Mol Neurobiol (2012) 45:388–402
DOI 10.1007/s12035-012-8250-4

Multiple Roles for Frequenin/NCS-1 in Synaptic Function and Development

Jeffrey S. Dason · Jesús Romero-Pozuelo · Harold L. Atwood · Alberto Ferrús

Received: 16 January 2012 / Accepted: 20 February 2012 / Published online: 7 March 2012

Abstract The calcium-binding protein frequenin (Frq), discovered in the fruit fly *Drosophila*, and its mammalian homologue neuronal calcium sensor 1 (NCS-1) have been reported to affect several aspects of synaptic transmission, including basal levels of neurotransmission and short- and long-term synaptic plasticities. However, discrepant reports leave doubts about the functional roles of these conserved proteins. In this review, we attempt to resolve some of these seemingly contradictory reports. We discuss how stimulation protocols, sources of calcium (voltage-gated channels versus internal stores), and expression patterns (presynaptic versus postsynaptic) of Frq may result in the activation of various protein targets, leading to different synaptic effects. In addition, the potential interactions of Frq's C-terminal and N-terminal domains with other proteins are discussed. Frq also has a role in regulating neurite outgrowth, axonal regeneration, and synaptic development. We examine whether the effects of Frq on neurotransmitter release and neurite outgrowth are distinct or interrelated through homeostatic mechanisms. Learning and memory are affected by manipulations of Frq probably through changes in synaptic transmission and neurite outgrowth, raising the possibility that Frq may be implicated in human pathological conditions, including schizophrenia, bipolar disorder, and X-linked mental retardation.

Keywords Neurotransmitter release · Calcium · Presynaptic · Postsynaptic · Facilitation · Short-term synaptic plasticity · LTP · Learning · Memory · Neurite outgrowth · Axon regeneration · Calcium-binding protein · *Drosophila*

J. S. Dason (✉) · H. L. Atwood
Department of Physiology, University of Toronto,
Toronto, ON, Canada M5S 1A8
e-mail: jeff.dason@utoronto.ca

J. Romero-Pozuelo · A. Ferrús
Instituto Cajal, CSIC,
Ave. Dr. Arce 37,
Madrid 28002, Spain

Present Address:
J. Romero-Pozuelo
Centro de Biología Molecular Severo Ochoa (C.S.I.C.-U.A.M),
Universidad Autónoma de Madrid,
c/ Nicolás Cabrera 1,
Madrid 28049, Spain

Introduction

Frequenin (Frq) and its mammalian counterpart, neuronal calcium sensor 1 (NCS-1), are members of a family of calcium-binding proteins called neuronal calcium sensors (NCS), which include neurocalcin, visinin-like protein (VILIP), hippocalcin, recoverin, guanylate cyclase activating protein (GCAP), and K^+ channel interacting protein (KChIP). These proteins are expressed primarily in the nervous system and are thought to be involved in a number of calcium signaling pathways [1, 2].

Frq is the most extensively studied and founding member of the NCS family. It is essential for survival in yeast [3], but not in worms [4], flies [5], and mice [6], although NCS-1 knockout mice have a 30% reduction in survival compared to controls. Frq has been implicated in a wide range of important functions. It is a regulator of ion channels and postsynaptic receptors, with consequent effects on neurite outgrowth and synaptic transmission [5, 7–11]. Through these effects, Frq affects higher functions, such as learning and memory [4, 11]. Frq has also been implicated in neuroprotection [12] and axonal regeneration [13] and may thus have potential as a therapeutic agent. Despite its importance, the significance of this Ca^{2+} sensor is not fully understood since the mechanisms

by which it exerts its effects are not entirely clear. Several good reviews on the entire family of NCS proteins exist [1, 2, 14–19]. A recent review on NCS-1 focused primarily on its role in chromaffin and PC12 cells [20]. Thus, a detailed review on Frq and its synaptic roles in particular is lacking. This review focuses on Frq's role in synaptic function and attempts to resolve conflicting reports.

The Discovery of Frq

Frq was first identified in *Drosophila melanogaster* and assigned a role in synaptic transmission [7]. The X-ray-induced *T(X;Y)V7* rearrangement was originally thought to be a *Shaker*-like mutant because the breakpoint was close to the *Shaker* gene, and *V7* mutants were found to shake their legs vigorously [21]. Subsequently, the *V7* rearrangement was shown to upregulate the expression of a novel calcium-binding protein that was named frequenin due to the frequency-dependent facilitation of neurotransmitter release observed in *V7* mutants [7]. Frq has since been identified in numerous organisms from yeast to humans. The mammalian counterpart was renamed NCS-1 [22]. A single *frq* gene has been found in all organisms with sequenced genomes except *Drosophila* [8] and the zebrafish *Danio rerio* [23] in which two *frq* genes have been found. The two Frq proteins in zebrafish and *Drosophila* differ by seven and ten amino acids, respectively [8, 23]. The protein sequences of *Drosophila* Frq1 and Frq2 is 100% conserved across 12 *Drosophila* species [24]. In zebrafish, NCS-1a is widely expressed in the nervous system and is involved in inner ear development, whereas the expression of NCS-1b is restricted to the olfactory bulb, and its function is unknown [23]. The two *Drosophila* Frq proteins are highly homologous and appear to have functionally similar roles in synaptic transmission and nerve terminal growth [8].

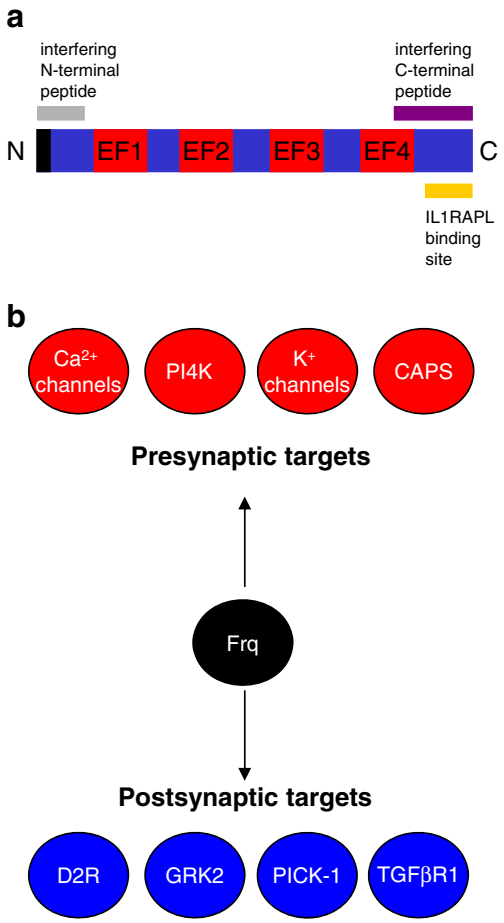

Fig. 1 Protein structure of Frq and possible binding partners. **a** Frq has an N-terminal myristoylation domain (*black*) and four EF-hands (*red*). An interfering N-terminal peptide (*gray*; first 27 amino acids) impairs PIP synthesis. An interfering C-terminal (*purple*) is thought to block an interaction with Ca^{2+} channels [5, 8, 9, 38, 39]. IL1RAPL binds to the last 16 amino acids of the C-terminus (*gold*; [72]). **b** Frq has been reported to interact directly or indirectly with several proteins and ion channels [87]. Some of the more prominent presynaptic (*red*) and postsynaptic (*blue*) targets are shown

Structure

The protein sequence of Frq across species is highly conserved with 100% conservation among mammals and 60% conservation between yeast and humans [25]. It contains four EF hand motifs (Fig. 1); two [7] or three [25] of which bind Ca^{2+} with high affinity. In vitro assays have shown that Frq binds calcium with a higher affinity than calmodulin and synaptotagmin [14, 26]. In addition, Frq has an N-terminal myristoylation domain, which allows it to be membrane bound. Myristoylated NCS-1 binds Ca^{2+} more strongly than non-myristoylated NCS-1, suggesting that myristoylation induces a change in protein conformation that enhances Ca^{2+} binding [27]. Myristoylation is also thought to be required for NCS-1 to activate some target proteins, such as phosphatidylinositol-4-OH kinase (PI4Kβ; [28, 29]). Some NCS proteins, such as recoverin, operate via a calcium/myristoyl switch [30]. The binding of Ca^{2+} to the EF hands of cytosolic protein results in the extrusion of the myristoyl group from a hydrophobic cavity of the protein, allowing it to interact with membrane targets [31–33]. However, Frq binds to membranes in the absence of Ca^{2+} [34], and membrane localization is not affected by mutations in all functional EF hands, indicating that in Frq, exposure of the myristoyl group and its localization is independent of Ca^{2+} [35]. However, some Frq is also reported in the cytosol [36]. This discrepancy was resolved in a recent study that used fluorescence recovery after photobleaching (FRAP) experiments to show that NCS-1 is transiently membrane associated at resting Ca^{2+} levels, with a fraction of NCS-1 becoming more stably associated with the membrane upon Ca^{2+} elevation [37]. Thus, Frq is primarily membrane bound due to myristoylation even

under resting conditions, but some cycling of Frq to the plasma membrane occurs.

The crystal structure of Frq revealed a large conformational shift of the C-terminal region in comparison to neurocalcin and recoverin, resulting in a wide hydrophobic crevice, which may accommodate an unknown protein ligand [25]. An interfering NCS-1 C-terminal peptide, consisting of the last 33 amino acids of NCS-1, has been used to disrupt binding of target proteins to this region [5, 8, 9, 38, 39]. This peptide consists of part of the fourth EF hand, but does not bind Ca^{2+} [40]. It is unknown which proteins or how many bind to this region. The interfering NCS-1 C-terminal peptide affects Ca^{2+} currents and signals; thus, it is possible that one of the target proteins is a Ca^{2+} channel or a protein that regulates Ca^{2+} channels.

In addition to myristoylation, the N-terminus of NCS-1 is also important for protein–protein interactions (Fig. 1). The first 71 residues of the N-terminus are required for an interaction with dopamine type-2 receptor (D2R; [41]). However, more recent work demonstrates that D2R binds to the hydrophobic crevice of NCS-1 with residues on both the N- and C-terminals being involved [42]. The N-terminus of NCS-1 has also been implicated in regulating phosphatidylinositol-4-phosphate (PIP) synthesis since an interfering NCS-1 N-terminal peptide and an antibody raised against the N-terminal of NCS-1 inhibited PIP synthesis [43].

Expression Patterns

Frq is widely expressed in the central nervous system (CNS) of flies [7], rats [44], mice [45], and humans [46], as evidenced by western blots and immunohistochemistry. It is expressed primarily in axons, dendrites, and astrocytes [47]. It is also expressed in the peripheral nervous system (PNS): Presynaptic terminals in neuromuscular junctions of *Drosophila* larvae [7], crayfish [48], frogs [49], and adult and developing rats [50] are all labeled with anti-Frq antibodies. Frq was originally thought to be exclusively expressed in the nervous system, but has since been detected in other non-neuronal tissue [51] including the heart [6, 52, 53]. By far, the strongest expression in mammalian species occurs in the central nervous system.

