

PYP-1, inorganic pyrophosphatase, is required for larval development and intestinal function in *C. elegans*

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Abstract Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate (PPi) into phosphate (Pi), which provides a thermodynamic driving force for important biosynthetic reactions. The nematode *Caenorhabditis elegans* gene C47E12.4 encodes a PPase (PYP-1) which shows 54% amino acid identity with human PPase. PYP-1 exhibits specific enzyme activity and is mainly expressed in the intestinal and nervous system. A null mutant of *pyp-1* reveals a developmental arrest at early larval stages and exhibits gross defects in intestinal morphology and function. The larval arrest phenotype was successfully rescued by reintroduction of the *pyp-1* gene, suggesting that PYP-1 is required for larval development and intestinal function in *C. elegans*.

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1. Introduction

Inorganic pyrophosphatases (PPases, EC 3.6.1.1) are ubiquitous enzyme catalyzing the hydrolysis of inorganic pyrophosphate (PPi) into two molecules of orthophosphate (Pi). This highly exergonic enzymatic reaction, which plays an important role in energy metabolism, provides a thermodynamic pull for many biosynthetic reactions [1], such as DNA, RNA, protein and polysaccharide synthesis. This enzyme may have an important role in evolution by affecting the accuracy during DNA replication processes [2]. Genes that encode PPases have been cloned from prokaryotes, yeast, *Drosophila melanogaster*, roundworm *Ascaris*, and as well as from mammals [3–7].

PPases fall under two major classes, soluble PPases and membrane-bound H⁺ translocating PPases (H⁺-PPase) and show no sequence similarity to each other [8]. Sequence alignment of Family I soluble PPases show that the structure of their active sites are evolutionarily well-conserved [9]. The enzyme has been shown to be essential for viability in bacteria

[10] and yeast [5]. In transgenic tobacco and potato plants, soluble PPase has been reported to alter metabolism, growth, and development [11]. In *Drosophila*, it was reported that PPase is a NURF-38 protein, which is a component of nucleosome remodeling complex [7]. However, little is known about the metabolic significance of PPases in humans, except that increased expression of PPase is associated with hyperthyroidism [12], and as well as many cancers [13–15].

In the parasitic roundworm *Ascaris*, PPase has shown to have an essential role in development and molting which has been demonstrated by disrupting the endogenous gene function by means of RNA-mediated interference [6]. However, an in-depth knowledge of a gene function comes from mutant studies and the nematode *Caenorhabditis elegans* has always been an excellent model system to analyze such gene functions *in vivo* and at the molecular level. Here, we report the characterization of a *C. elegans* PPase gene (*pyp-1*) by isolating a knock-out mutant and ascertained that the loss of PYP-1 causes developmental arrest at L2 larval stage. Furthermore, the mutant was found to have an aberrant intestinal morphology, reduced number of autofluorescent gut granules and fat deposits in the intestine. We show that PYP-1 is required for the larval development and intestinal functions in *C. elegans*.

2. Materials and methods

2.1. *C. elegans* strains and maintenance

The wild-type *C. elegans*, the Bristol strain (N2), CR185 *dpy-13(e184) unc-22(m52), uba-1(ok1374)/nT1[qIs51]* were obtained from *Caenorhabditis elegans* Genetic Center (CGC) at the University of Minnesota, USA. The knockout inorganic pyrophosphatase (*pyp-1*) mutant *pyp-1(jh123)* was isolated using reverse genetics method (this study) [16]. The genotype of *pyp-1(jh123)* was determined by nested PCR using following primers; outer upstream primer (5'-GGA-ATC-AAA-TAA-TCA-AAT-ACG-TTG-G-3') and outer downstream primer (5'-TAG-ATG-ATA-CTG-AGC-TGT-CGA-AGT-G-3'), inner upstream primer (5'-ACT-TAT-CAA-CAT-CTC-CTG-GTA-GTG-G-3') and inner downstream primers (5'-CAG-CAG-TTC-AAA-TAC-TTC-ACA-GAA-A-3') and (5'-CGA-GCT-ACA-CCC-TTC-TTC-TCA-TCT-TGC-3'). Since *pyp-1(jh123)* mutant exhibited a larval arrest phenotype, we maintained as a heterozygote [*pyp-1(jh123)/dpy-13(e184)unc-22(m52)IV*] and [*pyp-1(jh123)/nT1[qIs51](IV;V)*]. Worm breeding and handling were conducted as described [17].

2.2. GFP expression and rescue analysis

The genomic DNA fragment containing ~2 kb of 5' upstream region sequence and coding region up to 361 amino acids of the *pyp-1* gene was amplified by PCR using the cosmid, C47E12 as a template

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Abbreviations: *C. elegans*, *Caenorhabditis elegans*; PPase, inorganic pyrophosphatase

and cloned into promoterless GFP vector, pPD95.75 vector (kindly provided by A. Fire) using *Hind*III and *Bam*HI sites and micro-injected as described [18]. For rescue experiments, the *pyp-1* genomic DNA cloned in pGEM-T Easy vector (Promega) and *pyp-1::gfp* fusion construct as a transformation marker each at a final concentration of 100 ng/μl, were coinjected into the *pyp-1* heterozygous mutant [*pyp-1(jh123)/dpy-13(e184)unc-22(m52)IV*] as described [19]. Individual GFP-expressing adult progeny (genotypically heterozygote and phenotypically wild-type) was cloned and scored for larval-arrest phenotype.

