Focus Article

The inositol 1,4,5-trisphosphate receptor in *C. elegans*

Erik Allman,[1†] Baskaran Thyagarajan[2†] and Keith Nehrke[1,2*]

The soil nematode *Caenorhabditis elegans* is a genetic model organism whose cellular physiology is closely related to that of mammals, with many signaling cascades and second messengers mirroring those found in higher organisms. Due to the genetic, anatomical, and behavioral simplicity of worms, integrative physiological techniques are relatively straightforward and represent a powerful approach to understand the molecular mechanisms underlying more complex system functions. Studies of the nematode inositol 1,4,5-trisphosphate receptor ($InsP_3R$) have led to advances in our understanding of its role in development and behavior. Unlike mammals, which express three $InsP_3R$ isoforms, nematodes express only a single $InsP_3R$ coded for by the *itr-1* gene. Forward genetic screens have resulted in the generation of conditional loss- and gain-of-function alleles of *itr-1*, and genetic epistasis analyses have provided insight into how the $InsP_3R$ integrates various signals to regulate morphogenesis, sensation, and rhythmic behaviors. Novel techniques in the worm model have helped to define cell-specific regulatory pathways that control $InsP_3$ signaling and have shed light on new roles for the $InsP_3R$ itself in systems physiology. © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

How to cite this article:
WIREs Membr Transp Signal 2012, 1:321–328. doi: 10.1002/wmts.25

INTRODUCTION

The inositol 1,4,5-trisphosphate receptor ($InsP_3R$) is an endoplasmic reticular Ca^{2+} release channel that functions in Ca^{2+} signaling cascades. Mammals contain three genes that code for $InsP_3R$ isoforms, termed types I, II, and III. Differences in expression of these three isoforms in specific cell types may help to diversify the function of this well-conserved signaling pathway.[1,2] However, such diversity makes it more difficult to determine how the regulation of individual receptor isoforms controls specific physiological outputs.

Previous studies have clearly demonstrated that many cell signaling mechanisms are highly conserved between mammals and simpler organisms such as flies and worms. Given the ubiquitous nature of Ca^{2+} as a second messenger, it is unsurprising that forward genetic screens in model organisms such as *Caenorhabditis elegans* have generated unbiased evidence that $InsP_3R$ signaling regulates a wide variety of physiological functions. These include developmental morphogenesis, neuronal signaling, as well as rhythmic behaviors such as pharyngeal pumping, oocyte fertilization, and defecation.

In contrast to mammals, worms contain a single gene coding for the $InsP_3R$. This important difference has facilitated straightforward genotype–phenotype analyses with respect to $InsP_3R$ function in this genetic model organism. In addition, worms have a simplified body architecture in which adult hermaphrodites possess ~1000 somatic cells that comprise a limited number of tissues. This simplicity coupled with an abundance of tissue-specific promoters can be used to discriminate between cell-autonomous and non-autonomous signaling output when studying gene function. In short, cell- or tissue-specific expression of a rescuing transgene can be driven in a mutant background, and its ability to complement the loss-of-function phenotype can be assessed. This approach can answer the question of where a gene such as the $InsP_3R$ functions, to fulfill its role in a given

†These authors contributed equally to this work.
*Correspondence to: Keith_Nehrke@urmc.rochester.edu
[1]Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY, USA
[2]Department of Medicine, University of Rochester Medical Center, Rochester, NY, USA

 © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

physiological output. Further genetic approaches can be combined with this technique to decipher the precise cell types in which genes that regulate $InsP_3R$ activity function.[3] This is particularly important when studying systems physiology, where interactions between multiple cell types can contribute to a single biological process. With this in mind, the purpose of this article is to focus on how genetic and integrative approaches in worms have contributed to our understanding of the $InsP_3R$ and its role in systems physiology.

ITR-1, THE InsP3 RECEPTOR IN *C. ELEGANS*

In *C. elegans*, the $InsP_3R$ is the product of a single gene, *itr-1* (formerly termed *dec-4*). The *itr-1* gene product is overall ~42% identical to the human type II $InsP_3R$ isoform, and notable regions of identity occur in both the $InsP_3$ binding and Ca^{2+} pore domains (Figure 1). Smaller shared motifs may represent binding sites for proteins such as Homer, FKBP12, ankyrin, and chromogranin A, as well as small molecules such as ATP, suggesting that regulation and/or scaffolding by these factors may be conserved as well. The recombinant ITR-1 $InsP_3$ binding domain also exhibits high affinity binding of 1,4,5-$InsP_3$ [$K_d = 7(\pm 4)$ nM], similar to that of the mammalian type I $InsP_3R$.[4]

The work of Gower et al.[6] led to the idea that tissue-restricted expression of $InsP_3R$ variants in worms could help determine their specificity of Ca^{2+} signaling. The *itr*-1 mRNA has a complex 5′ end, containing 3 alternative splice variants.[4] The expression of three mRNA variants was shown to be regulated by different promoters, and the resulting proteins were predicted to contain unique N-terminal sequences.[6] Furthermore, each of the three promoters

