

# Voltage-Gated Calcium Channels

William A. Catterall

Department of Pharmacology, University of Washington, Seattle, Washington 98195-7280

Correspondence: wcatt@uw.edu

Voltage-gated calcium ( $\text{Ca}^{2+}$ ) channels are key transducers of membrane potential changes into intracellular  $\text{Ca}^{2+}$  transients that initiate many physiological events. There are ten members of the voltage-gated  $\text{Ca}^{2+}$  channel family in mammals, and they serve distinct roles in cellular signal transduction. The  $\text{Ca}_v1$  subfamily initiates contraction, secretion, regulation of gene expression, integration of synaptic input in neurons, and synaptic transmission at ribbon synapses in specialized sensory cells. The  $\text{Ca}_v2$  subfamily is primarily responsible for initiation of synaptic transmission at fast synapses. The  $\text{Ca}_v3$  subfamily is important for repetitive firing of action potentials in rhythmically firing cells such as cardiac myocytes and thalamic neurons. This article presents the molecular relationships and physiological functions of these  $\text{Ca}^{2+}$  channel proteins and provides information on their molecular, genetic, physiological, and pharmacological properties.

## PHYSIOLOGICAL ROLES OF VOLTAGE-GATED $\text{Ca}^{2+}$ CHANNELS

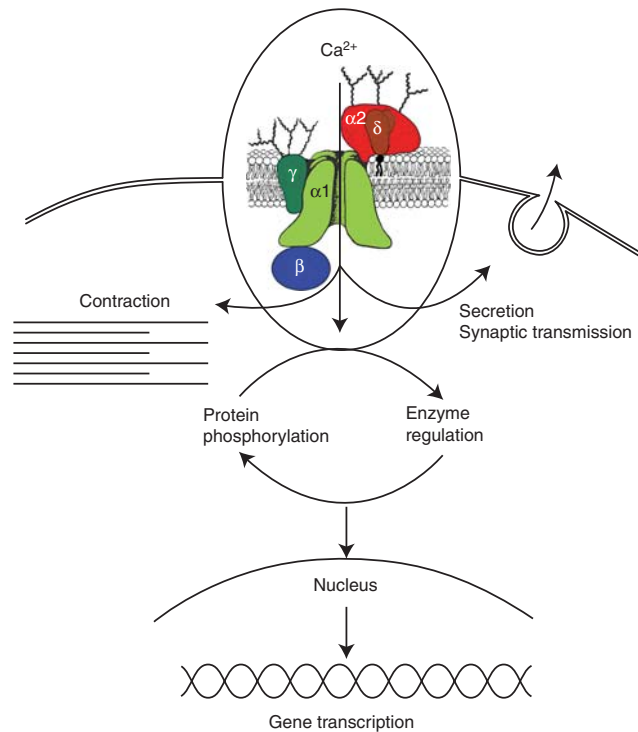
$\text{Ca}^{2+}$  channels in many different cell types activate on membrane depolarization and mediate  $\text{Ca}^{2+}$  influx in response to action potentials and subthreshold depolarizing signals.  $\text{Ca}^{2+}$  entering the cell through voltage-gated  $\text{Ca}^{2+}$  channels serves as the second messenger of electrical signaling, initiating many different cellular events (Fig. 1). In cardiac and smooth muscle cells, activation of  $\text{Ca}^{2+}$  channels initiates contraction directly by increasing cytosolic  $\text{Ca}^{2+}$  concentration and indirectly by activating calcium-dependent calcium release by ryanodine-sensitive  $\text{Ca}^{2+}$  release channels in the sarcoplasmic reticulum (Reuter 1979; Tsien 1983; Bers 2002). In skeletal muscle cells, voltage-gated  $\text{Ca}^{2+}$  channels in the transverse tubule membranes interact directly

with ryanodine-sensitive  $\text{Ca}^{2+}$  release channels in the sarcoplasmic reticulum and activate them to initiate rapid contraction (Catterall 1991; Tanabe et al. 1993). The same  $\text{Ca}^{2+}$  channels in the transverse tubules also mediate a slow  $\text{Ca}^{2+}$  conductance that increases cytosolic concentration and thereby regulates the force of contraction in response to high-frequency trains of nerve impulses (Catterall 1991). In endocrine cells, voltage-gated  $\text{Ca}^{2+}$  channels mediate  $\text{Ca}^{2+}$  entry that initiates secretion of hormones (Yang and Berggren 2006). In neurons, voltage-gated  $\text{Ca}^{2+}$  channels initiate synaptic transmission (Tsien et al. 1988; Dunlap et al. 1995; Catterall and Few 2008). In many different cell types,  $\text{Ca}^{2+}$  entering the cytosol via voltage-gated  $\text{Ca}^{2+}$  channels regulates enzyme activity, gene expression, and other biochemical processes (Flavell and Greenberg 2008). Thus, voltage-gated  $\text{Ca}^{2+}$  channels are

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**Figure 1.** Signal transduction by voltage-gated  $\text{Ca}^{2+}$  channels.  $\text{Ca}^{2+}$  entering cells initiates numerous intracellular events, including contraction, secretion, synaptic transmission, enzyme regulation, protein phosphorylation/dephosphorylation, and gene transcription. (*Inset*) Subunit structure of voltage-gated  $\text{Ca}^{2+}$  channels. The five-subunit complex that forms high-voltage-activated  $\text{Ca}^{2+}$  channels is illustrated with a central pore-forming  $\alpha 1$  subunit, a disulfide-linked glycoprotein dimer of  $\alpha 2$  and  $\delta$  subunits, an intracellular  $\beta$  subunit, and a transmembrane glycoprotein  $\gamma$  subunit (in some  $\text{Ca}^{2+}$  channel subtypes). As described in the text, this model is updated from the original description of the subunit structure of skeletal muscle  $\text{Ca}^{2+}$  channels. (Adapted from Takahashi et al. 1987).

the key signal transducers of electrical excitability, converting the electrical signal of the action potential in the cell surface membrane to an intracellular  $\text{Ca}^{2+}$  transient. Signal transduction in different cell types involves different molecular subtypes of voltage-gated  $\text{Ca}^{2+}$  channels, which mediate voltage-gated  $\text{Ca}^{2+}$  currents with different physiological, pharmacological, and regulatory properties.

### **$\text{Ca}^{2+}$ CURRENT TYPES DEFINED BY PHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES**

Since the first recordings of  $\text{Ca}^{2+}$  currents in cardiac myocytes (reviewed in Reuter 1979), it has become apparent that there are multiple

types of  $\text{Ca}^{2+}$  currents as defined by physiological and pharmacological criteria (Tsien et al. 1988; Bean 1989a; Llinás et al. 1992). In cardiac, smooth, and skeletal muscle, the major  $\text{Ca}^{2+}$  currents are distinguished by high voltage of activation, large single channel conductance, slow voltage-dependent inactivation, marked up-regulation by cAMP-dependent protein phosphorylation pathways, and specific inhibition by  $\text{Ca}^{2+}$  antagonist drugs including dihydropyridines, phenylalkylamines, and benzothiazepines (Table 1) (Reuter 1979; Tsien et al. 1988). These  $\text{Ca}^{2+}$  currents have been designated L-type, as they have slow voltage-dependent inactivation and therefore are long lasting when  $\text{Ba}^{2+}$  is the current carrier and there is no  $\text{Ca}^{2+}$ -dependent inactivation (Tsien et al.

**Table 1.** Subunit composition and function of Ca<sup>2+</sup> channel types

Ca <sup>2+</sup> current type	α1 Subunits	Specific blocker	Principal physiological functions	Inherited diseases
L	Ca <sub>v</sub> 1.1	DHPs	Excitation-contraction coupling in skeletal muscle, regulation of transcription	Hypokalemic periodic paralysis
	Ca <sub>v</sub> 1.2	DHPs	Excitation-contraction coupling in cardiac and smooth muscle, endocrine secretion, neuronal Ca <sup>2+</sup> transients in cell bodies and dendrites, regulation of enzyme activity, regulation of transcription	Timothy syndrome: cardiac arrhythmia with developmental abnormalities and autism spectrum disorders
	Ca <sub>v</sub> 1.3	DHPs	Endocrine secretion, cardiac pacemaking, neuronal Ca <sup>2+</sup> transients in cell bodies and dendrites, auditory transduction	
	Ca <sub>v</sub> 1.4	DHPs	Visual transduction	Stationary night blindness
N	Ca <sub>v</sub> 2.1	ω-CTx-GVIA	Neurotransmitter release, Dendritic Ca <sup>2+</sup> transients	
P/Q	Ca <sub>v</sub> 2.2	ω-Agatoxin	Neurotransmitter release, Dendritic Ca <sup>2+</sup> transients	Familial hemiplegic migraine, cerebellar ataxia
R	Ca <sub>v</sub> 2.3	SNX-482	Neurotransmitter release, Dendritic Ca <sup>2+</sup> transients	
T	Ca <sub>v</sub> 3.1	None	Pacemaking and repetitive firing	Absence seizures
	Ca <sub>v</sub> 3.2		Pacemaking and repetitive firing	
	Ca <sub>v</sub> 3.3			

Abbreviations: DHP, dihydropyridine; ω-CTx-GVIA, ω-conotoxin GVIA from the cone snail *Conus geographus*; SNX-482, a synthetic version of a peptide toxin from the tarantula *Hysterocrates gigas*.

