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Review

# Gene regulation by voltage-dependent calcium channels

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ABSTRACT

$Ca^{2+}$ is the most widely used second messenger in cell biology and fulfills a plethora of essential cell functions. One of the most exciting findings of the last decades was the involvement of $Ca^{2+}$ in the regulation of long-term cell adaptation through its ability to control gene expression. This finding provided a link between cell excitation and gene expression. In this review, we chose to focus on the role of voltage-dependent calcium channels in mediating gene expression in response to membrane depolarization. We illustrate the different pathways by which these channels are involved in excitation–transcription coupling, including the most recent $Ca^{2+}$ ion-independent strategies that highlight the transcription factor role of calcium channels.

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## 1. Introduction

Since the discovery of calcium as a second messenger, it has been implicated in an increasingly number of biological functions (reviewed in [1]). Indeed, calcium regulates basic cell processes such as proliferation, protein synthesis, and differentiation, but is also engaged in more specialized cell functions such as muscle contraction, neurotransmitter release, electrical excitability, and synaptic plasticity [2]. This implication in ever increasing cell functions reveals the amazing versatility in signaling properties of this second messenger. Moreover, $Ca^{2+}$ is not only a trophic factor, but is also involved in programmed cell death [3]. $Ca^{2+}$ differs from other second messengers in that, as all the elements of the periodic table, it cannot be metabolized. The second original property of $Ca^{2+}$ is that it is present in relatively high concentration in the extracellular space (1 to 2 mM). Consequently, a strict spatio-temporal control of intracellular $Ca^{2+}$ concentration is essential for its involvement in a wide variety of cell functions. To this end, $Ca^{2+}$ homeostasis is managed by a wealth of ion channels localized at both the plasma and the intracellular organelle membranes that permit elevation in cytosolic $Ca^{2+}$ concentration, as well as by $Ca^{2+}$-binding proteins and $Ca^{2+}$-pumps that restrict cytoplasmic $Ca^{2+}$ rise and organize its propagation in the cytosol and nucleus [4]. One amazing and unique property of $Ca^{2+}$ as a second messenger is its ability to control cellular events that develop on a large time scale, from milliseconds to hours [2]. The millisecond scale gives the full measure of the importance of fast gating $Ca^{2+}$ channels, unique pathways that promote localized $Ca^{2+}$ elevation. For instance, electrical excitability and neurotransmitter release entirely rely on this rapid signaling [5]. Due to the intricate balance between import, sequestration and export pathways, intracellular $Ca^{2+}$ signals are generally transient. Long-term $Ca^{2+}$ effects consequently occur thanks to the effective recruitment of various cytosolic and/or nuclear signaling pathways whose lifetimes are of longer durations than $Ca^{2+}$ signals themselves. $Ca^{2+}$ signal integration indeed explains how $Ca^{2+}$ can be involved in development, cell differentiation and synaptic plasticity. The ability of $Ca^{2+}$ to control both short- and long-term processes links cell differentiation and cell specialization to $Ca^{2+}$-regulated cell activity. As an example, late-phase of long-term potentiation in hippocampal CA1 neurons requires protein synthesis under the control of N-methyl-D-aspartate (NMDA) receptors and voltage-dependent calcium channels [6]. Many long-term effects of $Ca^{2+}$ as a second messenger imply a direct or indirect effect of $Ca^{2+}$ on gene regulation [7,8]. Recent development of high-throughput analyses methods has provided a vertiginous list of candidate genes whose expression is under the control of $Ca^{2+}$ homeostasis [9,10]. For instance, 70% of the gene expression modifications (up- or down-regulation of 111 genes) observed in T-lymphocytes from severe-combined immunodeficiency patients results from a defect of $Ca^{2+}$ entry [10]. In neuronal cells as well, exon expression profiling revealed modifications in the expression of several thousand transcripts in response to depolarization-induced $Ca^{2+}$ influx [9]. In spite of the impressive number of genes potentially affected by $Ca^{2+}$ signaling, only a small number of $Ca^{2+}$-dependent gene regulation pathways have been deciphered in details so far. This leaves ample room for discovering new signaling pathways and gene targets of $Ca^{2+}$ regulation.

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### 1.1. Molecular actors controlling $Ca^{2+}$-dependent gene regulation

The phenomenal buffering capacity of the cell strongly hampers $Ca^{2+}$ diffusion in the intracellular space [11]. While the extracellular space remains the main reservoir of $Ca^{2+}$, intracellular sources of $Ca^{2+}$ have been described as well that are essential in $Ca^{2+}$ signaling and gene regulation [12]. For instance, $Ca^{2+}$ released from the sarcoplasmic reticulum was shown to reduce the amount of mRNA coding for most nicotinic acetylcholine receptors, and thereby to control muscle fiber differentiation [13]. Although the best characterized intracellular $Ca^{2+}$ sources concerned with gene regulation are the endoplasmic reticulum and the nucleoplasmic reticulum, $Ca^{2+}$ from mitochondria, cytoplasmic secretion granules, and nuclear microvesicles [14] may deserve attention in the future. These $Ca^{2+}$ stores are spatially distributed in such a way that $Ca^{2+}$ can be mobilized in any domain of the cell allowing $Ca^{2+}$ signaling pathways to overcome the inherent buffering capacity of the cell. More recently, $Ca^{2+}$ nano- and microdomains have been proposed as important factors of the spatial specialization of $Ca^{2+}$ function [5,15]. These domains, corresponding to the opening of a few $Ca^{2+}$ channels, provide high $Ca^{2+}$ concentration environments restricted to the proteins associated to these channels. They have been shown to play a role in $Ca^{2+}$-dependent processes, including gene regulation. Obviously, the role of $Ca^{2+}$ in gene regulation will be as complex as its homeostasis. Regulation of intracellular $Ca^{2+}$ elevation implies many key factors that are all potential players in the field of gene regulation (Fig. 1). The first type of actors that intervene in $Ca^{2+}$ signaling and gene regulation are plasma membrane channels that sense various stimuli (voltage changes, extracellular ligand, loading level of intracellular $Ca^{2+}$ stores). These $Ca^{2+}$ channels comprise voltage-gated $Ca^{2+}$ channels (VGCC), receptor-activated $Ca^{2+}$ channels, such as the NMDA receptor, and store-operated $Ca^{2+}$ channels, such as ORAI and TRP members. The second type of players is composed of intracellular $Ca^{2+}$-release channels, namely ryanodine receptors and $IP_3$ receptors, which mobilize $Ca^{2+}$ from internal stores. An additional degree of complexity in $Ca^{2+}$ signaling resides in the intricate relationship between these calcium channels.

