FARL-11 (STRIP1/2) is required for sarcomere and sarcoplasmic reticulum organization in *C. elegans*

Sterling C. T. Martin, Hiroshi Qadota, Andres F. Oberhauser, Jeff Hardin, and Guy M. Benian

**ABSTRACT** Protein phosphatase 2A (PP2A) functions in a variety of cellular contexts. PP2A can assemble into four different complexes based on the inclusion of different regulatory or targeting subunits. The B''' regulatory subunit “striatin” forms the STRIPAK complex consisting of striatin, a catalytic subunit (PP2AC), striatin-interacting protein 1 (STRIP1), and MOB family member 4 (MOB4). In yeast and *Caenorhabditis elegans*, STRIP1 is required for formation of the endoplasmic reticulum (ER). Because the sarcoplasmic reticulum (SR) is the highly organized muscle-specific version of ER, we sought to determine the function of the STRIPAK complex in muscle using *C. elegans*. CASH-1 (striatin) and FARL-11 (STRIP1/2) form a complex in vivo, and each protein is localized to SR. Missense mutations and single amino acid losses in farl-11 and cash-1 each result in similar sarcomere disorganization. A missense mutation in farl-11 shows no detectable FARL-11 protein by immunoblot, disruption of SR organization around M-lines, and altered levels of the SR Ca$^{2+}$ release channel UNC-68.

**INTRODUCTION**

A major serine–threonine protein phosphatase that is conserved from yeast to humans is protein phosphatase 2A (PP2A; Shi, 2009). PP2A functions in a wide variety of cellular contexts and forms multiple specific protein complexes via different regulatory or targeting subunits. The PP2A core enzyme comprises a catalytic subunit C (PP2AC) and a scaffolding subunit A (PP2AA). This core forms a complex with a variety of regulatory B subunits, with the B subunits mediating subcellular localization and/or substrate recognition. There are four families of regulatory B subunits: B (B55 or PPP2R2), B’ (B56 or PPP2R5), B” (B72 or PPP2R3), and B’’’ (striatins).

The striatin-interacting phosphatase and kinase (STRIPAK) complex is a PP2A complex in which the regulatory subunit is the protein striatin. Recently, the structure of the STRIPAK complex was solved at 3.2 Å resolution using cryo-EM (Jeong et al., 2021). The complex consists of a catalytic subunit (PP2AC), a scaffolding subunit (PP2AA) called “striatin,” striatin-interacting protein 1 (STRIP1), and MOB family member 4 (MOB4). The striatin coiled-coil domain forms an elongated scaffold that links the complex together.

In the budding yeast, *Saccharomyces cerevisiae*, the “Far” complex, originally identified as crucial for pheromone-induced cell cycle arrest, is the yeast counterpart of the STRIPAK complex. The Far complex localizes to the endoplasmic reticulum (ER), and it has been shown that the six Far subunits (Far3, 7, 8, 9, 10, and 11) assemble at the ER in a specific sequence (Pracheil and Liu, 2013): Far9 and Far10 first attach to the ER membrane via their tail-anchor domains; Far3, Far7, and Far8 form a subcomplex that is recruited to the ER by Far9/10; and finally, Far11 binds to the Far3/7/8 subcomplex. Similarly, in *Caenorhabditis elegans*, the nematode orthologue of yeast Far11, called “FARL-11,” is localized to the ER and outer nuclear membrane of early embryos and the germ line.
FIGURE 1: Domain organization, tagging design, detection of expression of FARL-11 and CASH-1, and coIP of FARL-11 with CASH-1. (A) Domain organization of FARL-11 and CASH-1 and location of tags. CRISPR/Cas9 was used to create nematode strain SU853, in which consecutive mNeonGreen and Flag tags were added to the N-terminus of FARL-11, and strain SU854, in which RFP and myc tags were added to the N-terminus of CASH-1. “Antigen” refers to the 50 amino acid peptide that was used to generate rabbit antibodies to FARL-11. (B) Western blot demonstrating detection of FARL-11 and mNG-Flag-FARL-11. Nematode extracts were run on a gel, transferred to membrane, and reacted against antibodies to FARL-11. The protein bands detected using anti-FARL-11 ran at sizes expected for endogenous and tagged FARL-11. Western blot reaction on the left, Ponceau staining of the membrane on the right. The graph indicates that the presence of the tag does not significantly affect the level of FARL-11. For each strain, N = 3, and significance was tested by a one-tailed students t test. (C) Western blot demonstrating detection of RFP-myc-CASH-1 from strain SU854 of expected size (arrow) using antibodies to myc. An additional band of ~100 kDa (asterisk) is detected, but this is also detected in an extract from wild type (no myc tagged proteins expressed), suggesting cross-reactivity of anti-myc to a nematode or bacterial protein. (D) Immunoprecipitation of FARL-11 with CASH-1. As described in materials and methods, TagRFP-myc-CASH-1 was immunoprecipitated from a lysate of strain SU854, using nanobodies to RFP coupled to magnetic beads. The left half is a Western blot reacted against antibodies to myc showing that that RFP-myc-CASH-1 was immunoprecipitated from a total protein lysate. On the right is a Western blot reacted against antibodies to FARL-11 indicating that the immunoprecipitate also contains FARL-11. For parts (B), (C), and (D), the positions of molecular weight size markers are indicated.
the more severe effect seen with UNC-95 staining, especially at
seen in both strains containing the H121Y missense mutation, with
muscle cell boundaries. Mild disorganization of these structures is
for A-bands, and UNC-95 for bases of M-lines, dense bodies, and
immunostained with antibodies to UNC-89 for M-lines, myosin heavy chain A
type, (B) Representative images of several body wall muscle cells from wild
Cas9 in the RFP-myc tagged
The double missense mutant L82E/L86E was also created by CRISPR/
CRISPR/Cas9 in the RFP-myc tagged
originally found in the MMP allele cash-1(gk705529) and recreated by
CRISPR/Cas9 in the RFP-myc tagged cash-1 strain jc60, as jc60 jc100. The
double missense mutant L82E/L86E was also created by CRISPR/Cas9 in the RFP-myc tagged cash-1 strain jc60, as syb4646. (B) Representative images of several body wall muscle cells from wild
type, cash-1(gk705529), cash-1(jc60 jc100), and cash-1(jc60 syb4646), immunostained with antibodies to UNC-89 for M-lines, myosin heavy chain A for A-bands, and UNC-95 for bases of M-lines, dense bodies, and muscle cell boundaries. Mild disorganization of these structures is seen in both strains containing the H121Y missense mutation, with the more severe effect seen with UNC-95 staining, especially at

to represent cross-reactivity to another nematode protein or even a
bacterial protein. To seek further evidence of the evolutionary con-
servation of the STRIPAK complex, we asked whether an in vivo complex could be detected in nematodes that contains both
CASH-1 (STRN3) and FARL-11 (STRIP1/2). As shown in Figure 1D, and
detailed in Supplemental Figure 1, immunoprecipitation of
TagRFP-myc-CASH-1 from an extract of strain SU854, coimmuno-
precipitates FARL-11.