Expression levels of Frq vary in different neurons. For instance, it is expressed in the Purkinje cell layer of the mouse cerebellum but very little if at all in the granule cell layer [47]. Frq is also present in some glial cells such as astrocytes, but it is absent in Schwann cells and oligodendrocytes [47]. Higher levels of Frq are found in presynaptic terminals of crayfish and frog phasic motor neurons than in their tonic counterparts [48, 54], raising the possibility that the differential expression of Frq may contribute to functional differences at these synapses.

Given the high degree of sequence similarity between Frq and other NCS proteins (neurocalcin, VILIP, hippocalcin, recoverin, GCAP, and KChIP), caution should be taken in interpreting localization studies based on Frq antibodies. The ideal control to test for specificity of an antibody is to show that no staining is apparent in Frq knockouts. The only organisms in which Frq has been knocked out are *Saccharomyces cerevisiae* [3], *Caenorhabditis elegans* [4], *Drosophila melanogaster* [5], and *Mus musculus* [6, 55]. Localization studies have been conducted only in flies and mice. Frq was widely expressed in both CNS and PNS [7, 45, 47]. However, these studies were conducted before the Frq nulls were generated; thus, it is possible that some of the staining could be the result of cross reactivity with other NCS proteins (neurocalcin, VILIP, hippocalcin, recoverin, GCAP, and KChIP). Nevertheless, Frq does appear to be expressed widely in the *Drosophila* nervous system based on in situ hybridization studies for which highly specific probes for *frq* mRNA exist [8]. Future localization studies on Frq should use existing knockouts to confirm specificity of Frq antibodies.

Presynaptic Effects

The presynaptic role of Frq in regulating synaptic transmission is controversial (summarized in Table 1): Some studies have provided evidence suggesting that Frq is a calcium sensor that enhances both basal levels of neurotransmitter release in response to low-frequency stimulation [5, 8, 40, 56, 57], whereas other studies have indicated that Frq selectively enhances short-term synaptic plasticity elicited by paired-pulse or high-frequency stimulation [7, 28, 58, 59]. That Frq could be a sensor that promotes both functions is difficult to reconcile with since many synaptic investigations have shown that initial release probability (as evidenced by output of transmitter generated by the first impulse in a train of stimuli) is inversely related to facilitation [60]. Thus, if Frq enhances initial transmitter release, the expected outcome would be a decrease, rather than an increase, in short-term facilitation.

Overexpression of Frq due to an X-ray-induced chromosomal rearrangement in the *Drosophila V7* mutant was reported to enhance paired-pulse facilitation and transmission during high-frequency trains, but had no effect on spontaneous transmitter release or on neurotransmission evoked by low-frequency stimulation [7, 58, 59]. However, two more recent studies found that basal levels of neurotransmitter release were enhanced, and the paired-pulse ratio was reduced when either Frq1 or Frq2 was overexpressed using the GAL4/UAS system [5, 8]. Conversely, *frq* null mutants displayed reduced neurotransmitter release and an increase in the paired-pulse ratio [5]. The basis for the discrepancy between the more recent studies on *Drosophila* [5, 8] and the earlier work [7, 58, 59] may be due in part to the standard saline solution previously used, which does not maintain preparations in a stable condition at room temperature for a long time [61]. In addition, the earlier

Table 1 Summary of Frq's reported effects of basal levels of synaptic transmission and short-term synaptic plasticity

	Basal levels of evoked release	Spontaneous release	Facilitation/depression	References
Drosophila Frq overexpressers	No change	No change	Increased facilitation at low $[Ca^{2+}]_e$	[7, 58, 59]
Injection of Frq into *Xenopus* embryonic spinal neurons	Increased	Increased	Not tested	[56]
Frq upregulated in *Xenopus* nerve-muscle cocultured cells due to GDNF application	Increased	Increased	Decreased	[57]
Transfection of NCS-1 into cultured rat hippocampal neurons	No change	No change	Switched depression to facilitation	[28]
Drosophila Frq overexpressers	Increased	No change	Not tested	[8]
Drosophila Frq inhibitory C-terminal peptide	Reduced	No change	Not tested	[8]
Drosophila frq null mutants	Reduced	No change	Reduced depression at high $[Ca^{2+}]_e$ and no change at low $[Ca^{2+}]_e$	[5]
Drosophila Frq overexpressers	Increased	No change	Increased depression at high $[Ca^{2+}]_e$ and no change at low $[Ca^{2+}]_e$	[5]
Mouse NCS-1 inhibitory C-terminal peptide	Decreased	Not tested	Increased facilitation	[40]

studies did not take into account the fact that two types of axons (type 1b and 1s; [62]) with different facilitation properties innervate the muscle fibers from which EJPs were recorded. Type 1b motor-nerve terminals have physiological and morphological similarities with tonic motor-nerve terminals of other organisms (relatively large presynaptic boutons and facilitating synapses with low initial release probabilty), whereas type 1s terminals resemble phasic nerve terminals more closely (smaller boutons and depressing synapses with high release probability) [63]. Thus, variations in the contribution by the two types of recruited axons could account for the discrepancy.

A large facilitated response (LFR) was occasionally observed in *V7* mutants. This response was characterized by a failure of release in response to the first stimulus and LFR (10 to 12 times the number of quanta normally released) in response to the second stimulus [7, 58, 59]. In recent experiments with more stable preparations, this LFR phenotype was observed twice in *V7* mutants in several trials in hemolymph-like HL6 saline, but not when Frq1 or Frq2 was overexpressed using the GAL4/UAS system [5]. Thus, the X-ray-induced translocation that upregulates Frq2 in *V7* mutants may alter the expression of another protein that leads to the LFR. Consistent with this interpretation, *V7* mutants display a reduction in Shaker protein levels [64], but there is no change in Shaker levels when Frq is overexpressed using the GAL4/UAS system (J.R.-P. and A.F., unpublished data).

Findings in the two recent studies on *Drosophila* [5, 8] are consistent with two earlier studies on *Xenopus* [56, 57], which proposed a role for Frq in the regulation of basal levels of synaptic transmission. Injection of Frq into *Xenopus* embryonic spinal neurons caused an increase in both spontaneous and evoked neurotransmitter release [56]. Glial cell line-derived neurotrophic factor (GDNF), which upregulates Frq, reduces paired-pulse facilitation in *Xenopus* nerve-muscle cocultures by enhancing the amplitude of the first evoked synaptic response [57]. Thus, current evidence from both *Drosophila* and *Xenopus* indicates that Frq enhances the basal levels of synaptic transmission, with effects on paired-pulse facilitation or depression being a consequence of altered initial release probability for the first spike of the paired pulses.

Application of an interfering NCS-1 C-terminal peptide to mouse hippocampal slices impaired basal levels of synaptic transmission and enhanced paired-pulse facilitation [40], as in the *Drosophila* studies [5, 8]. However, an earlier study found that transfection of NCS-1 into cultured rat hippocampal neurons switched depression to facilitation in response to paired-pulse and high-frequency train stimulation without affecting basal levels of evoked release [28]. Examination of the newly created NCS-1 knockout mouse [6, 55] for neuronal phenotypes is needed to resolve the presynaptic role of Frq in mammals. Overall, the majority of evidence to date points to a role for Frq in regulating basal levels of neurotransmitter release, with effects on short-term synaptic plasticity consistent with alterations in initial release probability.

Overexpression of NCS-1 in PC12 cells was found to enhance growth hormone release in intact cells, demonstrating that NCS-1 is a positive regulator of evoked dense-core granule exocytosis [65]. However, overexpression had no effect on Ca^{2+}-induced exocytosis in permeabilized PC12 cells, suggesting that NCS-1 has a modulatory role on exocytosis instead of a direct action on the exocytotic machinery.

Presynaptic Mechanisms

Frq Regulates Voltage-Gated Ca^{2+} Channels

A physical interaction between Frq and Ca^{2+} channels has yet to be found. However, several studies have provided evidence

that Frq exerts its effects by either directly or indirectly regulating Ca^{2+} channels [5, 8, 9, 38, 39, 57, 66–68]. GDNF upregulates Frq and promotes synaptic transmission in *Xenopus* nerve-muscle cocultures by enhancing N-type Ca^{2+} channel activation, leading to increased Ca^{2+} influx [57]. The effects of GDNF were prevented by injection of a Frq antibody, demonstrating that Frq was either directly or indirectly responsible for the enhanced Ca^{2+} influx. *Drosophila frq* null mutants exhibit impaired Ca^{2+} influx in response to single action potentials [5], further suggesting a role for Frq in regulating presynaptic Ca^{2+} channels.

Several groups have used interfering peptides that provide evidence for an interaction between Frq and Ca^{2+} channels [5, 8, 9, 38, 39]. Loading an interfering NCS-1 C-terminal peptide into presynaptic terminals of the calyx of Held abolished activity-dependent facilitation of P/Q-type Ca^{2+} currents, whereas recombinant NCS-1 protein mimicked activity-dependent facilitation of Ca^{2+} currents. Facilitation of P/Q-type Ca^{2+} currents is similarly impaired in α_{1A}-subunit knockout mice [69]. Thus, the interfering NCS-1 C-terminal peptide may block activity-dependent facilitation of P/Q-type Ca^{2+} currents by disrupting an interaction between NCS-1 and the α_{1A}-subunit of voltage-gated Ca^{2+} channels. The same interfering peptide and knockdown of NCS-1 levels with RNA interference reduce Ca^{2+} signals and current in growth cones of cultured *Lymnaea* primary neurons [9, 39]. An interfering Frq C-terminal peptide based on the *Drosophila* sequence impairs Ca^{2+} entry in response to single action potentials with a concomitant reduction in quantal release at the *Drosophila* larval NMJ [5].

Further evidence for an interaction between Frq and Ca^{2+} channels comes from work with *Drosophila frq* and *cacophony* (*cac*; encodes the α_1-subunit of voltage-gated Ca^{2+} channels) null mutants. Trans-heterozygous genotypes can be used to determine if the two genes are functionally linked through participation in the same pathway. Such a functional link may occur through a direct protein–protein interaction or through one or more intermediary proteins. Impaired synaptic transmission (reduced neurotransmitter release) was observed in null *frq* and *cac* trans-heterozygotes, but not in *frq* or *cac* heterozygotes, demonstrating a synergistic relationship between Frq and the α_1-subunit of voltage-gated Ca^{2+} channels [5].

