



HAL
open science

New Insights in CaV β Subunits: Role in the Regulation of Gene Expression and Cellular Homeostasis

Amélie Vergnol, Massiré Traoré, France Pietri-Rouxel, Sestina Falcone

► To cite this version:

Amélie Vergnol, Massiré Traoré, France Pietri-Rouxel, Sestina Falcone. New Insights in CaV β Subunits: Role in the Regulation of Gene Expression and Cellular Homeostasis. *Frontiers in Cell and Developmental Biology*, 2022, 10, pp.880441. 10.3389/fcell.2022.880441 . hal-03829511

HAL Id: hal-03829511

<https://hal.science/hal-03829511v1>

Submitted on 25 Oct 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



New Insights in CaV β Subunits: Role in the Regulation of Gene Expression and Cellular Homeostasis

Amélie Vergnol, Massiré Traoré, France Pietri-Rouxel and Sestina Falcone*

INSERM U974, Center of Research in Myology-Sorbonne University, Paris, France

The voltage-gated calcium channels (CaVs or VGCCs) are fundamental regulators of intracellular calcium homeostasis. When electrical activity induces their activation, the influx of calcium that they mediate or their interaction with intracellular players leads to changes in intracellular Ca²⁺ levels which regulate many processes such as contraction, secretion and gene expression, depending on the cell type. The essential component of the pore channel is the CaV α_1 subunit. However, the fine-tuning of Ca²⁺-dependent signals is guaranteed by the modulatory role of the auxiliary subunits β , $\alpha_2\delta$, and γ of the CaVs. In particular, four different CaV β proteins (CaV β 1, CaV β 2, CaV β 3, and CaV β 4) are encoded by four different genes in mammals, each of them displaying several splice variants. Some of these isoforms have been described in regulating CaV α_1 docking and stability at the membrane and controlling the channel complex's conformational changes. In addition, emerging evidences have highlighted other properties of the CaV β subunits, independently of α_1 and non-correlated to its channel or voltage sensing functions. This review summarizes the recent findings reporting novel roles of the auxiliary CaV β subunits and in particular their direct or indirect implication in regulating gene expression in different cellular contexts.

Keywords: CaV β s, CaV subunits, gene expression, calcium, cell homeostasis, diseases

OPEN ACCESS

Edited by:

Marielle Saclier,
Institut Pasteur, France

Reviewed by:

Wenjun Xie,
Xi'an Jiaotong University, China

*Correspondence:

Sestina Falcone
s.falcone@institut-myologie.org

Specialty section:

This article was submitted to
Molecular and Cellular Pathology,
a section of the journal
Frontiers in Cell and Developmental
Biology

Received: 21 February 2022

Accepted: 16 March 2022

Published: 06 April 2022

Citation:

Vergnol A, Traoré M, Pietri-Rouxel F
and Falcone S (2022) New Insights in
CaV β Subunits: Role in the Regulation
of Gene Expression and
Cellular Homeostasis.
Front. Cell Dev. Biol. 10:880441.
doi: 10.3389/fcell.2022.880441

INTRODUCTION

Voltage-gated calcium channels (CaVs or VGCCs) are transmembrane ion channel proteins that act as major regulator of calcium-related cell functions. Their primary role is to mediate transmembrane calcium influx in response to membrane depolarization. Depending on their sensitivity to membrane depolarization, the activation of CaVs requires either a high or low threshold of membrane potential, dividing CaVs in High- and Low-voltage activated channels (HVA and LVA respectively) (Carbone and Lux, 1984; Fedulova et al., 1985). The crucial component of the channel pore is CaV α_1 , for which ten variants have been identified and classified based on their pharmacological properties and pore-opening kinetic: CaV1 and CaV2 for HVA, and CaV3 for LVA (Tsien et al., 1988; Catterall, 2011; Zamponi et al., 2015).

The CaV of skeletal muscle, also called dihydropyridine receptor (DHPR), was the first to be purified and cloned (Curtis and Catterall, 1984; Tanabe et al., 1987). In skeletal muscle fibers, CaV has a dual function of calcium channel and of voltage sensor of excitation-contraction coupling that controls, through a direct interaction, the opening of RyR1 (Ryanodine Receptor type 1), the Ca²⁺ release channel of the sarcoplasmic reticulum (Allard, 2018). Such a voltage sensor function for CaV and a direct interaction between CaV and RyR have also been described in neurons (Allard, 2018).

The main subunits of CaV, the α_1 subunits, are associated with auxiliary subunits that modulate expression and/or functional properties of the channel. In skeletal muscle, CaV1 is composed of five subunits: α_1S (or CaV1.1), β_1 , $\alpha_2\delta$, and γ (Hagiwara and Naka, 1964).

The function of both CaV1 and CaV2, members of HVA channels, needs the association of the auxiliary CaV β subunit for their plasma membrane docking and proper gating (Schredelseker et al., 2005; Dayal et al., 2013), while the function of LVA class of channel (CaV3) is independent of this subunit (Zhang et al., 2013).

CaV β are intracellular proteins that can either interact with the channel or be in their soluble form (Buraei and Yang, 2013).

Four different proteins, namely CaV β 1, CaV β 2, CaV β 3, and CaV β 4 encoded by four genes, exist in mammals, each of them having several splice variants (Buraei and Yang, 2010). All CaV β subunits are membrane-associated guanylate kinase (MAGUK) family members, with SH3 and GK domains as conserved domains whereas hook region, N- and C-terminal sequences are variables (Buraei and Yang, 2013). Hence, splice variants originate from alternative exon splicing and harbor different amino acid compositions of variable regions, leading to specificities in protein interaction (Subramanyam et al., 2009; Obermair et al., 2010), subcellular targeting properties, and cellular localization, all influencing channel complexes stability, and activity (Campiglio et al., 2013).

