Chapter 8
Voltage-Gated Calcium Channel Signaling to the Nucleus

Michel Bellis, Thierry Cens, Pierre Charnet, and Matthieu Rousset

Abstract Excitation-transcription coupling makes use of cellular excitability to produce intracellular signals to the nucleus to control activity-dependent gene expression. Voltage-gated calcium channels are presented here as a signaling platform able to redirect multiple signaling pathways toward the nucleus. Whilst several Ca_V subunits are implicated in excitation-transcription coupling, each type of Ca_V nevertheless possesses its own proteome and microenvironment able to promote individualized signaling pathways. L-type calcium channels have structural determinants that favor the initiation of MAPK and CamK pathways for example, but P/Q and N-type channels, in close proximity to the endoplasmic reticulum, promote calcium-induced calcium release-dependent mechanisms. Furthermore, auxiliary $Ca_V\beta4$ subunits or truncated C-termini of $Ca_V1.2$ and $Ca_V2.1$ channels can be targeted to the nucleus and become direct messengers involved in the regulation of gene expression. These later discoveries suggest that novel pathways must be inserted in the global description of excitation-transcription coupling and give new clues to the understanding of calcium channelopathies with interesting physiopathological perspectives.

Keywords Voltage gated calcium channels • Transcription • Nucleus • Ataxia • Epilepsy

8.1 Introduction

The nervous system undergoes a constant maturation induced by diverse types of cognitive, motor, sensory or accidental experiences. These experiences produce external signals which are integrated at the plasma membrane of neuronal cells

M. Bellis • T. Cens • P. Charnet • M. Rousset (✉)
CRBM, CNRS-UMR5237, Montpellier, France
e-mail: matthieu.rousset@crbm.cnrs.fr

G. Stephens and S. Mochida (eds.), *Modulation of Presynaptic Calcium Channels*,
DOI 10.1007/978-94-007-6334-0_8,

by numerous proteins responsible for signal reception and transduction. These transduction pathways induce various signaling cascades that regulate the activity of target proteins by direct post-translational modifications, and/or by modulation of their synthesis and degradation rates. A key pathway is the communication between these plasma membrane receptors and the nucleus, where the integration of external signals leads to the remodeling of the gene expression that promotes long-lasting modifications of neuronal activity and morphology. Here, our aim is to review the roles of the voltage-gated calcium channels (VGCCs) in such process as so-called excitation-transcription coupling (ETC), whereby neuronal depolarization due to opening of VGCCs activates specific activity-regulated transcription programs. ETC make reference to the well-described excitation-contraction coupling and excitation-secretion coupling processes, in which these VGCCs have central roles in linking electrical activity to muscle contraction (Bénitah et al. 2002) and synaptic vesicle release (Wojcik and Brose 2007; Eggermann et al. 2012), respectively.

8.2 Routes from Membrane to Nucleus

Communication between pre- and post-synapses and nucleus is well-established and pertain to two types of mechanisms (Saha and Dudek 2008; Fainzilber et al. 2011). One mechanism is characterized by a rapid transfer of external information through the axon or the dendritic tree that is ended by a somatic rise of the calcium concentration. This transfer is carried out by electrical activity of the neuron which *in fine* activate VGCCs located at the somatic compartment, or by a regenerative calcium wave that culminates in a large increase of the calcium concentration in the soma (Verkhratsky and Shmigol 1996; Rose and Konnerth 2001). The somatic calcium rise activates different types of calcium-sensitive transcription factors or calcium-sensitive transcriptional regulators that translocate into the nucleus to reach their targets. This "fast calcium track" seems to be implicated in general programs of activity-dependent gene expression such as expression of immediate early genes (IEG) involved in the synaptogenesis and neuronal plasticity processes (Alberini 2009; Barco and Marie 2011; Okuno 2011; Karpova et al. 2012; Middei et al. 2012).

Another mechanism which presents slower kinetics relies upon a physical translocation of pre or post-synaptically localized proteins to the nucleus. Usually activity-dependent post-translational modifications produce protein uncoupling from a transmembrane complex or their truncation, leading to the release of a soluble intracellular domain (ICD). In both cases, nuclear translocation occurs and the translocated functional protein regulates gene expression directly by acting as a transcription factor, or indirectly by interacting and regulating transcription factors. This "slow protein track" has been reported for several receptors such as APP, erbB4, Neuroligin or Notch, and has been proposed to be responsible for more specific responses of activity-controlled transcription (Jordan and Kreutz 2009; Ch'ng and Martin 2011; Ch'ng et al. 2012).

VGCCs are well-known activators of the fast track communication pathway, and, as new evidence demonstrates, VGCCs are also implicated in slow protein track

pathways in which VGCC subunits, or their ICDs, translocate from the plasma membrane to the nucleus. Therefore, it is tempting to speculate that VGCCs form nuclear signaling platforms able to drive different activity-regulated gene networks using specific signaling cascades, dependent on the stimulation patterns and the channel's environment (Hardingham et al. 1999; Mermelstein et al. 2001; Wu et al. 2001a).

8.3 Studies Implicating VGCCs in Fast Excitation-Transcription Coupling

Fast track gene regulation converges on a few transcription factors, such as Mef2, SRF or CREB (cAMP response element binding protein), the latter which represents the prototypical activity-regulated transcription factor. CREB contains a C-terminal DNA binding domain that recognizes the CRE (cAMP Response Element) site, and a transactivation domain. The transactivation domain contains Q1 and Q2, two glutamine-rich domains, able to interact with the transcription machinery, and a kinase-inducible domain that encloses multiple phosphorylation sites. The phosphorylation state of the kinase-inducible domain determines its ability to bind to the KIX domain of the co-factor CBP or p300 necessary to initiate transcription. In particular, the phosphorylation of serine 133 targeted by different types of kinases, including CamK, the cAMP-dependent protein kinase (PKA) or the mitogen/stress-activated protein kinase (MAPK), represents a key step of CREB activation (Dolmetsch 2003; Barco and Marie 2011; Sakamoto et al. 2011). CREB, which has been implicated, in particular, in synaptic plasticity and intrinsic plasticity, regulates the transcription of thousands of genes including immediate early genes (IEGs) like *c-fos* or *bdnf* (Sakamoto et al. 2011; Middei et al. 2012). Usually the phosphorylation state of the serine 133 of CREB and the induction of *c-fos* expression are regarded as classical markers of activity-dependent transcription. L-type, P/Q type and N-type calcium currents have all been reported to be able to activate CREB and to induce gene expression (Sutton et al. 1999; Brosenitsch and Katz 2001; Zhao et al. 2007; Wheeler et al. 2012). This coupling between VGCCs and CREB seems to be a general neuronal process as it occurs in a large diversity of neurons, from central nervous system (hippocampal formation, striatum, cortex and cerebellum), peripheral nervous system (sensory neurons) or endocrine system. However, this coupling can involve different pathways, using either propagating electrical waves or calcium regenerative waves.

8.3.1 Fast Excitation-Transcription Coupling Through Electrical Propagating Waves

Amongst the most studied properties of neurons prone to ECT are the forms of synaptic plasticity which allow the adaptation of the synaptic strength to the level of repetitive stimulation. For example, long-term potentiation and long-term

depression, which seem to be both regulated by AMPA receptor trafficking (Boehm et al. 2006; Bramham et al. 2008), are sensitive to transcription inhibitors (Squire and Barondes 1970; Abraham et al. 1991). Several lines of evidences suggest that activity-induced transcription must take place within a few minutes, if not seconds, after the stimulation (Saha and Dudek 2008). Actually, it has been demonstrated that many IEGs are induced less than 2 min after stimulation (Guzowski et al. 1999; Bottai et al. 2002; Pevzner et al. 2012). Biochemical and morphological constraints (low speed of protein transport and long distances from the nucleus) mean that membrane depolarization is one of the best ways to translate synaptic excitation to the somatic area within appropriate timeframes. As L-type calcium channels are mainly found in the somatic compartment and the proximal part of the dendritic tree, they are ideally placed to be key transducers of ECT. In fact, L-type calcium channels are implicated in CREB phosphorylation at serine 133 and in activity-dependent *c-fos* gene expression in different neuronal types and following diverse stimulations (Greenberg et al. 1986; Morgan and Curran 1986; Murphy et al. 1991; Hardingham et al. 1997; Liu and Graybiel 1996; Rajadhyaksha et al. 1999; Tolón et al. 2000; Dolmetsch et al. 2001; Macías et al. 2001). L-type calcium current induces activation of CamKII/IV less than 60 s after channel opening. CamK activation is an essential step of *c-fos* gene induction as it phosphorylates CREB at serine 133 (Bading et al. 1993). It has also been shown that N-type and P/Q type VGCCs are also able to phosphorylate CREB and activate *c-fos* expression (Brosenitsch and Katz 2001; Zhao et al. 2007; Wheeler et al. 2012).

8.3.2 Fast Excitation-Transcription Coupling Through Calcium Propagating Waves

An alternative route involves a synaptic calcium rise which triggers a calcium-induced-calcium-release mechanism along dendrites to produce a regenerative calcium wave that culminates in a large increase of the calcium concentration in the soma. The initial calcium increase arises from VGCC opening by membrane depolarization or by ligand binding to ionotropic receptors (for example, NMDA or AMPA receptors) or to metabotropic receptors that produces, respectively, calcium influx or calcium release from reticulum endoplasmic. Sutton et al. have shown that *syntaxin1a* gene transcription is controlled by such calcium-induced-calcium release mechanism (Sutton et al. 1999). Calcium entry through P/Q type calcium channels was able to trigger expression of syntaxin1a in $Ca_V2.1$-overexpressing HEK-293 cells and P/Q type calcium current block by ω-agatoxin IVA in cerebellar granule cells prevented *syntaxin1a* mRNA production. The pathway recruited was calcium dependent and required the integrity of the reticulum endoplasmic since xestospongin C, a specific inhibitor of IP3 receptors, and dantrolene, an inhibitor of store-operated calcium release, both blocked *syntaxin1a* gene transcription. It was also observed that P/Q type calcium current increased CREB phosphorylation and that gene expression required the activation of CamK, MAPK kinase and PKA (Sutton et al. 1999).