In our previous report of the PP2A complex in C. elegans muscle, we showed that RNAi knockdown of CASH-1 (STRN3) resulted in
adults that are slow-moving and sterile, and by immunostaining with antibodies to myosin, these animals have disorganized sarcomeric A-bands (Qadota et al., 2018). Inspection of the Million Mutation Project (MMP) collection of mutant strains (Thompson et al., 2013) revealed several strains that have missense mutations in conserved
residues of CASH-1, but one of them, cash-1(gk705529), which results in a H121Y mutation, had severe phenotypes: a high percent-
age of embryonic lethality, and, among the animals that reached adulthood, greatly reduced motility. To avoid the confounding influence of the many background mutations in an MMP strain, we recre-
ated the H121Y mutation by CRISPR in our RFP- and myc-tagged CASH-1 strain (jc100; Figure 2A). The crystal structure of human striatin 3 coiled-coil domain revealed that it forms a parallel dimer and that two highly conserved residues, L121 and L125, are critical for homodimerization and binding to the A subunit of PP2A (Chen et al., 2014). We used CRISPR to mutate the equivalent leucines (L82 and L86) to glutamates in our RFP- and myc-tagged CASH-1 strain (syb4646; Figure 2A). Strains containing the H121Y mutation were adult viable and fertile. However, nematodes homozygous for the L82E/L86E mutations reached adulthood but were sterile, and thus we maintained the mutant chromosome over a balancer chro-
mosome. We immunostained adults from each strain with antibod-
ies to UNC-89 (obscurin) to assess A-bands, myosin heavy chain A (MHC A) to assess A-bands, and UNC-95 to assess the bases of the
M-lines and dense bodies (Z-disks) and muscle cell boundaries. As
shown in Figure 2B, each of the mutants displays disorganization of
these structures, with a more severe phenotype in the L82E/L86E
mutant strain. It is interesting that for the L82E/L86E strain, some
regions of muscle cells show unusually broad localization of MHC A and especially UNC-95 (indicated by arrows). In addition, UNC-95 staining is moderately disorganized in each mutant strain, especially with respect to the dense bodies and muscle cell boundaries. To
assess to what extent the sarcomeric defects could be attributed to
the muscle cell boundaries. A more severe effect on sarcomere and
IAC organization is seen in the L82E/L86E double mutant. Yellow
arrows point out widening of the localization of UNC-89 and MHC A. Scale bar, 10 μm. (C) and (D) Western blot analysis of the level of
RFP-myc-CASH-1 in strain SU854, coimmunoprecipitation of
TagRFP-myc-CASH-1 from an extract of strain SU854, coimmuno-
precipitation of
CASH-1 (STRN3) and FARL-11 (STRIP1/2). As shown in Figure 1D,
immunoprecipitation of
CASH-1 (STRN3) and FARL-11 (STRIP1/2). As shown in Figure 1D,
immunoprecipitation of
CASH-1 (STRN3) and FARL-11 (STRIP1/2). As shown in Figure 1D,
immunoprecipitation of
CASH-1 (STRN3) and FARL-11 (STRIP1/2). As shown in Figure 1D,
a decreased level of mutant protein versus functionally defective proteins, we compared the level of the RFP-myc-CASH-1 in an otherwise wild-type background with the level of RFP-myc-CASH-1 H121Y, and RFP/myc-CASH-1 L82E/L86E. As shown in Figure 2C and D, the levels of RFP/myc-CASH-1 were similar in wild type as compared with either H121Y or L82E/L86E mutants. In fact, as indicated in the graph on the right side of Figure 2C, the level of RFP-myc-CASH-1 is increased in the H121Y mutant compared with wild type. Thus, in these missense mutants, the sarcomeric disorganization is most likely due to decreased function rather than decreased levels of the mutant CASH-1 proteins.

To obtain further insight into how these missense mutations affect the function of the CASH-1 protein, we generated a homology model of CASH-1 based on the cryoEM structure of the orthologous human protein, STRN3 (Jeong et al., 2021). Figure 2E displays the locations of the affected residues on this homology model. To predict the potential effects of the mutations, we used the rotamer/mutation tool in Chimera (Pettersen et al., 2004) and then energy minimized to visualize interatomic clashes and contacts based on van der Waals radii. In addition, we used the DynaMut2 (Rodrigues et al., 2021) to predict the effects of the point mutations on protein stability and dynamics. L82 and L86 lie along the surface of a long α-helix (as expected), and we predict that the L82E and L86E mutations will have no significant effect on structure. Nevertheless, as shown for the STRN3, these mutations are likely to affect homodimerization. H121 resides in a loop connecting two α-helices. Given that H121 lies on the surface of the protein, the H121Y could conceivably affect binding to FARL-11.

We selected one farl-11 MMP allele for analysis, farl-11(gk437008), which has a nonconservative L292 to P change in the N1221 domain (Figure 3A). This strain was outcrossed 3x to wild type. Although we could not create this mutation using CRISPR, we did generate two additional mutations from the CRISPR procedure at nearby residues in this same domain (Figure 3A): farl-11(jc93), which deletes the single amino acid T281 in frame, and farl-11(jc94), which deletes the single amino acid P280 in-frame. After outcrossing 2x to wild type and immunostaining, all three mutant alleles show severely disorganized sarcomeres (Figure 3B). Of particular note is the wider distribution of both UNC-89 and myosin, similar to what we observed in the cash-1 mutant containing the L82E/L86E mutations. For the farl-11 mutants, this wider distribution of UNC-89 and MHC A can be discerned more clearly in the zoomed insets (Figure 3C). To assess to what extent the sarcomeric defect is due to a decreased level of mutant protein versus a functionally defective mutant protein, we compared the levels of FARL-11 protein from all three farl-11 mutants with wild type. As shown in Figure 3D, Western blot analysis indicates no detectable FARL-11 protein in farl-11(gk437008) (L292P). Moreover, both jc93 and jc94 show decreased levels of FARL-11, as indicated in the graph in Figure 1D: jc93 has 48.9% of the wild-type level, and jc94 has 64.1% of the wild-type level (means, n = 4). We were somewhat surprised that...
CASH-1 and FARL-11 localize near dense bodies

