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Krajacic P, Hermanowski J, Lozynska O, Khurana TS, Lamitina T. *C. Elegans* dysferlin homolog *fer-1* is expressed in muscle, and *fer-1* mutations initiate altered gene expression of muscle enriched genes. *Physiol Genomics* 40: 8–14, 2009. First published September 15, 2009; doi:10.1152/physiolgenomics.00106.2009.— Mutations in the human dysferlin gene cause Limb Girdle Muscular Dystrophy 2B (LGMD2B). The *Caenorhabditis elegans* dysferlin homolog, *fer-1*, affects sperms development but is not known to be expressed in or have a functional roles outside of the male germline. Using several approaches, we show that *fer-1* mRNA is present in *C. elegans* muscle cells but is absent from neurons. In mammals, loss of muscle-expressed dysferlin causes transcriptional deregulation of muscle expressed genes. To determine if similar alterations in gene expression are initiated in *C. elegans* due to loss of muscle-expressed *fer-1*, we performed whole genome Affymetrix microarray analysis of two loss-of-function *fer-1* mutants. Both mutants gave rise to highly similar changes in gene expression and altered the expression of 337 genes. Using multiple analysis methods, we show that this gene set is enriched for genes known to regulate the structure and function of muscle. However, these transcriptional changes do not appear to be in response to gross sarcomeric damage, since genetically sensitized *fer-1* mutants exhibit normal thin filament organization. Our data suggest that processes other than sarcomere stability may be affected by loss of *fer-1* in *C. elegans* muscle. Therefore, *C. elegans* may be an attractive model system in which to explore new muscle-specific functions of the dysferlin protein and gain insights into the molecular pathogenesis of LGMD2B.

muscular dystrophy; LGMD2B; limb-girdle; microarray; *Caenorhabditis elegans*

LIMB-GIRDLE MUSCULAR DYSTROPHIES (LGMDs) are a diverse group of genetically determined muscle disorders. LGMD2B, Miyoshi myopathy (MM), and distal anterior compartment myopathy are clinically disparate autosomal recessive diseases caused by mutations in the dysferlin gene (17). Patients usually present in the second or third decade of life with proximal (LGMD2B) and/or distal (MM and distal anterior compartment myopathy) muscle weakness, elevated serum creatine kinase levels, and generally slow disease progression (26). While the onset and progression of the disease are highly variable, many individuals become wheelchair bound by their mid-30s (19). Currently, there are no treatments available to ameliorate LGMD2B phenotypes.

In mice and humans, loss-of-function mutations in the dysferlin gene give rise to visible pathology with relatively late onset. Recent studies have demonstrated that such pathology is preceded by alterations in muscle gene expression. In humans, loss of dysferlin function leads to the upregulation of genes involved in diverse processes, such as intracellular Ca^{2+} handling, immunity, muscle development, and protein synthesis (5). Dysferlin mutations in mice also give rise to distinct patterns of gene expression in muscle, including an overall upregulation of muscle structural genes and immune regulators (27). Some of these transcriptional responses are also observed in other genetically distinct forms of muscular dystrophy (20), suggesting that some changes in gene expression changes may represent a shared secondary response among the muscular dystrophies.

The *Caenorhabditis elegans* gene *fer-1* encodes the founding member of the dysferlin gene family (1). *fer-1* mutant animals are sterile due to defects in Ca^{2+}-dependent vesicle fusion during spermatogenesis. In *fer-1* mutants, specialized sperm vesicles called membranous organelles fail to fuse with the plasma membrane during sperm activation, resulting in nonmotile spermatozoa and sterility. As in mammalian muscle, the ability of *fer-1* to mediate sperm membrane fusion is calcium dependent, and missense mutations that disrupt the C2 calcium-binding domains are sufficient to cause this phenotype (29). Despite the obvious differences between *C. elegans* sperm and mammalian skeletal muscle, these findings have made a major contribution to our overall understanding of dysferlin's function in humans as a regulator of Ca^{2+}-dependent membrane fusion processes, such as membrane repair (11, 12). Despite these contributions, *C. elegans* has not made substantial contributions to our understanding of the muscle-specific functions of dysferlin. This is largely due to the assumption that *fer-1* is only expressed in sperm, although previous studies noted possible expression of *fer-1* in somatic tissues (1).

Here, we investigated whether the *C. elegans* dysferlin homolog *fer-1* might also be expressed in *C. elegans* muscle. Using purified *C. elegans* primary cell cultures, we demonstrate the *fer-1* mRNA is present in body wall muscle (BWM) cells, but not in neurons. Furthermore, we show that two independently derived *fer-1* loss-of-function alleles both cause alteration expression of genes known to be enriched in *C. elegans* muscle. Many of these genes promote muscle cell stability or attachment, suggesting that loss of *fer-1* in *C. elegans* might lead to destabilization of muscle function, as is the case in human LGMD2B patients. However, we find that loss of *fer-1* does not cause gross sarcomere disorganization, suggesting that *fer-1* may contribute to processes other than sarcomere stability in nematode muscle. Our findings establish *C. elegans* as a model to study the muscle-specific aspects of dysferlin function and suggest that dysferlin may regulate processes other than sarcomere stability in nematode muscle.