Intracellular Ca²⁺ changes account for eukaryotic cell adaptation to external stimuli by modifying gene expression. By controlling Ca²⁺ influx into the cell, CaVs are therefore at the key position to mediate excitation-transcription (E-T) coupling. In point of fact, the mechanisms leading to CREB (cyclic AMP response element-binding) or NFAT (nuclear factor of activated T-cells) activation require the CaVs-dependent Ca²⁺ signaling (Dolmetsch et al., 2001; Hernández-Ochoa et al., 2007; Zhao et al., 2007). Noteworthy, if the role of CaVs in E-T coupling is mainly restricted to the initiation of the subsequent transcriptional activity of Ca²⁺, other mechanisms have been demonstrated to initiate gene regulation by generating a shorter isoform of the CaV1.2 pore forming subunit, which relocalized to the nucleus and held a transcription factor activity, (Gomez-Ospina et al., 2006; Gomez-Ospina et al., 2013). or by mobilizing intracellular CaV β subunit after conformational changes upon membrane depolarization (Servili et al., 2018). Indeed, CaV β 2 subunit was recently demonstrated to be the mediator of CaV1.2-dependent E-T coupling, by interacting with H-Ras, which in turn activated MAPK (Mitogen Activated Protein Kinase)/ERK (Extracellular Signal-Regulated Kinase) pathway to induce CREB-directed gene expression in human neuronal SH-SY5Y cells (Servili et al., 2018). Nevertheless, the possibility that CaVs auxiliary subunits may be directly implicated as transcription factors has become an emerging hypothesis in the last 2 decades (Barbado et al., 2009).

This review will focus on CaV β s newly and less broadly described insights by illustrating their nuclei tracking in line with their role as regulators of gene expression.

CAV β AS A SELF-SUFFICIENT NUCLEAR PROTEIN

After the initial cloning of CaV β 1 in skeletal muscle [CaV β 1D formerly known as CaV β 1A (Traoré et al., 2019)] (Ruth et al., 1989), further works described several variants of CaV β 1 expressed in muscle and other tissues CaV β 1 (Hibino et al., 2003; Hullin et al., 2003; Foell et al., 2004; Harry et al., 2004; Cohen et al., 2005), lacking the domain required for its interaction with CaV α _{1S} at

the α -Interaction Domain (AID domain). These reports suggested for the first time that some CaV β subunits isoforms may have a CaV-independent function. Subsequently, several studies have demonstrated the capability of CaV β s to translocate to the nucleus, giving additional indications toward a role for CaV β distinct from its well-known function as modulator of CaV channels.

If not all, at least some isoforms of CaV β 1, CaV β 2, CaV β 3 and CaV β 4 proteins display nuclear localization properties upon appropriate conditions (Buraei and Yang, 2010). For both CaV β 1, in skeletal muscle (Traoré et al., 2019), and CaV β 4, in neurons (Subramanyam et al., 2009; Etemad et al., 2014), this nuclear localization has been demonstrated to be linked to electrical activity. Indeed, our recent study showed that in adult skeletal muscle, after nerve damage, the embryonic isoform CaV β 1E was expressed and localized to the nuclei and near the Z-lines, while the constitutive adult muscle variant, CaV β 1D, remained associated with CaV1.1 at the sarcolemma (Traoré et al., 2019). Similarly, it has been reported that in neurons, CaV β 4A and CaV β 4B translocated to the nuclei when electrical activity was aborted, whereas CaV β 4E did not display nuclear localization. Additionally, a decrease in nuclear targeting of CaV β 1E (Traoré et al., 2019) and CaV β 4 (isoforms A and B) (Etemad et al., 2014) has been correlated with the onset of electrical activity throughout development in muscle fibers and neurons. Interestingly, the proportion of CaV β 4 isoforms targeted to nuclei has been associated with their activity as gene regulators (CaV β 4B > CaV β 4A > CaV β 4E) (Etemad et al., 2014). The mechanism originating the nuclear localization of CaV β 2 and CaV β 3 has not been clearly characterized, however, it can be hypothesized that they follow the same depolarization-sensitive process.

As previously mentioned, the capacity to get to the nucleus is not held by all the CaV β 1, CaV β 2, CaV β 3 and CaV β 4 splicing variants. The mechanisms underlying the specificities of the nucleus-targeted proteins could be passive diffusion through nuclear membrane for small proteins, while large ones need a Nuclear Localization Sequence (NLS), allowing their binding to Importins, or require the association with nuclear proteins as a shuttle. The molecular aspects behind CaV β s translocation to the nucleus are still not fully understood. For CaV β 1 (Buraei and Yang, 2010; Taylor et al., 2014) and CaV β 4 (Tadmouri et al., 2012) the SH3 domain of the protein has been described to exhibit the functional features leading to nuclear shuttling. An additional aspect was highlighted for CaV β 1E which have been described to display a putative NLS signal in its sequence (Taylor et al., 2014; Traoré et al., 2019), suggesting that its nuclear targeting was occurring through its binding to Importin proteins. However, modified genetic constructions lacking the putative NLS sequence did not prevent CaV β 1 to enter the nucleus. As an example, Subramanyam and colleagues showed that a specific double-arginine motif at the N-terminal was necessary and sufficient to induce the recruitment of the CaV β 4B variant toward the nuclei in mouse brain (Subramanyam et al., 2009). Nevertheless, this domain was subsequently demonstrated to be only partially involved in CaV β 4B docking to the nucleus, and that SH3/GK protein interaction domain was required to control its nuclear targeting. If these data were confirmed by several studies, a supplemental and non-exclusive mechanism came up with the demonstration of a PxxP binding motif in the SH3 domain of CaV β 1, raising the possibility that

CaV β proteins might also bind to proteins that themselves shuttle to the nucleus (Buraei and Yang, 2010). An instance supporting this hypothesis is CaV β 4C, which interaction with HP1 γ has been shown as mandatory to localize to the nucleus in mammalian cells (Hibino et al., 2003), while the truncation of a large part of its GK domain cut off the exclusive requirement of SH3/GK interaction for nuclear docking of CaV β s proteins. The molecular aspects of nuclear targeting were less studied for CaV β 2 and CaV β 3, for which some studies mentioning their binding with chaperone proteins may be relevant in supporting their tackling to the nuclei (Zhang et al., 2010; Pickel et al., 2021).