2.3. Recombinant protein production, inorganic PPase assay and antibodies

The full coding sequence of cDNA of the *pyp-1* was subcloned into pGEX-4T3 (Amersham Pharmacia Biotech) vector using *Bam*HI and *Eco*RI restriction enzyme sites, and overexpressed as a GST::PYP-1 fusion protein. The purified protein (GST::PYP-1) was later used for PPase assay and used to immunize rabbits for generation of polyclonal antibodies. PPase assay was determined spectrophotometrically using a molybdate blue-based colorimetric assay as described [20].

2.4. Western analysis, immunofluorescence and immunogold staining

Total proteins obtained from wild-type worms and *pyp-1* homozygous mutants were prepared and used for Western blotting as described previously [21]. Immunostaining of wild-type *C. elegans* was performed as described [21]. Slides were mounted and observed under a fluorescence microscope (Olympus BX50, Carl Zeiss Axio Imager. A1). Immunogoldstaining with anti-PYP-1 antibody for N2 was carried out as previously described [16]. Samples were examined under a transmission electron microscope (Joel 1200 EXII, Peabody, MA).

2.5. Phenotypic assay

GFP expressing bacteria was seeded on NGM plates instead of OP50 for the worms to feed and investigated the intestinal contents thereafter. For Neutral Red (Sigma) feeding assay, experiments were carried out as described [22]. For Nile Red staining, worms were cultured 24 h on NGM containing 1 ng/ml Nile Red (5H-benzo [a] phenoxazine-5-one, 9-diethylamino) seeded with OP50. Autofluorescent gut granules and fat contents were monitored by fluorescence microscope (Olympus BX50). For the selective staining of intestinal cell nuclei, UV absorbing minor groove DNA binding stain Hoechst 33258 (Molecular Probes) was used and the staining was performed as described [23].

3. Results

3.1. Identification of an inorganic pyrophosphatase (*pyp-1*) in *C. elegans*

The *C. elegans* inorganic pyrophosphatase (*pyp-1*) is a single gene, physically mapped to the chromosome IV and located on the cosmid C47E12 (Fig. 1A). Wormbase annotation predicts the existence of four isoforms by alternative splicing. For our subsequent studies, we used yk1062b12 cDNA clone which contained the isoform C47E12.4c. The deduced PYP-1 protein showed 54% identity and 74% similarity with the human PPase at the amino acid levels (Fig. 1B). The variation between *C. elegans* PPase and human PPase was that the former had a longer N-terminal region in comparison to the human counterpart. This feature is highly conserved in nematodes given that *C. briggsae* homolog also has a longer N-terminal region like *C. elegans* PPase. The amino acid sequence DNDPIDV, defined as a signature sequence for PPase in human, yeast and prokaryotes [24,25], is shown to be well-conserved for the worms as well. Moreover, several potential phosphorylation sites on PYP-1 protein were predicted by computer analysis using PROSITE SCAN software (<http://us.expasy.org>) including protein kinase C, casein kinase II, and tyrosine kinase phosphorylation sites.

3.2. PPase enzyme activity is conserved in *C. elegans*

PPases are highly conserved enzymes, which hydrolyze inorganic pyrophosphate into two inorganic phosphates. To determine whether PYP-1 protein has PPase enzyme activity, we overexpressed GST::PYP-1 fusion protein, purified it, and then used to assay for PPase activity using molybdate blue-based colorimetric assay. As shown Fig. 1C, the recombinant PYP-1 protein was found to have relatively a strong PPase activity (~1570 μmole Pi/mg protein/min). However, in absence of enzyme, no change in color reaction was observed.

3.3. Expression pattern and subcellular localization of *pyp-1*

Reporter gene (GFP) expression under the control of the *pyp-1* promoter and *pyp-1* gene showed that *pyp-1* is mainly expressed in intestine (Fig. 2A–C) and nervous system, including nerve cords (Fig. 2C and F) and sensory neurons (Fig. 2G). Expression could be detected from larval stages through adult stages. *pyp-1* is also expressed in the coelomocytes (Fig. 2D and E). We further localized PYP-1 in the cytoplasm of 2-cell embryo (Fig. 2J) and robustly observed in the adult intestine (Fig. 2K). Furthermore, at the subcellular level, clustered signals of PYP-1 were observed in the intestinal cells of wild-type worms and more precisely around the intestinal granules and vacuoles (Fig. 2H and I).

3.4. Isolation of *pyp-1* deletion mutant

To characterize *in vivo* functions of *pyp-1*, we isolated a *pyp-1(jh123)* deletion mutant by PCR-based TMP/UV mutagenesis screening [26]. As shown in Fig. 3A, the deletion removed 744 bp of the *pyp-1* gene containing the third and fourth exons, which also contains the conserved PPase signature domain. The deletion region was further confirmed by nested PCR using the inner primers that produced no PCR band in the *pyp-1(jh123)* homozygous mutants but detected a 1.4 kb band in the wild-type and *pyp-1(jh123)* heterozygous animals (Fig. 3B). Interestingly, the *pyp-1* homozygous mutants were developmentally arrested prior to L3 larval stage. Because *pyp-1(jh123)* homozygous mutant exhibited a larval arrest phenotype, we maintained the mutant as a heterozygote [*pyp-1(jh123)/dpy-13(e184)unc-22(m52)IV*] and [*pyp-1(jh123)/nT1[qIs51](IV;V)*]. As evidenced from Fig. 3C, anti-PYP-1 antibodies detected a single band of approximately 45 kDa from protein extracts of a mixed stage population of wild-type animals, however, no PYP-1 protein was detected from *pyp-1(jh123)* homozygotes, indicating that deletion mutants are functionally null.

