Biochimica et Biophysica Acta 1853 (2015) 1921–1932

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Sense and specificity in neuronal calcium signalling☆

Robert D. Burgoyne *, Lee P. Haynes

Department of Cellular and Molecular Physiology, The Physiological Laboratory, Institute of Translational Medicine, University of Liverpool, Crown Street, Liverpool, L69 3BX, United Kingdom

ARTICLE INFO

Article history:
Received 19 September 2014
Received in revised form 25 October 2014
Accepted 29 October 2014
Available online 4 November 2014

Keywords:
Caenorhabditis elegans
Ca^{2+}-binding proteins
Ca^{2+} channel
Ca^{2+} sensors
NCS-1
Neuronal signalling

ABSTRACT

Changes in the intracellular free calcium concentration ($[Ca^{2+}]_i$) in neurons regulate many and varied aspects of neuronal function over time scales from microseconds to days. The mystery is how a single signalling ion can lead to such diverse and specific changes in cell function. This is partly due to aspects of the Ca^{2+} signal itself, including its magnitude, duration, localisation and persistent or oscillatory nature. The transduction of the Ca^{2+} signal requires Ca^{2+} binding to various Ca^{2+} sensor proteins. The different properties of these sensors are important for differential signal processing and determine the physiological specificity of Ca^{2+} signalling pathways. A major factor underlying the specific roles of particular Ca^{2+} sensor proteins is the nature of their interaction with target proteins and how this mediates unique patterns of regulation. We review here recent progress from structural analyses and from functional analyses in model organisms that have begun to reveal the rules that underlie Ca^{2+} sensor protein specificity for target interaction. We discuss three case studies exemplifying different aspects of Ca^{2+} sensor/target interaction. This article is part of a special issue titled the 13th European Symposium on Calcium.

1. Introduction

The intracellular free calcium concentration ($[Ca^{2+}]_i$) is tightly regulated through multiple mechanisms in neurons [1], and changes in $[Ca^{2+}]_i$ have crucial roles in the control of normal neuronal function [2]. In addition, abnormalities in Ca^{2+} signalling have been implicated in many aspects of neuropathology, neurodegeneration [3–5] and psychiatric disorders [6,7]. The mechanisms that elevate $[Ca^{2+}]_i$ include entry of extracellular Ca^{2+} through voltage-gated Ca^{2+} channels and release from intracellular stores such as the endoplasmic reticulum (ER), lysosomes and mitochondria. $[Ca^{2+}]_i$ can be reduced by sequestration into the same cellular organelles and by extrusion across the plasma membrane and these mechanisms have been well characterised [1,8]. Large numbers of studies on the localisation, magnitude and time course of $[Ca^{2+}]_i$ fluxes in neurons have been published. Changes in $[Ca^{2+}]_i$ can be local or global, highly transient, oscillatory or persistent. Localisation of $[Ca^{2+}]_i$ changes to particular neuronal compartments is important for the generation of specific neuronal responses. For example, certain stimuli strictly require nuclear rather than cytoplasmic changes in $[Ca^{2+}]_i$ [9] and also highly localised $[Ca^{2+}]_i$ changes restricted to dendritic spines may play an important role in activity-dependent synaptic plasticity [10,11]. The physiological effects of elevation of $[Ca^{2+}]_i$ can be manifest in microseconds (neurotransmitter release), milliseconds (channel facilitation or inactivation) or over much longer time scales leading to changes in gene expression [12,13] and effects on synaptic plasticity [14], neuronal development [15], learning [16], neuronal survival and cell death.

Clearly Ca^{2+} can influence many aspects of neuronal function with the same fundamental signalling ion being used to produce a variety of subtle and distinct changes. It is known, for example, that Ca^{2+} entry through different types of plasma membrane channels can affect changes in gene expression by different modes of signalling [17,18]. Of note here is the fact that even different classes of voltage-gated Ca^{2+} channels (VGCCs) in the same neurons are coupled through distinct signalling pathways to changes in gene expression [19]. Ca^{2+} signals are translated into changes in cellular function through various types of Ca^{2+} sensor proteins that in general terms detect increases in $[Ca^{2+}]_i$ by becoming loaded with Ca^{2+}, undergo a conformational change and then interact with and regulate various target proteins. The subtlety of neuronal Ca^{2+} signalling is underpinned by differential signal processing by these Ca^{2+} sensor proteins, which in turn determines the physiological specificity of Ca^{2+} signalling pathways.