We next sought to determine the localization of CASH-1 and FARL-11 in nematode body wall muscle. To localize CASH-1, we utilized our CRISPR strain, SU854, which expresses an RFP-myc-CASH-1 fusion protein. As shown in Figure 4A, in which CASH-1 was detected with antibodies to myc, and M-lines and dense bodies detected with antibodies to UNC-95, CASH-1 is localized between and surrounding dense bodies. To localize FARL-11, we used antibodies to FARL-11. As indicated in Figure 4B, in which we costained with antibodies to PAT-6 (α-parvin), which also localizes to the bases of M-lines and dense bodies, FARL-11 localizes between dense bodies and also surrounds dense bodies but not as broadly as CASH-1. Unfortunately, we were not able to address whether CASH-1 and FARL-11 colocalize, despite several attempts. This is probably because the anti-myc-CASH-1 staining produces a low signal and a high background. We also tried to localize the RFP-myc-CASH-1 fusion using antibodies to RFP, and, unfortunately, trials with three different commercial anti-RFP antibodies did not yield better results than anti-myc.

FARL-11 localizes to the SR

Transmission electron micrographs have revealed that the SR in C. elegans body wall muscle is restricted to thin membranous sacs closely adjacent to the dense bodies as well as near the bases of M-lines or the middle of the A-band region (Gieseler et al., 1997). Using confocal microscopy to image localization of antibodies to UNC-68 and various components of the myofilament lattice (MHC A, MHC B, vinculin, α-actinin), Maryon et al. (1998) concluded that UNC-68 resides primarily in flattened vesicular sacs adjacent to the outer muscle cell membrane in the A-band region. In contrast, Hamada et al. (2002), using antibodies to UNC-68 and either rhodamine-phalloidin or antibodies to the A-band protein paramyosin, concluded that UNC-68 resides in the l-band region. Although these two studies seem to give different localizations for UNC-68, together, the results include both known or suspected

presumed strength of mutant alleles. Also, it should be noted that both the penetrance and the severity of phenotype are lower for cash-1 than for farl-11.

To obtain additional understanding about how these mutations might affect the function of FARL-11, we generated an homology model of FARL-11 based on the cryoEM structure of the human orthologue, STRIP1 (Jeong et al., 2021). Figure 3E depicts the locations of the affected residues in this structure. P280 and T281 are located in a highly disordered 47 amino acid loop, and L292 is located in the center of an α-helix. Like for CASH-1, we used the rotamer/mutation tool in Chimera (Pettersen et al., 2004) and the DynaMut2 web tool (Rodrigues et al., 2021) to predict the impact of the mutations on protein stability.

We predict that deletion of either P280 or T281 will have minimal effect on the structure or stability of the protein. This prediction is consistent with the approximately normal abundance of these mutant proteins lacking either residue observed by Western blot (Figure 3D). In contrast, the L292P mutation leads to several interatomic clashes and hence is likely to break the α-helix and lead to a local and even global change in the structure of the protein and lower its stability ($\Delta G_{\text{Stability}} = -0.72$ kcal/mol). Again, our Western blot result on this L292P mutant is consistent with the prediction in that we cannot detect any FARL-11 L292P protein in worm lysates (Figure 3D).

FIGURE 4: Localization of CASH-1, FARL-11, and UNC-68 by confocal microscopy. Each panel shows a portion of a single body wall muscle cell immunostained with antibodies to the indicated proteins or to the myc or HA tags. Yellow arrowheads point to a row of dense bodies; yellow arrows to single M-lines. (A) Strain SU854 (expresses RFP-myc-CASH-1) showing localization of UNC-95 and myc-CASH-1. Myc-CASH-1 localizes between and surrounding dense bodies. (B) Wild type showing localization of PAT-6 and FARL-11. FARL-11 also localizes between and surrounding dense bodies. (C) Strain SU980 (expresses HA-UNC-68) showing localization of UNC-95 and HA-UNC-68. HA-UNC-68 localizes to puncta organized in a striated pattern roughly surrounding dense bodies and M-lines. (D) Strain SU980 showing localization of FARL-11 and HA-UNC-68. The two proteins show partial colocalization suggested by the white puncta created by overlap of green FARL-11 and magenta HA-UNC-68. (E) Strain SU1002 (expresses myc-CASH-1 and HA-UNC-68) showing localization of myc-CASH-1 and HA-UNC-68. The two proteins partially colocalize. Scale bar, 10 μm.
Localizes to rows of puncta, some large, some small, but the allele for localization in muscle. These authors created a split-GFP knock-in Piggott et al. (2021) provided the best confocal images of UNC-68 and their confocal images show that GFP::UNC-68 authors did not colocalize with any sarcomeric markers.

Given that FARL-11 has been localized to the ER of early nematode embryos and that the SR is a muscle-specific type of ER, we used CRISPR/Cas9 to create strain SU980, in which UNC-68 is tagged at its N-terminus with 3xHA. By conventional confocal microscopy, HA-UNC-68 exists in puncta, which are organized in a repeating striated pattern roughly surrounding both dense bodies and M-lines (Figure 4C). We next asked whether FARL-11 and UNC-68 might colocalize. As shown in Figure 4D, there is at least some colocalization of the two proteins, suggested by the white puncta created by the overlap of green FARL-11 and magenta HA-UNC-68 signals. Similarly, HA-UNC-68 and myc-CASH-1 partially colocalize (Figure 4E).

