

Decoding the L-Type Ca^{2+} Signal: A Story of Transcription Regulation

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L-type voltage-sensitive Ca^{2+} channels (Ca_v) on the plasma membrane of diverse cell types play a fundamental role in Ca^{2+} signaling and cellular function. In many cases, Ca_v channel signals are associated with gene transcription. This signal has distinguishing properties that include site of entry subcellular localization, amplitude and duration. This mini review briefly describes how regulation of the Ca^{2+} signal by Ca_v1 is used to transform specific firing patterns into qualitatively and quantitatively distinct nuclear functions.

Introduction

Many types of Ca^{2+} channels have been described in both the nervous system and peripheral tissues such as endocrine, skeletal, cardiac, and smooth muscle (Fig. 5-7). The Ca_v1 channel couples membrane depolarization to cell surface receptor stimuli in order to regulate a multitude of processes including gene expression, synaptic efficacy, mRNA stability, and cell survival. They are exclusively expressed in excitable cells such as skeletal, cardiac, smooth muscle, neurons and endocrine cells.¹⁻⁶ Increases in intracellular Ca^{2+} concentration is induced by electrical or receptor stimuli caused by Ca_v1 activation often occurs as repetitive Ca^{2+} spikes or Ca^{2+} oscillations. The parameters of Ca^{2+} entry such as amplitude, frequency, intracellular location and speed of Ca^{2+} waves are regulated by the type and the intensity of the stimulus in the same cell.^{7,8} These distinct modes of Ca^{2+} entry into a cell play a key role in exterminating which signaling pathways are activated and thus specify the cellular response and induced selective cellular function.⁹

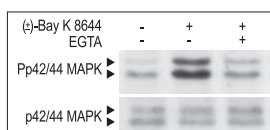
Ca^{2+} Initiates Transcription

Cells mainly employ two strategies to couple Ca^{2+} stimulus to transcription. First, Ca^{2+} (at the site of entry), activates cytoplasmic signaling molecules which conveys the signal to the nucleus. These signals can be blocked by the use of Ca_v1 -specific inhibitors (FS-2, Calciseptine and Calcicludine). In addition, the Ca_v1 activator (\pm)-Bay K 8644, has been shown to modulate the extracellular signal

regulated by the serine/threonine kinase (ERK)/mitogen-activated protein kinase (P42/44 MAPK) cascade in many cell types (Figs. 1, 2). This signaling pathway plays a fundamental role in cell proliferation, differentiation, motility, survival and apoptosis by transcription dependent and independent mechanisms in a variety of cell types.¹⁰⁻¹² For example, in human islets, glucose induced Ca^{2+} over stimulation is responsible for β cell dysfunction and induction of apoptosis (Fig. 2).¹³

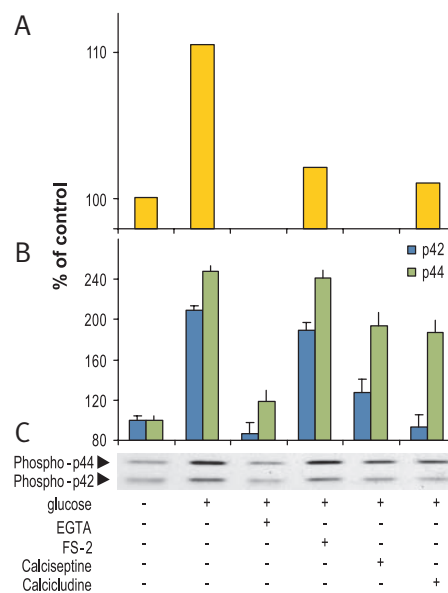
Secondly, Ca^{2+} can act directly in the nucleus. For instance, activation of Ca_v1 and Ca^{2+} influx has been shown to activate the transcription factor Cyclic Adenosine 3',5'-Monophosphate Response

Fig. 1: (\pm)-Bay K 8644, a Modulator of Ca_v1 (L-Type Channels) Promotes Phosphorylation of Mitogen Activated Protein Kinase (MAPK-ERK 1/2)



Jurkat T cells were preincubated with or without 2 mM EGTA to chelate Ca^{2+} for 20 min, then stimulated with 50 μM (\pm)-Bay K 8644 (#B-350) for 10 min. The cell proteins were resolved by SDS PAGE, probed with Anti-Phospho-p42/44 MAPK (upper panel) or with Anti-p42/44 MAPK (lower panel).

