody titers (453). These observations and considerations suggest that $Ca_v\beta$ autoantibodies do not directly contribute to the pathology of the disease, but their presence can serve as an additional diagnostic tool (368).

Finally, schistosomiasis, or bilharzia, is a parasitic disease caused by *Schistosoma* flatworms, which infect ~200 million people in the developing world, damaging the nervous system and internal organs. It is relatively successfully cured with Praziquantel (PZQ). The exact mechanism of action is still unclear, but PZQ seems to target a variant of *Schistostoma* $Ca_v\beta$ (reviewed in Refs. 124, 190, 240). *Schistosomas* have one “conventional” $Ca_v\beta$ and one, named β_{var}, with a long COOH terminus and nonconserved changes in both the SH3 and GK domains (240, 263,264, 334). When expressed with a mammalian $Ca_v\alpha_1$, β_{var} modulates gating as expected for a $Ca_v\beta$, but it causes a decrease in current amplitude (263). PZQ recovers current amplitude (263), consistent with results showing that PZQ causes a Ca^{2+} influx into worms, followed by a sustained muscular contraction and paralysis (240). It is not clear, however, whether β_{var} associates with *Schistosoma* $Ca_v\alpha_1$ since expressing them has been difficult (190). A new study shows that $Ca_v\beta$ knockdown in *Schistosomas*, using siRNA, confers resistance to PZQ, further implicating $Ca_v\beta$ (334). Thus PZQ likely targets

Schistosoma $Ca_v\beta$, but the downstream events remain to be elucidated (124, 190). They may involve an increase in Ca^{2+} channel currents but may also include other pathways. Recently, it was suggested that Ca^{2+} influx on its own is not sufficient to kill *Schistosomas*, because cytochalasin D, an inhibitor of actin polymerization, can rescue *Schistosomas* from PZQ, in spite of cellular Ca^{2+} overload (354).

XIV. PERSPECTIVES

Great strides have been made in the last two decades in our understanding of the molecular biology, structure, function, and regulation of $Ca_v\beta$. An emerging theme is that $Ca_v\beta$ is a multifunction protein, acting primarily as a Ca^{2+} channel regulatory subunit but also performing Ca^{2+} channel-independent functions. Although much is known, many important questions and issues remain to be elucidated, some of which we highlight here.

1. Although it is well established that $Ca_v\beta$ is essential for the surface expression of HVA Ca^{2+} channels, it is yet unclear why $Ca_v\beta$ is required. The traditional view is that $Ca_v\beta$ facilitates the export of $Ca_v\alpha_1$ from the ER. However, no definitive ER retention signals have been found on $Ca_v\alpha_1$ that are blocked by the binding of $Ca_v\beta$. An alternative possibility is that $Ca_v\alpha_1$ can traffic to the plasma membrane on its own, but its continued presence there requires $Ca_v\beta$. Further studies on the role of $Ca_v\beta$ in $Ca_v\alpha_1$ internalization, ubiquitination, and proteasomal degradation may shed light on this issue.
2. Given that many isoforms of $Ca_v\beta$ exist, that the association between $Ca_v\beta$ and $Ca_v\alpha_1$ is promiscuous, and that $Ca_v\beta$ regulates channel gating in a $Ca_v\alpha_1$-$Ca_v\beta$ pair-specific manner, there is enormous combinatorial complexity. Furthermore, the reversible nature of $Ca_v\alpha_1$-$Ca_v\beta$ association provides a means for dynamic regulation of HVA Ca^{2+} channel activity. Thus, to better understand the function and regulation of HVA Ca^{2+} channels in native cells, it is necessary to examine the spatial and temporal expression of different $Ca_v\beta$ isoforms, not only at cellular levels but also at subcellular levels, as exemplified by the work in neurons (317, 336). Currently, we know very little about the molecular mechanisms governing the splicing and expression of $Ca_v\beta$ in different tissues, cell types, and subcellular locations (e.g., soma vs. dendrites vs. axon terminals).
3. Although the high-affinity interaction between the $Ca_v\beta$ GK domain and the AID is essential for $Ca_v\beta$ regulation of HVA Ca^{2+} channel gating, it is the interactions among other regions of $Ca_v\beta$ and $Ca_v\alpha_1$ that confer distinct $Ca_v\alpha_1$-$Ca_v\beta$ pair-specific characteristics to $Ca_v\beta$ regulation. We do not yet have a full grasp of the molecular determinants involved in these interactions, owing to their intrinsically low affinity. Characterizing these low-affinity interactions will remain a difficult challenge, as conventional biochemical approaches may not be sufficient to uncover the underlying molecular components and mechanisms.
4. Useful knowledge has been gained from $Ca_v\beta$ knockouts; however, lethal phenotypes and/or compensation by other $Ca_v\beta$s have limited the amount of information gleaned from systemic knockouts. It would be desirable to achieve inducible and tissue-specific knockout, knockdown, or overexpression of a

particular $Ca_v\beta$. A recent study (90) suggests that such approaches may even become useful venues for gene therapy of certain forms of cardiovascular or neurological disorders.

5. The list of $Ca_v\beta$-interacting proteins continues to grow, but in most cases, the physiological importance of their interactions with $Ca_v\beta$ in native cells remains unclear. Some VGCC-independent functions of $Ca_v\beta$ are presented in this review, but almost certainly more are to be discovered. In this regard, it would be particularly interesting to investigate whether, and under what conditions, full-length $Ca_v\beta$s participate in regulating gene expression in native cells.
6. To better understand the function of $Ca_v\beta$, it would be valuable to obtain high-resolution structures of full-length $Ca_v\beta$s, by themselves and in complex with their various interacting partners. These are clearly long-term goals, and there will undoubtedly be technical challenges in such endeavors, but the successful determination of the crystal structure of three different $Ca_v\beta$ cores' and two different AID-$Ca_v\beta$ core complexes warrants optimism.

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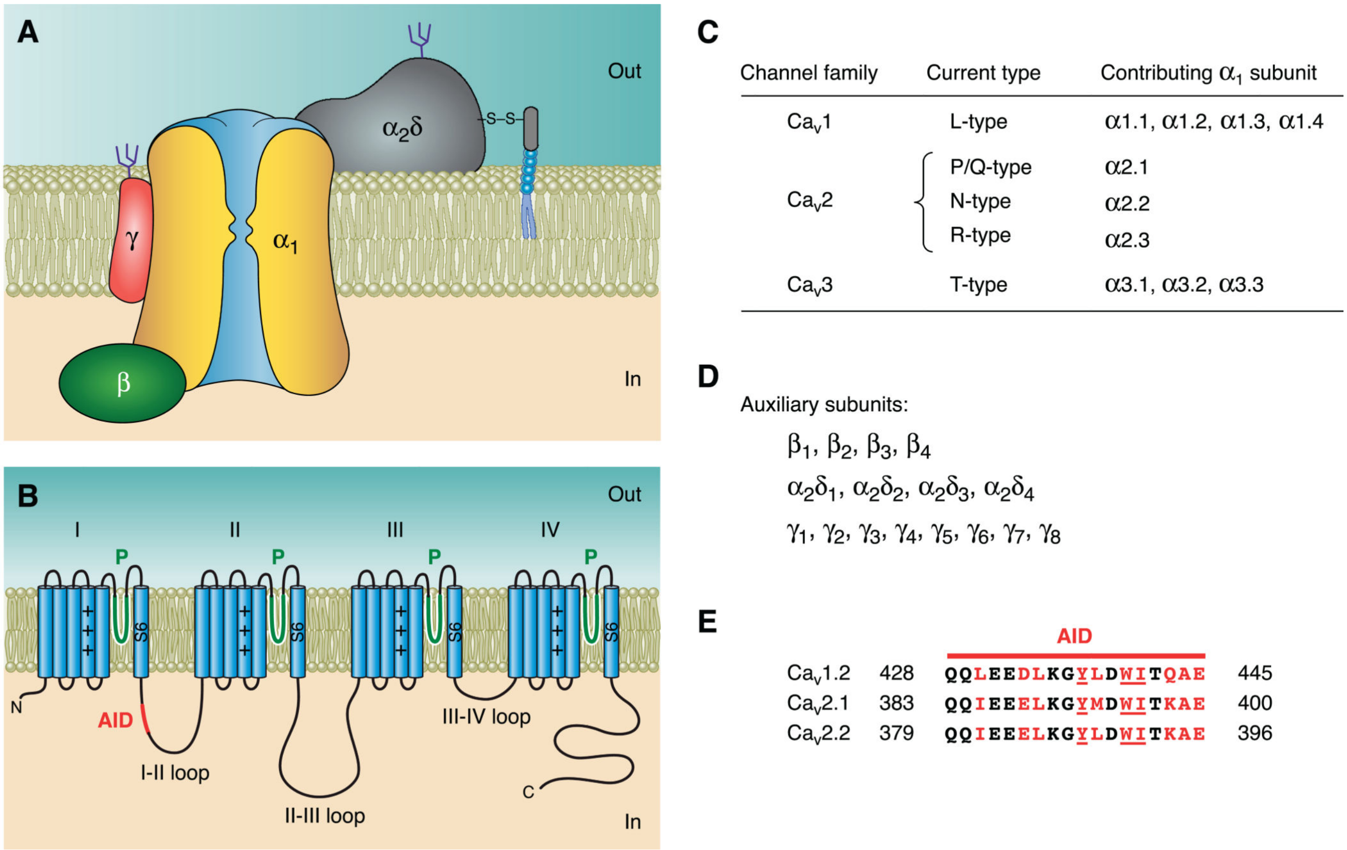