All these channel types have been involved in gene regulation with their own specificities. For instance, over-expression of the transient receptor potential canonical type 6 (TRPC6) calcium channel was shown to promote the formation of excitatory synapses *via* a calmodulin-dependent kinase IV (CaMKIV)/cAMP-response-element binding protein (CREB)-dependent pathway [16]. CaMKIV is a $Ca^{2+}$-dependent kinase that phosphorylates and activates the transcription factor CREB, similarly to other kinases such as protein kinase A (PKA), protein kinase C (PKC), CaMK kinase and mitogen-activated protein kinase (MAPK) [17,18]. CREB-mediated gene transcription is generally involved in synaptic plasticity and memory ([19] and see [20] for review), and accordingly, transgenic mice over-expressing TRPC6 show improved spatial learning and memory. As another illustration, the nuclear and cytosolic $Ca^{2+}$-rise induced by $IP_3$-receptor activation in skeletal muscle results in the activation of an extracellular signal-regulated kinase (Erk) and CREB phosphorylation, that ends by modification of gene transcription [21]. This signaling pathway is part of the process of excitation–transcription coupling whereby gene expression and thus cell differentiation is controlled by the electric activity of motor neurons [22]. In lymphocytes, activation of the $IP_3$-receptor has been shown to induce store-operated $Ca^{2+}$ entry through ORAI that in turn activates the phosphatase calcineurin. The transcription factor NFAT (nuclear factor of activated T-cells) is dephosphorylated by calcineurin, and then migrates to the nucleus where it activates gene transcription [23]. Although activation of each of these channels results in intracellular $Ca^{2+}$ rise, specific gene regulation pathways may be associated to the activation of a single channel type. The spatial [24] and temporal pattern of $Ca^{2+}$ elevation also matters with regard to $Ca^{2+}$-regulated gene transcription [25]. For instance, cytosolic $Ca^{2+}$ oscillations have been shown to reduce the $Ca^{2+}$ concentration threshold for activating the pro-inflammatory transcription factors NFAT, Oct/OAP and NFκB [26]. In the same trend of idea, the pathway of entry

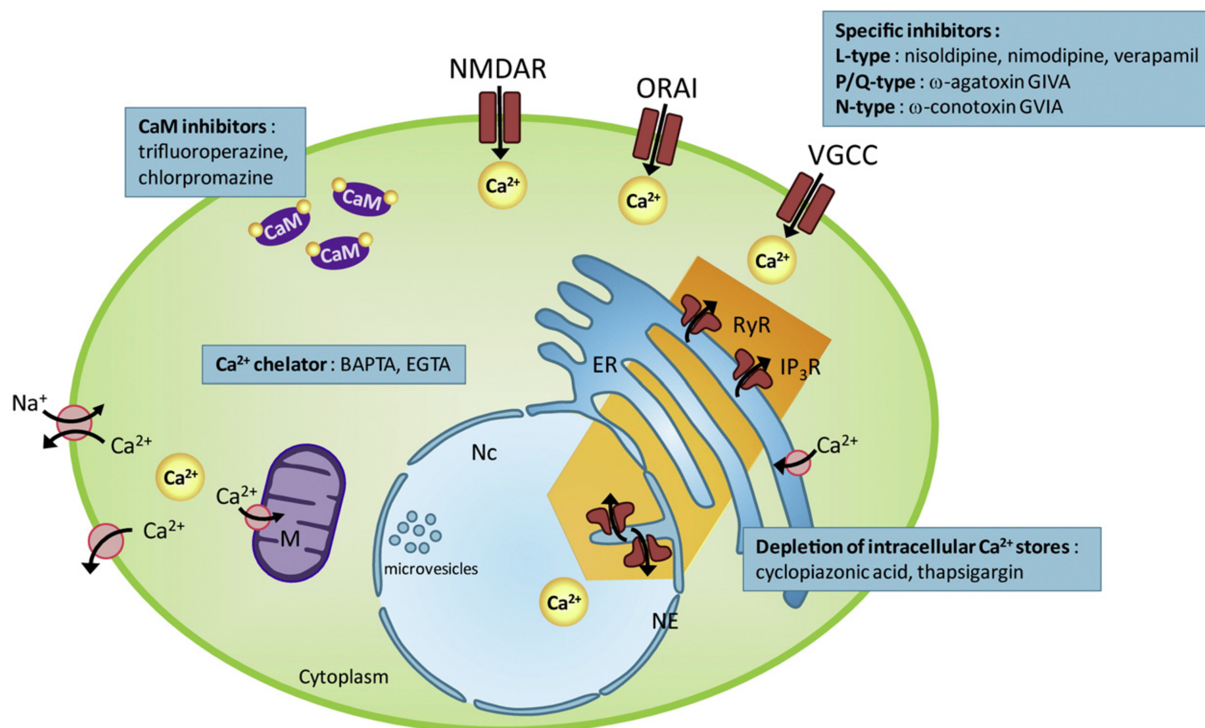


**Fig. 1.** Schematic representation of cytoplasmic and nuclear $Ca^{2+}$ elevation pathways. Inhibitors of $Ca^{2+}$ elevation pathways or $Ca^{2+}$-binding proteins are shown. Abbreviations are: CaM, calmodulin; ER, endoplasmic reticulum; $IP_3R$, $IP_3$ receptors; M, mitochondria; Nc, nucleoplasm; NE, nuclear envelope; NMDAR, NMDA receptors; RyR, ryanodine receptors; VGCC, voltage-gated calcium channels.

of $Ca^{2+}$ in hippocampal neurons, NMDA receptors *versus* voltage-dependent L-type channels, determines the kinetics of CREB phosphorylation and thereby the level of expression of the immediate early gene c-*fos* [27]. Finally, differences in amplitude and duration of the antigen-stimulated $Ca^{2+}$ responses in B lymphocytes translate into distinct patterns of activation of transcription factors such as NFAT, ATF-2 and NFκB [28].