MATERIALS AND METHODS

C. elegans strains and cell culture. All strains were maintained with standard culture methods and fed with the *Escherichia coli* strain

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OP50. The following strains were used: LS587 - *dys-1*(*cx18*) *I*; *hlh-1*(*cc561*) *II*; LGI: *fer-1*(*hc1ts*), *fer-1*(*hc24ts*), *glp-4*(*bn2*); LGIV *otIs117*[*unc-4*(*+*) + *unc-33p::GFP*]; *fem-3*(*e2006ts*). Prior to all functional assays, *fer-1* mutants were hatched at the restrictive temperature of 25°C and grown until they reached the young adult stage. For the RT-PCR experiments, *fem-3*(*e2006*) and *glp-4*(*bn2*) were hatched and grown at 25°. At this temperature, both mutants were completely sterile, as judged by the absence of fertilized embryos on the growth plates. The wild-type N2 strain was obtained from the Caenorhabditis elegans Genetic Stock Center.

Cultures of *C. elegans* muscle and neurons were carried out using previously described methods (6). Muscle cells were identified by culturing cells from animals expressing a *myo3p::dsRed2* transgene. Neurons were identified by culturing cells from animals expressing an *unc-33p::GFP* transgene. FACS sorting of GFP+ or RFP+ cells was carried out at the University of Pennsylvania Flow Cytometry Core Facility. RNA from >5,000 sorted cells was used for cDNA synthesis and subsequent RT-PCR experiments.

Microarray analysis. Hypochlorite synchronized L1 stage wild-type, *fer-1*(*hc1*), or *fer-1*(*hc24*) animals were grown to the young adult stage on standard NGM plates at 25°C. Worms were washed from the plates with M9 solution, and 1,000 adult worms were collected into 1.5 ml tubes according to gating criteria on the COPAS Biosort that excluded L4 and younger animals. Immediately following sorting, worms were pelleted at 2,000 rpm, and the supernatant was aspirated, leaving ~100 μl on top of the worm pellet. We added 400 μl of TRIzol, and the solution was vortexed for 2 min. Worms were stored at −80°C until RNA isolation. For each genotype, six individual replicates were performed and were hybridized to Affymetrix *C. elegans* Genechips per the manufacturer's recommended protocols at the University of Pennsylvania Microarray Core Facility. The best four preparations (as determined by the overall Pearson Correlation within a genotype) were used for RNA labeling and hybridization. The remaining two preparations were used for quantitative (q) PCR validation of microarray results. Each set of sample collections were performed independently, i.e., samples were collected on six separate occasions.

Microarray statistical analysis. Affymetrix .cel files for all arrays were uploaded into the Partek Genomics Suite and intensity values were normalized using the GC-RMA program. Data across the wild-type series was analyzed using the significance analysis of microarray data (SAM) algorithm to calculate the false discovery rate (FDR) (25). We calculated expression ratios of wild-type/*fer-1* mutants, and genes that exhibited a ≤0.1% FDR, ≥4-fold change in both *fer-1* mutants, and differed by ≤2-fold between *hc1* and *hc24* were considered to be "fer-1-regulated" genes. Microarray data have been deposited in the GEO database (accession number GSE16753).

qPCR. For microarray validation, RNA samples that were not utilized for microarray analysis were utilized for cDNA synthesis and qPCR. Briefly, cDNA was reverse synthesized from 1 μg total RNA (Superscript II kit; Invitrogen, Carlsbad, CA), and qPCR was performed using an ABI 7500 thermocycler and TaqMan probe sets (Applied Biosystems, Foster City, CA). Each reaction was performed in 20 μl reactions in technical quadruplicate from two biological replicates. Data were normalized to expression levels of *hmit-1.1*, which encodes one of three *C. elegans* proton (H+)-dependent myo-inositol transporters whose expression levels are not significantly affected in *fer-1* mutants. Data normalization using probes against the glycerol-3-phosphate dehydrogenase gene *gpdh-1*, whose expression was also unchanged in *fer-1* mutants, showed similar results.

Phalloidin staining. fer-1(*hc24*) was crossed into the *hlh-1*(*cc561ts*) background using standard crossing methods. Animals were cultured at 16°, since *hlh-1*(*cc561ts*) mutants exhibit significant lethality and muscle damage when grown at 20° or higher (10). Phalloidin staining was carried out as previously described (23). Image z-stacks were collected and deconvolved (blind algorithm for PSF determination) using a DMI4000B microscope, 63 × 1.4NA lens, DFC340 camera, and AF6000 software (Leica Microsystems, Bannockburn, IL).

Statistical analysis. For comparisons between overlapping sets of genes, *P* values were computed using the normal approximation of the hypergeometric probability (15).

