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Loss of dystrophin and the microtubule-binding protein ELP-1 causes progressive paralysis and death of adult *C. elegans*

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Abstract

EMAP-like proteins (ELPs) are conserved microtubule-binding proteins that function during cell division and in the behavior of post-mitotic cells. In *C. elegans*, ELP-1 is broadly expressed in many cells and tissues including the touch receptor neurons and body wall muscle. Within muscle, ELP-1 is associated with a microtubule network that is closely opposed to the integrin-based adhesion sites called dense bodies. To examine ELP-1 function we utilized an *elp-1* RNA interference assay and screened for synthetic interactions with mutated adhesion site proteins. We reveal a synthetic lethal relationship between ELP-1 and the dystrophin-like protein, DYS-1. Reduction of ELP-1 in a dystrophin [*dys-1(cx18)*] mutant results in adult animals with motility defects, splayed and hypercontracted muscle with altered cholinergic signaling. Worms fill with vesicles, become flaccid and die. We conclude that ELP-1 is a genetic modifier of a *C. elegans* model of muscular dystrophy.

Keywords

muscle physiology; cytoskeleton; cell adhesion; cholinergic agents; muscular dystrophy

Introduction

ELP-1 belongs to the EMAP-like protein family with orthologs such as sea urchin EMAP (Suprenant et al., 1993) and human EML2 (Eichenmuller et al., 2002). In *C. elegans*, there is a single *elp-1* gene that is required for normal touch sensation by the touch receptor neurons (TRNs) (Hueston et al., 2008). In addition to the six TRNs, ELP-1 is broadly expressed in body wall muscle, intestine, ciliated IL1 neurons, rays of the male tail, hypodermal seam cells, vulval muscles, spermatheca, head and tail neurons. Within body wall muscle, ELP-1 is associated with a criss-crossing array of microtubules that are aligned and concentrated along the dense bodies, integrin-based adhesion sites where the muscle tension is transmitted to the cuticle. ELP-1 may play an important role in the transmission of forces and signals between the extracellular matrix and the cytoskeleton (Hueston et al., 2008).

In vertebrate muscle, the transmembrane protein dystroglycan links the extracellular matrix to the actin cytoskeleton via the actin and dystroglycan binding domains of dystrophin (Ehmsen et al., 2002). This forms a dystrophin glycoprotein complex (DGC) that resists the stresses of repetitive contractions (Campbell, 1995). In addition to actin filaments, DGC components also interact with microtubules. For example, in non-muscle cells such as adhered platelets, β-dystroglycan alone interacts dynamically with both microtubules and actin filaments to modulate the formation of focal contacts (Cerecedo et al., 2008). Furthermore, both the vertebrate microtubule-actin cross-linking factor (MACF)—a hybrid

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of dystonin and a dystrophin-like plakin, and the *Drosophila* protein kakapo—a dystrophin-like plectin, each form essential links between microtubules, actin and integrin-based adhesion sites (Gregory and Brown, 1998; Leung et al., 1999; Leung et al., 2001; Sonnenberg and Liem, 2007).

Many of the components of the vertebrate DGC and integrin-based focal adhesion sites are conserved and expressed in *C. elegans* (Cox and Hardin, 2004), although not all of the DGC components are known to function in body wall muscle. For example dystroglycan (*dgn-1*) is expressed prominently in epithelia and neurons, but not in body wall muscle (Johnson et al., 2006). There is however a single dystrophin/utrophin homolog expressed in body wall muscle, and animals without a functional dystrophin homolog are hyperactive with an exaggerated bending of the head when moving forward and hypercontraction of the body when moving backwards (Bessou et al., 1998). This raises the possibility that DGC function in vertebrates is not conserved in *C. elegans* and that a better understanding of dystrophin-related functions in *C. elegans* could reveal additional roles for dystrophin and for understanding the muscular dystrophies in human.

