Review

The multiple faces of calcineurin signaling in *Caenorhabditis elegans*: Development, behaviour and aging

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Calcineurin, a well-conserved protein phosphatase 2B (PP2B), is a Ca^{2+}-calmodulin–dependent serine/threonine protein phosphatase that is known to be involved in a myriad of cellular processes and signal transduction pathways. The biological role of calcineurin has been extensively studied in diverse groups of organisms. Homologues of mammalian and *Drosophila* calcineurin subunits exist in the nematode, *Caenorhabditis elegans*. The *C. elegans* counterpart of the catalytic subunit, calcineurin A, *cna-1*/*tax-6*, and the regulatory subunit, calcineurin B, *cnb-1*, are known to express ubiquitously in multiple tissues including neurons. The characterization of *C. elegans* calcineurin mutants facilitates identification of its physiological functions and signaling pathways. Genetic interactions between *cna-1*/*tax-6* and *cnb-1* mutants with a number of mutants involved in several signaling pathways have exemplified the pivotal role of calcineurin in regulating nematode development, behaviour and lifespan (aging). The present review has been aimed to provide a succinct summary of the multiple functions of calcineurin in *C. elegans* relating to its development, fertility, proliferation, behaviour and lifespan. Analyses of *cna-1*/*tax-6* and *cnb-1* interacting proteins and regulators of the phosphatase in this fascinating worm model have an immense scope to identify potential drug targets in various parasitic nematodes, which cause many diseases inflicting huge economic loss; and also for many human diseases, particularly neurodegenerative and myocardial diseases.

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1. Introduction

Calcium functions in a multitude of cellular processes as a second messenger and is widely employed by all eukaryotic organisms to regulate gene expression (Clapham 1995). Ca^{2+} mediates intracellular signaling predominantly through the activity of Ca^{2+}-dependent protein kinases and phosphatases (Hunter 1995). One of the well-characterized effector molecules in the eukaryotic Ca^{2+}-regulated pathways is the serine/threonine-specific protein phosphatase, calcineurin. Also called protein phosphatase 2B (PP2B), calcineurin was first detected as a column fraction that inhibited the calmodulin (CaM)-dependent cyclic nucleotide phosphodiesterase (Wang and Desai 1976). It is known to have diverse cellular functions in different cell types and organisms (Klee *et al*. 1979, 1998; Stewart *et al*. 1982; Crabtree 1999). During the last three decades since isolation of this protein (Klee and Krinks 1978), the biological role of calcineurin has progressed from a putative inhibitor of the CaM-dependent phosphodiesterase to the revolutionary discovery that it is the target of immunosuppressant drugs, cyclosporin A (CsA) and FK506, pharmacological reagents that have been used to demonstrate it as a

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major player in Ca^{2+}-dependent eukaryotic signal transduction pathways (Liu *et al*. 1991).

Caenorhabditis elegans is a free-living nematode of ~1 mm length with a short generation cycle (~3 days) and lifespan (~3 weeks), and a transparent body that allows for the visualization of all cell types at all stages of development (Brenner 1974).

C. elegans has ~60–80% of human genes, ~40% of human disease-related genes and conserved neurotransmitter systems (Martinez-Finley *et al*. 2011). Therefore, over the years, it has emerged as an attractive model for investigating the basic biological processes as well as exploring the underlying mechanisms of various human diseases and drug screening.

In addition, *C. elegans* is a useful platform for the study of closely related parasitic nematodes and might lead to discovery of new methods to combat the debilitating effects of these pests.

2. Calcineurin-structural form and function

The biological functions of calcineurin have been extensively studied in the yeast, *Saccharomyces cerevisiae*, and also described in various other organisms including *Schizosaccharomyces pombe*, *Neurospora crassa*, *Aspergillus nidulans*, *Cryptococcus neoformans*, *Dictyostelium discoideum*, several human pathogens, plants and mammals (reviewed in Rusnak and Mertz 2000). Initially, calcineurin was found to be abundantly expressed in the nervous system (Goto *et al*. 1985; Kuno *et al*. 1992). In fact, the nomenclature of the protein itself signified its Ca^{2+}-binding properties and localization in the neuronal tissue (Klee *et al*. 1979). However, it was subsequently shown to be broadly distributed in non-neural tissues as well (Kincaid 1993).

From a structural point of view, calcineurin is a heterodimeric protein comprising a ~60 kDa catalytic subunit, calcineurin A (CnA), and a 19 kDa regulatory subunit, calcineurin B (CnB) (Klee *et al*. 1998). Homologues of mammalian and *Drosophila* calcineurin subunits exist in *C. elegans*, and represent a conserved branch of the PP2B family of protein phosphatases having important roles in normal physiology (Crabtree 2001). The *C. elegans* homologues of CnA and CnB have been annotated to *cna-1*/*tax-6* and *cnb-1*, respectively, and *in vivo* physiological functions of both the genes have been intensively described (Bandyopadhyay *et al*. 2004). The nematode CnA, encoded by a single *cna-1/tax-6* gene, possesses 77% overall amino acid identity with a human CnA isoform, whereas CnB is a small protein of 171 amino acids exhibiting 80% amino acid identity with human and *Drosophila* CnB and 58% with that of the yeast protein. CnB, encoded by the *cnb-1* locus, is characterized by four 'EF-hands' for Ca^{2+}-binding. Calcineurin is extensively expressed in *C. elegans* neurons as well as other tissues, including hypodermal seam cells, body-wall muscle, vulva muscle, sperm and spermatheca (Bandyopadhyay *et al*. 2002; Kuhara *et al*. 2002; Lee *et al*. 2004). Further, yeast two-hybrid screening has confirmed the interaction of the two calcineurin subunits in the presence of Ca^{2+}. While calcineurin subunits are encoded by multiple genes in vertebrates and *Drosophila*, the *C. elegans* genome contains a single gene encoding the catalytic subunit of calcineurin that was physically and genetically mapped to the gene cluster region of chromosome IV (Kuhara *et al*. 2002). CnA contains a catalytic phosphatase domain and a regulatory domain which, in turn, is composed of a CnB binding domain, a CaM-binding domain and a downstream autoinhibitory (AI) domain (figure 1). In the absence of Ca^{2+}/CaM, the AI domain occupies the active site, thereby inhibiting enzyme activity. However, upon binding of Ca^{2+}/CaM, the ensuing conformational changes displace the AI domain from the active site, leading to activation of the CnA phosphatase (Bandyopadhyay *et al*. 2004).