A genetic interaction between Frq and the Ca^{2+} channel was further shown using a cac^{ts2} mutation in the carboxyl tail of the α_1-subunit [5], which is critical for Ca^{2+} channel inactivation [70]. Gain-of-function Frq phenotypes (increased neurotransmitter release) were suppressed by cac^{ts2} [5]; thus, *cac* is epistatic to *frq*. Given the location of the cac^{ts2} mutation in the carboxyl tail of the α_1-subunit [70], Frq may negatively regulate Ca^{2+} channel inactivation. Visinin-like protein-2 (VILIP-2), another member of the NCS family, binds to the carboxyl tail of the α_1-subunit of Ca^{2+} channels, slowing inactivation [71]. This raises the possibility that Frq may also bind to the carboxyl tail of the α_1-subunit. However, a direct interaction has not been shown, leaving open the possibility that Frq is acting through an intermediary protein to regulate the channel (Fig. 2a).

In neuroendocrine cells, NCS-1 appears to be an inhibitory regulator of Ca^{2+} channels. Expression of a dominant negative form of NCS-1, in which the third EF hand is inactivated, in bovine adrenal chromaffin cells enhances non-L-type Ca^{2+} currents [66]. NCS-1 inhibits non-L-type Ca^{2+} channels by a voltage-independent mechanism that requires Src kinase activity [67].

A cDNA fragment encoding the last 16 amino acids of the C-terminus (amino acids 174-190) of the human *ncs-1* gene was found to interact with IL-1 receptor accessory protein-like (IL1RAPL) in a yeast two-hybrid screen [72]. This interaction was confirmed using GST pull-down assays and coimmunoprecipitation experiments. Expression of IL1RAPL in PC12 cells has an inhibitory effect on dense-core vesicle exocytosis and N-type Ca^{2+} currents, which is dependent on NCS-1 expression [73].

Heterologous expression of defined Ca^{2+} subunits and NCS-1 in *Xenopus* oocytes revealed that NCS-1 reduced L-, N-, and P/Q-type Ca^{2+} currents [68]. This effect was dependent on the expression of β subunit (β_1, β_2, or β_4), suggesting that NCS-1 may reduce trafficking of Ca^{2+} channels to the membrane by preventing an α_1/β association required for Ca^{2+} channel trafficking. The inhibitory role of NCS-1 in Ca^{2+} channel function is surprising, given that numerous studies have shown that NCS-1 has stimulatory effects on secretion. It's possible that the effects of NCS-1 on Ca^{2+} channels are cell-specific, with NCS-1 having a stimulatory role in neurons and an inhibitory role in neuroendocrine and non-neuronal cells. Alternatively, the different effects could be due to the different methods used to manipulate NCS-1 function (i.e., knockouts, overexpression, interfering peptides, and dominant-negatives).

Frq Interacts with Phosphatidylinositol-4-OH Kinase

Frq interacts with Pik1, the yeast orthologue of phosphatidylinositol-4-OH kinase (PI4Kβ), and is essential for survival in yeast [3]. Human NCS-1 is able to substitute for Frq in yeast and rescue the inviability of Frq-deficient yeast cells [74]. Frq binds to Pik1 on a binding site that includes as its core a 13-residue hydrophobic sequence (Ala-157 to Ala-169; [75]), and the structure for this complex in yeast has been resolved [76, 77]. However, this binding site is not conserved, and the complex has not been studied in higher organisms. Nevertheless, NCS-1 has been shown to coimmunoprecipitate with mammalian PI4Kβ from COS-7 cells [78], bovine chromaffin cells [79], and from rat neurosecretory cells [80]. In contrast, NCS-1 did not immunoprecipitate with PI4Kβ in cultured dorsal root ganglion neurons [81], and yeast-two-hybrid assays failed to show an interaction

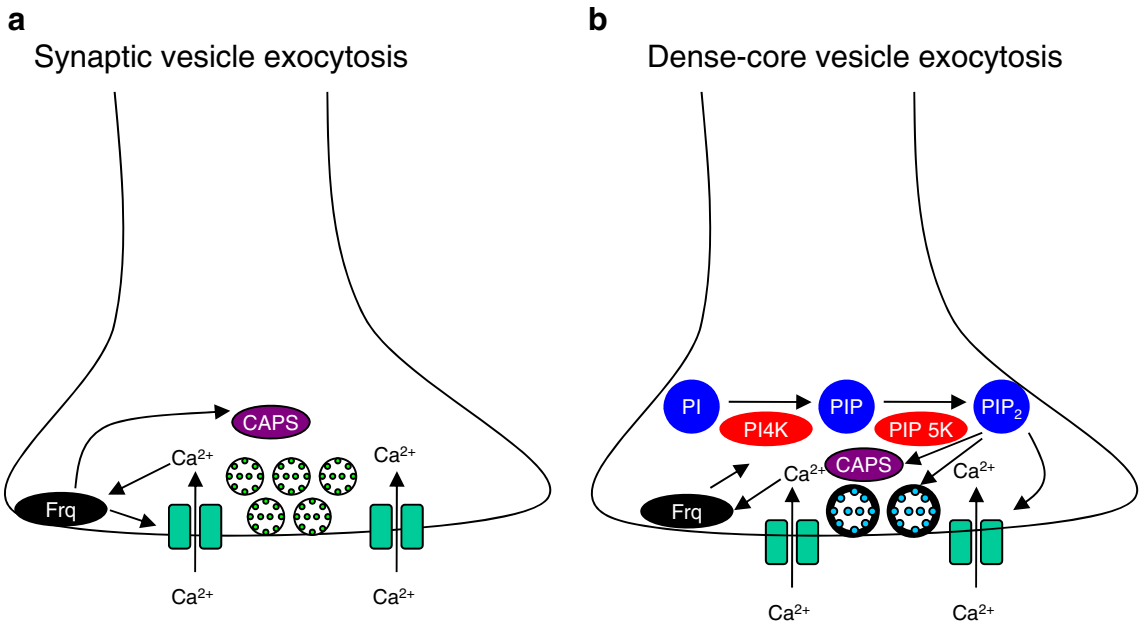

Fig. 2 Frq modulates both synaptic vesicle and dense-core vesicle exocytosis. **a** Frq binds Ca^{2+} and either directly or through intermediary proteins regulates Ca^{2+} channels, thereby modulating synaptic vesicle exocytosis [5, 38, 57]. **b** Frq binds Ca^{2+} and activates PI4Kβ, which leads to enhanced phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2) levels [29, 34, 36, 65, 80, 82–85]. The increased phosphoinositide levels (PIP and PIP_2) promote dense-core vesicle exocytosis

in *Drosophila* between Frq and PI4Kβ (J.R.-P. and A.F., unpublished data; Julie Brill, personal communication).

Dense-Core Vesicle Exocytosis

Numerous studies have shown that overexpression of NCS-1 in neuroendocrine cells enhances dense-core vesicle exocytosis [29, 34, 36, 65, 80, 82–85]. This enhanced exocytosis is likely due to the increased PI4Kβ activity, which leads to increased levels of phosphoinositides in PC12 cells overexpressing NCS-1 (Fig. 2b; [83]). Enhanced exocytosis is dependent on PI4Kβ levels since knock-down of PI4Kβ by RNAi prevents it [29]. In addition to increasing PI4Kβ activity, overexpression of NCS-1 in PC12 cells increased its association with membranes, suggesting that the interaction between NCS-1 and PI4Kβ may play a role in the translocation of PI4Kβ to the membrane [80]. The stimulatory effect of NCS-1 on exocytosis in PC12 cells can be abolished by overexpression of the small GTPase, ADP-ribosylation factor 1 (ARF1; [84]). This is likely due to the fact that while both NCS-1 and ARF1 can bind to and stimulate PI4Kβ independently, when present together, they form a complex that has impaired PI4Kβ binding [84, 85].

NCS-1 may play a role in vesicle priming or docking by enhancing PI4Kβ activity. Overexpression of NCS-1 in pancreatic β cells increased the number of secretory granules in the readily releasable pool, resulting in enhanced glucose-induced insulin secretion [86]. The authors propose that overexpression of NCS-1 increases PI4Kβ activity, which leads to increases in phosphoinositide levels. Phosphatidylinositol 4,5-bisphosphate (PIP_2) may then bind to Ca^{2+}-dependent activator protein for secretion (CAPS) and enhance the priming of secretory granules [86]. Frq has also been shown to directly interact with CAPS [87], which is required for exocytosis in pancreatic β cells [88], plays a critical role in regulating exocytosis of dense-core vesicles downstream of vesicle docking [89], and is required for synaptic vesicle priming [90]. A study on the interaction between Frq and CAPS may provide further insights into Frq's role in dense-core and synaptic vesicle release.

Synaptic Vesicle Exocytosis

Despite the importance of the Frq–PI4Kβ interaction for exocytosis of dense-core vesicles, it does not appear to regulate synaptic vesicle exocytosis. *Drosophila* PI4Kβ null mutants do not display the impaired synaptic transmission seen in Frq null mutants, suggesting that the two proteins likely function in separate pathways [5]. In addition, a point mutant that could not be myristoylated and thereby activate PI4Kβ enhanced short-term plasticity in cultured hippocampal neurons to the same extent as wild-type Frq, further suggesting that a Frq–PI4Kβ interaction does not regulate synaptic vesicle exocytosis [28]. The functional significance of the Frq–PI4Kβ interaction was further tested using a *Drosophila* PI4Kβ null. Overexpression of Frq at the *Drosophila* larval NMJ enhanced neurotransmitter release in the presence or absence of PI4Kβ, ruling out the possibility that a Frq–PI4Kβ interaction was enhancing release [5]. Thus, while an interaction between Frq

and PI4Kβ regulates dense-core vesicle release in neuroendocrine cells, this interaction does not appear to regulate synaptic vesicle exocytosis (Fig. 2).

Frq Regulates K^+ Channels

Type A K^+ currents operate in the subthreshold voltage range and play a role in the repolarization of action potentials. Coimmunoprecipitation experiments have shown that Frq interacts with Kv4 channels, the molecular components of type A K^+ currents [91]. Lobster Frq modifies type A K^+ shal-evoked currents in a Ca^{2+} dependent manner in *Xenopus* oocytes [92]. The amplitude of type A K^+-currents in *Drosophila* muscle fibers increases when external Ca^{2+} is increased, and this Ca^{2+}-dependent modulation of type A K^+ currents is absent in *V7* mutants [93]. However, a previous study did not detect any Frq in muscle fibers [7]. *V7* mutants do display a reduction in Shaker protein levels [64]. A reduction in K^+ currents in Frq overexpressers could increase action potential duration, leading to increased presynaptic Ca^{2+} entry and increased neurotransmitter release. However, we did not observe a change in Shaker levels when we overexpressed Frq using the GAL4/UAS system (J.R.-P. and A.F., unpublished data). Thus, as previously mentioned, the reduced Shaker levels in *V7* mutants may have been due to other effects of the X-ray-induced chromosomal rearrangement besides Frq overexpression. In addition, overexpression of Frq in COS cells results in an increase in Kv4 channel expression, suggesting that Frq may enhance the trafficking of Kv4 channels to the plasma membrane [91]. Nevertheless, some of the reported effects on action-potential-induced presynaptic Ca^{2+} entry could be due to an interaction between Frq and K^+ channels. Additional studies are needed to fully understand the functional significance of this putative interaction.