In this report, we carried out a pilot RNA-mediated interference screen to identify DGC and adhesion site genes that interacted synthetically with the microtubule-binding protein ELP-1 (Suprenant et al., 2000; Hueston et al., 2008). We identified the dystrophin-like protein-1 (DYS-1) as a potential interactor and showed that reduction of ELP-1 levels in a *dys-1(cx18)* animal resulted in a progressive paralysis with death as the final outcome. This study is consistent with our proposed role for ELP-1 in the reconciliation of the pushing and pulling forces that occur between the cytoskeleton and the extracellular environment.

Results

Evidence for a genetic interaction between *elp-1* and *dys-1*

By comparing the RNA-mediated interference (RNAi) phenotype in a wild-type worm and in a loss-of-function worm, RNAi can be used to identify redundant genes (Paradis and Ruvkun, 1998; Gotta and Ahringer, 2001) as well as multigene interactors (Ceron et al., 2007; Samuelson et al., 2007). In this report, we carried out a pilot RNAi screen to identify genes that may function redundantly with *elp-1* or act in a network to regulate microtubule function and cell adhesion sites.

Reduction of ELP-1 had little effect on the development, physiology, and behavior of wild-type worms (Fig 1 and Tables 1A & 2). There were no egg laying defects and all of the worms progressed normally through the larval stages and developed into adult animals of normal dimensions at both 20°C and 25°C.

For the pilot screen, a small number of candidate genes were selected from those that affect muscle function primarily through adhesion complexes. Several mutations are associated with the myopathies of the muscular dystrophies (Bessou et al., 1998; Gieseler et al., 1999; Chamberlain and Benian, 2000; Gieseler et al., 2001; Grisoni et al., 2003). Others, such as the *mua* mutations, result in catastrophic detachment of skeletal muscles from the body wall after normal muscle differentiation (Plenefisch et al., 2000). Several plakin and intermediate filament mutants were selected because these proteins play key roles in neuronal outgrowth, and the adhesion of muscle and epithelial cells (Bosher et al., 2003; Sonnenberg and Liem, 2007). Most strains showed no significant movement defects when fed *elp-1* dsRNA [hereafter *elp-1*(RNAi)]. For example, no effect on thrashing was observed in either the capon [*dyc-1(cx32)X*], dystrobrevin [*dyb-1(cx36)I*], or syntrophin [*stn-1(ok292)I*] mutant animals at 20°C or 25°C (Table 1A). For a summary of additional strains that showed mild or no synthetic movement defects see Supplemental Table 1B, online.

By contrast, loss of ELP-1 in a loss-of-function dystrophin strain, *dys-1(cx18)*, had visible effects on motility (Table 1A). When examined at ~48 hrs after the L4 stage, these worms thrashed ~20% less frequently than the *dys-1(cx18)* control worms at 25°C. To confirm these results, we reexamined these movement defects by a body bend assay on a solid substrate where we observed a time-dependent change in motility (Robatzek and Thomas, 2000). *dys-1(cx18); elp-1*(RNAi) worms were assayed at ~18 hrs after the L4 stage and showed no significant change in locomotion rate from the control *dys-1(cx18)* worms. When examined at ~ 48 hrs after L4, the animals had markedly fewer body bends per minute (Fig 2). In contrast, there were no locomotion defects observed with either RNAi-treated wild-type [N2] or dystrobrevin [*dyb-1(cx36)*] worms at either temperature or time point.

To confirm these RNAi results, we examined whether the previously characterized *elp-1(ok347)* deletion allele could exacerbate the dystrophin mutant phenotype. We crossed the *elp-1* deletion strain, *ok347*, into the *dys-1(cx18)* background and examined whether these animals showed any gross changes in physiology or motility which were not seen in the *dys-1(cx18)* animals. None was observed. This result is expected since *ok347* produces two transcripts predicted to encode nearly full-length ELP-1 proteins with both the HELP and WD domains (Hueston et al., 2008). Furthermore, when *ok347* is placed in *trans* to a deficiency that covers the *elp-1* allele (*ozDf1*), the animals are pale, scrawny and 25% shorter in length and width. A full-length $P_{elp\text{-}1}$*::elp-1::gfp* transgene rescues these worms such that the body size and density of the *trans* heterozygotes increase by approximately 50%. These results imply that loss of ELP-1 is at least partially responsible for the small and scrawny phenotype and that *ok347* is a partial loss-of-function allele (Hueston and Suprenant, unpublished results).