RESULTS

Fer-1 is expressed in cultured C. elegans muscle cells. In mammals, the expression of dysferlin is highly enriched in skeletal muscles (3). In *C. elegans*, 95 BWM cells control worm motility and are functionally homologous to vertebrate skeletal muscles. Previously, the expression of the *C. elegans* dysferlin homolog *fer-1* was reported to be restricted to sperm cells (1). However, using RT-PCR, we detected expression of *fer-1* in RNA from a purified population of L1 stage animals, which contain no germline and no sperm (Fig. 1*A*). Additionally, we found that the *fer-1* mRNA was present in both a genetically feminized and a germline-deficient genetic back-

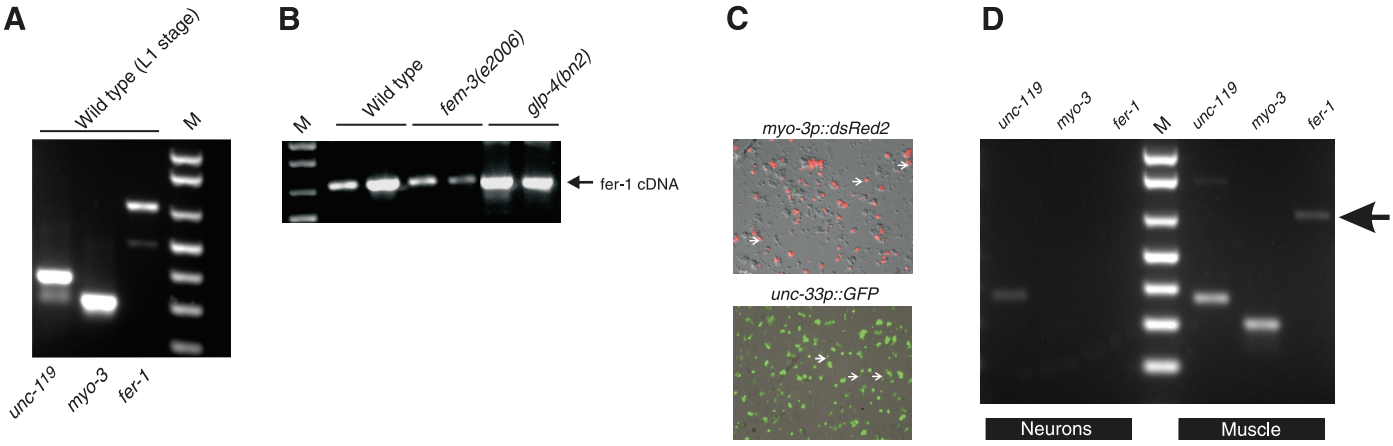

Fig. 1. *Caenorhabditis elegans fer-1* mRNA is expressed in the soma. *A*: RT-PCR of *unc-119* (neuron-specific control), *myo-3* (muscle-specific control), and *fer-1* from total RNA purified from wild-type L1 larvae, which do not contain sperm or germ cells. L1 stage larvae were purified by hypochlorite isolation of embryos followed by overnight hatching in the absence of food. *B*: RT-PCR of *fer-1* from wild type, genetically feminized hermaphrodites [*fem-3*(*e2006*)], and germline-deficient hermaphrodites [*glp-4*(*bn2*)]. Duplicate lanes indicate independently derived RNA samples. *C*: differentiated primary cell cultures from transgenic animals expressing *myo-3p::dsRed2* (*top*) or *unc-33p::GFP* (*bottom*). *D*: RT-PCR reactions from FACS-enriched neuronal cell cultures (*lanes 1–3*) or FACS-enriched muscle cell cultures (*lanes 5–7*). Arrow indicates the *fer-1* cDNA band.

ground (Fig. 1*B*). These data strongly suggested that *fer-1* may be expressed somatically, possibly in BWMs.

To determine whether *fer-1* is expressed in BWMs, we cultured BWM and neuronal cells isolated from transgenic animals expressing either a muscle specific reporter [*myo-3p::dsRed2* (18)] or a neuron-specific reporter [*unc-33::GFP* (24)] transgene. Cells in culture exhibited appropriate morphology for muscle (formation of muscle arms) or neurons (axonal extensions; Fig. 1*C*). Differentiated muscle or neurons were FACS sorted based on the expression of the dsRed or GFP marker, and RT-PCR for *fer-1*, as well as the muscle-enriched marker *myo-3* and the neuron-enriched marker *unc-119*, was performed on total RNA. FACS-sorted neurons exhibited expression of *unc-119* but not *myo-3*, suggesting that our neuronal cell preparation was extremely pure. FACS-sorted muscle exhibited expression of both *myo-3* and *unc-119*, suggesting the presence of some contaminating *unc-119*-expressing cells in the muscle cell population. Nevertheless, we only detected *fer-1* mRNA expression in the muscle-enriched but not the neuron-enriched RNA samples (Fig. 1*D*). DNA sequencing showed that this band was 100% identical to the predicted *fer-1* mRNA sequence (data not shown). Together, these data show that *fer-1* expression can be detected in developmental stages devoid of sperm as well as in genetic backgrounds lacking a germline and that at least one site of somatic *fer-1* expression is muscle.