Frq Regulates TRP Channels

A direct interaction between NCS-1 and TRPC5 has been shown using yeast two-hybrid and GST-pulldown assays [94]. NCS-1 also interacts with TRPC1 [95]. Expression of a dominant negative form of NCS-1, in which the third EF hand is inactivated, suppresses TRPC5 channel activity [94].

Other Possible Targets

Frq interacts with several other proteins, but the functional significance of many of these interactions is currently unknown. For instance, Frq interacts with adaptor protein 1 (AP1) and adaptor protein 2 (AP2) [87], raising the possibility that Frq may play a role in synaptic vesicle endocytosis. However, a role for Frq in synaptic vesicle endocytosis seems unlikely, given that no changes were found in the number of synaptic vesicles in *Drosophila frq* null mutants and overexpressers [5].

Frq may regulate cyclic nucleotide (cAMP and cGMP) levels. Frq activates guanylate cyclase [7, 96], which may lead to increased cGMP levels. Frq also binds to and activates cyclic nucleotide 3',5'-phosphodiesterase (PDE; [97]), which may result in reduced cAMP levels. However, studies in which cAMP and cGMP levels were directly measured yielded conflicting results. cAMP and cGMP levels in permeabilized chromaffin cells were not altered in the presence of NCS-1 [34], while cAMP levels were reduced in PC12 cells overexpressing NCS-1 [98].

Frq binds to calcineurin [87] and potentiates the activity of nitric oxide synthase in the presence of calmodulin [97]. However, these possible targets also interact with calmodulin and, in some cases, have a greater affinity for calmodulin [99], making it unlikely that they interact primarily with Frq in vivo.

Postsynaptic Effects

In addition to a presynaptic role in regulating synaptic transmission, some evidence indicates a postsynaptic role for Frq in regulating long-term synaptic plasticity in the mammalian CNS. It has been shown to regulate both long-term potentiation (LTP) and long-term depression (LTD; Fig. 3). NCS-1 is expressed in the cerebellum and hippocampus [44, 45]. Frq interacts with several proteins, including D2R, G-protein-coupled receptor kinase 2 (GRK2), and transforming growth factor β receptor 1 (TGFβR1), which are primarily present in postsynaptic neurons [41, 42, 87]. An interaction between *Drosophila* Frq and a type 1 TGFβ receptor was detected in a yeast 2-hybrid screen [100].

Induction of LTP in rats increased postsynaptic *ncs-1* mRNA levels in the dentate gyrus [101]. This transcriptional upregulation was dependent on NMDA receptor activation. Application of a metabotropic glutamate receptor (mGluR) agonist, (*R*, *S*)-3,5-dihydroxyphenylglycine, induces a form of LTP with slow onset and enhances the level of NCS-1 protein [102]. These studies raised the possibility that enhanced NCS-1 expression is at least in part responsible for the enhanced synaptic strength observed during LTP. However, it was unclear if the enhanced NCS-1 expression was the cause of the enhanced synaptic strength or a consequence of it.

A recent study shed considerable light on a postsynaptic role for NCS-1 in LTP. Overexpression of NCS-1 in the dentate gyrus of mice increased D2R surface expression, enhanced LTP, and lowered the stimulus threshold required for LTP induction [11]. These effects were blocked by either D2R antagonists or by an interfering peptide that was designed to block an interaction between NCS-1 and D2R. In HEK293 cells, NCS-1 attenuates D2R internalization in a Ca^{2+}-dependent manner by interacting with GRK2 and thereby inhibiting

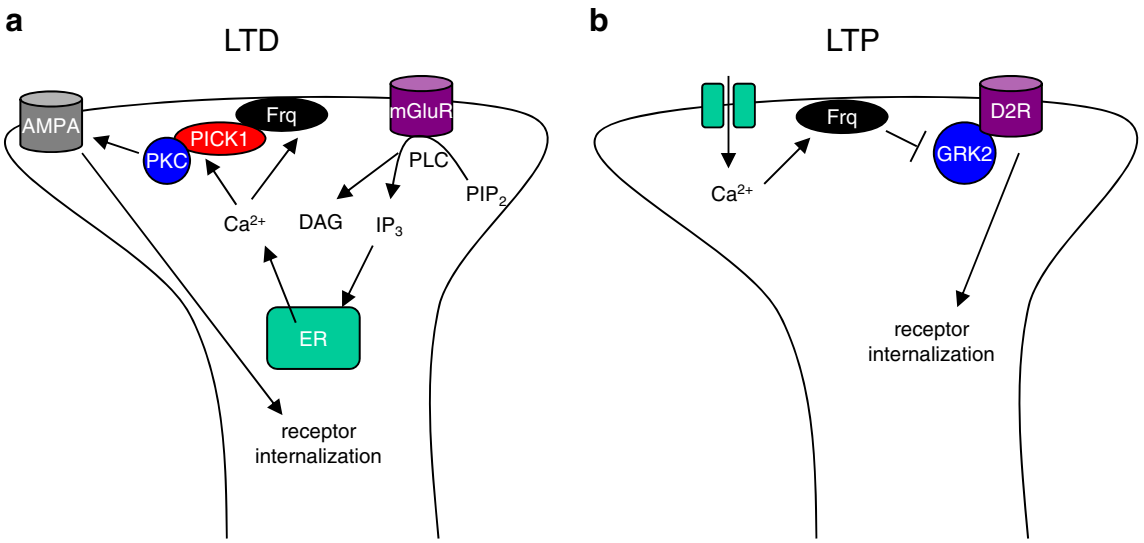

Fig. 3 Frq regulates both long-term depression (LTD) and long-term potentiation (LTP) through postsynaptic effects in the mammalian CNS. **a** Frq and protein interacting with C kinase (PICK1) bind to Ca^{2+} released from internal Ca^{2+} stores after mGluR activation. Frq may then form a complex with PICK1 and protein kinase C (PKC). This results in phosphorylation and internalization of AMPA receptors, resulting in LTD [10]. **b** Frq binds Ca^{2+} and then binds to G-protein-coupled receptor kinase 2 (GRK2), thereby inhibiting phosphorylation of dopamine type-2 receptor (D2R). This leads to an increase in D2R surface expression, resulting in LTP [11]. *DAG* diacylglycerol, *ER* endoplasmic reticulum, *IP_3* inositol trisphosphate, *PIP_2* phosphatidylinositol 4,5-bisphosphate, *PLC* phospholipase C

phosphorylation of D2R by GRK2 [41]. NCS-1 may regulate D2R surface expression in the dentate gyrus through a similar mechanism [11].

Another study found that NCS-1 also regulates LTD. RNAi knockdown of NCS-1 or the inclusion of dominant-negative NCS-1, in which the third EF hand is inactivated, blocks mGluR-dependent long-term depression (LTD) in the rat perirhinal cortex [10]. NCS-1 was activated by Ca^{2+} released from internal stores and binds to the BAR domain of protein interacting with C kinase (PICK-1). Inclusion of a PICK1 BAR domain peptide blocked mGluR-dependent LTD, likely by blocking AMPA receptor internalization [10].

A putative interaction between NCS-1 and AP1 or AP2 could also influence long-term synaptic plasticity by regulating postsynaptic receptor recycling. Hippocalcin, another NCS protein, acts as a Ca^{2+} sensor that binds to the β2-adaptin subunit of AP2 and forms a complex with GluR2 (AMPA receptor subunit) in response to NMDA receptor activation, leading to AMPA receptor internalization and induction of LTD. [103]. Additional studies are needed to determine if NCS-1 also regulates postsynaptic receptor recycling through a similar mechanism.

NCS-1 regulates both LTD and LTP because different stimulation protocols result in different sources of calcium activating NCS-1, which leads to NCS-1 binding to different target proteins (Fig. 3). When Ca^{2+} released from internal stores activates NCS-1, LTD is promoted through an interaction between NCS-1 and PICK-1 that leads to AMPA receptor internalization. On the other hand, when Ca^{2+} admitted through NMDA receptors activates NCS-1, LTP is enhanced due to an interaction between NCS-1 and GRK2. This inhibits phosphorylation of D2R resulting in increased surface expression of D2R. The expression of different target proteins in different cells may also contribute to the different effects of NCS-1.

Frq Regulates Neurite Outgrowth

Frq has a developmental role, as evidenced by experiments in fruit flies and snails in which neuron morphology was affected by different levels of Frq expression (summarized in Table 2). In *Drosophila*, Frq overexpressers had motor terminals with reduced number and length of branches, and fewer synaptic boutons [64, 104]. This effect was specific to phasic-like type 1s boutons [8]. Conversely, the number of these type 1s boutons was increased when an interfering C-terminal peptide was chronically expressed [8]. This effect was also observed in *frq* null mutants [5]. Application of the same interfering C-terminal peptide and knockdown of NCS-1 levels with RNAi interference enhanced neurite outgrowth of cultured *Lymnaea* primary neurons [9]. PC12 cells expressing a dominant negative form of NCS-1, in which the third EF hand is inactivated, also displayed enhanced neurite outgrowth [94]. Similarly, the number of neurites per cell, branches per neurite, and length of neurites were reduced in NG-108-15 cells transfected with NCS-1 [105]. All of these effects are consistent with a role for NCS-1 as a negative regulator of neurite outgrowth. In addition, due to its temporal and spatial expression patterns, NCS-1 has also

Table 2 Summary of Frq's reported effects on neurite outgrowth

	Effect	References
Drosophila Frq overexpressers	Reduction in nerve terminal growth	[8, 64, 104]
NG-108-15 cells transfected with NCS-1	Reduced number of neurites per cell, branches per neurite and length of neurites	[105]
PC12 cells expressing a dominant negative form of NCS-1	Increased neurite outgrowth	[94]
Drosophila Frq inhibitory C-terminal peptide	Increased nerve terminal growth	[8]
NCS-1 inhibitory C-terminal peptide or RNAi knockdown of NCS-1 in cultured *Lymnaea* primary neurons	Increased neurite outgrowth	[9]
Drosophila frq null mutants	Increased nerve terminal growth	[5]
Chromophore-assisted laser inactivation of NCS-1 in cultured chick dorsal root ganglion neurons	Growth arrest of neurites and retraction of lamellipodial and filopodial.	[114]
Lentiviral overexpression of NCS-1 in cultured rat cortical neurons	Increased neurite outgrowth	[13]

been implicated in synaptogenesis in the rat retina [106] and axon outgrowth in the chick retina [107], in the developing mouse olfactory pathway [108], and in the developing rat spinal cord [109].