In the remainder of this review, we shall focus on the implication of voltage-gated calcium channels in the control of gene expression. These channels represent a model of choice since they are at the core of the excitation–transcription process by transforming electrical activity in $Ca^{2+}$-signaling information. Since they are present at the plasma membrane, they use several original signaling pathways to vehicle information from the periphery of the cell towards the nucleus, the locus of the transcription. Voltage-dependent calcium channels being encoded by many genes, and fulfilling a wide variety of cell functions, from contraction to transmitter release, the excitation–transcription process in which each calcium channel type is involved may respond to specific functional needs (from contractile proteins to synaptic plasticity).

## 2. Voltage-dependent calcium channels and gene regulation

### 2.1. Voltage-dependent calcium channels: structure and molecular diversity

At the structural level, all voltage-dependent calcium channels share a common pore-forming subunit. Ten genes encode for low voltage-activated calcium channels (termed T-type channels, 3 types) and high voltage-activated calcium channels (7 types forming L-type channels (4 types), N-type channel (1 type), P/Q-type channel (1 type) or R-type channel (1 type)) [29]. So far, no auxiliary subunits have been clearly identified for low voltage-activated T-type calcium channels. In contrast, the subunit structure of high voltage-activated calcium channels is much better defined. All these channels possess, in addition to the pore-forming subunit, two associated auxiliary subunits: $\alpha_2\delta$, a glycosylated protein with a single transmembrane domain, and β, a regulatory subunit of the MAGUK family. The crystal structure of this latter protein has been resolved and shows that the protein is composed of an SH3 domain associated to a guanylate kinase (GK) domain. In some channels, a protein called γ subunit has been proposed to form the third auxiliary subunit of high voltage-activated channels. All these auxiliary subunits play a role in normalizing the biophysical properties of calcium channels and controlling their expression levels at the plasma membrane. These subunits are also encoded by several genes (4 for $\alpha_2\delta$ subunits, 4 for β subunits, and 8 for γ subunits) thereby greatly increasing the phenotype diversity of native calcium channels [30]. Neuronal tissues express many channel types, while other tissues express a poorer subset of voltage-gated calcium channels (one type only in skeletal muscles). In neurons, voltage-dependent calcium channels carry out several functions such as dendritic integration of incoming electrical signals and transmitter release at the synapse. This later function is accomplished mainly by P/Q-, N- and R-type channels. As a result of the importance of voltage-gated calcium channels in many physiological processes, genetic mutations of calcium channel genes induce severe pathologies such as epilepsy, autism, ataxia, migraine, myopathies, deafness and blindness [31] The link between these genetic mutations and defects in excitation–transcription coupling remains largely unexplored so far.

### 2.2. First evidences that voltage-dependent calcium channel are involved in gene regulation

The initial proof that voltage-gated calcium channels are implicated in gene regulation comes from two simultaneous studies published in 1986 [32,33]. In a first study, chronic depolarization of pheochromocytoma (PC12) cells was shown to induce an increase in c-*fos* expression levels. This effect was blocked by the L-type channel inhibitor nisoldipine, as well as by trifluoroperazine or chlorpromazine, two calmodulin antagonists. It was therefore concluded that L-type calcium channel-driven cytoplasmic $Ca^{2+}$ elevation turns on a calmodulin-dependent expression of c-*fos* [32]. This pathway differed from the earlier-described calmodulin-independent activation of c-*fos* expression observed after application of nerve growth factor (NGF) in PC12 cells. The second study demonstrated that, in PC12 cells, the c-*fos* expression induced by nicotinic receptor activation similarly results from the activation of L-type channels, this effect being blocked by verapamil, another L-type channel inhibitor [33]. Since this paradigm also induces a depolarization-independent expression of β-actin, it was concluded that activation of nicotinic receptor turns on two $Ca^{2+}$-dependent gene regulation pathways: one indirectly mediated by $Ca^{2+}$ entry through L-type channels and another one directly induced by $Ca^{2+}$ entry through the nicotinic receptor. This study perfectly illustrates that the origin of $Ca^{2+}$ entry defines the gene regulation process activated and consequently the nature of the modulated gene. Since these pioneering studies, different gene regulation pathways have been discovered to be under the control of voltage-dependent calcium channels. They are illustrated hereunder and summarized in Fig. 2.

### 2.3. Voltage-dependent calcium channels participate to several gene regulation pathways

Voltage-dependent calcium channels are increasingly depicted as multi-molecular complexes that regroup not only the channel itself and its auxiliary subunits, but also many key molecular players that translate depolarization-induced $Ca^{2+}$ influx and/or conformational changes of the channel into functional events. The excitation–transcription process in which calcium channels are involved does not escape this basic rule. Upon activation of voltage-dependent calcium channels, gene regulation can occur following several conceptually different pathways, that all lead to the activation of transcription factors. We will develop here example of pathways that end in the activation of CREB, NFAT and downstream regulator element antagonist modulator (DREAM) transcription factors. However, it should be mentioned that calcium channel activity has also been linked to MEF2 [34], MeCP2 [35], SRF [36] and NFκB [17,37] transcription regulators. These pathways are illustrated hereunder and key molecular players highlighted. Pathways were discriminated according to their channel-association state and their molecular nature (transcription factor or not), and the ability of $Ca^{2+}$ to propagate at distance of the site of influx (mouth of the channel). Controversy may arise from the real level of channel-association state of $Ca^{2+}$-sensitive proteins in each pathway described as this point is not always well resolved experimentally. The second point on which controversy will remain is whether $Ca^{2+}$ ions themselves have real diffusion capabilities to the nucleus or whether they require $Ca^{2+}$ binding proteins to reach the nucleus.