Research over the past years has implicated calcineurin in a variety of developmental and cellular processes in different organisms as well as in different cell types, viz. T-cell activation, skeletal and cardiac muscle growth and differentiation, memory and apoptosis (Kingsbury and Cunningham 2000). These signal transduction pathways mostly involve nuclear translocation and activation of the nuclear factor of activated T cells (NFAT) following its dephosphorylation by calcineurin in response to increased intracellular Ca^{2+} concentration, the mechanism being characterized initially in the T lymphocytes (Mattila *et al*. 1990; Flanagan *et al*. 1991). In fact, the regulation of cardiomyocyte maturation and hypertrophy, heart valvuloseptal development, early embryonic vascular development, skeletal muscle hypertrophy and fibre-type specialization by calcineurin-NFAT is well elucidated (Schulz and Yutzey 2004). Four nfatc genes, *nfatc1-c4*, have been identified (Rao *et al*. 1997). Thus, the central role executed by calcineurin-NFAT signaling in normal development, homeostasis and various pathological conditions in skin, cardiovascular system, skeletal muscle, immune system and central nervous system is clear. Nevertheless, in the context of *C. elegans*, the fact that *nfatc* homologues are absent in the genome of nematodes (Graef *et al*. 2001) is rather intriguing and the effectors of calcineurin signaling still remain elusive. Future experiments should be

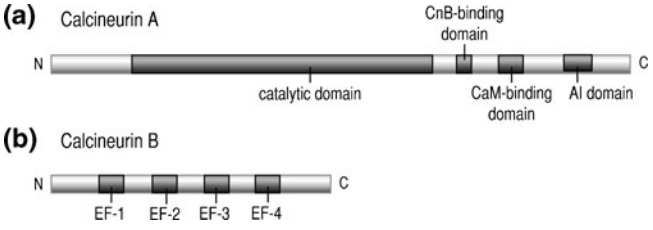

Figure 1. Structure of calcineurin subunits, (**a**) calcineurin A, the catalytic subunit having different domains viz. catalytic phosphatase domain, CnB-binding domain, a calmodulin (CaM)-binding domain and auto-inhibitory (AI) domain, (**b**) calcineurin B, the regulatory subunit having four Ca^{2+}-binding EF-hand motifs. (Adapted from Bandyopadhyay *et al*. 2004).

directed towards this aspect considering the multiple roles of calcineurin signaling in *C. elegans* pertaining to its development, behaviour and aging, which have been primarily established from mutant studies.

3. Role of calcineurin in *C. elegans* development

C. elegans completes its reproductive life cycle in 2.5 days at 25°C while progressing from a fertilized embryo through four larval stages to become an egg-laying adult. Under adverse conditions, such as starvation, over-crowding or high temperature, the larvae can enter an alternative life stage called the dauer (enduring) larva, during which animals move but do not feed (Samara and Tavernarakis 2003). When a dauer larva encounters favourable environmental conditions, it re-enters the life cycle at the fourth larval stage, progresses into adulthood to reproduce and then completes the final week or so of its lifespan. The developmental profile of *C. elegans* is exceptionally well characterized. The complete sequence of cell divisions that occur as the fertilized egg develops into the 959-cell adult worm has been recorded (Sulston and Horvitz 1977; Sulston *et al*. 1983).

The first report associating calcineurin with *C. elegans* development came in 2002 when it was observed that *C. elegans* animals bearing a loss-of-function mutation in TAX-6, a CnA subunit, exhibited pleiotropic abnormalities in body size, thermotaxis, growth and sensory behaviour (Kuhara *et al*. 2002). This was immediately followed by identification and detailed characterization of calcineurin genes in *C. elegans* (*cna-1* and *cnb-1*), and detection of both *cna-1* and *cnb-1* reporter transgenes in diverse tissues of the nematode along with their expression at all stages of development starting from the early comma stage embryos to the adult stages (Bandyopadhyay *et al*. 2002). Northern analysis revealed a single transcript of *tax-6* of approximately 3.2 kb at all stages of worm development. Mutants of *cnb-1* had cuticle defects, small body size, decreased brood size and egg-laying abnormalities, which appeared very similar to and typical of those observed in an *unc-43(gf)* encoding CaMKII protein kinase gain-of-function mutation.

3.1 *Egg-laying*

The hermaphrodite wild-type *C. elegans* normally lays around 300 eggs through the vulva over its lifetime. This is a well-regulated process that is affected by environmental sensory cues such as touch, food, and salts, and involves the coordination of 16 vulva muscles (Schafer 2006). Several exogenous agents are known to stimulate egg-laying in wild-type worms, viz. serotonin and imipramine (Trent *et al*. 1983). Levamisole, an agonist of the UNC-29 nicotinic acetylcholine receptor localized in the post-synaptic muscle, induces muscle hyper-contraction and subsequent egg-laying in wild-type *C. elegans* (Kim *et al*. 2001).

Serotonin is a known modulator of calcineurin signaling in cardiac growth and gene expression (Bush *et al*. 2004). The fact that calcineurin is involved in serotonin-regulated worm egg-laying is reinforced by our observation that while exogenous treatment of serotonin could stimulate egg-laying in wild-type worms, the *cnb-1(null)* mutants failed to respond to serotonin (Bandyopadhyay *et al*. 2002). The egg-laying phenotype of *cnb-1* was almost identical to those of *unc-43(gf)* with delayed egg-laying and resistance to serotonin (table 1). Conversely, *unc-43(null)* showed opposite phenotypes, such as hyperactive egg-laying and hypersensitivity to serotonin. These data clearly indicated an antagonistic relationship between CaMKII and calcineurin.

It has been shown that UNC-43 can regulate two Gα proteins, EGL-30 and GOA-1, that have opposing functions in egg-laying (Robatzek and Thomas 2000). EGL-30, a Gq-protein α-subunit, activated egg-laying behaviour via serotonin-dependent signaling in pre-synaptic neurons and post-synaptic muscles (Bastiani *et al*. 2003). In contrast, EGL-10 inhibited $G_o\alpha$ signaling in *C. elegans*, which, in turn, inhibited egg-laying (Koelle and Horvitz 1996). Both double mutants of *egl-30(ad805);tax-6(jh107)* and *egl-10(md176); tax-6(jh107)* exhibited severe egg retention, indicating that *egl-30* and *egl-10* are epistatic to *tax-6* (Lee *et al*. 2004). Double mutants of *egl-30(ad805);cnb-1(jh103)* displayed severe phenotypes likely due to synergistic effects. Therefore, it is suggested that calcineurin probably regulates egg-laying through EGL-30 and EGL-10 in *C. elegans*. It is imperative to mention here that G-proteins and serotonin modulate the functional states of neurons and muscles controlling *C. elegans* egg-laying behaviour (Shyn *et al*. 2003). Further, whereas *cnb-1(jh103)* null mutants showed strong serotonin resistance, a calcineurin gain-of-function mutant *cna-1(jh107)* displayed hypersensitivity to serotonin (Lee *et al*. 2003). Treatment of *cna-1(jh107)* worms with serotonin resulted in laying of almost all internal embryos, thus reaffirming the role of calcineurin phosphatase activity in worm egg-laying.