3.5. The *pyp-1* null mutants show developmental arrest at the larval stages and exhibit defects of intestinal development

Deletion mutation in *pyp-1* results in developmental arrest at L2 larval stages (Fig. 4B). The *pyp-1* heterozygous mutants produced ~25% of homozygous progeny that displayed arrest at the larval stages. Although the *pyp-1(jh123)* homozygous mutants were developmentally arrested, which means the L2-staged worms did not grow to L3 stage or beyond, the arrested worms were seen to continue to live the entire span of time alike the normal healthy N2 worms, however, with minimum activity. Additionally, these larval-arrested worms with time were gradually found to reduce their locomotory action and happen to be weak with no further development at all. Eventually they died the same time similar to N2 worms. However,

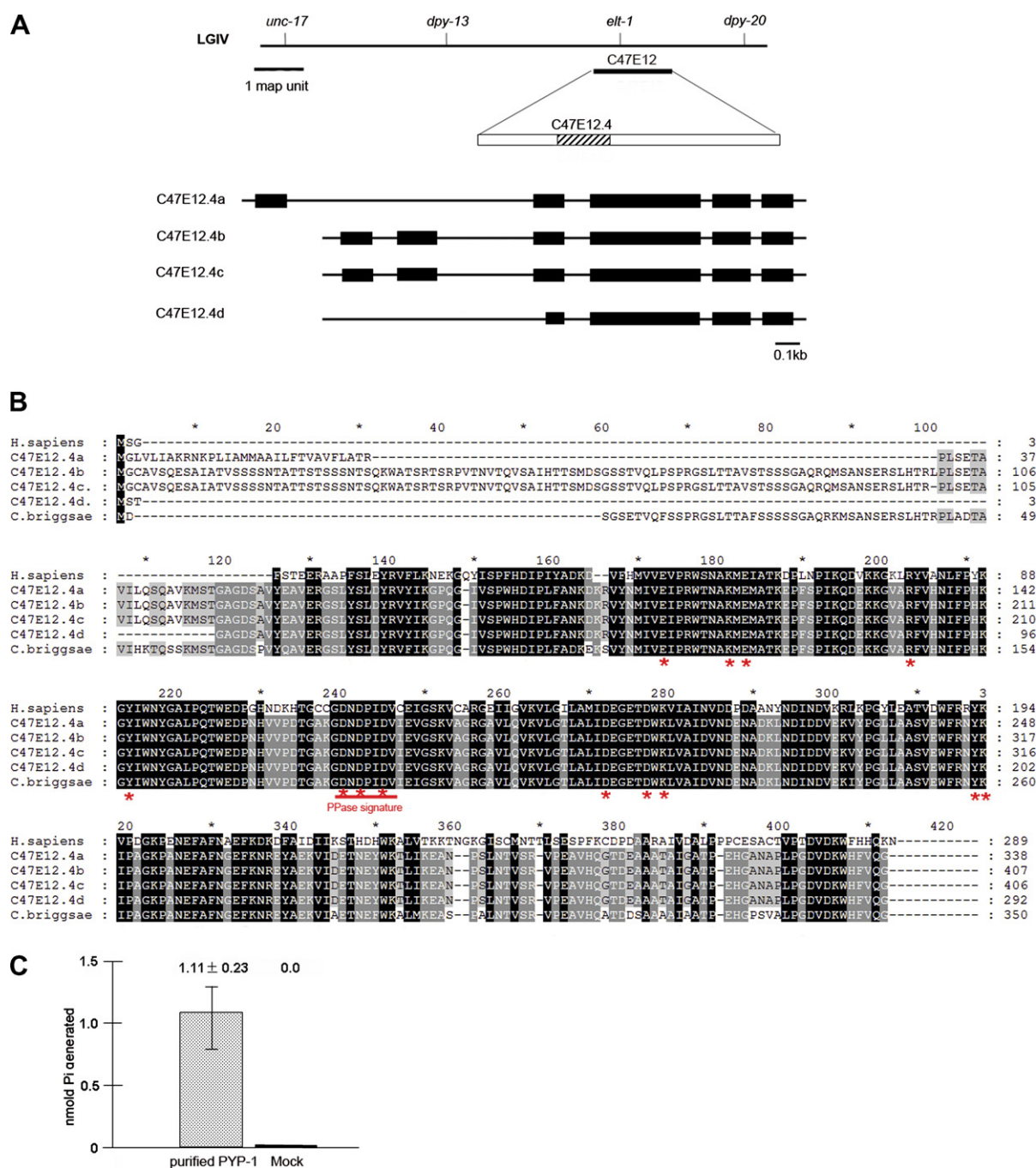


Fig. 1. (A) Genomic organization of *pyp-1* gene in *C. elegans*. Genetic and physical maps of the *pyp-1* region. The *pyp-1* locus relative to the nearby genetic markers on the gene cluster region of LG IV is shown. The *pyp-1* is mapped to the cosmid C47E12. The C47E12.4 gene (*pyp-1*) as predicted by Wormbase has four isoforms (C47E12.4a-d). (B) Amino acid sequence alignment of PYP-1. ClustalW alignments for the four isoforms of *C. elegans* inorganic pyrophosphatase (PYP-1) [Swiss-Prot:Q18680] with inorganic pyrophosphatase of *Homo sapiens* [Swiss-Prot:Q15181] and a *C. briggsae* counterpart. Dark shaded boxes represent identical amino acid residues. Red bar indicates the PPase signature, and the 13 conserved residues indicated by red asterisks are functionally important active sites. (C) Inorganic pyrophosphatase activity of recombinant PYP-1. PPase activity was measured spectrophotometrically for recombinant PYP-1 (70 pg) by measuring the rate of release of phosphate (Pi) from pyrophosphate (PPi) in the course of 10-min assay. (Mock) Mock indicates assay conducted in absence of PYP-1 protein.

this developmentally arrested phenotype was dramatically rescued to 75% by transformation of the heterozygote mutant with a *pyp-1* full-length genomic DNA (Table 1), suggesting once again that the larval arrest phenotype was indeed due to the *pyp-1* deletion.

