Regulation of voltage-gated calcium channels by proteolysis

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Abstract

Voltage gated calcium channels (VGCCs) are multi-subunit membrane proteins present in a variety of tissues and control many essential physiological processes. Due to their vital importance, VGCCs are regulated by a myriad of proteins and signaling pathways. Here we review the literature on the regulation of VGCCs by proteolysis of the pore-forming α_1 subunit, $Ca_v\alpha_1$. This form of regulation modulates channel function and degradation and affects cellular gene expression and excitability. L-type Ca^{2+} channels are proteolyzed in two ways, depending on tissue localization. In the heart and skeletal muscle, the distal C-terminus of $Ca_v\alpha_1$ is cleaved and acts as an autoinhibitor when it reassociates with the proximal C-terminus. Relief of this autoinhibition underlies the β-adrenergic stimulation-induced enhancement of cardiac and skeletal muscle calcium currents, part of the "fight or flight" response. Proteolysis of the distal C-terminus of L-type channels also occurs in the brain and is probably catalyzed by a calpain-like protease. In some brain regions, the entire C-terminus of L-type Ca^{2+} channels can be cleaved by an unknown protease and translocates to the nucleus acting as a transcription factor. The distal C-terminus of P/Q-channel $Ca_v\alpha_1$ is also proteolyzed and translocates to the nucleus. Truncated forms of the PQ-channel $Ca_v\alpha_1$ are produced by many disease-causing mutations and interfere with the function of full-length channels. Truncated forms of N-type channel $Ca_v\alpha_1$, generated by mutagenesis, affect the expression of full-length channels. New forms of proteolysis of VGCC subunits remain to be discovered and may represent a fruitful area of VGCC research.

Keywords

ion channels; calcium signaling; proteolytic processing; modulation; channelopathy; gene expression

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Proteolysis as a regulatory mechanism has been reported for a variety of ion channels. Epithelial Na^+ channels are targeted by the protease furin, which cleaves a small section of the α_1 subunit, releasing this inhibitory segment and activating the channel [1]. The AMPA receptor undergoes a C-terminal cleavage mediated by calpain which alters channel properties [2]. The voltage-gated Na^+ channel α_1 subunit is also proteolyzed by calpain[3], and its associated β_2 subunit is proteolyzed by β- and γ-secretase [4]. This review surveys studies on proteolysis of voltage-gated calcium channels (VGCCs), focusing on L-, N-, and P/Q-type channels.

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VGCCs control many critical physiological processes, including hormone and neurotransmitter release, cell migration, gene transcription, and muscle contraction [5]. In response to changes in membrane voltage, and under the regulation of many other proteins, these channels allow the heart to beat, the kidney to function, and neurons to communicate with one another. With such a diverse range of functions, it is of no surprise that VGCCs must be intricately regulated by a variety of mechanisms. For example, the G protein heterodimer, $G\beta\gamma$, acts to stabilize the channel in a closed conformation and it is only after $G\beta\gamma$ dissociates that the channel can return to a state willing to open [6]. Protein kinase C phosphorylates sites on the I–II linker of VGCC α_1 subunit ($Ca_v\alpha_1$), reversing G-protein inhibition, most likely by interfering with the $G\beta\gamma$ binding domains [7]. The RGK proteins, comprised of Rem, Rem2, Rad and Gem/Kir, are Ras-like small GTP-binding proteins [8] and act as inhibitors of VGCCs [9]. Calmodulin (CaM) assists with Ca^{2+} dependent inactivation by acting as a Ca^{2+} sensor to inactivate the channel when intracellular Ca^{2+} levels reach a certain concentration. Ca^{2+} dependent inactivation is a rapid process, occurring within milliseconds of Ca^{2+} influx [10]. CaM binds to the IQ region on the C-terminus, but once the two high-affinity Ca^{2+} binding sites on CaM are occupied, translocates to a different position on the α_1 subunit to inactivate the channel [11]. CaM also plays a role in Ca^{2+} dependent facilitation, whereby an increase in intracellular Ca^{2+} levels leads to channel opening [12]. Proteases are yet another subset of proteins that act on VGCCs to modulate their function, modify gene expression, or to prepare the channel for degradation.

