



Review

Functional Insights into Protein Kinase A (PKA) Signaling from *C. elegans*

Fereshteh Sadeghian ¹, Perla G. Castaneda ², Mustafi R. Amin ²  and Erin J. Cram ^{2,*} ¹ Department of Bioengineering, Northeastern University, Boston, MA 02115, USA² Department of Biology, Northeastern University, Boston, MA 02115, USA

* Correspondence: e.cram@northeastern.edu

Abstract: Protein kinase A (PKA), which regulates a diverse set of biological functions downstream of cyclic AMP (cAMP), is a tetramer consisting of two catalytic subunits (PKA-C) and two regulatory subunits (PKA-R). When cAMP binds the PKA-R subunits, the PKA-C subunits are released and interact with downstream effectors. In *Caenorhabditis elegans* (*C. elegans*), PKA-C and PKA-R are encoded by *kin-1* and *kin-2*, respectively. This review focuses on the contributions of work in *C. elegans* to our understanding of the many roles of PKA, including contractility and oocyte maturation in the reproductive system, lipid metabolism, physiology, mitochondrial function and lifespan, and a wide variety of behaviors. *C. elegans* provides a powerful genetic platform for understanding how this kinase can regulate an astounding variety of physiological responses.

Keywords: PKA; cAMP; calcium signaling; *C. elegans*; physiology; behavior



Citation: Sadeghian, F.; Castaneda, P.G.; Amin, M.R.; Cram, E.J. Functional Insights into Protein Kinase A (PKA) Signaling from *C. elegans*. *Life* **2022**, *12*, 1878. <https://doi.org/10.3390/life12111878>

Academic Editor: Xin Li

Received: 7 October 2022

Accepted: 11 November 2022

Published: 14 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Protein kinase A (PKA) is a 3'-5'-cyclic adenosine monophosphate (cAMP)-dependent kinase that is at the center of diverse biological functions in numerous systems. Inactive PKA is composed of two catalytic (PKA-C) and two regulatory (PKA-R) subunits. The binding of cAMP to the PKA-R subunits activates the enzyme, causing the release of PKA-C. The PKA-R subunits, therefore, inhibit PKA-C in the absence of cAMP. Once released, PKA-C interacts with multiple downstream effectors and regulates lipid metabolism [1], cell migration [2], and vasodilation [3], among many other functions [4]. While numerous genes encode the regulatory and catalytic subunits of PKA in mammals, complicating the study of PKA, *Caenorhabditis elegans* (*C. elegans*) only has a single gene encoding the regulatory subunit of PKA, *kin-2*, and one gene encoding the catalytic subunit, *kin-1*. This review focuses on the contribution *C. elegans* has made to our understanding of the function and biological role of PKA.

2. Isoforms

Humans have three genes encoding PKA-C: PRKACA, PRKACB, and PRKACG, and two types of PKA-R's: type PKA-RI (PKA-Ri α and PKA-Ri β) and type PKA-RII (PKA-RII α and PKA-RII β). Holoenzyme expression and distribution is largely determined by the type of regulatory subunit [5–7]. The PKA-RII β subunit is found in endocrine, brain, fat, and reproductive tissues. The PKA-RI α and PKA-RII α subunits are expressed ubiquitously [5,6] and PKA-RII β is enriched in the mitochondria. PKA-C can undergo post- and co-translational modifications [8], including myristoylation of the N-terminus, which increases PKA-C membrane affinity [9]. PKA-RI α has the highest binding affinity, followed by PKA-RII α and PKA-RII β [5,6].

In *C. elegans*, PKA is encoded by one catalytic subunit, KIN-1/PKA-C, and one regulatory subunit, KIN-2/PKA-R [10]. The PKA binding sites on *kin-2* are not very well conserved [11]. With only one PKA-C subunit for PKA-R to coordinate with, rather than

multiple forms encoded by different loci, the two subunits may have co-evolved; changes in the PKA-R locus could be compensated for by sequence changes in the PKA-C locus [11]. KIN-1/PKA-C is 82% identical to the mammalian catalytic subunit of PKA, and KIN-2 is most closely related to type I mammalian PKA regulatory subunit [12]. Proteomic analysis identified 419 potential PKA substrates with 630 potential PKA binding sites in *C. elegans* [11].

In *C. elegans*, expression of specific isoforms of the catalytic subunit *kin-1* could contribute to the specificity of PKA activity [13,14]. The *kin-1* gene has a total of 13 exons, including six 5' exons (N'1–N'6), and at least 12 different *kin-1* isoforms are expressed [14]. Depletion of N'3 *kin-1* isoform led to paralysis and egg-laying defects, while knockdown N'4 variants resulted in no apparent phenotypes [15]. Isoforms containing N'3 and N'4 are not targets of myristoylation [14], while the N'1 isoforms are N-myristoylated. This protein modification may prohibit docking of the N-terminal domain to a hydrophobic pocket in PKA-C, possibly affecting intracellular targeting and differentially regulating *kin-1* function [16]. Although one gene, *kin-2*, encodes PKA-R in *C. elegans*, diversity of function might be achieved through differential expression of its three isoforms. Some *kin-2* isoforms lack the typical dimerization/docking domains, implying they do not form the tetrameric PKA holoenzyme or interact with AKAP proteins, and suggesting these PKA-R isoforms might have other, unknown, functions [17].

3. AKAPs

PKA participates in many signaling pathways. A kinase anchoring proteins (AKAP) scaffold PKA and regulate signaling output by enabling association with specific effectors (Figure 1) [4,18]. Regulatory subunits are bound by AKAP until PKA is activated, allowing for spatial and temporal control of PKA [7,19,20]. AKAP-1 is the best characterized AKAP in *C. elegans* [21], and has a primarily neuronal expression pattern [22]. AKAP-1 has a high affinity for KIN-2/PKA-RI [21]. Although *C. elegans* expresses only one AKAP, other proteins, such as ERM-1, an ortholog of ezrin, which acts as an AKAP in mouse gastric parietal cells [23], may also function as an AKAP to PKA in *C. elegans*. ERM-1 is expressed in epithelial tissues such as the intestine and spermatheca, where it regulates apical polarization, junction formation [24], cortical actin organization, and lumen formation [25], processes in which PKA could play a role.

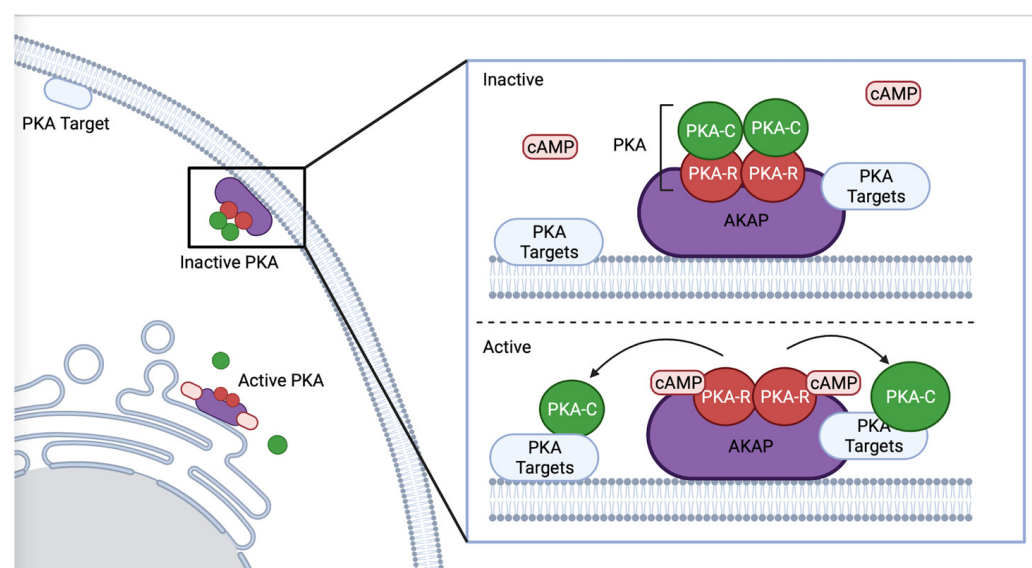


Figure 1. Schematic representation of AKAP binding PKA. AKAP binds the regulatory subunit of PKA, regulating PKA's subcellular localization and co-locating PKA with specific phosphorylation targets, such as proximal to the ER or plasma membrane. Created with BioRender.com (accessed on 31 October 2022).

4. Activation of PKA by G-Proteins

The second messenger, cyclic AMP (cAMP), is produced by adenylyl cyclase, which converts adenosine triphosphate (ATP) to cAMP. PKA is activated by the binding of cAMP to the PKA-R subunits, which releases PKA-C. cAMP levels are reduced by phosphodiesterases (PDEs), which convert cAMP into AMP [26]. Adenylyl cyclases are commonly regulated by G-protein signaling. Heterotrimeric G-proteins consist of an α and a $\beta\gamma$ subunit, which, when activated by an upstream G-protein coupled receptor (GPCR) or G-protein regulator (GPR), dissociate and independently activate signaling cascades [27–29]. Upon ligand binding, the GPCR acts as a guanine nucleotide exchange factor (GEF), exchanging GDP for GTP on the α subunit, and activating the heterotrimeric G-protein. Heterotrimeric G-proteins can also be activated via a receptor-independent mechanism facilitated by G-protein regulator proteins (GPRs) (Figure 2) [30,31].