In hippocampal neurons, L-type calcium channels are also able to target CREB directly to the nucleus by a calcium-induced-calcium-release mechanism. Using an elegant approach, the group of Bading has shown that calcium influx through VGCC triggers a calcium rise in the nucleus, which induces phosphorylation of CREB on serine 133 even in conditions where the nuclear pore complex had been previously blocked by injection of wheat germ agglutinin (Hardingham et al. 2001); treatment of wheat germ agglutinin is considered to completely prevent nucleocytoplasmic shuttling of proteins. Moreover, this group had previously demonstrated that chelation of nuclear calcium was sufficient to inhibit CREB phosphorylation and *c-fos* expression (Hardingham et al. 1997; Chawla et al. 1998). These experiments demonstrated the propagation of a calcium wave between the L-type channels and the nucleus which was able to trigger activity-dependent gene expression without any physical translocation of proteins into the nucleus. Moreover, these experiments have also shown that the nucleus contains all the elements required to activate CREB-dependent transcription and can operate independently under the occurrence of a nuclear calcium rise.

CREB is not the only transcription factor that can be activated by a regenerative calcium wave; other transcription factors contain calcium binding sites. DREAM, which is a transcription factor able to bind DNA on a DRE site and belongs to the recoverin calcium binding protein family, has four EF hands, of which three are functional. DREAM activity is under the control of nuclear calcium concentration. In low calcium concentrations DREAM forms a tetramer which is able to bind DNA and act as a repressor by preventing transcription initiation. When nuclear calcium concentration increases, the tetramer of DREAM is split into dimers which is unable to bind DNA and thereby releases transcription (Carrión et al. 1999; Savignac et al. 2005, 2007). Finally a regenerative calcium wave that culminates into the nucleus is clearly able to regulate DREAM activity. Leclerc et al. have demonstrated that *GnRH* gene expression in GT1-7 cells is under the control of both L-type VGCC activity and DREAM transcription factor, leaving open the possibility of a direct coupling between these components via a calcium-induced-calcium-release mechanism (Leclerc and Boockfor 2007).

8.3.3 Are There VGCC Specialized for Fast Excitation-Transcription Coupling?

L-type channels were the first discovered and best studied VGCC involved in ECT. Their somatodendritic localization and the lack of other critical neuronal functions led to the consideration that they were optimal for ETC. Other VGCC could be also implicated, but in a less direct way by contributing to the bulk cytoplasmic calcium pool that activates gene transcription in a nonspecific fashion. This idea was strengthened by the observation that in cortical and sensory neurons, despite a minor role in depolarization-induced increases in calcium, L-type channels play a

major role in activity-regulated gene expression (Mintz et al. 1991; Murphy et al. 1991; Brosenitsch et al. 1998; Dolmetsch et al. 2001; Zhao et al. 2007; Wheeler et al. 2012). However, the experimental conditions used to analyze L-type dependent ETC usually rely on the application of high extracellular potassium concentration, which induce a chronic membrane depolarization similar in amplitude to the depolarization observed during excitatory postsynaptic potentials. Such conditions are non-physiological and lead to a systematic inactivation of non-L-type VGCCs (Nowycky et al. 1985; Fox et al. 1987; Dolmetsch et al. 2001; Liu et al. 2003). Moreover, 40 mM KCl stimulation, which is the most commonly used KCl concentration, corresponds to an activation plateau maintained at −20 mV, meaning that Ca_V1 channels contribute to more than 75 % of the calcium current; this is mainly due to different biophysical properties between Ca_V1 and Ca_V2, as the latter are not activated at such potentials (Mermelstein et al. 2000; Wheeler et al. 2012). Together, this tends to underestimate the participation of Ca_V2 channels to ECT. Consequently, Ca_V2 channel contribution must be evaluated in presence of higher KCl concentrations (>60 mM) or especially using more physiological stimulations.

In primary sensory neurons, Brosenitsch and co-workers have shown that patterned electrical field stimulation at 5 Hz induces expression of the tyrosine hydroxylase *Th* gene (Brosenitsch and Katz 2001). In this case, ECT was neither sensitive to nimodipine nor CamK or MAPK inhibitors, but was sensitive to ω-conotoxin GVIA, a specific N-type VGCC blocker, and to protein kinase PKA/PKC inhibitors. Interestingly, in superior cervical ganglion neurons, 10 Hz electrical field stimulation induced an ETC that was completely blocked either by L-type blocker (nimodipine) or N-type blocker (ω-conotoxin GVIA) (Zhao et al. 2007). It is worth noting that, in the same preparation, KCl-induced-ETC is only sensitive to nimodipine; Increasing the frequency of stimulation to 50 Hz makes the ETC sensitive only to L-type blockers (Zhao et al. 2007). These results suggest that a large range of frequencies are able to induce ETC mediated by different VGCCs. Moreover, L-type VGCCs target CREB only after synaptic potentials while they are opened either by synaptic potentials or back propagating action potentials (Regehr and Tank 1992; Mermelstein et al. 2000; but see Dudek and Fields 2002). ETC is thus not an ON/OFF mechanism coupled to the opening of the channel but requires an adequate stimulation. Finally two different types of L-type channels present different abilities to target CREB depending on the amplitude of the stimulation. Using low potassium concentration, $Ca_V1.3$ is more effective than $Ca_V1.2$ in inducing CREB phosphorylation; however, increasing the extracellular potassium concentration make $Ca_V1.2$ more effective than $Ca_V1.3$ (Zhang et al. 2006). Together, these data suggest that neurons trigger different fast track ETCs corresponding to different types of electrical activity using specific VGCC and downstream signaling.

Overall, L-type channels seem to be more efficient in signaling CREB than Ca_V2 channels. It has been shown that at equal calcium influx through Ca_V1 and Ca_V2 channels, CREB phosphorylation level is 10 fold greater after Ca_V1-channels

activation (Wheeler et al. 2012). Moreover, in sympathetic neurons, ETC induced by electrical field stimulation at 10 Hz involves cooperatively between L and N-type VGCC; indeed, the ETC is completely blocked by L-type blockers or by N-type blockers, meaning that calcium influx through both channels is required (Zhao et al. 2007). It has been proposed recently that during an ECT episode, CamK activation requires a clustering step in close vicinity to the Ca_V1 VGCC, independently of the calcium source. Therefore calcium influx through Ca_V2 channels induces a translocation of CamKII near or in the macromolecular complex of Ca_V1 channels (Hudmon et al. 2005a; Wheeler et al. 2008, 2012). In this regard, the privileged ability of L-type channels for ETC seems to come from the specialization of the nanodomain around the mouth of the channel. The L-type channel-nanodomain regroups at the submicron scale the key signaling proteins required for the onset of ETC. Calmodulin and CamKII have been already shown to interact with $Ca_V1.2$ (Zühlke and Reuter 1998; Mori et al. 2004; Hudmon et al. 2005b; Xiong et al. 2005; Grueter et al. 2006, 2008; Fallon et al. 2009; Abiria and Colbran 2010). Moreover, $Ca_V1.2$ and $Ca_V1.3$ channels possess PDZ motifs on their C-terminal extremity that usually allows transmembrane proteins to bind to the cytoskeleton, thus contributing to the submembranous architecture organization. It has been shown that these domains are critical to L-type-dependent ETC (Weick et al. 2003; Zhang et al. 2005) and allow L-type channels to bind to scaffolding protein shank or neuronal-interleukin-16 (Kurschner and Yuzaki 1999; Zhang et al. 2005). These results suggest that PDZ sequences specifically found on L-type channels organize a macromolecular complex dedicated to ETC. Similarly, the Ca_V2 nanodomain seems to be buffered by endoplasmic reticulum and mitochondria (Akita and Kuba 2000; Wheeler et al. 2012). The functional consequences are double: first, a large part of Ca_V2 calcium influx is collected by the endoplasmic reticulum and mitochondria which limit the size of calcium nanodomain at the mouth of these channels; second, the close proximity of endoplasmic reticulum favors the activation of ryanodine receptors by Ca_V2-dependent calcium influx which, in turn, triggers a calcium-induced calcium release, phenomenon that can leads to the CREB phosphorylation (Sutton et al. 1999). Again, this specialized organization around the Ca_V2 channel mouth probably requires protein-protein interactions. Recent studies on the Ca_V2 proteome that point out multiple interactions with adaptors and cytoskeleton proteins could be a valuable source of data to study the Ca_V2-reticulum endoplasmic interaction (Muller et al. 2010).

Each class of VGCC displays specific biophysical properties which allow neurons to respond to diverse electrical stimulations, such as synaptic potentials and action potentials, for specific ECT processes. The partition of fast track ETC into use of electrical waves or calcium waves has origins in the nanodomain of each VGCC. Due to their close proximity with the endoplasmic reticulum, the Ca_V2 channel family is prone to calcium-induced-calcium-release mechanisms that culminate with a calcium rise in somatic space or directly within the nucleus, whilst the Ca_V1 family initiates CREB activation directly in response to electrical activity.