Since we observed that *pyp-1* was mainly expressed in the intestine, we therefore reasoned that PYP-1 may have an

important role in intestinal functions. In order to study this, we looked for intestinal malformation using DIC optics. In general, *pyp-1* mutants showed morphologically aberrant intestine (Fig. 4D and E) compared to wild-type control animals of equivalent stage (Fig. 4C). As shown in Fig. 4D, the *pyp-1(jh123)* mutants showed abnormally enlarged, empty dilated intestinal lumens and cavities throughout the worm

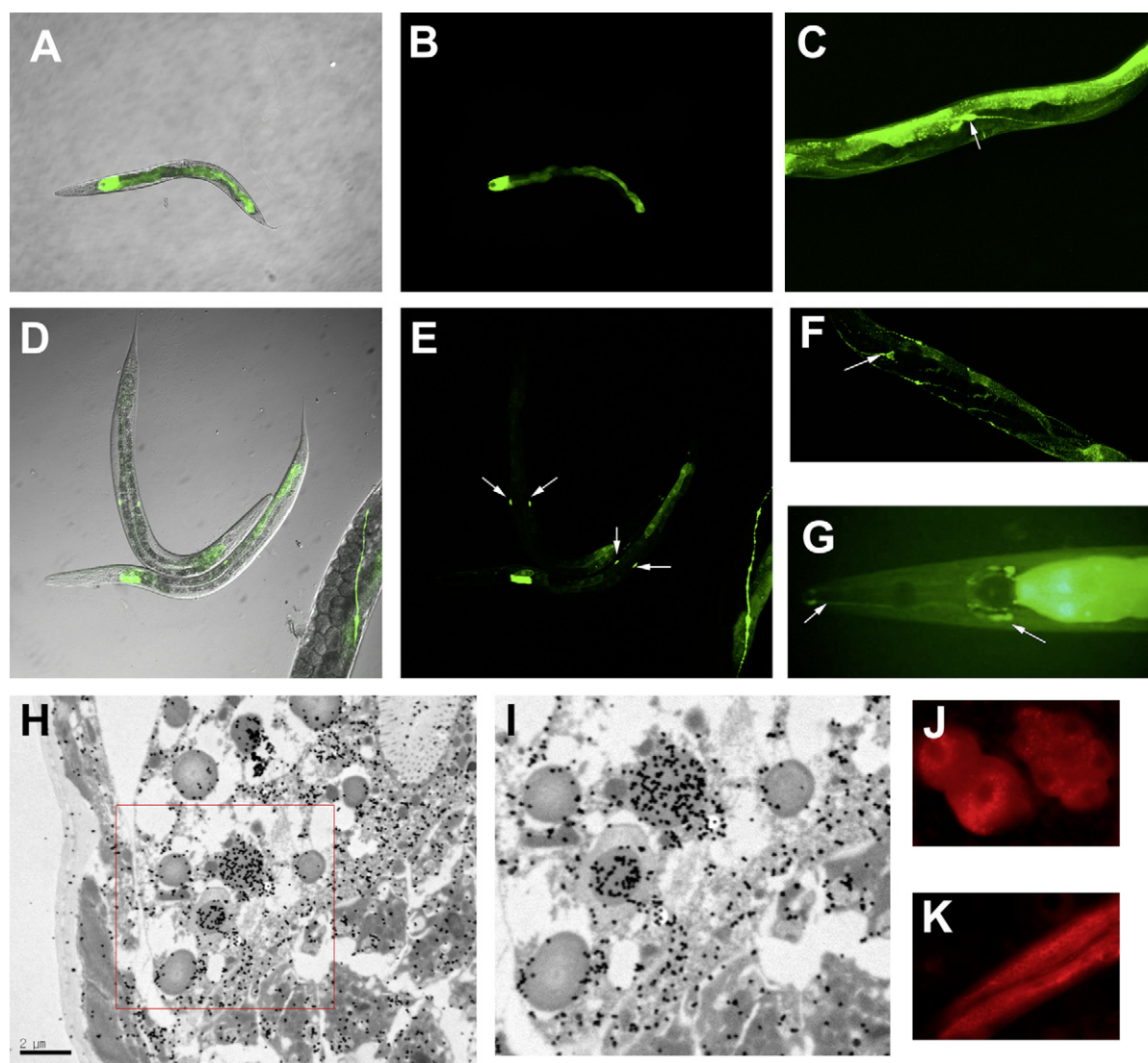


Fig. 2. Expression and localization of PYP-1. PYP-1::GFP expressed in intestine (A–C) from larval stage (A,B) to adult stage (C), (D,E) coelomocytes (arrows), (C,F) nerve cords (arrow), (G) sensory neurons (arrows). (H,I) Immunogold electron micrograph (EM) showed sub-cellular localization of PYP-1. (I) is the magnified view of the box shown in (H). Transverse section through the mid-body of wild-type worms showing PYP-1 localization by the signals of gold particles in the gut granules. Bar indicates 2 μm. (J,K) Immunostaining of wild-type worms with anti-PYP-1 antibodies detected PYP-1 at embryonic stage (J) and in the intestine of adult stages (K).

body. Furthermore, some *pyp-1(jh123)* mutants also had bigger but reduced number of gut granules (Fig. 4E).