Channel family	Current type	Contributing α_1 subunit
Ca_v1	L-type	$\alpha1.1$, $\alpha1.2$, $\alpha1.3$, $\alpha1.4$
Ca_v2	P/Q-type	$\alpha2.1$
	N-type	$\alpha2.2$
	R-type	$\alpha2.3$
Ca_v3	T-type	$\alpha3.1$, $\alpha3.2$, $\alpha3.3$

Auxiliary subunits:

β_1, β_2, β_3, β_4

$\alpha_2\delta_1$, $\alpha_2\delta_2$, $\alpha_2\delta_3$, $\alpha_2\delta_4$

γ_1, γ_2, γ_3, γ_4, γ_5, γ_6, γ_7, γ_8

		AID	
$Ca_v1.2$	428	QQLEEDLKGYLDWITQAE	445
$Ca_v2.1$	383	QQIEEELKGYMDWITKAE	400
$Ca_v2.2$	379	QQIEEELKGYLDWITKAE	396

Fig. 1.

Molecular organization of voltage-gated Ca^{2+} channels. *A*: subunit composition of high-voltage activated (HVA) Ca^{2+} channels. *B*: schematic representation of the predicted transmembrane topology of $Ca_v\alpha_1$, with the location of the α-interaction domain (AID) marked. *C*: Ca^{2+} channel current types and the corresponding α_1 subunits of the channels that produce them. *D*: list of all cloned auxiliary HVA Ca^{2+} channel subunits. *E*: amino acid sequence alignment of the AID from the indicated $Ca_v\alpha_1$. 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.

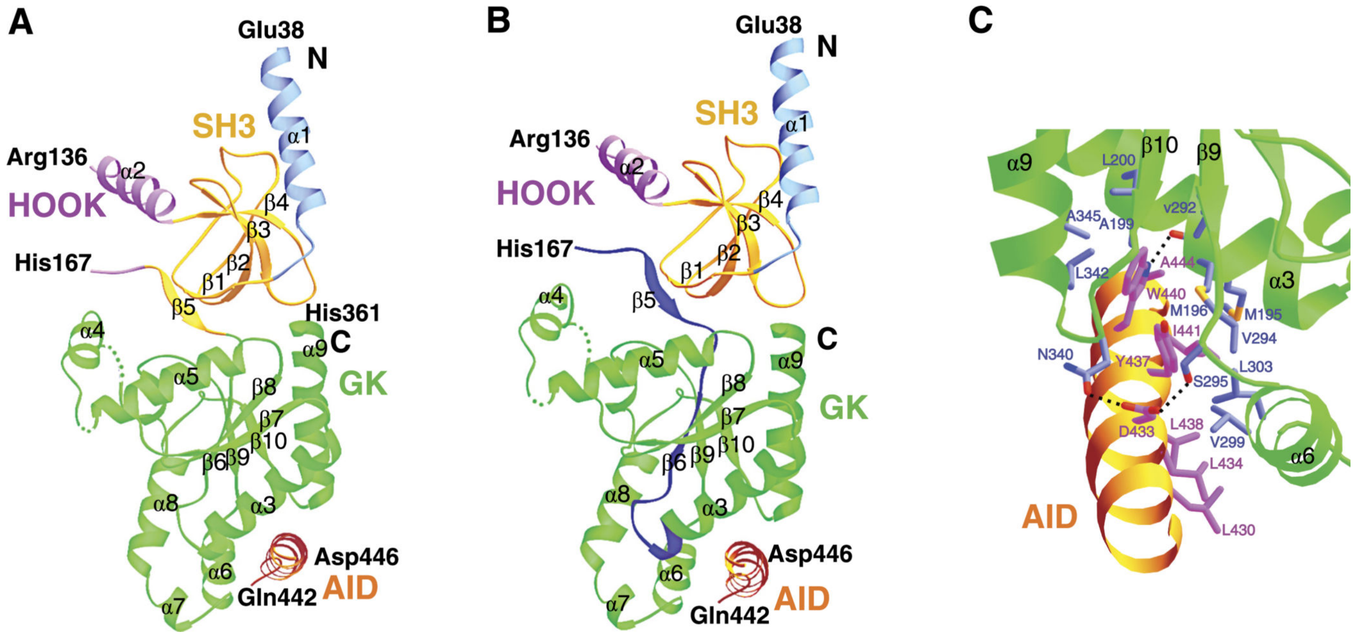
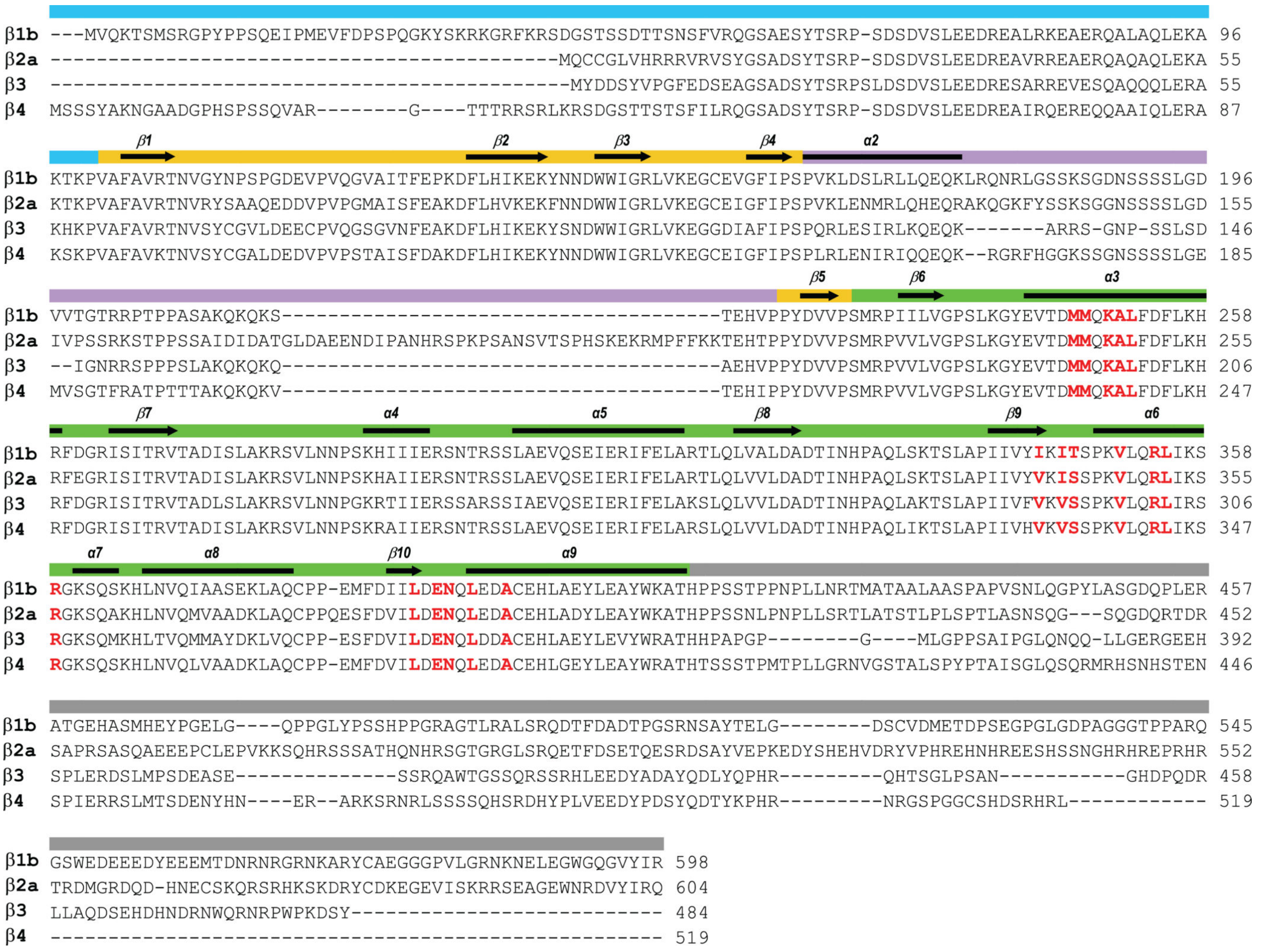


Fig. 2.