Microarray profiling of C. elegans fer-1 mutants. In mammals, loss of dysferlin alters global patterns of gene expression in muscle, even at presymptomatic ages (5, 27). In these studies, it was speculated that such alterations in muscle gene expression represents a compensatory response to the absence of dysferlin. Since our data demonstrate that *C. elegans fer-1* is expressed in muscle, we hypothesized that *fer-1* mutant worms might also exhibit alterations in muscle gene expression at the transcriptome level. To identify such transcriptional responses, we used whole genome microarray profiling to compare mRNA expression levels between purified young adult wild-type animals and *fer-1* loss-of-function mutants. To improve the specificity of this approach, we profiled transcriptional changes in two independently derived *fer-1* mutants, *fer-1*(*hc1*), and *fer-1*(*hc24*). We hybridized labeled RNA to whole genome Affymetrix *C. elegans* chips and calculated the fold changes in expression relative to wild-type animals. We considered genes to be *fer-1* regulated if they exhibited similar statistically significant fold changes in both *fer-1* mutant strains (see MATERIALS AND METHODS). Using this approach, we identified 337 unique *fer-1*-regulated genes (Fig. 2*A*, Supplemental Table S1);[1] 112 genes were upregulated, while 225 genes were downregulated in both mutants (Fig. 2*B*). Each of the independent replicates exhibited a high degree of within-genotype clustering, indicating a high level of reproducibly between samples (Fig. 2*A*). To further validate the quality of our microarray data, we performed qPCR analysis on 10 genes (5 upregulated and 5 downregulated) using independently derived RNA samples. We found concordant regulation in 80% (8/10) genes (Table 1), many of which are known to be expressed in or to functionally regulate muscle activity.

Since *fer-1* mutants have defective sperm and are therefore sterile, we considered the possibility that the observed transcriptional changes in *fer-1* mutants were due to secondary consequences of sperm-deficient sterility. If this were the case, genes upregulated in *fer-1* mutants might correspond to oocyte-enriched transcripts (i.e., more unfertilized oocytes in *fer-1* mutants than in wild type), while genes downregulated in *fer-1* mutants might correspond to embryo-enriched transcripts (i.e., fewer fertilized embryos in *fer-1* mutants than in wild type) or sperm-enriched transcripts (i.e., fewer mature sperm in *fer-1* mutants than in wild type). To test this possibility, we compared *fer-1* up- and downregulated genes to a previously