Hypercontraction and disorganization of striated and nonstriated muscle

The body wall muscles of *dys-1(cx18); elp-1*(RNAi)worms were hypercontracted with compressed sarcomeres (Fig 3A & B). To assess the degree of hypercontraction, measurements were made along two diagonal axes of individual muscle cells. In the dystrophin muscle cells, D1 and D2 measurements averaged 80.6±5.5 μm (n=6) and 25.3±1.5 μm (n=4), respectively. In contrast, D1 and D2 averaged 63.2±9.5 μm (n=14) and 17.4±1.0 μm (n=12) in ELP-1-depleted dystrophin muscle cells. This represents a compression of 22% and 31%, along the D1 and D2 axes, respectively ($p<0.01$ *T*-test).

Nonstriated, single-sarcomere muscles were also disrupted in *dys-1(cx18); elp-1*(RNAi) worms (Fig 3C & D). Uterine muscle fibers (um1) were crinkled and separated from one and another and from the uterine epithelium. Similarly, bundles of myofilaments within the vulval muscle (vm2) were separated and detached from the ventral margin of the body wall muscle quadrant.

Because these sex-specific muscles were disrupted, we examined worms for an egg-laying defect (Table 2). First, the dystrobrevin and dystrophin mutant worms showed an egg-laying defects in the absence of *elp-1*(RNAi) (Table 2). Each strain laid approximately 70% fewer embryos than wild-type worms. In addition, a larger fraction of the *dyb-1(cx36)* and *dys-1(cx18)* embryos were at the post-comma stage when compared to wild-type worms. However, when these worms were fed *elp-1* dsRNA, only the *dys-1(cx18)* worms responded by laying fewer eggs. Loss of ELP-1 in the *dys-1(cx18)* animals exacerbated this egg laying defect, but did not change the ratio of pre-comma and post-comma embryos. Furthermore, these worms did not show a “bag of worms” phenotype, whereby the larval worms hatch in the adult. These results imply that the vulval muscles retain partial function.

Cholinergic signaling is altered

ELP-1 is also found in the muscle feet (Hueston et al., 2008) where the neuromuscular junctions are located and the acetylcholine receptors are clustered (Rand, 2007). Furthermore, worms without a functional dystrophin are hyperactive with an exaggerated bending of the head when moving forward and a hypercontracted body when moving backwards (Bessou et al., 1998). These data imply that both ELP-1 and DYS-1 could be involved in cholinergic signaling.

To examine for defects in acetylcholine transmission, animals were examined for paralysis in the presence of the cholinergic drugs, aldicarb and levamisole. In Figure 4A & B, we confirmed that the *dys-1(cx18)* and *dyb-1(cx36)* mutants each showed an aldicarb-dependent Hypersensitivity to Inhibitors of Cholinesterase (Hic) phenotype (Bessou et al., 1998; Gieseler et al., 2001). These mutant worms remained 'Hic' when ELP-1 levels were reduced in both of these animals. In addition, animals were tested for their sensitivity to levamisole, a potent acetylcholine agonist that leads to paralysis and death of wild-type worms (Lewis et al., 1980). Both the *dys-1(cx18)* and *dyb-1(cx36)* mutant worms were more resistant to levamisole when compared to wild-type worms (Fig 4C & D). Loss of ELP-1 in *dys-1(cx18)* but not *dyb-1(cx36)* resulted in worms that were levamisole-hypersensitive (Fig. 4C & D). The levamisole resistance observed in the *dys-1(cx18)* worms alone was effectively suppressed by a loss of ELP-1. These data show that both ELP-1 and DYS-1 are involved in cholinergic signaling.