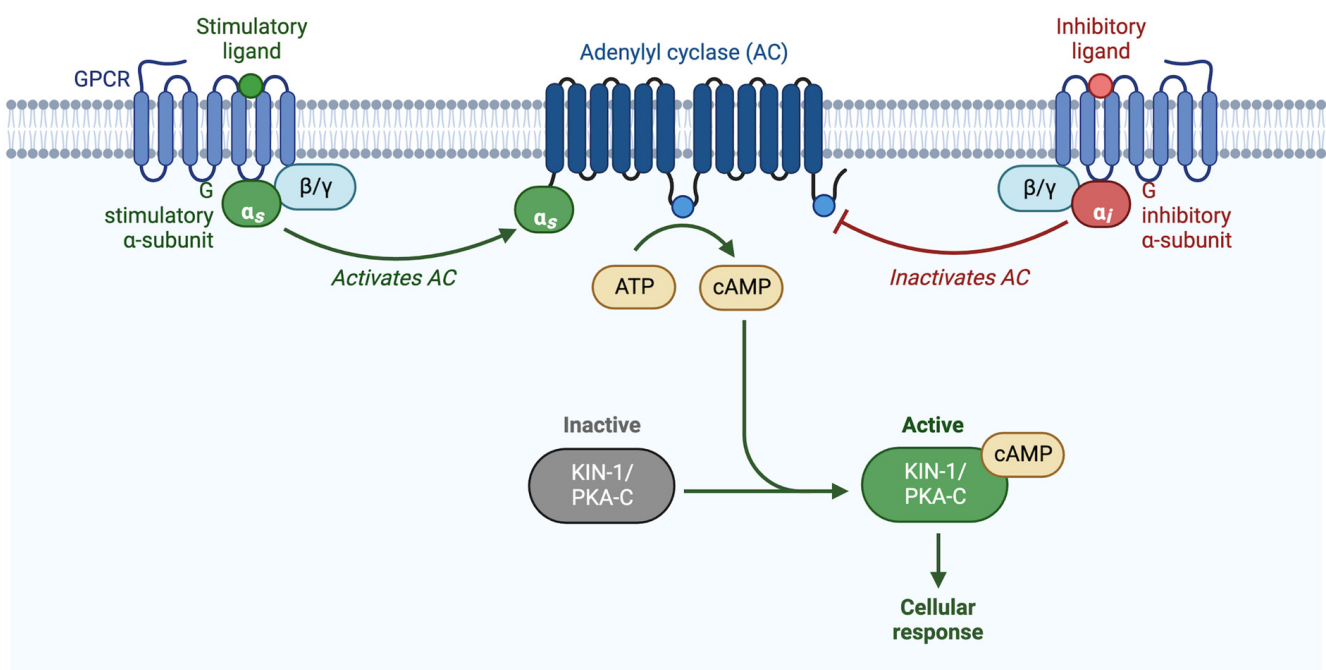


Figure 2. Schematic representation of G-protein activation of PKA. PKA is activated when the regulatory subunit binds to cAMP, releasing the catalytic subunit. cAMP is produced by adenylyl cyclase, which is either activated by G α_s , or inhibited by G α_i/o . Adapted from “Activation of Protein Kinase A (PKA)”, by BioRender.com (2022). Retrieved from <https://app.biorender.com/biorender-templates> (accessed on 27 October 2022).

Upon activation, the GTP-bound α subunit dissociates from the $\beta\gamma$ subunit, and both the α and $\beta\gamma$ subunits can initiate downstream signaling pathways [32]. *C. elegans* expresses 21 G α subunits [27] of the G_s, G_{i/o}, G_q, and G₁₂ families, including only one ortholog of G α_s (GSA-1) and G $\alpha_{i/o}$ (GOA-1) [33]; these G α subunits are typically upstream of adenylyl cyclase. *C. elegans* express two G β subunits, GPB-1 and GPB-2 and two γ subunits, GPC-1 and GPC-2. GPB-1 shares 86% homology with mammalian β subunits and interacts with all G α subunits in *C. elegans* [34,35]. GPC-1/ γ is expressed in sensory neurons, while GPC-2/ γ is expressed more broadly [36]. G $\beta\gamma$ subunits can regulate ion channels [37,38] including Ca²⁺ channels [39], as well as activate or inhibit adenylyl cyclase [40]. GPB-2 acts downstream of the G α subunit GOA-1 in pharyngeal pumping [41], and is required for egg-laying and locomotion [42,43], and works with GPB-1 and GSA-1 to regulate Ca²⁺ signaling and contractility in the spermatheca [44].

5. Ca²⁺ and cAMP Signaling Are Intertwined

The second messenger, Ca²⁺, is implicated in a variety of essential biological processes [45], making it critical to ensure correct concentration and localization. To maintain low Ca²⁺ concentrations in the cell, Ca²⁺ is pushed into the endoplasmic reticulum (ER) by SERCA pumps [46] or out of the cell by plasma membrane Ca²⁺ ATPases [47]. Gap junctions can mediate Ca²⁺ signaling between cells [48]. Channels located at the plasma membrane (PM), such as voltage-operated Ca²⁺ (VOCCs), receptor-operated Ca²⁺ channels (ROCCs), mechanically activated Ca²⁺ channels, transient receptor potential (TRP) ion channels, and store-operated Ca²⁺ channels (SOCCs) [49], also regulate the supply of Ca²⁺ from the extracellular space, and Ca²⁺ can be sequestered by the mitochondria [50]. Activation of phospholipase C (PLC) leads to the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) to produce inositol 1,4,5-trisphosphate (PIP₃) and diacyl glycerol (DAG). PIP₃ binds to the IP₃ receptor (IP₃R) on the ER to release Ca²⁺. G-proteins mediate this release primarily through phospholipase PLCβ [51].

Numerous studies describe the complex and intertwined relationship of PKA, cAMP, and Ca²⁺ signaling [52]. For example, PKA can activate the ITR-1/IP₃ receptor [53] and the ryanodine receptors (RyR), which mediate Ca²⁺ release from the ER in muscle and some non-muscle cell types [54,55]. PKA can regulate Ca²⁺ release by activating plasma membrane channels, such as stretch-sensitive TRPV channels [56]. In mouse cardiomyocytes, Gα activation can stimulate Ca²⁺ release through exchange protein directly activated by cAMP (EPAC) and Rap1 by stimulating PLCε [57,58]. However, PKA does not always stimulate Ca²⁺ release; in rat brain cells, phosphorylation of IP₃R by PKA-C decreases Ca²⁺ release [59]. PKA can lower cytosolic Ca²⁺ levels by increasing the activity of SERCA pumps, which pump Ca²⁺ back into the ER, by phosphorylating and dissociating phospholamban [60,61]. PKA can inhibit PLC-β [62], which would result in decreased Ca²⁺ release. Additionally, adenylyl cyclases can be regulated by Ca²⁺ signaling [63], and IP₃ receptors can be regulated by cAMP [64].

GPCRs regulate Ca²⁺ release through PKA in the *C. elegans* intestine. KIN-1/PKA-C plays a role in the *C. elegans* defecation cycle, which occurs rhythmically every 50 s [65]. The posterior, anterior, and enteric muscles contract sequentially to release waste [66]. GABAergic neurons (AVL and DVB) mediate the release of the neurotransmitter GABA, which prompts the release of gut contents by triggering muscle contractions [66–69]. KIN-1/PKA-C functions in the GABAergic neurons to regulate this expulsion step, acting downstream of the GPCR, AEX-2, and neuropeptide NLP-40. Constitutively active PKA in GABAergic neurons was sufficient to partially bypass loss of AEX-2, and PKA modulates muscle contraction and promotes Ca²⁺ influx into the DVB neurons through the voltage-gated Ca²⁺ channels UNC-2 and EGL-19 [65]. KIN-1/PKA-C stimulates the release of Ca²⁺ in neurons through specific voltage-gated calcium channels to control rhythmic defecation cycles.

Ca²⁺ and PKA signaling also coordinately regulate contractility in the *C. elegans* spermatheca, a smooth muscle-like tissue in the reproductive system and the site of fertilization in *C. elegans*. Cell contractility in the spermatheca is dependent on actin and myosin and is regulated, in part, by Ca²⁺ signaling through the phospholipase PLC-1 [70], which mediates Ca²⁺ release from the endoplasmic reticulum. GSA-1/Gα_s, KIN-1/PKA-C, and KIN-2/PKA-R, regulate Ca²⁺ release and contractility in the *C. elegans* spermatheca [44]. Without GSA-1/Gα_s or KIN-1/PKA-C, Ca²⁺ is not released, and oocytes become trapped in the spermatheca. Conversely, when PKA is activated through either a gain of function allele in GSA-1 or by depletion of KIN-2/PKA-R, Ca²⁺ pulses continuously propagate across the spermatheca, even in the absence of oocyte entry. In the spermathecal–uterine valve, which connects the spermatheca to the uterus, loss of GSA-1/Gα_s or KIN-1/PKA-C results in the opposite phenotype: sustained, high levels of Ca²⁺ and a loss of coordination between the spermathecal bag and sp-ut valve. The phospholipase PLC-1 is required for these Ca²⁺ pulses. These results suggest activation of PKA has tissue-specific effects on

the timing and intensity of Ca^{2+} release, and that KIN-1/PKA-C stimulates Ca^{2+} release downstream of GSA-1 in a PLC-1-dependent manner in the *C. elegans* spermatheca.

cAMP and Ca^{2+} signaling are also involved in neuronal regeneration in *C. elegans*. *C. elegans* neurons regenerate after laser axotomy [71,72]. PLM sensory neuron axotomy triggers a Ca^{2+} transient, which correlates with regenerative growth in late larval (L4) stage *C. elegans*. Genetically increasing Ca^{2+} or cAMP accelerates this growth, facilitates fusion of axonal fragments, and promotes branching; while inhibiting Ca^{2+} release reduces regrowth [73]. Inhibition of the regulatory subunit, through a loss of function allele *kin-2(ce179)* allele, promoted regeneration regrowth and elevated rates of fusion, while use of the PKA inhibitor H89 resulted in reduced regrowth in a dose-dependent manner [73]. In the ASJ neuron, cAMP signaling elevation or Ca^{2+} channel disruption improve DLK-independent regeneration [74]. Therefore, elevation of cAMP promotes neuronal regeneration in a PKA-dependent manner.