To obtain more information about the localization of UNC-68, we used structured illumination microscopy (SIM), which has an ~120-nm resolution in the XY plane. SIM followed by three-dimensional reconstruction (Figure 5A) shows that HA-UNC-68 localizes to a series of puncta, very similar to the images reported by Piggott et al. (2021). Costaining with PAT-6 (α-Parvin), which localizes to the bases of the M-lines and dense bodies, reveals that the HA-UNC-68 puncta localize on either side of the M-lines and surround the dense bodies, with the larger puncta being closer to the dense bodies. There is also accumulation of HA-UNC-68 at muscle cell boundaries (indicated by yellow arrows in Figure 5B). When a three-dimensional reconstruction of an entire muscle cell is viewed on its side, we observe that in addition to being localized like PAT-6 close to the muscle cell membrane, there is significant localization of HA-UNC-68 in the deeper portions of the muscle cell (Figure 5C). To determine how deeply this HA-UNC-68 is located, we costained anti-HA with anti-ATN-1 or with anti-UNC-89. ATN-1 localizes to the deepest portions of the dense bodies and defines the deepest portion of the myofilament lattice. As can be seen in Figure 5D, HA-UNC-68 is found mainly near the outer muscle cell membrane but also extends more deeply into the muscle cell than the myofilament lattice. A similar result was observed when HA-UNC-68 was costained with anti-UNC-89 (Figure 5E).

locations of the SR (surrounding dense bodies and adjacent to base of M-lines). Unfortunately, the antibodies generated to UNC-68 utilized in these studies are no longer available. A recent report by Piggott et al. (2021) provides the best confocal images of UNC-68 localization in muscle. These authors created a split-GFP knock-in allele for unc-68 and their confocal images show that GFP::UNC-68 localizes to rows of puncta, some large, some small, but the

To characterize the depth of HA-UNC-68 localization at the muscle cell boundary, we costained HA-UNC-68 and PAT-6. As can be seen at the bottom of Figure 5F, which presents part of one muscle cell boundary, HA-UNC-68 is located more deeply than PAT-6. Unfortunately, the anti-FARL-11 antibodies did not give a strong enough signal in immunostaining to allow SIM imaging. However, we were able to use confocal microscopy to acquire a Z-series using 36 | S.C.T. Martin et al.

Molecular Biology of the Cell
anti-FARL-11 and anti-SHA for HA-UNC-68, starting from the outer muscle cell membrane to deeper into muscle cells (Figure 6). FARL-11 is found between and around dense bodies throughout their depth and is also found at muscle cell boundaries (indicated by the arrows). HA-UNC-68 appears only at the muscle cell boundaries at the deepest portions of the muscle cells (second row from the bottom of the figure). Scale bar, 10 μm.

FIGURE 6: A confocal Z-series reveals that FARL-11 localization is similar to localization of HA-UNC-68. Strain SU980 was costained with antibodies to FARL-11 and to HA. From top to bottom are 0.5-μm thick sections from the outer portion of a body wall muscle cell consecutively deeper into the muscle cell. FARL-11 is found between and around dense bodies throughout their depth and is also found at muscle cell boundaries (indicated by the arrows). HA-UNC-68 appears only at the muscle cell boundaries at the deepest portions of the muscle cells (second row from the bottom of the figure). Scale bar, 10 μm.

In mammalian muscle, the UNC-89 homologue, obscurin, links myofibrils to the surrounding SR, through interaction of obscurin with the SR membrane proteins small ankyrin 1 and 2 (Bagnato et al., 2003; Kontrogianni-Kontstantopoulos et al., 2003). Moreover, knockout of the mouse obscurin gene results in disorganization of the SR (Lange et al., 2009). This role for obscurin/UNC-89 seems to be evolutionarily conserved. In C. elegans, there is genetic evidence for an UNC-89 to SR functional linkage. VAV-1 is a RacGEF that regulates the concentration of intracellular calcium and is expressed in body wall muscle; overexpression of vav-1 in muscle results in slow movement; mutagenesis resulted in suppressors that move better; the suppressor mutations are in egl-19 (an L-type calcium channel) and unc-89 (Spooner et al., 2012). Moreover, in unc-89 mutants, in addition to disorganization of the sarcomeres, there is disorganization of the SR as probed using transgenics overexpressing MYC-UNC-68 or SERCA-GFP (Spooner et al., 2012). Therefore, we wondered whether loss of function of unc-68, which has been shown to disrupt SR organization (Maryon et al., 1998), might also disrupt the organization of sarcomeres. As shown in Figure 7, unc-68(e540) shows disorganization of major structural components of the sarcomere—the A-bands (MHC, A), the M-lines (UNC-89), and the bases of M-lines and dense bodies (UNC-95).

If, as our data suggest, FARL-11 and CASH-1 are components of the SR, one straightforward prediction is that in a mutant in which the SR is disrupted, for example, an unc-68 mutant, FARL-11 and CASH-1 might be mislocalized. Curiously, however, this is not the case. As shown in Figure 8A, the localization of FARL-11 in unc-68(e540) and wild type are nearly identical. Because cash-1 and unc-68 are very close together on the genetic map, we used CRISPR to tag CASH-1 at the N terminus using TagRFP::3xmyc in an unc-68(e540) mutant background. As shown in Figure 8B, the localization of CASH-1 in unc-68(e540) and wild type are nearly identical.

We next tested the hypothesis that FARL-11 and CASH-1 proteins affect the organization of the SR or at least the SR marker UNC-68. To address this, we created two strains, one in which HA-UNC-68 is expressed in farl-11(gk437008), and one in which HA-UNC-68 is expressed in cash-1(jc100). As compared with the localization of HA-UNC-68 in a wild-type background (Figure 9A), the overall density of puncta is reduced, especially near the M-lines, in a farl-11(gk437008) mutant background (Figure 9B). However, in a cash-1(jc100) mutant background, we observed no change in overall pattern of HA-UNC-68 puncta (Figure 9C). A similar reduction in HA-UNC-68 puncta near the M-lines in farl-11(gk437008) was observed when we used the M-line marker UNC-89 (Supplemental Figure 2). To examine this perceived difference quantitatively, we counted the number of puncta around 10 M-lines from each wild type, and farl-11 and cash-1 mutants. As shown in Figure 9D, when expressed as the number of puncta per micrometer length of an M-line, farl-11 shows a substantial decrease compared with wild type (means of 0.75 vs. 1.66 puncta/μm).