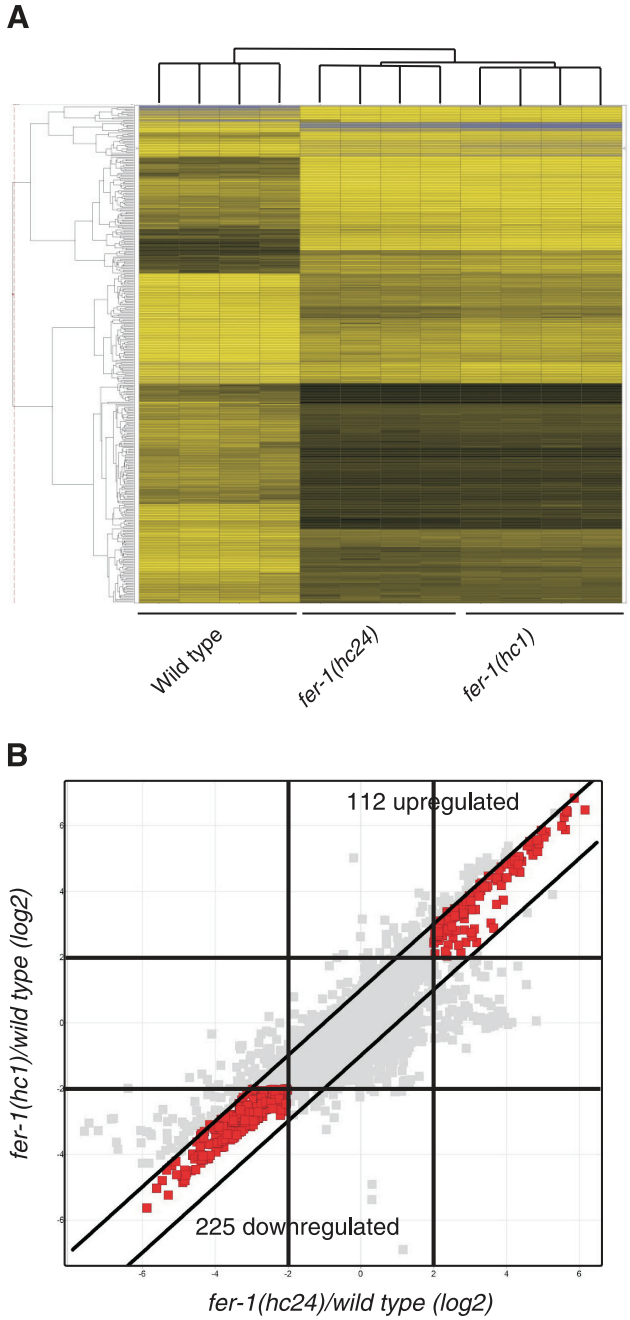

Fig. 2. Affymetrix transcriptional profiling of *fer-1*(*hc1*) and *fer-1*(*hc24*) mutants. *A*: hierarchically clustered heat map of probe intensities for all 337 *fer-1*-regulated genes. *B*: scatter plot of expression fold changes in *fer-1*(*hc1*) and *fer-1*(*hc24*) mutants compared with wild type. Genes with fold changes of ≥4 and <2-fold differences between both *fer-1* alleles are shown in red.

[1] The online version of this article contains supplemental material.

Table 1. *qPCR validation of fer-1 mutant microarray data*

Gene	Gene Name	KOG Description	*fer-1*(*hc1*) qPCR*	*fer-1*(*hc1*) Microarray	*fer-1*(*hc24*) qPCR	*fer-1*(*hc24*) Microarray
C17H12.9	*ceh-48*	CCAAT displacement protein and related homeoproteins	−16.3±0.6	−7.9	−16.7±4.0	−8.2
C43E11.6†	*nab-1*	protein phosphatase 1 binding protein spinophilin/neurabin II	−5.4±0.3	−6.8	−2.8±0.5	−10.2
F28F9.1†	*zag-1*	homeobox transcription factor SIP1	−18.5±2.2	−10.5	−17.5±3.6	−10.6
T22A3.8†	*lam-3*	ECM glycoprotein laminin	−15.3±1.2	−17.7	−12.4±2.7	−20.5
C52D10.9†	*skr-8*	SCF ubiquitin ligase, Skp1 component	4.8±0.4	8.8	4.4±0.4	5.5
F43G6.5		polyA polymerase	12.1±0.3	88.9	12.2±1.2	50.0
F44G3.2		creatine kinase	8.8±0.9	7.2	10.0±0.7	5.5
C12C8.1	*hsp-70*	heat shock protein	−11.3+2.1	−8.3	−2.56±0.1	−7.8

*All data are expressed as fold change ± SD relative to wild type. †Functionally associated with muscle via expression and/or phenotype.

described set of 1,030 oocyte-enriched mRNAs, 3,531 embryo-expressed mRNAs, and 865 sperm-enriched mRNAs (4, 21). We failed to observe any statistically significant enrichment between *fer-1*-regulated genes and either the oocyte dataset, the embryo dataset, or the sperm-enriched dataset [overlap between *fer-1* upregulated (112 genes) and oocyte enriched (1,030 genes) - 7 genes, $P > 0.05$ for overrepresentation; overlap between *fer-1*-downregulated (225 genes) and embryo enriched (3,531 genes) - 34 genes, $P > 0.05$ for overrepresentation; overlap between *fer-1*-downregulated (225) and sperm enriched (865) - 0 genes]. These data suggest that, in general, the transcriptional changes observed in *fer-1* mutants are not due to secondary consequences of sterility but rather might reflect compensatory somatic transcriptional responses to the loss of *fer-1* function.

Since our data suggested that at least one site of *fer-1* expression is muscle, we hypothesized that many of the *fer-1*-regulated genes might also be expressed in muscle and possibly represent a compensatory response to loss of *fer-1* function in this tissue. To test this hypothesis, we took three approaches. First, we compared the 337 *fer-1*-regulated genes identified in our microarray analysis to a set of 1,312 genes that have been previously found to be enriched in cultured *C. elegans* muscle cells (9) and found 35 genes in common. This represents a 1.5-fold enrichment above the number of overlapping genes exhibit to occur at random for this sized data set, which is statistically significant ($P < 0.012$). It should be noted that while the regulation of these genes is *fer-1* dependent, we cannot rule out the possibility that loss of *fer-1* causes misregulation of muscle enriched genes in other tissues. Nonetheless, these data suggest that the genes transcriptionally regulated in response to loss of *fer-1* are overrepresented for genes known to be enriched in expression in muscle.

Second, we analyzed the 337 *fer-1*-regulated genes for enriched Gene Ontology (GO) categories using the microarray analysis tool DAVID (8). *fer-1*-regulated genes were highly enriched in the GO Biological Process category for locomotion and locomotory activity, which include genes required for muscle-driven whole animal motility (Fig. 3). Of the 17 genes enriched for the GO locomotion category, 11 are either known to either be expressed in BWM cells or to be functionally required for normal muscle function. In every case, these genes were downregulated in *fer-1* mutants (Table 2). Several of these genes, including *ifa-3*, *unc-52*/Perlecan, and *sym-5,* are involved in stabilizing the attachment of muscle cell membranes to underlying substrates (7, 14, 22). Together, these data suggest that loss of *fer-1* induces significant changes in gene expression and some of these genes are known to function in *C. elegans* muscle cells.