A distinguishing feature of the *C. elegans* intestine is its developmental biology. Intestine is composed of 34 intestinal cells as evidenced by the selective staining of DNA of wild-type L2–L4 stage worms [23]. Staining displayed a characteristic distribution of intestinal cells along the length of the body. However, the *pyp-1* mutants, exhibited irregularly distributed and reduced number of cell nuclei (Fig. 4I–N). Besides, wild-type worms contained discrete and clear intestinal nuclei (Fig. 4F–H) that were rather diffused in the mutants (Fig. 4I–K). Such abnormal intestinal cellular morphology of the *pyp-1* mutants is therefore indicative of defective intestinal functions.

A function of intestinal cells is to secrete digestive enzymes (e.g. cysteine protease, endodeoxyribonuclease) into the lumen and to take up processed material and nutrients. This was

apparent when GFP-expressing bacteria fed to the *pyp-1* mutants failed to be taken up by the intestine (Fig. 5A–C). In case of N2 worms, the ingested GFP expressing bacteria were generally shown to spread along the intestine as evident by the fluorescence (Fig. 5A). However, there was little or no fluorescence observed within the lumen of the intestine suggesting that the intestinal contents were effectively absorbed from the lumen. On the other hand, the *pyp-1* homozygous mutants showed strong GFP expression along the pharyngeal region, and also towards the early part of the intestinal lumen, or sometimes along the entire length of the lumen which again explains the inability of absorption of intestinal contents by the intestinal cells (Fig. 5B and C).

In addition, intestine is also a large storage organ since it contains a large number of assorted storage gut granules [27], a marker of intestine-specific lysosomes [28]. These granules change in size, shape and number during various

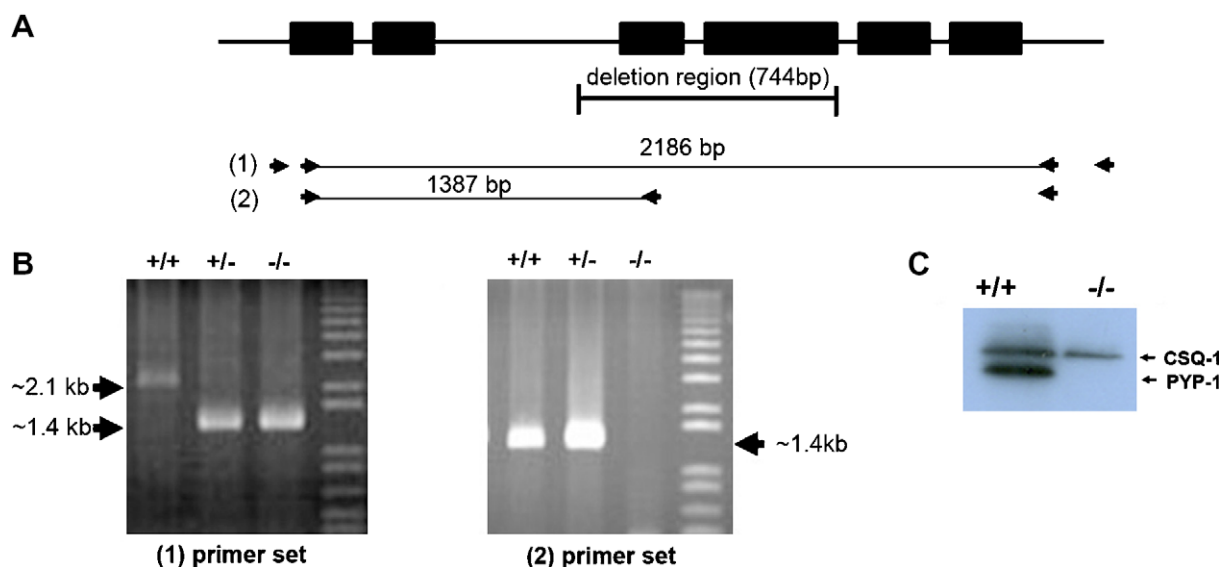


Fig. 3. Isolation of *pyp-1(jh123)* mutants. (A) The deleted region in *pyp-1* is shown (744 bp deletion). (1) and (2) indicate primer sets (shown by arrows) used for nested PCR during initial sib selection and for homozygosity checking, respectively. (B) PCR bands obtained from a single worm PCR of wild-type (+/+), heterozygote (+/-) and homozygote *pyp-1* (-/-) are marked by arrows. Absence of band in *pyp-1* (-/-) for primer set (2) confirms that the worm is a homozygote. (C) Western blotting analysis using anti-PYP-1 antibodies. A ~45 kDa PYP-1 protein was detected in mixed staged wild-type (+/+) worm lysates but, not in the *pyp-1* (-/-) L2 arrested homozygote lysates, indicating that *pyp-1(jh123)* is a null allele. CSQ-1 protein (calsequestrin as a positive internal control) was detected from both lysates (~64 kDa).

developmental stages of the animal. Neutral Red, a lysosomal marker, is known to be taken-up by the autofluorescent vesicles. We placed the *pyp-1* mutants on bacteria mixed with Neutral Red and allowed them to feed, and later examined their guts. Worms fed with bacteria mixed with Neutral Red showed the absence of autofluorescent vesicles in the *pyp-1* mutants (Fig. 5F and G) in comparison to the L2 stage N2 animals (Fig. 5D and E). The *pyp-1* mutants that lacked the gut granules also exhibited abnormal intestinal morphology (Fig. 5F and G). As evidenced from Fig. 5H and I, the *pyp-1(jh123)* mutants often showed the aberrant larger sized and diffused gut granules (data not shown).