The evidence of the nuclear localization/translocation of several CaV β variants spotted these proteins with an undeniable CaV α ₁ independent function and pinpointed their putative role in the modulation of gene expression. The next part of this review will summarize the mechanisms described for the CaV β auxiliary subunits in the regulation of gene transcription.

CAV β S AS FACTORS CONTROLLING GENE EXPRESSION

CaV-Independent Role of CaV β in Calcium-Mediated Gene Expression

As mentioned, a large set of intracellular processes are driven through Ca²⁺ signaling and therefore dependent on the free cytosolic calcium. Either CaVs-related Ca²⁺ entry from the extracellular space or mobilization of intracellular Ca²⁺ stock can modulate its cytosolic concentration and originate this signaling. As an auxiliary subunit, CaV β has been described to regulate Ca²⁺ influx into the cell by modulating CaVs activity (Buraei and Yang, 2010), however, this protein was also reported to regulate intracellular Ca²⁺ in a CaV-independent way by acting on Ca²⁺ stores. Indeed, in both pancreatic cells (Berggren et al., 2004; Becker et al., 2021) and fibroblasts (Belkacemi et al., 2018), it has been demonstrated that CaV β 3 could interfere with Inositol 3-Phosphate (IP3)-induced Ca²⁺ release from the Endoplasmic Reticulum (ER) by binding IP3 Receptor (IP3R), therefore desensitizing cells to low IP3 concentration (Berggren et al., 2004; Belkacemi et al., 2018; Becker et al., 2021). In this process, CaV β 3 acted as a “brake” on Ca²⁺ release, affecting glucose-triggered insulin exocytosis in β -pancreatic cells (Berggren et al., 2004; Becker et al., 2021) and cellular mobility in fibroblasts (Belkacemi et al., 2018). These studies illustrated an effect of CaV β subunits in affecting gene expression by regulating free cytosolic calcium concentration (Figure 1A).

Interaction With Various Transcription Factors

CaV β 3 has been reported by Zhang and colleagues to co-localize with Pax6(S) in the nucleus and the interaction of these two proteins has been described to account for a ~ 50% decrease in Pax6(S) transcriptional activity (demonstrated in *Xenopus* oocytes by reporter system *in vitro*) without impairing CaV channel properties (Zhang et al., 2010). More generally, Pax6 proteins are composed of two DNA-binding domains: a paired-domain (PD) and a homeodomain (HD), allowing the binding to the cis-elements of target genes to regulate their transcription

rate, and a proline/serine/threonine (PST)-rich C-terminal domain, holding a trans-activation function. The work of Zhang and colleagues highlighted that Pax6(S) presented intact PD and HD domains while its C-terminal domain was truncated, resulting in a weaker Pax6(S) trans-activity. This isoform also differed from canonical Pax6 by a unique S-tail, originating its interaction with CaV β 3. This work suggested a novel function of CaV β 3 in negatively regulating Pax6(S) protein activity, although the precise mechanism, supposed to occur either by CaV β 3 allosteric hindrance or by Pax6(S) removal from DNA binding sites, remained undefined. More importantly, this report showed for the first time a full-length CaV β protein having a role in the channel function, acting also directly as a modulator of gene transcription (Zhang et al., 2010) (Figure 1B).

In 2003, Hibino and colleagues identified from chicken cochlea the CaV β 4C variant, a truncated CaV β 4 isoform which is also expressed in the brain, eye, heart and lung, concomitantly with the full-length isoforms CaV β 4A and CaV β 4B. However, in contrast with these two isoforms, CaV β 4C was described to lack a large part of the GK domain necessary to associate with CaV α ₁ Chen et al., 2004, having therefore little effect on Ca²⁺ channel activity (Hibino et al., 2003). This -by then- newly identified variant showed a direct interaction with the chromo shadow domain (CSD) of the chromo box protein 2/heterochromatin protein 1 γ (CHCB2/HP1 γ), a nuclear protein that modulates the transcription of several genes by regulating heterochromatin conformation and therefore gene silencing. Noteworthy, the binding of HP1 γ to DNA regions of euchromatin was shown to correlate with gene repression. Hibino's study reported that when co-expressed with HP1 γ , CaV β 4C was recruited to the nucleus, dramatically lowering the CHCB2/HP1 γ gene-silencing activity *in vitro* (Hibino et al., 2003). This was the very first time that a CaV β protein was described to translocate to the nucleus and act as a transcriptional regulator.

A few years later, the existence of CaV β 4C was revealed in the human brain and observed to also interact with the CSD of HP1 γ (Xu et al., 2011). In addition, this interaction was shown to occur *via* a CSD binding motif, the PXXVL consensus sequence. Consistently with Hibino and colleagues' work, the binding of human CaV β 4C to HP1 γ was demonstrated to lead its nuclear translocation where it markedly reduces the gene-silencing activity of HP1 γ *in vitro* (Xu et al., 2011). These studies illustrated a first manner for CaV β subunits to indirectly modulate gene expression by affecting the activity of proteins involved in DNA compaction, like HP1 γ (Figure 1C).

The first report of nuclear localization of the CaV β 4B full-length isoform has been achieved by Subramanyam and colleagues in neurons and muscle cells, where this nuclear localization was reported to negatively relate on electrical activity (Subramanyam et al., 2009). The comprehension of the localization-related role of this protein has been realized later, with the demonstration that it acted as an organizing platform of a group of proteins which controlled transcription (Tadmouri et al., 2012). Among the complex-forming proteins, B56 δ , the regulatory subunit of the PP2A phosphatase induced histone dephosphorylation and HP1 γ restructured

heterochromatin. The last defined component of this complex was a transcription factor able to bind DNA at the promoter regions, allowing B56 δ and HP1 γ activity (Tadmouri et al., 2012). The gene that has been demonstrated to be modulated through this mechanism is Tyrosine hydroxylase (TH), the corresponding transcription factor being thyroid hormone receptor alpha (TR α) (Tadmouri et al., 2012). This situation was different from the previously described CaV β 4C effect on HP1 γ activity, since CaV β 4B was, in this case, the element enabling the complex B56 δ /HP1 γ /TR α to access their activity site rather than modulating their function itself (Figure 1D). If Subramanyam and colleagues demonstrated a negative correlation between neuronal excitability and V5-tagged CaV β 4B positioning at the nucleus, this report showed that the endogenous CaV β 4B association with B56 δ , originating their nucleus translocation, was consecutive to electrical activity, suggesting that the V5 tag might have hindered the pathways leading to CaV β 4B nuclear localization (Tadmouri et al., 2012).