Given that the overall density of HA-UNC-68 puncta is reduced in the farl-11 mutant, we wondered whether there would also be a decrease in the overall level of HA-UNC-68 protein. To address this question, we performed a Western blot in which protein extracts from wild type, farl-11(gk437008), and cash-1(jc100) were separated on a 5% polyacrylamide gel. As shown in Figure 10A, anti-HA detects two major protein bands, one running at the expected size of ∼557–572 kDa (indicated by a blue arrow) and the other running at 180 kDa (indicated by a yellow arrow). The origin of the ~180 kDa protein is unknown but we suspect that it results from either normal processing of UNC-68 or degradation during extract preparation. The ~180 kDa protein is likely not a nematode or bacterial protein that cross-reacts with antibodies to HA, because it is not detected by Western blot from an extract of wild-type worms (Figure 10B). Quantitation from three experiments shows that, as compared with wild type, 1) the level of the 560 kDa band is not changed in farl-11 but is increased in cash-1 (Figure 10C); 2) the level of the 180 kDa band is reduced in both farl-11 and cash-1 (Figure 10D); and 3) the combined levels of both the 560 and 180 kDa bands are reduced in
broadening of the distribution of myosin and especially UNC-89 (STRIP1), shows disorganized sarcomeres, including a variable et al. complex in vivo (Figure 1D, Supplemental Figure 1). We found that CASH-1 (STRN3) and FARL-11 (STRIP1) form the SR. Consistent with the known structure of the human STRIPAK to determine whether FARL-11 has a role in the muscle-specific ER, bryos and is required for normal ER organization. This prompted us to localize the CASH-1 protein. In the current study, we considered that striatin forms a special PP2A complex, associating not only with catalytic and scaffolding subunits of PP2A but also with STRIP1. The C. elegans orthologue of STRIP1 is called “FARL-11,” and FARL-11 localizes to the ER and the outer nuclear membrane of early em C. elegans cells detaching from the extracellular matrix (ECM), and that loss of function of various regulatory (B) subunits results in disorganization of sarcomeres. We had also demonstrated that various components of the PP2A complex localize to various sarcomeric structures (I-band, M-lines, dense bodies [Z-disks]). We had shown that RNAi for regulatory B” subunit CASH-1 (STRN3) results in disorganized sarcomeres; however, we did not have reagents available to localize the CASH-1 protein. In the current study, we considered that striatin forms a special PP2A complex, associating not only with catalytic and scaffolding subunits of PP2A but also with STRIP1. The C. elegans orthologue of STRIP1 is called “FARL-11,” and FARL-11 localizes to the ER and the outer nuclear membrane of early embryos and is required for normal ER organization. This prompted us to determine whether FARL-11 has a role in the muscle-specific ER, the SR. Consistent with the known structure of the human STRIPAK complex, we found that CASH-1 (STRN3) and FARL-11 (STRIP1) form a complex in vivo (Figure 1D, Supplemental Figure 1).

Similar to loss of function of canonical PP2A subunits (Qadota et al., 2018), loss of function of CASH-1 (STRN3), or FARL-11 (STRIP1), shows disorganized sarcomeres, including a variable broadening of the distribution of myosin and especially UNC-89 (obscurin) (Figures 2 and 3). We found that both CASH-1 and or CASH-1 (Figure 8); however, a farl-11 mutant shows overall reduced density of UNC-68 puncta, especially near the M-lines (Figure 9). Moreover, by Western blot, the overall level of UNC-68 is reduced in both farl-11 and cash-1 mutants as compared with wild type (Figure 10).

An interesting effect of mutations in farl-11 or cash-1 is a widening of the localization of UNC-89 and MHC A myosin (Figure 2B and Figure 3, B and C). The fact that both UNC-89 and MHC A are affected can be explained by considering that UNC-89 is located throughout the depth of the M-line (Warner et al., 2013), the M-line is the region of the sarcomeric A-band where thick filaments are cross-linked, and MHC A is the myosin isoform that is restricted to the middle of the thick filament in C. elegans (Miller et al., 1983). But how loss of function of farl-11 or cash-1 results in widening of the M-line region components is more difficult to explain, especially considering that neither FARL-11 nor CASH-1 is localized to the M-line (Figure 11A). One possibility is suggested by our finding that there are reduced numbers of UNC-68-containing puncta around the M-lines (Figure 9). Perhaps the SR sacs that lie on either side of the base of the M-line restrict the growth of the M-line laterally, either by physical hindrance or signaling. In support of this idea, in mammalian striated muscle, obscurin at the M-line links sarcomeres to the surrounding SR by interacting with SR membrane proteins small ankyrins 1 and 2 (Bagnato et al., 2003; Kontrogianni-Konstantopoulos et al., 2003). Another possibility is that with the reduced amount of SR in the farl-11 mutant, Ca^{2+} signaling is disturbed and this results in unregulated or asymmetric contraction, which ultimately results in disorganization of the sarcomeres. Figure 11 presents a summary of proteins examined in this study and their location in nematode body wall muscle (Figure 11A), and a simple model for results obtained in both farl-11 and cash-1 (Figure 10E). Thus, we can conclude that the status of farl-11 and cash-1 affects the level of UNC-68, consistent with the reduced number of puncta observed in the farl-11 mutant.

DISCUSSION

From this study, we have expanded the function of PP2A in striated muscle. We previously reported that loss of function of the catalytic subunit (LET-92) or the scaffolding subunit (PAA-1) results in muscle cells detaching from the extracellular matrix (ECM), and that loss of function of various regulatory (B) subunits results in disorganization of sarcomeres. We had also demonstrated that various components of the PP2A complex localize to various sarcomeric structures (I-band, M-lines, dense bodies [Z-disks]). We had shown that RNAi for regulatory B” subunit CASH-1 (STRN3) results in disorganized sarcomeres; however, we did not have reagents available to localize the CASH-1 protein. In the current study, we considered that striatin forms a special PP2A complex, associating not only with catalytic and scaffolding subunits of PP2A but also with STRIP1. The C. elegans orthologue of STRIP1 is called “FARL-11,” and FARL-11 localizes to the ER and the outer nuclear membrane of early embryos and is required for normal ER organization. This prompted us to determine whether FARL-11 has a role in the muscle-specific ER, the SR. Consistent with the known structure of the human STRIPAK complex, we found that CASH-1 (STRN3) and FARL-11 (STRIP1) form a complex in vivo (Figure 1D, Supplemental Figure 1).