1988). L-type Ca<sup>2+</sup> currents are also recorded in endocrine cells where they initiate release of hormones (Yang and Berggren 2006) and in neurons where they are important in regulation of gene expression, integration of synaptic input, and initiation of neurotransmitter release at specialized ribbon synapses in sensory cells (Tsien et al. 1988; Bean 1989a; Flavell and Greenberg 2008). L-type Ca<sup>2+</sup> currents are subject to regulation by second messenger-activated protein phosphorylation in several cell types as discussed below.

Electrophysiological studies of Ca<sup>2+</sup> currents in starfish eggs (Hagiwara et al. 1975) first revealed Ca<sup>2+</sup> currents with different properties from L-type, and these were subsequently characterized in detail in voltage-clamped dorsal root ganglion neurons (Carbone and Lux 1984; Fedulova et al. 1985; Nowycky et al. 1985). In comparison to L-type, these novel Ca<sup>2+</sup> currents activated at much more negative

membrane potentials, inactivated rapidly, deactivated slowly, had small single channel conductance, and were insensitive to conventional Ca<sup>2+</sup> antagonist drugs available at that time (Table 1). They were designated low-voltage-activated Ca<sup>2+</sup> currents for their negative voltage dependence (Carbone and Lux 1984) or T-type Ca<sup>2+</sup> currents for their transient openings (Nowycky et al. 1985).

Whole-cell voltage clamp and single-channel recording from dissociated dorsal root ganglion neurons revealed an additional Ca<sup>2+</sup> current, N-type (Table 1) (Nowycky et al. 1985). N-type Ca<sup>2+</sup> currents were initially distinguished by their intermediate voltage dependence and rate of inactivation—more negative and faster than L-type but more positive and slower than T-type (Nowycky et al. 1985). They are insensitive to organic L-type Ca<sup>2+</sup> channel blockers but blocked by the cone snail peptide ω-conotoxin GVIA and related peptide

toxins (Tsien et al. 1988; Olivera et al. 1994). This pharmacological profile has become the primary method to distinguish N-type  $\text{Ca}^{2+}$  currents, because the voltage dependence and kinetics of N-type  $\text{Ca}^{2+}$  currents in different neurons vary considerably.

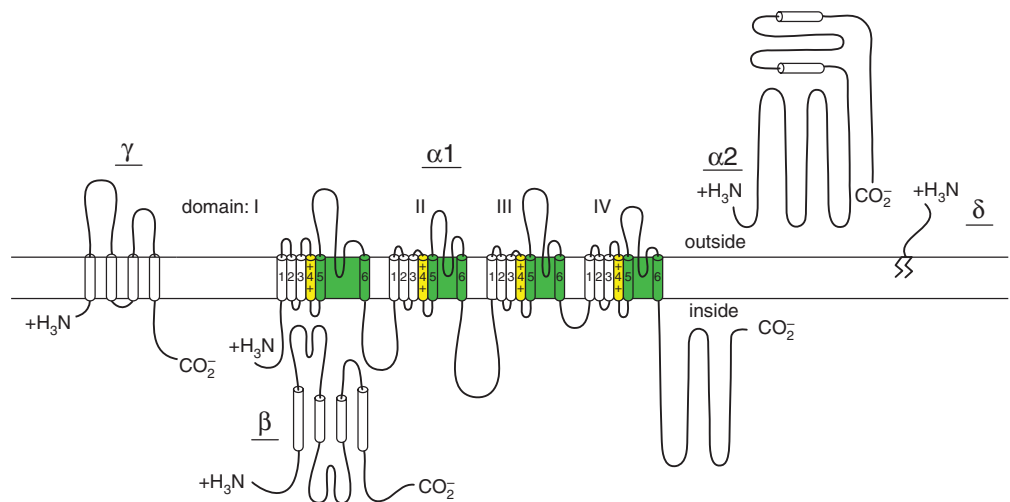
Analysis of the effects of other peptide toxins revealed three additional  $\text{Ca}^{2+}$  current types (Table 1). P-type  $\text{Ca}^{2+}$  currents, first recorded in Purkinje neurons (Llinás and Yarom 1981; Llinás et al. 1989), are distinguished by high sensitivity to the spider toxin  $\omega$ -agatoxin IVA (Mintz et al. 1992). Q-type  $\text{Ca}^{2+}$  currents, first recorded in cerebellar granule neurons (Randall and Tsien 1995), are blocked by  $\omega$ -agatoxin IVA with lower affinity. R-type  $\text{Ca}^{2+}$  currents in cerebellar granule neurons are resistant to most subtype-specific organic and peptide  $\text{Ca}^{2+}$  channel blockers (Randall and Tsien 1995) and may include multiple channel subtypes (Tottene et al. 1996). They can be blocked selectively in some cell types by the peptide SNX-482 derived from the tarantula *Hysterocrates gigas* (Newcomb et al. 1998). Although L-type and T-type  $\text{Ca}^{2+}$  currents are recorded in a wide range of cell types, N-, P-, Q-, and R-type  $\text{Ca}^{2+}$  currents are most prominent in neurons.

## MOLECULAR PROPERTIES OF $\text{Ca}^{2+}$ CHANNELS

### Subunit Structure

$\text{Ca}^{2+}$  channels purified from skeletal muscle transverse tubules are complexes of  $\alpha 1$ ,  $\alpha 2$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  subunits (Fig. 1) (Curtis and Catterall 1984, 1986; Flockerzi et al. 1986; Hosey et al. 1987; Leung et al. 1987; Striessnig et al. 1987; Takahashi et al. 1987). Analysis of the biochemical properties, glycosylation, and hydrophobicity of these five subunits led to a model comprising a principal transmembrane  $\alpha 1$  subunit of 190 kDa in association with a disulfide-linked  $\alpha 2\delta$  dimer of 170 kDa, an intracellular phosphorylated  $\beta$  subunit of 55 kDa, and a transmembrane  $\gamma$  subunit of 33 kDa (Fig. 1) (Takahashi et al. 1987).

The  $\alpha 1$  subunit is a protein of about 2000 amino acid residues in length with an amino acid sequence and predicted transmembrane structure like the previously characterized, pore-forming  $\alpha$  subunit of voltage-gated sodium channels (Fig. 2) (Tanabe et al. 1987). The amino acid sequence is organized in four repeated domains (I–IV), which each contains six transmembrane segments (S1–S6) and a



**Figure 2.** Subunit structure of  $\text{Ca}^{2+}$  channels. The structures of  $\text{Ca}^{2+}$  channel subunits are illustrated as transmembrane folding models; predicted  $\alpha$  helices are depicted as cylinders; the lengths of lines correlate approximately to the lengths of the polypeptide segments represented; and the zigzag line on the  $\delta$  subunit illustrates its glycophosphatidylinositol anchor.



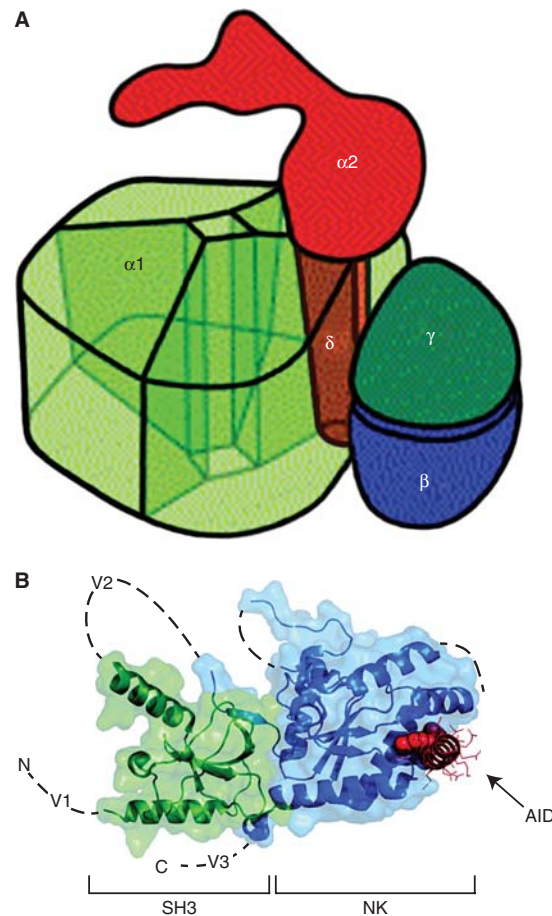
membrane-associated loop between transmembrane segments S5 and S6. As expected from biochemical analysis (Takahashi et al. 1987a), the intracellular  $\beta$  subunit has predicted  $\alpha$  helices but no transmembrane segments (Fig. 2) (Ruth et al. 1989), whereas the  $\gamma$  subunit is a glycoprotein with four transmembrane segments (Fig. 2) (Jay et al. 1990). The cloned  $\alpha 2$  subunit has many glycosylation sites and several hydrophobic sequences (Ellis et al. 1988), but biosynthesis studies indicate that it is an extracellular, extrinsic membrane glycoprotein, attached to the membrane through disulfide linkage to the  $\delta$  subunit (Fig. 2) (Gurnett et al. 1996). The  $\delta$  subunit is encoded by the 3' end of the coding sequence of the same gene as the  $\alpha 2$  subunit, and the mature forms of these two subunits are produced by posttranslational proteolytic processing and disulfide linkage (Fig. 2) (De Jongh et al. 1990). Although it was initially assumed that the  $\delta$  subunit was anchored to the membrane via a single membrane segment, recent work argues persuasively that further posttranslational processing actually cleaves the predicted transmembrane segment and replaces it with a glycosylphosphatidylinositol membrane anchor (Fig. 2) (Davies et al. 2010).