Two further studies, aimed at investigating the property of CaV β 4 variants in controlling the expression of cell cycle-related genes, demonstrated that nuclear CaV β 4 full-length was able to inhibit cell proliferation, while its epileptogenic mutant, lacking C-term, had no impact. The effects of CaV β 4 on cell cycle were

related to the ability of the CaV β 4 to interact to either B56 δ or T-cell factor 4 (TCF4) transcription factors. In the first case, B56 δ recruitment to the nuclei by CaV β 4 was suggested to mediate the repression of genes involved in cell proliferation (Rima et al., 2017a). On the other hand, the binding of CaV β 4 to TCF4 was demonstrated to prevent its interaction with β -catenin, as additional mechanism to inhibit the activation of β -catenin-Wnt-dependent gene expression and cell cycle (Rima et al., 2017b). These reports established the ability of a CaV β isoform to control gene expression, autonomously from CaVs, either dependently on or independently of electrical activity.

Interaction With Regulatory DNA Sequence

A study, published in 2014 by Taylor and colleagues, pinpointed that CaV β 1 was able to translocate to the nuclei and bind at the promoter region of 952 genes in muscle precursor cells (MPCs). Importantly, it showed that the absence of CaV β 1 resulted in changes in the expression of several genes, either positively or negatively misregulated, designating this subunit as a having a transcription factor function (Taylor et al., 2014). This role was more deeply confirmed for myogenin which was up-regulated in the absence of CaV β 1, preventing a correct myogenic development (Schuster-Gossler et al., 2007; Ho et al., 2011;

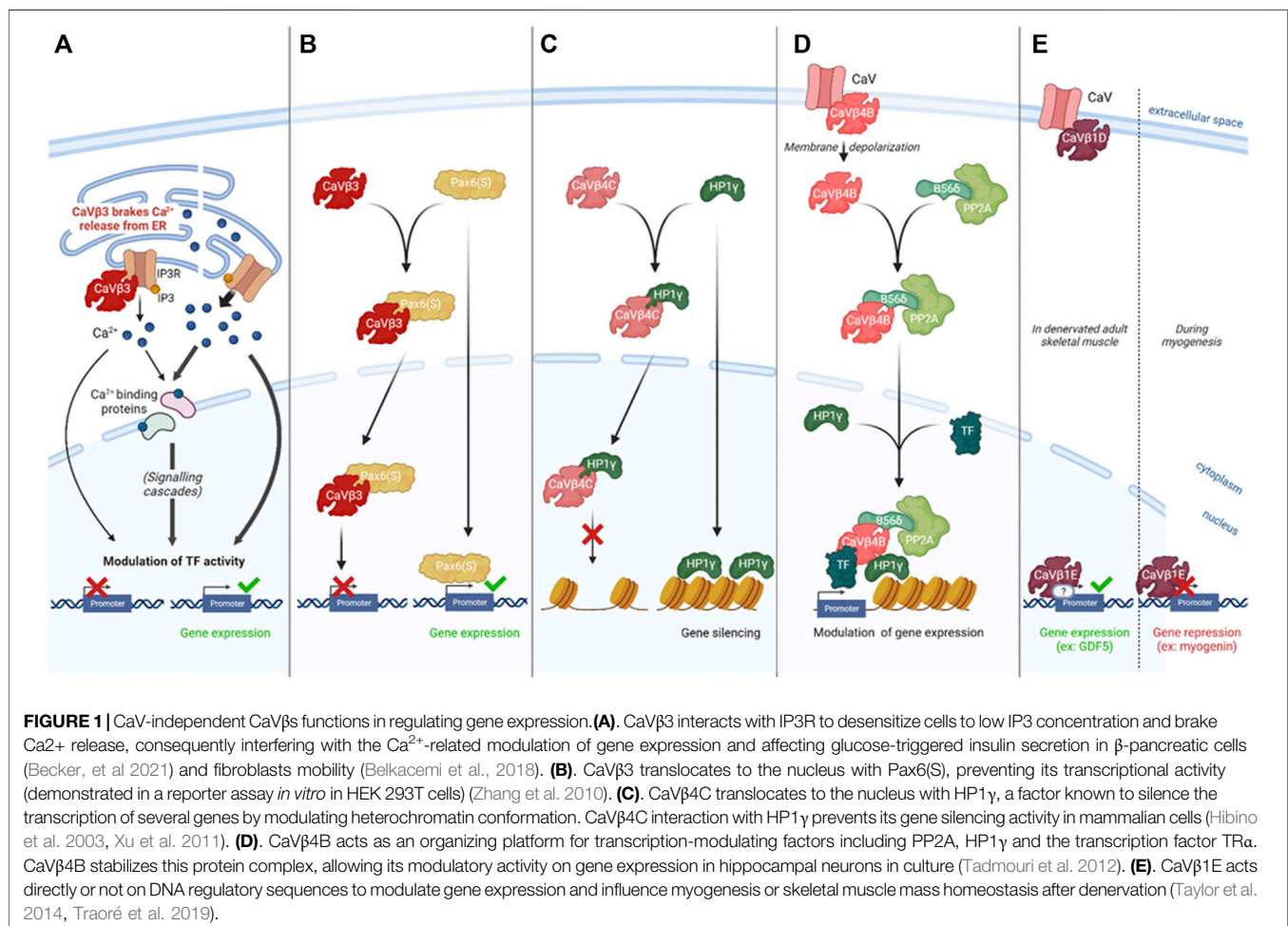


TABLE 1 | CaVβs associated disorders.