Earlier, a transgenic gain-of-function mutant of G_0-protein α-subunit, *goa-1*, showed egg retention phenotypes similar to *unc-43(gf)* mutants, while loss-of-function mutants of *unc-43(lf)* and *goa-1(lf)* displayed premature egg-laying (Mendel *et al*. 1995). Several mutant studies reiterated the involvement of calcineurin in similar aspects of *C. elegans* egg-laying as *unc-43* and *goa-1* (Bandyopadhyay *et al*. 2002; Lee *et al*. 2004). In brief, the *cnb-1* mutants exhibited defects in serotonin-induced egg-laying similar to those seen in *unc-43(gf)* and *goa-1(gf)*. The *cnb-1* and *unc-43(gf)* mutants also showed decreased sensitivity to imipramine compared with wild-type animals. In addition, the *cnb-1* mutants and the *unc-43(gf)*, *unc-43(lf)* and *goa-1(lf)* mutants were resistant to levamisole at all concentrations. It is interesting to note that the normal role of calcineurin is to negatively regulate GOA-1 since proteomic analysis revealed that

Table 1. Calcineurin-mediated regulation of worm brood size and egg-laying

Strains	Brood size (self progeny at 20°C)	Late stage embryos in uterus < comma[a]	Late stage embryos in uterus ≥ comma[b]
N2 (WT)	270±45 (10)	13±1.8	0 (17)
cnb-1	106±17 (15)	6±0.9	2±0.9 (20)
cnb-1;Ex[cnb-1]	146±27 (8)	13±3.3	0 (17)
sma-6	123±17 (10)	10±2.1	0 (20)
unc-43(gf)	61±12 (15)	10±1.7	12±1.9 (19)
unc-43(null)	173±29 (15)	6±2.8	0 (19)
unc-43(gf);cnb-1	8±7 (16)	4±1.3	3±1.6 (16)

Values are expressed as means ± SD; number in parentheses indicates sample sizes for worms.

[a] Number of embryos prior to comma stage inside a 1-d-old adult.

[b] Number of embryos from comma stage onwards (comma–3-fold stage) inside a 1-d-old adult. (Adapted from Bandyopadhyay *et al.* 2002).

GOA-1 was up-regulated more than two-fold in *cnb-1*-null mutants (Ahn *et al.* 2006). Therefore, it seems that calcineurin-mediated facilitation of worm egg-laying is achieved by down-regulating the opposing signal component, GOA-1.

Considering the expression of calcineurin in motor neurons and vulval muscles (Bandyopadhyay *et al.* 2002), and the fact that calcineurin mutants exhibited defects in serotonin-mediated egg-laying alike *egl-30* and *goa-1* mutants (Lee *et al.* 2004), it can be interpreted that calcineurin regulates worm egg-laying in a serotonin-dependent manner and has an opposing function to CaMKII that is mediated by G-protein signaling.

3.2 *Fertility*

The *cnb-1* mutants had significantly decreased brood size and defective sperm compared with wild-type (Bandyopadhyay *et al.* 2002). The defective sperm were smaller and smoother than wild-type sperm and displayed smaller pseudopods. Moreover, sperm, which normally gather in high numbers in the spermatheca, were scarcely found in the mutant worms. The *cnb-1*-deficient spermatheca was filled with oocyte debris, indicative of a possible endomitotic oocyte (*emo*)-like defect caused by spermatheca defects. To distinguish whether the small brood size phenotype observed in the mutant was a direct cause of defective sperm or spermatheca defects, we mated N2 male worms with *cnb*-1 hermaphrodites. The wild-type sperm only partially rescued the low brood size, and the percentage of outcrossed progeny of *cnb-1* hermaphrodites was almost the same as outcrossed of N2 hermaphrodites. This suggested that altered sperm alone cannot account for decreased fertility in *cnb-1* mutants, and that defective spermatheca and/or oocytes might also play a role in this phenotype. This is corroborated by the observation that calcineurin expresses in the spermatheca and sperm, and loss of calcineurin function resulted in burst oocytes derived from defects in spermatheca (Bandyopadhyay *et al.* 2002).

It was also observed that while wild-type worms had longer gonads with abundant mitotic germ cells, the *tax-6(jh107)* and *cnb-1(jh103)* mutants had shorter gonads with fewer mitotic germ cells (Ahn *et al.* 2006). The smaller brood size in calcineurin mutants was ascribed to decreased germ cells. In addition, the mutants showed low-penetrant embryonic lethality along with a marginal number of mutant progeny failing to complete the full developmental process. These data clearly assert the significant role of calcineurin in maintaining *C. elegans* fertility.

3.3 *Growth*

In multicellular organisms, the regulation of cell size is intimately linked to nutrient and growth factor availability and depends upon a well-controlled balance between macromolecule synthesis and degradation (Leevers and McNeill 2005). Several mutations in *C. elegans* result in abnormal body size, such as mutations that affect cuticle collagen and cause Dumpy (Dpy) phenotypes (Kramer 1997). *C. elegans* body length and cell size are determined by many different genetic pathways, including the TGF-β, insulin/IGF-1, spectrin and calcineurin (Morck and Pilon 2006). The importance of the calcineurin pathways in regulating worm body size is exemplified by the fact that both *tax-6* and *cnb-1* are expressed in many sensory neurons and most of the muscle cells, and the expression of *tax-6* specifically in neurons could rescue the small body phenotype in the *tax-6* mutant (Bandyopadhyay *et al.* 2002; Kuhara *et al.* 2002).

Besides body length, calcineurin also influences cuticle formation in the worms. The cuticle plays an important role in maintaining the overall morphology and also protects

from environmental adversities. The nematode undergoes a specialized moulting process and forms a new cuticle structure at the end of each developmental stage with distinct structural and biochemical characteristics. Several mutations have been identified that lead to various phenotypes viz. blister (Bli), dumpy (Dpy), long (Lon), and roller (Rol) involving cuticular defects (Kusch and Edgar 1986). Cuticle collagens, which are synthesized and secreted by the hypodermis, form a major component of nematode cuticle. Both CnA and CnB are expressed in hypodermal tissues, specifically in hypodermal seam cells that are required for cuticle formation (Bandyopadhyay *et al*. 2002). Alteration in cuticular structure was observed in calcineurin loss-of-function mutant worms, which appeared more transparent, compared to wild-type (Lee *et al*. 2004). Consistent with cuticle defects, *cnb-1(jh103)* and *tax-6(jh107)* mutants had small body size. In this context, it is notable that the largest, syncytial hypodermal cell, hyp7 has a major role in determining final body size (Flemming *et al*. 2000). Proteomic analysis revealed two proteins, encoded by the genes F01F1.12 and *acn-1*, specifically expressed in hyp7 hypodermal seam cell to be down-regulated in *tax-6(jh107)* mutants (Ahn *et al*. 2006). Earlier, RNAi of *acn-1* was shown to cause cuticle defects (Brooks *et al*. 2003). Possibly then, TAX-6-mediated maintenance and/or up-regulation of *acn-1* could be essential for proper cuticle formation. Moreover, mutations in *dpy* genes and *sqt* genes encoding cuticle collagens were reported to change nematode body size and shape; mutants of *dpy* and *sqt* genes exhibited short and fat bodies compared to wild-type animals (Kramer *et al*. 1988; Johnstone *et al*. 1992). Double mutants of *cnb-1;dpy-5*, *sma-6;cnb-1*, *sma-4;cnb-1* and *lon-2;cnb-1* were arrested at early larval stages (Lee *et al*. 2004). These data clearly implicate calcineurin in the regulation of body size via cuticle formation in *C. elegans*.