$Ca_V\beta$ crystal structure. *A*: crystal structure of the β_3 core in complex with the AID (PDB accession code 1VYT). This structure reveals the following regions: the NH_2 terminus (light blue, residues 38–59), an SH3 domain (gold, residues 60–120 and 170–175), a HOOK region (purple, residues 121–169), and a GK domain (green, residues 176–360). Residues 137–166 were disordered and are not included. Residues 226–244 (forming the α_4 helix of the GK domain) were disordered in this molecule but were well-resolved in another one in the same asymmetric unit. Residues 422–446 of $Ca_V1.2$ containing the entire AID are colored in orange. *B*: same structure as in *A* but with the BID (β_3 residues K163-T193) highlighted in dark blue. The BID spans parts of the SH3-HOOK-GK motif but is not directly involved in binding the AID. *C*: close-up of the interface between β_3 and AID. Some residues involved in the interactions are shown. [Adapted from Chen et al. (84)].



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β2a --------------------------------------------MQCCGLVHRRRVRVSYGSADSYTSRP-SDSDVSLEEDREAVRREAERQAQAQLEKA 55
β3  ---------------------------------------------MYDDSYVPGFEDSEAGSADSYTSRPSLDSDVSLEEDRESARREVESQAQQQLERA 55
β4  MSSSYAKNGAADGPHSPSSQVAR--------G----TTTRRSRLKRSDGSTTSTSFILRQGSADSYTSRP-SDSDVSLEEDREAIRQEREQQAAIQLERA 87

β1b KTKPVAFAVRTNVGYNPSPGDEVPVQGVAITFEPKDFLHIKEKYNNDWWIGRLVKEGCEVGFIPSPVKLDSLRLLQEQKLRQNRLGSSKSGDNSSSSLGD 196
β2a KTKPVAFAVRTNVRYSAAQEDDVPVPGMAISFEAKDFLHVKEKFNNDWWIGRLVKEGCEIGFIPSPVKLENMRLQHEQRAKQGKFYSSKSGGNSSSSLGD 155
β3  KHKPVAFAVRTNVSYCGVLDEECPVQGSGVNFEAKDFLHIKEKYSNDWWIGRLVKEGGDIAFIPSPQRLESIRLKQEQK-------ARRS-GNP-SSLSD 146
β4  KSKPVAFAVKTNVSYCGALDEDVPVPSTAISFDAKDFLHIKEKYNNDWWIGRLVKEGCEIGFIPSPLRLENIRIQQEQK--RGRFHGGKSSGNSSSSLGE 185

β1b VVTGTRRPTPPASAKQKQKS--------------------------------------TEHVPPYDVVPSMRPIILVGPSLKGYEVTDMMQKALFDFLKH 258
β2a IVPSSRKSTPPSSAIDIDATGLDAEENDIPANHRSPKPSANSVTSPHSKEKRMPFFKKTEHTPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKH 255
β3  --IGNRRSPPPSLAKQKQKQ--------------------------------------AEHVPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKH 206
β4  MVSGTFRATPTTTAKQKQKV--------------------------------------TEHIPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKH 247

β1b RFDGRISITRVTADISLAKRSVLNNPSKHIIIERSNTRSSLAEVQSEIERIFELARTLQLVALDADTINHPAQLSKTSLAPIIVYIKITSPKVLQRLIKS 358
β2a RFEGRISITRVTADISLAKRSVLNNPSKHAIIERSNTRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKS 355
β3  RFDGRISITRVTADLSLAKRSVLNNPGKRTIIERSSARSSIAEVQSEIERIFELAKSLQLVVLDADTINHPAQLAKTSLAPIIVFVKVSSPKVLQRLIRS 306
β4  RFDGRISITRVTADISLAKRSVLNNPSKRAIIERSNTRSSLAEVQSEIERIFELARSLQLVVLDADTINHPAQLIKTSLAPIIVHVKVSSPKVLQRLIKS 347

β1b RGKSQSKHLNVQIAASEKLAQCPP-EMFDIILDENQLEDACEHLAEYLEAYWKATHPPSSTPPNPLLNRTMATAALAASPAPVSNLQGPYLASGDQPLER 457
β2a RGKSQAKHLNVQMVAADKLAQCPPQESFDVILDENQLEDACEHLADYLEAYWKATHPPSSNLPNPLLSRTLATSTLPLSPTLASNSQG---SQGDQRTDR 452
β3  RGKSQMKHLTVQMMAYDKLVQCPP-ESFDVILDENQLDDACEHLAEYLEVYWRATHHPAPGP--------G----MLGPPSAIPGLQNQQ-LLGERGEEH 392
β4  RGKSQSKHLNVQLVAADKLAQCPP-EMFDVILDENQLEDACEHLGEYLEAYWRATHTSSSTPMTPLLGRNVGSTALSPYPTAISGLQSQRMRHSNHSTEN 446

β1b ATGEHASMHEYPGELG----QPPGLYPSSHPPGRAGTLRALSRQDTFDADTPGSRNSAYTELG--------DSCVDMETDPSEGPGLGDPAGGGTPPARQ 545
β2a SAPRSASQAEEEPCLEPVKKSQHRSSSATHQNHRSGTGRGLSRQETFDSETQESRDSAYVEPKEDYSHEHVDRYVPHREHNHREESHSSNGHRHREPRHR 552
β3  SPLERDSLMPSDEASE--------------SSRQAWTGSSQRSSRHLEEDYADAYQDLYQPHR---------QHTSGLPSAN-----------GHDPQDR 458
β4  SPIERRSLMTSDENYHN----ER--ARKSRNRLSSSSQHSRDHYPLVEEDYPDSYQDTYKPHR---------NRGSPGGCSHDSRHRL------------ 519

β1b GSWEDEEEDYEEEMTDNRNRGRNKARYCAEGGGPVLGRNKNELEGWGQGVYIR 598
β2a TRDMGRDQD-HNECSKQRSRHKSKDRYCDKEGEVISKRRSEAGEWNRDVYIRQ 604
β3  LLAQDSEHDHNDRNWQRNRPWPKDSY--------------------------- 484
β4  ----------------------------------------------------- 519
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Fig. 3.

Amino acid sequence alignment of $Ca_v\beta$ subtypes. The four included subtypes are β_{1b} (GenBank accession number, NP-000714), β_{2a} (M80545), β_3 (M88751), and β_{4a} (L02315). Light blue indicates the NH_2 terminus, gold the SH3 domain, purple the HOOK region, green the GK domain, and gray the COOH terminus. Secondary structure elements are indicated in the top line as arrows for β sheets and solid lines for α helices (based on the crystal structure of β_3). Residues involved in interactions with the AID are marked in red.

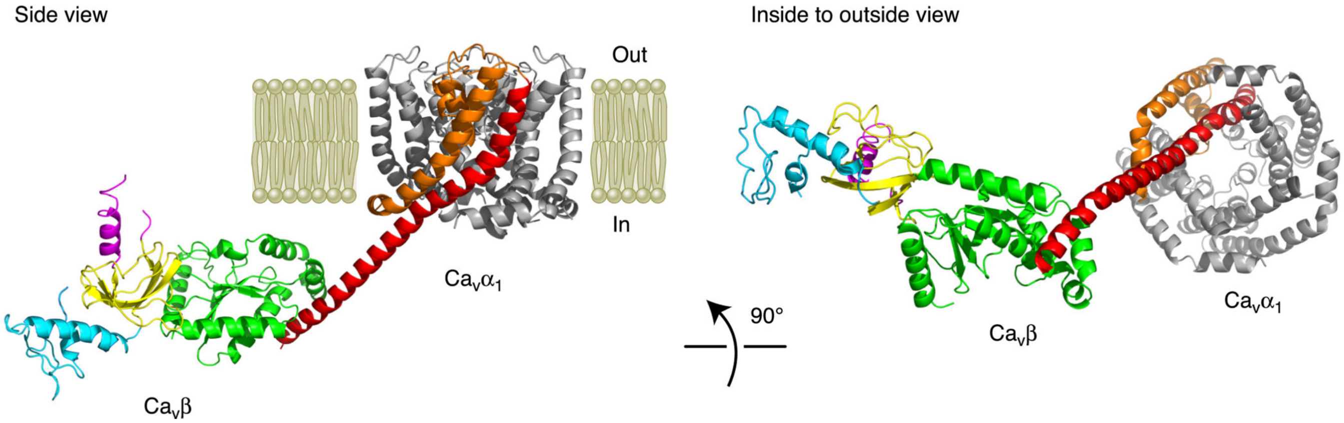


Fig. 4.