	Pathology/Pathological features	CACNB gene	References–CaVβ in the pathological context	
			Description	CaV-independent disorders
Heart	Brugada Syndrome (BrS), Type 4	CACNB2 (causal mutation)	Carbone and Lux (1984); Fedulova et al. (1985)	–
	Hypertrophic cardiomyopathy (HCM)	CACNB2 (gene modifier)	–	Catterall (2011) CaVβ2 regulates cardiomyocytes hypertrophy
Brain	Episodic Ataxia, Type 5	CACNB4 (causal mutation)	Tsien et al. (1988); Zamponi et al. (2015)	–
	Epilepsy, Idiopathic generalized 9		Tsien et al. (1988)	–
	Epilepsy, Myoclonic Juvenile		Tsien et al. (1988)	Curtis and Catterall (1984) The human CACNB4 mutation prevents CaVβ4 to get to the nucleus and modulates gene expression
Skeletal muscle	Epilepsy, Myoclonic Juvenile	CACNB1E (age-related decline of expression)	–	Tanabe et al. (1987) Restoration of CaVβ1E rescues GDF5 expression and prevents age-related skeletal muscle wasting

Taylor et al., 2014). In this study, the CaVβ1A was the isoform described as a transcription factor, while our study published in 2019, rather indicated that the capacity to localize to the nucleus and exert a transcription factor role was actually carried by CaVβ1E which was identified as the main CaVβ1 isoform in C2C12 myoblast cell line, consistent with what observed in MPCs (Traoré et al., 2019). In addition, our work showed that the CaVβ1E played a crucial role in adult muscle mass homeostasis, when electrical activity was impaired, by regulating directly or indirectly the GDF5 promoter to trigger its transcriptional activity (Traoré et al., 2019) (**Figure 1E**).

By all these studies, CaVβs proteins have emerged as key players in regulating gene expression through Ca²⁺ signaling, DNA remodeling, modification of transcription factors activity or by acting as transcription factors themselves. When these functions are lost, multiple cellular functions are disturbed, involving CaVβ proteins in pathological conditions independently of the CaV-related aspect.

IMPLICATION OF CAVβS IN PATHOLOGICAL CONDITIONS

Although isoforms of CaVβ1, CaVβ2, CaVβ3, and CaVβ4 are expressed in several regions of the brain (Buraei and Yang, 2013), ablation of CaVβ1, CaVβ2 and CaVβ3 have no major impact on neuronal function (Ball et al., 2002). On the contrary, the relevance of CaVβ4 in the nervous system physiology was shown in the *lethargic (lh)* mouse model, having ataxic and epileptic phenotype (Burgess et al., 1997). Its implication in the pathophysiology of neuronal disorders was confirmed in Humans, after the discovery that missense and coding mutations, affecting the N-terminal region of the protein, were associated with epilepsy and ataxia, respectively (Escayg et al., 2000; Tadmouri et al., 2012). In the cerebellum, CaVβ4 is the most expressed and the major auxiliary subunit, together with α2δ-2, of the CaV2.1 calcium channel. Interestingly, mutations in all three proteins have been reported to lead to an epileptic and

ataxic phenotype. Therefore, CaVβ4 involvement in such pathological conditions was first linked with its CaV-associated role (Escayg et al., 2000).

However, an additional mechanism to further elucidate such pathologies came from the CaV-independent role of CaVβ4. Indeed, at the molecular level, human epilepsy and ataxia-associated mutations were found to prevent CaVβ4 to shuttle toward nuclei by disrupting the SH3/GK domains interaction and indicated that mis-regulated CaVβ4-dependent gene transcription may have a key relevance in the pathophysiology of these neurological disorders (Tadmouri et al., 2012) (**Table 1**).

In 2017, the importance of *CACNB2* (gene coding for CaVβ2) as a genetic modifier of a Hypertrophic CardioMyopathy (HCM), in which the causal gene was *MYBPC3* (Myosin-Binding Protein C), has been described for the first time (Zhang et al., 2017). The authors hypothesized that the potential mechanism modifying disease phenotype was based on the attenuation of CaV-dependent Ca²⁺ current associated with *CACNB2* mutations. However, an additional possibility came out a few years later with a study that correlated the reduced cardiomyocyte hypertrophy to a CaV-independent CaVβ2 function (Pickel et al., 2021). CaVβ2 localization and activity in cardiomyocyte nuclei were shown to significantly regulate Calpain activity and Calpastatin expression (Pickel et al., 2021), a pro-hypertrophic protease and its inhibitor, respectively. Even though these events have not been explicitly linked to the reduction of cardiomyocyte hypertrophy, a correlation between the two might be hypothesized and would need to be further studied (**Table 1**).

Our recent work demonstrated the key role played by CaVβ1E in the context of age-related muscle wasting. We showed that CaVβ1E/GDF5 pathway counteracted the loss of muscle mass after denervation and that this signaling was defective in aged muscle fibers. Importantly, we overexpressed CaVβ1E in aged mouse muscles leading to increased GDF5 expression and activation of its signaling and therefore enabling the prevention of muscle mass loss and force decline during aging (Traoré et al., 2019). Importantly, the expression of an analogous of CaVβ1E has been also discovered in human muscle, decreasing

in an age-related manner, indicating that the defective hCaV β 1E signaling might also be impaired in sarcopenic patients and suggesting the CaV β 1E/GDF5 axis having a therapeutic potential in muscle aging and linked pathologies (Traoré et al., 2019) (Table 1).

Nevertheless, little is known about the causes behind the decrease of CaV β 1E expression in aged muscles. One hypothesis we assessed, was a damaged neuro-muscular junction (NMJ), but we did not detect any NMJ changes in the 78-week old mice involved in the study which could have testified toward changes in electrical activity and modifications in basal CaV β 1E levels (Traoré et al., 2019). Chromatin methylation/demethylation events or other epigenetic alterations could further explain the unbalanced CaV β 1E/GDF5 axis in old muscle and should be investigated in future works.