Animals become paralyzed and die prematurely

The motility and cholinergic defects observed in the *dys-1(cx18); elp-1*(RNAi) animals were coincident with a steady build up of large fluid-filled vesicles in the pseudocoelom and body wall muscle (Figure 5, Supplemental Movies 1-3). In body wall muscle, the vesicles are smaller and oblong in shape, with the long axis of the vesicle aligned along the rows of dense body attachment sites. The larger vesicles form near the cuticle and may be associated with the seam cells. The accumulation of vesicles began at the end of day one (between 20 to 30 hrs after the L4 stage), and continued until the worms died during day three. Greater than 95% of the synthetic double mutant worms were completely paralyzed (even after harsh prodding with a pick) 55-60 hours after the L4 stage. By comparison, N2 worms with reduced ELP-1 levels live two weeks longer than the synthetic double mutants at 25°C. This synthetic lethal phenotype was not seen in the RNAi-treated strains carrying mutations in *dyb-1(cx36)* (dystrobrevin), *stn-1(ok292)* (syntrophin), or *dyc-1(cx32)* (capon).

Discussion

In *C. elegans*, the sole member of a conserved family of ELP-1 microtubule-binding proteins interacts genetically with the sole member of the dystrophin/utrophin family to generate animals with altered cholinergic signaling and motility defects. Ultimately these animals fill with vesicles and die prematurely. This study is a critical first step in identifying a novel genetic modifier that synergistically aggravates the dystrophin phenotype in worms.

A temperature-dependent, synthetic lethal interaction between *elp-1* and *dys-1*

The severe modification of the dystrophin phenotype is not the sum of two sick animals but rather a classic genetic interaction where the combined effect is not exhibited by either mutation alone. Reduction of ELP-1 levels in a wild-type animal has little detectable effect on muscle contractility and importantly the animals are viable. The *dys-1(cx18)* animals are also viable with apparently normal muscle and subtle behavioral defects that include hyperactive head movements (Bessou et al., 1998; Grisoni et al., 2002). The synthetic lethal interaction between *elp-1* and *dys-1*, results in a temperature- and time-dependent

impairment of locomotion, both in liquid culture and on a solid substrate. Cholinergic signaling is altered. These animals are severely compromised by the formation of fluid-filled vesicles and the worms die during day three of adulthood. These results show that *elp-1* is a genetic modifier of *dys-1* in *C. elegans*. The rapid decline of the animals between day one and day three indicates that this interaction occurs at a critical period during the lifespan of the worms (Huang et al., 2004).

Modification of the dystrophin phenotype requires an elevated temperature of 25°C. This synthetic interaction between ELP-1 and DYS-1 could result from a temperature-induced protein destabilization similar to those that occur with certain *ts* mutations. Alternatively, there may be a threshold level of ELP-1 that maintains a near normal phenotype. Whether this could result from an increased efficiency of RNAi at 25°C or an allele-specific RNAi response is not known at this time.

Whether DYS-1 and ELP-1 work in parallel pathways or interact directly remains to be determined. ELP-1 and DYS-1 are broadly expressed in the adult worm. ELP-1 is located in body wall muscle, vulval muscle, seam cells, intestine, spermathecal valve, touch receptor neurons, ciliated IL1 neurons, ray neurons of the male tail, as well as a few unidentified head and tail neurons (Hueston et al., 2008). Similarly, DYS-1 is expressed in body wall muscle, vulval muscle, seam cells, intestine, reproductive system, pharynx, ventral nerve cord, and some unidentified head and tail neurons (Bessou et al., 1998; Dupuy et al., 2007; Hunt-Newbury et al., 2007). This overlapping pattern of protein expression implies that the synthetic lethal phenotype could arise from alterations in physiology and cellular architecture in a variety of cells and tissues.

Body wall muscle is the only tissue where the subcellular location of both of these proteins has been examined in detail. Within body wall muscle, ELP-1 is found within the muscle feet, in close association with the dense bodies, and along microtubules throughout the muscle cytosol (Hueston et al., 2008). The subcellular location of ELP-1 is consistent with a role in trafficking or anchoring membrane proteins/receptors, as well as force generation and sensing, as was previously suggested (Hueston et al., 2008). Similarly, DYS-1 is expressed within the body wall muscle, however DYS-1 does not strictly localize to the muscle feet, dense bodies, microtubules or actin filaments (Bessou et al., 1998). “In the body-wall muscles of *C. elegans*, the DYS-1 protein localizes in a broad region overlapping thin filaments and dense bodies (our unpublished results)” (Lecroisey et al., 2008). However, the truncated dystrophin construct used in these expression studies may not localize properly, leaving open the question as to whether ELP-1 and DYS-1 co-localize in muscle.