Structural model of a partial $Ca_v\alpha_1$/$Ca_v\beta$ complex on the plasma membrane. A side view and an inside-to-outside view are presented. The partial structure of $Ca_v\alpha_1$ includes only the S5, P-loop, and S6 segments and is based on a $Ca_v\alpha_1$ homology model developed in Stary et al. (411). IS5 is colored orange, and IS6 is red. The IS6-AID linker from Ca_v1.2 is modeled as an α-helix and is joined with IS6 at its NH_2 terminus and the AID at its COOH terminus. The structure of $Ca_v\beta$ is based on the crystal structure of the β_4 core region (84) and the NMR structure of the β_4 NH_2 terminus (451); there is no $Ca_v\beta$ COOH terminus. Since the structure of the β_4-AID complex is not available, we docked the AID to β_4 based on the crystal structure of the β_3 core-AID complex (84). The regions of $Ca_v\beta$ are color coded as in Figures 2 and 3 (NH_2 terminus in light blue, SH3 in gold, HOOK in purple, and GK in green).

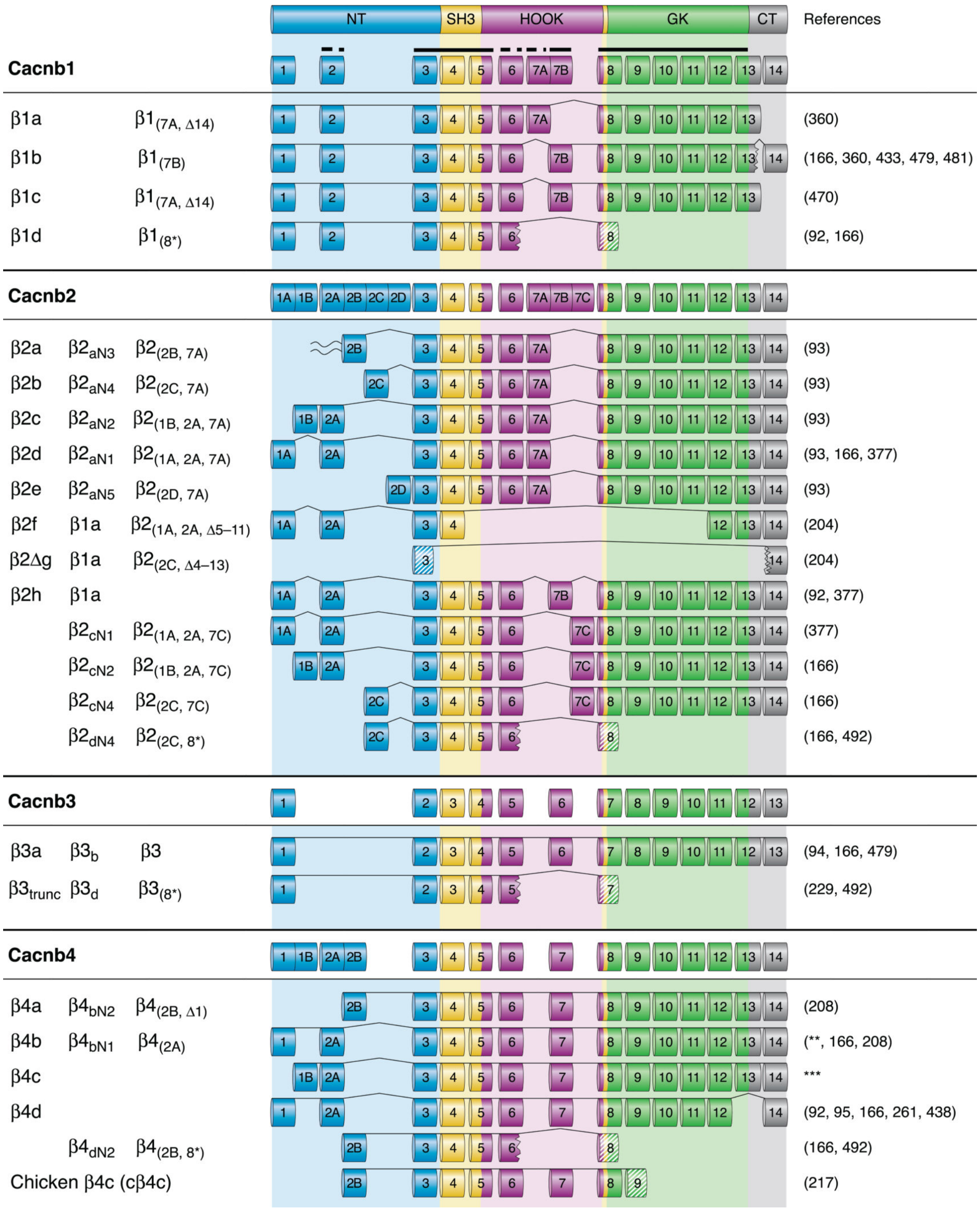
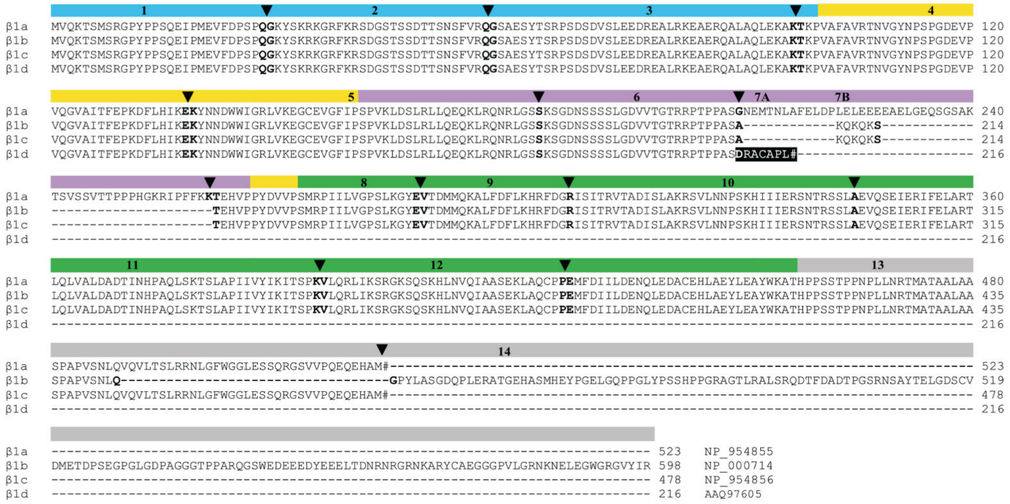


Fig. 5.

Human $Ca_v\beta$ splice variants. Fourteen $Ca_v\beta$ exons (13 for β_3) are color-coded based on the regions they give rise to: the NH_2 terminus (light blue), the SH3 domain (gold), the HOOK (purple), the GK domain (green), and the COOH terminus (gray). Exons are numbered, and some exons have additional letters to indicate alternatively spliced variants. The thick full and dashed lines at the very top indicate highly or somewhat conserved exons, respectively. Of the weakly conserved regions, similar exons are placed in the same column (e.g., β_1 exon 2 is homologous to β_2 exon 2A). Exons 13 and 14 of β_1 were originally designated as 13a and 13b, respectively (222). The names of splice variants are, from left to right columns, those used in this article, those proposed by Foell et al. (166), and those proposed by Yang and Berggren (486). β_{2a} is the only splice variant that can be palmitoylated (wave). The jagged edge (e.g., exon 6 of β_{1d}) indicates missing amino acids resulting from exon skipping and/or frame-shifts. Striped exons (e.g., exon 8 of β_{1d}) are translated with a frame shift; hence, their amino acid sequence is unrelated to the “conventional” sequence produced by that exon. **Direct submission by M. E. Williams, 1997. ***AK316045; direct submission by T. Isogai and J. Yamamoto, 2008.