Fig. 2: Ca_v1 (L-Type Channels) Promote Activation of p42/44 MAPK in RIN Cells



RIN beta cells were stimulated for 15 min with 20 mM glucose in presence or absence of 5mM EGTA, 2 μM FS-2 (#F-700), 10 μM Calcicludine (#C-650) or 10nM Calciseptine (#C-500). Graph A presents the intracellular levels of Ca^{2+} , 10 sec post stimulation, in Fura-2 AM loaded cells. Picture C shows the western blot analysis of active p42/44 MAP Kinase probed with an anti phospho p42/44 MAPK and graph B shows the intensity of p42 and p44 bands.

Element Binding protein (CREB) (Fig 3).¹⁴ This transcription factor regulates the expression of immediate early genes¹⁵, and is thought to be important in the formation of long-term memory in the brain.¹⁶

Calmodulin Mediates the Ca²⁺ Signal to the Nucleus

The intracellular receptor for Ca²⁺ is calmodulin. This protein is a very sensitive sensor of Ca²⁺ concentration, capable of discrimination between signals that differ in spike frequency, amplitude and duration, through its effects on a variety of calmodulin-binding proteins. Changes in intracellular Ca²⁺ concentration affect calmodulin in three distinct ways: by altering its subcellular distribution; by directing a variety of conformational states of calmodulin that result in target-specific activation and by creating different

modes of association with many target proteins.¹⁷

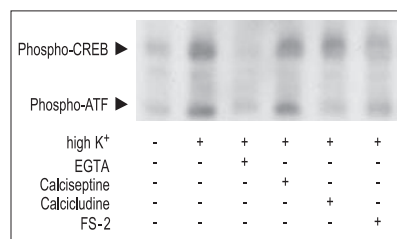
Binding of Ca²⁺ to calmodulin allows the cell to link Ca²⁺ changes with phosphorylation by activation of the multifunctional Ca²⁺/calmodulin-dependent protein kinase (CaMK, Fig 4). This kinase has the capability to directly anchor to the pore-forming α_{1C} subunit of Ca_v1. The frequency response and the state activation of CaMK are tightly modulated by Ca²⁺ and it reflects the frequency and the amplitude of Ca²⁺ spikes. CaMK has been shown to play a key role in synaptic plasticity, learning and memory and, in the heart has been implicated in the regulation of gene expression.¹⁸

The Ca_v1 contains two calmodulin binding sites, an "IQ" motif and an upstream calmodulin binding domain - "LA" motif within the carboxy terminus. At resting states, Ca²⁺-free calmodulin is tethered to the LA motif and lies close to the pore region. During Ca_v1 activation, when Ca²⁺ enters through the pore, it is captured by calmodulin.

The Ca²⁺/calmodulin complex switches to the downstream IQ motif, which has a high affinity site for the complex. The Ca²⁺/calmodulin complex is critical for conveying the signal from the mouth of the Ca_v1 pore to the nucleus. Binding of Ca²⁺/calmodulin to the IQ region of Ca_v1.2 is necessary for its activation and for trafficking of the Ras/P42/44 MAPK pathway, which conveys the signals to the nucleus by activation of transcription factors (Fig. 5).¹⁹⁻²¹ In the nervous system, this process is essential for neuronal survival, synaptic plasticity and formation of memory.^{20,22} For instance, in many cell types, voltage activation of Ca_v1 by K⁺ depolarization positively stimulates the ERK pathway. In the brain this activation is critical for re-phosphorylation of the synaptic vesicle protein synapsin I and for the activation of several transcription factors.²³

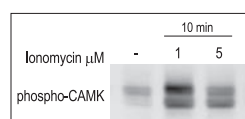
The transport of Ca²⁺ to the nucleus is likely to be mediated by its release from internal Ca²⁺ stores, although it may be a result of the translocation

Fig. 3: Ca_v1 (L-Type Channels) Promote Activation of Transcription Factors in PC12 Cells