Finally, we compared the list of 337 *fer-1*-regulated genes to a large-scale gene expression map for *C. elegans* (15). These data cluster *C. elegans* genes by similarity in mRNA expression level across hundreds of experimental conditions. In many cases, gene clusters within this map share similar function at either the molecular or cellular level (15). Therefore, mapping transcriptional responses onto the *C. elegans* gene expression map can provide insights into gene function. Upon mapping the *fer-1*-regulated genes onto this gene expression matrix, we observed highly significant enrichment of *fer-1*-regulated genes within specific biological clusters, or "mountains" (Fig. 4). For example, *fer-1*-regulated genes were highly enriched for gene expression *mountains 1, 12, 14,*

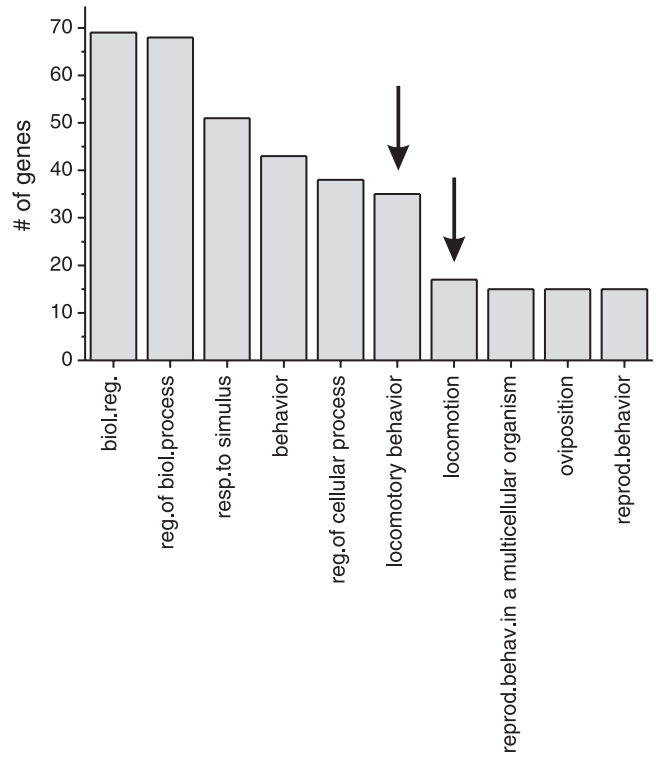

Fig. 3. *fer-1*-regulated genes show enrichment for Gene Ontology (GO) categories involved in locomotion and motility. *fer-1*-regulated genes were analyzed in using the microarray comparison tool DAVID (13). The top 10 best scoring GO: Biological Process categories with P values of <0.05 are shown.

Table 2. *fer-1 mutant regulated genes with known roles or expression in C. elegans muscle*

Gene	Sequence	Predicted Protein	Function	Change in *fer*-1 Mutants
ifa-3	F52E10.5	intermediate filament A	muscle positioning	↓
sup-12	T22B2.4	RNA-binding protein	muscle-specific RNA splicing	↓
sym-5	C44H4.2	leucine-rich repeat protein	locomotion, muscle attachment	↓
unc-6	F41C6.1	netrin	locomotion	↓
zag-1	F28F9.1	homeobox transcription factor	locomotion	↓
slo-1	Y51A2D.19	BK channel	locomotion	↓
lam-3	T22A3.8	laminin A2	locomotion	↓
unc-52	ZC101.2	perlecan	muscle structure	↓
unc-44	B0350.2	ankyrin-like	locomotion	↓
unc-120	D1081.2	MADS-box transcription factor	maintains actin/myosin expression	↓
unc-53	F45E10.1	NAV-2-like	locomotion	↓

and *36* (Table 3). Interestingly, *mount 1* has been previously shown to be enriched for genes involved in muscle function (15). These results suggest that many *fer-1*-regulated genes are transcriptionally co-regulated with genes known to function in *C. elegans* muscle. Together, these three lines of evidence (significant overlap with muscle-expressed genes, enrichment for GO locomotion genes, and expression clustering with muscle genes) are consistent with the hypothesis that loss of *fer-1* in *C. elegans* initiates alterations in the expression of muscle-expressed genes.