Another aspect of regulation of nematode body size includes autophagy, a highly regulated cellular pathway used by eukaryotic cells to degrade parts of their contents during development and to survive nutrient deprivation (Klionsky and Emr 2000). During development, these nematodes enter into a state of diapause called dauer, which is an arrested larval form specialized to survive unfavourable conditions. Dauer development in insulin/IGF-1 and TGF-β-signaling mutant nematodes requires the function of autophagy genes, and normal dauer morphogenesis is associated with increased autophagy (Melendez *et al*. 2003). Recently, it was reported that autophagy genes *unc-51* and *bec-1* are required for normal cell size in *C. elegans* (Aladzsity *et al*. 2007). In wild-type worms under normal conditions, calcineurin contributes to maintain the normal level of autophagy (Dwivedi *et al*. 2009). Therefore, it is apparent that there is an intricate relationship between calcineurin signaling and regulation of body size and development in *C. elegans*.

4. Role of calcineurin in *C. elegans* behaviour

Behaviour implies the ability of an organism to sense the ever-changing environment and then respond appropriately and quickly to these surrounding cues. The brain orchestrates the molecular circuitry involved in behaviour in an efficient and flexible manner. It is well known that at the neuronal level, phosphorylation and dephosphorylation of key molecules play an enormous role in the modulation of plastic behaviours. Several kinases such as protein kinase A, protein kinase C and CaMKII are critically involved in regulating animal behaviour (Milner *et al*. 1998; Shobe 2002). Calcineurin, the only Ca^{2+}-dependent phosphatase known to be expressed in the mammalian brain (Baumgartel and Mansuy 2012), seems to be the ideal candidate to play adversary to these kinases during behavioural signaling. Since the enzymatic activity of calcineurin is controlled by Ca^{2+} levels, dephosphorylation can be directly controlled by neuronal activity. The ability to modulate the phosphorylation status of key proteins involved in plastic behaviours is a huge advantage for organisms that constantly need to adjust their behaviours according to the environmental conditions.

A role for calcineurin in behaviour was first discovered in mice relating to learning and memory. When calcineurin activity was increased by genetic manipulation in the mouse brain, long-term potentiation in the hippocampus was altered, resulting in disruption of behaviours dependent on long-term memory (Mansuy *et al*. 1998; Winder *et al*. 1998). Quite akin to mammals, calcineurin unleashes a far-reaching impact on *C. elegans* behaviour as well. The *tax-6* mutants were first identified in a screen for thermotaxis defective animals (Hedgecock and Russell 1975), and later, the *tax-6* locus was mapped to the *cna-1* gene (Kuhara *et al*. 2002). Subsequently, roles for calcineurin were identified in many worm behaviours (table 2) including movement, osmotic avoidance, salt chemotaxis, defecation, quiescence and carbon dioxide avoidance, most of which will be discussed here.

4.1 *Locomotion/movement*

Previous reports had shown that *cnb-1(lf)* mutants were uncoordinated and exhibited defective movement (Bandyopadhyay *et al*. 2002), whereas *tax-6(gf)* mutants had hyperactive movement (Lee *et al*. 2004). A comparison of locomotory phenotypes of various mutants revealed that while wild-type worms move rapidly in a sinusoidal pattern, *cnb-1(null)* mutants showed severely uncoordinated movement. In contrast, *tax-6(gf)* worms displayed slightly hyperactive movement with more frequent tracks and increased amplitudes, phenotypes opposite to those of *cnb-1(null)* mutants. A transgenic gain-of-function mutant of G_0-protein

Table 2. Calcineurin-mediated regulation of several worm behaviours

Behaviour	Site of action	Calcineurin mutant or RNAi phenotype	Reference
Thermotaxis	AFD	(lf) - thermophilic, (gf) – cryophilic	Kuhara *et al*. 2002
Thermotaxis-associative learning	AIY, AIZ	(lf) - unable to associate feeding state with temperature, (gf) - loss of cryophilic behaviour	Kuhara and Mori 2006
Carbon dioxide avoidance	URX, BAG	(lf) - carbon dioxide avoidance defective	Hallem and Sternberg 2008
Swimming/behavioural quiescence	sensory neurons	(lf) - longer behavioural quiescence	Ghosh and Emmons 2010
Defecation	enteric muscle	(gf) - defective enteric muscle contraction	Lee *et al*. 2005
Movement	neurons and muscle	(lf) and RNAi - increased sensitivity to nicotineinduced paralysis, (gf) - decreased sensitivity to nicotineinduced paralysis	Gottschalk *et al*. 2005
Odour adaptation	AWC	(lf) - hyper-adaptation to AWC-sensed odorants	Kuhara *et al*. 2002
Osmotic avoidance	ASH?	(lf) - osmotic avoidance defective	Kuhara *et al*. 2002
Salt chemotaxis	ASE?	(lf) - defective for low concentrations of NaCl	Hukema *et al*. 2006
Starvation-regulated pharyngeal pumping	NSM?	(lf) - pharyngeal pumping normal after starvation	Donohoe *et al*. 2009
Serotonin-mediated egg-laying	vulva muscle?	(lf) - decreased serotoninmediated egg laying, (gf) - increased serotoninmediated egg laying	Bandyopadhyay *et al*. 2002; Lee *et al*. 2004
Lysine attraction	ASK?	(gf) - lysine avoidance	Jee *et al*. 2012

α-subunit, *goa-1*, had lethargic movement similar to *unc-43(gf)* mutants, while loss-of-function mutants of *unc-43(lf)* and *goa-1(lf)* displayed hyperactive movement (Mendel *et al*. 1995). Thus, the observation that *cnb-1* mutants exhibited defective locomotion similar to those seen in *unc-43(gf)* and *goa-1(gf)*, provides credence to the fact that, as in egg-laying, G-protein-coupled phosphorylation pathways also mediate the effect of calcineurin upon *C. elegans* locomotion. Thus, calcineurin regulates locomotion and egg-laying activity via GOA-1(Goα)/EGL-30(Gqα) by counteracting unc-43/CaMKII (figure 2).