6. Oocyte Maturation

In *C. elegans*, major sperm proteins (MSP), which also polymerize to drive sperm motility, are released from sperm and stimulate oocyte meiotic maturation and oocyte production [75–77]. MSP and $\text{G}\alpha$ -adenylyl cyclase signaling is required in the gonadal sheath cells and in the oocytes to regulate oocyte growth and meiotic maturation, possibly by antagonizing gap-junction communication between the sheath cells and oocytes [78]. Activating $\text{G}\alpha$ -adenylyl cyclase signaling is sufficient to drive oocyte meiotic maturation in the absence of sperm [78,79]. KIN-1/PKA is required for oocyte meiotic maturation and functions downstream of ACY-4/adenylyl cyclase [80,81]. SACY-1, a highly conserved DEAD-box helicase that functions downstream of PKA, a two-pore domain potassium (TWIK) channel, and multiple components of a CoREST-like complex suppress *acy-4(lf)* sterility [80], suggesting they act downstream of $\text{G}\alpha_s$ -ACY-4-PKA to regulate oocyte meiotic maturation.

7. PKA Regulates Lipid Metabolism

In mammalian thermogenesis, β 3-adrenergic receptor stimulation of G-proteins leads to the production of cAMP, and, therefore, PKA activation [82]. PKA activates hormone-sensitive lipase (HSL), which releases the glycerol and fatty acids required for the physiological activation of uncoupling protein 1 (UCP1), leading to increased production of heat [83]. Although *C. elegans* do not regulate body temperature [84], KIN-1/PKA-C does regulate *C. elegans* response to cold stress [85]. Under cold conditions, PKA signaling is activated in the intestine, where KIN-1 activity leads to increased expression of *hosl-1*/HSL, fat hydrolysis, increased glycerol availability, and increased cold tolerance. KIN-1 is also required in the neurons for cold tolerance. Although the mechanism is not known, perhaps neurons pass a signal to the intestine, which then upregulates *hosl-1* expression and lipid hydrolysis [85].

PKA activation also results in lipolysis of stored lipid droplets in response to food deprivation. KIN-1 phosphorylates and stimulates the adipose triglyceride lipase ATGL-1 to form a lipid droplet-localized protein complex containing ATGL-1 and the lipid droplet protein LID-1, leading to lipid hydrolysis. The suppression of *atgl-1* or *lid-1* hinders fasting-induced lipolysis in adult worms (Figure 3) [86]. Lipolysis is reduced in low-oxygen conditions. Hypoxia-inducible factor (HIF) is a transcription factor that drives adaptive responses to low-oxygen levels, including the suppression of lipolysis. In mammals and *C. elegans*, hypoxia reduces cAMP and PKA activity levels, although the mechanism by which HIF-1 regulates PKA signaling in *C. elegans* is not clear. Exposure of *C. elegans* to hypoxia (1% O_2) prevents PKA-stimulated lipolysis by targeting ATGL-1 for proteasomal degradation [87]. These studies are examples of the interaction between PKA activation and core metabolic processes.

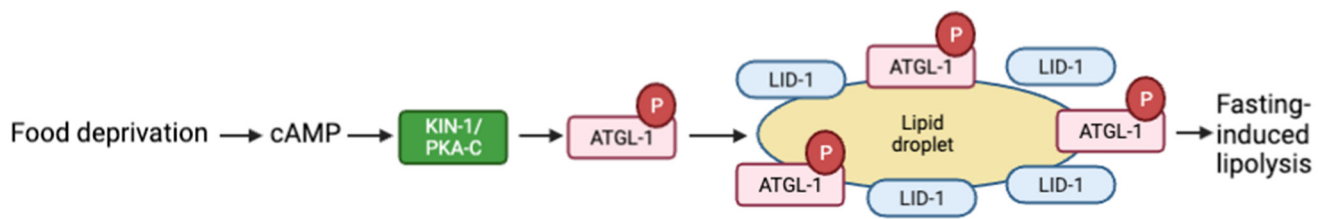


Figure 3. Schematic representation of fasting-induced lipolysis model via the cAMP pathway. Food deprivation increases cAMP and activates PKA. PKA-C activates the adipose triglyceride lipase ATGL-1, which then forms a liquid droplet with lipid droplet protein LID-1, which leads to lipid hydrolysis. Adapted with permission from [86], 2022, American Society for Microbiology. Created with BioRender.com (accessed on 23 October 2022).

PKA regulation of lipid metabolism impacts overall lifespan in most organisms [88–90]. In *C. elegans*, PKA activation of lipid catabolism in muscle cells and subsequent induction of AMPK/AAK-2 expression in non-muscle tissues, including neurons and intestinal cells, leads to enhanced mitochondrial metabolism and lifespan extension. Activating KIN-1/PKA-C and ATGL-1 in muscle cells led to a decrease in abundance of intramyocellular lipid and an extension of lifespan. Conversely, depletion of *atgl-1* in all tissues shortened lifespan [91].

8. PKA Regulates Mitochondrial Function and Lifespan

Mitochondrial fusion and fission are important for mitochondrial network morphology, biogenesis, embryonic development, metabolism, and apoptosis. Upon hypoxia or ischemia (low oxygen and glucose-deprived conditions), decreased availability of A-kinase anchoring protein 121 (AKAP121) by Siah2 (seven in absentia homolog (SIAH) family) leads to mitochondrial fission and cell death in mice [92]. Dynamin-related protein 1 (Drp1), a direct target of PKA, encodes a dynamin-like GTPase that controls mitochondrial fission [93]. AKAP121 binding of PKA blocks phosphorylation of Drp1, which prevents the formation of a complex between Drp1 and the outer mitochondrial membrane protein Fis1 (fission, mitochondrial 1) and subsequent mitochondrial fission. In *C. elegans*, inhibition of *siah-1* or *drp-1* during larval development shortens lifespan [92], presumably through effects on mitochondrial activity or dynamics.

PKA is required for *C. elegans* lifespan extension in a variety of contexts. For example, *C. elegans* with defective oxidative phosphorylation due to a mutation in the respiratory complex I subunit, *GAS-1*, are short lived. Inhibition of insulin/IGF signaling in these animals through loss of DAF-2/IGF1 receptor or AGE-1/PI3K rewires the animal's metabolism and extends lifespan [94]. This effect depends on PKA signaling; *kin-1(RNAi)* abrogates the lifespan extension of *age-1*; *gas-1* double mutants. The purine base xanthine increases when insulin signaling is inhibited. Treatment of animals with xanthine derivatives increases AAK-2/AMPK and KIN-1/PKA activity, enhances mitochondrial network remodeling, and induces the metabolic changes that give rise to the lifespan extension [95]. In addition, DAF-2/IGF1 receptor and AGE-1/PI3K inhibit KIN-1/PKA-C in dauer-stage animals [96].

In mammalian cells and *C. elegans*, PKA phosphorylates and activates SIRT1, a sirtuin protein, which leads to improved mitochondrial function and fatty acid oxidation. Hydralazine, a drug used for hypertension, heart failure, and cancer treatment, improves mitochondrial function and elevates SIRT1 levels in cells [97]. In *C. elegans*, hydralazine extends lifespan through a PKA-dependent mechanism. Hydralazine binds to the catalytic subunit KIN-1/PKA-C, enabling separation from the regulatory subunit and activating PKA. PKA activation contributes to both SIRT1 activation and to the stress regulatory SKN-1/NRF2 signaling pathway, resulting in increased lifespan through glucose-induced mitochondrial dysfunction. Further studies are required to discover the mechanism by which PKA regulates SIRT1 and NRF2 [97].

PKA is part of a signaling pathway that regulates nucleotide metabolism and reproductive development in response to nucleotide imbalance in the gut of *C. elegans*. During genotoxic stress, Nucleotide (NT) deficiency stimulates the nucleotide-sensing system that mediates mitotic germline proliferation and NT metabolism in the intestine. The poly(U)-specific endoribonuclease, ENDU-2, is a regulator that reacts to NT imbalance and genotoxic stresses. ENDU-2 regulates of CTPS-1, a cytidine triphosphate (CTP) synthase, by both inhibiting KIN-1/PKA-C signaling, possibly by repressing adenylyl cyclase activity, and by regulating histone deacetylase HDA-1 activity. This prevents activation of the cytidine triphosphate (CTP) synthase CTPS-1, which inhibits proliferation under genotoxic stress and increases lifespan [98]. Although these studies suggest an important role for PKA, many questions remain regarding the mechanisms by which PKA regulates lifespan extension, mitochondrial dynamics and metabolism.

9. PKA Signaling in Neurons Regulates *C. elegans* Behaviors

Several studies suggest PKA activity in neurons regulates locomotion. Activation of KIN-1/PKA-C, through depletion of *kin-2*, results in hyperactive movement of *C. elegans*; similar phenotypes are seen when the $G\alpha_s$ protein GSA-1 is activated or the phosphodiesterase PDE-4 is depleted [99]. Loss of function alleles in the two-pore-domain potassium (K_2P) channel TWK-7 increase locomotion. Genetic evidence suggests TWK-7 is downstream of the GSA-1-KIN-1/PKA pathway in B- and D-type motor neurons [99]. Activation of KIN-1/PKA-C inhibits TWK-7. When PKA is active, TWK-7 is repressed, leading to increased locomotion. Further studies are necessary to understand the mechanism by which KIN-1/PKA-C regulates TWK-7 in motor neurons (Figure 4) [99].

