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Review

Molecular structure and target recognition of neuronal calcium sensor proteins ☆

James B. Ames *, Sunghyuk Lim

University of California, Davis, Department of Chemistry, Davis, CA 95616, USA

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ABSTRACT

Background: Neuronal calcium sensor (NCS) proteins, a sub-branch of the calmodulin superfamily, are expressed in the brain and retina where they transduce calcium signals and are genetically linked to degenerative diseases. The amino acid sequences of NCS proteins are highly conserved but their physiological functions are quite distinct. Retinal recoverin and guanylate cyclase activating proteins (GCAPs) both serve as calcium sensors in retinal rod cells, neuronal frequenin (NCS1) modulate synaptic activity and neuronal secretion, K^+ channel interacting proteins (KChIPs) regulate ion channels to control neuronal excitability, and DREAM (KChIP3) is a transcriptional repressor that regulates neuronal gene expression.
Scope of review: Here we review the molecular structures of myristoylated forms of NCS1, recoverin, and GCAP1 that all look very different, suggesting that the sequestered myristoyl group helps to refold these highly homologous proteins into very different structures. The molecular structure of NCS target complexes have been solved for recoverin bound to rhodopsin kinase, NCS-1 bound to phosphatidylinositol 4-kinase, and KChIP1 bound to A-type K^+ channels.
Major conclusions: We propose the idea that N-terminal myristoylation is critical for shaping each NCS family member into a unique structure, which upon Ca^{2+}-induced extrusion of the myristoyl group exposes a unique set of previously masked residues, thereby exposing a distinctive ensemble of hydrophobic residues to associate specifically with a particular physiological target. This article is part of a Special Issue entitled Biochemical, biophysical and genetic approaches to intracellular calcium signaling.

1. Introduction

Intracellular calcium (Ca^{2+}) regulates a variety of neuronal signal transduction processes in the brain and retina [1,2]. The effects of changes in neuronal Ca^{2+} are mediated primarily by an emerging class of neuronal calcium sensor (NCS) proteins [3–7] that belong to the EF-hand superfamily [8–10]. The human genome encodes 14 members of the NCS family [11]. The amino acid sequences of NCS proteins are highly conserved from yeast to humans (Fig. 1). Recoverin, the first NCS protein to be discovered, and the guanylate cyclase activating proteins (GCAPs) are expressed exclusively in the retina where they serve as Ca^{2+} sensors in vision [12–16]. Other NCS proteins are expressed in the brain and spinal cord such as neurocalcin [17], frequenin (NCS1) [18,19], visinin-like proteins [20,21], K^+ channel interacting proteins (KChIPs) [22], DREAM/calsenilin [23,24] and hippocalcin [25–27]. Frequenin is also expressed outside of the central nervous system [28] as well as in invertebrates including flies [18], worms [29] and yeast (Frq1) [30–32]. The common features of these proteins are an approximately 200-residue chain containing four EF-hand motifs, the sequence CPXG in the first EF-hand that markedly impairs its capacity to bind Ca^{2+}, and an amino-terminal myristoylation consensus sequence.

The structurally similar NCS proteins have remarkably different physiologic functions (Table 1). Perhaps the best characterized NCS protein is recoverin that serves as a calcium sensor in retinal rod cells. Recoverin prolongs the lifetime of light-excited rhodopsin [33–35] by inhibiting rhodopsin kinase (RK) only at high Ca^{2+} levels [36–39]. Hence, recoverin makes receptor desensitization Ca^{2+}-dependent, and the resulting shortened lifetime of rhodopsin at low Ca^{2+} levels may promote visual recovery and contribute to the adaptation to background light. Recoverin may also function in the rod inner segment [40] and was identified as the antigen in cancer-associated retinopathy, an autoimmune disease of the retina caused by a primary tumor in another tissue [41,42]. Other NCS proteins in retinal rods include the guanylate cyclase activating proteins (GCAP1 and GCAP2) that activate retinal guanylate cyclase only at low Ca^{2+} levels and inhibit the cyclase at high Ca^{2+} [13,14,43]. GCAPs are important for regulating the recovery phase of visual excitation and particular mutants are linked to various forms of retinal degeneration [44–48]. Yeast and mammalian frequenins bind and activate a particular PtdIns 4-OH kinase isoform (Pik1 gene in yeast) [28,30,49,50] required for vesicular trafficking in the late secretory pathway [51,52]. Mammalian frequenin (NCS1) also regulates voltage-gated Ca^{2+} and K^+ channels [53,54]. The KChIPs regulate the gating kinetics of voltage-gated, A-type K^+ channels [22].