The alterations in muscle-enriched gene expression, as well as the phenotypes of mammalian dysferlin mutants, suggested that *fer-1* mutant worms might exhibit whole animal motility defects. To casual observation, *fer-1* mutants appear to move in a manner indistinguishable from wild-type animals. However, we have recently developed a novel imaging-based quantitative assay to measure *C. elegans* kinematics and biomechanics and demonstrated that *fer-1* mutants exhibit significant reductions in movement frequency, as well as whole animal force, power, and tissue mechanical properties (J. Sznitman, P. Purohit, P. Krajacic, T. Lamitina, P. Arratia, unpublished observations). To determine if the *fer-1* "uncoordinated" (Unc) phenotype was due to gross destabilization of sarcomere integrity, we visualized muscle thin filament organization in both *fer-1* and *dys-1* mutants. *dys-1* encodes the sole *C. elegans* dystrophin homolog and is known to destabilize sarcomere integrity (10). Both mutants were in a genetically sensitized MyoD mutant *hlh-1*(*cc561*) background, which is required to reveal *dys-1* mutant phenotypes (10). As was previously shown, *dys-1;hlh-1* mutants exhibit substantial disorganization of the myofilament lattice (Fig. 5*C*). However, *fer-1;hlh-1* mutants exhibit no significant thin filament damage compared with the control *hlh-1* background strain (Fig. 5*B*). Ageing of *fer-1* mutants did not enhance gross sarcomere damage beyond that seen in wild-type aged animals (data not shown). Together these data shown that while both *fer-1* and *dys-1* exhibit an Unc phenotype, the *fer-1* phenotype is distinct from the *dys-1* phenotype in that it does not involve gross sarcomeric disorganization or damage.

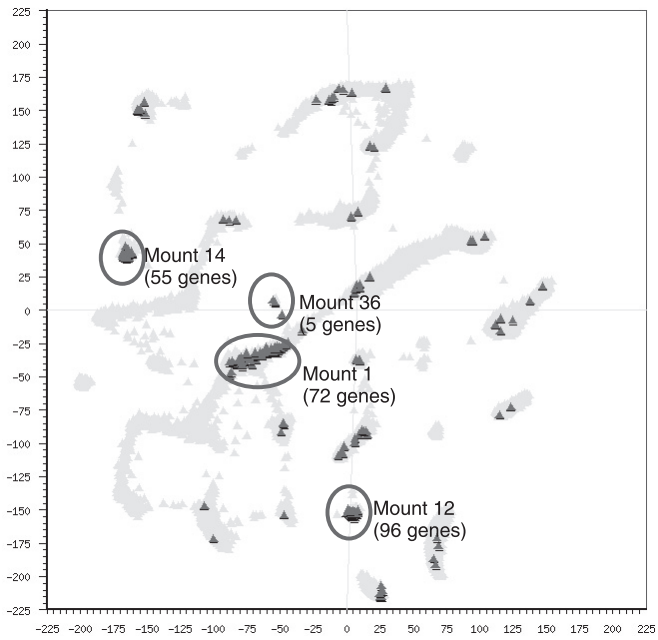

Fig. 4. *fer-1*-regulated genes exhibit expression clustering with other muscle-expressed genes. *fer-1*-regulated genes (triangles) were overlaid onto the *C. elegans* gene expression map (15). Enriched gene expression mountains and the number of *fer-1* regulated genes within that mountain are indicated.

DISCUSSION

The *fer-1* gene was first identified as a regulator of specialized membrane-fusion events during spermatogenesis in *C. elegans* (1, 2, 28). Subsequent studies in mammals have revealed similar roles in membrane fusion for dysferlin. However, the obvious differences between *C. elegans* sperm and mammalian skeletal muscle have limited the utility of *C. elegans* for understanding the muscle-specific functions of dysferlin that may contribute to LGMD2B disease phenotypes. Our finding that *fer-1* is expressed in *C. elegans* muscle cells suggests that the function(s) of *fer-1* and dysferlin may be even more evolutionarily conserved than has previously been appreciated. The numerous cell biological, functional, and physiological approaches to the study of *C. elegans* muscle should help to clarify the precise role of *fer-1* in muscle function. Given that *C. elegans* does not have either regenerative satellite cells or infiltrating immune cells, both of which are also regulated by dysferlin and are thought to play important roles in LGMD2B, worms may provide an important model to explore the muscle-autonomous functions of *fer-1*/dysferlin without the complications of immune infiltration or regeneration-based repair.

In mammals, loss of dysferlin has previously been shown to cause significant changes in muscle gene expression. Likewise, the enrichment for muscle-expressed genes

Table 3. *Comparison of fer-1-regulated genes to the C. elegans gene expression topomap*

Gene Expression Mountain	Known Functional Enrichment*	Genes in Mountain, *n*	Overlapping fer-1-regulated Genes, *n*	Representation Factor†	*P* Value‡
Mount 12	unknown	453	96	15.0	<0.001
Mount 14	collagens	352	55	11.1	<0.001
Mount 1	muscle expressed genes, neuron expressed genes, PDZ genes	1,698	72	3.0	<0.001
Mount 36	heat shock proteins	10	5	35.4	<0.001

*Functional annotation from Ref. 16. †Calculated as in Ref. 16. ‡Normal approximation of the hypergeometric probability.