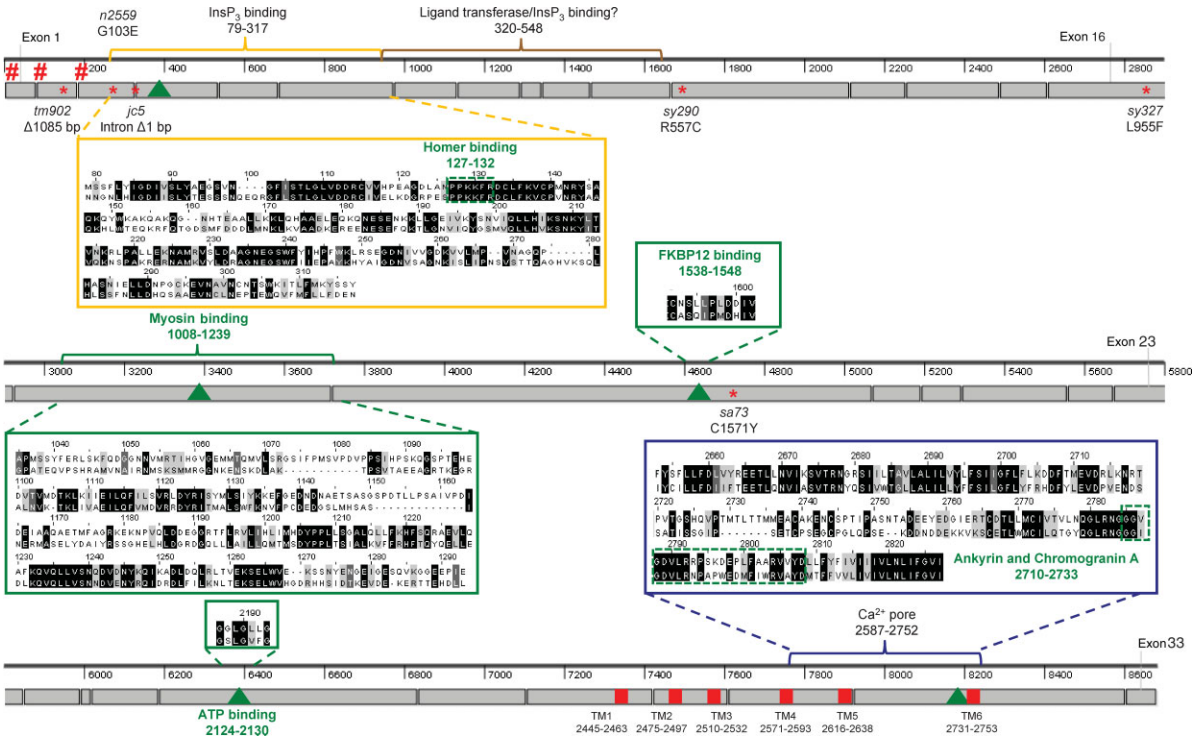

FIGURE 1 | The *C. elegans* $InsP_3R$. The *itr-1* gene product (isoform a) is depicted as a series of 33 exons, indicated in gray and shown to scale. The underlying genetic lesions associated with select *itr-1* mutant alleles are denoted by red asterisks together with brief descriptions of the resulting molecular alterations. For example, the tm902 allele is a 1085 nucleotide deletion, the jc5 allele is a single nucleotide deletion occurring in the third intron, and the n2559, sy290, sy327, and sa73 alleles reflect amino acid alterations as indicated. Alternative start sites at the N-terminal coding region are labeled with a pound sign. Conserved functional motifs such as core substrate binding and regulatory domains are distinguished by color. The minimal $InsP_3$ binding domain is denoted in yellow, while a putative extension of this domain is shown in brown (see Bosanac et al.[5] for crystallographic structure of the receptor/ligand pair). The Ca^{2+} pore is shown in blue and protein/ATP binding domains are shown in green (Homer, myosin, FKBP12, ATP, ankyrin, and chromogranin A). Most of these elements have been further expanded to the amino acid sequence level, using ClustalW2 to align them with their corresponding sequences in the human Type II isoform. The numbering scheme is based upon amino acid positions in ITR-1, and the ITR-1 sequences are below those from the human Type II isoform. Predicted transmembrane domains and their corresponding amino acid positions are designated by red boxes near the C-terminal coding sequences of the map.

© 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

was shown to direct the expression of an *itr-1*::GFP transgene to a unique repertoire of cell types. These results mirror those found using antibodies against the ITR-1 receptor,[4] with the caveat that immunological techniques may detect expression in cells where transgene expression is repressed in worms. Thus, despite the worm $InsP_3R$ being coded for by a single gene, signaling diversity may arise through alternative promoter and exon usage.