8.4 The Slow Protein Track: Long-Distance Physical Translocation of Ca_V-Bound Signaling Proteins to the Nucleus

As we have already mentioned, CREB can be activated by others kinases including MAPK and PKA. Cortical or hippocampal neurons stimulated by KCl induce a robust CREB phosphorylation lasting more than 1 h. It appears, in fact, that CREB activation follows two overlapping phases. The first phase is triggered within 1 min and lasts around 20 min, whereas the second phase starts 15 min after the stimulation and lasts for more than 1 h. Although the first phase seems not to be dependent on L-type channels and can use Ca_V2 channels (Murphy et al. 1991; Frödin and Gammeltoft 1999; Pearson et al. 2001), it appears that the second phase is clearly dependent on L-type channels, as nimodipine blocks its activation (Dolmetsch et al. 2001). The first phase, sensitive to KN-93, which is a potent inhibitor of CamK pathway, corresponds to the fast-track gene regulation pathway that we have already described. The second phase is sensitive to a dominant-negative form of Ras, to the MAPK kinase inhibitor PD98059 and proceeds with a sustained phosphorylation of ERK, indicating the activation of this kinase. Together, these data indicate that the second phase of CREB phosphorylation is performed by the MAPK cascades that have been extensively described elsewhere (Pearson et al. 2001). Using an elegant approach, Dolmetsch et al. demonstrated that the calcium sensor involved in MAPK activation is L-type channel bound calmodulin (Dolmetsch et al. 2001). The principle of the "functional knock in" technique they developed is to replace the endogenous channels by recombinant channels that have been mutated in order to investigate the role of a given functional site. Recombinant channels were also mutated at amino acids necessary for dihydropyridine block; using this approach, it was shown that the IQ domain, which allows the binding of calcium associated calmodulin to the channel, is necessary for ERK phosphorylation and the late phase of CREB activation (Dolmetsch et al. 2001). These results imply that the activation of the MAPK pathway occurred in the nanodomain of L-type channels.

Accordingly, it appears that activation of L-type channels is able to trigger two different signaling cascades from its macromolecular complex which both converge on the phosphorylation of CREB at serine 133. The differences between these two pathways lie on the stimulation strength and the kinetics of the cascades. The MAPK pathway requires a strong depolarization e.g. 90 mM KCl application, but is unresponsive to 20 mM KCl, which produces a smaller calcium rise; the key point is the lag time before CREB phosphorylation; whereas CamK pathway activates CREB almost immediately, the MAPK pathway requires more than 15 min (Wu et al.2001b). Why is there such a delay? Dolmetsch et al. (2001) demonstrated that the kinase ERK is activated less than 1 min after stimulation, suggesting that the period before CREB phosphorylation corresponds to the length required for

the activated ERK to reach the nucleus. Alternatively, activated ERK could act by targeting other kinases like Rsk1/2 or Msk, which in turn will directly phosphorylate CREB. In this case, it is possible that an extended time is needed for Rsk/Msk activation or for trafficking from the phosphorylated Rsk/Msk to CREB (Frödin and Gammeltoft 1999).

Another example of slow ETC mediated by uncoupling of Ca_V partners is illustrated by the NFAT signaling pathway. NFAT transcription factor represents five proteins, NFATc1-4 and NFAT5, which, with the exception of NFAT5, respond to cytoplasmic calcium rise by a dephosphorylation step mediated by calcineurin, a calcium-calmodulin activated phosphatase. NFATc1-4 are all highly expressed in the peripheral and central nervous system. The structure of NFAT is composed of two parts: an N-terminal regulatory domain called the NFAT homology region (NHR) which contains two calcineurin binding sites, the calcium-independent PXIXIT site, and the calcium-dependent LXVP site, and a DNA binding domain similar to the Rel/NFkappaB DNA binding domain. The NHR domain contains a nuclear localization sequence (NLS) controlled by the NFAT phosphorylation state. During basal conditions, cytoplasmic NFAT is highly phosphorylated at the NHR region, which probably masks the NLS. When the calcium concentration rises, calcium-calmodulin binds and activates calcineurin which, in turn, dephosphorylates NFAT allowing nuclear translocation of the transcription factor (Moore and Goldberg 2011).

In peripheral and central neurons, NFATc4 and NFATc3 are translocated to the nucleus after 3 min of 5 Hz stimulation or 90 mM KCl application; spontaneous synaptic activity of the neuron is also able to induce a NMDA-sensitive nuclear translocation (Graef et al. 1999; Ulrich et al. 2012). This translocation is associated with the transcription of specific genes like *IP3R1* gene and is sensitive to the calcineurin blockers FK506 and cyclosporine. Moreover, NFATc4/c3 nuclear translocation and *IP3R1* gene expression are increased by the L-type VGCC agonist BayK8644 and abolished by L-type antagonist nifedipine, but not by Ca_V2 blockers (Genazzani et al. 1999; Graef et al. 1999). It is worth noting that whereas both NFATc isoforms are able to enter the nucleus under strong depolarization, only NFATc3 is implicated in a nucleocytoplasmic shuttling under milder stimulation e.g. chronic membrane depolarization in response to 20 mM KCl. These dissimilar properties are explained by a differential sensitivity of each NFATc to GSK3β kinase; for example, GSK3β represses nuclear localization of NFATc4 by phosphorylating the C-terminal part of the NHR domain (Graef et al. 1999; Ulrich et al. 2012).

Oliveria et al. (2007) have shown using biochemical and FRET approaches that $Ca_V1.2$ channels interact with A-kinase anchoring protein 79/150 (AKAP79/150), and that AKAP79/150 binds to calcineurin. This macromolecular $Ca_V1.2$-calmodulin-AKAP79/150-calcineurin complex is required for NFAT signaling and AKAP79/150 knock-down abolishes KCl-induced NFATc4 nuclear translocation (Oliveria et al. 2007). The differential sensitivity to BAPTA and EGTA confirmed findings that calcineurin activation occurs in the nanodomain of $Ca_V1.2$. The most

likely scheme is that calcium influx through $Ca_V1.2$ channels binds to the IQ bound calmodulin that activates calcineurin, which, in turn, binds to NFATc. As a single calcineurin is probably unable to bind at the same time to both the PXIXIT site of AKAP79/150 and to NFATc, we can speculate that an uncoupling of calcineurin from the $Ca_V1.2$-calmodulin-AKAP79/150 complex occurs. NFATc starts to become located in the nucleus 15 min after the stimulation, and, in parallel, *IP3R1* expression become barely visible 1 h after the KCl stimulation (Genazzani et al. 1999; Graef et al. 1999). NFATc-dependent ETC is a slow process arising probably from NFAT4c nucleocytoplasmic shuttling.

Two additional nucleocytoplasmic shuttling processes that may be implicated in an ETC in neurons should also be mentioned. We have already described that DREAM is a transcriptional repressor when it is localized in the nucleus. However, DREAM is also present in the cytoplasm, mainly in the perinuclear area (Pruunsild and Timmusk 2012), and can translocate in the nucleus after sumoylation (Palczewska et al. 2011). In cardiomyocytes, CamKII regulates the DREAM nucleocytoplasmic ratio and, in parallel, DREAM regulates L-type channel expression by binding on the DRE site of $Ca_V1.2$ promoter (Ronkainen et al. 2011). Moreover, a clear correlation between the level of expression of CamKII and the expression of $Ca_V1.2$ channel has been shown (Xu et al. 2010; Ronkainen et al. 2011). An interesting point of this study is the modification of the calcium influx through L-type channels by BayK 8644 application, which favors the nuclear localization of DREAM (Ronkainen et al. 2011). Together, keeping in mind that CamKII interacts with L-type channels (Hudmon et al. 2005b; Grueter et al. 2008), these data suggest the existence of an ETC used to adapt $Ca_V1.2$ channels expression to intracellular calcium concentration via A calcium-calmodulin-CamKII sensor. This regulatory pathway also exists in neurons, since a calcium-insensitive dominant active form of DREAM induces a significant down-regulation of $Ca_V1.2$ channels in the cortex of transgenic mice (Naranjo and Mellström 2012). Finally, in cerebellar granule cells, it has been reported that DREAM interacts both with T-type calcium channels and K_V4 potassium channels(Anderson et al. 2010); these interactions allow calcium regulation of the K_V4 current. It would be worth testing to determine if specific electrical stimulation could unbind DREAM from T-type channels.

Presynaptic calcium channels have been shown to bind to several adaptor proteins. In particular, the PDZ protein MINT binds both to $Ca_V2.1$ and $Ca_V2.2$ channels, whilst CASK, a synaptic scaffolding protein, interacts exclusively with $Ca_V2.2$ channels. These interactions are implicated in the formation of the large macromolecular complexes which anchor the synaptic vesicle to the secretory machinery (Maximov et al. 1999). Moreover, it has been shown that MINT also interacts with CASK (Tabuchi et al. 2002; Zamponi 2003). Using imaging and biochemical approaches, CASK has been localized to the nucleus of neurons from embryonic brain. CASK regulates reelin gene expression by acting as a co-activator of the transcription factor Trb1 (Hsueh et al. 2000). It would be interesting to test if CASK is able to translocate from the synapse to the nucleus and if this process is triggered by calcium influx through Ca_V2 channels.

8.5 The Slow Protein Track: Unbinding of Ca_V Partners or Ca_V Fragments

A new aspect of VGCC signaling to the nucleus has been explored recently. VGCC subunits and truncated Ca_V channel subunits have been reported to be localized in the nucleus and to participate in transcription regulation. Here we will focus on the latest finding concerning $Ca_V\beta4$ subunits and Ca_V ICDs. However, it is noteworthy that $Ca_V\beta3$ subunits have been observed in the nucleus, where they bind to nuclear proteins and regulate transcription factors (Béguin et al. 2006; Zhang et al. 2010; Tadmouri et al. 2012).

8.5.1 Nuclear Ca_V Fragments

One of the findings from VGCC purification studies was that Ca_V1 and Ca_V2 channels can be cleaved at the C-terminus tail (Gerhardstein et al. 2000; Hell et al. 1993; De Jongh et al. 1991), releasing free C-terminal fragments that remain associated with the channel and maintain regulatory roles (Gao et al. 2001; Fuller et al. 2010). However, recently Gomez-Ospina et al. (2006) identified a 75 kDa C-terminal fragment of $Ca_V1.2$ called CCAT that translocates to the nucleus of inhibitory cortical neurons; CCAT interacts with nuclear proteins implicated in regulation of transcription-like thyroid hormone receptor, retinoic acid receptor and protein p54(nrd)/NonO. CCAT regulates the expression of numerous genes including the connexin *Cx31.1* gene (Gomez-Ospina et al. 2006). Moreover CCAT binds to the Cx31.1 promoter and is able to drive expression of a luciferase construction containing the promoter of *Cx31.1* gene. Finally, CCAT was shown to be a transcription factor in its own right, independently of $Ca_V1.2$ channels (Gomez-Ospina et al. 2006). How CCTA is generated is a remaining question, although a proteolytic cleavage of $Cav_V1.2$ seems to be the most likely process; however, the protease and the exact cleavage site needs to be found. Whatever the precise mechanism, it appears that the concentration of CCAT in the nucleus is regulated by calcium influx through L-type VGCCs and partially by others source of calcium such as NMDA receptors. Increasing electrical activity induces an export of CCAT from the nucleus (Gomez-Ospina et al. 2006). In cardiomyocytes, CCAT interacts with the *cacna1c* promoter and induces a repression of $Ca_V1.2$ expression, suggesting an autoregulatory mechanism of $Ca_V1.2$ channel expression (Schroder et al. 2009).

