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Functional interactions between voltage-gated Ca^{2+} channels and Rab3-interacting molecules (RIMs): New insights into stimulus–secretion coupling

María A. Gandini, Ricardo Felix *

Department of Cell Biology, Center for Research and Advanced Studies of the National Polytechnic Institute (Cinvestav-IPN), Mexico City, Mexico

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ABSTRACT

Stimulus–secretion coupling is a complex set of intracellular reactions initiated by an external stimulus that result in the release of hormones and neurotransmitters. Under physiological conditions this signaling process takes a few milliseconds, and to minimize delays cells have developed a formidable integrated network, in which the relevant molecules are tightly packed on the nanometer scale. Active zones, the sites of release, are composed of several different proteins including voltage-gated Ca^{2+} (Ca_V) channels. It is well acknowledged that hormone and neurotransmitter release is initiated by the activation of these channels located close to docked vesicles, though the mechanisms that enrich channels at release sites are largely unknown. Interestingly, Rab3 binding proteins (RIMs), a diverse multidomain family of proteins that operate as effectors of the small G protein Rab3 involved in secretory vesicle trafficking, have recently identified as binding partners of Ca_V channels, placing both proteins in the center of an interaction network in the molecular anatomy of the active zones that influence different aspects of secretion. Here, we review recent evidences providing support for the notion that RIMs directly bind to the pore-forming and auxiliary β subunits of Ca_V channels and with RIM-binding protein, another interactor of the channels. Through these interactions, RIMs regulate the biophysical properties of the channels and their anchoring relative to active zones, significantly influencing hormone and neurotransmitter release.

1. Introduction

There is hardly any cellular function that is not influenced directly or indirectly by transient changes in the intracellular concentration of Ca^{2+}. Stimulus–secretion coupling, a process that connects the receipt of a stimulus with the release of molecules from membrane bound vesicles by exocytosis, illustrates nicely the role of Ca^{2+} as a triggering and controlling event in cell behavior. A classical example of this process is the link between membrane depolarization at the presynaptic terminal and the release of neurotransmitter into the synaptic cleft. However, stimulus–secretion coupling is an essential process occurring not only in neurons but in all secretory cells including, neuroendocrine, endocrine, and exocrine cells.

Secretion of hormones and neurotransmitters involves an elaborate molecular dialogue between voltage-gated Ca^{2+} (Ca_V) channels and the exocytotic machinery. The temporal precision of exocytosis requires a tight spatial coupling between docked vesicles and plasma membrane Ca^{2+} channels. The opening and closing of these channels by depolarizing stimuli, such as action potentials, allows Ca^{2+} ions to enter cells down a steep electrochemical gradient, producing the transient intracellular Ca^{2+} signals that triggers exocytosis. As a consequence, regulation of Ca^{2+} channel activity through activation of second messenger cascades or by protein–protein interactions modulates hormone release and synaptic transmission.

Experimental evidence suggests that Ca^{2+} channels are indeed physically coupled to various proteins in the vesicle release machinery such as syntaxin 1, SNAP-25, and synaptotagmin [1] which enables a short diffusional distance between the channels and the Ca^{2+} sensor for vesicle fusion. However, the list of proteins and molecular interactions that enable an enrichment of Ca^{2+} channels at the active zones is far from complete. Indeed, the presynaptic active zone of synapses consists of a dense accumulation of cytomatrix proteins. Among them, the scaffolding Rab3-interacting molecules (RIMs), has received increasing attention because they appear to play a crucial role in Ca_V channel localization and regulation. Hence, the purpose of the present paper is to review recent advances in our understanding of the interaction between Ca_V channels and RIMs and its possible physiological significance.

2. Rab3-interacting molecules (RIMs)

RIM proteins were originally identified as putative effectors for the synaptic vesicle protein Rab3 [2]. Though a single gene has been detected in invertebrates, experimental data show that in vertebrates

* Corresponding author at: Departamento de Biología Celular, Cinvestav-IPN, Avenida IPN #2508, Col. Zacatenco, México D.F., CP 07300, México. Tel.: +52 55 57 47 39 88; fax: +52 55 57 47 33 93.
E-mail address: rfelix@cell.cinvestav.mx (R. Felix).