Motor coordination in *C. elegans* requires nicotinic acetylcholine receptors (nAChR) to mediate fast excitatory neurotransmission in motor neurons and muscles similar to other organisms. To identify proteins that interact with and may regulate nAChR function, tandem affinity purification of the levamisole nAChR receptor followed by mass spectrometry analysis of associated proteins was performed (Gottschalk *et al*. 2005). One of the more abundant proteins identified in this study was TAX-6. In order to investigate whether calcineurin affected movement through the levamisole nAChR receptor, the authors performed RNAi against *tax-6*, and subjected the animals to nicotine-sensitivity assay. Nicotine, an agonist of nAChRs, causes slow paralysis in wild-type worms, but either RNAi of *tax-6* or loss-of-function mutants of *tax-6* or *cnb-1* caused an increased sensitivity to nicotine and fast paralysis. On the other hand, *tax-6(gf)* mutant displayed decreased sensitivity to nicotine. Rescue analysis showed that calcineurin might be functioning both in neurons and muscle. This study highlighted the role of calcineurin in negative regulation of nicotine sensitivity of acetylcholine receptor possibly through a direct interaction with the receptor.

4.2 *Thermotaxis*

Worms can sense and are affected by temperature. When *C. elegans* are placed on a thermal gradient, they seek the temperature that they were grown at and continue to move along that specific temperature (Hedgecock and Russell 1975). Calcineurin mutants are defective for thermotaxis behaviour and, in fact, display a thermophilic behaviour in which they continually seek temperatures that are warmer than the one at which they were cultivated (Kuhara *et al*. 2002). Thermotaxis is a plastic behaviour. Worms seek their cultivation temperature if food is available at that temperature, but they avoid their cultivation temperature if they subsequently experience starvation conditions at that temperature. A circuitry for this feeding state-dependent thermotactic plasticity was identified involving the AFD sensory neuron along with AIZ and RIY interneurons, which are known to affect cryophilic behaviour (Kuhara and Mori 2006). It is notable that TAX-6 is expressed in the AFD thermosensory neuron where it negatively regulates AFD neuronal activity. Calcineurin is also important for associative learning. When TAX-6 is not functional in the AIZ and RIY interneurons, worms cannot learn to associate feeding

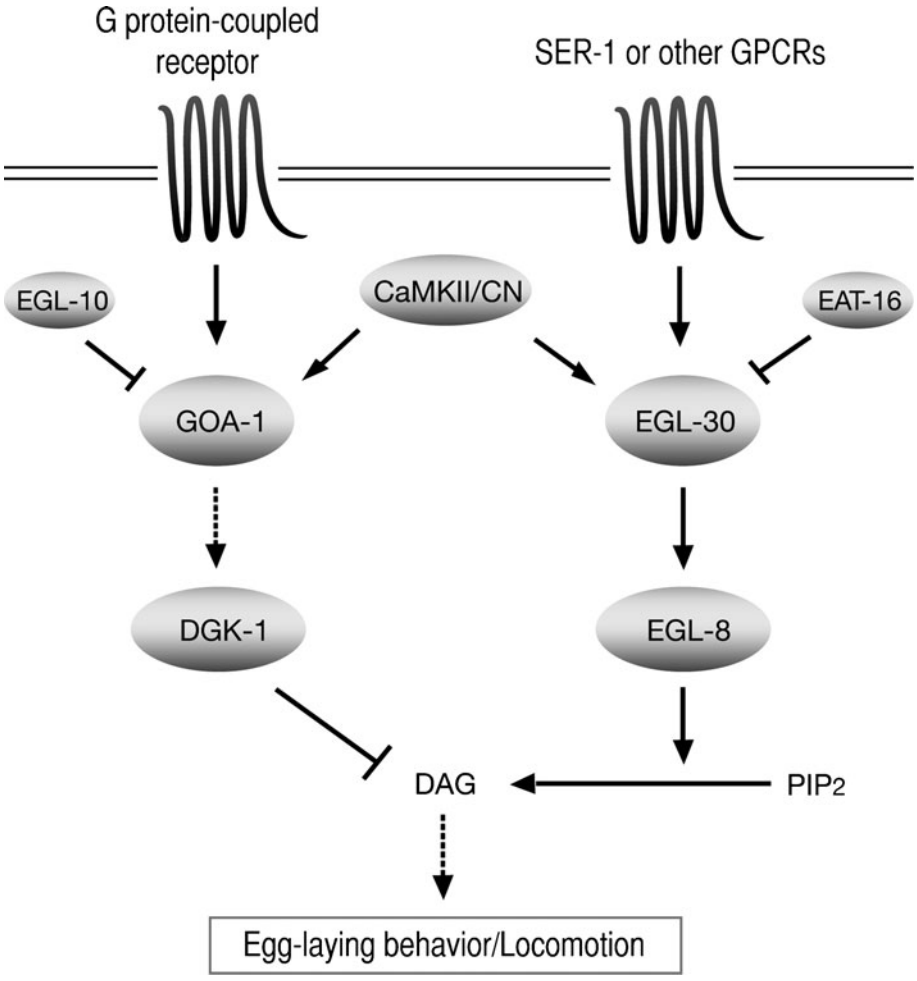

Figure 2. A model for calcineurin-mediated regulation of locomotion and egg-laying behaviour in *C. elegans*. GOA-1(Goα) and EGL-30(Gqα) are coupled to serotonin receptors and other G-protein-coupled receptors in the plasma membrane and both are regulated by RGS proteins (EGL-10 and EAT-16). Locomotion and egg-laying behaviour are activated through the EGL-30(Gqα) pathway. On the other hand, GOA-1(Goα) inhibits locomotion and egg-laying. Thus, calcineurin (CN) regulates locomotion and egg-laying activity *via* GOA-1(Goα)/EGL-30(Gqα) by counteracting *unc-43*/CaMKII in *C. elegans*. (Adapted from Bandyopadhyay *et al*. 2004).

state with temperature. On the other hand, increasing calcineurin activity in either of these neurons affects cryophilic behaviour. Possibly then, calcineurin negatively regulates the activities of these cryophilic interneurons. Using a genetically encoded Ca^{2+} indicator, the authors showed that AIZ neurons were activated with increasing temperature, and this activity was down-regulated by starvation. On the other hand, such an effect of starvation was not seen in calcineurin mutants. Therefore, it is logical to conclude that starvation down-regulates AIZ neuronal activity through TAX-6 calcineurin during feeding state-dependent thermotactic plasticity.

4.3 *Carbon dioxide avoidance*

The sensing of gases is highly important for animals as CO_2 and O_2 can serve as cues for finding food and avoiding predators or other environmental stress. *C. elegans* uses a pair of sensory neurons, URX and BAG neurons, to sense and move toward a preferred concentration of O_2 (Gray *et al*. 2004; Cheung *et al*. 2005). *C. elegans* can also sense and avoid CO_2 via BAG sensory neurons (Bretscher *et al*. 2008; Hallem and Sternberg 2008), which requires cGMP signaling and G-protein signaling (Hallem *et al*. 2011). The involvement of Ca^{2+} signaling through calcineurin is documented by *tax-6* mutants and *cnb-1* mutants that are both defective for CO_2 avoidance (Hallem and Sternberg 2008).