To summarize, mutations in CaV β s encoding genes have currently been associated with disorders and, even if the pathological mechanisms have not always been fully characterized, both CaV β s roles linked or unlinked to CaVs can be argued. Brugada Syndrome type 4 and Episodic ataxia type 5 present causative mutations in genes coding for CaV β s proteins and other CaV subunits (CACNA2D1 and CACNA1A, and CACNA1A respectively—MalaCard database), corroborating CaV β involvement in pathological mechanisms in a CaV-linked way (Table 1). Nevertheless, and as described above, CaV β may also originate or modify pathological features independently of CaVs and an underestimation of these situations can be hypothesized.

CONCLUSION

While CaV β s have long been considered to present exclusively CaVs' linked functions, we depicted in this review the elements

REFERENCES

- Allard, B. (2018). From Excitation to Intracellular Ca²⁺ Movements in Skeletal Muscle: Basic Aspects and Related Clinical Disorders. *Neuromuscul. Disord.* 28, 394–401. doi:10.1016/j.nmd.2018.03.004
- Ball, S. L., Powers, P. A., Shin, H.-S., Morgans, C. W., Peachey, N. S., and Gregg, R. G. (2002). Role of the β 2 Subunit of Voltage-dependent Calcium Channels in the Retinal Outer Plexiform Layer. *Invest. Ophthalmol. Vis. Sci.* 43, 1595–1603.
- Barbado, M., Fablet, K., Ronjat, M., and De Waard, M. (2009). Gene Regulation by Voltage-dependent Calcium Channels. *Biochim. Biophys. Acta (Bba) - Mol. Cell Res.* 1793, 1096–1104. doi:10.1016/j.bbamcr.2009.02.004
- Becker, A., Wardas, B., Salah, H., Amini, M., Fecher-Trost, C., Sen, Q., et al. (2021). Cav β 3 Regulates Ca²⁺ Signaling and Insulin Expression in Pancreatic β -Cells in a Cell-Autonomous Manner. *Diabetes* 70, 2532–2544. doi:10.2337/db21-0078
- Belkacemi, A., Hui, X., Wardas, B., Laschke, M. W., Wissenbach, U., Menger, M. D., et al. (2018). IP₃ Receptor-dependent Cytoplasmic Ca²⁺ Signals Are Tightly Controlled by Cav β 3. *Cel Rep.* 22, 1339–1349. doi:10.1016/j.celrep.2018.01.010
- Berggren, P.-O., Yang, S.-N., Murakami, M., Efanov, A. M., Uhles, S., Köhler, M., et al. (2004). Removal of Ca²⁺ Channel β 3 Subunit Enhances Ca²⁺ Oscillation Frequency and Insulin Exocytosis. *Cell* 119, 273–284. doi:10.1016/j.cell.2004.09.033
- Buraei, Z., and Yang, J. (2013). Structure and Function of the β Subunit of Voltage-Gated Ca²⁺ Channels. *Biochim. Biophys. Acta (Bba) - Biomembranes* 1828, 1530–1540. doi:10.1016/j.bbamem.2012.08.028

pointing towards a far more extensive significance in organ and cell homeostasis. CaV β s proteins have been described as efficient modulators of gene expression, either through their effect on Ca²⁺ signaling or through their DNA-related activities enabled by their nuclear localization. Regardless of the molecular mechanisms in which CaV β s are implicated, these proteins have been shown to influence cell and tissue capability to respond to different stimuli and to adapt following environmental changes. In the light of the findings of these two last decades, the impact of CaV β 1 and CaV β 4 on gene expression has been demonstrated several times, whereas CaV β 2 and CaV β 3 are less deeply characterized. A better establishment and appreciation of CaV β s relevance in cell biology will probably strengthen our understanding in a plethora of physiological and pathological mechanisms.

AUTHOR CONTRIBUTIONS

FP-R and SF proposed the concept for the review, the first draft and revised the manuscript; AV wrote the manuscript and drawn figure and table; MT read the manuscript and contributed to the article conception.

FUNDING

First author fellowship is funded by Sorbonne University.

ACKNOWLEDGMENTS

We thank B. Allard for helpful scientific discussion and the reading of the review; A. Muchir and D. Cardoso to have shared their Biorender Licence.

- Buraei, Z., and Yang, J. (2010). The β Subunit of Voltage-Gated Ca²⁺ Channels. *Physiol. Rev.* 90, 1461–1506. doi:10.1152/physrev.00057.2009
- Burgess, D. L., Jones, J. M., Meisler, M. H., and Noebels, J. L. (1997). Mutation of the Ca²⁺ Channel β Subunit Gene Cchb4 Is Associated with Ataxia and Seizures in the Lethargic (Lh) Mouse. *Cell* 88, 385–392. doi:10.1016/s0092-8674(00)81877-2
- Campiglio, M., Di Biase, V., Tuluc, P., and Flucher, B. E. (2013). Stable Incorporation versus Dynamic Exchange of β Subunits in a Native Ca²⁺ Channel Complex. *J. Cel. Sci.* 126, 2092–2101. doi:10.1242/jcs.jcs124537
- Carbone, E., and Lux, H. D. (1984). A Low Voltage-Activated Calcium Conductance in Embryonic Chick Sensory Neurons. *Biophysical J.* 46, 413–418. doi:10.1016/s0006-3495(84)84037-0
- Catterall, W. A. (2011). Voltage-gated Calcium Channels. *Cold Spring Harbor Perspect. Biol.* 3, a003947. doi:10.1101/cshperspect.a003947
- Chen, Y.-H., Li, M.-h., Zhang, Y., He, L.-l., Yamada, Y., Fitzmaurice, A., et al. (2004). Structural Basis of the α 1- β Subunit Interaction of Voltage-Gated Ca²⁺ Channels. *Nature* 429, 675–680. doi:10.1038/nature02641
- Cohen, R. M., Foell, J. D., Balijepalli, R. C., Shah, V., Hell, J. W., and Kamp, T. J. (2005). Unique Modulation of L-type Ca²⁺ channels by Short Auxiliary β 1 subunit Present in Cardiac Muscle. *Am. J. Physiology-Heart Circulatory Physiol.* 288, H2363–H2374. doi:10.1152/ajpheart.00348.2004
- Curtis, B. M., and Catterall, W. A. (1984). Purification of the Calcium Antagonist Receptor of the Voltage-Sensitive Calcium Channel from Skeletal Muscle Transverse Tubules. *Biochemistry* 23, 2113–2118. doi:10.1021/bi00305a001
- Dayal, A., Bhat, V., Franzini-Armstrong, C., and Grabner, M. (2013). Domain Cooperativity in the β 1a Subunit Is Essential for Dihydropyridine Receptor