Similar to loss of function of canonical PP2A subunits (Qadota et al., 2018), loss of function of CASH-1 (STRN3), or FARL-11 (STRIP1), shows disorganized sarcomeres, including a variable broadening of the distribution of myosin and especially UNC-89 (obscurin) (Figures 2 and 3). We found that both CASH-1 and

FIGURE 7: Loss of function of unc-68, which encodes the SR calcium release channel, results in disorganized sarcomeres. Wild type and mutant unc-68(e540) animals were immunostained with antibodies to MHC A myosin, UNC-89, and UNC-95. All the structures labeled by these antibodies (A-bands, M-lines, dense bodies, and muscle cell boundaries) show significant disorganization in unc-68(e540), especially in the organization of UNC-89 and UNC-95. Scale bar, 10 μm.
FIGURE 8: Loss of function of unc-68 does not affect the localization of FARL-11 or CASH-1. (A) Wild type and unc-68(e540) were costained with antibodies to FARL-11 and PAT-6. Note that the localization of FARL-11 in unc-68(e540) is no different from the localization of FARL-11 in wild type. (B) SU854, which expresses RFP and myc tagged CASH-1 and also has the unc-68(e540) mutation, designated “wild type,” and SU1086, which expresses RFP and myc tagged CASH-1 and also has the unc-68(e540) mutation, designated “unc-68(e540)”. Arrowheads point to rows of dense bodies; arrows point to M-lines. Scale bar, 10 μm.

this study (Figure 11B). In this model, the small blue arrow indicates that FARL-11 is important for the normal organization or assembly of the SR (at least near the M-line). The large blue arrow indicates that the SR structure affects sarcomere organization; the orange arrow indicates that alternatively, FARL-11 and CASH-1, are required independently of the SR for sarcomere organization.

We also found that FARL-11 and for the first time that UNC-68 are located at the muscle cell boundaries (Figures 5 and 6), in addition to their locations surrounding M-lines and dense bodies. In addition, a Z-series indicates that UNC-68 appears only at the muscle cell boundaries at the deepest portions of the muscle cell. It should be noted that C. elegans spindle-shaped body wall muscle cells are arranged cell to ECM to cell, where attachment plaques of each cell anchor the cell to a thin layer of ECM that lies between the adjacent cells. These attachment plaques are integrin adhesion complexes and contain a subset of proteins found at dense bodies (Qadota et al., 2017). Electron microscopy reveals that the muscle boundaries also contain gap junctions and fingerlike projections of one cell into another cell (Qadota et al., 2017). We had previously speculated that the gap junctions and fingerlike projections provide electrical conductivity, signaling, and structural integrity allowing multiple smaller cells to function as larger units. Although membranous sacs reminiscent of SR have not been found at muscle cell boundaries, perhaps UNC-68 embedded in the cell membranes might facilitate movement of Ca\textsuperscript{2+} between muscle cells.

Finally, we would like to point out that we took advantage of the MMP to identify and study missense mutants in conserved residues in cash-1 and farl-11 that developed into adults and were fertile. This allowed us to study adult muscle phenotypes more easily. For example, an intragenic deletion of farl-11, tm6233, is noted on WormBase to be lethal or sterile.

METHODS
Request a protocol through Bio-protocol.

C. elegans strains

N2 (wild type, Bristol)
SU853 farl-11 (jc61[mNG::TEV::3xflag::farl-11+LoxP] III
SU854 cash-1 (jc60[TagRFP::TEV::3xmyc::cash-1 +Loox 2272]) V
SU980 unc-68(jc78[3xha::unc-68]) V
SU1002 cash-1 (jc60[TagRFP::TEV::3xmyc::cash-1 +Loox 2272]; unc-68(jc78[3xha::unc-68]) V -outcrossed to wild type 2X (o.c. 2x)
SU1047 farl-11 (jc93 [farl-11 ΔT281 *jc61]) II - o.c. 2x
SU1048 farl-11 (jc94 [farl-11 ΔP280 *jc61]) II
SU1054: cash-1 (jc100[cash-1 h121y *jc60]) V o.c. 2x
SU1086 cash-1 (jc104[3xmyc::cash-1]); unc-68 (e540)-o.c. 2x
SU1070 cash-1 (jc100[cash-1 h121y *jc60]); unc-68 (jc78 [3xha::unc-68a])-V-o.c. 2x
CB540 unc-68 (e540) V
VC40583 cash-1 (gk705529[cash-1 H121Y]) V
VC30204 farl-11 (gk437008 [farl-11 L292P]) II
GB351 farl-11 (gk437008 [FARL-11 L292P]) II o.c. 3x
GB352 farl-11 (gk437008 [FARL-11 L292P]) II o.c. 3x; unc-68 (jc78 [3xha::unc-68a])-V
SU1030 cash-1[syb4646][TagRFP::CASH-1 L82E/L86E *jc60]/tmC12[egl-9(tmIs1197)] V
PHX4646 cash-1[syb4646] [TagRFP::cash-1 L82E/L86E *jc60]/tm1(nT1)(qs51)] (IV; V)

CRISPR/Cas9

To generate endogenous insertions, a self-excising cassette was used as described (Dickinson et al., 2015). tagrfp::SEC::CASH-1/- was crossed into the tmC12 (Dejima et al., 2018) balancer to facilitate SEC removal. mng::SEC::farl-11/+ was crossed into the mln1 balancer to facilitate SEC removal. The balanced progeny were heat shocked at 34°C to remove the SEC. SEC excision and correct knock-in were confirmed by PCR of nonrolling, nonbalanced progeny. Worms were outcrossed with N2 a minimum of 2x.

SunyBiotech generated the PHX4646 cash-1[syb4646][TagRFP::cash-1 L82E/L86E *jc60]/tm1(nT1)(qs51)] (IV; V) strain. This strain was outcrossed with N2 and then crossed into the tmC12[egl-9(tmIs1197)] strain to create SU1030 cash-1[syb4646][TagRFP::cash-1 L82E/L86E *jc60]/tmC12[egl-9(tmIs1197)] V

Single point mutants were generated as described (Arribere et al., 2014).

Guide RNA sequences were cloned into the Cas9 containing plasmid (pJW1219).