Using a specific antibody against the $Ca_V2.1$ C-terminal, Kordasiewicz et al. (2006) have clearly established that in neurons and in heterogeneous systems $Ca_V2.1$ is cleaved and produces a 60 kDa C-terminal fragment which is translocated to the nucleus. The $Ca_V2.1$ C-terminus has four successive putative NLS, of which only the first three seems to be required for nuclear localization (Kordasiewicz et al. 2006). Like CCAT, it has been suggested that the $Ca_V2.1$ C-terminal fragment is

implicated in gene regulation (Du et al. 2009), even if the mechanism of such regulation remain unclear. However, the $Ca_V\beta4$ is reported to be the $Ca_V\beta$ subunit with the highest affinity for $Ca_V2.1$ channels and to co-localize with them (De Waard et al. 1995; Bichet et al. 2000; Wittemann et al. 2000; Xie et al. 2007). In particular, the $Ca_V2.1$ C-terminal interacts with a specific $Ca_V\beta4$ region (Walker et al. 1998, 1999). The fact that $Ca_V\beta$ subunits are able to unbind from the Ca_V subunits (Bichet et al. 2000; Cantí et al. 2001; Restituito et al. 2001) and that $Ca_V\beta4$ subunits have a nuclear localization, leaves open the possibility that, after $Ca_V2.1$ cleavage, the $Ca_V2.1$ ICD remains associated with $Ca_V\beta4$. Considering the formation of any $Ca_V2.1$ ICD/$Ca_V\beta4$ dimer could facilitate the understanding of the mechanism of nuclear translocation and the role in the transcription of the $Ca_V2.1$ ICD.

8.5.2 Nuclear $Ca_V\beta$ Subunits

The $Ca_V\beta$ subunit is necessary for numerous functions of Ca_V. In particular, the $Ca_V\beta$ subunit is mandatory for proper targeting, regulation of activity and modulation of regulatory pathways which adapt the activity of Ca_V to the cell demand (Cens et al. 1998; Restituito et al. 2000; Rousset et al. 2003; Leyris et al. 2009; Buraei and Yang 2010). This explains why $Ca_V\beta$ subunits were first considered pure cytoplasmic and sub-plasma membrane proteins. However, later experiments showed that overexpressed GFP tagged $Ca_V\beta1$, $Ca_V\beta3$ and $Ca_V\beta4$ subunits are localized in the nucleus of adult cardiomyocytes (Colecraft et al. 2002). Subsequently, overexpressed and endogenous $Ca_V\beta$ subunits have been found in the nucleus of multiple excitable cells. In particular, nuclear localization of endogenous $Ca_V\beta4$ has been observed in NG108 cells, Purkinje neurons, cerebellar granular cells, dorsal cochlear nucleus neurons, medial vestibular nuclei neurons, hippocampal neurons and myotubes (Subramanyam et al. 2009; Xu et al. 2011; Tadmouri et al. 2012). A systematic comparison of nuclear targeting of $Ca_V\beta$ subunits showed that the $Ca_V\beta4b$ subunit has the highest nucleocytoplasmic ratio (Subramanyam et al. 2009). Analysis of $Ca_V\beta$ subunits sequences showed that they possess a nuclear export sequence (NES), but are devoid of a NLS. These analyses failed to disclose any advantageous structural element, which could explain the strong nuclear tropism of $Ca_V\beta4b$. However, truncation of the $Ca_V\beta4b$ N-terminal reduced nuclear targeting, pointing the importance of this region; conversely, addition of the $Ca_V\beta4b$ N-terminal segment (amino acids 1–48) to $Ca_V\beta2a$ increased its nucleocytoplasmic ratio to values similar to those of $Ca_V\beta4b$ (Subramanyam et al. 2009). A stretch of basic residues (RRSRLKR) located between the position 28 and 34 of $Ca_V\beta4b$ played a key role as the mutation of amino acids R28-R29-S20 in A28-A29-A30 induced a drastic reduction of the nucleocytoplasmic ratio, close to the level observed with $Ca_V\beta4a$ and $Ca_V\beta3$ (Subramanyam et al. 2009); therefore, this sequence contributes to the large nuclear translocation specifically observed with the $Ca_V\beta4b$ subunit.

Nevertheless, other sequences are equally important to elicit nuclear targeting of $Ca_V\beta4b$ subunits. The truncation of the last 38 amino acids decreased drastically the $Ca_V\beta4b$ subunit nucleocytoplasmic ratio (Tadmouri et al. 2012). This sequence is in fact one of the binding sites for the protein B56δ, a binding partner of $Ca_V\beta4b$ and $Ca_V\beta3$ identified in a two-hybrid screen (Tadmouri et al. 2012). B56δ is a regulatory subunit of the protein phosphatase 2a (PP2a), which is a heterotrimeric serine/threonine phosphatase. B56δ has a NLS in its C-terminal part, which is sufficient to translocate endogenous $Ca_V\beta4b$ to the nucleus of undifferentiated NG108 cells. Moreover in a context of B56δ down regulation, the nuclear localization of $Ca_V\beta4b$ is impaired. It is interesting to note that the integrity of the two conserved domains SH3 and MAGUK of $Ca_V\beta4b$ is required to allow its interaction with B56δ. These two domains bind to each other and the alteration of this interaction prevented B56δ binding to $Ca_V\beta4b$ (Tadmouri et al. 2012). Thus, it appears that multiple structural determinants are essential for nuclear translocation of $Ca_V\beta4b$. This suggests the possibility that these sequences do not work together, but are more probably recruited individually according to conditions and cell types. $Ca_V\beta4b$ subunits may use different pathways to enter the nucleus, some being specific and some being shared with others $Ca_V\beta$ subunits.

What is the role of $Ca_V\beta4$ subunits in the nucleus? A first clue was provided by studies on a short isoform of the $Ca_V\beta4$ subunit, the $Ca_V\beta4c$ subunit, expressed in cochlear hair cells, brainstem neurons and heart (Hibino et al. 2003; Xu et al. 2011). $Ca_V\beta4c$ is produced by skipping exon 9 of *cacnb4* gene which creates a frameshift and a premature stop codon. The corresponding protein is truncated at the beginning of the conserved guanylate-kinase (GK) domain and exhibits an additional specific sequence of 13 amino acids at the C-terminus. Using two-hybrid approaches, $Ca_V\beta4c$ have been shown to interact with the three members of the HP1 family (Hibino et al. 2003). HP1 are heterochromatin binding proteins structured in three parts: a chromodomain (CD) and a chromo shadow domain (CSD) separated by a linker that interacts with DNA. While CSD is a protein-protein interaction domain which binds to various nuclear proteins including SUV39H1/2, CD binds the methylated Lysine in position 9 on the histone H3 C-terminal. The lysine H3K9 is tri-methylated by diverse methytransferases including SETDB1 and SUV39H1/2; binding of HP1 to H3K9me is a critical step in the formation and maintenance of heterochromatin structure (Zeng et al. 2010). Heterochromatin is a specific organization of the DNA-histone complex which is inaccessible to the transcriptional machinery; accordingly, this chromatin state represses transcription of the large chromosomal domain. HP1 proteins, which are also able to silence individual genes by H3K9me binding, are markers of epigenetic silencing. Interestingly, all four $Ca_V\beta$ subunits possess a binding site for HP1, namely the short sequence PVVLV; for example, located at position 187–191 of $Ca_V\beta4b$, which is very similar to the HP1 binding consensus motif, PxVxL, found on others HP1 partners, such as the chromatin assembly factor 1. However $Ca_V\beta4c$ is the only $Ca_V\beta$ subunit shown to interact with HP1γ. This differential affinity of $Ca_V\beta$ subunits has been confirmed using the GAL4-CAT reporter assay, and it has been shown that $Ca_V\beta4c$, but not

$Ca_V\beta4a$, diminished the HP1γ silencing effect on a GAL4-CAT artificial gene (Hibino et al. 2003). It has been proposed that PVVLV sequence in full length $Ca_V\beta$ subunit is buried in a β-strand of the GK domain and is not accessible to HP1 interaction, explaining why the $Ca_V\beta4a$ subunit is not able to interact with HP1 (Xu et al. 2011). An important point is that binding between HP1 and $Ca_V\beta4c$ is mandatory for the nuclear targeting of $Ca_V\beta4c$.

Is the full-length $Ca_V\beta4b$ also implicated in gene silencing? Imaging experiments using electronic microscopy indicate that ∼50 % of the nuclear $Ca_V\beta4b$ is associated with heterochromatin, suggesting a potential role in the regulation of chromatin state (Tadmouri et al. 2012). Tadmouri et al. have shown that the interaction between HP1γ and the full-length beta subunit is more subtle than thought previously. The $Ca_V\beta4b$ is in fact able to bind to HP1γ, but only if B56δ is already attached to the $Ca_V\beta4b$ subunit. This indicates that the binding of B56δ opens the secondary structure of the $Ca_V\beta4b$ MAGUK domain, which correlates with the requirement of an intact SH3/MAGUK interaction for the B56δ binding to $Ca_V\beta4b$.