#### 2.3.1. Regulation of transcription factors by $Ca^{2+}$-binding proteins not directly associated to the channel

In 1993, $Ca^{2+}$ entry through L-type calcium channels was shown to induce activation of multifunctional CaMK in hippocampal neurons [38]. This process was linked to the activation of c-*fos* promoter. At the same time, it was demonstrated that CaMKI, CaMKII and CaMKIV share the ability to phosphorylate Ser133 of the transcription factor CREB [39,40]. While phosphorylation of CREB on Ser133 is generally considered as an activating event, the situation turns out to be more complex since CREB is also phosphorylated at Ser142 by CaMKII leading to its inhibition [41]. CaMKIV is considered as the most reliable stimulating enzyme of the CREB pathway since (i) it allows the recruitment of CREB binding protein (CBP) on CREB, and (ii) its

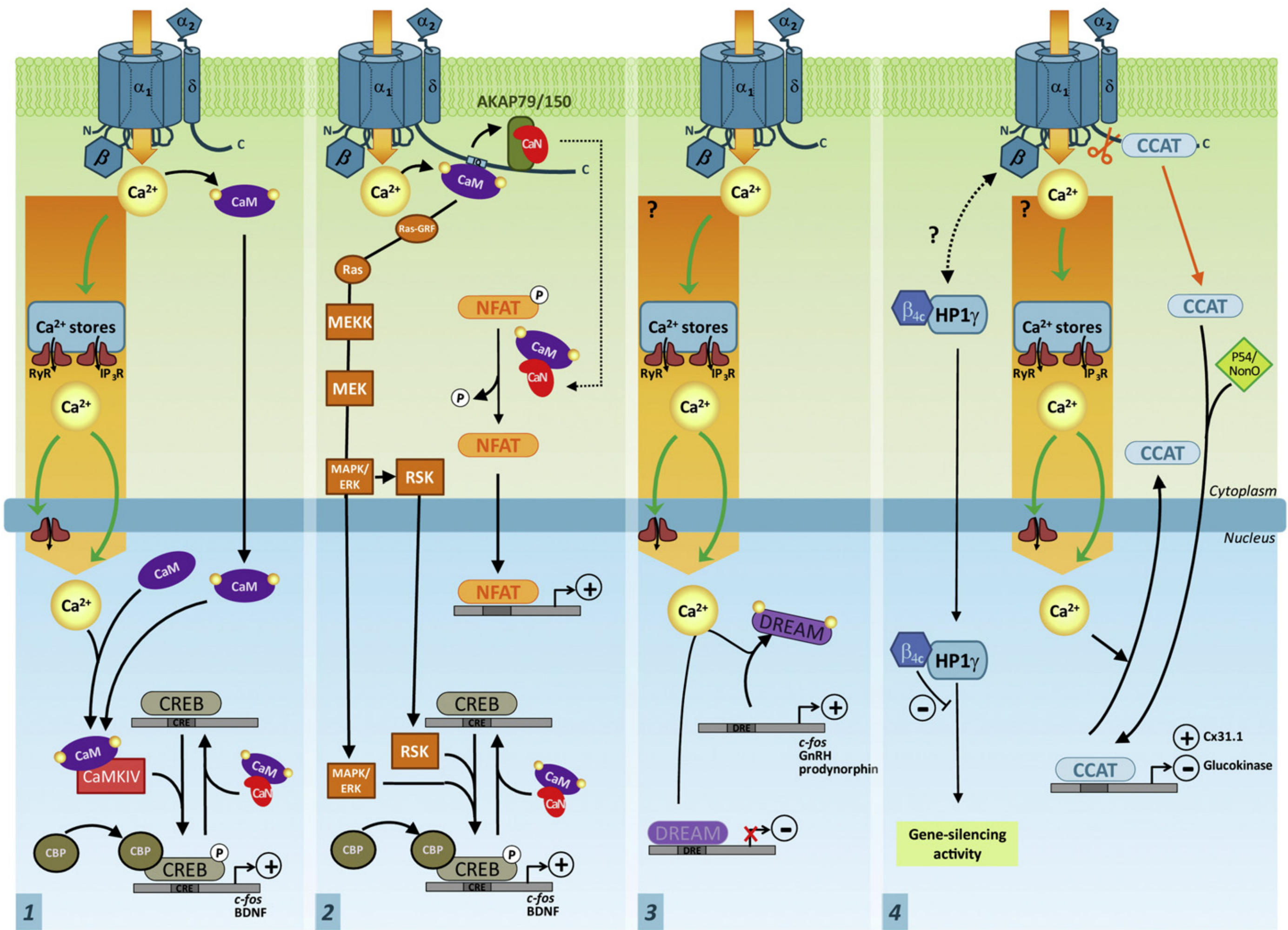


**Fig. 2.** Schematic gene transcription pathways regulated by voltage-gated calcium channels. 1. Activation of transcription factors by $Ca^{2+}$-binding proteins. The example of CREB phosphorylation by CaMKIV following activation of calmodulin by $Ca^{2+}$ entry is shown. Calmodulin activation can occur consecutively to $Ca^{2+}$ entry through voltage-gated calcium channels, or after an amplification cascade through $IP_3R$- or RyR-sensitive internal stores. 2. Regulation of transcription factors by $Ca^{2+}$-activated proteins that are part of the calcium channel signaling complex. Two examples are illustrated: MAPK regulation by calmodulin bound on the IQ domain of L-type channels, and calmodulin-activated calcineurin (CaN) regulation of cytoplasmic NFAT. Whether CaN dissociates from the channel to activate NFAT or whether NFAT is in the immediate vicinity of the channel remains unknown. 3. Regulation of $Ca^{2+}$-binding transcription factors, such as DREAM, by voltage-gated $Ca^{2+}$ channels. Whether the $Ca^{2+}$ that enters voltage-dependent channels activates directly DREAM by propagating to the nucleus, or whether it is relayed and amplified by $Ca^{2+}$ stores as depicted here is not established. 4. Calcium channel fragments or subunits acting as transcription factors. Two examples are detailed. $\beta_{4c}$ is a short splice variant of $\beta_4$ subunit whose association with voltage-dependent calcium channels is challenged by the lack of well-defined guanylate kinase domain required for its association with the pore-forming subunit. It binds onto HP1γ and inhibits its gene silencing function. CCAT is a transcription factor directly derived from the carboxy-terminus of the pore-forming subunit of L-type channels. It translocates to the nucleus where it regulates gene activity. Its export out of the nucleus is controlled by nuclear $Ca^{2+}$. Again, whether internal $Ca^{2+}$ stores relay $Ca^{2+}$ entry through voltage-dependent channels is not yet established.