Genetic loss of *itr-1* or targeting of *itr-1* expression by double-stranded RNA-mediated gene interference (RNAi) causes sterility. The absence of viable progeny makes it difficult to study the role of the $InsP_3R$ during development. Fortunately, however, forward genetic screens have resulted in the generation of a wide variety of viable strains containing *itr-1* mutant alleles. These include loss-of-function (lf) alleles such as n2559, cold-sensitive weak reduction-of-function (rf) alleles such as jc5, heat-sensitive conditional alleles such as sa73, and gain-of-function (gf) suppressor alleles such as sy290 and sy337 (Figure 1). These alleles are those most often employed to study $InsP_3$ signaling in worms. $InsP_3R$ function has also been disrupted using tissue-specific overexpression of the ITR-1 $InsP_3$ binding domain,[7] which disrupts endogenous signaling in a dominant negative fashion. These approaches have allowed researchers to circumvent lethality associated with global disruption of $InsP_3$ signaling and to study the role of the $InsP_3R$ in a wide variety of developmental and behavioral processes.

PHYSIOLOGICAL FUNCTIONS OF $InsP_3$ RECEPTORS

Epidermal Morphogenesis

Morphogenesis is a fundamental aspect of developmental biology that controls the organization and spatial distribution of cells during embryonic development. Early work in *C. elegans* suggested that $InsP_3$ signaling regulates the rearrangement of epidermal cells during embryonic morphogenesis.[8] Polarized migration and spreading of epithelial cells are critical steps during embryogenesis and a cold-sensitive loss of function allele jc5 which results from a single bp deletion between exons 3 and 4 of *itr-1* compromises this process.[8] The jc5 allele causes reduced expression of ITR-1 protein and at the restrictive temperature of 15°C results in either misdirected migration or a premature cessation of epidermal cell migration. The net effect is ~95% embryonic lethality. Furthermore, the sa73 temperature-sensitive mutant of *itr-1* causes ~20% embryonic lethality as well as ventral enclosure defects at the partially restrictive temperature of 20°C, supporting a role for the receptor in embryogenesis. Additional evidence for the role of the $InsP_3R$ in epidermal morphogenesis comes from the observation that the *itr-1* mutant phenotype could be mimicked by pharmacological treatment with the $InsP_3R$ antagonist xestospongin.[8]

The identity of molecules that function in the $InsP_3$ signaling pathway to regulate epithelial morphogenesis was further studied by genetic epistasis. Epistasis analysis examines two mutations in combination to determine how they interact genetically to influence a common physiological output. The ability of one of the mutants to alter the phenotype induced by another mutant suggests a shared pathway. The hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to $InsP_3$ and diacylglycerol (DAG) by the enzyme phospholipase-C (PLC) is a critical step in the initiation of Ca^{2+} signaling. The worm genome codes for five PLC isoforms and one ortholog of the inactive PLC-like family.[9] A survey of these genes revealed that mutations which disrupted *plc-1* (PLCε) resulted in a substantial number of arrested embryos and defective morphogenesis, akin to *itr-1* rf mutations.[10] *itr-1(sy290)* has a missense mutation that has been suggested to increase affinity for $InsP_3$ and is at a position which also has this effect in mammalian $InsP_3Rs$.[11] *Itr-1(sy290)* can suppress defects in upstream signaling pathways, as it has been shown to partially compensate for the loss of *plc-1*, while further analysis demonstrated that RNAi targeting of *egl-8* (PLCβ) or heterozygosity of the *plc-3* (PLCγ) tm1340 deletion allele resulted in an increased number of arrested embryos, but only when performed in a *plc-1* mutant background.[10] The ability of *plc-3* and *egl-8* to partially compensate for the loss of *plc-1* suggests a redundancy of function for different isoforms of PLC in the activation of *itr-1* during epithelial morphogenesis. However, it is possible that these different PLC isoforms do not normally play a role in this process but are compensatory in the absence of *plc-1*. These results were confirmed by the following observations: (1) a transgene overexpressing *plc-1* in an *itr-1(jc5)* lf background could partially complement embryonic lethality, (2) cell-specific expression in the epidermis sufficed to complement the *itr-1*(rf) phenotype, and (3) overexpression of *egl-8*/PLCβ could also rescue embryonic lethality.[10]

Mammalian PLCε interacts with small GTPases of the Ras and Rho families and can couple small GTPase with $InsP_3$-mediated Ca^{2+} signaling. Collectively, these data support the hypotheses that intracellular Ca^{2+} signaling mediated through *itr-1* may be linked with Rho and Ras family GTPases that function to regulate epidermal morphogenesis (Figure 2a).

 © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

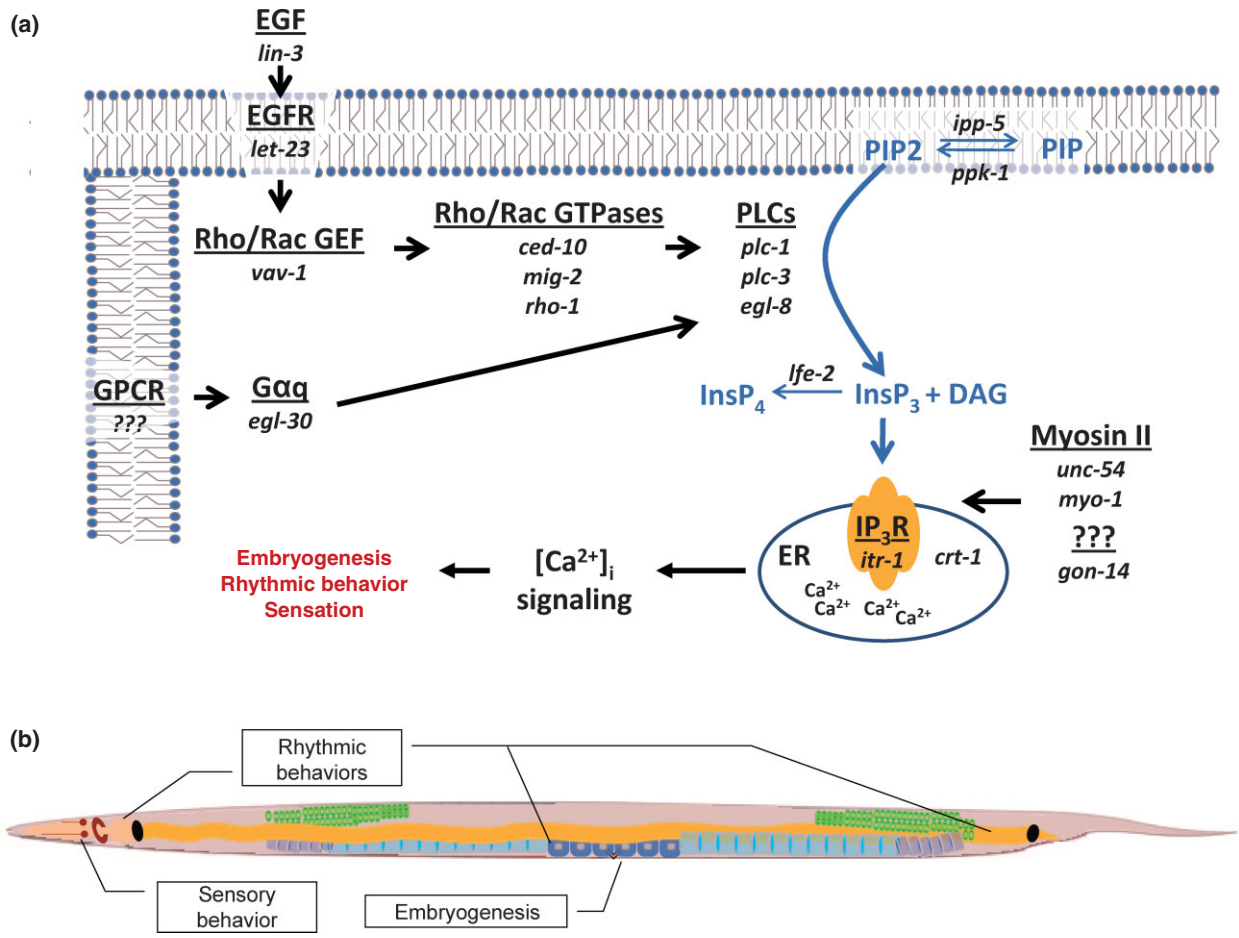

FIGURE 2 | $InsP_3R$-mediated signaling pathways in *C. elegans*. (a) Schematic denoting genes that have been shown to regulate the $InsP_3R$ in worms (italics) as well as functional descriptions of each genes activity (underlined). Note that not each of the genes in the schematic has been shown to regulate all of the behavioral or developmental outputs detailed in this article, and that the relationships indicated are based upon a combination of epistasis and predicted function of the gene products. Abbreviations are as follows: GPCR, G-protein coupled receptor; EGFR, epidermal growth factor receptor; GEF, guanidine nucleotide exchange factor; PLC, phospholipase C; ER, endoplasmic reticulum; PIP2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; $InsP_4$, inositol 1,3,4,5-tetrakisphosphate; $InsP_3R$, inositol 1,4,5 trisphosphate receptor. (b) The organs mediating the $InsP_3R$-dependent behaviors and developmental processes discussed in this review are indicated on a schematic anatomical diagram of the worm, with the relevant tissues shown in color, such as the GI tract, including the pharyx (tan) and the intestine (yellow), the amphid ASH polymodal neurons (red), and the gonad, where the germline cells (green) undergo meiotic maturation (light blue), fertilization in the spermatheca, and the first stages of embryogenesis (dark blue).

However to date no study has directly measured Ca^{2+} oscillations in the *C. elegans* epidermis. Interestingly, a recent report has shown that $InsP_3R$ activity is also important for establishing epithelial cell polarity during development.[12] Similarly intriguing is substantial evidence for the role of Rho GTPases in this process. Further genetic analyses in worms may help to shed light on whether PLCε links these divergent signaling pathways.

Behavioral Rhythmicity

C. elegans exhibit a number of rhythmic behaviors such as pharyngeal pumping, contraction of the gonadal sheath cells during ovulation, and execution of the defecation motor program (DMP) (Figure 2b shows a schematic depiction of the tissues that mediate these behaviors). The time scale on which these individual behaviors are repeated is vastly different. The pharynx contracts ~100 times per minute, which increases to ~225 times per minute in the presence of food. Basal and peak sheath cell contractions occur on a slower time-scale and range between 8 and 22 per minute, respectively. The slowest of the rhythmic behaviors is the DMP, which occurs every ~45 seconds in well-fed animals. Mutations in *itr-1* impact each of these rhythmic behaviors in a specific way. Thus, studying the genetics of $InsP_3$ signaling should

 © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

provide insight as to how the period and regularity of rhythmic behaviors is determined. Furthermore, since the period of these rhythmic behaviors can be modulated by external cues, the $InsP_3R$ is a likely target for coordinating metabolic or environmental stimuli with the rate of behavioral output.