As HP1 proteins bind to the nucleosome, it would be interesting to determine if the $Ca_V\beta4b$/B56δ/HP1γ complex also binds to the nucleosome. Immunoprecipitation experiments have revealed that overexpressed and endogenous $Ca_V\beta4b$ are able to interact with histones H2, H3 and H4. This interaction is mediated by B56δ as the strength of the histone/$Ca_V\beta4b$ interaction is correlated with the level of B56δ expression (Tadmouri et al. 2012). Additionally $Ca_V\beta4b$ lacks capacity to interact with the histone H3 in B56δ knockout mice. It is known that the binding of HP1γ to chromatin during the cell cycle requires a tri-methylation of H3K9 and is regulated by the phosphorylation state of H3S10 (Fischle et al. 2005; Hirota et al. 2005; Terada 2006). AuroraB which phosphorylates H3S10 at the start of mitosis, lowers the affinity of HP1γ for chromatin; however, during interphase H3S10phos is dephosphorylated, which induces re-association of HP1γ with chromatin, a mechanism termed a "binary methylation-phosphorylation switch" (Dormann et al. 2006). B56δ is a regulatory subunit of the PP2A phosphatase and PP2A may dephosphorylate H2S10phos (Nowak et al. 2003; Simboeck et al. 2010). Moreover, $Ca_V\beta4b$ is able to immunoprecipitate PP2A in the presence of B56δ and immunoprecipitation of the $Ca_V\beta4b$/B56δ/PP2A/HP1γ complex induces dephosphorylation of an 8 amino acid histone3 peptide that contains a phosphorylated serine 10 site (Tadmouri et al. 2012). Together, these data suggest that $Ca_V\beta4b$/B56δ/PP2A/HP1γ is a functional complex in which PP2A allows HP1γ binding to the nucleosome, whilst HP1γ mediates heterochromatization.

Is the $Ca_V\beta4b$/B56δ/PP2A/HP1γ complex acting in a broad, non-specific way or at a specific site on DNA? The *lethargic* mouse, considered as a spontaneous knock out of the $Ca_V\beta4$ subunit, displays neurological disorders including ataxia and epilepsy (Burgess et al. 1997). The analysis of *lethargic* mice cerebellum and forebrain transcriptomic profiles revealed the expression of more than 50 genes showing a significantly change, the *Th* gene being the most increased (Tadmouri et al. 2012). Since 80 % of genes are up-regulated, $Ca_V\beta4$ seems to have an overall silencing impact. Their large distribution over all of the genome suggests that the $Ca_V\beta4b$/B56δ/PP2A/HP1γ complex may inhibit individual genes, rather

than acting by a regional silencing effect. Since the $Ca_V\beta$ subunit has no DNA binding sequence, we speculate that $Ca_V\beta4$ must bind others proteins, such as transcription factors able to target specific genes. During a two-hybrid screen, an interaction of $Ca_V\beta4b$ with the transcription factor thyroid hormone receptor alpha (TRα) has been found (Tadmouri et al. 2012). TRα usually binds to DNA via a TRE consensus site in the absence of the hormone and represses gene expression; binding of T3 hormone to TRα induce a conformational change of the receptor which become able to recruit the transcription machinery and to initiate gene expression (Cheng et al. 2010). TRα can bind constitutively to the *Th* promoter, even in absence of $Ca_V\beta4$ or B56δ. Contrary to the canonical view, T3 hormone application induced a repression of luciferase expression under the control of the *Th* promoter. Interestingly co-expression of the $Ca_V\beta4$ subunit turns TRα receptor into a mere repressor, independently of the presence of T3 hormone (Tadmouri et al. 2012). Chromatin immunoprecipitation experiments demonstrate that beside TRα, $Ca_V\beta4b$, HP1γ, B56δ and PP2A interact with the *Th* promoter (Tadmouri et al. 2012). Using the "Promoter Analysis and Interaction Network Generation Tool" called PAINT (Vadigepalli et al. 2003; Gonye et al. 2007), we retrieved the 5' sequence from the entire *Lh* mice modulated gene set and listed all transcriptional regulatory elements present on these cis-regulatory regions. We found that several predicted transcription factor binding sites are over-represented. C-rel and Pax-6 were the most significantly over-represented transcription factor. Pax-6 has been already reported to bind to $Ca_V\beta$ subunits (Zhang et al. 2010) and we confirmed the interaction of $Ca_V\beta4$ with c-rel using imaging and biochemical approaches (Bellis et al. in preparation). Our theory is that binding of $Ca_V\beta4$ to transcription factors brings the silencing machinery B56δ/PP2A/HP1γ to specific site(s) on DNA and represses expression of the corresponding genes.

Is nuclear $Ca_V\beta$ subunit due to $Ca_V\beta$ acting alone or are there messengers between VGCC and nucleus? In cerebellar granular neurons, *Th* expression is repress by activity; this effect is mediated by the $Ca_V\beta4$ subunit since *Th* gene expression become activity-independent in *Lh* mice (Tadmouri et al. 2012). Moreover, the nuclear localization of $Ca_V\beta4b$ is regulated by calcium influx and the VGCC (Subramanyam et al. 2009; Tadmouri et al. 2012). Biochemical studies have shown that B56δ and PP2A binding to $Ca_V\beta4b$ is sensitive to activity in cultured neurons and occurs only after strong chronic depolarization in heterogeneous systems which over-express $Ca_V\alpha$ subunits (Tadmouri et al. 2012). These results suggest a binding competition to $Ca_V\beta$ subunits between $Ca_V\alpha$ and B56δ that is regulated by the excitability state of the neuron, bringing new credence to the possible unbinding of $Ca_V\beta$ from $Ca_V\alpha$ subunits (Restituito et al. 2001). We speculate therefore that $Ca_V\beta4$ is a messenger which, during certain excitability episodes, unbinds from the $Ca_V\alpha$ subunit, translocates to the nucleus with B56δ and targets specific transcription factors associated with gene promoters.

The physiological significance of $Ca_V\beta4$ nuclear localization is still under investigation. In some case, the nuclear localization of $Ca_V\beta4b$ is developmentally regulated. For example, in NG108 cells, the $Ca_V\beta4$ nucleocytoplasmic ratio increases gradually as differentiation takes place. Heterochromatin plays a pivotal

role during the development and differentiation of cells. Recent data obtained in zebrafish point out such roles of $Ca_V\beta4$ in development, with the appearance of a lethal phenotype when $Ca_V\beta4$ is down regulated (Ebert et al. 2008).

8.6 Concluding Remarks

Chronic depolarization of hippocampal neurons using >60 mM KCl induces synchronously nuclear translocation of NFAT4c, MAPK/CREB pathway activation, CAMK/CREB activation and the nuclear translocation of the $Ca_V\beta4b$ subunit released from the VGCC. This example illustrates the role of VGCC as a nuclear signaling platform able to trigger a large diversity of signals to the nucleus. However, such KCl stimulation protocols mask a probable finer correlation between excitability events and the signals triggered by VGCC. Each depolarization amplitude or stimulation frequency generates specific signaling pathway, as suggested by the differential activation thresholds of MAPK/CREB and CamK/CREB pathways. Another element participating in the decoding of signals issued from the VGCC is the proteome of the channel. VGCC are not only a calcium source, but also a signaling hub where numerous calcium sensors and signaling proteins are part of the macromolecular complex organized around the channel. However, it is likely that VGCCs can be divided into several sub-groups, dependent on function, subcellular localization and their proteome. In consequence, proteomes of $Ca_V1.2$ channels localized at the dendritic shaft or in the soma should have a large number of common partners, but also few specific key partners. As already suggested, a calcium channel inserted into specific slot receives specific electrical stimulations and is surrounded by a specific set of proteins, combinations of which define a specific type of biological response (Cao et al. 2004). Subsequently, in theory, if NFAT4c nuclear translocation, MAPK/CREB activation, CAMK/CREB activation and/or nuclear translocation of $Ca_V\beta4b$ subunits could occur synchronously from a single $Ca_V1.2$ channel, it would be more realistic that each $Ca_V1.2$ channel slot is specialized in coupling excitation to a restricted number of signaling cascades.

At the same time, it is clear that several types of VGCCs are able to initiate the same pathway, or pathways, which converge on a common transcriptional effector, such as CREB. Again, by integrating the biophysical specificity of each channel type, the neuron is able to respond to a range of potential signals by recruiting signaling cascades common to most gene remodeling events. The key differences come from the coupling efficiency of the signaling molecules. Both Ca_V1 and Ca_V2 calcium channels are able to initiate the CamK/CREB pathway, but different spatial modes of activation differentiate them. Whilst Ca_V1 channels group all the proteins required to activate the CamK/CREB signaling cascade in their nanodomain, Ca_V2 channels act in less specific way at the micrometer scale via local calcium increases. Accordingly, Ca_V2 channels require a larger, more sustained stimulation to induce a sufficient local calcium rise required to activate local CamK. In consequence, the activated CamK pool will be dependent on the strength of the stimulation which,

in turn, will be interpreted differentially at the nuclear level and lead to different biological outputs.

The neuronal nucleus receives multiple waves of signals which add a temporal dimension to VGCC-nucleus communication. From an initial stimulation episode, several messengers are translocated to the nucleus with different kinetics and thus arrive at the nucleus at different times. The best known example is the L-type VGCC which lead to CREB phosphorylation at serine 133 via two independent signaling pathways with different time frames. The first pathway, which uses CamK activation and is carried by calcium waves, is very quick and lasts less than 15 min, whilst the second pathway, which uses MAPK is slower and requires nuclear translocation of signaling proteins. The distinct properties between these two pathways are certainly of primary importance for the computational properties of neurons. For example, these superposed signals can provide information about the strength of the electrical stimulation. For small amplitude depolarizations, only CamK/CREB can be activated, whilst larger depolarization involve both signaling pathways which, in turn, changes the life time of phosphorylated CREB and results in different activity-dependent transcription programs (Liu and Graybiel 1996).

This temporal distinction can also provide information about the spatial scope of the stimulation. It is assumed that calcium wave propagation is a mechanism that causes an amplification of the signal, unlike the physical nuclear translocation system which de facto reveals the number of VGCC recruited by the stimulation. Accordingly, fast track signaling gives information regarding the local stimulation input that requires a rapid but non-specific change of gene expression, whilst slow track signaling works in a cooperative fashion to integrate multiple channel recruitment and converges on the nucleus, where the signal leads to a more profound gene remodeling. $Ca_V\beta$ subunits translocate to the nucleus by inducing change in chromatin status, which is believe to represent longer term changes to gene expression.