Purification of cardiac  $\text{Ca}^{2+}$  channels labeled by dihydropyridine  $\text{Ca}^{2+}$  antagonists identified subunits of the sizes of the  $\alpha 1$ ,  $\alpha 2\delta$ ,  $\beta$ , and  $\gamma$  subunits of skeletal muscle  $\text{Ca}^{2+}$  channels (Chang and Hosey 1988; Schneider and Hofmann 1988; Kuniyasu et al. 1992), whereas immunoprecipitation of  $\text{Ca}^{2+}$  channels from neurons labeled by dihydropyridine  $\text{Ca}^{2+}$  antagonists revealed  $\alpha 1$ ,  $\alpha 2\delta$ , and  $\beta$  subunits but no  $\gamma$  subunit (Ahlijanian et al. 1990). Purification and immunoprecipitation of N-type and P/Q-type  $\text{Ca}^{2+}$  channels labeled by  $\omega$ -conotoxin GVIA and  $\omega$ -agatoxin IVA, respectively, from brain membrane preparations also revealed  $\alpha 1$ ,  $\alpha 2\delta$ , and  $\beta$  subunits but not  $\gamma$  subunits (McEnery et al. 1991; Martin-Moutot et al. 1995; Witcher et al. 1995a; Liu et al. 1996). More recent experiments have unexpectedly revealed a novel  $\gamma$  subunit (stargazin), which is the target of the *stargazer* mutation in mice (Letts et al. 1998), and a related series of seven  $\gamma$  subunits is expressed in brain and

other tissues (Klugbauer et al. 2000). These  $\gamma$ -subunit-like proteins can modulate the voltage dependence of  $\text{Ca}_v 2.1$  channels expressed in nonneuronal cells, so they may be associated with these  $\text{Ca}^{2+}$  channels in vivo. However, the stargazin-like  $\gamma$  subunits (also called transmembrane AMPA receptor modulators [TARPs]) are the primary modulators of glutamate receptors in the postsynaptic membranes of brain neurons (Nicoll et al. 2006), and it remains to be determined whether they are also associated with voltage-gated  $\text{Ca}^{2+}$  channels in brain neurons in vivo.

### Three-Dimensional Structure of $\text{Ca}^{2+}$ Channels

The three-dimensional structure of  $\text{Ca}^{2+}$  channels is not known at high resolution. Low-resolution structural models have been developed from image reconstruction analysis of  $\text{Ca}_v 1.1$  channels purified from skeletal muscle membranes (Serysheva et al. 2002; Wang et al. 2002; Wolf et al. 2003), and some of the structural features have been associated with the  $\alpha 1$ ,  $\beta$ , and  $\alpha 2\delta$  subunits (Fig. 3A). Further high-resolution structural analysis will be required to confirm these initial structural models. The three-dimensional structure of the  $\text{Ca}_v \beta$  subunits has been determined at high resolution by X-ray crystallography (Fig. 3B) (Chen et al. 2004; Van Petegem et al. 2004). These subunits contain conserved SH3 and guanylate kinase domains like the MAGUK family of scaffolding proteins. These two domains are arrayed side-by-side in the  $\text{Ca}_v \beta$  subunit (Fig. 3B). The  $\text{Ca}_v \beta$  subunits bind to a single site in the  $\alpha 1$  subunits (the  $\alpha$  interaction domain, AID) (Pragnell et al. 1994), which is located in the first half of the intracellular loop connecting domains I and II. The AID forms an  $\alpha$  helix that is bound tightly to a groove in the guanylate kinase domain of the  $\text{Ca}_v \beta$  subunit. This tight, multipoint binding interaction likely sustains the association between  $\text{Ca}^{2+}$  channel  $\alpha 1$  and  $\beta$  subunits throughout the lifetime of the  $\text{Ca}^{2+}$  channel complex at the cell surface membrane. MAGUK proteins often bind more than one protein partner, so  $\text{Ca}_v \beta$  subunits may



**Figure 3.** Three-dimensional architecture of  $\text{Ca}^{2+}$  channels. (A) Illustration of the skeletal muscle  $\text{Ca}_V1.1$  channel based on cryo-electron microscopy. This drawing assumes pseudo-fourfold symmetry of the  $\alpha 1$  subunit. The view shows the extracellular side with the  $\alpha 2$  subunit. The  $\alpha 1$ ,  $\gamma$ , and  $\delta$  subunits are embedded into the lipid membrane (not shown), which separates the extracellular  $\alpha 2$  subunit from the cytosol.  $\alpha 2$  is anchored via the disulfide-linked  $\delta$  subunit within the  $\alpha 1$  subunit. The proposed model allows for a tight interaction between  $\alpha 1$  and  $\delta$  as well as  $\alpha 1$  and  $\gamma$ . (B) Structure of the  $\text{Ca}_V\beta$  subunit with the  $\alpha$  interaction domain (AID). Coordinates are for the  $\text{Ca}_V\beta 2a$ – $\text{Ca}_V1.2$  AID complex with SH3 (green) and NK (blue) domains are indicated. V1, V2, and V3 show the locations of the three variable domains that are absent from the structure. The AID (red) binds to a deep groove in the NK domain. AID residues tyrosine, tryptophan, and isoleucine are shown as CPK. The remaining residues are shown as lines.

also interact with other intracellular proteins, and several potential binding partners are under active investigation.

### Functions of $\text{Ca}^{2+}$ Channel Subunits

Expression of the  $\alpha 1$  subunit is sufficient to produce functional skeletal muscle  $\text{Ca}^{2+}$  channels, but with low expression level and abnormal

kinetics and voltage dependence of the  $\text{Ca}^{2+}$  current (Perez-Reyes et al. 1989). Coexpression of the  $\alpha 2\delta$  subunit and especially the  $\beta$  subunit enhanced the level of expression and conferred more normal gating properties (Lacerda et al. 1991; Singer et al. 1991). As for skeletal muscle  $\text{Ca}^{2+}$  channels, coexpression of  $\beta$  subunits has a large effect on the level of expression and the voltage dependence and kinetics of gating of





cardiac and neuronal  $\text{Ca}^{2+}$  channels (reviewed in Hofmann et al. 1994; Dolphin 2003). In general, the level of expression is increased and the voltage dependence of activation and inactivation is shifted to more negative membrane potentials, and the rate of inactivation is increased. However, these effects are different for the individual  $\beta$  subunit isoforms. For example, the  $\beta 2a$  subunit slows channel inactivation in most subunit combinations. Coexpression of  $\alpha 2\delta$  subunits also increases expression and enhances function of  $\text{Ca}^{2+}$  channels, but to a lesser extent and in a more channel-specific way than do  $\beta$  subunits (Arikath and Campbell 2003; Davies et al. 2007). In general,  $\gamma$  subunits have smaller effects.

### $\text{Ca}^{2+}$ Channel Diversity

The different types of  $\text{Ca}^{2+}$  currents are primarily defined by different  $\alpha 1$  subunits, and ten different ones have been characterized by cDNA cloning and functional expression in mammalian cells or *Xenopus* oocytes (Table 1). These subunits can be divided into three structurally and functionally related families ( $\text{Ca}_v1$ ,  $\text{Ca}_v2$ , and  $\text{Ca}_v3$ ) (Snutch and Reiner 1992; Ertel et al. 2000). L-type  $\text{Ca}^{2+}$  currents are mediated by the  $\text{Ca}_v1$  type of  $\alpha 1$  subunits, which have about 75% amino acid sequence identity among them. The  $\text{Ca}_v2$  type  $\text{Ca}^{2+}$  channels form a distinct subfamily with <40% amino acid sequence identity with  $\text{Ca}_v1$   $\alpha 1$  subunits but >70% amino acid sequence identity among themselves. Cloned  $\text{Ca}_v2.1$  subunits (Mori et al. 1991; Starr et al. 1991) conduct P- or Q-type  $\text{Ca}^{2+}$  currents, which are inhibited by  $\omega$ -agatoxin IVA.  $\text{Ca}_v2.2$  subunits conduct N-type  $\text{Ca}^{2+}$  currents blocked with high affinity by  $\omega$ -conotoxin GVIA (Dubel et al. 1992; Williams et al. 1992). Cloned  $\text{Ca}_v2.3$  subunits form R-type  $\text{Ca}^{2+}$  channels, which are resistant to both organic  $\text{Ca}^{2+}$  antagonists specific for L-type  $\text{Ca}^{2+}$  currents and the peptide toxins specific for N-type or P/Q-type  $\text{Ca}^{2+}$  currents (Soong et al. 1994). T-type  $\text{Ca}^{2+}$  currents are mediated by the  $\text{Ca}_v3$   $\text{Ca}^{2+}$  channels (Perez-Reyes et al. 1998). These  $\alpha 1$  subunits are only distantly related to the other known homologs,

with <25% amino acid sequence identity. These results reveal a surprising structural dichotomy between the T-type, low-voltage-activated  $\text{Ca}^{2+}$  channels and the high-voltage-activated  $\text{Ca}^{2+}$  channels. Evidently, these two lineages of  $\text{Ca}^{2+}$  channels diverged very early in evolution of multicellular organisms. Single representatives of the  $\text{Ca}_v1$ ,  $\text{Ca}_v2$ , and  $\text{Ca}_v3$  subfamilies are present in invertebrate genomes, including the worm *Caenorhabditis elegans* and the fruit fly *Drosophila*.