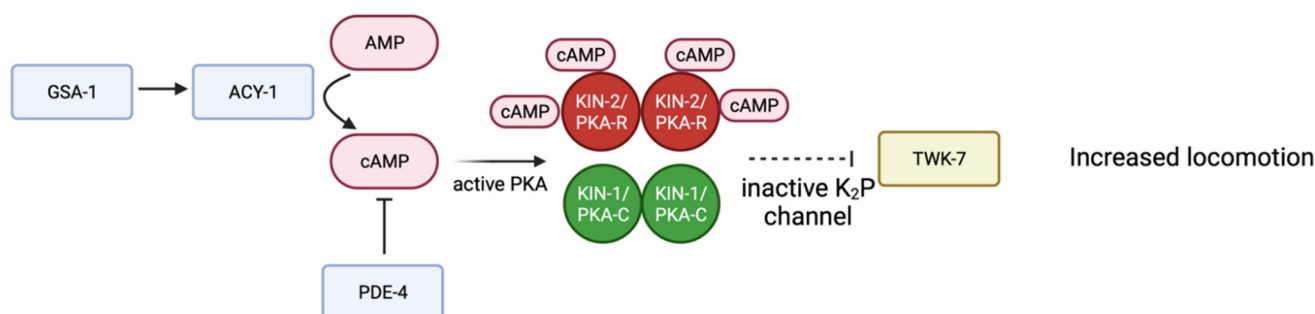


Figure 4. Schematic representation of proposed signaling pathway in B- and D-type motor neurons. $G\alpha_s$ GSA-1 activates adenylyl cyclase ACY-1, producing more cAMP and therefore activating PKA. TWK-7 is a K_2P channel that normally decreases locomotion. Through a mechanism that is not entirely understood (dotted line) active PKA inhibits TWK-7, therefore increasing locomotion. Adapted with permission from [99], 2022, Oxford University Press. Created with BioRender.com (accessed on 23 October 2022).

The neurotransmitter acetylcholine stimulates *C. elegans* locomotion. Several studies have revealed a role for PKA in acetylcholine release in motor neurons [100]. Exposure of *C. elegans* to 0.1% ethanol also increases locomotion. Although the precise mechanism by which ethanol increases locomotion remains unclear, the $G\alpha_s$ -cAMP-PKA signaling pathway can be activated by ethanol in the IL2 sensory neurons, which release acetylcholine and link to locomotor circuits by intermediary neurons [101]. This study identified a key downstream effector of PKA signaling, UNC-18/Sec1-Munc18. UNC-18 plays an essential role in synaptic vesicle exocytosis. $G\alpha_s$ signaling activates KIN-1/PKA-C, which phosphorylates UNC-18 thereby increasing neurotransmitter release and stimulating *C. elegans* locomotion [101]. A similar pathway may be involved in the response to isoflurane, a general anesthetic, which results in erratic and diminished neuronal activity in motor neurons and physical quiescence of the nematode [102]. Sensitivity to isoflurane is related to levels of acetylcholine release. Activating KIN-1/PKA-C through loss of *kin-2* or a gain

of function mutation in adenylyl cyclase *acy-1(js127)* results in resistance to isoflurane [103]. Aldicarb treatment increases acetylcholine release and leads to sustained muscle activation and eventual paralysis. Activation of KIN-1 further increases acetylcholine release and increases sensitivity to aldicarb-induced paralysis [104].

In addition to locomotion, PKA signaling in neurons regulates other behaviors, including wakefulness. In *C. elegans*, *Drosophila*, and mice, increased PKA-1 activity promotes wakefulness [105–108], via the transcriptional activator cAMP response element-binding protein (CREB) [108]. When KIN-1/PKA-C activity is increased by deletion of KIN-2/PKA-R or ACY-1(GF), the worms are more active. PKA-C activates the transcription factor CRH-1/CREB and promotes neuropeptide release to promote active wakefulness. CRH-1 is the CREB ortholog in *C. elegans* [105,109]. The Ca²⁺-dependent activator protein for secretion UNC-31/CAPS is necessary for neuropeptide release from dense core vesicles (DCV). By enhancing mobilization and priming, cAMP/PKA signaling augments synaptic vesicle (SV) fusion [110]. Activation of PKA can bypass the requirement for UNC-31 in the docking of DCVs in exocytosis. KIN-1/PKA-C phosphorylates the syntaxin-1-binding protein, TOM-1, which downregulates synaptic transmission and UNC-31/CAPS-dependent neuropeptide release, resulting in locomotion regulation and stabilized wakefulness [105,111].

CREB is a common downstream effector of PKA. PKA activation of CRH-1/CREB also regulates the level of the FMRFamide-related neuropeptide FLP-19 in BAG sensory neurons, contributing to CO₂ sensing and response [112]. PKA signaling through CREB also enhances neural circuit excitability and improves memory. *Eleutheroside E*, a sterol glycoside extracted from Siberian ginseng, *Eleutherococcus senticosus* [113], has a neuromodulatory effect and protects radiation-damaged nerves. This compound signals through Gαq and PLC to activate cAMP-PKA, improving performance on associative learning assay and memory tasks. Through downstream activation of the transcriptional regulator CREB and expression of neuropeptides, *Eleutheroside E* increases long-term memory of radiation-damaged *C. elegans* in AIM and AWC neurons, respectively [114].

Signaling through cAMP/PKA also modulates axonal regeneration in many systems. In response to injury, cAMP/PKA-dependent phosphorylation activates the transcription factor ETS-4, which interacts with CEBP-1 to upregulate the expression of the receptor tyrosine kinase SHV-1. Activation of *svh-2* expression requires simultaneous Ca²⁺ signaling and activation of the p38 MAPK pathway. SVH-2 then activates the JNK MAPK pathway, which stimulates axon regeneration (Figure 5) [115].

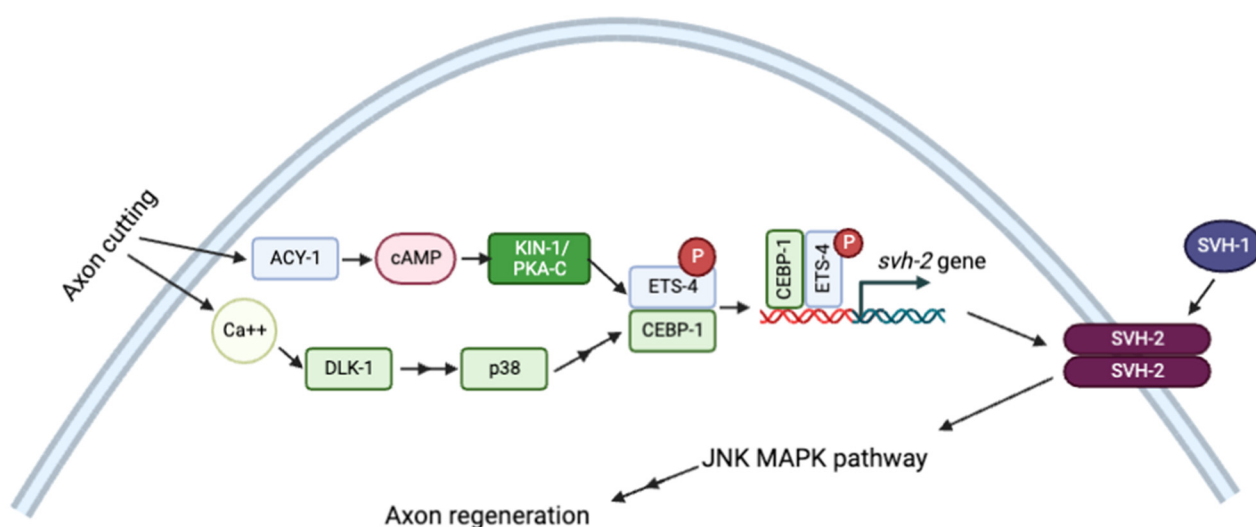


Figure 5. Schematic representation of axon regeneration model through JNK MAPK pathway by Ca²⁺ and cAMP signaling pathways. Adapted with permission from [115], 2022, Creative Commons. Created with BioRender.com (accessed on 23 October 2022).

10. PKA Action in Neurons Regulates *C. elegans* Physiology

In addition to the effects on lifespan, movement, and behavior, PKA action in neurons regulate a wide variety of different processes in *C. elegans*. For example, the neuromodulator serotonin (5-hydroxytryptamine, 5-HT), released by maternal neurons upon stress, can activate the transcription factor heat shock factor 1 (HSF-1) through PKA signaling in the germline, mediating the histone chaperone FACT (facilitates chromatin transcription) and promoting viability and future stress tolerance. For example, embryos produced from the heat-shocked mothers have more protective mRNA and are better able to tolerate high temperatures as larvae. [116].

In vertebrates, melatonin, which influences circadian rhythms, is produced by arylalkylamine N-acetyltransferase (AA-NAT) and N-acetylserotonin methyltransferase (ASMT) [117]. The AA-NATs are broadly expressed, including in many neurons. Light inhibits AA-NAT activity, allowing for a day/night rhythm. In dark–light conditions, *C. elegans* also produces a rhythmic pattern of melatonin levels [118]. Nine putative *C. elegans* AA-NATs were found with PKA phosphorylation sites [118], providing a possible mechanism by which PKA could regulate circadian rhythms.

KIN-29 is a serine/threonine kinase of the SIK (salt-inducible kinase) family that regulates chemoreceptor gene expression by phosphorylating and inhibiting histone deacetylases [119]. cAMP is produced in the CAN (canal-associated neurons) and dissociates from the CANs through gap junctions to the target cells to regulate PKA and KIN-29, which in turn, regulates larval development. When KIN-29 is present, larval development is inhibited. PKA inhibition of KIN-29 is necessary for larval development to proceed [120].

11. Immunity

The KIN-1/PKA-C pathway is critical for *C. elegans* immune response to infection by *S. enterica*, *P. aeruginosa*, and *S. aureus*. The adenylyl cyclase ACY-1 regulates the innate immune response to pathogens through activation of KIN-1/PKA-C [121]. Neuronal-specific knockdown of *kin-1* by RNAi contributes to a decline in the survival rate of WT worms infected with *S. enterica* and inhibition in the expression of some antimicrobial and lysosomal genes. KIN-1 upregulates antimicrobial genes including lysozymes, caenopores, C-type lectins, caenacins, and genes of the pqn family, among other factors. The lysosomal pathway mediates the downstream effects of PKA/KIN-1 signaling and controls autophagic flux and the lysosomal degradation rate. KIN-1/PKA-C action in the nervous system is critical for innate immunity, perhaps via release of an unknown signal that triggers these pathways in the intestine and epidermis [121].