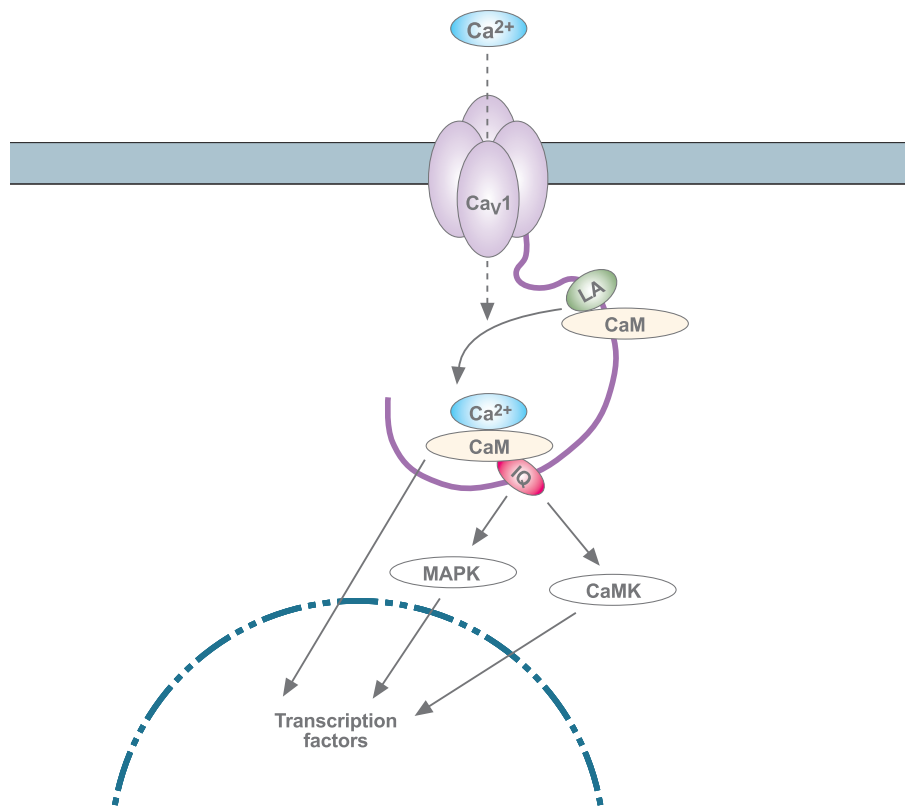
PC12 cells stimulated for 10 min with 35 mM High K⁺ in presence or absence of 5mM EGTA, 2 μ M FS-2 (#F-700), 10 μ M Calciclude (#C-650) or 10nM Calciseptine (#C-500). The cell extracts were blotted and probed with anti-phospho-CREB which also recognized phospho-ATF.

Fig. 4: Increase of Intracellular Ca²⁺ Induces Activation of Ca²⁺ Calmodulin Dependent Kinase II (CAMK II) Phosphorylation in 3T3-L1 Cells



3T3-L1 cells were starved for 2h and then stimulated with 1 or 5 μ M Ionomycin (#I-700) for 10 min. The cell extracts were blotted and probed with an antibody for phospho-(Thr₂₆₈)-CAMKII antibody.

Fig. 5: Schematic Representation of Ca_v1 Activated Signal Transduction



Activation of Ca_v channel and Ca²⁺ entry through the pore, promotes the binding of calmodulin to Ca²⁺ and causes calmodulin conformational changes which allow it to migrate downstream from the LA motif to the IQ motif in the COOH terminus. This process in turn regulates the opening of the channel and simultaneously facilitates the activation of signaling pathways.

of the Ca²⁺/calmodulin complex from the cell surface to the nucleus¹⁴ (particularly in nerves by retrograde dendritic action potentials) to support activation of CREB, which is strongly associated with dendritic development, neuronal survival and cognition.^{23,24} The nuclear mobilization of Ca²⁺/calmodulin is exclusively promoted by Ca_v1 and glutamate receptor activity,¹⁴ but it is modulated by other cellular signals which control calmodulin's phosphorylation state. The actions of the phosphocalmodulin differ from those of the non-phosphorylated species.²⁵⁻²⁶

Conclusion

In biological systems the Ca²⁺ signal serves as an universal intracellular messenger which modulates multiple Ca²⁺-regulated process. The intracellular mechanisms which decode this Ca²⁺ signal are complicated and depend upon processes which engage a wide range

of enzymatic activity. The Ca_v1 signal is a unique message which involves intracellular Ca²⁺ fluctuations together with direct control of Ca²⁺ binding protein-calmodulin by initiating specific signal transduction pathways and gene transcription.