Neurons express a large number of Ca^{2+} sensor proteins [20] ranging from synaptotagmin I, which is the essential Ca^{2+}-sensor for fast (microsecond) neurotransmitter release [21], through the annexins [22], to many different EF hand containing proteins [20]. The EF hand is a highly conserved Ca^{2+}-binding motif [23] which is present, for

Abbreviations: CaBP, calcium-binding protein; DREAM, downstream regulatory element antagonist modulator; CDF, Ca^{2+}-dependent facilitation; CDI, Ca^{2+}-dependent inhibition; GCAP, guanylyl cyclase activating protein; IL1RAPL1, interleukin 1 receptor accessory protein-like 1 protein 1; KChIP, potassium channel interacting protein; NCS, neuronal calcium sensor; NCX, sodium/calcium exchanger; VGCC, voltage-gated Ca^{2+} channel; VILIP, visinin-like protein

☆ This article is part of a Special Issue entitled: 13th European Symposium on Calcium.

* Corresponding author. Tel.: +44 151 794 5305.
E-mail address: burgoyne@liv.ac.uk (R.D. Burgoyne).

http://dx.doi.org/10.1016/j.bbamcr.2014.10.029

example, in the ubiquitous Ca^{2+} sensor protein calmodulin [24]. Calmodulin has numerous targets for regulation and is known to have multiple functions in neurons acting via various targets including Ca^{2+}/calmodulin-dependent protein kinase II [25]. Other EF hand containing Ca^{2+} sensors expressed in neurons include the calcium-binding protein (CaBP) family [26,27] and the neuronal calcium sensor (NCS) proteins [28–30].

The differential processing of neuronal Ca^{2+}-signals is affected by multiple aspects of the properties of Ca^{2+} sensors, and we suggest that they have a key role in determining signalling specificity. Factors that influence differential Ca^{2+} signalling include varied expression levels of sensors between neuronal cell types [31], differences in subcellular localisation, affinity and dynamics of Ca^{2+}-binding [32], association with protein signalling complexes [33,34], regulation and variations in stoichiometry of binding with target proteins [35,36] and specificity of target protein interaction. Appreciation of the contribution of Ca^{2+}-sensors to signalling specificity requires analysis of both the Ca^{2+}-sensors and their target interactions at a structural level [24,37]. The rules underlying the sensing and specificity of Ca^{2+} signalling in neurons have begun to emerge in recent years but still remain to be fully understood. In this review, we will present three case studies to illustrate current understanding of the molecular and structural basis of the contribution of the properties of Ca^{2+} sensors to differential neuronal signal processing.

2. Case Study 1: NCS-1, a Ca^{2+} sensor with multiple specific targets

NCS-1 is a member of the neuronal calcium sensor family that is encoded by 14 genes in mammals. Of these, recoverin and the guanylyl cyclase activating protein (GCAP) 1–3 have specialised roles in the Ca^{2+} regulation of phototransduction. One other subfamily, consisting of the visinin-like protein (VILIP) 1–3, neurocalcin δ and hippocalcin has less well-defined functions. Hippocalcin, as suggested by its name, has a very restricted pattern of expression mainly in hippocampal neurons where it shows dynamic membrane association (the Ca^{2+} myristoyl switch [38]) in response to Ca^{2+}-elevation [39] and neuronal activity [40,41], including activity-dependent translocation into dendritic spines. Hippocalcin has been implicated as a Ca^{2+} sensor in long-term depression [42,43] and the gating of channels underlying a slow after-hyperpolarisation current [44]. VILIPs also show the Ca^{2+} myristoyl switch [45] and may have multiple roles, including the regulation of receptor trafficking [46]. The potassium-channel interacting proteins (KChIPs) have all been implicated in the gating [47] and trafficking [48] of A-type potassium channels but show differential expression in different classes of neurons [49,50]. One of them, KChIP3 also known as calsenilin/DREAM, regulates presenilin function [51] and can act as a transcriptional repressor [52]. The other KChIPs may share the DREAM activity [53], but KChIP3 is specific among the KChIPs in increasing regulated secretion and down regulating the Na^{+}/Ca^{2+} exchanger NCX3 [54].