Apart from muscles, intestine plays a major organ for fatty acid metabolism. In this regard, we wished to investigate the fat deposit in the *pyp-1* mutants to see if the fat content correlates with the larval arrest phenotype. Wild-type worms and *pyp-1* mutants were fed with bacteria mixed with the vital dye Nile Red, which effectively labels fat deposits. We observed that the *pyp-1(jh123)* mutants showed a clear reduction in Nile Red staining compared to wild-type (Fig. 5J and K), indicating that fat stores are depleted in the mutants (Fig. 5L and M).

4. Discussion

Inorganic pyrophosphatase (PPase) is an essential enzyme in living cells, providing a thermodynamic sink for many biosynthetic reactions. However, most of the previous studies have focused on biochemical approaches in microorganisms. Yet again using RNAi technology, a recent report on the PPase function in the parasitic roundworm *Ascaris* demonstrates that PPase plays an important role in molting and development [6]. In *Ascaris*, double-stranded RNA against PPase inhibited the larval molting without affecting the larval viability or causing

any morphological defect. We however, made a further attempt to carry out an in-depth study of PPase function *in vivo* by isolating and characterizing a deletion mutant of PPase (*pyp-1*) in the nematode *C. elegans*. This is the first comprehensive report on PPase function in a PPase knockout (null) metazoan, like the *C. elegans*.

The PPases catalyze the hydrolysis of inorganic PPi, a by-product of numerous important biosynthetic reactions that utilize ATP [1]. Therefore, it is found to be active in energy, protein synthetic and degradation pathways in growing cells that have increased rate of PPi production [29]. The over-expression of PPases led to the stimulation of sucrose degradation and starch synthesis, and increased nucleotide levels in growing potato tubers [30]. Several recent reports also showed that PPase was significantly overexpressed in various tumor tissues [13–15]. It seems likely that PPase may be required for both energy and protein synthetic and degradation pathways in rapidly growing tumor tissues. In view of this, we sought to overexpress PYP-1 under the control of a heat shock promoter to increase the metabolic rates, anticipating that it would affect growth rate and life span of the worms. Unexpectedly, we found no defects with respect to life span, brood size, growth rates and morphology, suggesting that the overexpression of PYP-1 apparently does not affect the development in the nematode *C. elegans*.

A function of PPase has been shown to be crucial for mitochondria genome replication during *S. cerevisiae* cell division [31], and also growth of *E. coli* [10]. In this study, abolishing PPase in *C. elegans* causes developmental arrest at L2 stage. Conversely, a different allele *pyp-1(n4599)* showed larval lethality prior to the third larval stage [32]. However, RNAi of *pyp-1* caused embryonic lethality [32], which also suggests that maternal expression of *pyp-1* is indispensable. It is likely that PYP-1, as a consequence of maternal effect, is able to maintain the survival ability of the embryonic and larval