These developmental effects raise the possibility that Frq's effects on transmitter release could be interrelated to its effects on neurite outgrowth. However, chronic expression of an interfering C-terminal peptide affects both nerve terminal growth and neurotransmitter release, while acute application of the peptide produces a rapid reduction of neurotransmitter release (comparable in magnitude to that obtained with transgenic expression) but without an effect on nerve terminal morphology [8]. While this proves that Frq's effects on neurotransmitter release are not dependent on morphological changes, it could be argued that Frq's effects on nerve terminal growth are a consequence of its effects on neurotransmission. Presynaptic terminal retraction has been shown to occur at both wild-type *Drosophila* larval NMJ [110] and the frog NMJ [111, 112]. When presynaptic terminal retraction occurs, the remaining postsynaptic density can be used as a footprint for presynaptic terminal retraction [110]. There was no postsynaptic footprint observed in Frq overexpressers [113]; thus, the reduced innervation was probably not due to presynaptic terminal retraction. In addition, Frq regulates transmitter release from both 1b and 1s boutons, but only affects the number of 1s boutons [5, 8], suggesting that the effects on nerve terminal growth and neurotransmission are not tightly linked, implying activation of different mechanisms in the two types of motor neuron. In totality, these observations indicate that Frq has a developmental role in regulating nerve terminal growth.

Conversely, two recent studies have suggested that Frq may be a positive regulator of neurite outgrowth [13, 114]. NCS-1 and 1,4,5-trisphosphate receptor ($InsP_3R1$) colocalized in growth cones of cultured chick dorsal root ganglion neurons [114]. Application of $InsP_3R1$ inhibitors (2-APB) reduced NCS-1 labeling in growth cones and inhibited neurite outgrowth. The authors used chromophore-assisted laser inactivation (CALI) to inactivate NCS-1 bound to a specific NCS-1 antibody and found that growth of neurites was arrested and lamellipodial and filopodial were retracted. One caveat of this method is the assumption that the antibody is binding only to the protein of interest. Since NCS proteins (neurocalcin, VILIP, hippocalcin, recoverin, GCAP, and KChIP) are highly similar in structure, it is possible that NCS proteins other than NCS-1 could have been inactivated.

Lentiviral overexpression of NCS-1 in cultured rat cortical neurons enhanced neurite outgrowth and phosphorylation of Akt [13]. The increased neurite outgrowth and phosphorylation of Akt were both blocked by a phosphatidylinositol-3-OH kinase (PI3K) inhibitor (ly249002), which did not reduce NCS-1 expression, demonstrating that PI3K is downstream of NCS-1. It is unclear how NCS-1 activates PI3K. Frq does not interact with PI3K in yeast [3], and no evidence for an interaction exists in mammals. The discrepancy between these two studies and previous studies may be due to the activation of NCS-1 by Ca^{2+} from internal stores, as opposed to Ca^{2+} entering the cell via voltage-gated Ca^{2+} channels. Different sources of Ca^{2+} may result in NCS-1 binding to different protein targets. In addition, NCS-1 may have differential effects in different neurons, possibly due to the expression of different target proteins. A striking example occurs in *Drosophila*: Frq regulates the number of 1s boutons, but has no effect on the number of 1b boutons [5, 8].

Frq Enhances Learning and Memory

By regulating long-term synaptic plasticity [10, 11], NCS-1 may also affect higher functions such as learning and memory. A role for NCS-1 in learning and memory was first demonstrated in a study on *C. elegans*, in which NCS-1 knockout worms showed defects in associative memory, while overexpression of NCS-1 resulted in accelerated learning [4]. In mice, a recent study showed that overexpression of NCS-1 in the dentate gyrus enhanced D2R surface expression and

spatial memory [11]. Both types of enhancement were blocked by infusion of D2R antagonists or an interfering peptide that disrupted an interaction between NCS-1 and D2R receptors, demonstrating that overexpression of NCS-1 enhances spatial memory by regulating D2R surface expression.

Neuroprotective Effects of Frq

A trauma or injury to the brain can often result in neuronal apoptosis. NCS-1 appears to promote neuronal survival since NCS-1 expression in cultured cortical neurons increased after the application of GDNF and overexpression of NCS-1 in the absence of GDNF mimicked the survival-promoting effects of GDNF [12]. In addition, overexpression of NCS-1 in PC12 cells and cultured cortical neurons has neuroprotective effects under apoptotic conditions. Similarly, lentiviral overexpression of NCS-1 in axotomized corticospinal neurons in living rats reduced cell atrophy [13]. NCS-1 is upregulated in injured neurons and can activate antiapoptotic pathways that may prevent neuronal loss after a trauma by activating PI3K/Akt pathway [12].

NCS-1 has also been implicated in regeneration. Lentiviral overexpression of NCS-1 in the rat corticospinal tract induced axonal sprouting and regeneration from the lesioned corticospinal tract and collateral sprouting from the intact corticospinal tract [13]. Both behavioral and electrophysiological tests indicated improved forelimb function in injured rats that were overexpressing NCS-1 in the intact corticospinal tract.

Role of Frq in Cardiac Function

While NCS-1 is primarily expressed in the nervous system, it has also been detected in the mammalian heart [52]. NCS-1 expression in the heart is highest at the fetal and neonatal stages; it then gradually decreases [6, 53]. NCS-1 interacts and colocalizes with K^+ channels in the heart [52, 53]. NCS-1 has also been shown to interact with IP_3 receptors. Ca^{2+} levels were reduced in NCS-1 knockout myocytes, leading to a reduction in Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) activity [6]. Whether NCS-1 regulates CAMKII activation in neurons is currently unknown.

Links Between Frq and Human Diseases

Connections have been made between NCS-1 and a number of human diseases. Elevated levels of NCS-1 were found in the dorsolateral prefrontal cortex in schizophrenic and bipolar disorder patients [115, 116]. It is thought that dysregulation of dopaminergic neurotransmission in schizophrenia may be due to altered levels of dopamine receptor interacting proteins [15, 116]. NCS-1 has been shown to colocalize [41, 117] and interact with D2R [41, 42]. In addition, NCS-1 attenuates D2R internalization in a Ca^{2+}-dependent manner by interacting with GRK2 and thereby inhibits phosphorylation of D2R by GRK2 [41]. Therefore, NCS-1 may couple dopamine and calcium signaling pathways and play a role in neuropathologies characterized by a dysregulation in dopaminergic neurotransmission [41].

NCS-1 interacts through its C-terminus with IL1RAPL, which is involved in X-linked mental retardation [72]. Expression of IL1RAPL in PC12 cells has an inhibitory effect on dense-core vesicle exocytosis, N-type Ca^{2+} currents, and neurite outgrowth [73]. These phenotypes were dependent on NCS-1 expression, as RNAi mediated knockdown of NCS-1 rescued all the phenotypes. Thus, an interaction between NCS-1 and IL1RAPL may play a role in X-linked mental retardation by regulating exocytosis and neurite outgrowth. Mutations in IL1RAPL have been linked to autism, and a point mutation in NCS-1 (R102Q) was identified in one autistic patient [118]. This mutation does not affect binding of NCS-1 to IL1RAPL or Ca^{2+}, but affects cycling of NCS-1 to the plasma membrane [37].

A recent study on *Drosophila* has also linked Frq to fragile X syndrome (FXS). *frq1* and *frq2* mRNA levels were mildly reduced (15–25%) in null *fragile X mental retardation 1* (*fmr1*) mutants [119]. It remains to be seen whether this reduction in *frq* levels is responsible for the cognitive defects associated with FXS. Understanding the role of Frq in these various diseases would be aided by using existing fly or mouse knockouts in disease models of these two organisms.

Given these links to disease, several studies have attempted to find compounds that bind to or alter NCS-1 levels. The neuroleptic drug chlorpromazine binds to NCS-1 [120], while several antipsychotic drugs (haloperidol, clozapine, olanzapine, and aripiprazole) do not alter NCS-1 levels [121]. Interestingly, Paclitaxel (also called Taxol), a chemotherapeutic drug, reduced NCS-1 levels [122–124]. Paclitaxel is used to treat several types of cancer and solid tumors. Prolonged exposure to Paclitaxel activates calpain, which degrades NCS-1, leading to a reduction in IP_3-mediated Ca^{2+} signaling [123, 125].

Conclusions and Future Directions

Over the last two decades, we have come a long way in our understanding of Frq's role in synaptic function and development. Early evidence suggested a presynaptic role for Frq as a Ca^{2+} sensor affecting short-term synaptic plasticity, but recent studies favor a role for Frq in regulating basal levels of synaptic transmission, with effects on short-term synaptic plasticity being a consequence of altered initial release probability. Several studies have disputed whether the presynaptic

effects of Frq were due to Ca^{2+} channels or an interaction with PI4Kβ. We propose that a functional interaction with Ca^{2+} channels is required for synaptic vesicle release, whereas an interaction with PI4Kβ is required for dense-core vesicle release (Fig. 2).

Clearly, Frq has a significant role in regulating synaptic transmission and neurite outgrowth. However, a complete mechanistic account of its actions remains elusive. For instance, does Frq bind to Ca^{2+} channels and how precisely does it regulate Ca^{2+} channel function? The functional significance of many of Frq's binding partners remains unknown.

Frq also has a postsynaptic role in regulating both LTP and LTD in the mammalian CNS. The different stimulation protocols used to induce LTP and LTD result in different sources of Ca^{2+} activating NCS-1, which leads to NCS-1 binding to different target proteins (Fig. 3). NCS-1 may also have differential effects in different neurons due to the expression of different target proteins. These effects on long-term synaptic plasticity likely contribute to the learning and memory phenotypes reported in worms and mice.

A question that remains unanswered is why the absence of Frq is lethal in yeast [3], but not in worms [4], flies [5], or mice [6]. One possibility is functional complementation between Frq and other NCS proteins (neurocalcin, VILIP, hippocalcin, recoverin, GCAP, and KChIP). Frq is the only NCS protein found in yeast, whereas additional NCS proteins are found in worms, flies, and mice. The study of multiallelic combinations of Frq and other NCS mutants could be a means to test this hypothesis. This could be done in genetically tractable organisms such as worms and flies.

Acknowledgements This work was supported by grants from CIHR, Canada MOP-37774 (H.L.A.) and MEC, Spain BFU2006-10180, and MYORES European Network CE: 511978 (A.F.). We would like to thank Dr. Milton Charlton for his help and advice on past and ongoing research projects related to this review.