Pharyngeal Pumping

In worms, pharyngeal pumping controls feeding by grinding and forcing food into the intestine. The rate of pumping is tightly regulated by both neuronal inputs as well as the presence of food itself, which increases the rate of pumping two-fold. Fundamental contractility in this tissue is driven primarily by ryanodine receptors and plasma membrane voltage-gated Ca^{2+} channels. However, *itr-1* is highly expressed in the terminal bulb and the isthmus of the pharynx. When $InsP_3$ signaling is disrupted either by expression of a dominant-negative $InsP_3R$ $InsP_3$-binding domain (commonly referred to as an $InsP_3$ 'sponge'), by the presence of mutations in *itr-1* itself, or when *itr-1* expression is ablated by RNAi, the upregulation of pharyngeal pumping that occurs upon exposure to food is suppressed.[7] However, the basal pumping rate is unaffected by the loss of ITR-1 activity. Thus it is likely that the role of the $InsP_3R$ in this tissue is primarily to translate environmental cues rather than to set the basal contractile period. Epistasis indicates that this environmental regulation of ITR-1 may be mediated through parallel EGF[13] and G-protein coupled receptor signaling[14] as shown in Figure 2a.

Clues as to how this occurs have arisen from yeast two-hybrid screens. ITR-1 interacts with myosin heavy chain II proteins, and a transient disruption of this interaction suppresses the effect of *itr-1* on pharyngeal pumping.[15] The absence of effect on other rhythmic behaviors suggested that this interaction is specific to *itr-1*-dependent adaptive changes that occur in response to food in the pharyngeal muscle. It has been suggested that a redistribution of the cytoskeleton may be important for fine tuning local Ca^{2+} signals in a variety of physiological contexts as well.

ITR-1 has also been shown to interact and colocalize with GON-14 (formerly termed IRI-1), a homolog of LIN-15B, which is a negative regulator of ectopic vulval induction.[16] As is the case with the myosin II-GON-14 interaction, suppressing GON-14 function reduces the rate of pharyngeal pumping in the presence of food, without affecting the basal rate.[17] However, in contrast to myosin II, disrupting GON-14 function also influences the period and, more significantly, the variability of the defecation cycle. Since the precise cellular role of GON-14 is unknown, it is difficult to hypothesize how this protein might couple to $InsP_3R$ signaling, though SH domain structures suggest that it may have an adaptor role and help to anchor the receptor to other signaling components. It is also possible that GON-14 affects ITR-1-regulated processes more indirectly by affecting gene expression, as a rescuing transgene can localize to the nucleus and *gon-14* itself interacts genetically with *lin-35*, the sole worm Rb ortholog.[18]

Ovulation

Ovulation is driven by rhythmic, coordinated contraction of the gonadal sheath cells in worms. The $InsP_3R$ has been shown to function in two distinct processes that facilitate ovulation, spermathecal dialation as well as the basal and ovulatory contractile cycle of the myoepithelial sheath cells. An epidermal growth factor (EGF) receptor homolog, *let-23,* and its ligand *lin-3* are core regulators of ovulation, and a genetic screen for genes that act downstream of *let-23* identified *itr-1* as an essential component of this signaling pathway.[19] Evidence for this is threefold: first, gf mutations in *itr-1* partially suppress the reduced brood size deficiency in *let-23* and *lin-3* mutants; second, lf mutations in an $InsP_3$ kinase, *lfe-2*, likewise partially suppress the brood-size defect[19]; and third, worms containing a mutant allele of *ipp-5*, a phosphatase that recognizes and reduces $InsP_3$ levels, produce two oocytes per cycle through hyperextension of the spermatheca.[20] Mutations in *plc-1* and *plc-3* also result in ovulation defects, which occur through regulation of spermathecal dilation or sheath cell contractions, respectively.[21,22] Finally, *vav-1,* a guanine nucleotide exchange factor (GEF) that activates the Rho GTPases, has been shown through epistasis to act downstream of *let-23* and upstream of *itr-1* in the gonad. Homozygous *vav-1* mutants arrest at the first larval (L1) stage, while transgenic *vav-1* mutants exhibit reduced fertility.[23]

Defecation

The stereotypical DMP consists of three motor steps that are temporally coordinated and requires the involvement of the intestine, two neurons, and several sets of muscles. The behavior itself helps to mix and clear the intestinal lumen, and worms that lack the DMP become constipated. The DMP is executed at ~45 second intervals and this period can be modulated through environmental stimuli.[24] Weak rf mutations such as the *itr-1(sa73)* allele slow down the cycle, while null mutations suppress the cycle entirely. To determine where the $InsP_3R$ functions to time defecation, a technique known as mosaic analysis was employed. Mosaic analysis is a method for performing cell-specific genotype–phenotype analysis. This

 © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

method relies on the fact that rescuing transgenes are sometimes lost during cell division. Transgenic worms can be grouped by phenotype and then further analyzed to determine the repertoire of cells containing the rescue marker (which can be followed by tagging with GFP). Using this technique, Dal Santo and colleagues demonstrated that intestinal expression of *itr-1* was sufficient to time the DMP, that intestinal loss of *itr-1* was sufficient to suppress the DMP, and that overexpression of ITR-1 reduced the cycle period.[25] Further evidence that ITR-1 functions cell-autonomously in the intestine to regulate defecation timing came from expressing the dominant negative $InsP_3$ sponge from an intestinal promoter, which was also sufficient to suppress defecation.[7] Thus, the $InsP_3R$ has been hypothesized to be a central component of the molecular pacemaker that controls defecation. The defecation model has been studied extensively and other components of the signaling pathway that appear to work upstream of *itr-1* in the intestine include PLCγ (*plc-3*), the $InsP_3$ kinase *lfe-2*, the phosphatase *ipp-5*, Rho/Rac GTPases (*ced-10*, *mig-2*, and *rho-1*), the guanidine nucleotide exchange factor *vav-1*, and the lipid kinase *ppk-1*, as well as downstream Ca^{2+} binding proteins such as *crt-1* (Figure 2a). Although mutations in the PLCβ ortholog *egl-8* can cause arrhythmia, epistasis analysis suggested that this does not occur through ITR-1 but through an unrelated signaling pathway.[26] Finally, RNAi of *ppk-1* (PIP 5-kinase) has been shown to cause defects in the defecation cycle. As both Rac1 and RhoA have been shown to activate PIP5K in mammals,[27,28] this is consistent with the idea that in *C. elegans* Rho/Rac GTPases help modulate $InsP_3$ levels. These studies suggest a model where VAV-1 functionally couples to Rho/Rac GTPases and modulates the $InsP_3$ signaling process that regulates the *itr-1* rhythmic output.

In all three of the above examples, oscillatory Ca^{2+} signaling is hypothesized to underlie behavioral rhythmicity. Direct confirmation of this hypothesis, at least with regard to pharyngeal pumping, was provided by Kerr and colleagues in 2000. The genetically encoded yellow cameleon YC2.1 biosensor was expressed in the pharynx using a cell-specific promoter. Dynamic fluorescent imaging was then used to measure changes in the YC2.1 FRET ratio upon Ca^{2+} binding and to demonstrate that Ca^{2+} oscillations occur coincidentally with pharyngeal contractions. Similar techniques were used to study defecation, demonstrating that rhythmic initiation and propagation of a Ca^{2+} wave in the worm intestine was necessary for proper timing and execution of the DMP.[26,29,30] Interestingly, the *egl-8* mutation that caused arrhythmia independent of *itr-1* also resulted in aberrant Ca^{2+} wave initiation and the propagation of backward intestinal waves. Thus, this combination of transgenic biosensors and genetics can help to illuminate the physiological basis of behavior.

ITR-1 Function in the Nervous System

The polymodal amphid ASH sensory neurons mediate the behavioral response to a range of environmental stimuli. Of these, the locomotory reversal response that occurs following nose touch (mechanosensation) or following exposure to benzaldehyde (chemosensation) has been shown to require ITR-1.[31] Interestingly, disrupting ITR-1 function does not affect the ability of the worms to respond to strong stimulation, but instead raises the threshold required to elicit avoidance behavior. Even more intriguing is the observation that only the nose touch response has been shown to be mediated through $InsP_3$ signaling.[31] Work in other systems has suggested that the $InsP_3R$ can function independent of its canonical ligand through activation by other proteins.[32] Certainly, additional studies in *C. elegans* will be necessary to determine whether this is also true in nematode polymodal neurons. If so, however, then using an established and well-characterized genetic model may help to quickly identify components of the signaling pathway that contribute to InsP3-independent function of the receptor.

In addition, the worm $InsP_3R$ functions to regulate male mating behavior.[9] Male mating in worms occurs through a stereotyped sequence of behavioral steps that integrate chemosensory and mechanosensory cues. Specifically, ITR-1 has been demonstrated to contribute to turning behavior, spicule insertion, and sperm transfer, and acts downstream of PLCβ.[9]

Finally, Ghosh-Roy et al.[33] have found that Ca^{2+} signaling via the $InsP_3R$ is critical for axon regeneration. Importantly, this data suggests that the $InsP_3R$ may have a role in the axonal migration and repair. Additionally, though not a behavior per se, the worm $InsP_3R$ has also been shown to contribute to necrotic cell death.[34] The idea that calcium overload is central to necrosis is supported by the fact that mutations in genes involved in calcium homeostasis such as ITR-1 can suppress necrosis in worm neurons.[34]

CONCLUDING REMARKS

Advantages of studying the roles and regulation of the $InsP_3R$ using the nematode model include its amenability to genetic approaches, the availability of a variety of *itr-1* mutant alleles, the ability to perform tissue-specific complementation and/or RNAi, and the established use of dominant negative approaches such as overexpression of $InsP_3$ sponges. These types

 © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

of approaches have led to a better understanding of signaling pathways that act upstream of *itr-1* in specific cells to regulate diverse physiological outputs. In addition, new roles for the $InsP_3R$ are emerging from ongoing genetic research in worms. For example, a study performed recently asked the question of what genes contribute to the quality of youthfulness in worms. Youthfulness is distinct from lifespan and is quantified by measuring physiological traits that are associated with aging, such as locomotory decline and the appearance of age pigments. One novel finding in this study was that the $InsP_3R$ appears to act through the EGF signaling pathway to modulate youthfulness or 'healthspan'.[35] This observation will undoubtedly provide a new focus on understanding the physiology of aging.

ACKNOWLEDGMENTS

The authors acknowledge support from NSF grant IOS 0919848 (K.N.). E.A. was supported by the Ruth L. Kirschtein National Research Service Institutional Training Grant T32 GM068411. We would like to thank Drs. Paul Brookes and David Yule for critical comments.

REFERENCES

1. Duncan RS, Hwang SY, Koulen P. Differential inositol 1,4,5-trisphosphate receptor signaling in a neuronal cell line. *Int J Biochem Cell Biol* 2007, 39:1852–1862.
2. Mikoshiba K. IP3 receptor/Ca^{2+} channel: from discovery to new signaling concepts. *J Neurochem* 2007, 102: 1426–1446.
3. Chi W, Reinke V. DPL-1 (DP) acts in the germ line to coordinate ovulation and fertilization in *C. elegans*. *Mech Dev* 2009, 126:406–416.
4. Baylis HA, Furuichi T, Yoshikawa F, Mikoshiba K, Sattelle DB. Inositol 1,4,5-trisphosphate receptors are strongly expressed in the nervous system, pharynx, intestine, gonad and excretory cell of *Caenorhabditis elegans* and are encoded by a single gene (itr-1). *J Mol Biol* 1999, 294:467–476.
5. Bosanac I, Alattia JR, Mal TK, Chan J, Talarico S, Tong FK, Tong KI, Yoshikawa F, Furuichi T, Iwai M, et al. Structure of the inositol 1,4,5-trisphosphate receptor binding core in complex with its ligand. *Nature* 2002, 420:696–700.
6. Gower NJ, Temple GR, Schein JE, Marra M, Walker DS, Baylis HA. Dissection of the promoter region of the inositol 1,4,5-trisphosphate receptor gene, itr-1, in *C. elegans*: a molecular basis for cell-specific expression of IP3R isoforms. *J Mol Biol* 2001, 306:145–157.
7. Walker DS, Gower NJ, Ly S, Bradley GL, Baylis HA. Regulated disruption of inositol 1,4,5-trisphosphate signaling in *Caenorhabditis elegans* reveals new functions in feeding and embryogenesis. *Mol Biol Cell* 2002, 13:1329–1337.
8. Thomas-Virnig CL, Sims PA, Simske JS, Hardin J. The inositol 1,4,5-trisphosphate receptor regulates epidermal cell migration in *Caenorhabditis elegans*. *Curr Biol* 2004, 14:1882–1887.
9. Gower NJ, Walker DS, Baylis HA. Inositol 1,4,5-trisphosphate signaling regulates mating behavior in *Caenorhabditis elegans* males. *Mol Biol Cell* 2005, 16:3978–3986.
10. Vazquez-Manrique RP, Nagy AI, Legg JC, Bales OA, Ly S, Baylis HA. Phospholipase C-epsilon regulates epidermal morphogenesis in *Caenorhabditis elegans*. *PLoS Genet* 2008, 4:e1000043.
11. Yoshikawa F, Morita M, Monkawa T, Michikawa T, Furuichi T, Mikoshiba K. Mutational analysis of the ligand binding site of the inositol 1,4,5-trisphosphate receptor. *J Biol Chem* 1996, 271:18277–18284.
12. Pilipiuk J, Lefebvre C, Wiesenfahrt T, Legouis R, Bossinger O. Increased IP3/Ca^{2+} signaling compensates depletion of LET-413/DLG-1 in *C. elegans* epithelial junction assembly. *Dev Biol* 2009, 327:34–47.
13. Van Buskirk C, Sternberg PW. Epidermal growth factor signaling induces behavioral quiescence in *Caenorhabditis elegans*. *Nat Neurosci* 2007, 10:1300–1307.
14. Bastiani CA, Gharib S, Simon MI, Sternberg PW. *Caenorhabditis elegans* Galphaq regulates egg-laying behavior via a PLCbeta-independent and serotonin-dependent signaling pathway and likely functions both in the nervous system and in muscle. *Genetics* 2003, 165:1805–1822.
15. Walker DS, Ly S, Lockwood KC, Baylis HA. A direct interaction between IP(3) receptors and myosin II regulates IP(3) signaling in *C. elegans*. *Curr Biol* 2002, 12: 951–956.
16. Clark SG, Lu X, Horvitz HR. The *Caenorhabditis elegans* locus lin-15, a negative regulator of a tyrosine kinase signaling pathway, encodes two different proteins. *Genetics* 1994, 137:987–997.
17. Walker DS, Ly S, Gower NJ, Baylis HA. IRI-1, a LIN-15B homologue, interacts with inositol-1,4,5-triphosphate receptors and regulates gonadogenesis, defecation, and pharyngeal pumping in *Caenorhabditis elegans*. *Mol Biol Cell* 2004, 15:3073–3082.

 © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

18. Chesney MA, Kidd AR III, Kimble J. gon-14 functions with class B and class C synthetic multivulva genes to control larval growth in *Caenorhabditis elegans*. *Genetics* 2006, 172:915–928.
19. Clandinin TR, DeModena JA, Sternberg PW. Inositol trisphosphate mediates a RAS-independent response to LET-23 receptor tyrosine kinase activation in *C. elegans*. *Cell* 1998, 92:523–533.
20. Bui YK, Sternberg PW. *Caenorhabditis elegans* inositol 5-phosphatase homolog negatively regulates inositol 1,4,5-triphosphate signaling in ovulation. *Mol Biol Cell* 2002, 13:1641–1651.
21. Kariya K, Bui YK, Gao X, Sternberg PW, Kataoka T. Phospholipase cepsilon regulates ovulation in *Caenorhabditis elegans*. *Dev Biol* 2004, 274:201–210.
22. Yin X, Gower NJ, Baylis HA, Strange K. Inositol 1,4,5-trisphosphate signaling regulates rhythmic contractile activity of myoepithelial sheath cells in *Caenorhabditis elegans*. *Mol Biol Cell* 2004, 15:3938–3949.
23. Norman KR, Fazzio RT, Mellem JE, Espelt MV, Strange K, Beckerle MC, Maricq AV. The Rho/Rac-family guanine nucleotide exchange factor VAV-1 regulates rhythmic behaviors in *C. elegans*. *Cell* 2005, 123:119–132.
24. Thomas JH. Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics* 1990, 124:855–872.
25. Dal Santo P, Logan MA, Chisholm AD, Jorgensen EM. The inositol trisphosphate receptor regulates a 50-second behavioral rhythm in *C. elegans*. *Cell* 1999, 98: 757–767.
26. Espelt MV, Estevez AY, Yin X, Strange K. Oscillatory Ca^{2+} signaling in the isolated *Caenorhabditis elegans* intestine: role of the inositol-1,4,5-trisphosphate receptor and phospholipases C beta and gamma. *J Gen Physiol* 2005, 126:379–392.
27. O'Rourke LM, Tooze R, Turner M, Sandoval DM, Carter RH, Tybulewicz VL, Fearon DT. CD19 as a membrane-anchored adaptor protein of B lymphocytes: costimulation of lipid and protein kinases by recruitment of Vav. *Immunity* 1998, 8:635–645.
28. Saci A, Carpenter CL. RhoA GTPase regulates B cell receptor signaling. *Mol Cell* 2005, 17:205–214.
29. Teramoto T, Iwasaki K. Intestinal calcium waves coordinate a behavioral motor program in *C. elegans*. *Cell Calcium* 2006, 40:319–327.
30. Nehrke K, Denton J, Mowrey W. Intestinal Ca^{2+} wave dynamics in freely moving *C. elegans* coordinate execution of a rhythmic motor program. *Am J Physiol Cell Physiol* 2008, 294:C333–C344.
31. Walker DS, Vazquez-Manrique RP, Gower NJ, Gregory E, Schafer WR, Baylis HA. Inositol 1,4,5-trisphosphate signalling regulates the avoidance response to nose touch in *Caenorhabditis elegans*. *PLoS Genet* 2009, 5:e1000636.
32. Foskett JK, White C, Cheung KH, Mak DO. Inositol trisphosphate receptor Ca^{2+} release channels. *Physiol Rev* 2007, 87:593–658.
33. Ghosh-Roy A, Wu Z, Goncharov A, Jin Y, Chisholm AD. Calcium and cyclic AMP promote axonal regeneration in Caenorhabditis elegans and require DLK-1 kinase. *J Neurosci* 2010, 30:3175–3183.
34. Xu K, Tavernarakis N, Driscoll M. Necrotic cell death in *C. elegans* requires the function of calreticulin and regulators of Ca(2+) release from the endoplasmic reticulum. *Neuron* 2001, 31:957–971.
35. Iwasa H, Yu S, Xue J, Driscoll M. Novel EGF pathway regulators modulate *C. elegans* healthspan and lifespan via EGF receptor, PLC-gamma, and IP3R activation. *Aging Cell* 2010, 9:490–505.

FURTHER READING

Wormbase entry on the *itr-1* gene: http://www.wormbase.org/db/gene/gene?name=WBGene00002173;class=Gene.

Chisholm AD, Hardin J. Epidermal morphogenesis (December 01, 2005), In: WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.35.1, http://www.wormbook.org.

Greenstein D. Control of oocyte meiotic maturation and fertilization (December 28, 2005), WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.53.1, http://www.wormbook.org.

 © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.