It is also known that CREB can be phosphorylated at others sites. In particular, serine 142 and serine 143 are phosphorylated specifically after calcium influx, an effect which occurs with a delay after serine 133 phosphorylation (Gau et al. 2002; Kornhauser et al. 2002). This delayed phosphorylation inhibits the binding of the CBP protein and down regulates CRE-dependent transcription. Phosphorylation of serine 142 is sensitive to KN-93, but not to PD-98059, which means that CamKs are specifically implicated (Gau et al. 2002; Kornhauser et al. 2002). We can speculate that different kinase pathways triggered by L-type channels activation may target different phosphorylation sites on CREB, additional to serine 133, and thus regulate CRE-dependent transcription. Finally, Dolmetsch et al. have demonstrated that MAPK pathway activation by calcium influx through L-type channels was also able to activate MEF, another activity-dependent transcription factor (Dolmetsch et al. 2001). Besides CREB, the two delayed kinase pathways activated by L-type channels can also target different sets of activity-regulated transcription factors that activate, in turn, specific and non-overlapping transcription programs (Benito et al. 2011).

8.7 Physiopathological Perspectives

If the different pathways implicated in activity-dependent gene regulations are now relatively well-defined, the gene programs initiated and their effects on neurons are still in debate. An entry point is to consider the physiopathological aspects of VGCC-nucleus communication. It has been mentioned elsewhere that many inherited neurological disorders present mutations in proteins engaged in activity-dependent-transcription pathways (Deisseroth and Tsien 2002; Greer and Greenberg 2008; Ulrich et al. 2012). Rubenstein-Taybi syndrome is a mental retardation disease caused by mutation in the CBP gene (Petrij et al. 1995). Coffin-Lowry syndrome is due to a mutation in the gene coding for rsk2, one of multiple kinases participating to the phosphorylation of CREB at the serine 133 (Trivier et al. 1996). The calcineurin/NFATc4 pathway has been implicated in β-amyloid-neurotoxicity (Wu et al. 2010; Hudry et al. 2012). Some polymorphisms in the *bdnf* gene, which is an IEG gene, results in memory deficit (Chen et al. 2006). Similarly, it has been shown that mutations in the gene *cacnb4* coding for $Ca_V\beta4$ are responsible of epilepsy and ataxia. At least two mutations have been identified, the mutation L125P in the middle of the SH3 domain and the R481X at the C-terminus of the protein (Escayg et al. 2000). Electrophysiological characterization did not give any clues in the pathogenesis of the disease; however, Tadmouri et al. have demonstrated that $Ca_V\beta4b$ harboring the identified mutation lost their ability to bind to B56δ and to be translocated to the nucleus (Tadmouri et al. 2012). Undeniably, this lead has to be investigated more deeply to determine the potential link between the chromatin remodeling mediated by $Ca_V\beta4b$ and the neuronal change required to avoid epileptic events. One initial approach would be to determine all the binding sites on DNA for the $Ca_V4\beta$/B56δ/PP2A/HP1γ complex, this would open the door to a systematic determination of the genes that are under the control of this complex. Moreover, the identification of the physiological conditions that drives the unbinding of $Ca_V\beta4b$ from the VGCC will provide pivotal knowledge to understand the pathogenesis of these diseases.

The $Ca_V2.1$ C-terminal fragment observed in the neuronal nucleus also constitutes a serious candidate for elucidation of spinocerebellar ataxia type 6 (SCA6) pathogenesis. This debilitating disease, characterized by a late onset and progressive Purkinje neurons loss, is due to an abnormal polyglutamine (poly-Q) expansion in the C-terminus of a $Ca_V2.1$ isoform. Interestingly, this poly-Q sequence is present in the $Ca_V2.1$ cleaved fragment identified in the nucleus. Recent results indicate that the $Ca_V2.1$ fragment is able to bind to the promoter sequence of at least three genes: BTG1, progranulin and PMCA2 (Du et al. 2009). This $Ca_V2.1$ fragment has been also shown to drive BTG1 expression; however, a $Ca_V2.1$ fragment version containing a pathological poly-Q sequence (33 successive glutamines) is unable to regulate BTG1 expression (Du et al. 2009). Despite the current absence of evidence supporting a link between ataxia pathogenesis and transcriptional regulatory role of the $Ca_V2.1$ fragment, it is clear that investigation of $Ca_V2.1$ fragment activity in the nucleus is warranted.

References

Abiria SA, Colbran RJ (2010) CaMKII associates with $Ca_V1.2$ L-type calcium channels via selected beta subunits to enhance regulatory phosphorylation. J Neurochem 112:150–161

Abraham WC, Dragunow M, Tate WP (1991) The role of immediate early genes in the stabilization of long-term potentiation. Mol Neurobiol 5:297–314

Akita T, Kuba K (2000) Functional triads consisting of ryanodine receptors, Ca^{2+} channels, and Ca^{2+}-activated K^+ channels in bullfrog sympathetic neurons. Plastic modulation of action potential. J Gen Physiol 116:697–720

Alberini CM (2009) Transcription factors in long-term memory and synaptic plasticity. Physiol Rev 89:121–145

Anderson D, Mehaffey WH, Iftinca M et al (2010) Regulation of neuronal activity by Cav3-Kv4 channel signaling complexes. Nat Neurosci 13:333–337

Bading H, Ginty DD, Greenberg ME (1993) Regulation of gene expression in Hippocampal neurons by distinct calcium signaling pathways. Science 260:181–186

Barco A, Marie H (2011) Genetic approaches to investigate the role of CREB in neuronal plasticity and memory. Mol Neurobiol 44:330–349

Béguin P, Mahalakshmi RN, Nagashima K et al (2006) Nuclear sequestration of beta-subunits by Rad and Rem is controlled by 14-3-3 and calmodulin and reveals a novel mechanism for Ca^{2+} channel regulation. J Mol Biol 355:34–46

Bénitah JP, Gómez AM, Fauconnier J et al (2002) Voltage-gated Ca^{2+} currents in the human pathophysiologic heart: a review. Basic Res Cardiol 97(Suppl 1):I11–I18

Benito E, Valor LM, Jimenez-Minchan M et al (2011) cAMP response element-binding protein is a primary hub of activity-driven neuronal gene expression. J Neurosci 31:18237–18250

Bichet D, Lecomte C, Sabatier JM et al (2000) Reversibility of the Ca^{2+} channel alpha1-beta subunit interaction. Biochem Biophys Res Commun 277:729–735

Boehm J, Kang M-G, Johnson RC et al (2006) Synaptic incorporation of AMPA receptors during LTP is controlled by a PKC phosphorylation site on GluR1. Neuron 51:213–225

Bottai D, Guzowski JF, Schwarz MK et al (2002) Synaptic activity-induced conversion of intronic to exonic sequence in homer 1 immediate early gene expression. J Neurosci 22:167–175

Bramham CR, Worley PF, Moore MJ, Guzowski JF (2008) The immediate early gene arc/arg3.1: regulation, mechanisms, and function. J Neurosci 28:11760–11767

Brosenitsch TA, Katz DM (2001) Physiological patterns of electrical stimulation can induce neuronal gene expression by activating N-type calcium channels. J Neurosci 21:2571–2579

Brosenitsch TA, Salgado-Commissariat D, Kunze DL, Katz DM (1998) A role for L-type calcium channels in developmental regulation of transmitter phenotype in primary sensory neurons. J Neurosci 18:1047–1055

Buraei Z, Yang J (2010) The ß subunit of voltage-gated Ca^{2+} channels. Physiol Rev 90: 1461–1506

Burgess DL, Jones JM, Meisler MH, Noebels JL (1997) Mutation of the Ca^{2+} channel beta subunit gene Cchb4 is associated with ataxia and seizures in the lethargic (lh) mouse. Cell 88:385–392

Cantí C, Davies A, Berrow NS et al (2001) Evidence for two concentration-dependent processes for beta-subunit effects on alpha1B calcium channels. Biophys J 81:1439–1451

Cao Y-Q, Piedras-Rentería ES, Smith GB et al (2004) Presynaptic Ca^{2+} channels compete for channel type-preferring slots in altered neurotransmission arising from Ca^{2+} channelopathy. Neuron 43:387–400

Carrión AM, Link WA, Ledo F et al (1999) DREAM is a Ca^{2+}-regulated transcriptional repressor. Nature 398:80–84

Cens T, Restituito S, Vallentin A, Charnet P (1998) Promotion and inhibition of L-type Ca^{2+} channel facilitation by distinct domains of the subunit. J Biol Chem 273:18308–18315

Ch'ng TH, Martin KC (2011) Synapse-to-nucleus signaling. Curr Opin Neurobiol 21:345–352

Ch'ng TH, Uzgil B, Lin P et al (2012) Activity-dependent transport of the transcriptional coactivator CRTC1 from synapse to nucleus. Cell 150:207–221

Chawla S, Hardingham GE, Quinn DR, Bading H (1998) CBP: a signal-regulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. Science 281:1505–1509

Chen Z-Y, Jing D, Bath KG et al (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140–143

Cheng S-Y, Leonard JL, Davis PJ (2010) Molecular aspects of thyroid hormone actions. Endocr Rev 31:139–170

Colecraft HM, Alseikhan B, Takahashi SX et al (2002) Novel functional properties of Ca^{2+} channel beta subunits revealed by their expression in adult rat heart cells. J Physiol 541: 435–452

De Jongh KS, Warner C, Colvin AA, Catterall WA (1991) Characterization of the two size forms of the alpha 1 subunit of skeletal muscle L-type calcium channels. Proc Natl Acad Sci U S A 88:10778–10782

De Waard M, Witcher DR, Pragnell M et al (1995) Properties of the alpha 1-beta anchoring site in voltage-dependent Ca^{2+} channels. J Biol Chem 270:12056–12064