References

1. Burgoyne RD, O'Callaghan DW, Hasdemir B, Haynes LP, Tepikin AV (2004) Neuronal Ca2+-sensor proteins: multitalented regulators of neuronal function. Trends Neurosci 27(4):203–209
2. Burgoyne RD (2007) Neuronal calcium sensor proteins: generating diversity in neuronal Ca2+ signalling. Nat Rev Neurosci 8(3):182–193
3. Hendricks KB, Wang BQ, Schnieders EA, Thorner J (1999) Yeast homologue of neuronal frequenin is a regulator of phosphatidylinositol-4-OH kinase. Nat Cell Biol 1(4):234–241
4. Gomez M, De Castro E, Guarin E, Sasakura H, Kuhara A, Mori I, Bartfai T, Bargmann CI, Nef P (2001) Ca2+ signaling via the neuronal calcium sensor-1 regulates associative learning and memory in *C. elegans*. Neuron 30(1):241–248
5. Dason JS, Romero-Pozuelo J, Marin L, Iyengar BG, Klose MK, Ferrús A, Atwood HL (2009) Frequenin/NCS-1 and the Ca^{2+} channel α_1-subunit co-regulate synaptic transmission and nerve terminal growth. J Cell Sci 122(22):4109–4121
6. Nakamura TY, Jeromin A, Mikoshiba K, Wakabayashi S (2011) Neuronal calcium sensor-1 promotes immature heart function and hypertrophy by enhancing Ca2+ signals. Circ Res 109(5):512–523
7. Pongs O, Lindemeier J, Zhu XR, Theil T, Engelkamp D, Krah-Jentgens I, Lambrecht HG, Koch KW, Schwemer J, Rivosecchi R, Mallart A, Galceran J, Canal I, Barbas A, Ferrus A (1993) Frequenin—a novel calcium-binding protein that modulates synaptic efficacy in the *Drosophila* nervous system. Neuron 11(1):15–28
8. Romero-Pozuelo J, Dason JS, Atwood HL, Ferrús A (2007) Chronic and acute alterations in the functional levels of frequenins 1 and 2 reveal their roles in synaptic transmission and axon terminal morphology. Eur J Neurosci 26(9):2428–2443
9. Hui K, Fei GH, Saab BJ, Su J, Roder JC, Feng ZP (2007) Neuronal calcium sensor-1 modulation of optimal calcium level for neurite outgrowth. Development 134(24):4479–4489
10. Jo J, Heon S, Kim MJ, Son GH, Park Y, Henley JM, Weiss JL, Sheng M, Collingridge GL, Cho K (2008) Metabotropic glutamate receptor-mediated LTD involves two interacting Ca(2+) sensors, NCS-1 and PICK1. Neuron 60(6):1095–1111
11. Saab BJ, Georgiou J, Nath A, Lee FJS, Wang M, Michalon A, Liu F, Mansuy IM, Roder JC (2009) NCS-1 in the dentate gyrus promotes exploration, synaptic plasticity, and rapid acquisition of spatial memory. Neuron 63(5):643–656
12. Nakamura TY, Jeromin A, Smith G, Kurushima H, Koga H, Nakabeppu Y, Wakabayashi S, Nabekura J (2007) Novel role of neuronal Ca2+ sensor-1 as a survival factor up-regulated in injured neurons. J Cell Biol 172(7):1081–1091
13. Yip PK, Wong LF, Sears TA, Yáñez-Muñoz RJ, McMahon SB (2010) Cortical overexpression of neuronal calcium sensor-1 induces functional plasticity in spinal cord following unilateral pyramidal tract injury in rat. PLoS Biol 8(6):e1000399
14. Burgoyne RD, Weiss JL (2001) The neuronal calcium sensor family of Ca2+-binding proteins. Biochem J 353(1):1–12
15. Braunewell KH (2005) The darker side of Ca2+ signaling by neuronal Ca2+-sensor proteins: from Alzheimer's disease to cancer. Trends Pharmacol Sci 26(7):345–351
16. McCue HV, Haynes LP, Burgoyne RD (2010) The diversity of calcium sensor proteins in the regulation of neuronal function. Cold Spring Harb Perspect Biol 2(8):a004085
17. Mikhaylova M, Hradsky J, Kreutz MR (2011) Between promiscuity and specificity: novel roles of EF-hand calcium sensors in neuronal Ca(2+) signalling. J Neurochem 118(5):695–713
18. Ames JB, Lim S (2011) Molecular structure and target recognition of neuronal calcium sensor proteins. Biochim Biophys Acta, in press
19. Burgoyne RD, Haynes LP (2012) Understanding the physiological roles of the neuronal calcium sensor proteins. Mol Brain 5(1):2
20. Weiss JL, Hui H, Burgoyne RD (2010) Neuronal calcium sensor-1 regulation of calcium channels, secretion, and neuronal outgrowth. Cell Mol Neurobiol 30(8):1283–1292
21. Tanouye MA, Ferrus A, Fujita SC (1981) Abnormal action potentials associated with the Shaker complex locus of Drosophila. Proc Natl Acad Sci USA 78(10):6548–6552
22. Nef S, Fiumelli H, de Castro E, Raes MB, Nef P (1995) Identification of neuronal calcium sensor (NCS-1) possibly involved in the regulation of receptor phosphorylation. J Recept Signal Transduct Res 15(1–4):365–378
23. Blasiole B, Kabbani N, Boehmler W, Thisse B, Thisse C, Canfield V, Levenson R (2005) Neuronal calcium sensor-1 gene ncs-1a is essential for semicircular canal formation in zebrafish inner ear. J Neurobiol 64(3):285–297
24. Sanchez-Gracia A, Romero-Pozuelo J, Ferrús A (2010) Two frequenins in *Drosophila*: unveiling the evolutionary history of an unusual neuronal calcium sensor (NCS) duplication. BMC Evol Biol 10:54
25. Bourne Y, Dannenberg J, Pollmann V, Marchot P, Pongs O (2001) Immunocytochemical localization and crystal structure of human

frequenin (neuronal calcium sensor 1). J Biol Chem 276(15):11949–11955
26. Cox JA, Durussel I, Comte M, Nef S, Nef P, Lenz SE, Gundelfinger ED (1994) Cation binding and conformational changes in VILIP and NCS-1, two neuron-specific calcium-binding proteins. J Biol Chem 269(52):32807–32813
27. Jeromin A, Muralidhar D, Parameswaran MN, Roder J, Fairwell T, Scarlata S, Dowal L, Mustafi SM, Chary KV, Sharma Y (2004) N-terminal myristoylation regulates calcium-induced conformational changes in neuronal calcium sensor-1. J Biol Chem 279(26):27158–27167
28. Sippy T, Cruz-Martin A, Jeromin A, Schweizwer FE (2003) Acute changes in short-term plasticity at synapses with elevated levels of neuronal calcium sensor-1. Nat Neurosci 6(10):1031–1038
29. de Barry J, Janoshazi A, Dupont JL, Procksch O, Chasserot-Golaz S, Jeromin A, Vitale N (2006) Functional implication of neuronal calcium sensor-1 and phosphoinositol 4-kinase-beta interaction in regulated exocytosis of PC12 cells. J Biol Chem 281(26):18098–18111
30. O'Callaghan DW, Burgoyne RD (2003) Role of myristoylation in the intracellular targeting of neuronal calcium sensor (NCS) proteins. Biochem Soc Trans 31(5):963–965
31. Tanaka T, Ames JB, Harvey TS, Stryer L, Ikura M (1995) Sequestration of the membrane targeting myristoyl group of recoverin in the calcium-free state. Nature 376:444–447
32. Ames JB, Ishima R, Tanaka T, Gordon JI, Stryer L, Ikura M (1997) Molecular mechanics of calcium–myristoyl switches. Nature 389:198–202
33. Meyer T, York JD (1999) Calcium–myristoyl switches turn on new lights. Nat Cell Biol 1(4):E93–E95
34. McFerran BW, Weiss JL, Burgoyne RD (1999) Neuronal Ca(2+) sensor 1. Characterization of the myristoylated protein, its cellular effects in permeabilized adrenal chromaffin cells, Ca(2+)-independent membrane association, and interaction with binding proteins, suggesting a role in rapid Ca(2+) signal transduction. J Biol Chem 274(42):30258–30265
35. O'Callaghan DW, Ivings L, Weiss JL, Ashby MC, Tepikin AV, Burgoyne RD (2002) Differential use of myristoyl groups on neuronal calcium sensor proteins as a determinant of spatio-temporal aspects of Ca2+ signal transduction. J Biol Chem 277 (16):14227–14237
36. Koizumi S, Rosa P, Willars GB, Challiss RA, Taverna E, Francolini M, Bootman MD, Lipp P, Inoue K, Roder J, Jeromin A (2002) Mechanisms underlying the neuronal calcium sensor-1-evoked enhancement of exocytosis in PC12 cells. J Biol Chem 277 (33):30315–30324
37. Handley MT, Lian LY, Haynes LP, Burgoyne RD (2010) Structural and functional deficits in a neuronal calcium sensor-1 mutant identified in a case of autistic spectrum disorder. PLoS One 5(5):e10534
38. Tsujimoto T, Jeromin A, Saitoh N, Roder JC, Takahashi T (2002) Neuronal calcium sensor 1 and activity-dependent facilitation of P/Q-type calcium currents at presynaptic nerve terminals. Science 295(5563):2276–2279
39. Hui K, Feng ZP (2008) NCS-1 differentially regulates growth cone and somata calcium channels in Lymnaea neurons. Eur J Neurosci 27(3):631–643
40. Saab BJ (2010). The synaptic role of neuronal calcium sensor 1 in dentate gyrus plasticity, curiosity and spatial memory. PhD thesis, University of Toronto.
41. Kabbani N, Negyessy L, Lin R, Goldman-Rakic P, Levenson R (2002) Interaction with neuronal calcium sensor NCS-1 mediates desensitization of the D2 dopamine receptor. J Neurosci 22 (19):8476–8486
42. Lian L-Y, Pandalaneni SR, Patel P, McCue HV, Haynes LP, Burgoyne RD (2011) Characterisation of the interaction of the C-terminus of the dopamine D2 receptor with neuronal calcium sensor-1. PLoS One 6(11):e27779
43. Zheng Q, Bobich JA, Vidugiriene J, McFadden SC, Thomas F, Roder J, Jeromin A (2005) Neuronal calcium sensor-1 facilitates neuronal exocytosis through phosphatidylinositol 4-kinase. J Neurochem 92(3):442–451
44. Martone ME, Edelmann VM, Ellisman MH, Nef P (1999) Cellular and subcellular distribution of the calcium-binding protein NCS-1 in the central nervous system of the rat. Cell Tissue Res 295(3):395–407
45. Jinno S, Jeromin A, Roder J, Kosaka T (2002) Immunocytochemical localization of neuronal calcium sensor-1 in the hippocampus and cerebellum of the mouse, with special reference to presynaptic terminals. Neuroscience 113(2):449–461
46. Chen C, Yu L, Zhang P, Jiang J, Zhang Y, Chen X, Wu Q, Wu Q, Zhao S (2002) Human neuronal calcium sensor-1 shows the highest expression level in cerebral cortex. Neurosci Lett 319(2):67–70
47. Olafsson P, Soares HD, Herzog KH, Wang T, Morgan JI, Lu B (1997) The Ca2+ binding protein, frequenin is a nervous system-specific protein in mouse preferentially localized in neurites. Brain Res Mol Brain Res 44(1):73–82
48. Jeromin A, Shayan AJ, Msghina M, Roder J, Atwood HL (1999) Crustacean frequenins: molecular cloning and differential localization at neuromuscular junctions. J Neurobiol 41(2):165–175
49. Werle MJ, Roder J, Jeromin A (2000) Expression of frequenin at the frog (Rana) neuromuscular junction, muscle spindle and nerve. Neurosci Lett 284(1–2):33–36
50. Garcia N, Lanuza MA, Besalduch N, Santafe MM, Jeromin A, Tomas J (2005) Localization of neuronal calcium sensor-1 at the adult and developing rat neuromuscular junction. J Neurosci Res 82(1):1–9
51. Gierke P, Zhao C, Brackmann M, Linke B, Heinemann U, Braunewell KH (2004) Expression analysis of members of the neuronal calcium sensor protein family: combining bioinformatics and western blot analysis. Biochem Biophys Res Commun 323(1):38–43
52. Guo W, Malin SA, Johns DC, Jeromin A, Nerbonne JM (2002) Modulation of Kv4-encoded K(+) currents in the mammalian myocardium by neuronal calcium sensor-1. J Biol Chem 277 (29):26436–26443
53. Nakamura TY, Sturm E, Pountney DJ, Orenzoff B, Artman M, Coetzee WA (2003) Developmental expression of NCS-1 (frequenin), a regulator of Kv4 K+channels, in mouse heart. Pediatr Res 53(4):554–557
54. Bélair EL, Vallée J, Robitaille R (2005) Long-term in vivo modulation of synaptic efficacy at the neuromuscular junction of *Rana pipiens* frogs. J Physiol 569(1):163–178
55. Hermainski J, Stockebrand M, Pongs O (2009). Synaptic plasticity in NCS-1 knock-out and NCS-1-EGFP overexpressing mice. 2nd European Calcium Society Workshop 2009 Annexins, targets and calcium-binding proteins in pathology June 3–6, 2009, Smolenice, Slovakia
56. Olafsson P, Wang T, Lu B (1995) Molecular cloning and functional characterization of the Xenopus Ca^{2+}-binding protein frequenin. Proc Natl Acad Sci USA 92(17):8001–8005
57. Wang CY, Yang F, He X, Chow A, Du J, Russell JT, Lu B (2001) Ca(2+) binding protein frequenin mediates GDNF-induced potentiation of Ca2+ channels and transmitter release. Neuron 32 (1):99–112
58. Mallart A, Angaut-Petit D, Bourret-Poulain C, Ferrus A (1991) Nerve terminal excitability and neuromuscular transmission in T (X;Y)V7 and Shaker mutants of *Drosophila melanogaster*. J Neurogenet 7(2–3):75–84
59. Rivosecchi R, Pongs O, Theil T, Mallart A (1994) Implication of frequenin in the facilitation of transmitter release in Drosophila. J Physiol 474(2):223–232
60. Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Annu Rev Physiol 64:355–405