NCS-1 appears to be expressed in most neuronal cell types [55,31], and studies in various organisms have determined that it has multiple physiological functions [29,56–58]. Some of the roles of NCS-1 may be specific to certain organisms such as its particular role in temperature-dependent behaviours in *Caenorhabditis elegans* as a consequence of its restricted neuronal expression [59–61]. One of two encoded NCS-1 proteins generated through gene duplication (ncs-1a) is required for semi-circular canal formation in the zebrafish inner ear [62] and NCS-1 (frequenin, Frq) is required for the development of synaptic boutons in *Drosophila* [56], the organism in which NCS-1 was first discovered [63]. In addition, in *Saccharomyces cerevisiae* NCS-1 (Frq1) is essential for survival as a consequence of its requirement for activation of the phosphatidylinositol-4-kinase Pik1 [64] despite its absence not being lethal in other organisms. In mammalian cells, NCS-1 regulates Ca^{2+}-dependent exocytosis [65], long-term depression [66], axonal growth and neuronal regeneration [67] and channel function [68]. In mice, knock-out of NCS-1 has relatively subtle effects but results in an increase in anxiety and depressive behaviour [69]. Selective overexpression of NCS-1 in adult mouse dentate gyrus neurons promoted a form of exploratory behaviour and enhanced acquisition of spatial memory [70].

NCS-1 has many known interacting partners [71] (Fig. 1), some of which are unique for NCS-1 but some that are also regulated by other Ca^{2+} sensors particularly calmodulin [72]. Some of the interactions are known only from *in vitro* binding assays, and so their biological importance is unclear. It is possible that those binding partners that are also calmodulin targets *in vitro* [72] are not regulated by NCS-1 under physiological conditions [73]. There are, however, several physiological effects of NCS-1 that can be attributed directly to one of its identified target proteins (Table 1).Two documented NCS-1 interactions are of possible clinical significance. The potential importance of the regulation of dopamine D2 receptors by NCS-1 whereby NCS-1 inhibits D2 receptor internalisation (Fig. 2) [74] stems from the fact that dopamine is of key importance for signalling within the CNS and in addictive behaviour [75,76]. The regulation of D2 receptors by NCS-1 has been shown to underlie the effect of overexpression of NCS-1 on spatial memory acquisition [70]. Importantly, dopamine D2 receptors are the targets for all known effective antipsychotic drugs [77]. Interestingly, NCS-1 is up-regulated in patients with bipolar disorder or schizophrenia [78] and in response to anti-psychotic drugs [79] and is genetically associated with cocaine addiction [80] believed to be linked to effects of cocaine on dopamine transporters [81]. Recently, NCS-1 has been shown to be required for an adaptive response to dopaminergic agonists in substantia nigra neurons, and coupled with its up-regulation in the substantia nigra from patients with Parkinson's disease, this has resulted in the suggestion that it could be a target for modifying the vulnerability of neurons in the substantia nigra to neurodegeneration [82]. The binding of NCS-1 to the D2 receptor involves the very short cytoplasmic C-terminal domain of the receptor [74]. This interaction has been partially characterised using structural approaches [83] and this may allow exploration of the interaction as a therapeutic drug target.

The other clinically important interaction is with the interleukin 1 receptor accessory protein-like 1 protein (IL1RAPL1), which appears to be specific for NCS-1 [84]. Mutations in ILIRAPL1 have been shown

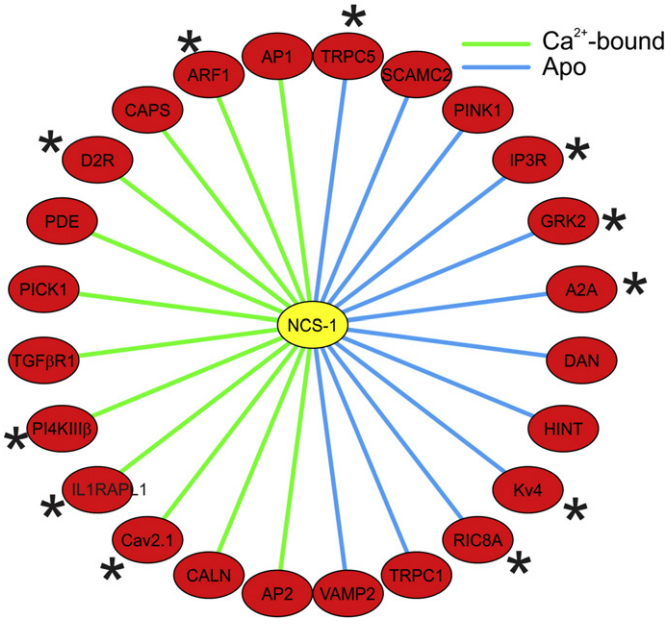

Fig. 1. Known target proteins for NCS-1 indicating interactions that require the Ca^{2+}-bound or the apo form of NCS-1. The interactions shown include ones that are based only *in vitro* binding assays as well as interactions that have been substantiated and shown to have physiological relevance in functional studies (marked with an asterisk).