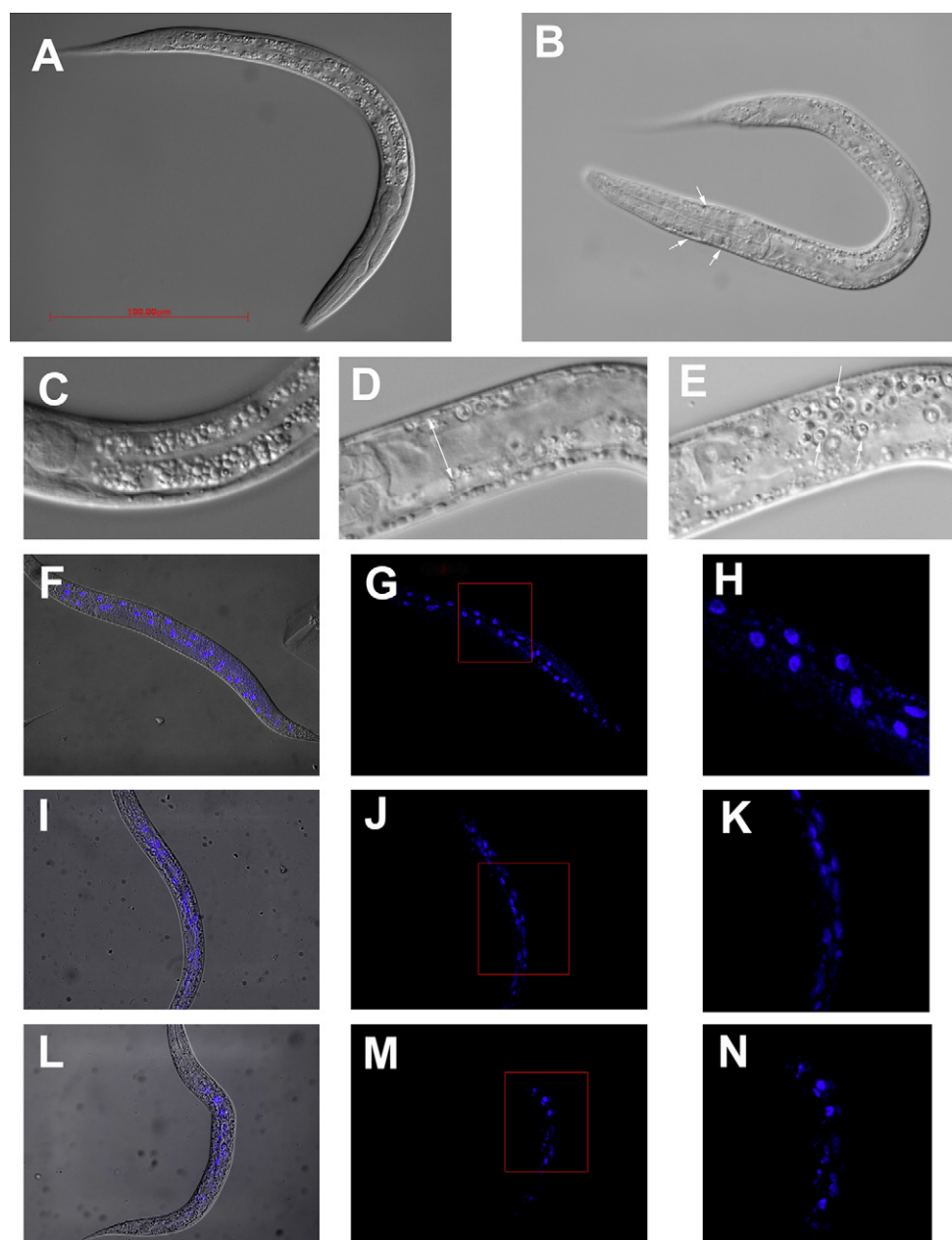


Fig. 4. The larval arrested phenotypes of *pyp-1(jh123)* deletion mutants. (A–E) Images by Nomarski microscopy. (A) Wild-type at L2 stage, (B) a *pyp-1(jh123)* developmental arrested L2 stage mutant showing cavities (indicated by arrows) with aberrant intestinal morphology. (C) Magnified view of intestine of wild-type at L2 stage showing normal intestine morphology. (D,E) Magnified view of intestine of *pyp-1(jh123)* mutant showing aberrant enlarged intestinal lumen (D), enlarged, reduced number of gut granules (E). Scale bar indicates 100 μm. (F–N) Selective staining (Hoechst 33258) of intestinal cell nuclei. Lateral view of the *C. elegans* larvae showing (F,G) wild-type worm with a characteristic distribution pattern of clean and discrete intestinal cell nuclei and (I–N) *pyp-1* homozygote with irregularly distributed reduced number of diffused nuclei. H, K, N are the magnified view of boxes shown in G, J, M, respectively.

stages, but not beyond L2 stage of the worms, which perhaps explains in a way that maternally derived PYP-1 falls short of the requirement for the growth of the animals. It is also possible that the cause of the developmental arrest of *pyp-1(jh123)* may be due to the deficiency of energy precursors required for the development of the nematode. Besides, it is indeed interesting to note that the larval-arrested mutants (*pyp-1*) could survive as long as the wild-type worms. A partial reduction in food intake (caloric restriction) has been found to increase the lifespan in many different organisms by reducing

basal metabolic rates and energy production [33]. For instance, *C. elegans eat* mutants exhibit defects in pharyngeal pumping that lead to a reduction in food uptake, a starved appearance, and an increased longevity [33,34]. Therefore, we may presume that the developmentally arrested mutants (*pyp-1*) that lived as long as the normal worms is perhaps a consequence of caloric restriction owing to the defects of digestion and absorption of bacteria (Fig. 5B and C).

In the parasitic nematode *Ascaris*, PPase expresses in the larval and as well as in the adult hypodermis, suggesting the

Table 1
Rescue analysis of *pyp-1* mutant

Genotype of P ₀	Larval arrest (%) ^a	N ^b
<i>pyp-1(jh123)/dpy-13(e184)unc-22(m52)</i>	24(±3.6)	4781
<i>pyp-1(jh123)/dpy-13(e184)unc-22(m52); Ex[pyp-1]</i>	6.5(±3.5)	7004

^aPercentage of larval arrest worms among total progeny.

^bIndicates the number of progeny examined in each experiment.

