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Review

Genetic analysis of IP_3 and calcium signalling pathways in *C. elegans* ☆

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ABSTRACT

Background: The nematode, *Caenorhabditis elegans* is an established model system that is particularly well suited to genetic analysis. *C. elegans* is easily manipulated and we have an in depth knowledge of many aspects of its biology. Thus, it is an attractive system in which to pursue integrated studies of signalling pathways. *C. elegans* has a complement of calcium signalling molecules similar to that of other animals.
Scope of review: We focus on IP_3 signalling. We describe how forward and reverse genetic approaches, including RNAi, have resulted in a tool kit which enables the analysis of IP_3/Ca^{2+} signalling pathways. The importance of cell and tissue specific manipulation of signalling pathways and the use of epistasis analysis are highlighted. We discuss how these tools have increased our understanding of IP_3 signalling in specific developmental, physiological and behavioural roles. Approaches to imaging calcium signals in *C. elegans* are considered.
Major conclusions: A wide selection of tools is available for the analysis of IP_3/Ca^{2+} signalling in *C. elegans*. This has resulted in detailed descriptions of the function of IP_3/Ca^{2+} signalling in the animal's biology. Nevertheless many questions about how IP_3 signalling regulates specific processes remain.
General significance: Many of the approaches described may be applied to other calcium signalling systems. *C. elegans* offers the opportunity to dissect pathways, perform integrated studies and to test the importance of the properties of calcium signalling molecules to whole animal function, thus illuminating the function of calcium signalling in animals. This article is part of a Special Issue entitled Biochemical, biophysical and genetic approaches to intracellular calcium signalling.

1. Introduction—*Caenorhabditis elegans* as a model system for the integrated study of calcium signalling

C. elegans is a widely used model system. It was initially established as a model in which to address fundamental questions about animal development and neurobiology using the power of genetic analysis [1]. However, over time it has become a system in which a wide range of biological questions are being addressed and in which a wide range of approaches can be utilised (see www.wormbook.org and [2,3]). *C. elegans* also has advantages as a screening system for therapeutic and other compounds of value (reviewed in [4,5]). In this review we focus on genetic and other approaches that have been used to investigate intracellular calcium signalling pathways based on the second messenger IP_3, its receptor (IP_3R) and the enzyme responsible for it production, phospholipase C. The experimental tractability and biological characteristics of *C. elegans* make it an attractive system in which to achieve an integrated analysis of calcium signalling, ranging from the importance of specific amino acids within a protein to the analysis of developmental, physiological and behavioural outputs. Fundamental to this approach is the ability to define and quantify many whole animal phenotypes.

C. elegans is a small nematode worm. Adults are approximately 1 mm in length and 80 μm in diameter (www.wormatlas.org). It belongs to the Rhabditida group of nematodes which includes both

Abbreviations: AM, acetoxymethyl; CaM, calmodulin; DAG, diacylglycerol; dsRNA, double stranded RNA; DIC, differential interference contrast (microscopy); EGF, epidermal growth factor; EMC, enteric muscle contraction in *C. elegans* defecation; EMS, ethyl methanesulphonate; ENU, N-ethyl N-nitrosourea; Eph, ephrin; GEF, guanine nucleotide exchange factor; *gf*, gain-of-function; GFP, green fluorescent protein; GPCR, G-protein coupled receptor; IP_2, inositol 1,4-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; IP_3R, IP_3 receptor; IP_4, inositol 1,3,4,5-tetrakisphosphate; IR, inverted repeat; *lf*, loss-of-function; LGIC, ligand gated ion channel; MSP, major sperm protein; MAPK, mitogen activated protein kinase; miRNA, microRNA; MosSCI, Mos1 mediated Single Copy gene Insertion; NAADP, Nicotinic acid adenine dinucleotide phosphate; *pBoc*, the posterior body muscle contraction in *C. elegans* defecation; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; PLL, phospholipase C like; PMCA, plasma membrane Ca^{2+}-ATPase; RFP, red fluorescent protein; RNAi, RNA interference; RTK, receptor tyrosine kinase; SERCA, sarco/endoplasmic reticulum; SNP, single nucleotide polymorphism; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRP, transient receptor potential (channel); ts, temperature sensitive; VOCC, voltage operated calcium channel

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free living and parasitic species [6]. *C. elegans* itself is a free living nematode which feeds on microorganisms [7]. In the laboratory it is routinely cultured on *E. coli* lawns grown on agar Petri dishes at between 15 and 25 °C. It may also be grown in liquid culture in, for example, 96 well plates. Until recently little has been known about the animal's natural habitat, but recent work suggests that it is found in microorganism rich environments especially on rotting plant material [7,8].

C. elegans has a relatively simply and rapid life cycle (Fig. 1). The entire life cycle can be completed within 3.5 days. Thus *C. elegans* can be cultured rapidly and with ease in the laboratory and, large numbers of animals can be readily produced and maintained. The majority of adult animals are self fertilising hermaphrodites with an XX sex chromosome complement. Males (XO) occur at a frequency of about 0.1% due to random non-disjunction of the X chromosome. The frequency of males can be artificially increased by a number of means (for example, heat stress or "high incidence of males" (*him*) mutants) to provide animals for crosses. Oocytes are produced in the adult hermaphrodite and are fertilised internally by sperm derived from the hermaphrodite or by mating with a male. Embryos develop through several cell divisions before being deposited on the growth medium and finishing their development. Embryos hatch to give the so called L1 larvae and then progress through a series of larval stages, L1 to L4 before adulthood (Fig. 1). Prior to the L3 stage animals choose either to progress through the normal L3 larval stage or to form a Dauer larva (Fig. 1), a long lived and stress resistant stage. The culture, storage and maintenance of *C. elegans* is described in detail in several excellent books [3,9–13] and on-line resources (e.g. www.wormbook.org).