nuclear depletion disrupts CREB phosphorylation in hippocampal neurons [42]. These results raised the question on how nuclear CaMK are activated by $Ca^{2+}$ entering through L-type channels. All these studies highlight the importance of both the nuclear translocation of calmodulin and the ratio between apo-calmodulin and $Ca^{2+}$-calmodulin for the activation of nuclear CaMK. Two modes of nuclear accumulation of $Ca^{2+}$-calmodulin have been demonstrated. On one hand, $Ca^{2+}$ entering the cell through L-type channels activates surrounding calmodulin that translocates and accumulates within the nucleus [43,44]. Indeed, nuclear translocation is supported by the 20-fold higher diffusion coefficient of $Ca^{2+}$-calmodulin compared to apo-calmodulin [43]. However, nuclear accumulation of $Ca^{2+}$-calmodulin cannot be explained simply by diffusion, but is likely to require a cofactor for both $Ca^{2+}$-calmodulin stabilization and transport. On the other hand, a CREB activation pathway, independent of $Ca^{2+}$-calmodulin nuclear translocation, has been identified in electrically-stimulated hippocampal neurons [45]. In this study, CREB activation induced by L-type channel stimulation was still observed in the presence of wheat germ agglutinin, a nuclear pore blocker precluding protein transfer to the nucleus. In this case, CREB activation correlates with increases in nuclear $Ca^{2+}$ concentration that are inhibited by depletion of intracellular $Ca^{2+}$ stores with cyclopiazonic acid or thapsigargin. The mechanism behind this regulation relies on the propagation of the $Ca^{2+}$ signal from the cell periphery to the nucleus which is made possible by $Ca^{2+}$ release from internal stores induced by $Ca^{2+}$ influx following electric stimulation. This study supports earlier reports showing that nuclear injection of BAPTA-D70, a $Ca^{2+}$ chelator specifically addressed to the nucleus, completely inhibits CREB activation following L-type channel opening [39,46]. While the importance of $Ca^{2+}$-activated kinases in CREB phosphorylation and CRE-mediated transcription cannot be dismissed, evidence is building up for a critical role of $Ca^{2+}$-dependent phosphatases (phosphatase 2B or calcineurin). In neurons, $Ca^{2+}$ entry through L-type channels controls inositol 1,4,5-trisphosphate type 1 receptor expression through the activation of calcineurin [47]. Conceptually, since calcineurin is mostly present within the cytoplasm, it is assumed

that it may dephosphorylate and thereby induce the nuclear translocation of a cytoplasmic transcription factor. However, despite the proven existence of nuclear calcineurin, it was found that calcineurin dephosphorylates CREB Ser133 raising suspicion that it may also act directly within the nucleus [48]. Interestingly, L-type-dependent calcineurin activation has also been involved in the temporal control of CREB phosphorylation through a mechanism that involves a dephosphorylation-induced inhibition of L-type channel activity [49]. The dual regulation of nuclear signaling pathways by kinases and phosphatases is a general feature of the cell surface to nucleus communication [50].

Although L-type channels are the best characterized voltage-dependent calcium channels with regard to gene regulation, some studies also suggest the implication of non-L-type channels in excitation–transcription coupling. Investigation of NFATc1 nuclear translocation in response to specific patterns of electrical activity in rat sympathetic neurons shows that 10 Hertz stimulation trains promotes its $Ca^{2+}$-dependent nuclear translocation while 1 Hertz trains are ineffective [51]. Importantly, this activity-dependent translocation of NFATc1 strictly relies on the activation of N-type calcium channels since this effect is blocked by ω-conotoxin GVIA, a specific inhibitor of these channels. In another example, depolarization of superior cervical ganglion neurons induces both CREB phosphorylation and increases in c-*fos* mRNA and protein levels [52]. This N-type channel-dependent CREB phosphorylation occurs only at low frequency stimulation, while, at high frequency stimulation, CREB regulation exclusively involves L-type channels. This selective frequency-dependent implication of calcium channel types seems to be based on inactivation kinetic differences between N- and L-type channels. Finally, specific activation of N-type calcium channels in primary sensory neurons by electric stimulation results in tyrosine hydroxylase mRNA expression [53]. This effect is reduced by inhibitors of PKA and PKC, while blockers of MAPK and CaMKII have no effect. Importantly, in all these studies, action potential-like electric stimulations were used instead of KCl-induced depolarization since this latter paradigm produces N-type calcium channel inactivation that may mask their implication in excitation–transcription coupling. P/Q-type calcium channels have also been implicated in an important gene regulation pathway. Indeed, blocking depolarization-activated P/Q-type calcium channels in cerebellar granule cells using the selective blocker ω-agatoxin IVA reduces the expression of syntaxin-1A mRNA to undetectable levels [54]. This effect is specifically linked to P/Q calcium channels since L- and N-type channel blockers have no effect on the $Ca^{2+}$-dependent expression of syntaxin-1A. In addition, the effect of P/Q calcium channel activation on syntaxin-1A expression requires intact intracellular $Ca^{2+}$ stores as well as functional CaMKII and IV, PKA and MAPK kinase pathways. Interestingly, syntaxin-1A is a critical SNARE protein component for neurotransmitter release that is physically associated to P/Q calcium channels. This example therefore represents a positive feedback mechanism linking synaptic signaling and gene expression.

### *2.3.2. Regulation of transcription factors by $Ca^{2+}$-activated proteins associated to voltage-dependent calcium channel complexes*