CASH-1 TagRFP 5’- guide- 5’- CGGATTCGAGTAATCCAAATGG CASH-1 H121Y guide- 5’- ATTTATGTATGCATCAAGGT CASH-1 L82E/L86E guide- 1-GCTGATTTGTTCTCTAACAGG CASH-1 L82E/L86E guide 2- AATTATCGACTAACCACACCGG
FIGURE 9: The organization of UNC-68 is adversely affected by loss of function of farl-11. Confocal imaging of body wall muscle costained with antibodies to HA to detect HA-UNC-68 and antibodies to PAT-6. Scale bar, 10 μm. (A) Imaging of SU980, which expresses HA-UNC-68 in a wild-type background. The two thin rectangles at the bottom are zoomed-in images of the areas denoted by the orange arrows. Note the accumulation of HA-UNC-68 small puncta around M-lines (magenta lines of PAT-6 staining). (B) Imaging of GB352, which expresses HA-UNC-68 in a farl-11(gk437008) mutant background. Note that in the zoomed-in images there are fewer HA-UNC-68 small puncta surrounding M-lines, as compared with what is observed in a wild-type background shown in (A). (C) Imaging of SU1070, which expresses HA-UNC-68 in a cash-1(jc100) mutant background. Note that in the zoomed-in images UNC-68 small puncta have the same density and arrangement of distribution around M-lines as in the wild-type background shown in (A). (D) Quantitation of HA-UNC-68 puncta near M-lines in wild type and the two mutants. For each strain, 5–6 confocal images were taken and 10 M-lines were chosen randomly. A rectangle was placed around each M-line, with dimensions 600 × 40 pixels (with length ∼ 34.3 μm) and the number of puncta recorded, and this number divided by the length of the rectangle. As indicated on the graph, farl-11(gk437008) shows a statistically significant (p < 0.0001) reduction in the number of these HA-UNC-68 puncta as compared with wild type.

FARL-11-mNG 5′ guide: - 5′GGCCCTGATTCCCATTACATT
FARL-11 L290P guide-5′ ATTTCATTATCAACCATAGT
UNC-68-5′guide-5′ TCCTCCCTGCTCCTCCTTG

Homology searches/protein sequence analysis
CASH-1 and FARL-11 protein sequences were run through a Hydrophobic Cluster analysis program (mboyle@RPBS) to generate a Hydrophobic Cluster Plot (Néron et al., 2009). jc93, jc94, and jc100 were generated at sites of predicted secondary structure (Callebaut et al., 1997). cash-1 L82E/L86E mutations were designed to mimic the mutations in the homodimerization domain of STRN-3 (Chen et al., 2014).

Protein structure modeling
For FARL-11 (uniprot Q19300) and CASH-1 (uniprot G5EE12) protein structure modeling, CLUSTALW version 1.2.2 (https://www.ebi.ac.uk/Tools/msa/clustalw2/), SWISS-MODEL version July 2021 (https://swissmodel.expasy.org/; Waterhouse et al., 2018), and Phyre2 version 2.0 (http://www.sbg.bio.ic.ac.uk.phyre2/html/page.cgi?id=index; Kelley et al., 2015) online tools were used.

For FARL-11, we used the cryoEM structure of human orthologue of STRIP1 as reference for modeling (7K36.pdb; Jeong et al., 2021). For CASH-1, we used the human orthologue STRN3 as reference for modeling (7K36.pdb; Jeong et al., 2021). Molecular graphics were generated by using Chimera version 1.15 (https://www.cgl.ucsf.edu/chimera/; Pettersen et al., 2004). Single amino acid mutations were inserted using the rotamer tool and then energy minimized to minimize interatomic clashes and contacts based on van der Waals radii. In addition, we used the DynaMut2 (Rodrigues et al., 2021) to predict the effects of the point mutations on protein stability and dynamics.

FARL-11 antibody production
Antibody production was performed by Li International (Denver, CO). A 50 amino acid chemically synthesized peptide corresponding to the extreme C-terminus of FARL-11 (aa 929-978 TCAHSVLGANLKLGRHFKKDYEKWLEQVFNASIDWDKLLETRGVDEL) was injected into New Zealand rabbits. Li International performed immunization and provided lyophilized antibody, which was resuspended to 1 mg/ml with 1× PBS.

expresses HA-UNC-68 in a wild-type background. The two thin rectangles at the bottom are zoomed-in images of the areas denoted by the orange arrows. Note the accumulation of HA-UNC-68 small puncta around M-lines (magenta lines of PAT-6 staining). (B) Imaging of GB352, which expresses HA-UNC-68 in a farl-11(gk437008) mutant background. Note that in the zoomed-in images there are fewer HA-UNC-68 small puncta surrounding M-lines, as compared with what is observed in a wild-type background shown in (A). (C) Imaging of SU1070, which expresses HA-UNC-68 in a cash-1(jc100) mutant background. Note that in the zoomed-in images UNC-68 small puncta have the same density and arrangement of distribution around M-lines as in the wild-type background shown in (A). (D) Quantitation of HA-UNC-68 puncta near M-lines in wild type and the two mutants. For each strain, 5–6 confocal images were taken and 10 M-lines were chosen randomly. A rectangle was placed around each M-line, with dimensions 600 × 40 pixels (with length ∼ 34.3 μm) and the number of puncta recorded, and this number divided by the length of the rectangle. As indicated on the graph, farl-11(gk437008) shows a statistically significant (p < 0.0001) reduction in the number of these HA-UNC-68 puncta as compared with wild type.
Coimmunoprecipitation of FARL-11 with CASH-1

For Supplemental Figure 1, coimmunoprecipitation was performed essentially as described (Zaidel-Bar et al., 2010). Twenty, 10-cm NGM plates with gravid adults were harvested and frozen in Lysis (−) buffer (50-mM HEPES pH 7.4, 150-mM NaCl, 0.05-mM DTT). Worms were lysed by sonication in Lysis (+) buffer (50-mM HEPES pH 7.4, 150-mM NaCl, 0.05-mM DTT, 1% NP-40) and total lysates were centrifuged at 4°C. The supernatant was then precleared using ChromoTek control magnetic agarose beads (ChromoTek, cat. no. gmab-20) for 30 min at 4°C. Precleared lysates were then incubated with ChromoTek RFP-Trap magnetic agarose beads (cat. no. rtm-20) for 1 h at 4°C to bind to TagRFP-myc-CASH-1. Beads were washed 3x with 1 ml of Lysis (−) buffer and eluted with 50 μl of Lysis (−) buffer at 90°C for 10 min. Samples were resolved on 10% SDS-PAGE and transferred to PVDF membrane (Immobilon-FL, Millipore, cat. no. IPFL00010) using a ThermoFisher Powerblotter (cat. no. PB0013), and immunoblotting was performed using ThermoFisher Scientific (Waltham, MA) iBindFlex (cat. no. SLF2000). FARL-11 was detected at 1:500 dilution using the rabbit anti-FARL-11 antibody described above. LiCor 2° goat anti-rabbit antibody (cat. no. 925-3211) was used for detection at 1:4000 dilution.