1 VGCC subunits

VGCCs consist of an assembly of subunits that together form a functional channel. The main $Ca_v\alpha_1$ subunit associates with auxiliary subunits β, α_2-δ and sometimes γ to form the VGCC complex. Although α_1 alone is able to conduct current, the auxiliary subunits allow for proper activation and inactivation properties [13]. Co-expression of β is necessary for α_1 trafficking from the endoplasmic reticulum to the plasma membrane; in heterologous systems little α_1 is expressed on the cell surface without β present. $Ca_v\alpha_1$ contains the voltage sensor and the ion conduction pore and is responsible for most of the biophysical and pharmacological properties of VGCCs [5]. $Ca_v\alpha_1$ consists of four homologous repeats, each containing six transmembrane segments (S1–S6). The cytoplasmic N- and C-termini and the intracellular linkers connecting the repeats contain binding sites for a multitude of proteins responsible for channel modulation and regulation. The pore-lining loops (P loops) between S5 and S6 contain highly conserved glutamic acid residues and are responsible for the high Ca^{2+} selectivity of VGCCs [14]. The S4 segments contain positively charged lysines and arginines that act as the voltage sensor to open and close the channel [15]. The S6 segment is involved in channel inactivation, whereby the I–II linker connecting the first two repeats presumably docks to the intracellular end of S6 in response to depolarization, essentially blocking the pore [16].

Ten different genes for α_1 have been identified [5]. The α_1 subunit dictates the type of currents conducted through the channel and whether the channel is high-voltage activated (HVA) or low-voltage activated (LVA). $Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$, and $Ca_v1.4$ form L-type channels, $Ca_v2.1$ forms P/Q-type channels, $Ca_v2.2$ forms N-type channels, and $Ca_v2.3$ forms

R-type channels, all of which constitute HVA channels. $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ form LVA, T-type channels.

The cytosolic β subunit is a multifunctional protein [17]. It acts as a chaperone to $α_1$, trafficking it out of the endoplasmic reticulum and to the plasma membrane. The β subunit also greatly affects activation and inactivation properties of the channel. There are four known β subunit genes, each with splice variants, termed $β_1$, $β_2$, $β_3$ and $β_4$. Each subtype of VGCC associates with one specific β. β directly interacts with $α_1$ on the I–II linker through high-affinity interactions with a binding site called the $α_1$-interaction domain, or AID. This high-affinity binding is responsible for β's chaperone function. It also enables otherwise intrinsically low-affinity interactions between other regions of $α_1$ and β to fine tune channel biophysical properties.

The $α_2$ and δ subunits are generally considered one subunit as they are encoded by the same gene [18]. However, posttranslational cleavage of the single polypeptide produces a 143-kDa $α_2$ and a 27-kDa δ subunit, which are then linked by a disulfide bond to form the $α_2$-δ complex. Several splice variants of $α_2$-δ are known, many with specific tissue expression. The $α_2δ$ subunit assists with $α_1$ trafficking to the plasma membrane, increases currents of $α_1/β$ recombinant channels, and influences channel inactivation. The $α_2$-δ subunit is a target for pain and epilepsy drugs such as pregabalin and gabapentin.

The γ subunit is a hydrophobic glycoprotein with four transmembrane domains with cytoplasmic N- and C-termini [17]. There are eight known γ genes. Only the non-neuronal L-type channels associate with the γ subunit. The most robust functional effect of γ on VGCCs is a small reduction of currents.

2 Subtypes of VGCCS

Two types of VGCCs exist, HVA and LVA, and they are further differentiated based on the type of $α_1$ subunit they contain and on their biophysical and pharmacological properties. T-type currents are classified as LVA channels, and L-, R-, N-, and P/Q-type currents are classified as HVA channels. This review focuses on proteolysis of HVA channels.