While this pathway also implies a modification of transcription factors by $Ca^{2+}$-activated phosphatases or kinases, it differs from the preceding by the specific implication of regulatory proteins directly interacting with voltage-dependent calcium channel components. The first example of such a mechanism again concerns the CREB transcription factor family. It was shown that L-type channel activation by depolarization in cortical neurons induces CREB Ser133 phosphorylation by the MAPK pathway [55]. This effect is specific of L-type channel activation since blockers of other calcium channel types (N- and P/Q-types) have no effect on this depolarization-induced activation of CREB. In this case, $Ca^{2+}$ activation of calmodulin bound to the IQ domain of $Ca_v1.2$ (L-type channel pore-forming subunit) is required for the recruitment of the MAP kinase pathway. This result also highlights the importance of $Ca^{2+}$ microdomains. Indeed, EGTA that buffers cytoplasmic $Ca^{2+}$ without affecting $Ca^{2+}$ elevation at the immediate proximity of the channel mouth has no effect on the L-type channel-dependent CREB phosphorylation [44]. Therefore, this CREB phosphorylation pathway involves both the L-type channel complex itself (*via* its associated calmodulin) and the $Ca^{2+}$ permeating through the channel. A mutation of the IQ domain, that prevents calmodulin binding onto $Ca_v1.2$, without affecting $Ca^{2+}$ currents, inhibits depolarization-induced activation of CREB-dependent genes indicating that free $Ca^{2+}$-calmodulin not bound to the channel is unable to induce CREB phosphorylation. Activation of MAPK/Erk involves activation of Ras, a small G protein, followed by MEK1 phosphorylation by a MEK kinase. In turn, activated MEK1 phosphorylates and activates MAPK/Erk [56]. Activated MAPK/Erk translocates to the nucleus where it phosphorylates CREB. Alternatively, MAPK/Erk can phosphorylate RSK (p90 kDa ribosomal S6 kinase) which translocates to the nucleus and acts as a CREB kinase [57]. Ras activation may occur through the $Ca^{2+}$-calmodulin-sensitive Ras-GRF exchange factor in neurons [58]. The prevailing view is that CREB is already bound to CREs within the promoter of CREB-regulated genes and that its phosphorylation on Ser133 by MAPK favors the recruitment of the transcriptional coactivator CREB binding protein (CBP). The role of CBP in histone acetylation and further recruitment of the RNA polymerase II transcription machinery permits chromatin remodeling and transcription.

Recently, it has been described that CaMKII directly interacts with the C-terminal domain of the pore-forming subunit of L-type channels [59]. CaMKII tethering onto L-type channels requires $Ca^{2+}$ entry through the ionic pore and calmodulin activation. Activated CaMKII phosphorylates L-type channels and contributes to channel facilitation, but it appears also to activate the CREB pathway through a mechanism that remains to be determined [60]. Interestingly, the dual action of CaMKII on L-type channels and CREB phosphorylation may temporally adjust L-type channel signaling input on gene transcription.

In another example, by using a plasmid encoding green fluorescence protein with a promoter under the control of NFATc4, it was shown that activation of NFATc4 is triggered by neuronal depolarization [61]. This process is inhibited by blockers of L-type channels while those of N- and P/Q-type channels have no effect. The authors show that activation of NFATc4 relies on activation of calcineurin, a $Ca^{2+}$-calmodulin-dependent phosphatase that by dephosphorylating NFATc4 allows its nuclear activation [62]. Recently, Oliveria et al. have shown that calcineurin anchoring to $Ca_v1.2$ *via* A-kinase anchoring proteins AKAP79/150 is required to turn on the NFATc4-dependent gene regulation pathway [63]. This group demonstrates that AKAP150 specific RNAi inhibits depolarization-induced NFATc4 nuclear translocation suggesting that the absence of AKAP150 precludes calcineurin-dependent NFATc4 dephosphorylation.

These examples demonstrate that voltage-dependent calcium channels are also involved in gene regulation *via* $Ca^{2+}$-sensing elements interacting with the calcium channel complex. $Ca^{2+}$-sensing proteins may be bound directly to the channel itself or indirectly through a connecting protein. This new route of $Ca^{2+}$ signaling that originates in the close molecular vicinity of the channel complex strongly reinforce the importance of strictly restricted local $Ca^{2+}$ increases (nano/microdomains).

### *2.3.3. Voltage-gated calcium channels and $Ca^{2+}$-regulated transcription factors*

In the former studies described above, transcription factors were under the control of $Ca^{2+}$-binding proteins such as calmodulin. Here, we illustrate the case of transcription factors that directly bind $Ca^{2+}$, providing a more straightforward link between intracellular $Ca^{2+}$ elevation and transcription regulation. However, the same

questioning is raised in this case with regard to the origin of the required $Ca^{2+}$ elevation, nuclear *versus* cytoplasmic. In a search for proteins interacting with the downstream regulatory element (DRE) of the prodynorphin gene, a 29 kDa protein, DREAM, was identified that unexpectedly possesses four EF-hands [64,65]. DREAM binds to DRE as a tetramer and acts as repressor. Binding of $Ca^{2+}$ to DREAM promotes its dissociation from DRE, possibly by favoring its conformational transition from a tetrameric state to a dimeric state [66]. In this scheme, $Ca^{2+}$ relieves transcriptional repression of DRE-containing promoters. Other DRE-regulated genes have been identified, including c-*fos* and gonodotropin-releasing hormone (GnRH) [64,67]. Using immortalized GT1-7 GnRH neurons, it was shown that nimodipine, a L-type channel blocker, prevents GnRH expression and release. Real-time monitoring of DREAM action on luciferase reporter under the control of the GnRH promoter shows that nimodipine blocks luciferase expression [67]. These data demonstrate the link between $Ca^{2+}$ entry through L-type channels and DREAM activation. Similar inhibition of luciferase expression was observed when DREAM was neutralized using specific antibodies. DREAM possesses sequence identity with calsenilin, a presinilin-2 interacting protein, and KChIP-3 (for potassium channel interacting protein 3), which interacts with the $K_v4$ amino-terminus domain. Three other members of this family have been identified since then (KChIP-1, -2 and -4) whose DNA-binding activities have not yet been established (for review see [68]). These data indicate that KChIP proteins have pleiotropic functions in cells, both in the cytoplasm and in the nucleus.