The diversity of  $\text{Ca}^{2+}$  channel structure and function is substantially enhanced by multiple  $\beta$  subunits. Four  $\beta$  subunit genes have been identified, and each is subject to alternative splicing to yield additional isoforms (reviewed in Hofmann et al. 1994; Dolphin 2003). In  $\text{Ca}^{2+}$  channel preparations isolated from brain, individual  $\text{Ca}^{2+}$  channel  $\alpha 1$  subunit types are associated with multiple types of  $\beta$  subunits, although there is a different rank order in each case (Pichler et al. 1997; Witcher et al. 1995b). The different  $\beta$  subunit isoforms cause different shifts in the kinetics and voltage dependence of gating, so association with different  $\beta$  subunits can substantially alter the physiological function of an  $\alpha 1$  subunit. Genes encoding four  $\alpha 2\delta$  subunits have been described (Klugbauer et al. 1999), and the  $\alpha 2\delta$  isoforms produced by these different genes have selective effects on the level of functional expression and the voltage dependence of different  $\alpha 1$  subunits (Davies et al. 2007).

### Molecular Basis for $\text{Ca}^{2+}$ Channel Function

Intensive studies of the structure and function of the related pore-forming subunits of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{K}^+$  channels have led to identification of their principal functional domains (reviewed in Catterall 2000a,b; Yi and Jan 2000; Bichet et al. 2003; Yu et al. 2005). Each domain of the principal subunits consists of six transmembrane  $\alpha$  helices (S1–S6) and a membrane-associated loop between S5 and S6 (Fig. 2). The S4 segments of each homologous domain serve as the voltage sensors for activation, moving outward and rotating under the influence of the electric field and initiating a

conformational change that opens the pore. The S5 and S6 segments and the membrane-associated pore loop between them form the pore lining of the voltage-gated ion channels. The narrow external end of the pore is lined by the pore loop, which contains a pair of glutamate residues in each domain that are required for  $\text{Ca}^{2+}$  selectivity, a structural feature that is unique to  $\text{Ca}^{2+}$  channels (Heinemann et al. 1992). Remarkably, substitutions that add only three glutamate residues in the pore loops between the S5 and S6 segments in domains II, III, and IV of sodium channels are sufficient to confer  $\text{Ca}^{2+}$  selectivity (Heinemann et al. 1992; Sather and McCleskey 2003). The inner pore is lined by the S6 segments, which form the receptor sites for the pore-blocking  $\text{Ca}^{2+}$  antagonist drugs specific for L-type  $\text{Ca}^{2+}$  channels (Hockerman et al. 1997a,b). All  $\text{Ca}^{2+}$  channels share these general structural features, but the amino acid residues that confer high affinity for the organic  $\text{Ca}^{2+}$  antagonists used in therapy of cardiovascular diseases are present only in the  $\text{Ca}_v1$  family of  $\text{Ca}^{2+}$  channels, which conduct L-type  $\text{Ca}^{2+}$  currents.

### **$\text{Ca}_v1$ CHANNELS AND EXCITATION-RESPONSE COUPLING**

$\text{Ca}_v1$  channels serve to couple depolarization of the plasma membrane to a wide range of cellular responses (Fig. 1). Three widely studied examples are excitation-contraction coupling in muscle, excitation-transcription coupling in nerve and muscle, and excitation-secretion coupling in endocrine cells and at specialized ribbon synapses.

### **$\text{Ca}_v1$ CHANNELS AND EXCITATION-CONTRACTION COUPLING**

#### **Mechanisms of Excitation-Contraction Coupling**

$\text{Ca}_v1$  channels initiate excitation-contraction coupling in skeletal, cardiac, and smooth muscle. There are striking mechanistic differences between excitation-contraction coupling in skeletal muscle and cardiac muscle. In skeletal muscle, entry of external  $\text{Ca}^{2+}$  is not required

for initiation of contraction (Armstrong et al. 1972).  $\text{Ca}_v1.1$  channels in the transverse tubules are thought to interact directly with the ryanodine-sensitive  $\text{Ca}^{2+}$  release channels (RyR1) of the sarcoplasmic reticulum (Numa et al. 1990), as observed in high-resolution electron microscopy (Block et al. 1988), and the voltage-driven conformational changes in their voltage-sensing domains are thought to directly induce activation of RyR1 (Numa et al. 1990). Reconstitution of excitation-contraction coupling in myocytes from mutant mice requires both  $\text{Ca}_v1.1$  and RyR1 proteins and their relevant sites of protein-protein interaction (Tanabe et al. 1990; Nakai et al. 1998), and functional expression of the  $\text{Ca}_v1.1$  channel in skeletal muscle requires its RyR1 binding partner (Nakai et al. 1996).

In contrast to skeletal muscle, entry of  $\text{Ca}^{2+}$  is required for excitation-contraction coupling in cardiac myocytes, and  $\text{Ca}^{2+}$  entry via  $\text{Ca}_v1.2$  channels triggers activation of the RyR2 and initiates  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release, activation of actomyosin, and contraction (Fabiato 1983; Bers 2002). Release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum via RyR2 greatly amplifies the cellular  $\text{Ca}^{2+}$  transient and is required for effective initiation of contraction. All three steps in the cascade of  $\text{Ca}^{2+}$  transport processes— $\text{Ca}^{2+}$  entry via  $\text{Ca}_v1.2$  channels,  $\text{Ca}^{2+}$  release via RyR, and  $\text{Ca}^{2+}$  uptake into the sarcoplasmic reticulum by SERCA  $\text{Ca}^{2+}$  pumps—are tightly regulated by second messenger signaling networks (Bers 2002). The section below considers the regulation of  $\text{Ca}_v1$  channels in excitation-contraction coupling.

#### **Regulation of Excitation-Contraction Coupling via $\text{Ca}_v1$ Channels**

As part of the flight-or-flight response, the rate and force of contraction of both skeletal and cardiac muscle are increased through the activity of the sympathetic nervous system. Release of catecholamines stimulates  $\beta$ -adrenergic receptors ( $\beta$ -ARs), which increases the force of skeletal and cardiac muscle contraction and the heart rate (Reuter 1983; Tsien et al. 1986). In





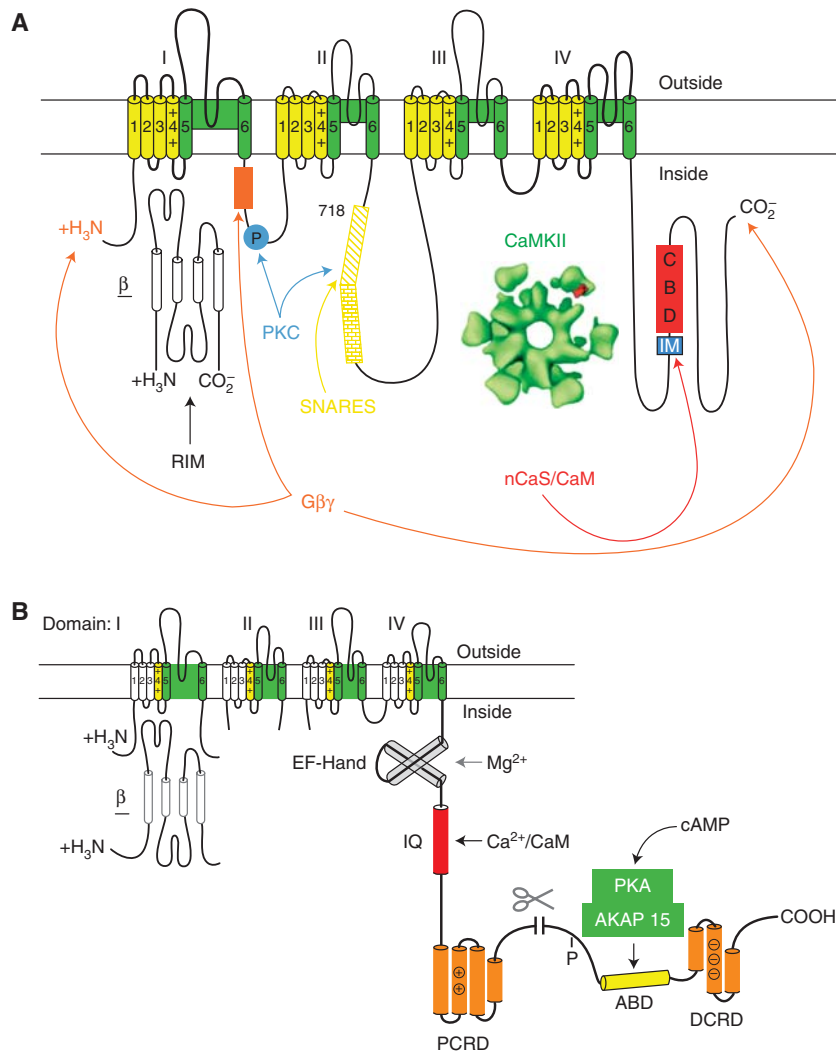
cardiac muscle,  $\text{Ca}^{2+}$  influx through  $\text{Ca}_v1.2$  channels is responsible for initiating excitation-contraction coupling, and increased  $\text{Ca}^{2+}$  channel activity via the PKA pathway is primarily responsible for the increase in contractility.  $\text{Ca}_v1.2$  channels are modulated by the  $\beta$ -adrenergic receptor/cAMP signaling. Activation of  $\beta$ -adrenergic receptors increases L-type  $\text{Ca}^{2+}$  currents through PKA-mediated phosphorylation of the  $\text{Ca}_v1.2$  channel protein and/or associated proteins (Tsien 1973; Reuter and Scholz 1977; Osterrieder et al. 1982; McDonald et al. 1994).