4.4 *Swimming behaviour and behavioural quiescence*

Behavioural quiescence, the intermittent ceasing of behaviour, at first may seem to be a random break of activity for the animal. This actually is a regulated event most notably

observed during lethargy, which occurs between each of the larval moults during *C. elegans* development. cGMP signaling regulates behavioural quiescence during lethargus. Mutations in EGL-4, the *C. elegans* cGMP-dependent protein kinase, modify the length and timing of lethargus during molts. Behavioural quiescence also occurs during swimming activity (Raizen *et al*. 2008). Although *C. elegans* do not show periods of inactivity during normal movement on solid agar media, over long periods of time worms periodically pause during swimming in liquid media (Ghosh and Emmons 2008). As in lethargus, EGL-4 is required for behavioural quiescence during swimming (Ghosh and Emmons 2010), and calcineurin plays a direct role in this behaviour. Whereas EGL-4 promotes behavioural quiescence, TAX-6 and CNB-1 both promote swimming behaviour. Behavioural quiescence is lengthened in *tax-6* and *cnb-1* mutants, particularly in the first 90 minutes of swimming behaviour. Rescue analysis showed that calcineurin expression in sensory neurons promotes swimming behaviour, whereas expression in muscle could not restore quiescent behaviour defects in calcineurin mutants.

4.5 *Defecation*

Calcineurin is involved in another behaviour that is characterized as a periodic behaviour in *C. elegans*. Defecation in worms is a cyclical behaviour that occurs every 50 s. The defecation cycle consists of three sequential muscle contractions: a posterior muscle contraction followed by an anterior contraction, and ending with an enteric muscle contraction that expels the intestinal contents. The neurotransmitter GABA is synthesized in the AVL and DVB motor neurons (Jin *et al*. 1994). It can activate enteric muscle contraction through the excitatory GABA receptor EXP-1, which is expressed in enteric muscle, and loss-of-function mutants of *exp-1* are defective for enteric muscle contraction (Beg and Jorgensen 2003). TAX-6 is also expressed in enteric muscle and binds directly to EXP-1 (Lee *et al*. 2005). This is consistent with studies in mouse brain, where calcineurin A was shown to bind GABA receptors during long-term depression in CA1 hippocampal neurons (Wang *et al*. 2003). In addition, a gain-of-function mutant of *tax-6* is defective for enteric muscle contraction similar to *exp-1* mutants (Lee *et al*. 2005). Finally, genetic epistasis analysis revealed that TAX-6 might negatively regulate the EXP-1 receptor. Thus, TAX-6 binds to EXP-1 to regulate the excitatory GABA receptor and inhibit enteric muscle contraction.

4.6 *Other behaviours*

C. elegans calcineurin, unlike mammalian calcineurin, is not required developmentally for the survival of the animal. This makes the worm very useful for identifying the wide breadth of behaviors that the Ca^{2+}-dependent phosphatase is involved in. Along with all the behaviors discussed above, calcineurin is involved in several other behaviors (table 2) including osmotic avoidance (Kuhara *et al*. 2002), salt chemotaxis (Kuhara *et al*. 2002; Hukema *et al*. 2006), pharyngeal pumping during starvation (Donohoe *et al*. 2009), serotonin-mediated egg-laying (Bandyopadhyay *et al*. 2002; Lee *et al*. 2003, 2004), lysine avoidance (Jee *et al*. 2012), olfactory adaptation (Kuhara *et al*. 2002) and male mating behaviour (Hu *et al*. 2006). The involvement of calcineurin in such a wide range of behaviours might be attributed to its unique biochemical function as one of the only Ca^{2+}-dependent phosphatases in the genome.

5. Role of calcineurin in *C. elegans* aging

Aging is a complex multi-factorial process involving natural decline in the fitness of an organism over time due to progressive accumulation of unrepaired cellular damage, primarily oxidized, misfolded, cross-linked or aggregated macromolecules with abnormal structure and function (Hekimi and Guarente 2003). Cellular damage is generated continuously during the entire life of an organism. For example, ionizing radiation of biological molecules, endogenous factors including specific enzymes, and mitochondria produce reactive oxygen species, which in turn oxidize macromolecules, leading to their abnormal function (Scherz-Shouval and Elazar 2007). In this manner, life-long accumulation of aberrant cellular constituents hampers the ability of the organism to survive. Certain conserved signaling pathways and regulatory proteins are implicated in the processes of aging, cell division and cell death in various eukaryotic organisms (Pinkston *et al*. 2006; Matheu *et al*. 2007). The damaged macromolecules and organelles, or their aggregates, can actively compromise cellular function, presumably by interfering with signaling pathways (Vellai *et al*. 2009).

5.1 *Lifespan*

With a relatively short lifespan of roughly 13 days at 25.5°C (Larsen *et al*. 1995), *C. elegans* offers an attractive platform for the investigation of aging. Lifespan has traditionally been thought of as a trait controlled by environmental conditions such as nutrition, dietary restriction or stress. The idea that lifespan could be regulated by single gene mutations as is the case for eye color or blood type was almost inconceivable until the late 20th century when *C. elegans* geneticists isolated *age-1* and *daf-2* mutants that significantly extended worm lifespan (Klass 1983; Dorman *et al*. 1995). These studies revealed that *age-1*, which encodes a phosphoinositide-3-kinase, and *daf-2*, which encodes the only insulin/IGF-1 receptor in *C. elegans*,

were players in a larger insulin/IGF-1 signaling pathway that can regulate lifespan (Kenyon 2010). To date, there are more than 60 such known *C. elegans* genes which, when mutated, can extend lifespan. Some of the best studied are *age*/*daf* mutants, 'clock' (*clk*) mutants in which development and rhythmic behaviours of the nematode are slowed, mutants with defects in sensory perception, and *eat* mutants defective in pharyngeal pumping, thought to experience dietary restriction effects (Samara and Tavernarakis 2003), and several mitochondrial (*mit*) mutants (Butler *et al*. 2010). Genetic and molecular analyses have implicated several gene classes in lifespan determination and have indicated that aging is affected by alteration of several biological processes, namely, dormancy, physiological rates, food intake and reproduction (Hekimi *et al*. 2001).

Aging in *C. elegans* is known to be controlled by several signaling pathways and regulatory proteins, prominent among which are insulin/IGF-1 (insulin-like growth factor 1) signaling (Hekimi and Guarente 2003); JNK (c-Jun N-terminal kinase) and TGF-β (transforming growth factor-β) signaling (Oh *et al*. 2005; Shaw *et al*. 2007); cellular energy sensor TOR (target of rapamycin) kinase signaling (Vellai *et al*. 2003); Sirtuin-type chromatin remodeling factors (Berdichevsky *et al*. 2006); tumour suppressor protein p53 (Tavernarakis *et al*. 2008); SNF-1, the nematode ortholog of NRF-1 (nuclear respiratory factor 1) type transcription factors (Ahn and Blackwell 2003) and FoxA-like transcription factor PHA-4 (Panowski *et al*. 2007).