#### *2.3.4. Voltage-dependent calcium channel domains that act as transcription factors*

Since the pioneering studies on calcium channel implication in gene regulation, a novel concept has emerged centered around the idea that calcium channel domains themselves may act as transcription factors. The very first evidence for such an implication of calcium channel domains comes from a study of Hibino et al. [69]. In this work, the authors demonstrate that a short particular splice variant of $\beta_4$, termed $\beta_{4c}$, interacts with the chromobox protein 2/heterochromatin protein 1 γ (CHCB2/HP1γ), a nuclear protein involved in gene silencing and transcriptional regulation [70]. The interaction between $\beta_{4c}$ and CHCB2/HP1γ is required for nuclear translocation of $\beta_{4c}$. β subunits are normally auxiliary subunits of voltage-gated calcium channels but this short isoform lacks most of the guanylate kinase domain of β subunits and should therefore not associate with the pore-forming subunit of voltage-gated calcium channels [71]. Nevertheless, the authors report a mild regulation of the channel gating in the presence of this subunit suggesting a form of loose connection between $\beta_{4c}$ and the channel moiety. CHCB2/HP1γ recognizes and binds a lysine residue near the carboxy-terminus of histone H3 that is specifically methylated by SUV39H1. Binding of SUV39H1 and CHCB2/HP1γ onto histone H3 favors the formation of hetechromatin, a higher order chromatin state, thereby repressing gene activity (for review see [72]). Using a GAL4-CAT reporter system, the authors show that CHCB2/HP1γ inhibits CAT expression, and that $\beta_{4c}$ decreases the gene silencing activity of CHCB2/HP1γ. The mechanism of this regulation remains unexplained but highlights for the first time the direct implication of calcium channel sequences in the regulation of gene expression. The mechanism of this regulation remains unexplained but highlights for the first time the direct implication of calcium channel sequences in the regulation of gene expression.

More recently, a proteolytic fragment of the C-terminus of the pore-forming subunit of L-type channels ($Ca_v1.2$) was shown to act as a transcription factor [73]. This domain was called calcium channel-associated transcriptional regulator (CCAT). Using antibodies directed towards the C-terminus of $Ca_v1.2$, the authors show the presence of CCAT in the nucleus of neurons from developing and adult brains. CCAT contains a nuclear retention domain but no nuclear localization signal. In agreement, the rise of intracellular $Ca^{2+}$ concentration, resulting from L-type calcium channel opening, triggers the export of CCAT from the nucleus. CCAT was found associated with p54(nrb)/NonO, a transcriptional regulator of, among others, the retinoic acid and thyroid hormone receptors. Using oligonucleotide microarray, the authors identified several genes that are down- or up-regulated by CCAT. Among these genes, a gene coding for gap junction protein connexin Cx31.1 is up-regulated as shown by the effect of CCAT on the Cx31.1 promoter associated to a luciferase reporter. This protein plays an essential role in neurite extension. However, the protease responsible for CCAT production in neurons remains to be identified, as well as the mechanism of its regulation by L-type channels. Alternatively, CCAT could result from the alternative splicing of the $Ca_v1.2$ gene, a hypothesis that would be worth investigating.

Lately, we evidenced, in a study that has been the object of a communication at the 10th European Calcium Society meeting in Leuven, that the full-length $\beta_{4a}$ isoform of voltage-dependent calcium channels is addressed to the nucleus of neurons. This process requires the association of $\beta_4$ with B56δ, an auxiliary subunit of phosphatase 2A (PP2A). The complex $\beta_{4a}$/B56δ/PP2A associates to nucleosomes and probably plays a role in the dephosphorylation of histones. The association of B56δ to $\beta_4$ is sensitive to the conformation of $\beta_4$ and requires the interaction of the SH3 domain of $\beta_4$ with its guanylate kinase domain. This conformation-dependent interaction leaves room for various regulation of $\beta_4$ nuclear targeting. Interestingly, a mutation of $\beta_4$, associated to human epilepsy, leads to the loss of the 38 last carboxyl-terminal amino acids of $\beta_4$ [74]. We have shown that this mutant is defective for nuclear localization because of a lack of association with B56δ. The relevance of PP2A in transcription regulation has been previously documented [75–77]. Accordingly, a transcriptomic study evidenced that $\beta_4$ nuclear translocation induces up- or down-regulation of several genes. Some of these genes are differently regulated if the human epileptic $\beta_4$ mutant is expressed instead of wild-type $\beta_4$. Overall, these data indicate for the first time that a full-length subunit of a calcium channel can regulate gene transcription. The signaling route whereby $\beta_4$ subunit dissociates from the channel and associate to B56δ, a new protein partner that takes it to the nucleus, is still not understood. However, these data open exciting new perspectives for the role of voltage-dependent calcium channels in excitation–transcription coupling. The universality of the uncovered regulation (β isoforms concerned and calcium channels implicated) and the intimate process of gene regulation by $\beta_4$ will require independent investigations.

### *2.4. Role of voltage-dependent calcium channels in post-transcriptional regulation*

Although studies on voltage-dependent calcium channel implication in regulation of gene transcription have mostly focused on transcription, $Ca^{2+}$ also controls post-transcriptional events by influencing mRNA splicing and stability. Indeed, depleting $Ca^{2+}$ stores with thapsigargin produces alterations in the relative abundance of no less than 3,489 different transcripts in human IMR32 neuroblastoma cells, as assessed by high density exon-centric microarray [9]. If depolarization is used instead of thapsigargin treatment, no less than 1,505 transcripts vary in their copy number illustrating again the crucial role of $Ca^{2+}$ in gene expression. However, if one looks at the effect of thapsigargin or KCl treatment on alternative splicing, then 8,533 (thapsigargin) or 5,139 (KCl) transcripts appear to carry a modified number of copies for one or more exons. Increase in cytoplasmic calcium concentration results in (i) alteration of the abundance of a given transcript (transcriptional regulation and/or mRNA stability) and (ii) preferential alternative splicing that result in the appearance of new transcripts (post-transcriptional regulation). Interestingly, $Ca^{2+}$-binding proteins, plasma membrane proteins and ion transporters are part of the transcripts up-regulated by elevated intracellular $Ca^{2+}$ concentration indicating that $Ca^{2+}$ itself controls the

expression of proteins involved in $Ca^{2+}$ homeostasis. In a more specific study on IMR32 cells, a transcript variant encoding a new form of the plasma membrane $Ca^{2+}$ ATPase PMCA is detected exclusively in response to KCl depolarization [78]. In the same trend, chronic KCl depolarization of cortical neurons modulates the splicing of neurexin 2a [79], a synaptic adhesion protein. This splicing produces the selective exclusion of exon 11 of neurexin 2α and necessitates calcium channel activity as witnessed by its blockade by $CdCl_2$. Other studies have been conducted on the mechanisms of the alternative splicing regulation by $Ca^{2+}$. Exon 21 of the NMDA receptor type 1 gene encodes a protein sequence that controls several functions of the receptor, among which is targeting to the plasma membrane. The splicing of this exon varies during development [80]. Depolarization of P19 embryonic carcinoma cells results in the exclusion of exon 21 from the transcript. This effect requires the activation of CaMKIV and depends of two CaRRE (CaMKIV-Responsive RNA Element) motifs of exon 21 [81].