Ca$_v$β1



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β1c   MVQKTSMSRGPYPPSQEIPMEVFDPSPQGKYSKRKGRFKRSDGSTSSDTTSNSFVRQGSAESYTSRPSDSDVSLEEDREALRKEAERQALAQLEKAKTKPVAFAVRTNVGYNPSPGDEVP 120
β1d   MVQKTSMSRGPYPPSQEIPMEVFDPSPQGKYSKRKGRFKRSDGSTSSDTTSNSFVRQGSAESYTSRPSDSDVSLEEDREALRKEAERQALAQLEKAKTKPVAFAVRTNVGYNPSPGDEVP 120

β1a   VQGVAITFEPKDFLHIKEKYNNDWWIGRLVKEGCEVGFIPSPVKLDSLRLLQEQKLRQNRLGSSKSGDNSSSSLGDVVTGTRRPTPPASGNEMTNLAFELDPLELEEEEAELGEQSGSAK 240
β1b   VQGVAITFEPKDFLHIKEKYNNDWWIGRLVKEGCEVGFIPSPVKLDSLRLLQEQKLRQNRLGSSKSGDNSSSSLGDVVTGTRRPTPPASA------------KQKQKS------------ 214
β1c   VQGVAITFEPKDFLHIKEKYNNDWWIGRLVKEGCEVGFIPSPVKLDSLRLLQEQKLRQNRLGSSKSGDNSSSSLGDVVTGTRRPTPPASA------------KQKQKS------------ 214
β1d   VQGVAITFEPKDFLHIKEKYNNDWWIGRLVKEGCEVGFIPSPVKLDSLRLLQEQKLRQNRLGSSKSGDNSSSSLGDVVTGTRRPTPPASDRACAPL#----------------------- 216

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β1c   ---------------------TEHVPPYDVVPSMRPIILVGPSLKGYEVTDMMQKALFDFLKHRFDGRISITRVTADISLAKRSVLNNPSKHIIIERSNTRSSLAEVQSEIERIFELART 315
β1d   ------------------------------------------------------------------------------------------------------------------------ 216

β1a   LQLVALDADTINHPAQLSKTSLAPIIVYIKITSPKVLQRLIKSRGKSQSKHLNVQIAASEKLAQCPPEMFDIILDENQLEDACEHLAEYLEAYWKATHPPSSTPPNPLLNRTMATAALAA 480
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β1c   LQLVALDADTINHPAQLSKTSLAPIIVYIKITSPKVLQRLIKSRGKSQSKHLNVQIAASEKLAQCPPEMFDIILDENQLEDACEHLAEYLEAYWKATHPPSSTPPNPLLNRTMATAALAA 435
β1d   ------------------------------------------------------------------------------------------------------------------------ 216

β1a   SPAPVSNLQVQVLTSLRRNLGFWGGLESSQRGSVVPQEQEHAM#---------------------------------------------------------------------------- 523
β1b   SPAPVSNLQ-----------------------------------GPYLASGDQPLERATGEHASMHEYPGELGQPPGLYPSSHPPGRAGTLRALSRQDTFDADTPGSRNSAYTELGDSCV 519
β1c   SPAPVSNLQVQVLTSLRRNLGFWGGLESSQRGSVVPQEQEHAM#---------------------------------------------------------------------------- 478
β1d   ------------------------------------------------------------------------------------------------------------------------ 216

β1a   ------------------------------------------------------------------------------ 523   NP_954855
β1b   DMETDPSEGPGLGDPAGGGTPPARQGSWEDEEEDYEEELTDNRNRGRNKARYCAEGGGPVLGRNKNELEGWGRGVYIR 598   NP_000714
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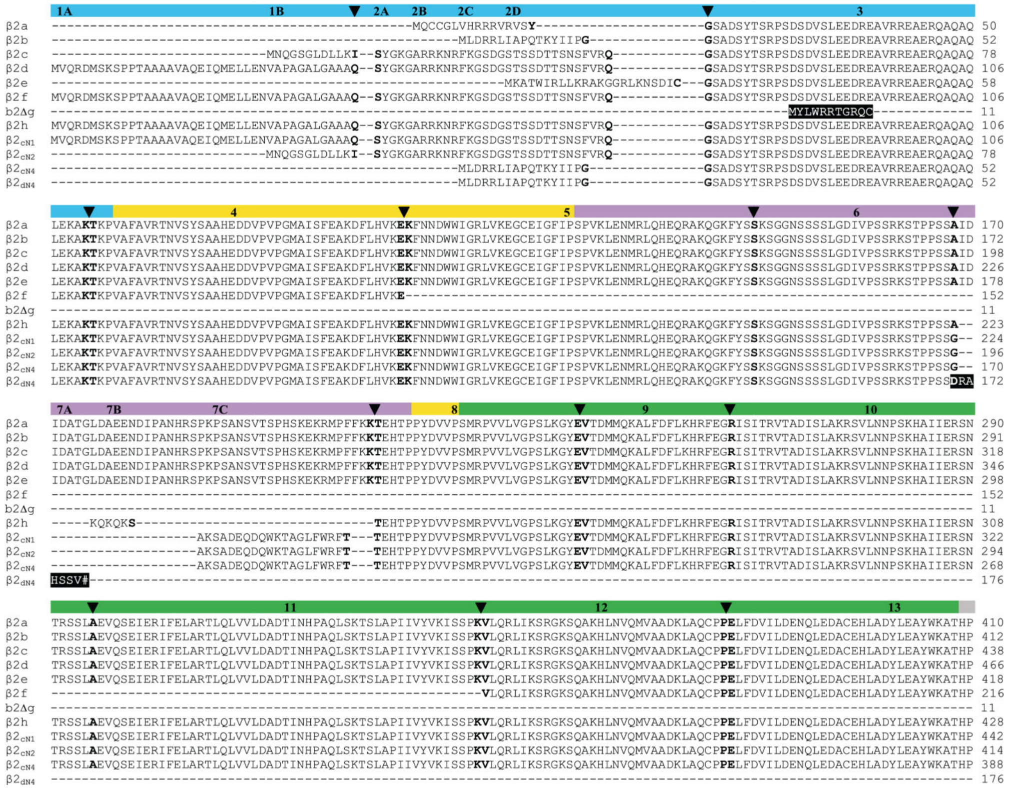
Ca$_v$β2



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β2c   ----------------------------MNQGSGLDLLKI--SYGKGARRKNRFKGSDGSTSSDTTSNSFVRQ------------GSADSYTSRPSDSDVSLEEDREAVRREAERQAQAQ 78
β2d   MVQRDMSKSPPTAAAAVAQEIQMELLENVAPAGALGAAAQ--SYGKGARRKNRFKGSDGSTSSDTTSNSFVRQ------------GSADSYTSRPSDSDVSLEEDREAVRREAERQAQAQ 106
β2e   -----------------------------------------------------------MKATWIRLLKRAKGGRLKNSDIC---GSADSYTSRPSDSDVSLEEDREAVRREAERQAQAQ 58
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b2Δg  ------------------------------------------------------------------------------------------------MYLWRRTGRQC------------- 11
β2h   MVQRDMSKSPPTAAAAVAQEIQMELLENVAPAGALGAAAQ--SYGKGARRKNRFKGSDGSTSSDTTSNSFVRQ------------GSADSYTSRPSDSDVSLEEDREAVRREAERQAQAQ 106
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β2cN2 ----------------------------MNQGSGLDLLKI--SYGKGARRKNRFKGSDGSTSSDTTSNSFVRQ------------GSADSYTSRPSDSDVSLEEDREAVRREAERQAQAQ 78
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β2dN4 -----------------------------------------------------MLDRRLIAPQTKYIIPG---------------GSADSYTSRPSDSDVSLEEDREAVRREAERQAQAQ 52

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β2f   LEKAKTKPVAFAVRTNVSYSAAHEDDVPVPGMAISFEAKDFLHVKE-------------------------------------------------------------------------- 152
b2Δg  ------------------------------------------------------------------------------------------------------------------------ 11
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β2c   IDATGLDAEENDIPANHRSPKPSANSVTSPHSKEKRMPFFKKTEHTPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKHRFEGRISITRVTADISLAKRSVLNNPSKHAIIERSN 318
β2d   IDATGLDAEENDIPANHRSPKPSANSVTSPHSKEKRMPFFKKTEHTPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKHRFEGRISITRVTADISLAKRSVLNNPSKHAIIERSN 346
β2e   IDATGLDAEENDIPANHRSPKPSANSVTSPHSKEKRMPFFKKTEHTPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKHRFEGRISITRVTADISLAKRSVLNNPSKHAIIERSN 298
β2f   ------------------------------------------------------------------------------------------------------------------------ 152
b2Δg  ------------------------------------------------------------------------------------------------------------------------ 11
β2h   -----KQKQKS-------------------------------TEHTPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKHRFEGRISITRVTADISLAKRSVLNNPSKHAIIERSN 308
β2cN1 -------------------AKSADEQDQWKTAGLFWRFT---TEHTPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKHRFEGRISITRVTADISLAKRSVLNNPSKHAIIERSN 322
β2cN2 -------------------AKSADEQDQWKTAGLFWRFT---TEHTPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKHRFEGRISITRVTADISLAKRSVLNNPSKHAIIERSN 294
β2cN4 -------------------AKSADEQDQWKTAGLFWRFT---TEHTPPYDVVPSMRPVVLVGPSLKGYEVTDMMQKALFDFLKHRFEGRISITRVTADISLAKRSVLNNPSKHAIIERSN 268
β2dN4 HSSV#------------------------------------------------------------------------------------------------------------------- 176