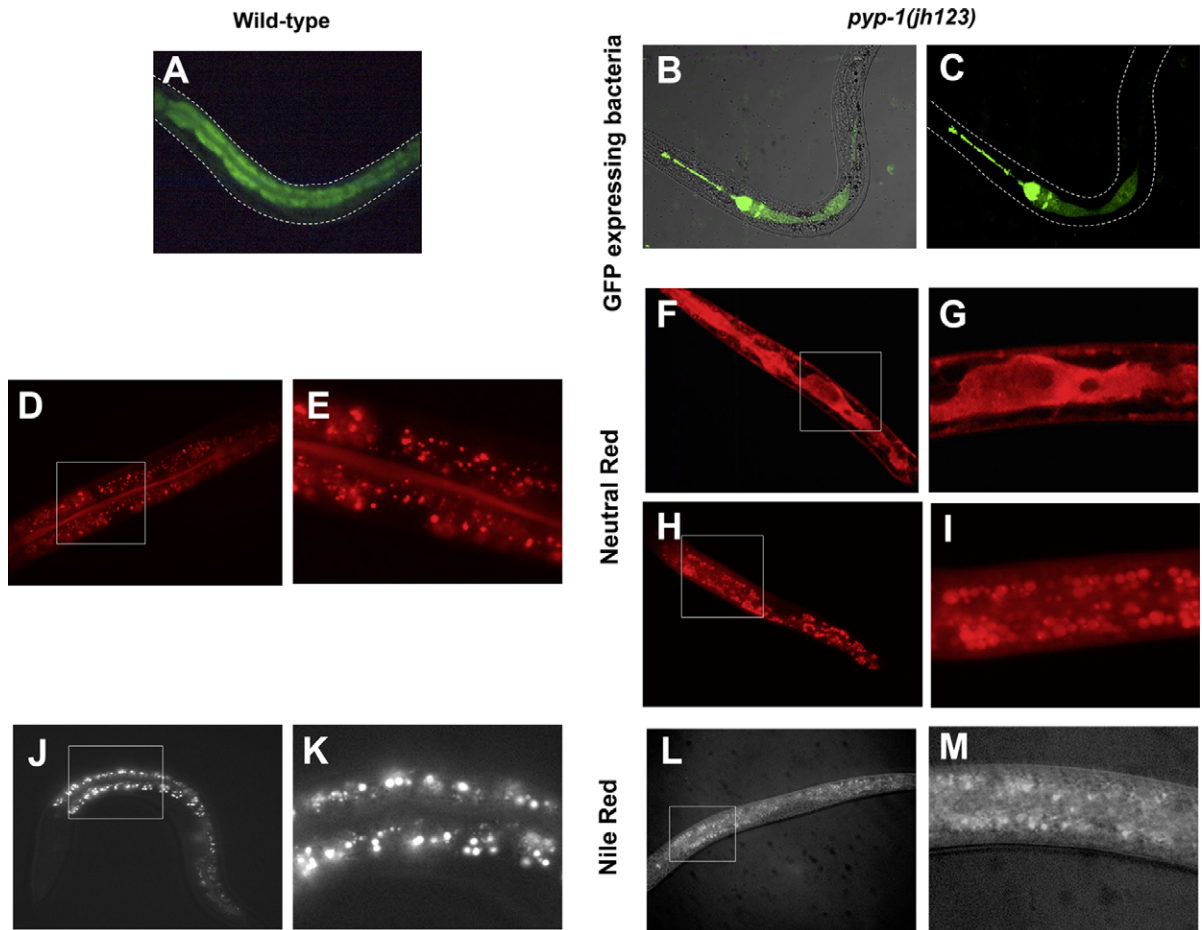


Fig. 5. Abnormal intestinal development and function in the *pyp-1(jh123)* mutant. (A–C) GFP-expressing bacteria feeding assay. Wild-type animals show evenly distributed GFP signals in the entire intestine (A), whereas, GFP-expressing bacteria have accumulated in the intestinal lumen in the *pyp-1(jh123)* mutants (B,C). (B) is the merged DIC image of (C). (D–I) Neutral Red staining. Wild-type animals show normal autofluorescent gut granules (D). (E) is the magnified view of the box shown in (D). (F,G) *pyp-1(jh123)* mutants show completely reduced autofluorescent gut granules. (G) is the magnified view of the box shown in (F). (H,I) The *pyp-1(jh123)* mutants also show the larger sized gut granules. (J,K) The L2 stage animals of wild-type show fat deposits. (K) is the magnified view of the box shown in (J). (L,M) Absence of fluorescence in the *pyp-1(jh123)* mutants, indicative of complete reduction in the levels of fat deposits. (M) is the magnified view of the box shown in (L).

enzyme's functional role in the synthesis of new cuticular components necessary for molting [6]. However, in *C. elegans*, no expression was detected in hypodermis which again implies that the *C. elegans* PYP-1 albeit having its role in larval development and gut function, may perhaps directly or indirectly be necessary for molting. On the other hand, in *Drosophila*, the inorganic PPase is a component of the nucleosome remodeling factor complex, NURF [7]. It has been shown that NURF-38, besides having an essential role in driving critical biosynthetic reactions is also involved in transcription, replication, and DNA repair. Also another nucleosome remodeling factor,

NURF-140, in the flies is involved in larval development [7]. We therefore cannot rule out the possibility that the larval arrest phenotype in the *pyp-1* mutants may perhaps be a consequence of abnormal chromosomal integrity or improper maintenance.

PYP-1 is mainly expressed in the intestine and nervous system in *C. elegans*. Since *C. elegans* intestine is one of the most highly metabolically active organ, which is involved not merely in digestion but also in storage and macromolecule synthesis, PYP-1 seems to play a significant role in energy metabolism in the intestine. In fact, the mutation of *pyp-1* causes

developmental arrest before L3 stage, and leads to morphological defects of intestine and consequently exhibits defects in the formation of gut granules. It is assumed that few gut granules or malformation of these birefringent granules in the *pyp-1* mutants are not likely to be enough to perform normal secretory, digestive, or absorptive functions in the gut. But, on the other hand, it has also been shown that worms with mutations in gut granule loss gene (*glo*), that result in defective gut granule biogenesis and therefore have loss or significant reduction in these autofluorescent granules, have near normal growth to adulthood [35]. However, our data hitherto in connection with loss of autofluorescent gut granules might be correlated with the reduced intestinal function, such as defects of intestinal absorption observed in the *pyp-1* mutants.

PYP-1 is probably involved in fat metabolism in view of the fact that lipid metabolism also requires PPase activity wherein PPi has to be hydrolyzed by PPase to support these metabolic reactions. A genome-wide RNAi screen for several fat regulatory genes have shown that reduction of fat contents is not only accompanied by larval arrest but also by embryonic lethality and sterility [36]. Therefore, it is probable that reduced fat contents in the *pyp-1* mutants may be merely a characteristic feature of the larval arrested worms. But, there are many genes that are yet to be identified for regulating fat contents in the worms. Therefore, further study is needed to establish a correlation of body fat contents with the PYP-1 activity.

In summary, we have shown the PPase function *in vivo* and our results demonstrate that PPase is required for the larval development and intestinal function in *C. elegans*. This report may also provide valuable insights into the functions of conserved PPases in higher animals.

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