12. Conclusions

PKA is a pleiotropic cellular regulator that wields powerful effects on diverse biological processes. Much has been done to elucidate the role of PKA in *C. elegans*. PKA plays vital roles in fertility, lipid metabolism, mitochondrial function and lifespan, and *C. elegans* behaviors and physiology. Because PKA activity is required for such a broad set of roles, PKA activity is tightly controlled. Several different mechanisms contribute to specificity in PKA signaling, including expression of specific isoforms, protein modifications that affect intracellular targeting, and binding of PKA-R subunits to A kinase-anchoring proteins (AKAPs), which control signaling output by enabling association with specific effectors, facilitating spatial and temporal compartmentalization of PKA signaling.

The relative simplicity and genetic tractability of the *C. elegans* offers an opportunity for further discovery of novel regulators and effectors of PKA signaling. For example, the large, polarized, and easily visible cells of the spermatheca offer an opportunity to observe KIN-2/PKA-R localization during ovulation and oocyte transit, and to assess the dependency of this localization on AKAP-1, ERM-1, and/or yet to be identified factors that may function as AKAPs in *C. elegans*. In addition, the *C. elegans* somatic gonad, comprised of gonadal sheath cells, spermathecal cells, sp-ut valve and uterus, offers an excellent system for genetic screens that will improve our understanding how PKA regulates coordinated

Ca²⁺ signaling between and among cell types in a tissue. Of particular interest is how Ca²⁺ release is inhibited by PKA in some cell types and stimulated in others. As in previous work, paradigms identified in *C. elegans* may apply across organisms in this well conserved signaling pathway.

Funding: This work was supported by a grant from the National Institutes of Health National Institute of General Medical Sciences (GM110268) to E.J.C. (<https://www.nigms.nih.gov/>).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Lee, J.H.; Han, J.S.; Kong, J.; Ji, Y.; Lv, X.; Lee, J.; Li, P.; Kim, J.B. Protein Kinase a Subunit Balance Regulates Lipid Metabolism in *Caenorhabditis elegans* and Mammalian Adipocytes. *J. Biol. Chem.* **2016**, *291*, 20315–20328. [[CrossRef](#)]
- Howe, A.K. Regulation of Actin-Based Cell Migration by CAMP/PKA. *Biochim. Biophys. Acta Mol. Cell Res.* **2004**, *1692*, 159–174. [[CrossRef](#)] [[PubMed](#)]
- Billington, C.K.; Ojo, O.O.; Penn, R.B.; Ito, S. CAMP Regulation of Airway Smooth Muscle Function. *Pulm. Pharmacol. Ther.* **2013**, *26*, 112–120. [[CrossRef](#)]
- Torres-Quesada, O.; Mayrhofer, J.E.; Stefan, E. The Many Faces of Compartmentalized PKA Signalosomes. *Cell. Signal.* **2017**, *37*, 1–11. [[CrossRef](#)] [[PubMed](#)]
- Taylor, S.S.; Knighton, D.R.; Zheng, J.; Ten Eyck, L.F.; Sowadski, J.M. Structural Framework for the Protein Kinase Family. *Annu. Rev. Cell Biol.* **1992**, *8*, 429–462. [[CrossRef](#)] [[PubMed](#)]
- Edelman, A.M.; Blumenthal, D.K.; Krebs, E.G. Protein Serine/Threonine Kinases. *Annu. Rev. Biochem.* **1987**, *56*, 567–613. [[CrossRef](#)]
- Feliciello, A.; Gottesman, M.E.; Avvedimento, E.V. The Biological Functions of A-Kinase Anchor Proteins. *J. Mol. Biol.* **2001**, *308*, 99–114. [[CrossRef](#)]
- Johnson, D.A.; Akamine, P.; Radzio-Andzelm, E.; Madhusudan; Taylor, S.S. Dynamics of CAMP-Dependent Protein Kinase. *Chem. Rev.* **2001**, *101*, 2243–2270. [[CrossRef](#)]
- Gangal, M.; Clifford, T.; Deich, J.; Cheng, X.; Taylor, S.S.; Johnson, D.A. Mobilization of the A-Kinase N-Myristate through an Isoform-Specific Intermolecular Switch. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12394–12399. [[CrossRef](#)]
- Gross, R.E.; Bagchi, S.; Lu, X.; Rubin, C.S. Cloning, Characterization, and Expression of the Gene for the Catalytic Subunit of CAMP-Dependent Protein Kinase in *Caenorhabditis elegans*. Identification of Highly Conserved and Unique Isoforms Generated by Alternative Splicing. *J. Biol. Chem.* **1990**, *265*, 6896–6907. [[CrossRef](#)]
- Gao, X.; Jin, C.; Ren, J.; Yao, X.; Xue, Y. Proteome-Wide Prediction of PKA Phosphorylation Sites in Eukaryotic Kingdom. *Genomics* **2008**, *92*, 457–463. [[CrossRef](#)] [[PubMed](#)]
- Lu, X.; Gross, R.E.; Bagchi, S.; Rubin, C.S. Cloning, Structure, and Expression of the Gene for a Novel Regulatory Subunit of CAMP-Dependent Protein Kinase in *Caenorhabditis elegans*. *J. Biol. Chem.* **1990**, *265*, 3293–3303. [[CrossRef](#)]
- Bowen, L.C.; Bicknell, A.V.; Tabish, M.; Clegg, R.A.; Rees, H.H.; Fisher, M.J. Expression of Multiple Isoforms of the CAMP-Dependent Protein Kinase (PK-A) Catalytic Subunit in the Nematode, *Caenorhabditis elegans*. *Cell. Signal.* **2006**, *18*, 2230–2237. [[CrossRef](#)]
- Tabish, M.; Clegg, R.A.; Rees, H.H.; Fisher, M.J. Organization and Alternative Splicing of the *Caenorhabditis elegans* CAMP-Dependent Protein Kinase Catalytic-Subunit Gene (Kin-1). *Biochem. J.* **1999**, *339*, 209–216. [[CrossRef](#)] [[PubMed](#)]
- Murray, P.; Clegg, R.A.; Rees, H.H.; Fisher, M.J. SiRNA-Mediated Knockdown of a Splice Variant of the PK-A Catalytic Subunit Gene Causes Adult-Onset Paralysis in *C. elegans*. *Gene* **2008**, *408*, 157–163. [[CrossRef](#)] [[PubMed](#)]
- Clegg, R.A.; Bowen, L.C.; Bicknell, A.V.; Tabish, M.; Prescott, M.C.; Rees, H.H.; Fisher, M.J. Characterisation of the N¹ Isoform of the Cyclic AMP-Dependent Protein Kinase (PK-A) Catalytic Subunit in the Nematode, *Caenorhabditis elegans*. *Arch. Biochem. Biophys.* **2012**, *519*, 38–45. [[CrossRef](#)]
- Pastok, M.W.; Prescott, M.C.; Dart, C.; Murray, P.; Rees, H.H.; Fisher, M.J. Structural Diversity of the CAMP-Dependent Protein Kinase Regulatory Subunit in *Caenorhabditis elegans*. *Cell. Signal.* **2013**, *25*, 168–177. [[CrossRef](#)]
- Horvat, S.J.; Deshpande, D.A.; Yan, H.; Panettieri, R.A.; Codina, J.; DuBose, T.D.; Xin, W.; Rich, T.C.; Penn, R.B. A-Kinase Anchoring Proteins Regulate Compartmentalized CAMP Signaling in Airway Smooth Muscle. *FASEB J.* **2012**, *26*, 3670–3679. [[CrossRef](#)]
- Edwards, A.S.; Scott, J.D. A-Kinase Anchoring Proteins: Protein Kinase A and Beyond. *Curr. Opin. Cell Biol.* **2000**, *12*, 217–221. [[CrossRef](#)]
- Langeberg, L.K.; Scott, J.D. A-Kinase-Anchoring Proteins. *J. Cell Sci.* **2005**, *118*, 3217–3220. [[CrossRef](#)]