β2a   TRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 410
β2b   TRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 412
β2c   TRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 438
β2d   TRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 466
β2e   TRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 418
β2f   --------------------------------------------------------VLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 216
b2Δg  ------------------------------------------------------------------------------------------------------------------------ 11
β2h   TRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 428
β2cN1 TRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 442
β2cN2 TRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 414
β2cN4 TRSSLAEVQSEIERIFELARTLQLVVLDADTINHPAQLSKTSLAPIIVYVKISSPKVLQRLIKSRGKSQAKHLNVQMVAADKLAQCPPELFDVILDENQLEDACEHLADYLEAYWKATHP 388
β2dN4 ------------------------------------------------------------------------------------------------------------------------ 176
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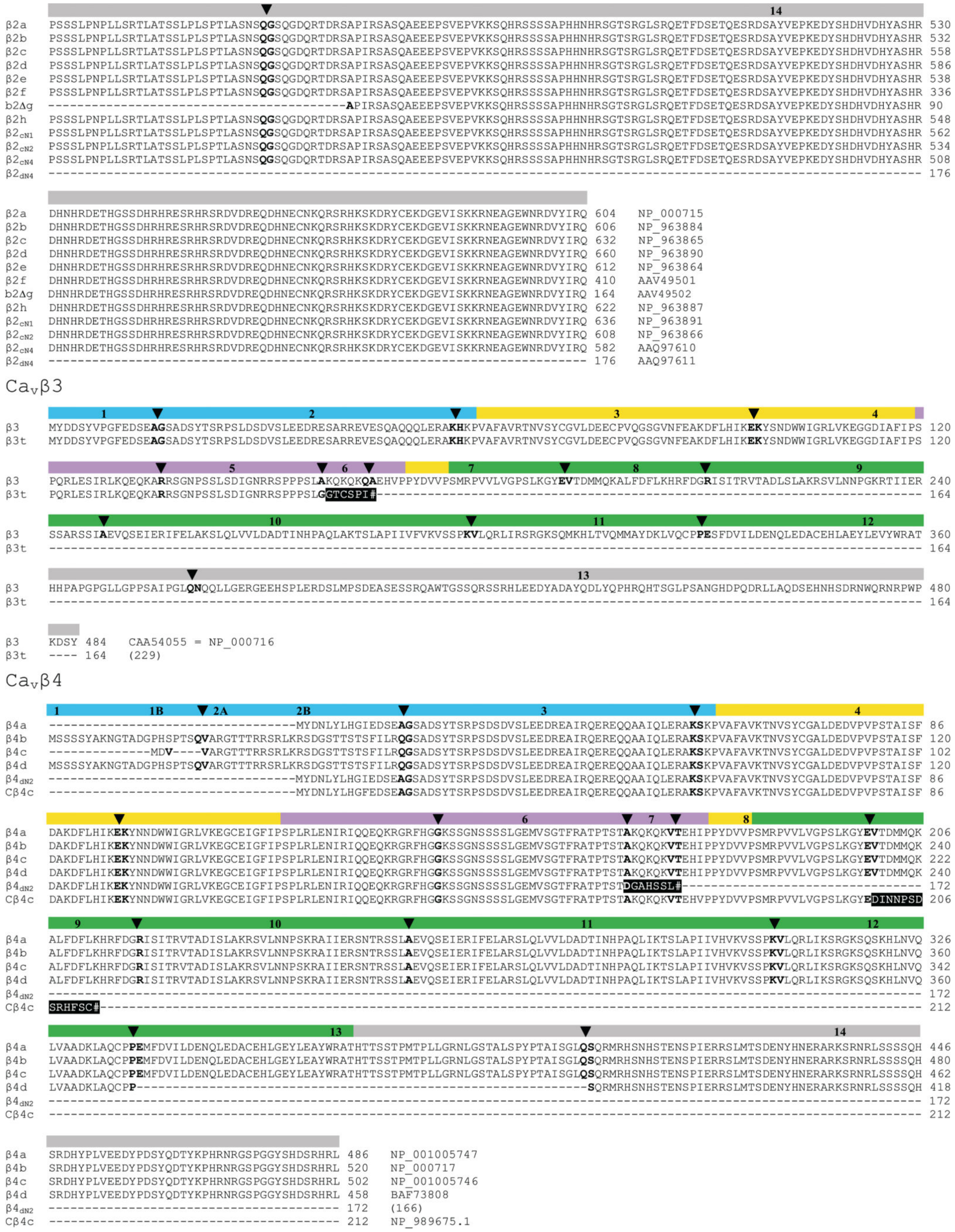

Fig. 6.

Amino acid sequence alignment of Ca$_v$β splice variants. The 5 Ca$_v$β regions, their corresponding exons, and the exon boundaries are marked. Color coding follows the same scheme as in previous figures, with the NH_2 terminus in light blue, the SH3 domain in gold, the HOOK in purple, the GK domain in green, and the COOH terminus in gray. Exon numbers are indicated in the color bar, and some exons have additional letters to indicate alternatively spliced variants. Arrows and bold amino acids mark exon boundaries. A single bold residue indicates that exon splicing occurs within its codon, whereas two bold residues indicate that splicing occurs between their codons. Shaded in black are missense sequences resulting from a frame-shift. # Indicates a premature stop codon. The GenBank accession number of each sequence is indicated at the end of the sequence, except for two sequences where the original reference is given. All sequences are from human except Cβ_{4c}, which is a chicken isoform. In regions where alternative splicing occurs (e.g., the NH_2 terminus of β_2), the amino acid sequence is aligned with its parent exon; thus the alignment in these regions does not necessarily indicate amino acid sequence similarity.

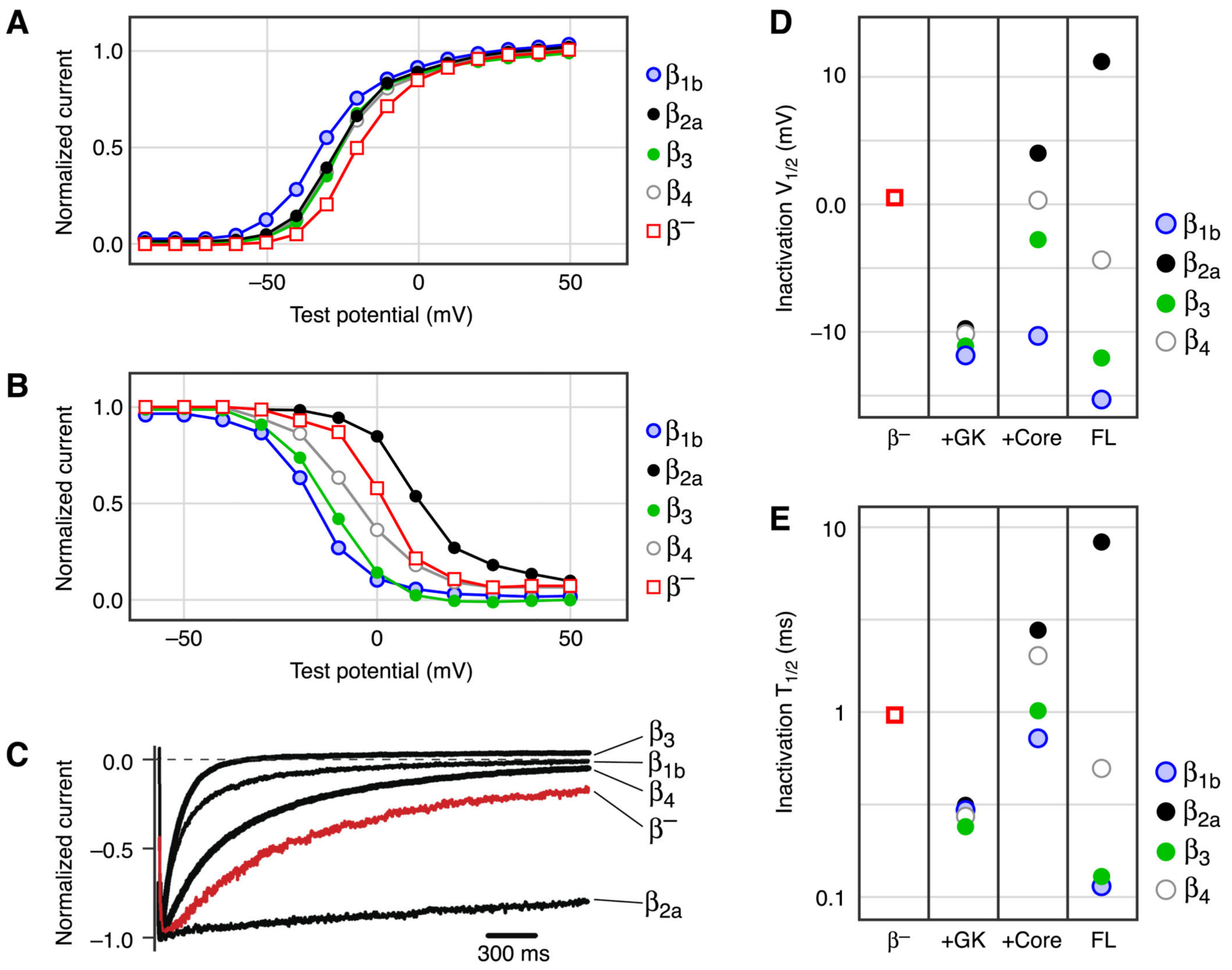

Fig. 7.