The pore-forming  $\alpha 1$  subunit and the auxiliary  $\beta$  subunits of skeletal muscle  $\text{Ca}_v1.1$  channels (Curtis and Catterall 1985; Flockerzi et al. 1986; Takahashi et al. 1987) and cardiac  $\text{Ca}_v1.2$  channels (Hell et al. 1993b; De Jongh et al. 1996; Haase et al. 1996; Puri et al. 1997) are phosphorylated by PKA. These  $\alpha 1$  subunits are also truncated by proteolytic processing of the carboxy-terminal domain (Fig. 4) (De Jongh et al. 1989, 1991, 1996; Hulme et al. 2005). Voltage-dependent potentiation of  $\text{Ca}_v1.1$  channels on the 50-msec time scale requires PKA phosphorylation (Sculptoreanu et al. 1993) as well as PKA anchoring via an A kinase anchoring protein (AKAP) (Johnson et al. 1994, 1997), suggesting close association of PKA and  $\text{Ca}^{2+}$  channels. A novel, plasma membrane-targeted AKAP (AKAP15) is associated with both  $\text{Ca}_v1.1$  channels (Gray et al. 1997, 1998) and  $\text{Ca}_v1.2$  channels (Hulme et al. 2003), and may mediate their regulation by PKA. This AKAP (also known as AKAP18 [Fraser et al. 1998]) binds to the carboxy-terminal domain of  $\text{Ca}_v1.1$  channels (Hulme et al. 2002) and  $\text{Ca}_v1.2$  channels (Hulme et al. 2003) via a novel modified leucine zipper interaction near the primary sites of PKA phosphorylation. Block of this interaction by competing peptides prevents PKA regulation of  $\text{Ca}^{2+}$  currents in intact skeletal and cardiac myocytes (Hulme et al. 2002, 2003, 2006b). These physiological results suggest that a  $\text{Ca}^{2+}$  channel signaling complex containing AKAP15 and PKA is formed in both skeletal and cardiac muscle, and this conclusion is supported by specific colocalization of these

proteins in both skeletal and cardiac myocytes and specific coimmunoprecipitation of this complex from both tissues (Hulme et al. 2002, 2003, 2006a). Remarkably, block of kinase anchoring is as effective as block of kinase activity in preventing  $\text{Ca}_v1.1$  and  $\text{Ca}_v1.2$  channel regulation, consistent with the conclusion that PKA targeting via leucine zipper interactions is absolutely required for regulation of  $\text{Ca}_v1$  channels in intact skeletal and cardiac myocytes.

### Proteolytic Processing and Regulation via the Carboxy-Terminal Domain

The distal carboxy-terminal domains of skeletal muscle and cardiac  $\text{Ca}^{2+}$  channels are proteolytically processed in vivo (Fig. 4B) (De Jongh et al. 1991, 1996). Nevertheless, the most prominent in vitro PKA phosphorylation sites of both proteins are located beyond the site of proteolytic truncation (Rotman et al. 1992, 1995; De Jongh et al. 1996; Mitterdorfer et al. 1996), and interaction of AKAP15 and PKA with the distal carboxy-terminal domain through a leucine zipper motif is required for regulation of cardiac  $\text{Ca}^{2+}$  channels in intact myocytes (Hulme et al. 2003). These results imply that the distal carboxy-terminal domain remains associated with the proteolytically processed cardiac  $\text{Ca}_v1.2$  channel, and this is supported by evidence that the distal carboxyl-terminus can bind to the truncated  $\text{Ca}_v1.1$  and  $\text{Ca}_v1.2$  channels in vitro (Gerhardstein et al. 2000; Gao et al. 2001; Hulme et al. 2005) and in transfected cells (Hulme et al. 2002; Hulme et al. 2006b). Moreover, formation of this complex dramatically inhibits cardiac  $\text{Ca}^{2+}$  channel function in intact mammalian cells (Hulme et al. 2006b). Deletion of the distal carboxy-terminal near the site of proteolytic processing increases  $\text{Ca}^{2+}$  channel activity (Wei et al. 1994; Hulme et al. 2006b). However, noncovalent association of the cleaved distal carboxy-terminal reduces channel activity more than 10-fold, to a level much below that of channels with an intact carboxyl-terminus (Hulme et al. 2006b). Thus, proteolytic processing produces an autoinhibited  $\text{Ca}^{2+}$  channel complex containing noncovalently bound distal



**Figure 4.**  $\text{Ca}^{2+}$  channel signaling complexes. (A) The presynaptic  $\text{Ca}^{2+}$  channel signaling complex. A presynaptic  $\text{Ca}^{2+}$  channel  $\alpha 1$  subunit is illustrated as a transmembrane folding diagram as in Figure 2. Sites of interaction of SNARE proteins (the synprint site),  $\text{G}\beta\gamma$  subunits, protein kinase C (PKC), CaMKII, and CaM and CaS proteins are illustrated. IM, IQ-like motif; CBD, CaM binding domain. (B) The cardiac  $\text{Ca}^{2+}$  channel signaling complex. The carboxy-terminal domain of the cardiac  $\text{Ca}^{2+}$  channels is shown in expanded presentation to illustrate the regulatory interactions clearly. ABD, AKAP15 binding domain; DCRD, distal carboxy-terminal regulatory domain; PCRD, proximal carboxy-terminal regulatory domain; scissors, site of proteolytic processing. The DCRD binds to the PCRD through a modified leucine zipper interaction.

carboxyl-terminus with AKAP15 and PKA associated through a modified leucine zipper interaction (Fig. 4B). This autoinhibited complex appears to be the primary substrate for regulation of cardiac  $\text{Ca}^{2+}$  channels by the  $\beta$ -adrenergic receptor/PKA pathway in vivo,

and PKA up-regulation results from phosphorylation of a single site near the end of the proximal carboxy-terminal domain at the interface with the distal carboxy-terminal domain (Fig. 4B) (Hulme et al. 2006b; Emrick et al. 2010; Fuller et al. 2010).



### Ca<sup>2+</sup> Binding Proteins

In addition to their regulation by the PKA/AKAP15 signaling complex, cardiac Ca<sup>2+</sup> channels have calmodulin bound to their carboxy-terminal domain through an IQ motif (Fig. 4B), and Ca<sup>2+</sup> binding to calmodulin causes Ca<sup>2+</sup>-dependent inactivation (Peterson et al. 1999; Qin et al. 1999; Zühlke et al. 1999). Activation of Ca<sub>v</sub>1.2 channels in the presence of Ba<sup>2+</sup> as the permeant ion results in inward Ba<sup>2+</sup> currents that activate rapidly and inactivate slowly via a voltage-dependent inactivation process. In contrast, in the presence of Ca<sup>2+</sup> as the permeant ion, Ca<sup>2+</sup> currents are rapidly inactivated via Ca<sup>2+</sup>/calmodulin-dependent inactivation. The Ca<sup>2+</sup>-dependent inactivation process is crucial for limiting Ca<sup>2+</sup> entry during long cardiac action potentials. In light of these results, it is evident that both the cAMP and Ca<sup>2+</sup> second messenger pathways regulate Ca<sub>v</sub>1.2 channels locally, dependent on associated regulatory proteins in Ca<sup>2+</sup> channel signaling complexes.