Regarding the mRNA stability issue, it was shown that the long-form mRNA stability of $Ca_v2.2$ pore-forming subunit of N-type calcium channels, is increased by KCl-induced depolarization and L-type channel activation [82]. This results in the increase of N-type channel density at the plasma membrane of sympathetic neurons. This process, whereby the activity of one calcium channel type controls the expression level of another calcium channel, may represent an important feedback mechanism during development and/or adaptation and should be carefully considered in the interpretation of data coming from calcium channel knock-out or transgenic mice.

### 2.5. Altered gene regulation due to pathological mutations of voltage-dependent calcium channels

Genes coding for calcium channel subunits are known to carry mutations associated to various pathologies [83,84]. Researches on channelopathies generally focus on biophysical defects of the mutated calcium channels, miss-folding and/or miss-targeting issues, and more rarely on possibly associated gene expression deregulation [85]. However, considering the numerous pathways implicating voltage-dependent calcium channels in the regulation of gene expression, the causal link between calcium channel mutations and pathologies may also reside in transcription or post-transcription defects. Some preliminary evidences illustrate that pathologies associated to voltage-dependent calcium channel gene mutations result from the alteration of gene expression. The rolling mouse Nagoya, carrying a mutation of the gene coding for $Ca_v2.1$ of P/Q calcium channels, is an ataxic mutant mouse with severe ataxic gait. It shows altered mRNA and protein expression levels of the ryanodine receptors type 1 and 3 [86]. mRNA levels of ryanodine receptor type 1 are also found to be altered in tottering mice that carry another type of $Ca_v2.1$ mutation [87]. Considering the role of voltage-dependent calcium channels in gene regulation, transcriptomic alterations are likely to be at the basis of an ever growing list of channelopathies.

## 3. Perspectives

In this review, we illustrated the different routes that link without ambiguity voltage-dependent calcium channels and gene expression regulation, at both the transcriptional and post-transcriptional levels. In some cases, these routes have been linked to the expression of specific genes involved in physiological functions such as circadian rhythms, learning and memory, and control of neuronal survival and death (for review [88]). The understanding of the relationship between specific extracellular stimuli, $Ca^{2+}$-signaling pathway and specific physiological response represents the ultimate goal of these studies.

Other pathways exist between $Ca^{2+}$ ions and gene regulation that are not connected so far to voltage-gated calcium channels. Indeed, several transcription factors are known to be regulated by $Ca^{2+}$, directly or indirectly, or to be part of the calcium channel signaling complexes. For instance, a specific class of transcription factors, termed basic helix–loop–helix (bHLH) proteins is regulated by the EF-hand-containing $Ca^{2+}$-binding proteins calmodulin and S100. Seven classes of bHLH transcription factors have been described, some acting as transcription factors (e.g. class I) and others as repressors (e.g. class IV and VI) [89]. bHLH proteins bind to DNA onto a consensus E-box sequence, CANNTG, through their basic DNA-binding domain and following the formation of dimers or higher oligomerization states. $Ca^{2+}$-calmodulin may regulate bHLH transcription factors according to one of the following mechanisms: i) by precluding class I and II bHLH binding to DNA, or ii) by preventing the oligomerization of bHLH required for their DNA-binding activity [68]. Since several $Ca^{2+}$-calmodulin-dependent gene regulation pathways rely on $Ca^{2+}$ entry through voltage-dependent calcium channels, it will be interesting to investigate the possible link between $Ca^{2+}$-calmodulin regulation of bHLH factors and voltage-gated calcium channel activity.

In another series of observations, CASK, a member of membrane-associated guanylate kinase family, has been shown to interact with Tbr-1, a T-box transcription factor [90] that is implicated in cerebellar development. When associated to Tbr-1, CASK redistributes to the nucleus and is present in a T element complex that binds to a specific T-box DNA sequence. Interestingly, CASK interacts also with the C-terminal domain of $Ca_v2.2$, the pore-forming subunit of N-type calcium channels, but not with the homologous domain of L- or R-type calcium channels [91]. CASK association with one isoform of $Ca_v2.2$ directs the channel towards presynaptic nerve terminals in hippocampal neurons, suggesting that, besides its role as transcription factor, it also influences channel targeting [92]. The importance of this CASK/channel complex in excitation–transcription coupling, specifically from synapse to nucleus, is still not established but will merit further investigation.

While regulation of transcription factors by channel activity has been widely illustrated, a completely new signaling pathway linking transcription factors to ion channels has recently been discovered. Caraveo et al. showed that TFII-I, a ubiquitously expressed transcription factor present in both the cytoplasm and the nucleus, regulates the agonist-induced $Ca^{2+}$-entry through TRPC3 calcium channels [93]. Down-expression of TFII-I using siRNA technology results in an increase of agonist-induced $Ca^{2+}$ influx. This effect of TFII-I is maintained upon replacement of wild-type TFII-I by a nuclear localization-deficient TFII-I mutant; a result indicating that TFII-I regulates TRPC3 by a gene transcription-independent mechanism. The authors identified phospholipase Cγ as a specific partner of TFII-I. Phospholipase Cγ was shown to play a role in the agonist-induced plasma membrane incorporation of *de novo* TRPC3 channels [93], a process that would be modulated by its association with TFII-I.

This review illustrates the amazing interplay existing between calcium channels, $Ca^{2+}$ ions, $Ca^{2+}$-binding proteins and transcription factors during the processing of information between the plasma membrane, sometimes as remotely as the synapse, and the nucleus. The presence of some transcription factors directly in the calcium channel molecular complexes indicates the incredible level of local integration in information processing. Conversely, the idea that channel fragments may directly act as transcription factors implies a simplification of the signaling scheme underlying excitation–transcription coupling. We bet that novel conceptually interesting pathways linking calcium channels to gene transcription will soon emerge to further enrich our understanding of this fascinating research world.

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