21. Angelo, R.G.; Rubin, C.S. Characterization of Structural Features That Mediate the Tethering of *Caenorhabditis elegans* Protein Kinase A to a Novel A Kinase Anchor Protein. Insights into the Anchoring of PKAI Isoforms. *J. Biol. Chem.* **2000**, *275*, 4351–4362. [[CrossRef](#)] [[PubMed](#)]
22. Curran, S.P.; Ruvkun, G. Lifespan Regulation by Evolutionarily Conserved Genes Essential for Viability. *PLoS Genet.* **2007**, *3*, e56. [[CrossRef](#)] [[PubMed](#)]
23. Dransfield, D.T.; Bradford, A.J.; Smith, J.; Martin, M.; Roy, C.; Mangeat, P.H.; Goldenring, J.R. Ezrin Is a Cyclic AMP-Dependent Protein Kinase Anchoring Protein. *EMBO J.* **1997**, *16*, 35–43. [[CrossRef](#)] [[PubMed](#)]
24. van Furden, D.; Johnson, K.; Segbert, C.; Bossinger, O. The *C. elegans* Ezrin-Radixin-Moesin Protein ERM-1 Is Necessary for Apical Junction Remodelling and Tubulogenesis in the Intestine. *Dev. Biol.* **2004**, *272*, 262–276. [[CrossRef](#)]
25. Ramalho, J.J.; Sepers, J.J.; Nicolle, O.; Schmidt, R.; Cravo, J.; Michaux, G.; Boxem, M. C-Terminal Phosphorylation Modulates ERM-1 Localization and Dynamics to Control Cortical Actin Organization and Support Lumen Formation during *Caenorhabditis elegans* Development. *Development* **2020**, *147*, dev188011. [[CrossRef](#)]
26. Sassone-Corsi, P. The Cyclic AMP Pathway. *Cold Spring Harb. Perspect. Biol.* **2012**, *4*, a011148. [[CrossRef](#)]
27. Bastiani, C.; Mendel, J. *Heterotrimeric G Proteins in C. elegans*; WormBook, Ed.; The *C. elegans* Research Community; WormBook: Pasadena, CA, USA, 2016. Available online: www.wormbook.org (accessed on 23 October 2022).
28. Oldham, W.M.; Hamm, H.E. Heterotrimeric G Protein Activation by G-Protein-Coupled Receptors. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 60–71. [[CrossRef](#)]
29. DiGiacomo, V.; Marivin, A.; Garcia-Marcos, M. When Heterotrimeric G Proteins Are Not Activated by G Protein-Coupled Receptors: Structural Insights and Evolutionary Conservation. *Biochemistry* **2018**, *57*, 255–257. [[CrossRef](#)]
30. Syrovatkina, V.; Alegre, K.O.; Dey, R.; Huang, X.Y. Regulation, Signaling, and Physiological Functions of G-Proteins. *J. Mol. Biol.* **2016**, *428*, 3850–3868. [[CrossRef](#)]
31. Srinivasan, D.G.; Fisk, R.M.; Xu, H.; Van den Heuvel, S. A Complex of LIN-5 and GPR Proteins Regulates G Protein Signaling and Spindle Function in *C. elegans*. *Genes Dev.* **2003**, *17*, 1225–1239. [[CrossRef](#)]
32. Koelle, M.R. Heterotrimeric G Protein Signaling: Getting inside the Cell. *Cell* **2006**, *126*, 25–27. [[CrossRef](#)] [[PubMed](#)]
33. Neves, S.R.; Ram, P.T.; Iyengar, R. G Protein Pathways. *Science* **2002**, *296*, 1636–1639. [[CrossRef](#)] [[PubMed](#)]
34. van der Voorn, L.; Gebbink, M.; Plasterk, R.H.A.; Ploegh, H.L. Characterization of a G-Protein β -Subunit Gene from the Nematode *Caenorhabditis elegans*. *J. Mol. Biol.* **1990**, *213*, 17–26. [[CrossRef](#)]
35. Zwaal, R.R.; Ahringer, J.; van Luenen, H.G.A.M.; Rushforth, A.; Anderson, P.; Plasterk, R.H.A. G Proteins Are Required for Spatial Orientation of Early Cell Cleavages in *C. elegans* Embryos. *Cell* **1996**, *86*, 619–629. [[CrossRef](#)]
36. Jansen, G.; Weinkove, D.; Plasterk, R.H.A. The G-Protein γ Subunit Gpc-1 of the Nematode *C. elegans* Is Involved in Taste Adaptation. *EMBO J.* **2002**, *21*, 986–994. [[CrossRef](#)] [[PubMed](#)]
37. Krapivinsky, G.; Krapivinsky, L.; Wickman, K.; Clapham, D.E. $G\beta\gamma$ Binds Directly to the G Protein-Gated K^+ Channel, I(KACH). *J. Biol. Chem.* **1995**, *270*, 29059–29062. [[CrossRef](#)]
38. Dascal, N. Ion-Channel Regulation by G Proteins. *Trends Endocrinol. Metab.* **2001**, *12*, 391–398. [[CrossRef](#)]
39. Herlitze, S.; Garcla, D.E.; Mackle, K.; Hille, B.; Scheuer, T.; Catterall, W.A. Modulation of Ca^{2+} Channels by G-Protein By Subunits. *Nature* **1996**, *380*, 258–262. [[CrossRef](#)]
40. Tang, W.J.; Gilman, A.G. Type-Specific Regulation of Adenylyl Cyclase by G Protein By Subunits. *Science* **1991**, *254*, 1500–1503. [[CrossRef](#)]
41. van der Linden, A.M.; Simmer, F.; Cuppen, E.; Plasterk, R.H. The G-Protein Beta-Subunit GPB-2 in *Caenorhabditis elegans* Regulates the G(o)Alpha-G(q)Alpha Signaling Network through Interactions with the Regulator of G-Protein Signaling Proteins EGL-10 and EAT-16. *Genetics* **2001**, *158*, 221–235. [[CrossRef](#)]
42. Koelle, M.R.; Horvitz, H.R. EGL-10 Regulates G Protein Signaling in the *C. elegans* Nervous System and Shares a Conserved Domain with Many Mammalian Proteins. *Cell* **1996**, *84*, 115–125. [[CrossRef](#)]
43. Shyn, S.I.; Kerr, R.; Schafer, W.R. Serotonin and Go Modulate Functional States of Neurons and Muscles Controlling *C. elegans* Egg-Laying Behavior. *Curr. Biol.* **2003**, *13*, 1910–1915. [[CrossRef](#)] [[PubMed](#)]
44. Castaneda, P.G.; Cecchetelli, A.D.; Pettit, H.N.; Cram, E.J. $G\alpha/GSA-1$ Works Upstream of PKA/KIN-1 to Regulate Calcium Signaling and Contractility in the *Caenorhabditis elegans* Spermatheca. *PLoS Genet.* **2020**, *16*, e1008644. [[CrossRef](#)] [[PubMed](#)]
45. Berridge, M.J.; Lipp, P.; Bootman, M.D. The Versatility and Universality of Calcium Signalling. *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 11–21. [[CrossRef](#)] [[PubMed](#)]
46. Primeau, J.O.; Armanious, G.P.; Fisher, M.L.E.; Young, H.S. The Sarcoplasmic Reticulum Calcium ATPase. In *Subcellular Biochemistry*; Springer: New York, NY, USA, 2018; Volume 87, pp. 229–258.
47. Burdyga, T.; Paul, R.J. Calcium Homeostasis and Signaling in Smooth Muscle. In *Muscle*; Elsevier Inc.: Amsterdam, The Netherlands, 2012; Volume 2, pp. 1155–1171, ISBN 9780123815101.
48. Evans, W.H.; Martin, P.E.M. Gap Junctions: Structure and Function (Review). *Mol. Membr. Biol.* **2002**, *19*, 121–136. [[CrossRef](#)]
49. Bootman, M.D.; Collins, T.J.; Peppiatt, C.M.; Prothero, L.S.; MacKenzie, L.; De Smet, P.; Travers, M.; Tovey, S.C.; Seo, J.T.; Berridge, M.J.; et al. Calcium Signalling—An Overview. *Semin. Cell Dev. Biol.* **2001**, *12*, 3–10. [[CrossRef](#)]
50. Szabadkai, G.; Duchen, M.R. Mitochondria: The Hub of Cellular Ca^{2+} Signaling. *Physiology* **2008**, *23*, 84–94. [[CrossRef](#)]