61. Stewart BA, Atwood HL, Renger JJ, Wang J, Wu CF (1994) Improved stability of *Drosophila* larval neuromuscular preparations in haemolymph-like physiological solutions. Comp Physiol [A] 175(2):179–191
62. Atwood HL, Govind CK, Wu CF (1993) Differential ultrastructure of synaptic terminals on ventral longitudinal abdominal muscles in *Drosophila* larvae. J Neurobiol 24(8):1008–1024
63. Kurdyak P, Atwood HL, Stewart BA, Wu CF (1994) Differential physiology and morphology of motor axons to ventral longitudinal muscles in larval *Drosophila*. J Comp Neurol 350(3):463–472
64. Angaut-Petit D, Toth P, Rogero O, Faille L, Tejedor FJ, Ferrús A (1998) Enhanced neurotransmitter release is associated with reduction of neuronal branching in a Drosophila mutant overexpressing frequenin. Eur J Neurosci 10(2):423–434
65. McFerran BW, Graham ME, Burgoyne RD (1998) Neuronal Ca2 + sensor 1, the mammalian homologue of frequenin, is expressed in chromaffin and PC12 cells and regulates neurosecretion from dense-core granules. J Biol Chem 273(35):22768–22772
66. Weiss JL, Archer DA, Burgoyne RD (2000) Neuronal Ca2+ sensor-1/frequenin functions in an autocrine pathway regulating Ca2+ channels in bovine adrenal chromaffin cells. J Biol Chem 275(51):40082–40087
67. Weiss JL, Burgoyne RD (2001) Voltage-independent inhibition of P/Q-type Ca2+ channels in adrenal chromaffin cells via a neuronal Ca2+ sensor-1-dependent pathway involves Src family tyrosine kinase. J Biol Chem 276(48):44804–44811
68. Rousset M, Cens T, Gavarini S, Jeromin A, Charnet P (2003) Down-regulation of voltage-gated Ca2+ channels by neuronal calcium sensor-1 is beta subunit-specific. J Biol Chem 278(9):7019–7026
69. Ishikawa T, Kaneko M, Shin HS, Takahashi T (2005) Presynaptic N-type and P/Q-type Ca2+ channels mediating synaptic transmission at the calyx of Held of mice. J Physiol 568(Pt 1):199–209
70. Macleod GT, Chen L, Karunanithi S, Peloquin JB, Atwood HL, McRory JE, Zamponi GW, Charlton MP (2006) The Drosophila cacts2 mutation reduces presynaptic Ca2+ entry and defines an important element in Cav2.1 channel inactivation. Eur J Neurosci 23(12):3230–3244
71. Lautermilch NJ, Few AP, Scheuer T, Catterall WA (2005) Modulation of CaV2.1 channels by the neuronal calcium-binding protein visinin-like protein-2. J Neurosci 25(30):7062–7070
72. Bahi N, Friocourt G, Carrié A, Graham ME, Weiss JL, Chafey P, Fauchereau F, Burgoyne RD, Chelly J (2003) IL1 receptor accessory protein like, a protein involved in X-linked mental retardation, interacts with neuronal calcium sensor-1 and regulates exocytosis. Hum Mol Genet 12(12):1415–1425
73. Gambino F, Pavlowsky A, Béglé A, Dupont JL, Bahi N, Courjaret R, Gardette R, Hadjkacem H, Skala H, Poulain B, Chelly J, Vitale N, Humeau Y (2007) IL1-receptor accessory protein-like 1 (IL1RAPL1), a protein involved in cognitive functions, regulates N-type Ca2+-channel and neurite elongation. Proc Natl Acad Sci USA 104(21):9063–9068
74. Strahl T, Grafelmann B, Dannenberg J, Thorner J, Pongs O (2003) Conservation of regulatory function in calcium-binding proteins: human frequenin (neuronal calcium sensor-1) associates productively with yeast phosphatidylinositol 4-kinase isoform, Pik1. J Biol Chem 278(49):49589–49599
75. Huttner IG, Strahl T, Osawa M, King DS, Ames JB, Thorner J (2003) Molecular interactions of yeast frequenin (Frq1) with the phosphatidylinositol 4-kinase isoform, Pik1. J Biol Chem 278 (7):4862–4874
76. Strahl T, Huttner IG, Lusin JD, Osawa M, King D, Thorner J, Ames JB (2007) Structural insights into activation of phosphatidylinositol 4-kinase (pik1) by yeast frequenin (Frq1). J Biol Chem 282(42):30949–30959
77. Lim S, Strahl T, Thorner J, Ames JB (2011) Structure of a Ca^{2+}–myristoyl switch protein that controls activation of a phosphatidylinositol 4-kinase in fission yeast. J Biol Chem 286(14):12565–12577
78. Zhao X, Várnai P, Tuymetova G, Balla A, Tóth ZE, Oker-Blom C, Roder J, Jeromin A, Balla T (2001) Interaction of neuronal calcium sensor-1 (NCS-1) with phosphatidylinositol 4-kinase beta stimulates lipid kinase activity and affects membrane trafficking in COS-7 cells. J Biol Chem 276(43):40183–40189
79. Pan CY, Jeromin A, Lundstrom K, Yoo SH, Roder J, Fox AP (2002) Alterations in exocytosis induced by neuronal Ca2+ sensor-1 in bovine chromaffin cells. J Neurosci 22(7):2427–2433
80. Taverna E, Francolini M, Jeromin A, Hilfiker S, Roder J, Rosa P (2002) Neuronal calcium sensor 1 and phosphatidylinositol 4-OH kinase beta interact in neuronal cells and are translocated to membranes during nucleotide-evoked exocytosis. J Cell Sci 115 (20):3909–3922
81. Bartlett SE, Reynolds AJ, Weible M, Jeromin A, Roder J, Hendry IA (2000) PtdIns 4-kinasebeta and neuronal calcium sensor-1 co-localize but may not directly associate in mammalian neurons. J Neurosci Res 62(2):216–224
82. Scalettar BA, Rosa P, Taverna E, Francolini M, Tsuboi T, Terakawa S, Koizumi S, Roder J, Jeromin A (2002) Neuronal calcium sensor-1 binds to regulated secretory organelles and functions in basal and stimulated exocytosis in PC12 cells. J Cell Sci 115(11):2399–2412
83. Rajebhosale M, Greenwood S, Vidugiriene J, Jeromin A, Hilfiker S (2003) Phosphatidylinositol 4-OH kinase is a downstream target of neuronal calcium sensor-1 in enhancing exocytosis in neuroendocrine cells. J Biol Chem 278(8):6075–6084
84. Haynes LP, Thomas GM, Burgoyne RD (2005) Interaction of neuronal calcium sensor-1 and ADP-ribosylation factor 1 allows bidirectional control of phosphatidylinositol 4-kinase beta and trans-Golgi network-plasma membrane traffic. J Biol Chem 280 (7):6047–6054
85. Haynes LP, Sherwood MW, Dolman NJ, Burgoyne RD (2007) Specificity, promiscuity and localization of ARF protein interactions with NCS-1 and phosphatidylinositol-4 kinase-III beta. Traffic 8(8):1080–1092
86. Gromada J, Bark C, Smidt K, Efanov AM, Janson J, Mandic SA, Webb DL, Zhang W, Meister B, Jeromin A, Berggren PO (2005) Neuronal calcium sensor-1 potentiates glucose-dependent exocytosis in pancreatic beta cells through activation of phosphatidylinositol 4-kinase beta. Proc Natl Acad Sci USA 102(29):10303–10308
87. Haynes LP, Fitzgerald DJ, Wareing B, O'Callaghan DW, Morgan A, Burgoyne RD (2006) Analysis of the interacting partners of the neuronal calcium-binding proteins L-CaBP1, hippocalcin, NCS-1 and neurocalcin delta. Proteomics 6(6):1822–1832
88. Loyet KM, Kowalchyk JA, Chaudhary A, Chen J, Prestwich GD, Martin TF (1998) Specific binding of phosphatidylinositol 4,5-bisphosphate to calcium-dependent activator protein for secretion (CAPS), a potential phosphoinositide effector protein for regulated exocytosis. J Biol Chem 273(14):8337–8343
89. Fujita Y, Xu A, Xie L, Arunachalam L, Chou TC, Jiang T, Chiew SK, Kourtesis J, Wang L, Gaisano HY, Sugita S (2007) Ca2+-dependent activator protein for secretion 1 is critical for constitutive and regulated exocytosis but not for loading of transmitters into dense core vesicles. J Biol Chem 282(29):21392–21403
90. Jockusch WJ, Speidel D, Sigler A, Sørensen JB, Varoqueaux F, Rhee JS, Brose N (2007) CAPS-1 and CAPS-2 are essential synaptic vesicle priming proteins. Cell 131(4):796–808
91. Nakamura TY, Pountney DJ, Ozaita A, Nandi S, Ueda S, Rudy B, Coetzee WA (2001) A role for frequenin, a Ca2+-binding protein, as a regulator of Kv4 K+-currents. Proc Natl Acad Sci USA 98 (22):12808–12813
92. Zhang Y, MacLean JN, An WF, Lanning CC, Harris-Warrick RM (2003) KChIP1 and frequenin modify shal-evoked potassium