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* Corresponding author at: University of California, Davis Department of Chemistry, One Shields Avenue, Davis, CA 95616, USA. Tel.: +1 530 752 6358.
E-mail address: ames@chem.ucdavis.edu (J.B. Ames).

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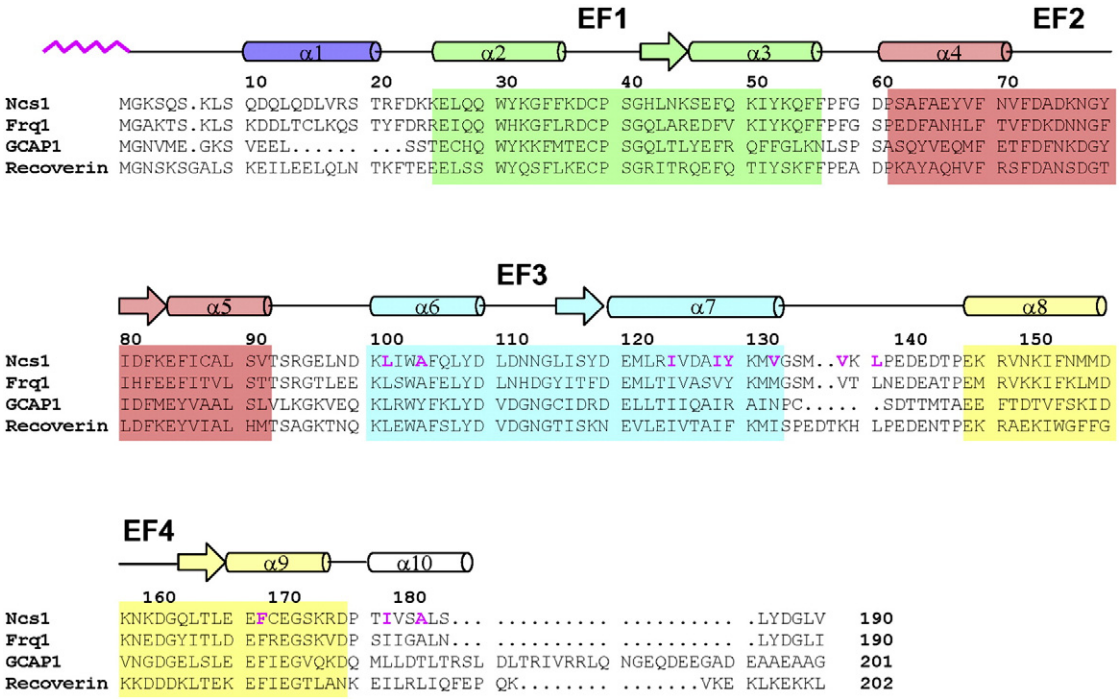

Fig. 1. Amino acid sequence alignment of selected NCS proteins (sequence numbering is for *S. pombe* NCS1). Secondary structure elements (helices and strands), EF-hand motifs (EF1 green, EF2 red, EF3 cyan and EF4 yellow), and residues that interact with the myristoyl group (highlighted magenta) are indicated. Swiss Protein Database accession numbers are Q09711 (*S. pombe* Ncs1), Q06389 (*S. cerevisiae* Frq1), P21457 (bovine recoverin), and P43080 (human GCAP1).