To understand how insulin/IGF-1 signaling can modify lifespan, researchers used several molecular and biochemical approaches to find changes in the expression profile of genes and proteins in long-lived *C. elegans*. In one of these studies using a quantitative proteomic approach, 86 proteins were found to be differentially expressed out of a total of 1685 proteins in long-lived *daf-2* mutants (Dong *et al*. 2007). Interestingly, TAX-6 was identified as one of the proteins in higher abundance in *daf-2* long-lived mutants compared to wild-type worms, confirmed by higher TAX-6 protein levels in *daf-2* mutants and increased brightness of TAX-6:GFP in the intestine of *daf-2* RNAi animals. Both *tax-6* and *cnb-1* mutants displayed longer lifespan. Genetic analysis of *tax-6* with *daf-2*, *daf-16*, and *age-1* mutants suggest that calcineurin facilitates DAF-2 signaling and acts in parallel and/or upstream to DAF-16. Because TAX-6 levels are also regulated by DAF-2, it is thought to be a part of a feedback loop that maintains DAF-2 signaling. This implicates a new role for the protein phosphatase apart from its roles in development and behaviour in regulating longevity.

In mammals, calcineurin and the kinase AMPK antagonistically target and regulate a family of cofactors called the CREB-regulated transcriptional coactivators (CRTCs) to modulate energy homeostasis and ER stress (Screaton *et al*. 2004; Koo *et al*. 2005; Wang *et al*. 2009). CRTCs associate with transcription factors belonging to the cAMP response element binding protein (CREB) family of transcription factors (Mayr and Montminy 2001). Recently, a single CRTC homologue called CRTC-1 was identified in *C. elegans* (Mair *et al*. 2011). These authors reasoned that since both TAX-6 and AAK-2, the *C. elegans* AMPK catalytic subunit, modulate lifespan pathways, it is possible that TAX-6 and AAK-2 are targeting CRTC-1 to regulate its phosphorylation state and alter longevity pathways. They showed that AAK-2 and TAX-6 indeed target CRTC-1 to regulate its sub-cellular localization. This can affect CRTC-1's ability to associate with nuclear CRH-1, the *C. elegans* orthologue of the CREB transcription factor family. In addition, *crtc-1* RNAi results in longer lifespan similar to *tax-6* RNAi and *aak-2* overexpression. The increase in lifespan in *tax-6*-deficient worms or *aak-2* over-expression was eliminated when conserved phosphorylation/dephorphorylation sites were mutated in CRTC-1, demonstrating that calcineurin's and AMPK's role in longevity is dependent on the phosphorylation state of CRTC-1. Looking further downstream to the target of CRTC-1, CRH-1, it was found that a null mutation of *crh-1* suppressed the long lifespan of CRTC-1 RNAi, indicating that CRTC-1's role in longevity is mediated by *crh-1* (Mair *et al*. 2011). When the authors compared transcriptional profiles of *crh-1* mutants, *tax-6* mutants, and *aak-2* overexpressing worms, they saw remarkably similar transcriptional profile changes in all three of these long-living strains. Thus, it seems apparent that the effect of calcineurin on lifespan might be occurring through CREB-dependent transcriptional changes.

5.2 *Autophagy*

The cellular recycling process of autophagy is emerging as a central player in many of the conserved longevity pathways in *C. elegans*, but the underlying mechanisms that link autophagy and lifespan are yet to be fully understood (Lapierre *et al*. 2012). Autophagy is a process of cellular self-cannibalism in which portions of cytoplasm are sequestered in double membrane vesicles called autophagosomes and then transferred to lysosomes for bulk degradation. This process of self cannibalization is a cytoprotective process and the cytoplasmic macromolecules can be mobilized to generate energy-rich compounds that are needed to fulfil the bio-energetic demand of the cell. During *C. elegans* development, autophagy plays an important role in many physiological processes, including survival under starvation conditions, modulation of lifespan, and regulation of necrotic cell death caused by toxic ion-channel variants (Kovacs and Zhang 2010). The role of autophagy in adaptation of individual cells or organs to changing conditions is well implicated and its prominent role in determining lifespan of various model organisms is now being established (Cuervo 2008; Vellai *et al*. 2009).

The first genetic connection between autophagy and aging was established in *C. elegans*, in addition to the identification of genes that mediate lifespan extension (Johnson 2008). The insulin/IGF-1 receptor DAF-2 depends on the activity of certain autophagy genes. The knockdown of essential autophagy proteins in *daf-2* mutants drastically reduced their lifespan extension (Melendez *et al*. 2003; Aladzsity *et al*. 2007; Hars *et al*. 2007). Thus, the autophagic machinery appears to function downstream of DAF-2. Further, nematode longevity triggered by increased dosage of JNK or reduced TGF-β receptor activity is mediated by DAF-16/FoxO, which thereby links the insulin/IGF-1, TGF-β and JNK pathways and autophagy in aging control. For example, BEC-1/Atg6/Beclin 1, which is an essential component of the worm autophagic machinery, mediates the effects of TGF-β signaling both on reproductive growth and cell size (Aladzsity *et al*. 2007).

The existing evidence suggests that all pathways leading to extended lifespan converge at autophagy (Toth *et al*. 2008). While investigating the role of calcineurin in the autophagic process in *C. elegans*, we found that in addition to extended lifespan in calcineurin loss-of-function/null mutants, enhanced autophagy was also observed in the *cnb-1(jh103)* CnB null mutant and the *tax-6(ok2065)* loss-of-function mutant of CnA when compared to wild-type and the *tax-6(jh107)* gainof-function mutant. This extended lifespan phenotype and enhanced autophagy phenomenon was lost in the *cnb-1(jh103)* CnB null mutant after the treatment with *bec-1* and *atg-7* (two autophagy genes) RNAi (Dwivedi *et al*. 2009). This study provided the first definitive evidence of autophagy genes being essential factors in the regulation of lifespan in the calcineurin-defective *C. elegans* strains.