L-type channels have four different gene transcripts, all signifying different $α_1$ subtypes. These are labeled $Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$, and $Ca_V1.4$, which express $α_{1S}$, $α_{1C}$, $α_{1D}$, and $α_{1F}$ respectively. $Ca_V1.1$ is predominately found in skeletal muscle and $Ca_V1.2$ is located in cardiac tissue and brain. $Ca_V1.3$ is generally in the central nervous system [5] and auditory hair cells [19]. $Ca_V1.4$ is expressed in the retina and is involved in phototransduction [20]. Neuronal L-type channels ($Ca_V1.2$ and $Ca_V1.3$) are found at postsynaptic synapses and in the soma. They are also recruited to microdomains along distal dendrites and spines, which allows for easier synapse-to-nucleus communication [21]. L-type channels play a role in gene transcription in neurons [22] and are involved in hormone secretion in endocrine cells [23]. They are known to be sensitive to phenylalkylamines, benzothiazapines, and dihydropyridines [24] and are blocked by the spider toxin ω-agatoxin IIIA [25]. L-type channels are implicated in many disease states, including Duchenne muscular dystrophy [26], arrhythmias and hypertension [27], Timothy syndrome [28], hypokalemic periodic paralysis and night blindness [29].

P/Q-, R- and N-type Ca^{2+} channels make up the Ca_V2 family and are widely expressed in neurons and chromaffin cells [30]. P/Q-type channels are classified as $Ca_V2.1$ and express α_{1A}, N-type channels are classified as $Ca_V2.2$ and express α_{1B}, and R-type channels are classified as $Ca_V2.3$ and express α_{1E}. P- and Q-type channels are derived from alternative splice variants of the same gene [31], and can be distinguished based on their sensitivities to various toxins. P-type channels are more sensitive to ω-AgaIVA, whereas Q-type channels are sensitive to ω-CMVIIC [32]. N-type channels are specifically blocked by the marine snail toxin, ω-conotoxin GVIA [33]. R-type channels are resistant to these toxins, thus termed "R" for resistant. P/Q- and N-type channels are abundant in presynaptic terminals and dendrites and are involved in neurotransmitter release [34]. While $Ca_V2.2$ knockouts do not display an adverse phenotype besides higher threshold for pain [35], $Ca_V2.1$ knockout mice survive for only a few weeks, and display seizures and ataxia while alive [36]. Also, a lack of N- and R-type channels can be compensated for, whereas deficiencies in P/Q-type channels cannot [35].

P/Q-type channels ($Ca_V2.1$) are found throughout the entire brain but are expressed at particularly high levels in the cerebellum [37]. $Ca_V2.1$ channels are also found in brain regions associated with the perception of pain, such as the trigeminal ganglia and brainstem nuclei [37]. They play a major role at excitatory synapses (glutamate) and a lesser role at inhibitory synapses (GABA) [38]. There are restrictions on the number of P/Q-type channels that can occupy specific slots at synapses, therefore if some channels are of mutated form, a decrease in activity will result [39].

N-type channels are found in neurons and neuroendocrine cells, and they are predominately involved in neurotransmitter release [40]. They have also been identified in saccular hair cells [41]. In the spinal cord [42] they are located in dorsal root ganglia and at synaptic terminals in dorsal horn neurons that connect to afferent sensory fibers [43]. In the dorsal horn, they are found predominately in substance P containing cells [42, 44]. N-type channels are responsible for the majority of synaptic processing of nociceptive information in dorsal horn neurons [45, 46]. The natural N-type channel antagonists, ω-conotoxins, inhibit $Ca_V2.2$ channels and prevent release of pronociceptive neurotransmitters from central nerve terminals of primary afferent neurons [47].

3 L-type channel proteolysis

Two forms of $Ca_V1.2$ channel exist in the heart, full length and truncated. Approximately 80% of $Ca_V1.2$ channels are truncated in the heart [48]; thus, the truncated channel is the predominant physiological form. This truncated version is produced by a proteolytic cleavage of the full length protein in the C-terminus between residues 1 685 and 1 699, producing a distal C-terminal fragment of ~45 kDa [48]. In skeletal muscle, the C-terminus of $Ca_V1.1$ is cleaved between A1664 and A1665, producing a similar size fragment [49]. The C-terminus is an important element for VGCC function, affecting channel properties through interactions with calmodulin [50], the β subunit [51], cAMP-dependent protein kinase (PKA) [52], and by being involved in targeting the channel to the plasma membrane [53]. What purpose would this proteolysis serve if the C-terminus is so important for channel function?