Modulation of Ca^{2+} channel gating by $Ca_v\beta$. *A*: voltage dependence of activation of P/Q-type Ca^{2+} channels containing β_{1b}, β_{2a}, β_3, or β_4 or no β (β^-). In this and all other panels, currents were recorded in cell-attached macropatches from oocytes expressing $Ca_v2.1$ and $\alpha_2\delta$, without or with the indicated β subunit. *B*: voltage dependence of inactivation. *C*: representative current traces evoked by a depolarization to ~30 mV, showing the kinetics of voltage-dependent inactivation. Currents are shown only from the first 2.5 s of a 25-s pulse. *D* and *E*: comparison of $V_{1/2}$ and $t_{1/2}$ of voltage-dependent inactivation of P/Q-type Ca^{2+} channels containing no β (β^-) or the indicated β module: the GK domain, β core (SH3-HOOK-GK), or full-length (FL) β. $V_{1/2}$ is the membrane voltage at the midpoint of voltage-dependent inactivation, and *t*1/2 is the time for the current to inactivate to 50% of the peak value in *C*. Note the logarithmic scale of the *y*-axis in *E*. [All data from He et al. (206).]

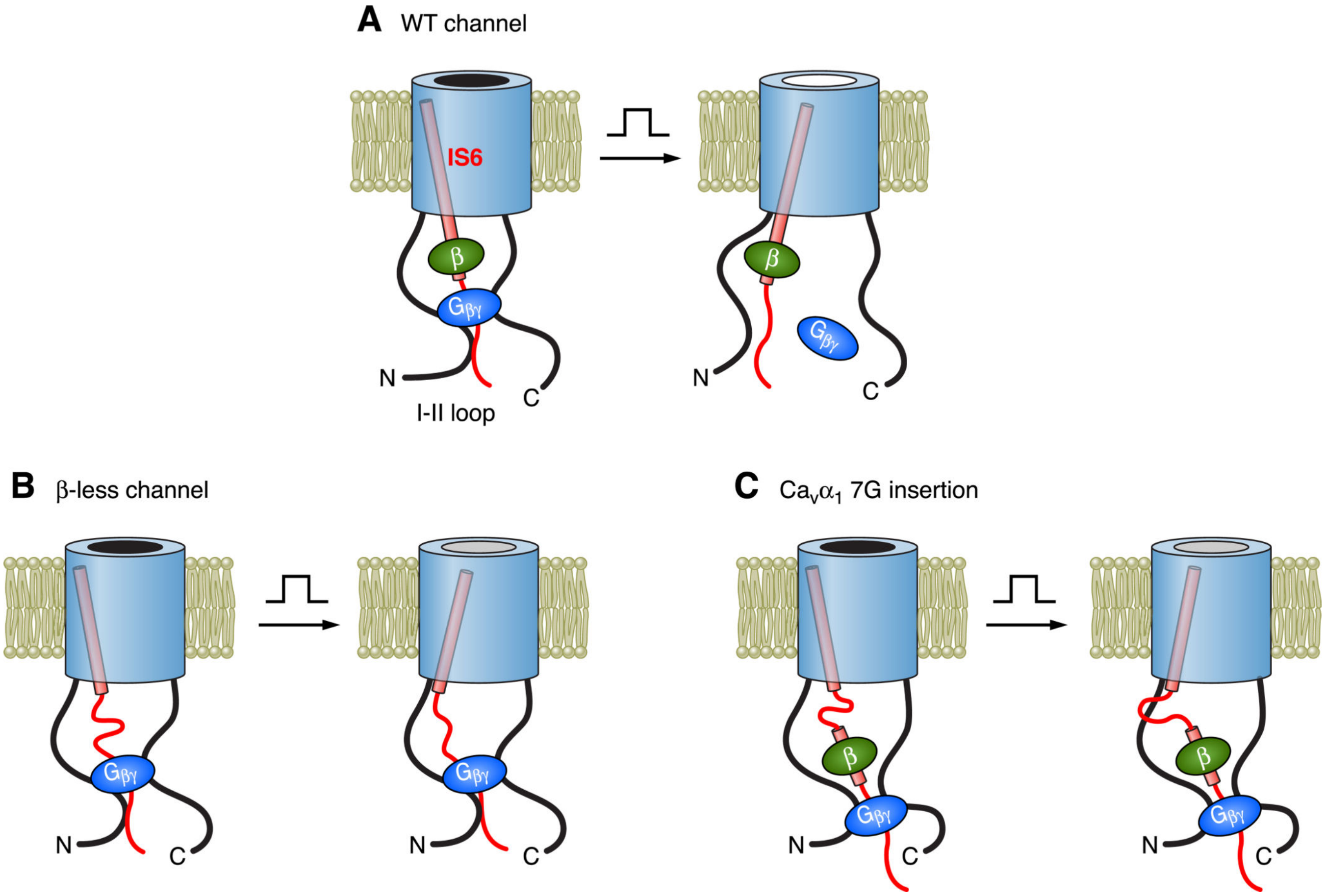

Fig. 8.

Model for the voltage dependence of $G_{\beta\gamma}$ inhibition. The $G_{\beta\gamma}$-binding pocket in the holo-channel is postulated to be formed by a region of the I–II loop distal to the AID, the NH_2 terminus, and the COOH terminus of $Ca_v\alpha_1$. *A*: WT channel: depolarization moves IS6; this movement is propagated through the rigid IS6-AID α-helix, consequently altering the conformation of the $G_{\beta\gamma}$-binding pocket and resulting in $G_{\beta\gamma}$ dissociation. *B*: β-less channel: the AID relaxes into a random coil in the absence of $Ca_v\beta$, uncoupling IS6 from the $G_{\beta\gamma}$-binding pocket. $G_{\beta\gamma}$ can bind and inhibit the channel but does not dissociate in a voltage-dependent way. *C*: channel containing $Ca_v\beta$ but with a flexible IS6-AID linker: insertion of 3–7 glycine residues in the IS6-AID linker disrupts the α-helix, uncoupling IS6 from the $G_{\beta\gamma}$-binding pocket and abolishing voltage-dependent dissociation of $G_{\beta\gamma}$. [Adapted from Zhang et al. (502)].

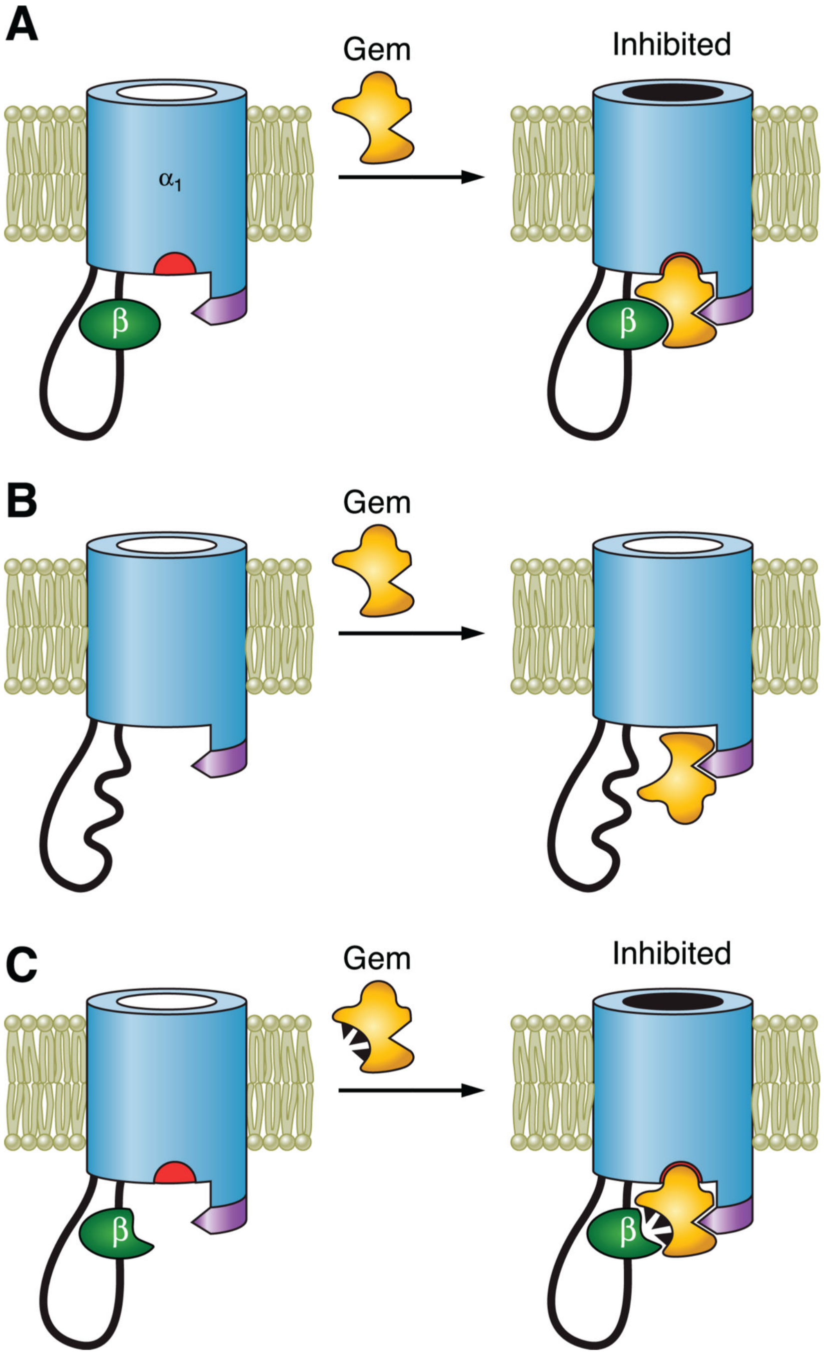

Fig. 9.