6. Calcineurin-interacting proteins in *C. elegans*

In general, calcineurin accomplishes multiple functions in different eukaryotes through a variety of mechanisms such as dephosphorylation of its substrates (NFAT, cdk-4, GABA) (Garcia-Cozar *et al*. 1998; Baksh *et al*. 2000; Wang *et al*. 2003) and binding with its activators (Hsp 70) (Someren *et al*. 1999) and inhibitors (RCAN-1, cain/cabin1) (Lai *et al*. 1998; Sun *et al*. 1998; Lee *et al*. 2003). In the worm, using yeast two-hybrid screening of TAX-6 as bait, KIN-29 was identified as a specific calcineurin-interacting protein (Singaravelu *et al*. 2007). KIN-29 is a Ser/Thr kinase involved in regulating gene expression of a subset of chemoreceptors in specific neurons. Both TAX-6 and KIN-29 were expressed in hypodermis, muscles and neurons. Moreover, both calcineurin and *kin-29* mutants exhibited similar phenotypes, namely, small body size, small brood size and slow growth. Another such interacting protein was identified as Y46G5A.10, named as *cnp-2*, where '*cnp*' denotes calcineurin-binding protein and the succeeding numeral indicates the order of its discovery (Xianglan *et al*. 2008). The *cnp-2* gene of *C. elegans* was physically mapped to chromosome II, and corresponds to the region between the *rol-1* and *sup-6* loci on the genetic map. The genomic DNA of *cnp-2* is 6422 bp in size having 20 exons and 19 introns, and encodes a nematode-specific protein that was strongly expressed in the intestine of *C. elegans*. However, *cnp-2* RNAi knock-down did not reveal any gross phenotypic defects. Thus, CNP-2 was proven to be a calcineurin-binding protein whose role remains to be elucidated. Another calcineurin-interacting protein, CNP-3 was identified in the nuclei of intestine, hypodermis, dorsal uterine regions and spermatheca (Kim *et al*. 2008). Although the *cnp-3(jh145)* single mutant had no gross defects compared to wild-type, the phenotypes of the double mutants, *tax-6(p675);cnp-3(jh145)* and *cnb-1(jh103);cnp-3(jh145)*, were more severe in terms of brood size, body size and serotonin-mediated egg-laying defects than *tax-6(p675)* and *cnb-1(jh103)*, respectively. These results suggested that *cnp-3* dysfunction accentuated certain calcineurin loss-of-function phenotypes.

Another novel calcineurin-binding protein was identified, namely, HLH-11, a member of basic helix-loop-helix (bHLH) proteins (Lee *et al*. 2009). HLH-11 is a putative counterpart of human AP4 transcription factor. bHLH transcription factors have been implicated to regulate many developmental processes such as cell proliferation and differentiation, sex determination, neurogenesis and myogenesis in various metazoans including flies, nematodes and vertebrates (Jan and Jan 1993; Hallam *et al*. 2000; Massari and Murre 2000). Interestingly, in *C. elegans*, *hlh-11* expression in the pharynx, intestine, nerve cords, anal depressor and vulval muscles coincides with calcineurin expression and genetic epistasis implicated *hlh-11* in the regulation of serotonin-mediated egg-laying at the downstream of *tax-6* (Lee *et al*. 2009).

Therefore, much importance is being given at present to understand the novel proteins that physically interact with *C. elegans* calcineurin so that it would allow identification of additional signaling pathways in which calcineurin might directly participate and also specify potential drug targets.

7. Regulators of *C. elegans* calcineurin

Calcineurin signaling is tightly regulated by a number of proteins that bind to and inhibit calcineurin such as Cain, RCAN, AKAP79, and CHP (Sun *et al*. 1998; Lin *et al*. 1999; Fuentes *et al*. 2000). This lends evidence to the significance of calcineurin's biological functions and the importance of specific and sensitive control of downstream responses. It also raises the possibility of more precise calcineurin inhibition for clinical purposes by targeting endogenous proteins

that regulate calcineurin. Among these, the RCAN family of calcineurin regulating proteins spans vertebrate, invertebrate and fungal genomes. RCAN was originally identified as a gene located in an area of human chromosome 21 critical for the effects of Down's Syndrome, and RCAN was highly expressed in the brain of Down's Syndrome patients. RCAN was characterized in yeast and humans as a calcineurin-binding protein that is transcriptionally regulated by yeast calcineurin and can inhibit calcineurin phosphatase activity (Fuentes *et al*. 2000; Kingsbury and Cunningham 2000).

We identified a single RCAN gene in the *C. elegans* genome, which we named *rcn-1* (Lee *et al.* 2003). We showed that RCN-1 protein binds directly to *C. elegans* calcineurin, inhibits calcineurin phosphatase activity, and that the *rcn-1* gene is regulated by calcineurin activity. When we drove RCN-1 overexpression with a *tax-6* promoter, we observed multiple phenotypes that resembled loss of calcineurin function. For instance, RCN-1 overexpression resulted in serotonin-mediated egg-laying defects similar to *tax-6* and *cnb-1* loss of function mutants. We also observed smaller body size, smaller brood size, and cuticle defects similar to *tax-6* and *cnb-1* mutants. To confirm whether RCN-1 functionally inhibits calcineurin activity *in vivo,* we overexpressed RCN-1 in *tax-6* gain of function mutants and suppressed the hyperactive egg-laying phenotype back to normal levels. In addition *rcn-1* RNAi resulted in defects in male mating behaviour similar to those observed in *tax-6* mutants (Hu *et al*. 2006).

8. Conclusion and future perspective

Since calcineurin is highly conserved from yeast to human, studying components of the calcineurin pathway in a model organism likely provides insights into the human situation. *C. elegans* is an attractive model, since its genome contains single *tax-6* and *cnb-1* genes encoding CnA and CnB, which facilitates genetic studies (Ahn *et al*. 2006). Moreover, the high percentage of identity between *C. elegans* and human calcineurin allows study of the structure and function of *C. elegans* calcineurin by homology modelling with the use of human CnA and CnB structures as templates (Ke and Huai 2003).

Calcineurin plays a pivotal role in regulating a wide variety of cellular processes relating to development, fertility, proliferation, and behaviour and lifespan regulation in *C. elegans*. The physiological significance of calcineurin is further highlighted by the fact that many of the proteins that physically bind with calcineurin are critical for maintaining normal homeostasis and are associated with numerous diseases upon dysfunction that include cardiac hypertrophy (Molkentin *et al*. 1998), Alzheimer's disease (Ladner *et al*. 1996; Lian *et al*. 2001), schizophrenia (Miyakawa *et al*. 2003), diabetes (Gooch *et al*. 2004), Duchenne muscular dystrophy (Sundaram *et al*. 2007) and others. Substantial evidences have associated calpain-calcineurin signaling with Ca^{2+}-dependent disorders, such as Alzheimer's disease and cardiac hypertrophy. The calpain inhibitors that block calpain-dependent calcineurin activation have been suggested as potential therapeutics for certain neurodegenerative and myocardial diseases (Wu *et al*. 2007). In addition, calcineurin's major role in signaling pathways involved in growth and development, reproduction, movement, egg-laying and sensory behaviours makes it an attractive target for the discovery of drugs and anthelmintics to combat the debilitating effects of parasitic nematodes. In fact, a model involving Ca^{2+} release from sarcoplasmic reticulum, CaM kinase, calcineurin, muscarinic receptors and AF2 receptors was proposed to develop a pharmacological approach to counter resistance of parasitic nematodes to cholinergic anthelmintics (Trailovic *et al*. 2005). Several drugs are already known to inhibit calcineurin activity, such as cyclosporin A and FK-506. It would be interesting to study the effects that these drugs have upon *C. elegans* and various destructive parasitic nematodes.

The characterization of *C. elegans* calcineurin mutants provides a simple but excellent genetic model system for studying the *in vivo* functions of calcineurin, identifying its interacting proteins and regulators, and delineating the multiple signaling pathways in which calcineurin participates.

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