Deisseroth K, Tsien RW (2002) Dynamic multiphosphorylation passwords for activity-dependent gene expression. Neuron 34:179–182

Dolmetsch R (2003) Excitation-transcription coupling: signaling by ion channels to the nucleus. Science's STKE: signal transduction knowledge environment Sci STKE 2003:PE4

Dolmetsch RE, Pajvani U, Fife K et al (2001) Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. Science 294:333–339

Dormann HL, Tseng BS, Allis CD et al (2006) Dynamic regulation of effector protein binding to histone modifications: the biology of HP1 switching. Cell Cycle 5:2842–2851

Du X, Wang H, Zhu D et al (2009) Regulatory function of alpha1A C termini of P/Q calcium channels in neuronal gene expression and differentiation. SFNmeeting _poster 699.7

Dudek SM, Fields RD (2002) Somatic action potentials are sufficient for late-phase LTP-related cell signaling. Proc Natl Acad Sci U S A 99:3962–3967

Ebert AM, McAnelly CA, Srinivasan A et al (2008) Ca^{2+} channel-independent requirement for MAGUK family CACNB4 genes in initiation of zebrafish epiboly. Proc Natl Acad Sci U S A 105:198–203

Eggermann E, Bucurenciu I, Goswami SP, Jonas P (2012) Nanodomain coupling between Ca^{2+} channels and sensors of exocytosis at fast mammalian synapses. Nat Rev Neurosci 13:7–21

Escayg A, De Waard M, Lee DD et al (2000) Coding and noncoding variation of the human calcium-channel beta4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. Am J Hum Genet 66:1531–1539

Fainzilber M, Budnik V, Segal RA, Kreutz MR (2011) From synapse to nucleus and back again—communication over distance within neurons. J Neurosci 31:16045–16048

Fallon JL, Baker MR, Xiong L et al (2009) Crystal structure of dimeric cardiac L-type calcium channel regulatory domains bridged by Ca^{2+} * calmodulins. Proc Natl Acad Sci U S A 106:5135–5140

Fischle W, Tseng BS, Dormann HL et al (2005) Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature 438:1116–1122

Fox AP, Nowycky MC, Tsien RW (1987) Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. J Physiol 394:149–172

Frödin M, Gammeltoft S (1999) Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. Mol Cell Endocrinol 151:65–77

Fuller MD, Emrick MA, Sadilek M et al (2010) Molecular mechanism of calcium channel regulation in the fight-or-flight response. Sci Signal 3:ra70

Gao T, Cuadra AE, Ma H et al (2001) C-terminal fragments of the alpha 1C ($Ca_V1.2$) subunit associate with and regulate L-type calcium channels containing C-terminal-truncated alpha 1C subunits. J Biol Chem 276:21089–21097

Gau D, Lemberger T, von Gall C et al (2002) Phosphorylation of CREB Ser142 regulates light-induced phase shifts of the circadian clock. Neuron 34:245–253

Genazzani AA, Carafoli E, Guerini D (1999) Calcineurin controls inositol 1,4,5-trisphosphate type 1 receptor expression in neurons. Proc Natl Acad Sci U S A 96:5797–5801

Gerhardstein BL, Gao T, Bünemann M et al (2000) Proteolytic processing of the C terminus of the alpha(1C) subunit of L-type calcium channels and the role of a proline-rich domain in membrane tethering of proteolytic fragments. J Biol Chem 275:8556–8563

Gomez-Ospina N, Tsuruta F, Barreto-Chang O et al (2006) The C terminus of the L-type voltage-gated calcium channel $Ca_V1.2$ encodes a transcription factor. Cell 127:591–606

Gonye GE, Chakravarthula P, Schwaber JS, Vadigepalli R (2007) From promoter analysis to transcriptional regulatory network prediction using PAINT. Methods Mol Biol 408:49–68

Graef IA, Mermelstein PG, Stankunas K et al (1999) L-type calcium channels and GSK-3 regulate the activity of NF-ATc4 in hippocampal neurons. Nature 401:703–708

Greenberg ME, Ziff EB, Greene L (1986) Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. Science 234:80–83

Greer PL, Greenberg ME (2008) From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. Neuron 59:846–860

Grueter CE, Abiria SA, Dzhura I et al (2006) L-type Ca^{2+} channel facilitation mediated by phosphorylation of the beta subunit by CaMKII. Mol Cell 23:641–650

Grueter CE, Abiria SA, Wu Y et al (2008) Differential regulated interactions of calcium/calmodulin-dependent protein kinase II with isoforms of voltage-gated calcium channel beta subunits. Biochemistry 47:1760–1767

Guzowski JF, McNaughton BL, Barnes CA, Worley PF (1999) Environment-specific expression of the immediate-early gene arc in hippocampal neuronal ensembles. Nat Neurosci 2:1120–1124

Hardingham GE, Chawla S, Johnson CM, Bading H (1997) Distinct functions of nuclear and cytoplasmic calcium in the control of gene expression. Nature 385:260–265

Hardingham GE, Chawla S, Cruzalegui FH, Bading H (1999) Control of recruitment and transcription-activating function of CBP determines gene regulation by NMDA receptors and L-type calcium channels. Neuron 22:789–798

Hardingham GE, Arnold FJ, Bading H (2001) Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity. Nat Neurosci 4:261–267

Hell JW, Yokoyama CT, Wong ST et al (1993) Differential phosphorylation of two size forms of the neuronal class C L-type calcium channel alpha 1 subunit. J Biol Chem 268:19451–19457

Hibino H, Pironkova R, Onwumere O et al (2003) Direct interaction with a nuclear protein and regulation of gene silencing by a variant of the Ca^{2+}-channel beta 4 subunit. Proc Natl Acad Sci U S A 100:307–312

Hirota T, Lipp JJ, Toh B-H, Peters J-M (2005) Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. Nature 438:1176–1180

Hsueh YP, Wang TF, Yang FC, Sheng M (2000) Nuclear translocation and transcription regulation by the membrane-associated guanylate kinase CASK/LIN-2. Nature 404:298–302

Hudmon A, Lebel E, Roy H et al (2005a) A mechanism for Ca^{2+}/calmodulin-dependent protein kinase II clustering at synaptic and nonsynaptic sites based on self-association. J Neurosci 25:6971–6983

Hudmon A, Schulman H, Kim J et al (2005b) CaMKII tethers to L-type Ca^{2+} channels, establishing a local and dedicated integrator of Ca^{2+} signals for facilitation. J Cell Biol 171:537–547

Hudry E, Wu H-Y, Arbel-Ornath M et al (2012) Inhibition of the NFAT pathway alleviates amyloid β neurotoxicity in a mouse model of Alzheimer's disease. J Neurosci 32:3176–3192

Jordan BA, Kreutz MR (2009) Nucleocytoplasmic protein shuttling: the direct route in synapse-to-nucleus signaling. Trends Neurosci 32:392–401

Karpova A, Bär J, Kreutz MR (2012) Long-distance signaling from synapse to nucleus via protein messengers. Adv Exp Med Biol 970:355–376

Kordasiewicz HB, Thompson RM, Clark HB, Gomez CM (2006) C-termini of P/Q-type Ca^{2+} channel alpha1A subunits translocate to nuclei and promote polyglutamine-mediated toxicity. Hum Mol Genet 15:1587–1599

Kornhauser JM, Cowan CW, Shaywitz AJ et al (2002) CREB transcriptional activity in neurons is regulated by multiple, calcium-specific phosphorylation events. Neuron 34:221–233

Kurschner C, Yuzaki M (1999) Neuronal interleukin-16 (NIL-16): a dual function PDZ domain protein. J Neurosci 19:7770–7780

Leclerc GM, Boockfor FR (2007) Calcium influx and DREAM protein are required for GnRH gene expression pulse activity. Mol Cell Endocrinol 267:70–79

Leyris J-P, Gondeau C, Charnet A et al (2009) RGK GTPase-dependent $Ca_V2.1$ Ca^{2+} channel inhibition is independent of CaVbeta-subunit-induced current potentiation. FASEB J 23:2627–2638

Liu FC, Graybiel AM (1996) Spatiotemporal dynamics of CREB phosphorylation: transient versus sustained phosphorylation in the developing striatum. Neuron 17:1133–1144

Liu Z, Ren J, Murphy TH (2003) Decoding of synaptic voltage waveforms by specific classes of recombinant high-threshold Ca^{2+} channels. J Physiol 553:473–488

Macías W, Carlson R, Rajadhyaksha A et al (2001) Potassium chloride depolarization mediates CREB phosphorylation in striatal neurons in an NMDA receptor-dependent manner. Brain Res 890:222–232

Maximov A, Südhof TC, Bezprozvanny I (1999) Association of neuronal calcium channels with modular adaptor proteins. J Biol Chem 274:24453–24456

Mermelstein PG, Bito H, Deisseroth K, Tsien RW (2000) Critical dependence of cAMP response element-binding protein phosphorylation on L-type calcium channels supports a selective response to EPSPs in preference to action potentials. J Neurosci 20:266–273

Mermelstein PG, Deisseroth K, Dasgupta N et al (2001) Calmodulin priming: nuclear translocation of a calmodulin complex and the memory of prior neuronal activity. Proc Natl Acad Sci U S A 98:15342–15347

Middei S, Spalloni A, Longone P et al (2012) CREB selectively controls learning-induced structural remodeling of neurons. Learn Mem 19:330–336

Mintz IM, Venema VJ, Adams ME, Bean BP (1991) Inhibition of N- and L-type Ca^{2+} channels by the spider venom toxin omega-aga-IIIA. Proc Natl Acad Sci U S A 88:6628–6631

Moore DL, Goldberg JL (2011) Multiple transcription factor families regulate axon growth and regeneration. Dev Neurobiol 71:1186–1211

Morgan JI, Curran T (1986) Role of ion flux in the control of c-fos expression. Nature 322: 552–555

Mori MX, Erickson MG, Yue DT (2004) Functional stoichiometry and local enrichment of calmodulin interacting with Ca^{2+} channels. Science 304:432–435