The “Ca$_V$β-priming” model of Gem inhibition of surface HVA Ca^{2+} channels. Gem associates directly with Ca$_V$α$_1$ via an anchoring site in Ca$_V$α$_1$ (indicated by the purple patch). *A*: WT channel: binding of Ca$_V$β to Ca$_V$α$_1$ induces an inhibitory site in Ca$_V$α$_1$ (indicated by the red patch), where Gem binds to induce inhibition. *B*: β-less channel: Gem can still associate with Ca$_V$α$_1$ via the anchoring site, but it does not inhibit the channel because Ca$_V$α$_1$ lacks the Ca$_V$β-induced inhibitory site. *C*: WT channel with mutually noninteracting Ca$_V$β and Gem: disrupting the interaction between Ca$_V$β/Gem with mutations in the $Ca_V\beta$/Gem interface does not affect Gem inhibition, since the interactions between $Ca_V\beta$ and $Ca_V\alpha_1$ and between Gem and $Ca_V\alpha_1$ remain intact. [Modified from Fan et al. (144).]

TABLE 1

Tissue distribution of $Ca_V\beta$

$Ca_V\beta$	Tissue Distribution	Reference Nos.
β_1	Expressed in brain (cerebral cortex, habenula, hippocampus, and olfactory bulb), heart, skeletal muscle, spleen, and T cells, but not in kidney, liver, or stomach.	360, 419, 435
β_{1a}	Expressed only in skeletal muscle (but see Ref. 89). Exclusive partner of $Ca_V1.1$ and irreplaceable for excitation-contraction coupling.	360, 381, 395, 472
β_{1b}	Expressed in brain (cerebellum and cerebral cortex), nerve endings at the NMJ, and pancreas. Expression is detected at P0 in rat brains and increases from P7 to adulthood by ~3-fold.	311, 344, 353, 355, 360, 395, 456, 472
β_{1c}	Expressed in brain and spleen, but not in kidney, liver, muscle, or stomach.	360
β_{1d}	Expressed in heart.	92
β_2	Expressed in brain (hippocampus–becoming the most abundant isoform there, cerebellum, pontine nucleus, susbtantia nigra, central grey, habenula, pineal gland, thalamic nuclei, cerebrum), heart, lung, nerve endings at the NMJ, T cells, and osteoblasts, but not in kidney, liver, pancreas, or spleen. Brain expression is constant during development, but see hippocampus data (391).	121, 294, 311, 344, 353, 355, 391, 398, 419, 435, 456
β_{2a}	Expressed in brain, heart, and aorta; its heart and brain levels seem lower than other β subunits and isoforms.	215, 229, 230
β_{2b}	Expressed in brain, heart, and aorta. It is the most abundant $Ca_V\beta$ in human heart.	89, 215, 230
β_{2c}	Expressed in brain and heart, where it is the second most abundant $Ca_V\beta$. Its expression declines in adults.	89, 215, 230
$\beta_{2d,e}$	Expressed in heart. β_{2e} expression is robust only in young animals.	89, 215
β_3	Expressed mostly in brain (cerebellum, cerebral cortex, habenula, hippocampus, olfactory bulb, and striatum), but also in heart, aorta, kidney, lung, skeletal muscle, smooth muscle, spleen, thalamus, T cells, and trachea, but not in liver, pancreas, or testis. Expression remains constant in the brain and heart during development. It is the most predominant partner of $Ca_V2.2$ (N-type) channels in the brain, and it pairs with ~40% of brain L-type channels.	58, 68, 89, 230, 294, 310, 329, 344, 355, 395, 419, 435, 449, 456, 472, 473
β_{3trunc}	Expressed in brain, heart, and aorta.	230
β_4	Expressed in brain (cerebellum–the most abundant Cavβ there, brain stem, cerebral cortex, dentate gyrus, habenula, hippocampus, olfactory bulb, striatum, thalamus, and hypothalamus), kidney, nerve endings at the NMJ, ovary, skeletal muscle, spinal cord, T cells, and testis, but not detected in heart (except in young animals, Ref. 89), liver, lung, spleen, or thymus. The expression increases in rat brain by 10-fold from P0 to adult. It is the most prevalent partner of $Ca_V2.1$ (P/Q-type) channels in brain, and, like β_3, it pairs with ~40% of brain L-type channels.	55, 67, 294, 311, 355, 391, 395, 419, 435, 449, 456, 472
β_{4a}	Expressed in spinal cord and cerebellum.	208, 209, 452
β_{4b}	Expressed in spinal cord and forebrain.	209

NMJ, neuromuscular junction.

TABLE 2

Effect of Ca$_V$β on HVA Ca^{2+} channel gating properties

Ca$_V$β	**Kinetics of Activation**	**Voltage Dependence of Activation**	**Kinetics of Inactivation**	**Voltage Dependence of Inactivation**
β$_1$	Accelerates activation by 2- to 100-fold; acceleration is weaker for Ca$_V$2 compared with Ca$_v$1 channels (40, 119, 206, 245, 268, 406, 412, 443, 450, 467).	Hyperpolarizing shift of −10 to −15 mV (40, 44, 92, 206, 245, 417, 450, 467).	Accelerates inactivation by 2- to 10-fold (40, 206, 218, 276, 340, 406, 417, 443, 450). α$_2$δ may be required in some instances.	Hyperpolarizing shift of −5 to −30 mV (40, 44, 119, 206, 218, 245, 276, 314, 340, 406, 443). α$_2$δ or γ may promote larger shifts.
β$_{2a}$	Accelerates activation by 2- to 4-fold; acceleration is less evident with Ca$_V$2 channels (40, 119, 206, 230, 245, 353).	Hyperpolarizing shift of −5 to −20 mV (40, 206, 230, 245, 276, 340, 353, 417).	Slows inactivation by ~10-fold (except for the unaffected Ca$_V$1.4 channels) (40, 58, 206, 218, 265, 276, 340, 369).	Depolarizing shift of 10 to 40 mV with Ca$_V$2 channels; much less or no effect on L-type channels (40, 119, 132, 206, 218, 245, 276, 314, 340, 369).
Other β$_2$	Accelerate activation kinetics ~2- to 4-fold (110, 215, 230, 433)	Hyperpolarizing shift of −7 to −20 mV (40, 110, 215, 230, 340, 433).	Inactivation is accelerated except with β2e (58, 215, 340).	Hyperpolarizing shift of approximately −10 mV except β$_{2e}$ (215, 340, 433).
β$_3$	Accelerates activation by 0 to 3.5-fold; acceleration is less evident with Ca$_V$2 channels (68, 119, 206, 230, 245).	Hyperpolarizing shift of −6 to −15 mV (44, 68, 206, 245, 276, 386, 417, 477).	Accelerates inactivation by 2- to 7-fold (68, 206, 265, 329).	Hyperpolarizing shift of −5 to −30 mV; α2δ may lessen the shift (44, 68, 119, 206, 245, 276, 386).
β$_4$	Accelerates activation by ~2- to 3-fold; acceleration is less evident with Ca$_V$2 channels (67, 119, 206, 208, 209, 245).	Hyperpolarizing shift of −5 to −25 mV (67, 159, 179, 206, 208, 245, 386, 452). For differences between splice variants, see Refs. 208, 209.	Accelerates inactivation by 2- to 4-fold (67, 206, 208), but see (417).	Hyperpolarizing shift of −5 to −30 mV; less evident for L-type channels (67, 119, 179, 206, 245, 386). For some differences between splice variants, see Refs. 208, 209.

Reference numbers are given in parentheses.