Loss of ELP-1 and DYS-1 alters muscle excitability

Previously, it was shown that cholinergic signaling is altered in *dys-1* mutant animals as demonstrated by an aldicarb-dependent Hic phenotype (Bessou et al., 1998; Gieseler et al., 1999). Herein we confirm these results and additionally show that the *dys-1* mutant is levamisole resistant and that the resistance is suppressed by loss of ELP-1. These results imply that ELP-1 and DYS-1 share a related function in cholinergic transmission in body wall muscle.

There are at least two pharmacological subtypes of AChRs in *C. elegans* body wall muscle, a levamisole-sensitive type of nicotinic receptor (hereafter “Lev-type”) and a nicotine-sensitive receptor that does not respond to the acetylcholine agonist, levamisole (hereafter “Nic-type”) (Richmond and Jorgensen, 1999). The Lev-type receptor is comprised of three essential subunits, UNC-29, UNC-38, and UNC-63 (Fleming et al., 1997; Richmond and Jorgensen, 1999; Culetto et al., 2004) while the Nic-type receptor is comprised of a single essential α-type ACR-16 subunit (Francis et al., 2005; Touroutine et al., 2005). Both Nic-

and Lev-type receptors are concentrated at the neuromuscular junctions of the muscle feet. One explanation for the results is that *dys-1(cx18)* mutants have fewer or less active Lev-type receptors than Nic-type receptors. Nic-type receptors are more sensitive to acetylcholine and could generate increased excitability. By contrast, the synthetic mutant *dys-1(cx18); elp-1*(RNAi) could have increased numbers of or more active Lev-type receptors. This model could account for the *elp-1*(RNAi) suppression of the *dys-1* levamisole resistance.

The model presented above is one explanation for the cholinergic effects observed, but not the only possible explanation. Considering that both DYS-1 and ELP-1 are expressed in body wall muscle, ELP-1 and DYS-1 could modifying the extent of muscle excitability by regulating (1) the trafficking of ACh receptors between the Golgi and the plasma membrane, (2) the aggregation or anchoring of receptors at the synapse, or (3) the modulation of receptor activity. In addition, it is conceivable that this synthetic interaction could misregulate the novel acetylcholine/choline transporter, SNF-6 (Kim et al., 2004), the voltage-regulated calcium channel of muscle, EGL-19 (Mariol and Segalat, 2001), or even the large conductance K+ channel, SLO-1 (Carre-Pierrat et al., 2006). All of these transport proteins play critical roles in muscle excitation.

Disease connections

There are seven genes that encode EMAP-like proteins (EMLs) in humans: EML1-5, ENST00000398629_HUMAN, and Q6ZMW3_HUMAN. All EMAP-like proteins are characterized as having a Hydrophobic ELP (HELP) domain/motif and a WD domain (Eichenmuller et al., 2002). The HELP domain is required for microtubule binding and it is assumed that the WD repeat domain is required for additional protein-protein interactions (Eichenmuller et al., 2001; Tegha-Dunghu et al., 2008).

Several of the human EMLs are directly involved in human diseases. EML1 was originally a “strong candidate for the Usher syndrome type 1A gene” (Eudy et al., 1997) but was demoted when the locus for this autosomal recessive disorder of deafness and blindness was found not to exist (Gerber et al., 2006). EML1's biomedical importance was upheld when it was discovered that in certain T-cell leukemias, the protein kinase c-ABL1 is fused to the EML1 gene resulting in the expression of a dysregulated tyrosine kinase (De Keersmaecker et al., 2005). Similarly, EML4 was initially identified as a protein that was overexpressed during mitosis (Heidebrecht et al., 2000). Recently, it was demonstrated that a chromosomal inversion that produces an EML4-ALK fusion-type protein tyrosine kinase results in a novel, transforming gene in non-small-lung cancer (Soda et al., 2007). Further, expression of EML4-ALK in lung epithelia of mice produces hundreds of adenocarcinoma nodules within a few weeks of birth (Soda et al., 2008). EML hybrid oncogenes are thus associated with both blood related cancers and solid tumors of the lung.