51. Choi, S.; Kim, H.J.; Ko, Y.S.; Jeong, S.W.; Kim, Y.I.; Simonds, W.F.; Oh, J.W.; Nah, S.Y. $G\alpha_q/11$ Coupled to Mammalian Phospholipase C B3-like Enzyme Mediates the Ginsenoside Effect on Ca^{2+} -Activated Cl^- Current in the Xenopus Oocyte. *J. Biol. Chem.* **2001**, *276*, 48797–48802. [[CrossRef](#)]
52. Hofer, M.A. Interactions Between Calcium and CAMP Signaling. *Curr. Med. Chem.* **2012**, *19*, 5768–5773. [[CrossRef](#)]
53. Desouza, N.; Reiken, S.; Ondrias, K.; Yang, Y.M.; Matkovich, S.; Marks, A.R. Protein Kinase A and Two Phosphatases Are Components of the Inositol 1,4,5-Trisphosphate Receptor Macromolecular Signaling Complex. *J. Biol. Chem.* **2002**, *277*, 39397–39400. [[CrossRef](#)]
54. Dulhunty, A.F.; Beard, N.A.; Casarotto, M.G. Recent Advances in Understanding the Ryanodine Receptor Calcium Release Channels and Their Role in Calcium Signalling. *F1000Research* **2018**, *7*. [[CrossRef](#)] [[PubMed](#)]
55. Marx, S.O.; Reiken, S.; Hisamatsu, Y.; Jayaraman, T.; Burkhoff, D.; Rosembli, N.; Marks, A.R. PKA Phosphorylation Dissociates FKBP12.6 from the Calcium Release Channel (Ryanodine Receptor): Defective Regulation in Failing Hearts. *Cell* **2000**, *101*, 365–376. [[CrossRef](#)]
56. van Goor, M.K.; Verkaart, S.; van Dam, T.J.; Huynen, M.A.; van der Wijst, J. Interspecies Differences in PTH-Mediated PKA Phosphorylation of the Epithelial Calcium Channel TRPV5. *Pflug. Arch. Eur. J. Physiol.* **2017**, *469*, 1301–1311. [[CrossRef](#)] [[PubMed](#)]
57. Oestreich, E.A.; Wang, H.; Malik, S.; Kaproth-Joslin, K.A.; Blaxall, B.C.; Kelley, G.G.; Dirksen, R.T.; Smrcka, A.V.; Goonasekera, S.A. Epac and Phospholipase Cepsilon Regulate Ca^{2+} Release in the Heart by Activation of Protein Kinase Cepsilon and Calcium-Calmodulin Kinase II. *J. Biol. Chem.* **2007**, *282*, 1514–1522.
58. Oestreich, E.A.; Wang, H.; Malik, S.; Kaproth-Joslin, K.A.; Blaxall, B.C.; Kelley, G.G.; Dirksen, R.T.; Smrcka, A.V. Epac-Mediated Activation of Phospholipase C ϵ Plays a Critical Role in β -Adrenergic Receptor-Dependent Enhancement of Ca^{2+} Mobilization in Cardiac Myocytes. *J. Biol. Chem.* **2007**, *282*, 5488–5495. [[CrossRef](#)] [[PubMed](#)]
59. Supattapone, S.; Danoff, S.K.; Theibert, A.; Joseph, S.K.; Steiner, J.; Snyder, S.H. Cyclic AMP-Dependent Phosphorylation of a Brain Inositol Trisphosphate Receptor Decreases Its Release of Calcium. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 8747–8750. [[CrossRef](#)]
60. Tada, M.; Toyofuku, T. SR Ca^{2+} -ATPase/Phospholamban in Cardiomyocyte Function. *J. Card. Fail.* **1996**, *2*, S77–S85. [[CrossRef](#)]
61. Nakagawa, T.; Yokoe, S.; Asahi, M. Phospholamban Degradation Is Induced by Phosphorylation-Mediated Ubiquitination and Inhibited by Interaction with Cardiac Type Sarco(Endo)Plasmic Reticulum Ca^{2+} -ATPase. *Biochem. Biophys. Res. Commun.* **2016**, *472*, 523–530. [[CrossRef](#)]
62. Nalli, A.D.; Kumar, D.P.; Al-Shboul, O.; Mahavadi, S.; Kuemmerle, J.F.; Grider, J.R.; Murthy, K.S. Regulation of $G\beta\gamma$ -Dependent PLC-B3 Activity in Smooth Muscle: Inhibitory Phosphorylation of PLC-B3 by PKA and PKG and Stimulatory Phosphorylation of $G\alpha_i$ -GTPase-Activating Protein RGS2 by PKG. *Cell Biochem. Biophys.* **2014**, *70*, 867–880. [[CrossRef](#)]
63. Halls, M.L.; Cooper, D.M.F. Regulation by Ca^{2+} -Signaling Pathways of Adenylyl Cyclases. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, 1–22. [[CrossRef](#)]
64. Taylor, C.W. Regulation of IP3 Receptors by Cyclic AMP. *Cell Calcium* **2017**, *63*, 48–52. [[CrossRef](#)]
65. Wang, H.; Sieburth, D. PKA Controls Calcium Influx into Motor Neurons during a Rhythmic Behavior. *PLoS Genet.* **2013**, *9*, e1003831. [[CrossRef](#)]
66. Branicky, R.; Hekimi, S. What Keeps *C. elegans* Regular: The Genetics of Defecation. *Trends Genet.* **2006**, *22*, 571–579. [[CrossRef](#)] [[PubMed](#)]
67. Beg, A.A.; Jorgensen, E.M. EXP-1 Is an Excitatory GABA-Gated Cation Channel. *Nat. Neurosci.* **2003**, *6*, 1145–1152. [[CrossRef](#)] [[PubMed](#)]
68. Wang, H.; Girskis, K.; Janssen, T.; Chan, J.P.; Dasgupta, K.; Knowles, J.A.; Schoofs, L.; Sieburth, D. Neuropeptide Secreted from a Pacemaker Activates Neurons to Control a Rhythmic Behavior. *Curr. Biol.* **2013**, *23*, 746–754. [[CrossRef](#)] [[PubMed](#)]
69. Dal Santo, P.; Logan, M.A.; Chisholm, A.D.; Jorgensen, E.M. The Inositol Trisphosphate Receptor Regulates a 50-Second Behavioral Rhythm in *C. elegans*. *Cell* **1999**, *98*, 757–767. [[CrossRef](#)]
70. Kovacevic, I.; Orozco, J.M.; Cram, E.J. Filamin and Phospholipase C- ϵ Are Required for Calcium Signaling in the *Caenorhabditis elegans* Spermatheca. *PLoS Genet.* **2013**, *9*, e1003510. [[CrossRef](#)]
71. Yanik, M.F.; Cinar, H.; Cinar, H.N.; Chisholm, A.D.; Jin, Y.; Ben-Yakar, A. Functional Regeneration after Laser Axotomy. *Nature* **2004**, *432*, 822. [[CrossRef](#)]
72. Wu, Z.; Ghosh-Roy, A.; Yanik, M.F.; Zhang, J.Z.; Jin, Y.; Chisholm, A.D. *Caenorhabditis elegans* Neuronal Regeneration Is Influenced by Life Stage, Ephrin Signaling, and Synaptic Branching. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15132–15137. [[CrossRef](#)]
73. Ghosh-Roy, A.; Wu, Z.; Goncharov, A.; Jin, Y.; Chisholm, A.D. Calcium and Cyclic AMP Promote Axonal Regeneration in *Caenorhabditis elegans* and Require DLK-1 Kinase. *J. Neurosci.* **2010**, *30*, 3175–3183. [[CrossRef](#)]
74. Chung, S.H.; Awal, M.R.; Shay, J.; McLoed, M.M.; Mazur, E.; Gabel, C.V. Novel DLK-Independent Neuronal Regeneration in *Caenorhabditis elegans* Shares Links with Activity-Dependent Ectopic Outgrowth. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E2852–E2860. [[CrossRef](#)]
75. Miller, M.A.; Nguyen, V.Q.; Lee, M.H.; Kosinski, M.; Schedl, T.; Caprioli, R.M.; Greenstein, D. A Sperm Cytoskeletal Protein That Signals Oocyte Meiotic Maturation and Ovulation. *Science* **2001**, *291*, 2144–2147. [[CrossRef](#)]
76. McCarter, J.; Bartlett, B.; Dang, T.; Schedl, T. On the Control of Oocyte Meiotic Maturation and Ovulation in *Caenorhabditis elegans*. *Dev. Biol.* **1999**, *205*, 111–128. [[CrossRef](#)] [[PubMed](#)]
77. Kosinski, M.; McDonald, K.; Schwartz, J.; Yamamoto, I.; Greenstein, D.C. *elegans* Sperm Bud Vesicles to Deliver a Meiotic Maturation Signal to Distant Oocytes. *Development* **2005**, *132*, 3357–3369. [[CrossRef](#)] [[PubMed](#)]

78. Govindan, J.A.; Nadarajan, S.; Kim, S.; Starich, T.A.; Greenstein, D. Somatic CAMP Signaling Regulates MSP-Dependent Oocyte Growth and Meiotic Maturation in *C. elegans*. *Development* **2009**, *136*, 2211–2221. [[CrossRef](#)] [[PubMed](#)]
79. Govindan, J.A.; Cheng, H.; Harris, J.E.; Greenstein, D. $G\alpha/i$ and $G\alpha/s$ Signaling Function in Parallel with the MSP/Eph Receptor to Control Meiotic Diapause in *C. elegans*. *Curr. Biol.* **2006**, *16*, 1257–1268. [[CrossRef](#)] [[PubMed](#)]
80. Kim, S.; Govindan, J.A.; Tu, Z.J.; Greenstein, D. SACY-1 DEAD-Box Helicase Links the Somatic Control of Oocyte Meiotic Maturation to the Sperm-to-Oocyte Switch and Gamete Maintenance in *Caenorhabditis elegans*. *Genetics* **2012**, *192*, 905–928. [[CrossRef](#)]
81. Huelgas-Morales, G.; Greenstein, D. Control of Oocyte Meiotic Maturation in *C. elegans*. *Semin. Cell Dev. Biol.* **2018**, *84*, 90–99. [[CrossRef](#)] [[PubMed](#)]
82. Cannon, B.; Nedergaard, J.A. Brown Adipose Tissue: Function and Physiological Significance. *Physiol. Rev.* **2004**, *84*, 277–359. [[CrossRef](#)]
83. van Marken Lichtenbelt, W.D.; Schrauwen, P. Implications of Nonshivering Thermogenesis for Energy Balance Regulation in Humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *301*, R285–R296. [[CrossRef](#)]
84. Xiao, R.; Zhang, B.; Dong, Y.; Gong, J.; Xu, T.; Liu, J.; Xu, X.S. A Genetic Program Promotes *C. elegans* Longevity at Cold Temperatures via a Thermosensitive TRP Channel. *Cell* **2013**, *152*, 806–817. [[CrossRef](#)] [[PubMed](#)]
85. Liu, F.; Xiao, Y.; Ji, X.L.; Zhang, K.Q.; Zou, C.G. The CAMP-PKA Pathway-Mediated Fat Mobilization Is Required for Cold Tolerance in *C. elegans*. *Sci. Rep.* **2017**, *7*, 638. [[CrossRef](#)]
86. Lee, J.H.; Kong, J.; Jang, J.Y.; Han, J.S.; Ji, Y.; Lee, J.; Kim, J.B. Lipid Droplet Protein LID-1 Mediates ATGL-1-Dependent Lipolysis during Fasting in *Caenorhabditis elegans*. *Mol. Cell. Biol.* **2014**, *34*, 4165–4176. [[CrossRef](#)]
87. Han, J.S.; Lee, J.H.; Kong, J.; Ji, Y.; Kim, J.; Choe, S.S.; Kim, J.B. Hypoxia Restrains Lipid Utilization via Protein Kinase A and Adipose Triglyceride Lipase Downregulation through Hypoxia-Inducible Factor. *Mol. Cell. Biol.* **2018**, *39*, e00390-18. [[CrossRef](#)] [[PubMed](#)]
88. Lin, S.J.; Defosse, P.A.; Guarente, L. Requirement of NAD and SIR2 for Life-Span Extension by Calorie Restriction in *Saccharomyces cerevisiae*. *Science* **2000**, *289*, 2126–2128. [[CrossRef](#)] [[PubMed](#)]
89. Tong, J.J.; Schriener, S.E.; McCleary, D.; Day, B.J.; Wallace, D.C. Life Extension through Neurofibromin Mitochondrial Regulation and Antioxidant Therapy for Neurofibromatosis-1 in *Drosophila melanogaster*. *Nat. Genet.* **2007**, *39*, 476–485. [[CrossRef](#)]
90. Enns, L.C.; Morton, J.F.; Treuting, P.R.; Emond, M.J.; Wolf, N.S.; McKnight, G.S.; Rabinovitch, P.S.; Ladiges, W.C. Disruption of Protein Kinase A in Mice Enhances Healthy Aging. *PLoS ONE* **2009**, *4*, e5963. [[CrossRef](#)]
91. Schmeisser, S.; Li, S.; Bouchard, B.; Ruiz, M.; Des Rosiers, C.; Roy, R. Muscle-Specific Lipid Hydrolysis Prolongs Lifespan through Global Lipidomic Remodeling. *Cell Rep.* **2019**, *29*, 4540–4552.e8. [[CrossRef](#)]
92. Kim, H.; Scimia, M.C.; Wilkinson, D.; Trelles, R.D.; Wood, M.R.; Bowtell, D.; Dillin, A.; Mercola, M.; Ronai, Z.A. Fine-Tuning of Drp1/Fis1 Availability by AKAP121/Siah2 Regulates Mitochondrial Adaptation to Hypoxia. *Mol. Cell* **2011**, *44*, 532–544. [[CrossRef](#)]
93. Hoppins, S.; Lackner, L.; Nunnari, J. The Machines That Divide and Fuse Mitochondria. *Annu. Rev. Biochem.* **2007**, *76*, 751–780. [[CrossRef](#)]
94. Venz, R.; Pekec, T.; Katic, I.; Ciosk, R.; Ewald, C.Y. End-of-Life Targeted Degradation of DAF-2 Insulin/IGF-1 Receptor Promotes Longevity Free from Growth-Related Pathologies. *eLife* **2021**, *10*, e71335. [[CrossRef](#)]
95. Gioran, A.; Piazzesi, A.; Bertan, F.; Schroer, J.; Wischhof, L.; Nicotera, P.; Bano, D. Multi-omics Identify Xanthine as a Pro-survival Metabolite for Nematodes with Mitochondrial Dysfunction. *EMBO J.* **2019**, *38*, e99558. [[CrossRef](#)] [[PubMed](#)]
96. Vanfleteren, J.R.; De Vreese, A. Modulation of Kinase Activities in Dauers and in Long-Lived Mutants of *Caenorhabditis elegans*. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **1997**, *52*, B212–B216. [[CrossRef](#)] [[PubMed](#)]
97. Dehghan, E.; Goodarzi, M.; Saremi, B.; Lin, R.; Mirzaei, H. Hydralazine Targets CAMP-Dependent Protein Kinase Leading to Sirtuin1/5 Activation and Lifespan Extension in *C. elegans*. *Nat. Commun.* **2019**, *10*, 4905. [[CrossRef](#)]
98. Jia, F.; Chi, C.; Han, M. Regulation of Nucleotide Metabolism and Germline Proliferation in Response to Nucleotide Imbalance and Genotoxic Stresses by EndoU Nuclease. *Cell Rep.* **2020**, *30*, 1848–1861.e5. [[CrossRef](#)] [[PubMed](#)]
99. Gottschling, D.C.; Döring, F.; Lüersen, K. Locomotion Behavior Is Affected by the $G\alpha/s$ Pathway and the Two-Pore-Domain K^+ Channel TWK-7 Interacting in GABAergic Motor Neurons in *Caenorhabditis elegans*. *Genetics* **2017**, *206*, 283–297. [[CrossRef](#)] [[PubMed](#)]
100. Reynolds, N.K.; Schade, M.A.; Miller, K.G. Convergent, RIC-8-Dependent $G\alpha$ Signaling Pathways in the *Caenorhabditis elegans* Synaptic Signaling Network. *Genetics* **2005**, *169*, 651–670. [[CrossRef](#)] [[PubMed](#)]
101. Johnson, J.R.; Edwards, M.R.; Davies, H.; Newman, D.; Holden, W.; Jenkins, R.E.; Burgoyne, R.D.; Lucas, R.J.; Barclay, J.W. Ethanol Stimulates Locomotion via a $G\alpha/s$ -Signaling Pathway in IL2 Neurons in *Caenorhabditis elegans*. *Genetics* **2017**, *207*, 1023–1039. [[CrossRef](#)]
102. Awal, M.R.; Austin, D.; Florman, J.; Alkema, M.; Gabel, C.V.; Connor, C.W. Breakdown of Neural Function under Isoflurane Anesthesia: In Vivo, Multineuronal Imaging in *Caenorhabditis elegans*. *Anesthesiology* **2018**, *129*, 733–743. [[CrossRef](#)]
103. Saifee, O.; Metz, L.B.; Nonet, M.L.; Crowder, C.M. A Gain-of-Function Mutation in Adenylate Cyclase Confers Isoflurane Resistance in *Caenorhabditis elegans*. *Anesthesiology* **2011**, *115*, 1162–1171. [[CrossRef](#)]