In the heart and skeletal muscle, excitation-contraction coupling is initiated by the opening of L-type channels, whose activity is greatly increased by the stimulation of the sympathetic nervous system [54]. This current potentiation is thought to underlie the "fight or flight" phenomenon. Basically, when faced with a threat, epinephrine and norepinephrine are released and activate the β-adrenergic system, which in turn enhances L-type channel activity allowing cardiac and skeletal muscle contraction to increase and the heart to beat faster [55]. This mechanism has now been parsed out and the cleaved Ca_V1 C-terminal fragment plays a major role in the "fight or flight" phenomenon.

Ca_V1 potentiation in both the heart and skeletal muscle is controlled by a rapid phosphorylation by PKA [52, 56]. Two conserved phosphorylation sites (S1575 and T1579 in $Ca_V1.1$ and S1700 and T1704 in $Ca_V1.2$) are regulated by the β-adrenergic system. S1575 phosphorylation is increased with isoproterenol, a β-adrenergic receptor agonist, and decreased with propranolol, an antagonist [57]. These experiments were more relevant than past *in vitro* phosphorylation assays [58, 59] because they were done using isolated rabbit skeletal muscle, whereby the animal was injected with these drugs while moving and breathing, and therefore are more physiologically relevant. While PKA has been primarily implicated in the β-adrenergic system, it was found that S1575 can be phosphorylated by both PKA and CAMKII [57]. On the other hand, T1579 is phosphorylated by casein kinase 2 [57]. PKA phosphorylation dramatically increases Ca_V1 current [59], presumably by relieving the autoinhibition produced by the proteolytically cleaved distal C-terminal fragment, as will be discussed below.

PKA phosphorylation occurs rapidly due to the fact that PKA is stationed very close to the channel. An anchoring protein, AKAP-15, was found to be critical for PKA phosphorylation. When a peptide corresponding to a 24-amino acid segment of a human AKAP protein was applied into myotubes through a patch electrode, it was able to inhibit channel current to the same extent as PKA inhibitors did [56]. AKAP-15 co-immunoprecipitated with $Ca_V1.1$ from skeletal muscle [60] and from tsa-201 cells [61], and confocal microscopy revealed it colocalized with $Ca_V1.1$ in distinct areas [61]. Yeast two-hybrid screen showed that AKAP-15 bound to the distal C-terminal domain of $Ca_V1.1$ (residues 1 774–1 841) [62]. This interaction occurs through a leucine zipper (LZ) motif on AKAP-15 and a LZ-like motif on the $Ca_V1.1$ C-terminus. When either of the two regions was mutated to alanine, or a synthetic peptide comprising the LZ motif of AKAP-15 was introduced to compete with AKAP-15, PKA was unable to anchor to the channel, disrupting L-type channel phosphorylation and potentiation [61, 62].

$Ca_V1.2$ is targeted by AKAP-15 and PKA and is phosphorylated in the same manner as $Ca_V1.1$ is. This mechanism has recently been reconstituted in tsa-201 cells with $Ca_V1.2$ [58] after transfection of the necessary molecular players, namely AKAP-15, PKA, $Ca_V1.2$ (1–1 800) and the distal C-terminus. Furthermore, mice with $Ca_V1.2$ lacking the distal C-terminus developed cardiac hypertrophy and their β-adrenergic pathway was disabled due to an inability of PKA to regulate $Ca_V1.2$ function [63]. Cardiac myocytes from these mice also showed decreased surface expression of this truncated $Ca_V1.2$, consistent with a role of the C-terminus in trafficking the channel to the plasma membrane. These experiments

confirmed the importance of $Ca_V1.2$ proteolysis and $Ca_V1.2$-S1700 phosphorylation in the β-adrenergic signaling pathway.

It seems strange that the sites of phosphorylation and AKAP-15 interaction occur in the distal C-terminus, a region that is cleaved from the channel under physiological conditions. It turns out that the cleaved C-terminus reassociates with the channel. The distal and proximal C-terminus co-immunoprecipitated when co-expressed in HEK-293 cells and also directly interacted in a yeast-two hybrid screen [49]. Functional studies revealed that when the C-terminus was removed from the channel by mutagenesis, an increase in peak current amplitude resulted [64]. This increase has also been demonstrated using tsa-201 cells with cardiac $Ca_V1.2$ channels truncated at 1 821 [65] or 1 905 [66]. When truncated channels were co-expressed with the C-terminal fragment, a 20-fold inhibition occurred; even full-length channels were further inhibited by the C-terminal fragment [65]. Channels missing only their last 147 residues displayed an increase in peak current amplitude compared to full-length channels, which could be reversed by the addition of the missing C-terminal fragment [67]. Arginines 1 696 and 1 697 on the proximal C-terminus interact with negatively charged residues (E2103, E2106, and D2110) on the distal C-terminal fragment to exert this inhibitory effect [65]. Thus, the distal C-terminal fragment cleaved from the $α_1$ subunit can reassociate with the channel and act as an autoinhibitor. Besides an overall decrease in peak current amplitude, the distal C-terminal fragment shifts the voltage dependence of activation causing the channels open at higher voltages [65].