In this report we show that *elp-1* acts as a modifier gene in a *C. elegans* model of muscular dystrophy. While mutation of the human dystrophin gene is recognized as a hallmark event in defining the X-linked myopathy in both Duchenne muscular dystrophy and the milder form of the disease, Becker muscular dystrophy, accumulating evidence indicates that additional genes contribute to the modulation of the dystrophin mutant phenotype. We speculate that mutations in one or more of the seven human EMAP-like genes could also act as a modifier gene(s) because mutations in the dystrophin gene alone cannot account for the variability in the onset, severity and progression of muscular dystrophy (Heydemann et al., 2007).

As indicated above, Duchenne muscular dystrophy is a complex and multivariate disease. The pioneering neurobiologist, Guillaume-Benjamin-Armand Duchenne De Boulogne,

recognized in 1868, that five of his patients had poor cognitive and language skills (Duchenne, 1868). A hundred and twenty years later there is overwhelming evidence supporting Duchenne de Boulogne's original observation that there is significant cognitive impairment of some DMD patients. These clinical observations are supported by histological, biochemical, and electrophysiological evidence for an involvement of the central nervous system and the retina (Blake and Kroger, 2000; Anderson et al., 2002). Although we initiated this study to reveal ELP-1 and microtubule-related functions in muscle cell adhesion, we uncovered a broader synthetic interaction with dystrophin that indicates that these proteins could function together in a variety of cell types. Our results with *C. elegans* could play an important role in identifying conserved roles for EMAP-like proteins and dystrophin and for shedding light on the variable nature of the muscular dystrophies.

Experimental Procedures

C. elegans strains and culture

Wild type worms (N2 Bristol) were grown on NGM agar medium seeded with the *Escherichia coli* strain OP50 at 20°C unless otherwise indicated (Brenner, 1974). Worm strains were provided by the *Caenorhabditis* Genetics Center, Minneapolis, MN. Genes and mutations used in this study are listed by linkage group as follows: LG I: dys-1(cx18), dyb-1(cx36), stn-1(ok292), anc-1(e1802), vab-10(e698); LG II: *hlh-1(cc561), mua-6(rh85);* LG III: *mua-2(rh119), mua-3(rh195);* LG V: *unc-23(e25), unc-83(e1408);* LG X: dyc-1(cx32), unc-27(e155), mua-10(rh267), unc-84(e1174).

Molecular Biology

The full-length $P_{elp\text{-}1}$*::elp*-1::*gfp* construct (pKA99-2) contains 9 kb of the *elp-1* gene with the endogenous *elp-1* promoter and expresses the full-length ELP-1 protein (exons 1-16) fused to GFP (Hueston et al., 2008).

RNA mediated interference (RNAi)

A feeding-based RNAi assay was used to decrease the expression of *elp-1* (Timmons et al., 2001). Two L4 hermaphrodites were transferred onto each feeding plate and incubated at either 20 or 25°C. The development of the F2 progeny was staged by transferring L4 stage worms onto new RNAi feeding plates at 20 or 25°C. These F2 worms were then examined at 18, 24 and 48 hrs after being transferred at the L4 stage.

Phalloidin Staining

The actin cytoskeleton of muscle cells was visualized by means of fluorescence after staining with a 1:750 dilution Oregon Green 488 phalloidin (Costa et al., 1997).

Thrashing Assay

Individual worms were transferred onto a microscope slide containing a 5μL drop of M9 buffer. After a 30 s recovery period, we counted as one beat, each time the worm swung its head to one specific side over a 1-min period (Miller et al., 1996).

Body Bend Assay

A single animal was transferred directly from an RNAi plate to a NGM plate with a fresh lawn of OP50 *E. coli*. After 1-minute, the number of body bends were measured during a subsequent 3-minute period. A single body bend was scored when “the vulva reached the peak or trough of the sine wave” (Robatzek and Thomas, 2000).