### Ca<sub>v</sub>1 CHANNELS IN EXCITATION-TRANSCRIPTION COUPLING

Ca<sup>2+</sup> entering neurons through L-type Ca<sup>2+</sup> currents conducted by Ca<sub>v</sub>1 channels has a privileged role in regulation of gene transcription, compared to similar amounts of Ca<sup>2+</sup> entering via other voltage-gated or ligand-gated ion channels (Flavell and Greenberg 2008). This unique access of Ca<sub>v</sub>1 channels to regulation of transcription might arise from preferential localization, which could provide Ca<sup>2+</sup> in the vicinity of transcriptional regulators, preferential interaction with binding partners, which could be activated by local Ca<sup>2+</sup> entry and carry the regulatory signal to the nucleus, or nuclear targeting of a subunit or domain of the Ca<sub>v</sub>1 channel itself, which would serve to regulate transcription directly. It is likely that all three of these mechanisms are involved based on recent experiments.

Ca<sub>v</sub>1 channels are localized in higher density in the cell bodies and proximal dendrites of neurons compared to Ca<sub>v</sub>2 and Ca<sub>v</sub>3

channels, which are more prevalent in nerve terminals and dendrites, respectively (Westenbroek et al. 1990; Hell et al. 1993a). This preferential localization would favor Ca<sup>2+</sup> entry through these channels in control of transcription in the nucleus. However, this effect seems insufficient to fully account for the dominance of this Ca<sup>2+</sup> entry pathway.

Studies with selective Ca<sup>2+</sup> buffers indicate that only a local increase in Ca<sup>2+</sup> is required for up-regulation of transcription in neurons (Wheeler et al. 2008). These findings suggest that specifically bound Ca<sup>2+</sup>-dependent regulatory proteins may respond to local Ca<sup>2+</sup> entering via Ca<sub>v</sub>1 channels and regulate transcription. Calmodulin is a resident Ca<sup>2+</sup>-dependent regulator of Ca<sub>v</sub>1 channels (Pitt et al. 2001), and calmodulin binding to the proximal carboxy-terminal domain of Ca<sub>v</sub>1.2 channels is required for regulation of transcription in neurons (Bito et al. 1996; Dolmetsch et al. 2001). Thus, calmodulin itself might serve as a regulator by binding local Ca<sup>2+</sup>, changing conformation to the active form, and moving to the nucleus (Bito et al. 1996; Deisseroth et al. 1998). However, there are large pools of free and Ca<sup>2+</sup>-bound calmodulin throughout the cell, so additional mechanisms must be engaged to specifically move Ca<sup>2+</sup>/calmodulin complexes from the Ca<sub>v</sub>1 channels to the nucleus in the context of this mode of regulation. Calcineurin bound to the distal carboxy-terminal domain of Ca<sub>v</sub>1 channels also is a potential transcriptional regulator through dephosphorylation of regulatory proteins (Oliveria et al. 2007). In cultured hippocampal neurons, dephosphorylation of the nuclear factor of activated T cells (NFAT) by calcineurin bound to Ca<sub>v</sub>1.2 channels induces its dissociation, movement to the nucleus, and regulation of transcription (Oliveria et al. 2007). This pathway appears to have all of the necessary elements for selective regulation of gene transcription by Ca<sup>2+</sup> entering neurons via Ca<sub>v</sub>1.2 channels and has the precedent that it is a crucial element in gene regulation in lymphocytes by a similar mechanism.

The distal carboxy-terminal domain of the Ca<sub>v</sub>1 channel itself has also been proposed as

a transcriptional regulator (Gomez-Ospina et al. 2006). The large carboxy-terminal domain of  $\text{Ca}_v1.1$  and  $\text{Ca}_v1.2$  channels is proteolytically processed in vivo near its center (De Jongh et al. 1991, 1996), leaving a noncovalently associated distal carboxy-terminal domain of more than 300 residues intact to regulate channel activity (Fig. 4B) (Hulme et al. 2006b). In neurons, this proteolytic cleavage process is regulated by  $\text{Ca}^{2+}$  and blocked by calpain inhibitors (Hell et al. 1996). The distal carboxy-terminal domain can be detected in the nuclei of a subset of neurons in the developing brain and in neurons in cell culture (Gomez-Ospina et al. 2006), opening the possibility of direct effects on transcription in the nucleus. Indeed, the distal carboxy-terminal domain can regulate the transcription of a substantial set of other genes in neurons (Gomez-Ospina et al. 2006), as well as the transcription of the gene encoding the  $\text{Ca}_v1.2$  channel itself in cardiac myocytes (Schroder et al. 2009). This regulatory mechanism also has all of the necessary elements to give selective regulation of gene expression by  $\text{Ca}_v1.2$  channels, but it remains unknown how the parallel effects of the distal carboxyl-terminus on regulation of channel activity versus migration to the nucleus and regulation of transcription are controlled. At least in neurons, it seems that only a small fraction of the distal carboxy-terminal is located in the nucleus (Gomez-Ospina et al. 2006), so it may be that most of the proteolytically processed distal carboxy-terminal domain remains associated with  $\text{Ca}_v1.2$  channels as an autoinhibitory regulator of channel activity while a small fraction dissociates and moves to the nucleus to regulate transcription.

### $\text{Ca}_v1$ CHANNELS IN EXCITATION-SECRETION COUPLING

$\text{Ca}^{2+}$  entry via  $\text{Ca}_v1$  channels initiates secretion of hormones from endocrine cells (Artalejo et al. 1994; Yang and Berggren 2006) and release of neurotransmitters at specialized ribbon synapses in sensory-transduction neurons (Table 1) (Kollmar et al. 1997; Barnes and Kelly 2002). The relative role of individual  $\text{Ca}_v1$  channel

subtypes in secretion, as well as the contribution of  $\text{Ca}_v2$  channels, differs among cell types and species. In the pancreas, the requirement for L-type  $\text{Ca}^{2+}$  currents for insulin secretion is greater in mouse than in human  $\beta$  cells (Eliason et al. 2008; Braun et al. 2009). In adrenal chromaffin cells, L-type  $\text{Ca}^{2+}$  currents conducted by  $\text{Ca}_v1.2$  and  $\text{Ca}_v1.3$  channels trigger secretion of catecholamines, and their activity is strongly regulated by second messenger signaling pathways, including cAMP (Marcantoni et al. 2007).

Neurotransmitter release at specialized ribbon synapses is continuous, similar to hormone secretion in some physiological circumstances, and  $\text{Ca}_v1$  channels are specifically required for this mode of synaptic transmission. In photoreceptors,  $\text{Ca}_v1.4$  channels are primarily responsible for  $\text{Ca}^{2+}$  entry that triggers exocytosis of neurotransmitters (Table 1) (Barnes and Kelly 2002). Mutations in the  $\text{Ca}_v1.4$  channel in humans lead to stationary night blindness (Bech-Hansen et al. 1998; Striessnig et al. 2010). In auditory hair cells,  $\text{Ca}_v1.3$  channels conduct the L-type  $\text{Ca}^{2+}$  currents that trigger neurotransmitter release (Kollmar et al. 1997). Deletion of the gene encoding  $\text{Ca}_v1.3$  channels causes deafness in mice (Platzer et al. 2000). The distal carboxy-terminal domain plays an auto-regulatory role in both  $\text{Ca}_v1.3$  and  $\text{Ca}_v1.4$  channels (Singh et al. 2006, 2008), but it is not known whether it is subject to proteolytic processing in vivo.  $\text{Ca}_v1.3$  channels are regulated by multiple interacting proteins (Cui et al. 2007; Jenkins et al. 2010), which may be important in tuning their activity to fit the specific requirements of hair cells transmitting auditory information at different frequencies.

### $\text{Ca}_v2$ CHANNELS IN SYNAPTIC TRANSMISSION

Presynaptic  $\text{Ca}^{2+}$  channels conduct P/Q-, N-, and R-type  $\text{Ca}^{2+}$  currents, which initiate synaptic transmission (Table 1). The efficiency of neurotransmitter release depends on the third or fourth power of the entering  $\text{Ca}^{2+}$ . This steep dependence of neurotransmission on  $\text{Ca}^{2+}$  entry makes the presynaptic  $\text{Ca}^{2+}$  channel an

exceptionally sensitive and important target of regulation. In the nervous system,  $\text{Ca}_v2.1$  channels conducting P/Q-type  $\text{Ca}^{2+}$  currents and  $\text{Ca}_v2.2$  channels conducting N-type  $\text{Ca}^{2+}$  currents are the predominant pathways for  $\text{Ca}^{2+}$  entry initiating fast release of classical neurotransmitters like glutamate, acetylcholine, and GABA. Extensive studies indicate that they are controlled by many different protein interactions with their intracellular domains, which serve as a platform for  $\text{Ca}^{2+}$ -dependent signal transduction (Fig. 4A).