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RIMs are encoded by a complex gene family of four members (RIM1–4). Of these, the RIM2 gene includes three independent promoters that specify the three isoforms of the RIM2 protein, whereas the other three genes appear to have only a single promoter that directs transcription of either RIM1 or RIM3 and RIM4 [3,4]. However, the multiplicity of RIMs in vertebrates is also related to extensive alternative splicing. Three canonical sites of alternative splicing referred to as sites A, B and C has been identified in the sequence analyses of the RIM genes. In addition to any intrinsic activity of the alternatively spliced sequences, alternative splicing may alter the way Rab3 and RIM-binding proteins (RBPs) bind to distinct RIM domains [3,4].

All known RIM proteins share a common structural and functional domain architecture (Fig. 1A), with a C2B module at their C termini [3] which serves as an interaction site with multiple binding partners. RIM1α and RIM2α (hereafter referred to as RIM1 and RIM2) are highly homologous proteins expressed in distinct patterns in the brain and other tissues (Table 1), and are the only members of the protein family that bind Rab3 [5,6]. They are formed by an N-terminal Zn^{2+} finger, a central PDZ domain and two "degenerated" C2-domains which fail to bind Ca^{2+}. These functional domains confer RIM1 and RIM2 a central role as scaffold proteins able to interact with a variety of proteins involved in exocytosis (Fig. 1A) including SNAP25, synaptotagmin, 14-3-3, ELSK/CAST, RBPs and Ca_V channels [7-11]. These molecular interactions regulate vesicle fusion, and therefore contribute to determine the process of hormone and neurotransmitter release triggered by depolarization-induced Ca^{2+} influx. Furthermore, at least one of these two RIM variants is required for effective synaptic transmission [5].

The RIM protein family also includes RIM1β and RIM2β members, which differ from the α-RIMs by the lack of the zinc finger, and RIM2γ–4γ proteins comprising only the C-terminal C2 domain and a flanking N-terminal region [4]. These proteins have been also shown to regulate exocytosis [3]. Excellent reviews have been written on RIM proteins with different emphases, mostly on their role in synaptic plasticity and neurotransmitter release, which are the most studied molecular functions of these proteins [12-14].

3. Voltage-gated Ca^{2+} (Ca_V) channels

Ca_V channels are transmembrane proteins that mediate Ca^{2+} ions to enter to the cell from the extracellular space in response to membrane depolarization, coupling the electrical signals in the cell surface to intracellular processes such as muscle contraction, hormone secretion or neurotransmitter release, among many others [15-18]. According to their electrophysiological properties, Ca_V channels have been classified into low voltage-activated (LVA or T-type) and high voltage-activated (HVA) channels, a class that includes the L-, N-, P/Q- and R-types, which can be distinguished using pharmacological approaches [16,17,19]. Ca^{2+} channels are also named using the chemical symbol of the permeating ion (Ca) with the principal physiological regulator (voltage) indicated as a subscript (Ca_V). The numerical identifier corresponds to the Ca_V channel transmembrane pore-forming α_1-subunit gene subfamily (1 to 3 at present) and the order of discovery of the α_1-subunit within that subfamily. Hence, the Ca_V1 subfamily ($Ca_V1.1$ to $Ca_V1.4$) includes channels containing α_{1S}, α_{1C}, α_{1D}, and α_{1F} which mediate L-type currents. The Ca_V2 family ($Ca_V2.1$ to $Ca_V2.3$)