Egg-staging assay

Fifteen animals were placed onto NGM plates with a fresh lawn of OP50 bacteria and allowed to 'calm' for 30 minutes. Embryos from this 'calming' period were removed and not counted. Subsequently, embryos were staged and counted at 10-minute intervals over a 2-hr period.

Light Microscopy

Worms were mounted on 2 % agar pads on glass slides in 1% 1-phenoxy-2-propanol or 10 mM levamisole in M9 buffer. Images were collected with a 63X 1.4 NA DIC Plan-Apochromat DIC objective and captured with a Hamamatsu Orca-ER camera (Hamamatsu City, Japan) driven by the OpenLab software version 2.1A (Improvision, Lexington, MA). Quicktime™ movies were compiled from 0.5 μm optical sections along the Z-axis spanning the circumference of the worm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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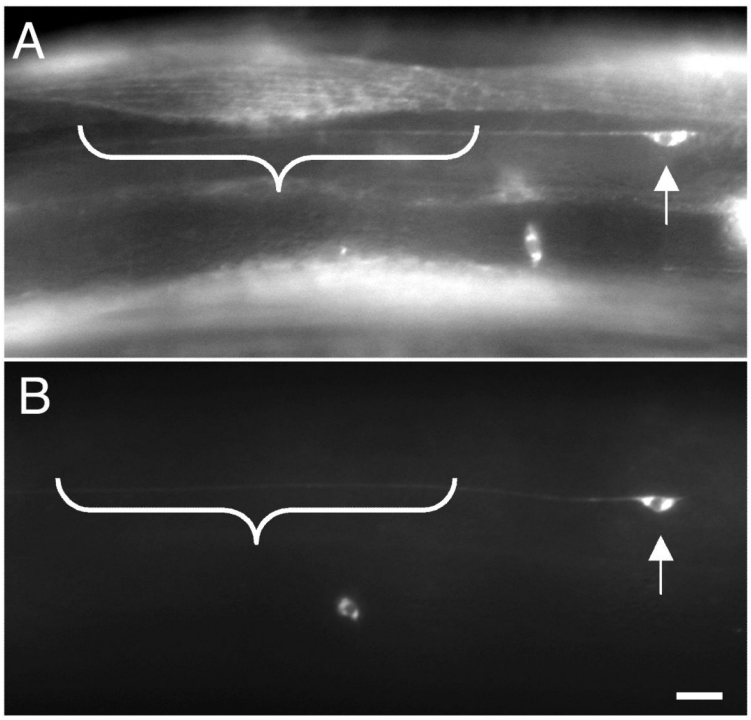

Figure 1.
Reduced expression of ELP-1 by RNAi was observed by fluorescence microscopy. A. ELP-1::GFP animal fed the control bacteria. B. ELP-1::GFP animal fed *elp-1* dsRNA. ELP-1 is lost in the body wall muscle (bracket) but a significant amount of ELP-1 remains in the touch receptor neurons (arrow, ALM cell body is shown). Bar represents 10 μm.

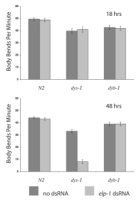

Figure 2.

The locomotion rate of the *dys-1(cx18)*;*elp-1*(rnai)worms decreased with age. Animals were grown and scored at 25°C and the average number of body bends per minute was plotted +/− the standard error of the mean for N2 (n=16), (*dys-1(cx18)*(n=38), and *dyb-1(cx36)* (n=26) worms. Measurements were taken at 18 hr (A) and 48 hr (B) after the L4 stage (see Materials and Methods).

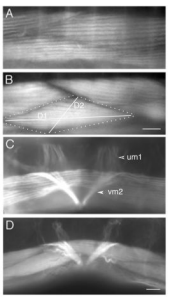

Figure 3.

Visualization of body wall and vulval muscle defects by means of 488 Oregon Green-conjugated phalloidin. Adult *dys-1(cx18)* hermaphrodites worms were grown at 25°C on either control bacteria (A & C) or *elp-1* dsRNA producing bacteria (B & D). The worms shown were photographed 48 hrs after the L4 stage. Body wall muscles are shaped similar to an irregular parallelogram (dotted outline). To estimate the degree of hypercontraction, length measurements were made along the long axis of the muscle cell (D1) and between the two remaining vertices of the parallelogram (D2). In addition, both the uterine muscle (um1) and vulval muscle (vm2) exhibited significant defects. Both the um1 and vm2 muscles area were hypercontracted with a squigglier outline than untreated muscle. The bars represent 10 μm.