### SNARE Proteins

$\text{Ca}^{2+}$  entry through voltage-gated  $\text{Ca}^{2+}$  channels initiates exocytosis by triggering the fusion of secretory vesicle membranes with the plasma membrane through actions on the SNARE protein complex of syntaxin, SNAP-25, and VAMP/synaptobrevin (reviewed in Bajjalieh and Scheller 1995; Sudhof 1995, 2004). The function of the SNARE protein complex is regulated by interactions with numerous proteins, including the synaptic vesicle  $\text{Ca}^{2+}$ -binding protein synaptotagmin. Presynaptic  $\text{Ca}_v2.1$  and  $\text{Ca}_v2.2$  channels interact directly with the SNARE proteins through a specific synaptic protein interaction (synprint) site in the large intracellular loop connecting domains II and III (Fig. 4A) (Sheng et al. 1994; Rettig et al. 1996). This interaction is regulated by  $\text{Ca}^{2+}$  and protein phosphorylation (Sheng et al. 1996; Yokoyama et al. 1997, 2005). Synaptotagmin also binds to the synprint site of  $\text{Ca}_v2$  channels (Charvin et al. 1997; Sheng et al. 1997; Wisner et al. 1997). Injection into presynaptic neurons of peptides that block SNARE protein interactions with  $\text{Ca}_v2$  channels inhibits synaptic transmission, consistent with the conclusion that interaction with SNARE proteins is required to position docked synaptic vesicles near  $\text{Ca}^{2+}$  channels for fast exocytosis (Mochida et al. 1996; Rettig et al. 1997). These results define a second functional activity of the presynaptic  $\text{Ca}^{2+}$  channel—targeting docked synaptic vesicles to a source of  $\text{Ca}^{2+}$  for effective transmitter release.

In addition to this functional role of interaction between  $\text{Ca}^{2+}$  channels and SNARE

proteins in the anterograde process of synaptic transmission, these interactions also have retrograde regulatory effects on  $\text{Ca}^{2+}$  channel function. Coexpression of the plasma membrane SNARE proteins syntaxin or SNAP-25 with  $\text{Ca}_v2.1$  or  $\text{Ca}_v2.2$  channels reduces the level of channel expression and inhibits  $\text{Ca}^{2+}$  channel activity by shifting the voltage dependence of steady-state inactivation during long depolarizing prepulses toward more negative membrane potentials (Bezprozvanny et al. 1995; Wisner et al. 1996; Zhong et al. 1999). The inhibitory effects of syntaxin are relieved by coexpression of SNAP-25 and synaptotagmin to form a complete SNARE complex (Wisner et al. 1997; Tobi et al. 1999; Zhong et al. 1999), which has the effect of enhancing activation of  $\text{Ca}_v2$  channels with nearby docked synaptic vesicles that have formed complete SNARE complexes and are ready for release. These processes fine-tune the efficiency of neurotransmitter release at frog neuromuscular junctions, where peptide and cDNA reagents can be used to modify synaptic function in vivo (Keith et al. 2007).

### G Protein Modulation

N-type and P/Q-type  $\text{Ca}^{2+}$  currents are regulated through multiple G protein coupled pathways (Hille 1994; Jones et al. 1997; Ikeda and Dunlap 1999). Although there are several G protein signaling pathways that regulate these channels, one common pathway that has been best studied at both cellular and molecular levels is voltage dependent and membrane delimited (i.e., a pathway without soluble intracellular messengers whose effects can be reversed by strong depolarization). Inhibition of  $\text{Ca}^{2+}$  channel activity is typically caused by a positive shift in the voltage dependence and a slowing of channel activation (Bean 1989b). These effects are relieved by strong depolarization resulting in facilitation of  $\text{Ca}^{2+}$  currents (Marchetti et al. 1986; Bean 1989b). Synaptic transmission is inhibited by neurotransmitters through this mechanism. G-protein  $\alpha$  subunits are thought to confer specificity in receptor coupling, but  $\text{G}\beta\gamma$  subunits are responsible for modulation of  $\text{Ca}^{2+}$  channels. Cotransfection of cells with





the  $\text{Ca}^{2+}$  channel  $\alpha 1$  and  $\beta$  subunits plus  $\text{G}\beta\gamma$  causes a shift in the voltage dependence of  $\text{Ca}^{2+}$  channel activation to more positive membrane potentials and reduces the steepness of voltage-dependent activation, effects that closely mimic the actions of neurotransmitters and guanyl nucleotides on N-type and P/Q-type  $\text{Ca}^{2+}$  channels in neurons and neuroendocrine cells (Herlitze et al. 1996). In contrast, transfection with a range of  $\text{G}\alpha$  subunits does not have this effect. This voltage shift can be reversed by strong positive prepulses resulting in voltage-dependent facilitation of the  $\text{Ca}^{2+}$  current in the presence of  $\text{G}\beta\gamma$ , again closely mimicking the effects of neurotransmitters and guanyl nucleotides on  $\text{Ca}^{2+}$  channels. Similarly, injection or expression of  $\text{G}\beta\gamma$  subunits in sympathetic ganglion neurons induces facilitation and occludes modulation of N-type channels by norepinephrine, but  $\text{G}\alpha$  subunits do not (Herlitze et al. 1996; Ikeda 1996). These results point to the  $\text{G}\beta\gamma$  subunits as the primary regulators of presynaptic  $\text{Ca}^{2+}$  channels via this voltage-dependent pathway through direct protein–protein interactions (Fig. 4A).

Possible sites of G protein  $\beta\gamma$  subunit interaction with  $\text{Ca}^{2+}$  channels have been extensively investigated by construction and analysis of channel chimeras, by G protein binding experiments, and by site-directed mutagenesis and expression (Fig. 4A). Evidence from G protein binding and site-directed mutagenesis experiments points to the intracellular loop between domains I and II ( $\text{L}_{\text{I-II}}$ ) as a crucial site of G protein regulation, and peptides from this region of  $\text{Ca}_v2.2$  prevent inhibition of channel activity by  $\text{G}\beta\gamma$ , presumably by binding to  $\text{G}\beta\gamma$  and competitively inhibiting its access to  $\text{Ca}^{2+}$  channels (De Waard et al. 1997; Herlitze et al. 1997; Zamponi et al. 1997). This region of the channel binds  $\text{G}\beta\gamma$  in vitro as well as in vivo in the yeast two-hybrid assay (De Waard et al. 1997; Zamponi et al. 1997; Garcia et al. 1998). Increasing evidence also points to segments in the amino- and carboxy-terminal domains of  $\text{Ca}^{2+}$  channels that are also required for G protein regulation (Zhang et al. 1996; Page et al. 1997, 1998; Qin et al. 1997; Canti et al. 1999; Li et al. 2004). As the amino- and carboxy-terminal domains

are likely to interact with each other in the folded channel protein, a second site of interaction for G proteins may be formed at their intersection.

### $\text{Ca}^{2+}$ Binding Proteins

$\text{Ca}^{2+}$ -dependent facilitation and inactivation of presynaptic  $\text{Ca}^{2+}$  channels was observed in patch clamp recordings of presynaptic nerve terminals in the rat neurohypophysis (Branchaw et al. 1997) and the calyx of Held synapse in the rat brainstem (Forsythe et al. 1998b). During tetanic stimulation at this synapse,  $\text{Ca}_v2.1$  channel currents show both  $\text{Ca}^{2+}$ -dependent facilitation and inactivation (Borst and Sakmann 1998; Cuttle et al. 1998; Forsythe et al. 1998a), which results in facilitation and depression of excitatory postsynaptic responses (Borst and Sakmann 1998; Cuttle et al. 1998; Forsythe et al. 1998b).  $\text{Ca}^{2+}$ -dependent facilitation and inactivation are also observed for cloned and expressed  $\text{Ca}_v2.1$  channels expressed in mammalian cells (Lee et al. 1999, 2000). A novel CaM-binding site was identified by yeast two-hybrid screening in the carboxy-terminal domain of the pore-forming  $\alpha_{1.2.1}$  subunit of  $\text{Ca}_v2.1$  channels (Lee et al. 1999). This CaM-binding domain (CBD) (Fig. 4A) is located on the carboxy-terminal side of the sequence in  $\alpha_{1.2.1}$  that corresponds to the IQ-domain that is required for CaM modulation of cardiac  $\text{Ca}_v1.2$  channels (Peterson et al. 1999; Qin et al. 1999; Zühlke et al. 1999). The modified IQ domain of  $\alpha_{1.2.1}$  begins with the amino acid sequence IM instead of IQ and has other changes that would be predicted to substantially reduce its affinity for CaM. CaM binding to the CBD is  $\text{Ca}^{2+}$ -dependent. Both  $\text{Ca}^{2+}$ -dependent facilitation and inactivation are blocked by coexpression of a CaM inhibitor peptide (Lee et al. 1999), suggesting that  $\text{Ca}^{2+}$ -dependent modulation of  $\text{Ca}_v2.1$  channels in neurons is caused by two sequential interactions with CaM or a related  $\text{Ca}^{2+}$ -binding protein.