among *fer-1*-regulated transcripts in our microarray data suggests that loss of *fer-1* in *C. elegans* also causes alterations in muscle gene expression. The genes misregulated in *C. elegans fer-1* mutants appear to be distinct from genes misregulated in human and mouse dysferlin deficiency models, as we failed to observe any overlap among orthologous genes from these three datasets (data not shown). Such discordance in microarray data is not uncommon. Even for microarray profiles of dysferlin-deficient mouse models, virtually no overlapping genes were identified (16, 27). A similar lack of overlap was also observed when profiles among dysferlin-deficient muscles from mice and humans were compared (27). In these cases, it should be noted that the mouse model of dysferlin deficiency that was profiled (SJL-Dysf) is know to be affected by numerous mutations, one of which affects the dysferlin gene. Such differences in genetic background, as well as differences in sample handling, the pathological state examined, or the muscle groups studied likely all contribute to the distinct gene expression patterns described in these studies. Our studies of *C. elegans fer-1* mutants are largely free of confounding genetic background issues, since the strains utilized in this study are highly congenic. Moreover, our data are largely reflective of muscle-autonomous responses to loss of *fer-1* since *C. elegans* lacks an immune system and regenerative satellite cells.

Despite the clear muscle defects of human dysferlin patients, previous qualitative observations of *C. elegans fer-1* mutants have not revealed similarly striking defects in either muscle organization or whole animal motility. However, we have recently developed a novel quantitative biomechanical platform to analyze nematode movement and have demonstrated clear and consistent defects in *fer-1* mutants, as well as in other previously characterized muscle mutants (J. Sznitman, P. Purohit, P. Krajacic, T. Lamitina, P. Arratia, unpublished observations). Surprisingly, we show here that the motility defects of *fer-1* mutants are not associated with gross sarcomeric damage, as has been observed in *C. elegans dys-1* mutants (10). These findings suggest that *C. elegans* may provide an important model system in which to explore new functional properties of dysferlin, outside of its previously studied roles in sarcolemmal membrane maintenance. Given that the mechanism of LGMD2B is not well understood, such functions may provide new insights into the mechanisms leading to dysferlinopathy, as well as potentially novel therapeutic targets for treatment.

Summary and Conclusions

Our data show for the first time that the dysferlin homolog *fer-1* is expressed in *C. elegans* muscle. Using microarray analysis we demonstrate the loss of *fer-1* alters the expression of muscle-enriched genes. Finally, we show that *fer-1* mutants do not grossly destabilize sarcomere integrity, even in a sensitized genetic background. Future investigation of muscle functional properties in *C. elegans fer-1* mutants, such as damaged-induced sarcolemmal membrane repair, sarcomere assembly, and synaptic function, may give new insights into the function(s) of the ferlin family of proteins and the pathogenesis of LGMD2B.

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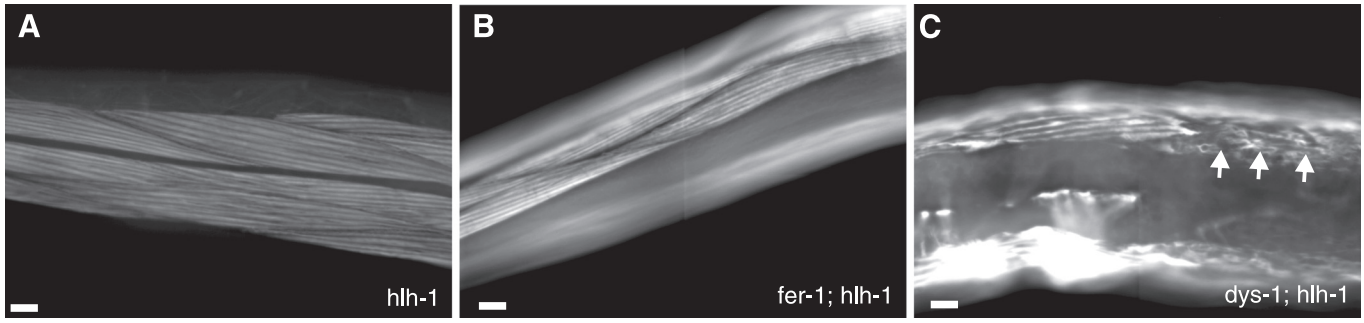

Fig. 5. *fer-1*(*hc24*) mutants do not exhibit dystrophic-like myofiber damage in a sensitized MyoD mutant genetic background. 1 day old synchronized adult *hlh-1*(*cc561ts*) (*A*), *fer-1*(*hc24*); *hlh-1*(*cc561ts*) (*B*), and *dys-1*(*cx18*); *hlh-1*(*cc561ts*) (*C*) hermaphrodites were fixed and stained with Rhodamine phalloidin. Arrows in *C* indicate damaged muscle cells. Scale bar = 10 μm.

DISCLOSURES

P. Krajacic, T. S. Khurana, and T. Lamitina are authors of a patent relating to new therapeutic strategies for treatment of LGMD2B.

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