104. Schade, M.A.; Reynolds, N.K.; Dollins, C.M.; Miller, K.G. Mutations That Rescue the Paralysis of *Caenorhabditis elegans* Ric-8 (Synembryon) Mutants Activate the G α s Pathway and Define a Third Major Branch of the Synaptic Signaling Network. *Genetics* **2005**, *169*, 631–649. [[CrossRef](#)] [[PubMed](#)]
105. Nagy, S.; Wright, C.; Tramm, N.; Labello, N.; Burov, S.; Biron, D. A Longitudinal Study of *Caenorhabditis elegans* Larvae Reveals a Novel Locomotion Switch, Regulated by G α s Signaling. *eLife* **2013**, *2013*, e00782. [[CrossRef](#)]
106. Belfer, S.J.; Chuang, H.S.; Freedman, B.L.; Yuan, J.; Norton, M.; Bau, H.H.; Raizen, D.M. Caenorhabditis-in-Drop Array for Monitoring *C. elegans* Quiescent Behavior. *Sleep* **2013**, *36*, 689–698. [[CrossRef](#)] [[PubMed](#)]
107. Trojanowski, N.F.; Raizen, D.M. Call It Worm Sleep. *Trends Neurosci.* **2016**, *39*, 54. [[CrossRef](#)] [[PubMed](#)]
108. Cianciulli, A.; Yoslov, L.; Buscemi, K.; Sullivan, N.; Vance, R.T.; Janton, F.; Szurgot, M.R.; Buerkert, T.; Li, E.; Nelson, M.D. Interneurons Regulate Locomotion Quiescence via Cyclic Adenosine Monophosphate Signaling During Stress-Induced Sleep in *Caenorhabditis elegans*. *Genetics* **2019**, *213*, 267–279. [[CrossRef](#)]
109. Mackiewicz, M.; Naidoo, N.; Zimmerman, J.E.; Pack, A.I. Molecular Mechanisms of Sleep and Wakefulness. *Ann. N. Y. Acad. Sci.* **2008**, *1129*, 335–349. [[CrossRef](#)]
110. Steuer Costa, W.; Yu, S.C.; Liewald, J.F.; Gottschalk, A. Fast CAMP Modulation of Neurotransmission via Neuropeptide Signals and Vesicle Loading. *Curr. Biol.* **2017**, *27*, 495–507. [[CrossRef](#)]
111. Zhou, K.M.; Dong, Y.M.; Ge, Q.; Zhu, D.; Zhou, W.; Lin, X.G.; Liang, T.; Wu, Z.X.; Xu, T. PKA Activation Bypasses the Requirement for UNC-31 in the Docking of Dense Core Vesicles from *C. elegans* Neurons. *Neuron* **2007**, *56*, 657–669. [[CrossRef](#)]
112. Rojo Romanos, T.; Petersen, J.G.; Pocock, R. Control of Neuropeptide Expression by Parallel Activity-Dependent Pathways in *Caenorhabditis elegans*. *Sci. Rep.* **2017**, *7*, 38734. [[CrossRef](#)]
113. Davydov, M.; Krikorian, A.D. *Eleutherococcus senticosus* (Rupr. and Maxim.) Maxim. (Araliaceae) as an Adaptogen: A Closer Look. *J. Ethnopharmacol.* **2000**, *72*, 345–393. [[CrossRef](#)]
114. Liu, M.; Xiong, Y.; Shan, S.; Zhu, Y.; Zeng, D.; Shi, Y.; Zhang, Y.; Lu, W. Eleutheroside e Enhances the Long-Term Memory of Radiation-Damaged *C. elegans* through G-Protein-Coupled Receptor and Neuropeptide Signaling Pathways. *J. Nat. Prod.* **2020**, *83*, 3315–3323. [[CrossRef](#)] [[PubMed](#)]
115. Li, C.; Hisamoto, N.; Matsumoto, K. Axon Regeneration Is Regulated by Ets-C/EBP Transcription Complexes Generated by Activation of the CAMP/Ca²⁺ Signaling Pathways. *PLoS Genet.* **2015**, *11*, e1005603. [[CrossRef](#)] [[PubMed](#)]
116. Das, S.; Ooi, F.K.; Corchado, J.C.; Fuller, L.C.; Weiner, J.A.; Prahlad, V. Serotonin Signaling by Maternal Neurons upon Stress Ensures Progeny Survival. *eLife* **2020**, *9*, e55246. [[CrossRef](#)] [[PubMed](#)]
117. Berra, B.; Rizzo, A.M. Melatonin: Circadian Rhythm Regulator, Chronobiotic, Antioxidant and Beyond. *Clin. Dermatol.* **2009**, *27*, 202–209. [[CrossRef](#)] [[PubMed](#)]
118. Migliori, M.L.; Romanowski, A.; Simonetta, S.H.; Valdez, D.; Guido, M.; Golombek, D.A. Daily Variation in Melatonin Synthesis and Arylalkylamine N-Acetyltransferase Activity in the Nematode *Caenorhabditis elegans*. *J. Pineal Res.* **2012**, *53*, 38–46. [[CrossRef](#)] [[PubMed](#)]
119. Van Der Linden, A.M.; Wiener, S.; You, N.J.; Kim, K.; Avery, L.; Sengupta, P. The EGL-4 PKG Acts with KIN-29 Salt-Inducible Kinase and Protein Kinase A to Regulate Chemoreceptor Gene Expression and Sensory Behaviors in *Caenorhabditis elegans*. *Genetics* **2008**, *180*, 1475–1491. [[CrossRef](#)] [[PubMed](#)]
120. Chien, J.; Wolf, F.W.; Grosche, S.; Yosef, N.; Garriga, G.; Mörck, C. The Enigmatic Canal-Associated Neurons Regulate *Caenorhabditis elegans* Larval Development through a CAMP Signaling Pathway. *Genetics* **2019**, *213*, 1465–1478. [[CrossRef](#)]
121. Xiao, Y.; Liu, F.; Zhao, P.J.; Zou, C.G.; Zhang, K.Q. PKA/KIN-1 Mediates Innate Immune Responses to Bacterial Pathogens in *Caenorhabditis elegans*. *Innate Immun.* **2017**, *23*, 656–666. [[CrossRef](#)]