The proteolytic cleavage described above for cardiac and skeletal muscle Ca_V1 channels also takes place in neurons. In response to NMDA receptor activation, which leads to increases in intracellular Ca^{2+}, $Ca_V1.2$ channels in the hippocampus underwent extensive C-terminal cleavage, while other classes of VGCCs were unaffected [68]. Thus, proteolytic cleavage of the distal C-terminus of L-type Ca^{2+} channels appears to be a widespread phenomenon.

There is evidence suggesting that calpain-like proteases may be responsible for Ca_V1 proteolysis. In hippocampal neurons, cleavage of $Ca_V1.2$ C-terminus is induced by Ca^{2+} influx through NMDA receptors and is blocked by calpain inhibitors [68]. Purified calpain increased Ca_V1 C-terminal proteolysis *in vitro* [68]. Cleavage of $Ca_V1.1$ at A1664 [49] is also consistent with the catalytic specificity of calpain. Calpain requires Ca^{2+} for its catalytic activity and is stimulated by membrane depolarization [69], presumably through depolarization-induced intracellular Ca^{2+} increase. Calpain-mediated Ca_V1 proteolysis may thus represent a feedback regulation of Ca_V1 channel activity.

To summarize, under normal physiological conditions, Ca_V1 is proteolyzed in the C-terminus (see Fig. 1), perhaps by calpain-like proteases. The cleaved distal C-terminus reassociates with the channel to inhibit its function. AKAP-15 binds to the C-terminal fragment and recruits PKA to the scene. In a "fight or flight" scenario the β-adrenergic system is activated, in turn activating PKA. PKA phosphorylates the channel, which dispels the distal C-terminus from the channel, relieving inhibition and greatly enhancing channel activity.

Proteolysis of Ca$_V$1.3 has not been reported, but a truncated form of the channel exists naturally due to alternative splicing [70]. The truncated Ca$_V$1.3 incorporates an alternative exon that encodes a stop codon after the IQ domain in the C-terminus. Truncated Ca$_V$1.3 channels inactivate much faster, due mainly to enhanced calcium-dependent inactivation, and they activate at more negative potentials than full length Ca$_V$1.3 does.

It has been well established that one of the many functions of L-type channels is to control gene expression; however, it was always thought to occur through calcium-mediated activation of transcription factors or signaling proteins [22]. Several years ago, the C-terminus of neuronal Ca$_V$1.2 itself was found to act as a transcription factor [71]. The C-terminal fragment, termed calcium channel associated transcriptional regulator (CCAT), was found to translocate to the nucleus of mainly inhibitory neurons. CCAT is larger than the cleaved distal C-terminal fragment described above; it appears as a ~75 kDa band in Western blots and comprises virtually the entire C-terminus of Ca$_V$1.2 (Fig. 1). The appearance of this fragment is developmentally regulated, gradually becoming more prominent in the nucleus starting at age P1 through adulthood. In addition, another 150 kDa fragment began to appear in rats around P21, suggesting that other forms of L-type channel proteolysis might exist. While it is assumed that the full-length channel is proteolyzed to generate CCAT, neither the cleavage site nor the protease was identified. CCAT translocation to the nucleus appeared to be activity-dependent. When L-type channels were stimulated with either KCl or glutamate, less CCAT was detected in the nucleus. Conversely, chelating intracellular calcium caused a significant increase in the amount of CCAT in the nucleus. The expression of 16 genes was upregulated and 31 genes was downregulated in Neuro2A cells transfected with CCAT, and CCAT itself bound to the enhancer of the Connexin 3.1 gene. In addition, CCAT appeared to increase neurite growth in cerebellar granule neurons.