Fig. 7: Detection of Ca_v1.2a in Rat Ventricular Membranes

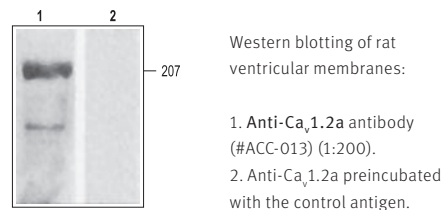
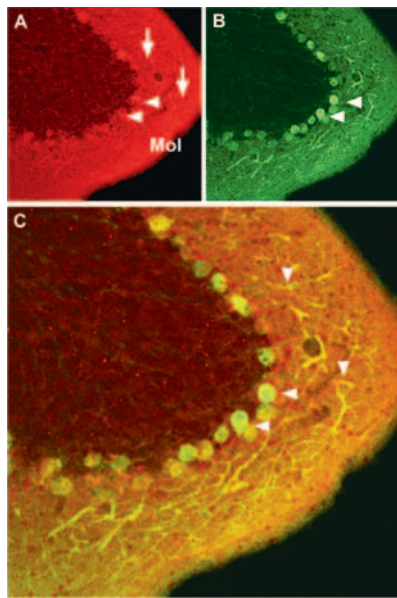


Fig. 6: Expression of Ca_v1.2 in Mouse Brain



Immunohistochemical staining of Ca_v1.2 channel with Anti-Ca_v1.2 antibody (#ACC-003) in mouse cerebellum. (A) Ca_v1.2 channel (red) appears in Purkinje cells (horizontal arrows) and is distributed diffusely in the molecular layer (Mol) including in Purkinje dendrites (vertical arrows). (B) staining of Purkinje nerve cells with mouse anti-calcium binding protein antibody (green) in the section demonstrates the location of dendrites in the molecular layer. (C) Confocal merge of Ca_v1.2 and CBD28K.

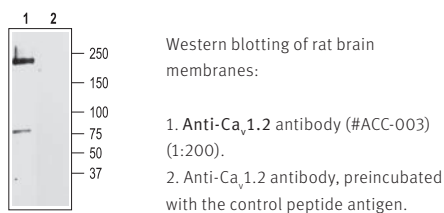
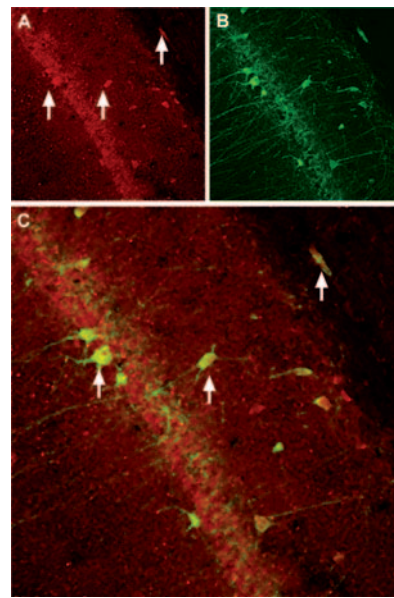
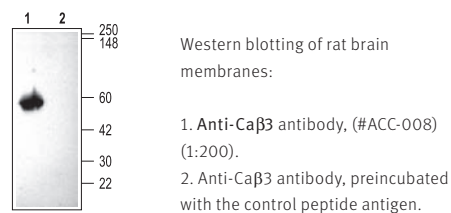


Fig. 8: Expression of Caβ3 in Rat Hippocampus



Immunohistochemical staining of Caβ3 channel with Anti-Caβ3 antibody (#ACC-008) in rat hippocampus. (A) Caβ3 channel (red) appears in neurons (arrows). (B) Staining of nerve cells with mouse anti-parvalbumin (a calcium binding protein, green) demonstrates the restriction of Caβ3 to cell bodies. (C) Confocal merge of Caβ3 and parvalbumin demonstrates some co-localization of these proteins.



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Related Products

Compound	Product #
Voltage-Gated Ca²⁺ Channel Activator	
(±)-Bay K 8644	B-350
Voltage-Gated Ca²⁺ Channel Blockers	
Calcicludine	C-650
Calciseptine	C-500
FS-2	F-700
TaiCatoxin	T-800
Ca²⁺ Ionophores	
Ionomycin	I-700
A23187	A-600
MAPK and MAPK Homolog Inhibitors	
PD98059	P-260
SB203580	S-370
U0126	U-400
MAPK and MAPK Homolog Activator	
Anisomycin	A-520
Voltage-Gated Ca²⁺ Channel Antibodies	
Anti-Ca _v 1.2	ACC-003
Anti-Ca _v 1.2a	ACC-013
Anti-Ca _v 1.3	ACC-005
Anti-human Ca _v 1.2	ACC-022
Anti-Caβ3	ACC-008