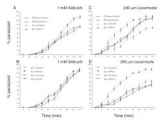

Figure 4.
Loss of ELP-1 suppresses the levamisole resistant phenotype of *dys-1(cx18)* worms. Effect of 1 mM aldicarb (A & B) or 200 μM levamisole (C & D) on the motility of wild-type, *dys-1(cx18)* and dyb-1(cx36) worms fed control bacteria or *elp-1* dsRNA producing bacteria.

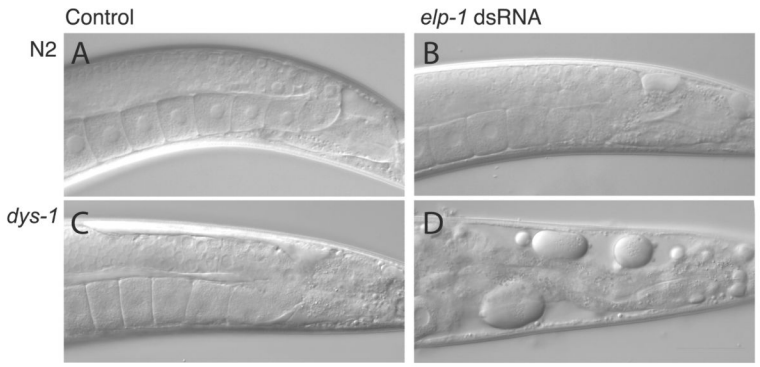

Figure 5.

ELP-1 knockdown causes synthetic lethality in *dys-1(cx18)* animals N2 (A, B) and *dys-1(cx18)* worms (C, D) were fed either control bacterial (A, C) or *elp-1* dsRNA producing bacteria at 25°C (B, D). At approximately 55-60 hours past the L4 stage the *dys-1(cx18); elp-1*(rnai)worms were flaccid and presumed to be dead (D). Bar represents 50 μm.

Table 1A

Effects of *elp-1*(RNAi) on thrashing (bends/min)

Genotype	Description	20°C		25°C	
		Control	**elp-1(RNAi)**	**Control**	**elp-1(RNAi)**
Bristol N2	wild-type	87.0 ± 10.3 (51)	83.5 ± 9.2 (53)	94.0 ± 7.3 (15)	95.4 ± 8.0 (15)
dys-1(cx18)I	dystrophin-like	70.8 ± 12.5 (24)	69.7 ± 9.1 (23)	47.4 ± 23.3 (75)	38.5 ± 26.7 (72)*
dyc-1(cx32)X	capon-like	104.9 ± 8.5 (8)	96.3 ± 8.6 (8)	76.5 ± 30.1 (30)	81.0 ± 20.4 (30)
dyb-1(cx36)I	α-dystrobrevin	73.8 ± 19.5 (22)	77.4 ± 7.7 (22)	79.8 ± 21.8 (30)	74.0 ± 21.9 (30)
stn-1(ok292)I	β-syntrophin	51.8 ± 2.7 (41)	57.9 ± 17.5 (41)	61.0 ± 24.0 (30)	58.6 ± 23.0 (30)

Results are reported as averages +/− standard deviation. Number of worms assayed is in parentheses.

* Significantly different from the *dys-1(cx18)* controls (P<0.05, *T*-test)

Table 2

Effect of *elp-1(RNAi)*on egg laying

Genotype	% eggs laid		
	Pre-comma	**Post-comma**	**Total eggs laid**
Wild-type N2	97	3	349
Wild-type N2; *elp-1(RNAi)*	94	6	345
dys-1(cx18)	41	59	237
dys-1(cx18); elp-1(RNAi)	38	62	173
dyb-1(cx36)	61	39	247
dyb-1(cx36); elp-1(RNAi)	54	46	238

Worms were grown on RNAi feeding plates at 25°C and the embryos of the F2 progeny were observed.