currents in pyloric neurons in the lobster stomatogastric ganglion. J Neurophysiol 89(4):1902–1909
93. Poulain C, Ferrús A, Mallart A (1994) Modulation of type A K^+ current in *Drosophila* larval muscle by internal Ca2+; effects of the overexpression of frequenin. Pflugers Arch 427 (1–2):71–79
94. Hui H, McHugh D, Hannan M, Zeng F, Xu SZ, Khan SU, Levenson R, Beech DJ, Weiss JL (2006) Calcium-sensing mechanism in TRPC5 channels contributing to retardation of neurite outgrowth. J Physiol 572(1):165–172
95. Hannan MA, Kabbani N, Paspalas CD, Levenson R (2008) Interaction with dopamine D2 receptor enhances expression of transient receptor potential channel 1 at the cell surface. Biochim Biophys Acta 1778(4):974–982
96. Fik-Rymarkiewicz E, Duda T, Sharma RK (2006) Novel frequenin-modulated Ca2+-signaling membrane guanylate cyclase (ROS-GC) transduction pathway in bovine hippocampus. Mol Cell Biochem 291(1–2):187–204
97. Schaad NC, De Castro E, Nef S, Hegi S, Hinrichsen R, Martone ME, Ellisman MH, Sikkink R, Rusnak F, Sygush J, Nef P (1996) Direct modulation of calmodulin targets by the neuronal calcium sensor NCS-1. Proc Natl Acad Sci USA 93(17):9253–9258
98. Souza BR, Torres KC, Miranda DM, Motta BS, Caetano FS, Rosa DV, Souza RP, Giovani A Jr, Carneiro DS, Guimarães MM, Martins-Silva C, Reis HJ, Gomez MV, Jeromin A, Romano-Silva MA. (2010). Downregulation of the cAMP/PKA pathway in PC12 cells overexpressing NCS-1. Cell Mol Neurobiol. Sep 14. [Epub ahead of print]
99. Fitzgerald DJ, Burgoyne RD, Haynes LP (2008) Neuronal calcium sensor proteins are unable to modulate NFAT activation in mammalian cells. Biochim Biophys Acta 1780(2):240–248
100. Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E, Vijayadamodar G, Pochart P, Machineni H, Welsh M, Kong Y, Zerhusen B, Malcolm R, Varrone Z, Collis A, Minto M, Burgess S, McDaniel L, Stimpson E, Spriggs F, Williams J, Neurath K, Ioime N, Agee M, Voss E, Furtak K, Renzulli R, Aanensen N, Carrolla S, Bickelhaupt E, Lazovatsky Y, DaSilva A, Zhong J, Stanyon CA, Finley RL Jr, White KP, Braverman M, Jarvie T, Gold S, Leach M, Knight J, Shimkets RA, McKenna MP, Chant J, Rothberg JM (2003) A protein interaction map of *Drosophila melanogaster*. Science 302 (5651):1727–1736
101. Genin A, Davis S, Meziane H, Doyere V, Jeromin A, Roder J, Mallet J, Laroche S (2001) Regulated expression of the neuronal calcium sensor-1 gene during long-term potentiation in the dentate gyrus in vivo. Neuroscience 106(3):571–577
102. Brackmann M, Zhao C, Kuhl D, Manahan-Vaughan D, Braunewell KH (2004) MGluRs regulate the expression of neuronal calcium sensor proteins NCS-1 and VILIP-1 and the immediate early gene arg3.1/arc in the hippocampus in vivo. Biochem Biophys Res Commun 322(3):1073–1079
103. Palmer CL, Lim W, Hastie PG, Toward M, Korolchuk VI, Burbidge SA, Banting G, Collingridge GL, Isaac JT, Henley JM (2005) Hippocalcin functions as a calcium sensor in hippocampal LTD. Neuron 47(4):487–494
104. Angaut-Petit D, Ferrús A, Faille L (1993) Plasticity of motor nerve terminals in Drosophila T (X, Y)V7 mutant: effect of deregulation of the novel calcium-binding protein frequenin. Neurosci Lett 153(2):227–231
105. Chen XL, Zhong ZG, Yokoyama S, Bark C, Meister B, Berggren PO, Roder J, Higashida H, Jeromin A (2001) Overexpression of rat neuronal calcium sensor-1 in rodent NG108-15 cells enhances synapse formation and transmission. J Physiol 532(3):649–659
106. Reynolds AJ, Bartlett SE, Morgans C (2001) The distribution of neuronal calcium sensor-1 protein in the developing and adult rat retina. Neuroreport 12(4):725–728
107. Bergmann M, Grabs D, Roder J, Rager G, Jeromin A (2002) Differential expression of neuronal calcium sensor-1 in the developing chick retina. J Comp Neurol 449(3):231–240
108. Treloar HB, Uboha U, Jeromin A, Greer CA (2005) Expression of the neuronal calcium sensor protein NCS-1 in the developing mouse olfactory pathway. J Comp Neurol 482(2):201–216
109. Kawasaki T, Nishio T, Kurosawa H, Roder J, Jeromin A (2003) Spatiotemporal distribution of neuronal calcium sensor-1 in the developing rat spinal cord. J Comp Neurol 460(4):465–475
110. Eaton BA, Fetter RD, Davis GW (2002) Dynactin is necessary for synapse stabilization. Neuron 34(5):729–741
111. Letinsky MS, Fischbeck KH, McMahan UJ (1976) Precision of reinnervation of original postsynaptic sites in frog muscle after a nerve crush. J Neurocytol 5(6):691–718
112. Wernig A, Pécot-Dechavassine M, Stover H (1980) Sprouting and regression of the nerve at the frog neuromuscular junction in normal conditions and after prolonged paralysis with curare. J Neurocytol 9(3):278–303
113. Dason JS (2009). Role of frequenin1 and frequenin2 in regulating neurotransmitter release and nerve terminal growth at the *Drosophila* neuromuscular junction. PhD thesis, University of Toronto.
114. Iketani M, Imaizumi C, Nakamura F, Jeromin A, Mikoshiba K, Goshima Y, Takei K (2009) Regulation of neurite outgrowth mediated by neuronal calcium sensor-1 and inositol 1,4,5-trisphosphate receptor in nerve growth cones. Neuroscience 161 (3):743–752
115. Koh PO, Undie AS, Kabbani N, Levenson R, Goldman-Rakic PS, Lidow MS (2003) Up-regulation of neuronal calcium sensor-1 (NCS-1) in the prefrontal cortex of schizophrenic and bipolar patients. Proc Natl Acad Sci USA 100(1):313–317
116. Bai J, He F, Novikova SI, Undie AS, Dracheva S, Haroutunian V, Lidow MS (2004) Abnormalities in the dopamine system in schizophrenia may lie in altered levels of dopamine receptor-interacting proteins. Biol Psychiatry 56(6):427–440
117. Negyessy L, Goldman-Rakic PS (2007) Subcellular localization of the dopamine D2 receptor and coexistence with the calcium-binding protein neuronal calcium sensor-1 in the primate prefrontal cortex. J Comp Neurol 488(4):464–475
118. Piton A, Michaud JL, Peng H, Aradhya S, Gauthier J, Mottron L, Champagne N, Lafrenière RG, Hamdan FF, S2D team, Joober R, Fombonne E, Marineau C, Cossette P, Dubé MP, Haghighi P, Drapeau P, Barker PA, Carbonetto S, Rouleau GA (2008) Mutations in the calcium-related gene IL1RAPL1 are associated with autism. Hum Mol Genet 17(24):3965–3974
119. Tessier CR, Broadie K (2011) The fragile X mental retardation protein developmentally regulates the strength and fidelity of calcium signaling in Drosophila mushroom body neurons. Neurobiol Dis 41(1):147–159
120. Muralidhar D, Kunjachen Jobby M, Jeromin A, Roder J, Thomas F, Sharma Y (2004) Calcium and chlorpromazine binding to the EF-hand peptides of neuronal calcium sensor-1. Peptides 25 (6):909–917
121. Souza BR, Motta BS, Rosa DV, Torres KC, Castro AA, Comim CM, Sampaio AM, Lima FF, Jeromin A, Quevedo J, Romano-Silva MA (2008) DARPP-32 and NCS-1 expression is not altered in brains of rats treated with typical or atypical antipsychotics. Neurochem Res 33(3):533–538
122. Boehmerle W, Splittgerber U, Lazarus MB, McKenzie KM, Johnston DG, Austin DJ, Ehrlich BE (2006) Paclitaxel induces calcium oscillations via an inositol 1,4,5-trisphosphate receptor

and neuronal calcium sensor 1-dependent mechanism. Proc Natl Acad Sci USA 103(48):18356–18361

123. Boehmerle W, Zhang K, Sivula M, Heidrich FM, Lee Y, Jordt SE, Ehrlich BE (2007) Chronic exposure to paclitaxel diminishes phosphoinositide signaling by calpain-mediated neuronal calcium sensor-1 degradation. Proc Natl Acad Sci USA 104(26):11103–11108
124. Blachford C, Celić A, Petri ET, Ehrlich BE (2009) Discrete proteolysis of neuronal calcium sensor-1 (NCS-1) by mu-calpain disrupts calcium binding. Cell Calcium 46(4):257–262
125. Benbow JH, Degray B, Ehrlich BE. (2011). Protection of neuronal calcium sensor 1 in cells treated with Taxol. J Biol Chem. 2011 Aug 1.