A ~37 kDa C-terminal fragment of Ca$_V$1.2 has also been found to exhibit transcriptional activity in cardiac myocytes [72]. This C-terminal fragment was found to localize to the nucleus, interact with the Ca$_V$1.2 promoter, and ultimately act as a repressor, downregulating Ca$_V$1.2 levels. It is unclear how this fragment relates to the two C-terminal fragments described above. Nevertheless, it seems that the C-terminus of Ca$_V$1.2 is cleaved from the full-length channel and has the ability to enter the nucleus, affecting the expression of various genes, including its own gene.

4 PQ- AND N-type channel proteolysis

Two P/Q-type channels of different sizes, 220 kDa and 170 kDa, have been observed [73, 74]. In addition, a 95 kDa short form of the channel has been discovered that comprises the N-terminus to the middle of the II–III linker [75]. This short form interacts with β and is glycosylated. It has yet to be determined whether this 95 kDa form of Ca$_V$2.1 is a splice variant or the result of post-translational proteolysis, although a splice variant causing a truncation in the II–III linker has been reported [76]. However, the 170 kDa fragment could be the result of C-terminal proteolysis. A 60–75 kDa fragment was detected from mouse cerebellar lysates and was confirmed to include the C-terminus of Ca$_V$2.1 after staining with two different C-terminal antibodies [74]. The 60–75 kDa fragment appeared in cell nuclei,

suggesting that it might also affect gene expression. $Ca_V2.1$ C-terminus contains a histidine repeat (11 copies) that is common among transcription factors, serving as a nuclear localization signal [77]. In addition, three other putative nuclear localization signals were identified and when mutated decreased nuclear localization of the 60–75 kDa fragment by almost 60% [74].

Many P/Q-type channel truncations resulting from premature stop codon mutations have been implicated in disease. In episodic ataxia type 2 (EA2) there are over 50 different mutations leading to truncated channels [78]. When co-expressed with full-length channels, $Ca_V2.1$ truncation mutants markedly repressed peak current amplitude. This dominant-negative effect was dependent on the AID being present in the truncated construct [79]. One possibility is that the truncated channels compete with full-length channels for the β subunit. The dominant-negative effect of the truncated channels sheds light on a possible pathogenic mechanism for episodic ataxias. It also demonstrates a possible physiological effect of fragment channels that might be produced by proteolysis of $Ca_V2.1$.

Two main N-type α1 subunits have been identified, one 240 kDa and the other 210 kDa, that differ in their C-terminus [80]. While they are both substrates for PKA, protein kinase C and c-GMP dependent protein kinase, only the 240 kDaa isoform is phosphorylated by camodulin-dependent protein kinase II, indicating that the two isoforms are independently regulated [81]. A 140 kDa form of $Ca_V2.2$ has also been reported [82]. It is unknown whether the smaller forms are due to post-translational proteolysis or are specific splice variants.

While specific regulatory proteolysis has not been identified for $Ca_V2.2$ channels, truncated versions of the channel have been generated by mutagenesis and studied. When truncated $Ca_V2.2$ channels containing either the I–II repeat or the III–IV repeat were co-expressed with full-length $Ca_V2.2$, they markedly inhibited channel currents without changing their biophysical properties [83]. The truncated fragment containing the I–II repeat, $Ca_V2.2$(I–II), almost abolished membrane expression of full-length channels. This effect was not due to the sequestration of β since co-expression of the I–II linker alone had no effect and overexpression of β did not rescue full-length membrane expression [83]. Instead, the decreased expression of full-length $Ca_V2.2$ was due to $Ca_V2.2$(I–II) triggering the unfolded protein response by activating the RNA-dependent kinase PERK and inhibiting $Ca_V2.2$ exit from the endoplasmic reticulum [84]. The region of $Ca_V2.2$(I–II) responsible for this inhibition and decreased surface expression has now been identified as the first 95 residues of the N-terminus, specifically the arginines at position 52 and 54[85]. When these two residues are mutated to alanines, full-length $Ca_V2.2$ protein levels actually surpassed that of wild-type $Ca_V2.2$ when coexpressed with $α_2δ$ and $β_1$. These experiments further highlight how short forms of VGCCs can interfere or regulate full-length forms.