- Voltage Sensing in Skeletal Muscle. *Proc. Natl. Acad. Sci. U.S.A.* 110, 7488–7493. doi:10.1073/pnas.1301087110
- Dolmetsch, R. E., Pajvani, U., Fife, K., Spotts, J. M., and Greenberg, M. E. (2001). Signaling to the Nucleus by an L-type Calcium Channel-Calmodulin Complex through the MAP Kinase Pathway. *Science* 294, 333–339. doi:10.1126/science.1063395
- Escayg, A., De Waard, M., Lee, D. D., Bichet, D., Wolf, P., Mayer, T., et al. (2000). Coding and Noncoding Variation of the Human Calcium-Channel β 4-Subunit Gene CACNB4 in Patients with Idiopathic Generalized Epilepsy and Episodic Ataxia. *Am. J. Hum. Genet.* 66, 1531–1539. doi:10.1086/302909
- Etemad, S., Obermair, G. J., Bindreither, D., Benedetti, A., Stanika, R., Di Biase, V., et al. (2014). Differential Neuronal Targeting of a New and Two Known Calcium Channel 4 Subunit Splice Variants Correlates with Their Regulation of Gene Expression. *J. Neurosci.* 34, 1446–1461. doi:10.1523/jneurosci.3935-13.2014
- Fedulova, S. A., Kostyuk, P. G., and Veselovsky, N. S. (1985). Two Types of Calcium Channels in the Somatic Membrane of New-Born Rat Dorsal Root Ganglion Neurons. *J. Physiol.* 359, 431–446. doi:10.1113/jphysiol.1985.sp155594
- Foell, J. D., Balijepalli, R. C., Delisle, B. P., Yunker, A. M. R., Robia, S. L., Walker, J. W., et al. (2004). Molecular Heterogeneity of Calcium Channel β -subunits in Canine and Human Heart: Evidence for Differential Subcellular Localization. *Physiol. Genomics* 17, 183–200. doi:10.1152/physiolgenomics.00207.2003
- Gomez-Ospina, N., Panagiotakos, G., Portmann, T., Pasca, S. P., Rabah, D., Budzillo, A., et al. (2013). A Promoter in the Coding Region of the Calcium Channel Gene CACNA1C Generates the Transcription Factor CCAT. *PLOS ONE* 8, e60526. doi:10.1371/journal.pone.0060526
- Gomez-Ospina, N., Tsuruta, F., Barreto-Chang, O., Hu, L., and Dolmetsch, R. (2006). The C Terminus of the L-type Voltage-Gated Calcium Channel CaV1.2 Encodes a Transcription Factor. *Cell* 127, 591–606. doi:10.1016/j.cell.2006.10.017
- Hagiwara, S., and Naka, K.-i. (1964). The Initiation of Spike Potential in Barnacle Muscle Fibers under Low Intracellular Ca $^{++}$. *J. Gen. Physiol.* 48, 141–162. doi:10.1085/jgp.48.1.141
- Harry, J. B., Kobrin, E., Abernethy, D. R., and Soldatov, N. M. (2004). New Short Splice Variants of the Human Cardiac Cav β 2 Subunit. *J. Biol. Chem.* 279, 46367–46372. doi:10.1074/jbc.m409523200
- Hernández-Ochoa, E. O., Contreras, M., Cseresnyés, Z., and Schneider, M. F. (2007). Ca $^{2+}$ Signal Summation and NFATc1 Nuclear Translocation in Sympathetic Ganglion Neurons during Repetitive Action Potentials. *Cell Calcium* 41, 559–571. doi:10.1016/j.ceca.2006.10.006
- Hibino, H., Pironkova, R., Onwumere, O., Rousset, M., Charnet, P., Hudspeth, A. J., et al. (2003). Direct Interaction with a Nuclear Protein and Regulation of Gene Silencing by a Variant of the Ca $^{2+}$ -channel β 4 Subunit. *Proc. Natl. Acad. Sci. U.S.A.* 100, 307–312. doi:10.1073/pnas.0136791100
- Ho, A. T. V., Hayashi, S., Bröhl, D., Auradé, F., Rattenbach, R., and Relaix, F. (2011). Neural Crest Cell Lineage Restricts Skeletal Muscle Progenitor Cell Differentiation through Neuregulin1-ErbB3 Signaling. *Develop. Cell* 21, 273–287. doi:10.1016/j.devcel.2011.06.019
- Hullin, R., Khan, I. F. Y., Wirtz, S., Mohacs, P., Varadi, G., Schwartz, A., et al. (2003). Cardiac L-type Calcium Channel β -Subunits Expressed in Human Heart Have Differential Effects on Single Channel Characteristics. *J. Biol. Chem.* 278, 21623–21630. doi:10.1074/jbc.m211164200
- Obermair, G. J., Schlick, B., Di Biase, V., Subramanyam, P., Gebhart, M., Baumgartner, S., et al. (2010). Reciprocal Interactions Regulate Targeting of Calcium Channel β Subunits and Membrane Expression of α 1 Subunits in Cultured Hippocampal Neurons. *J. Biol. Chem.* 285, 5776–5791. doi:10.1074/jbc.m109.044271
- Pickel, S., Cruz-Garcia, Y., Bandleon, S., Barkovits, K., Heindl, C., Völker, K., et al. (2021). The β 2-Subunit of Voltage-Gated Calcium Channels Regulates Cardiomyocyte Hypertrophy. *Front. Cardiovasc. Med.* 8, 692. doi:10.3389/fcvm.2021.704657
- Rima, M., Daghni, M., De Waard, S., Gaborit, N., Fajloun, Z., Ronjat, M., et al. (2017). The β 4 Subunit of the Voltage-Gated Calcium Channel (Cacnb4) Regulates the Rate of Cell Proliferation in Chinese Hamster Ovary Cells. *Int. J. Biochem. Cell Biol.* 89, 57–70. doi:10.1016/j.biocel.2017.05.032