Muller SC, Haupt A, Bildl W et al (2010) Quantitative proteomics of the Ca_V2 channel nano-environments in the mammalian brain. PNAS

Murphy TH, Worley PF, Baraban JM (1991) L-type voltage-sensitive calcium channels mediate synaptic activation of immediate early genes. Neuron 7:625–635

Naranjo JR, Mellström B (2012) Ca^{2+}-dependent transcriptional control of Ca^{2+} homeostasis. J Biol Chem 287:31674–31680

Nowak SJ, Pai C-Y, Corces VG (2003) Protein phosphatase 2A activity affects histone H3 phosphorylation and transcription in drosophila melanogaster. Mol Cell Biol 23:6129–6138

Nowycky MC, Fox AP, Tsien RW (1985) Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature 316:440–443

Okuno H (2011) Regulation and function of immediate-early genes in the brain: beyond neuronal activity markers. Neurosci Res 69:175–186

Oliveria SF, Dell'Acqua ML, Sather WA (2007) AKAP79/150 anchoring of calcineurin controls neuronal L-type Ca^{2+} channel activity and nuclear signaling. Neuron 55:261–275

Palczewska M, Casafont I, Ghimire K et al (2011) Sumoylation regulates nuclear localization of repressor DREAM. Biochim Biophys Acta 1813:1050–1058

Pearson G, Robinson F, Beers Gibson T et al (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 22:153–183

Petrij F, Giles RH, Dauwerse HG et al (1995) Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature 376:348–351

Pevzner A, Miyashita T, Schiffman AJ, Guzowski JF (2012) Temporal dynamics of arc gene induction in hippocampus: relationship to context memory formation. Neurobiol Learn Mem 97:313–320

Pruunsild P, Timmusk T (2012) Subcellular localization and transcription regulatory potency of KCNIP/calsenilin/DREAM/KChIP proteins in cultured primary cortical neurons do not provide support for their role in CRE-dependent gene expression. J Neurochem 123:29–43

Rajadhyaksha A, Barczak A, Macías W et al (1999) L-type Ca^{2+} channels are essential for glutamate-mediated CREB phosphorylation and c-fos gene expression in striatal neurons. J Neurosci 19:6348–6359

Regehr WG, Tank DW (1992) Calcium concentration dynamics produced by synaptic activation of CA1 hippocampal pyramidal cells. J Neurosci 12:4202–4223

Restituito S, Cens T, Barrere C et al (2000) The beta2a subunit is a molecular groom for the Ca^{2+} channel inactivation gate. J Neurosci 20:9046–9052

Restituito S, Cens T, Rousset M, Charnet P (2001) Ca^{2+} channel inactivation heterogeneity reveals physiological unbinding of auxiliary beta subunits. Biophys J 81:89–96

Ronkainen JJ, Hänninen SL, Korhonen T et al (2011) Ca^{2+}-calmodulin-dependent protein kinase II represses cardiac transcription of the L-type calcium channel alpha1C-subunit gene (Cacna1c) by DREAM translocation. J Physiol 589:2669–2686

Rose CR, Konnerth A (2001) Stores not just for storage. Intracellular calcium release and synaptic plasticity. Neuron 31:519–522

Rousset M, Cens T, Gavarini S et al (2003) Down-regulation of voltage-gated Ca^{2+} channels by neuronal calcium sensor-1 is beta subunit-specific. J Biol Chem 278:7019–7026

Saha RN, Dudek SM (2008) Action potentials: to the nucleus and beyond. Exp Biol Med 233: 385–393

Sakamoto K, Karelina K, Obrietan K (2011) CREB: a multifaceted regulator of neuronal plasticity and protection. J Neurochem 116:1–9

Savignac M, Pintado B, Gutierrez-Adan A et al (2005) Transcriptional repressor DREAM regulates T-lymphocyte proliferation and cytokine gene expression. EMBO J 24:3555–3564

Savignac M, Mellström B, Naranjo JR (2007) Calcium-dependent transcription of cytokine genes in T lymphocytes. Pflugers Arch 454:523–533

Schroder E, Byse M, Satin J (2009) L-type calcium channel C terminus autoregulates transcription. Circ Res 104:1373–1381

Simboeck E, Sawicka A, Zupkovitz G et al (2010) A phosphorylation switch regulates the transcriptional activation of cell cycle regulator p21 by histone deacetylase inhibitors. J Biol Chem 285:41062–41073

Squire LR, Barondes SH (1970) Actinomycin-D: effects on memory at different times after training. Nature 225:649–650

Subramanyam P, Obermair GJ, Baumgartner S et al (2009) Activity and calcium regulate nuclear targeting of the calcium channel beta4b subunit in nerve and muscle cells. Channels 3:343–355

Sutton KG, McRory JE, Guthrie H et al (1999) P/Q-type calcium channels mediate the activity-dependent feedback of syntaxin-1A. Nature 401:800–804

Tabuchi K, Biederer T, Butz S, Sudhof TC (2002) CASK participates in alternative tripartite complexes in which mint 1 competes for binding with caskin 1, a novel CASK-binding protein. J Neurosci 22:4264–4273

Tadmouri A, Kiyonaka S, Barbado M et al (2012) Cacnb4 directly couples electrical activity to gene expression, a process defective in juvenile epilepsy. EMBO J 31:3730–3744

Terada Y (2006) Aurora-B/AIM-1 regulates the dynamic behavior of HP1alpha at the G2-M transition. Mol Biol Cell 17:3232–3241

Tolón R, Franco FS, Villuendas G et al (2000) Potassium depolarization-induced cAMP stimulates somatostatin mRNA levels in cultured diencephalic neurons. Brain Res 868:338–346

Trivier E, De Cesare D, Jacquot S et al (1996) Mutations in the kinase Rsk-2 associated with Coffin-Lowry syndrome. Nature 384:567–570

Ulrich JD, Kim M-S, Houlihan PR et al (2012) Distinct activation properties of the nuclear factor of activated T-cells (NFAT) isoforms NFATc3 and NFATc4 in neurons. J Biol Chem

Vadigepalli R, Chakravarthula P, Zak DE et al (2003) PAINT: a promoter analysis and interaction network generation tool for gene regulatory network identification. OMICS 7:235–252

Verkhratsky A, Shmigol A (1996) Calcium-induced calcium release in neurones. Cell Calcium 19:1–14

Walker D, Bichet D, Campbell KP, De Waard M (1998) A beta 4 isoform-specific interaction site in the carboxyl-terminal region of the voltage-dependent Ca^{2+} channel alpha 1A subunit. J Biol Chem 273:2361–2367

Walker D, Bichet D, Geib S et al (1999) A new beta subtype-specific interaction in alpha1A subunit controls P/Q-type Ca^{2+} channel activation. J Biol Chem 274:12383–12390

Weick JP, Groth RD, Isaksen AL, Mermelstein PG (2003) Interactions with PDZ proteins are required for L-type calcium channels to activate cAMP response element-binding protein-dependent gene expression. J Neurosci 23:3446–3456

Wheeler DG, Barrett CF, Groth RD et al (2008) CaMKII locally encodes L-type channel activity to signal to nuclear CREB in excitation-transcription coupling. J Cell Biol 183:849–863

Wheeler DG, Groth RD, Ma H et al (2012) Ca_V1 and Ca_V2 channels engage distinct modes of Ca^{2+} signaling to control CREB-dependent gene expression. Cell 149:1112–1124

Wittemann S, Mark MD, Rettig J, Herlitze S (2000) Synaptic localization and presynaptic function of calcium channel beta 4-subunits in cultured hippocampal neurons. J Biol Chem 275: 37807–37814

Wojcik SM, Brose N (2007) Regulation of membrane fusion in synaptic excitation-secretion coupling: speed and accuracy matter. Neuron 55:11–24

Wu GY, Deisseroth K, Tsien RW (2001a) Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology. Nat Neurosci 4:151–158

Wu GY, Deisseroth K, Tsien RW (2001b) Activity-dependent CREB phosphorylation: convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. Proc Natl Acad Sci U S A 98:2808–2813

Wu H-Y, Hudry E, Hashimoto T et al (2010) Amyloid beta induces the morphological neurodegenerative triad of spine loss, dendritic simplification, and neuritic dystrophies through calcineurin activation. J Neurosci 30:2636–2649

Xie M, Li X, Han J et al (2007) Facilitation versus depression in cultured hippocampal neurons determined by targeting of Ca^{2+} channel Ca_Vbeta4 versus Ca_Vbeta2 subunits to synaptic terminals. J Cell Biol 178:489–502

Xiong L, Kleerekoper QK, He R et al (2005) Sites on calmodulin that interact with the C-terminal tail of $Ca_V1.2$ channel. J Biol Chem 280:7070–7079

Xu L, Lai D, Cheng J et al (2010) Alterations of L-type calcium current and cardiac function in CaMKIIdelta knockout mice. Circ Res 107:398–407

Xu X, Lee YJ, Holm JB et al (2011) The Ca^{2+} channel beta4c subunit interacts with heterochromatin protein 1 via a PXVXL binding motif. J Biol Chem 286:9677–9687

Zamponi GW (2003) Regulation of presynaptic calcium channels by synaptic proteins. J Pharmacol Sci 92:79–83

Zeng W, Ball AR, Yokomori K (2010) HP1: heterochromatin binding proteins working the genome. Epigenetics 5:287–292

Zhang H, Maximov A, Fu Y et al (2005) Association of $Ca_V1.3$ L-type calcium channels with shank. J Neurosci 25:1037–1049

Zhang H, Fu Y, Altier C et al (2006) Ca1.2 and $Ca_V1.3$ neuronal L-type calcium channels: differential targeting and signaling to pCREB. Eur J Neurosci 23:2297–2310

Zhang Y, Yamada Y, Fan M et al (2010) The beta 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

Zhao R, Liu L, Rittenhouse AR (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

Zühlke RD, Reuter H (1998) Ca^{2+}-sensitive inactivation of L-type Ca^{2+} channels depends on multiple cytoplasmic amino acid sequences of the alpha1C subunit. Proc Natl Acad Sci U S A 95:3287–3294