Whilst the ease and low cost of *C. elegans* culture are not be undervalued, more striking is our exquisitely detailed knowledge of many aspects of this animal. Perhaps most famously the entire cell lineage of the animals is known. *C. elegans* has a nearly invariant cell lineage and the position and progenitor history of each of the 959 somatic cells found in the adult hermaphrodite are known [14–16]. Behind this astonishing level of knowledge lies a key advantage of *C. elegans*, every stage of the animal is transparent so that the internal detail of the animal can be observed using techniques such as DIC microscopy. Further, and importantly, fluorescent markers are readily observed. Detailed visualisation of the anatomy of the worm is thus readily achieved in live animals. *C. elegans* has a relatively simply anatomy (Fig. 2A) but encompasses a range of tissue and cell types that is typical of metazoan animals, including muscle, neurones and associated support cells, and epithelia. Although many tissue types are present in *C. elegans* it clearly lacks the tissue complexity of, for example, a mammal. In addition to the cell lineage the full wiring diagram of the hermaphrodite nervous system has been determined [17]. Several findings of critical biological importance such as apoptosis, RNAi, the role of miRNAs and the use of transgenic GFP were made in *C. elegans*, further highlighting the power of this nematode as a research tool in modern biology.

At the molecular level *C. elegans* was the first animal to have its entire genome sequenced [18]. Associated with the full genome sequence are annotated databases (wormbase.org) and a wide selection of molecular resources. This has also been followed by high throughput approaches to analysis of the transcriptome, proteome and interactome. More recently the genomes of a number of other Caenorhabditids including *C. briggsae*, *C. japonica*, *C. brenneri*, *C remanii* and others [19] have been determined enabling the comparative analysis of calcium signalling genes. For example a comparison of the IP_3 receptor gene (*itr-1* see below) from *C. elegans* and *C. briggsae* enabled Gower et al., to identify putative tissue specific regulatory elements within the promoter regions and to identify novel splice variants [20].

This brief survey of the biology and experimental tools available in *C. elegans* points to the potential of this organism for integrated approaches to understanding cell signalling pathways [21–23]. As, for any organism, biochemical studies may be performed on purified proteins or sub-cellular preparations. Protein interactions may be studied by a variety of means. Genetic, reverse genetic and transgenic approaches mean that it is possible to identify or manipulate genes in order to change individual amino acids or defined structures in proteins based on such biochemical, biophysical or molecular studies of either the *C. elegans* protein or its mammalian homologues. For example one might mutate binding sites for modulatory ligands or protein interactors or alter an alternative splice or phosphorylation site. The effect of such changes may then be observed at the cellular and tissue level by measuring calcium or other signals. But the effect can also be observed at the whole animal level by measuring developmental, physiological or behavioural outputs. Thus one can establish whether a residue or interaction is really important for animal function and whether such changes have specific effects on sub-sets of processes mediated by the protein.

Pre-genomic studies of calcium signalling in *C. elegans* relied on the fortuitous discovery of calcium signalling mutants in genetic screens or on the isolation by directed cloning of calcium signalling

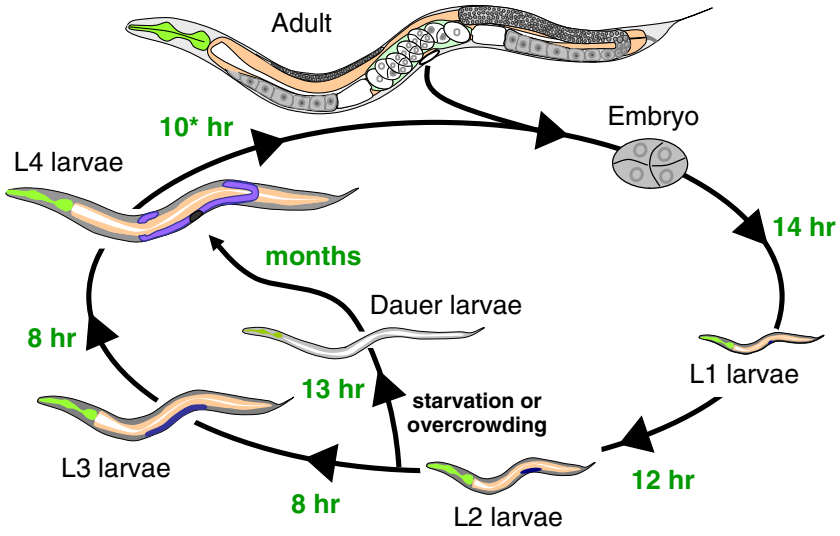

Fig. 1. The life cycle of *C. elegans*. Approximate times (in hours) are given for each stage. Values represent times for growth at 22 °C in standard laboratory conditions. *Time to reach the young adult stage, the fertile stage will be produced 8 h later. After Altun, Z.F. and Hall, D.H. 2008. Handbook of *C. elegans* Anatomy. In *WormAtlas*. (www.wormatlas.org).