5 Concluding remarks

It is becoming increasingly apparent that proteolysis of HVA Ca^{2+} channels may be responsible for specific physiological functions. The proteolysis of the C-terminus of cardiac and skeletal muscle Ca_V1 channels resulting in autoinhibition and β-adrenergic regulation exemplifies the importance of VGCC proteolysis in channel regulation and in the fight or

flight response. The C-terminal proteolysis of neuronal $Ca_V1.2$ illustrates the potential importance of VGCC proteolysis in gene expression. Although proteolysis of Ca_V2 channels has not been extensively observed, many short forms of Ca_V2 channels are known to exist as a result of alternative splicing or disease-causing mutations. These truncated forms of Ca_V2 channels markedly change the function and expression of their full-length counterparts. With improved molecular biology tools and finer detection methods, future studies will likely uncover new forms of proteolytic processing of VGCCs. A major challenge is to distinguish regulated proteolysis from non-specific protein degradation. Another challenge is to demonstrate the physiological significance of VGCC proteolysis, as has been done for the proteolysis of cardiac L-type Ca^{2+} channels.

Acknowledgments

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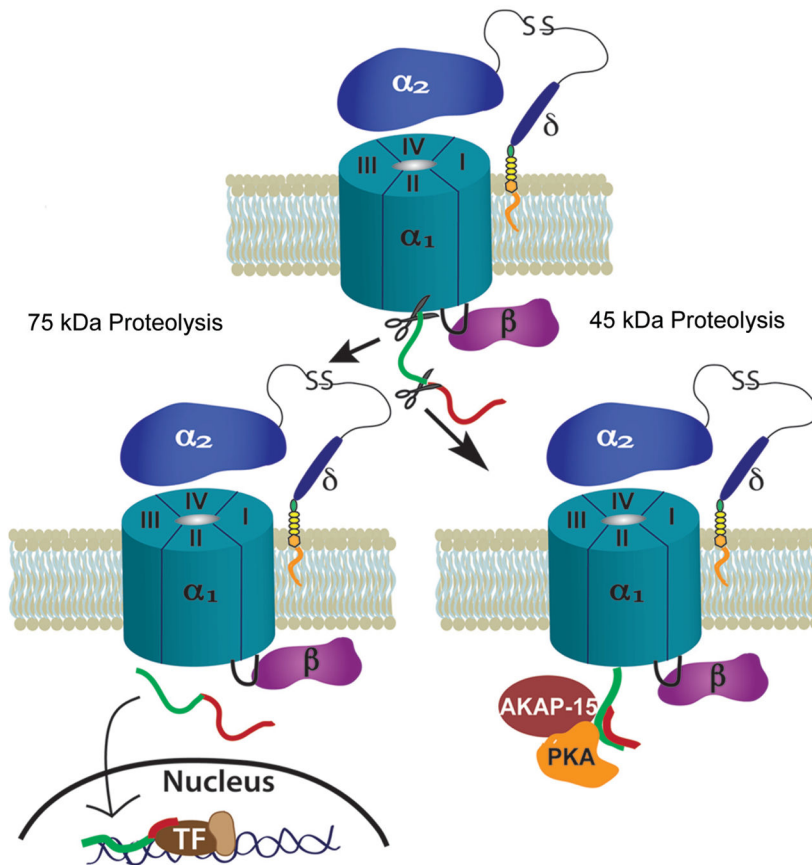**Fig. 1.**
Two forms of proteolytic processing of L-type voltage-gated calcium channels. Top: A schematic of an L-type VGCC complex on the plasma membrane. The α_1 subunit is the pore-forming subunit and contains four homologous repeats (I–IV). $\alpha_2\delta$ and β are auxiliary subunits. The red and green regions represent the C-terminus of α_1. Two sites of proteolysis are indicated. Bottom left: The 75 kDa cut: the C-terminus of α_1 is cleaved from the channel and translocates to the nucleus to interact with transcription factors (TF) and influence gene expression. Bottom right: The 45 kDa cut: the distal C-terminus (red) of α_1 is cleaved from the channel and reassocciates with the proximal C-terminus (green) causing inhibition of the channel. AKAP-15 and PKA are docked close to this site. When activated by the β-adrenergic pathway, PKA phosphorylates a site to dislodge the distal C-terminus, relieving the inhibition.