The DREAM/calsenilin/KChIP3 protein binds to specific DNA sequences in the prodynorphin and c-fos genes [23,55] and serves as a calcium sensor and transcriptional repressor for pain modulation [56,57]. Hence, the functions of the NCS proteins appear to be quite diverse and non-overlapping.

Mass spectrometric analysis of retinal recoverin and some of the other NCS proteins revealed that they are myristoylated at the amino terminus [26,58,59]. Recoverin contains an N-terminal myristoyl (14:0) or related fatty acyl group (12:0, 14:1, 14:2). Retinal recoverin and myristoylated recombinant recoverin, but not unmyristoylated recoverin, bind to membranes in a Ca^{2+}-dependent manner [60,61]. Likewise, bovine neurocalcin and hippocalcin contain an N-terminal myristoyl group and both exhibit Ca^{2+}-induced membrane binding [59]. These findings led to the proposal that NCS proteins possess a Ca^{2+}-myristoyl switch (Fig. 2). The covalently attached fatty acid is highly sequestered in recoverin in the calcium-free state. The binding of calcium to recoverin leads to the extrusion of the fatty acid, making it available to interact with lipid bilayer membranes or other hydrophobic sites. The Ca^{2+}-myristoyl switch function by recoverin also enables its light-dependent protein translocation in retinal rods [40].

In this review, the atomic-level structures of various NCS proteins and their target complexes will be discussed and compared with that of calmodulin. We begin by examining the large effect of N-terminal myristoylation on the structures of recoverin, GCAP1 and NCS1. Ca^{2+}-induced extrusion of the myristoyl group exposes unique hydrophobic binding sites in each protein that in turn interact with various target proteins. An emerging theme is that N-terminal myristoylation is critical for shaping each NCS family member into a unique structure, which upon Ca^{2+}-induced extrusion of the myristoyl group exposes a unique set of previously masked residues, thereby exposing a distinctive ensemble of hydrophobic residues to associate specifically with a particular physiological target.

Table 1
Function of NCS proteins.

NCS protein	Function	Reference
Recoverin	Inhibit rhodopsin kinase in retinal rods.	[33,35,37]
GCAP1	Activate guanylate cyclase in retinal cones.	[14,43]
GCAP2	Activate guanylate cyclase in retinal rods.	[13]
GCIP	Inhibit guanylate cyclase in frog photoreceptors.	[101]
KChIP1	Regulate K^+ channel gating kinetics in brain.	[22,102]
KChIP2	Regulate K^+ channel gating kinetics in cardiac cells.	[22,103]
Calsenilin/ DREAM	Repress transcription of prodynorphin and c-fos genes.	[22–24]
NCS1	Activate PI(4) kinase; regulate Ca^{2+} and K^+ channels.	[18,30,53,54]
Neurocalcin δ	Activate membrane guanylate cyclase	[17,104]
Hippocalcin	Activate phospholipase D; MAP kinase signaling.	[25,105,106]
VILIP-1	Activate guanylate cyclase; traffic nicotinic receptors.	[92,107]

2. Structure of recoverin's calcium-myristoyl switch

The X-ray crystal structure of recombinant unmyristoylated recoverin [62,63] showed it to contain a compact array of EF-hand motifs, in contrast to the dumbbell shape of calmodulin [64] and troponin C [65]. The four EF-hands are organized into two domains: The first EF-hand, EF-1 (residues 27–56, colored green in Figs. 1 and 3), interacts

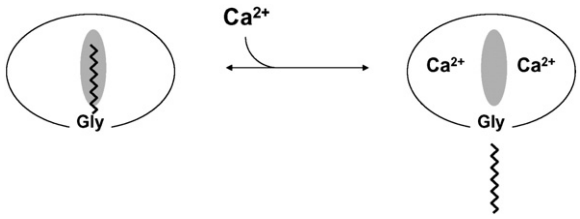

Fig. 2. Schematic diagram of calcium-myristoyl switch in recoverin. The binding of two Ca^{2+} ions promotes the extrusion of the myristoyl group and exposure of other hydrophobic residues (marked by the shaded oval). This figure was adapted from and originally published by [61].