The mechanism for  $\text{Ca}^{2+}$ -dependent facilitation and inactivation of  $\text{Ca}_v2.1$  channels involves CaM binding to two adjacent subsites—the CBD and the upstream IQ-like motif

(Lee et al. 2003). The IQ-like motif is required for facilitation, whereas the CBD is required for inactivation. In addition, the two lobes of CaM are also differentially involved in these two processes. Mutation of the two EF hands in the carboxy-terminal lobe primarily prevents facilitation, whereas mutation of the EF hands in the amino-terminal lobe primarily prevents inactivation (DeMaria et al. 2001; Erickson et al. 2001; Lee et al. 2003). FRET studies indicate that apo-calmodulin can bind to Ca<sub>v</sub>2.1 channels in intact cells and binding is enhanced by Ca<sup>2+</sup> binding to calmodulin (Erickson et al. 2001). Altogether, these results support a model in which the two lobes of CaM interact differentially with the modified IQ domain and the CBD to effect bi-directional regulation, with the high-affinity carboxy-terminal lobe primarily controlling facilitation through interactions with the IQ-like domain and the lower-affinity amino-terminal lobe primarily controlling inactivation through interactions with the CBD. This biphasic regulation of Ca<sub>v</sub>2.1 channels causes synaptic facilitation and depression in transfected sympathetic ganglion neuron synapses in which neurotransmission is initiated by transfected Ca<sub>v</sub>2.1 channels (Mochida et al. 2008).

CaM is the most well-characterized member of a superfamily of Ca<sup>2+</sup> sensor (CaS) proteins, many of which differ from CaM in having neuron-specific localization, amino-terminal myristoylation, and amino acid substitutions that prevent Ca<sup>2+</sup> binding to one or two of the EF hands (Haeseleer and Palczewski 2002). The CaS protein CaBP1 binds to the CBD, but not the IQ-like domain, of α<sub>1</sub>2.1 and its binding is Ca<sup>2+</sup>-independent (Lee et al. 2002). CaBP1 causes a strong enhancement of the rate of inactivation, a positive shift in the voltage-dependence of activation, and a loss of Ca<sup>2+</sup>-dependent facilitation of Ca<sub>v</sub>2.1 channels, which would combine to reduce the activity of these channels. Because it coimmunoprecipitates and colocalizes with Ca<sub>v</sub>2.1 channels in the brain (Lee et al. 2002), CaBP1 may be an important determinant of Ca<sub>v</sub>2.1 channel function in neurons and may contribute to the diversity of function of these channels in the

nervous system. Visinin-like protein 2 (VILIP-2) is a neuronal Ca<sup>2+</sup>-binding protein that is distantly related to CaBP-1 (Haeseleer and Palczewski 2002). Consistent with these structural differences, VILIP-2 has opposite effects on Ca<sub>v</sub>2.1 channels than CaBP-1 (Lautermilch et al. 2005). Coexpression of VILIP-2 causes slowed inactivation and enhanced facilitation, but its binding and effects are Ca<sup>2+</sup>-independent like CaBP-1. VILIP-2 may serve as a positive modulator of synaptic transmission, prolonging Ca<sup>2+</sup> channel opening, and enhancing facilitation. Differential expression of CaBP1 and VILIP-2 at synapses would lead to opposite modulation of synaptic transmission in response to trains of action potentials and opposing input–output functions at the synapse.

## Ca<sub>v</sub>3 CHANNELS AND FREQUENCY MODULATION

### Molecular Properties of Ca<sub>v</sub>3 Channels

Ca<sup>2+</sup> channels of the Ca<sub>v</sub>3 subfamily conduct T-type Ca<sup>2+</sup> currents (Catterall et al. 2005). These Ca<sup>2+</sup> currents are activated at comparatively negative membrane potentials, in the same range as Na<sup>+</sup> currents in most cells, and they have fast voltage-dependent inactivation compared to other Ca<sup>2+</sup> currents (Nowycky et al. 1985). These Ca<sup>2+</sup> currents are therefore well-suited for rhythmic firing of action potentials. They are also well-suited for generation of large Ca<sup>2+</sup> transients because they are activated at negative membrane potentials where the driving force for Ca<sup>2+</sup> entry is large. A family of three Ca<sub>v</sub>3 channel α1 subunits have been characterized by cDNA cloning and sequencing (Catterall et al. 2005). Remarkably, these Ca<sup>2+</sup> channel subunits have the same molecular organization as Ca<sub>v</sub>1 and Ca<sub>v</sub>2 channels but are only ~25% identical in amino acid sequence (Catterall et al. 2005). This is a similar level of amino acid sequence identity as Ca<sup>2+</sup> channels have with Na<sup>+</sup> channels, indicating that these subfamilies of Ca<sup>2+</sup> channels separated from each other at the same point of evolution as Na<sup>+</sup> channels separated from Ca<sup>2+</sup> channels. Although Ca<sub>v</sub>3 channels are similar

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in structure to  $Ca_v1$  and  $Ca_v2$  channels, there is no clear evidence at present that they interact with the same set of auxiliary subunits. In fact, the prevailing view is that the  $\alpha 1$  subunits function independently of other subunits. This would be unique among the families of  $Na^+$  and  $Ca^{2+}$  channels.

### Functional Roles of $Ca_v3$ Channels

As expected from their functional properties,  $Ca_v3$  channels are important in repetitively firing tissues. In the sino-atrial node of the heart, they conduct an important component of the pacemaker current that generates the heartbeat (Mangoni et al. 2006). In the relay neurons of the thalamus, they are crucial for generation of the rhythmic bursts of action potentials that drive sleep spindles and control sleep (Lee et al. 2004). Moreover, mutations in  $Ca_v3$  channels cause absence epilepsy, in which the affected individuals transiently enter a sleep-like state that interrupts their normal activities (Kim et al. 2001; Song et al. 2004). In the adrenal cortex, they are important in regulation of synthesis and secretion of aldosterone (Welsby et al. 2003).

### Regulation of $Ca_v3$ Channels

In neurons, dopamine and other neurotransmitters inhibit T-type  $Ca^{2+}$  currents via a pathway that is specific for the  $G\beta 2$  subunit (Wolfe et al. 2003). As for  $Ca_v2$  channels, G protein  $\beta\gamma$  subunits bind directly to  $Ca_v3$  channels and regulate them (DePuy et al. 2006). The site of interaction is in the intracellular loop connecting domains II and III (DePuy et al. 2006). In addition, in adrenal glomerulosa cells, angiotensin II regulates aldosterone secretion via enhanced activation of  $Ca_v3.2$  channels (Welsby et al. 2003). This regulation is mediated by a signaling complex of CaMKII bound to the intracellular loop connecting domains II and III (Yao et al. 2006). Phosphorylation of a single serine residue in this intracellular loop negatively shifts the voltage dependence of activation and thereby substantially increases  $Ca^{2+}$  current at negative membrane potentials (Yao et al.

2006). It is unknown at this stage whether binding of CaMKII is required for physiological regulation or whether binding of the kinase per se has any regulatory effect.

### THE EFFECTOR CHECKPOINT MODEL OF $Ca^{2+}$ CHANNEL REGULATION

In closing this article on  $Ca^{2+}$  signaling via voltage-gated  $Ca^{2+}$  channels, it is interesting to introduce an emerging theme that unites several aspects of the localized regulation of these proteins.  $Ca^{2+}$  channel signaling complexes are formed when the effectors and regulators of the  $Ca^{2+}$  signal bind to the intracellular domains of  $Ca^{2+}$  channels to effectively receive and respond to the local  $Ca^{2+}$  signal. In four cases, binding of the effectors of the  $Ca^{2+}$  signal has been shown to enhance the activity of the  $Ca_v1$  and  $Ca_v2$  channels. First, in skeletal muscle, interactions of the plasma membrane  $Ca_v1.1$  channel with the ryanodine-sensitive  $Ca^{2+}$  release channel in the sarcoplasmic reticulum, which serves as the effector of excitation-contraction coupling, greatly increase the functional activity of the  $Ca_v1.1$  channels (Nakai et al. 1996a). Second, as described above, interaction with individual plasma membrane SNARE proteins inhibits the activity of  $Ca_v2$  channels, but formation of complete SNARE complex containing synaptotagmin, the effector of exocytosis, relieves this inhibition and enhances  $Ca^{2+}$  channel activity (Bezprozvanny et al. 1995; Wiser et al. 1996, 1997; Zhong et al. 1999). Third, binding of  $Ca^{2+}$ /CaM-dependent protein kinase II, an effector of  $Ca^{2+}$ -dependent regulatory events, to a site in the carboxy-terminal domain of  $Ca_v2.1$  channels substantially increases their activity (Jiang et al. 2008). Finally, binding of RIM, a regulator of SNARE protein function, to the  $Ca_v\beta$  subunits substantially increases  $Ca_v2$  channel activity (Kiyonaka et al. 2007). The common thread in all of these diverse examples of  $Ca^{2+}$  channel regulation by interacting proteins is that binding of an effector ready to respond to the  $Ca^{2+}$  signal enhances the activity of the  $Ca^{2+}$  channel. Thus, this mechanism provides a functional checkpoint of the fitness of a

Ca<sup>2+</sup> channel to carry out its physiological role, and enhances its activity if it passes this checkpoint criterion. This “effector checkpoint” mechanism would serve to focus Ca<sup>2+</sup> entry on the Ca<sub>V</sub> channels that are ready to use the resulting Ca<sup>2+</sup> signal to initiate a physiological intracellular signaling process. It seems likely that further studies will reveal more examples of this form of regulation and that it may be a unifying theme in the regulation of Ca<sup>2+</sup> signaling by Ca<sub>V</sub> channels.

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