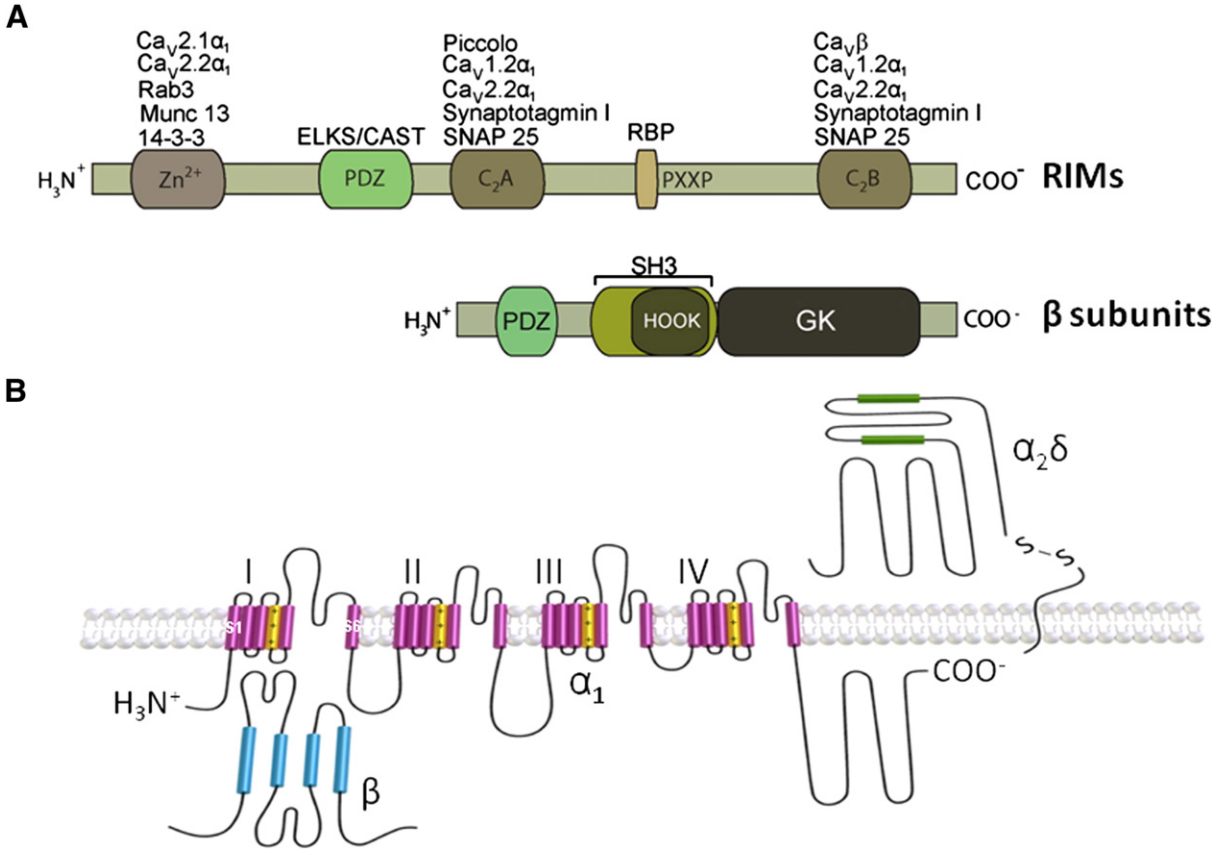

Fig. 1. Molecular structure of RIM proteins, $Ca_V\beta$ subunits, and the HVA Ca_V channel complex. A) RIM 1/2α is formed by one Zn^{2+}-finger-like domain (Zn^{2+}), one PDZ domain (PDZ), two C2 domains (C2A and C2B) and a proline-rich region (PXXP). RIMs 1/2β lack the Zn^{2+}-finger-like domain and RIMs 2γ–4γ have comprised only the C2 domains. $Ca_V\beta$ subunits are formed by three conserved domains: PDZ, Scr homology 3 (SH3) and guanylate kinase (GK). The β-interaction domain (BID) is one of the regions involved in the interaction of the protein with the $Ca_V\alpha_1$ pore-forming subunit. Both protein structures are depicted in N- to C-terminal direction. B) HVA Ca_V channels are oligomeric proteins of a pore-forming α_1 subunit, and auxiliary subunits β and $\alpha_2\delta$. The α_1 subunit is formed by four homologous membrane-spanning repeated domains (I–IV), each with six transmembrane-helixes (S1–S6) and a pore-forming reentrant loop between S5 and S6. The voltage sensor is located in S4 and is indicated in yellow.