- Rima, M., Daghni, M., Lopez, A., Fajloun, Z., Lefrançois, L., Dunach, M., et al. (2017). Down-regulation of the Wnt/ β -Catenin Signaling Pathway by Cacnb4. *MBoC* 28, 3699–3708. doi:10.1091/mbc.e17-01-0076
- Ruth, P., Röhrkasten, A., Biel, M., Bosse, E., Regulla, S., Meyer, H. E., et al. (1989). Primary Structure of the β Subunit of the DHP-Sensitive Calcium Channel from Skeletal Muscle. *Science* 245, 1115–1118. doi:10.1126/science.2549640
- Schredelseker, J., Di Biase, V., Obermair, G. J., Felder, E. T., Flucher, B. E., Franzini-Armstrong, C., et al. (2005). The β 1a Subunit Is Essential for the Assembly of Dihydropyridine-Receptor Arrays in Skeletal Muscle. *Proc. Natl. Acad. Sci. U.S.A.* 102, 17219–17224. doi:10.1073/pnas.0508710102
- Schuster-Gossler, K., Cordes, R., and Gossler, A. (2007). Premature Myogenic Differentiation and Depletion of Progenitor Cells Cause Severe Muscle Hypotrophy in Delta1 Mutants. *Proc. Natl. Acad. Sci. U.S.A.* 104, 537–542. doi:10.1073/pnas.0608281104
- Servili, E., Trus, M., Maayan, D., and Atlas, D. (2018). β -Subunit of the Voltage-Gated Ca $^{2+}$ Channel Cav1.2 Drives Signaling to the Nucleus via H-Ras. *Proc. Natl. Acad. Sci. U.S.A.* 115, E8624–E8633. doi:10.1073/pnas.1805380115
- Subramanyam, P., Obermair, G. J., Baumgartner, S., Gebhart, M., Striessnig, J., Kaufmann, W. A., et al. (2009). Activity and Calcium Regulate Nuclear Targeting of the Calcium Channel Beta4b Subunit in Nerve and Muscle Cells. *Channels* 3, 343–355. doi:10.4161/chan.3.5.9696
- Tadmouri, A., Kiyonaka, S., Barbado, M., Rousset, M., Fablet, K., Sawamura, S., et al. (2012). Cacnb4 Directly Couples Electrical Activity to Gene Expression, a Process Defective in Juvenile Epilepsy. *EMBO J.* 31, 3730–3744. doi:10.1038/emboj.2012.226
- Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., et al. (1987). Primary Structure of the Receptor for Calcium Channel Blockers from Skeletal Muscle. *Nature* 328, 313–318. doi:10.1038/328313a0
- Taylor, J., Pereyra, A., Zhang, T., Messi, M. L., Wang, Z.-M., Hereñú, C., et al. (2014). The Cav β 1a Subunit Regulates Gene Expression and Suppresses Myogenin in Muscle Progenitor Cells. *J. Cell Biol.* 205, 829–846. doi:10.1083/jcb.201403021
- Traoré, M., Gentil, C., Benedetto, C., Hogrel, J.-Y., De la Grange, P., Cadot, B., et al. (2019). An Embryonic CaV β 1 Isoform Promotes Muscle Mass Maintenance via GDF5 Signaling in Adult Mouse. *Sci. Translational Med.* 11, eaaw1131. doi:10.1126/scitranslmed.aaw1131
- Tsien, R. W., Lipscombe, D., Madison, D. V., Bley, K. R., and Fox, A. P. (1988). Multiple Types of Neuronal Calcium Channels and Their Selective Modulation. *Trends Neurosciences* 11, 431–438. doi:10.1016/0166-2236(88)90194-4
- Xu, X., Lee, Y. J., Holm, J. B., Terry, M. D., Oswald, R. E., and Horne, W. A. (2011). The Ca $^{2+}$ Channel β 4c Subunit Interacts with Heterochromatin Protein 1 via a PXVXL Binding Motif. *J. Biol. Chem.* 286, 9677–9687. doi:10.1074/jbc.m110.187864
- Zamponi, G. W., Striessnig, J., Koschak, A., and Dolphin, A. C. (2015). The Physiology, Pathology, and Pharmacology of Voltage-Gated Calcium Channels and Their Future Therapeutic Potential. *Pharmacol. Rev.* 67, 821–870. doi:10.1124/pr.114.009654
- Zhang, X., Xie, J., Zhu, S., Chen, Y., Wang, L., and Xu, B. (2017). Next-generation Sequencing Identifies Pathogenic and Modifier Mutations in a Consanguineous Chinese Family with Hypertrophic Cardiomyopathy. *Medicine (Baltimore)* 96, e7010. doi:10.1097/md.0000000000007010
- Zhang, Y., Jiang, X., Snutch, T. P., and Tao, J. (2013). Modulation of Low-Voltage-Activated T-type Ca $^{2+}$ Channels. *Biochim. Biophys. Acta (Bba) - Biomembranes* 1828, 1550–1559. doi:10.1016/j.bbame.2012.08.032
- Zhang, Y., Yamada, Y., Fan, M., Bangaru, S. D., Lin, B., and Yang, J. (2010). The β Subunit of Voltage-Gated Ca $^{2+}$ Channels Interacts with and Regulates the Activity of a Novel Isoform of Pax6. *J. Biol. Chem.* 285, 2527–2536. doi:10.1074/jbc.m109.022236
- Zhao, R., Liu, L., and Rittenhouse, A. R. (2007). Ca $^{2+}$ Influx through Both L- and N-type Ca $^{2+}$ Channels Increases C-Fos Expression by Electrical Stimulation of Sympathetic Neurons. *Eur. J. Neurosci.* 25, 1127–1135. doi:10.1111/j.1460-9568.2007.05359.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Vergnol, Traoré, Pietri-Rouxel and Falcone. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.