For Figure 1D, coimmunoprecipitation was performed as follows. A large quantity of worms (−3 ml packed) from strain SU854 was grown on 19, 15-cm high-peptone NGM plates seeded with E. coli strain NA22, and a “worm powder” was generated by grinding the worms extensively in a mortar and pestle in liquid nitrogen. A total protein lysate (Lysate) was prepared by adding worm powder to 1 ml of Lysis Buffer (50-mM Tris pH 7.5, 150-mM NaCl, 1% NP40, 0.05-mM DTT, 1-mM EDTA, Complete Mini protease inhibitor cocktail [Roche], 1-mM PMSF) at a ratio of approximately 20% volume to volume, vortexing for 1 min, incubating on ice for 2 min, vortexing for 2 min, and then spinning at top speed in a microfuge at 4° for 10 min, and saving the supernatant. A portion was saved and diluted 1:1 with 2X Laemmli loading buffer to run on a gel. To the remainder of the supernatant was added 30 μl of RFP-Trap Magnetic Particles M-270 (cat. no. rtd-20, ChromoTek), incubating on a rotating wheel at 4° for 50 min, removing the beads from the solution using a rack containing neodymium magnets, and then washing the beads with 1-ml Wash Buffer (50-mM Tris pH 7.5, 150-mM NaCl, 0.05-mM DTT, 1-mM EDTA, Complete Mini protease inhibitor cocktail [Roche], 1-mM PMSF) 3×. To the washed beads was added 33 μl of 2X Laemmli sample buffer, vortexing for 5 s, heating at 95° for 5 min, vortexing for 5 s, and then separating out the beads on the magnetic stand, the resulting liquid designated as “IP.” Multiple lanes containing either 20 μl of Lysate or IP were separated on a 10% SDS-PAGE, transferred to nitrocellulose membrane, and as shown in Figure 1D, one portion reacted against anti-C-Myc (catalogue number M4439, Sigma-Aldrich, St. Louis, MO at 1:3000 dilution), or affinity-purified anti-FARL-11 at 1:1000 dilution, reacted with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies, and visualized with enhanced chemiluminescence (ECL) reagents (cat. no. 32106, Thermo Scientific) and exposure to film.

Western blots

The method of Hannak et al. (2002) was used to prepare total protein lysates from mixed-stage populations of wild-type, SU853, SU854, SU1054, farl-11(jc93), farl-11(jc94), farl-11(gk437008), SU980, GB352, and cash-1 mutants. For each strain N = 3, and significance was tested by a one-tailed Student’s t test.
amide-SDS-Laemmli gels, transferred to nitrocellulose membranes, equal amounts of total protein were separated on 10% polyacrylamide-SDS-Laemmli gels, transferred to nitrocellulose membranes, and SU1070 worms. For the Western blots presented in Figures 1–3, equal amounts of total protein were separated on 10% polyacrylamide-SDS-Laemmli gels, transferred to nitrocellulose membrane for 2 h, and reacted with anti-HA (rabbit monoclonal antibody from Cell Signaling Technologies, cat. no. 37245) at 1:1000 dilution, followed by reaction with antirabbit immunoglobulin G conjugated to HRP (GE HealthCare) at 1:10,000 dilution and visualized by ECL. For protein molecular weight size markers, we used the HiMark Pre-Stained Protein Standard (cat. no. LC5699 from Life Technologies). For comparison of protein levels between wild type and mutants, samples were normalized based on total protein per lane visualized by Ponceau S staining.

**Immunostaining, confocal, and SIM microscopy of body wall muscle**

Adult nematodes were fixed and immunostained using the method described by Nonet et al. (1993) with further details described in Wilson et al. (2012). The following primary antibodies were used at 1:200 dilution except as noted: anti-UNC-89 (mouse monoclonal MH42; Hresko et al., 1994; Benian et al., 1996), anti-MHC A (mouse monoclonal 5-6; Miller et al., 1983), anti-UNC-95 (rabbit polyclonal Benian-13; Qadota et al., 2007), anti-PAT-6 (rat polyclonal; Warner et al., 2013), anti-ATN-1 (mouse monoclonal MH153; Francis and Waterston, 1991), anti-HA (rabbit monoclonal from Cell Signaling Technology, cat. no. 37245; and mouse monoclonal from Sigma-Aldrich, cat. no. H3663), anti-myc (mouse monoclonal clone 9E 10 from the University of Iowa Hybridoma Bank), and anti-FARL-11 (this study). Secondary antibodies, used at 1:200 dilution, included antirabbit Alexa 488, antirat Alexa 594, and antimouse Alexa 594, all purchased from Invitrogen. Images were captured at room temperature with a Zeiss confocal system (LSM510) equipped with an Axiovert 100M microscope and an Apochromat x63/1.4 numerical aperture oil immersion objective, in 1× and 2.5× zoom mode. For imaging Alexa 488, the excitation was conducted using a 488-nm Argon laser, and emission was captured using a 505–530 nm bandpass filter. For imaging Alexa 594, the excitation was conducted using a 543-nm Helium–Neon laser, and emission >560 nm was captured using a longpass filter. Super-resolution microscopy was conducted with a Nikon N-SIM system in three-dimensional structured illumination mode on an Eclipse Ti-E microscope equipped with a 100× 1.49 NA oil immersion objective, 488- and 561-nm solid-state lasers, and an EM-CCD camera (DU-897, Andor Technology). For imaging Alexa 488, we used a 470–490 nm excitation filter and a 500–545 nm emission filter. For imaging Alexa 594, we used a 556–566 nm excitation filter and a 570–640 nm emission filter. Super-resolution images were reconstructed using the N-SIM module in NIS-Elements software. For all images, confocal and SIM, color balance was adjusted by using Adobe Photoshop (Adobe, San Jose, CA).

**ACKNOWLEDGMENTS**

H.Q. and G.M.B. were supported by the National Institutes of Health (NIH) grant R01HL160693 and the National Science Foundation (NSF) grant 2050009. J.H. and S.C.T.M. were supported by NIH grants R01GM058038 and MIRA R35GM145312. A.F.O. acknowledges support from The Cecil and Ida Green Endowment. In addition, S.C.T.M. was supported by an NIH T32 (GM130550) Biophysics...
Training Grant, a Gilliam Fellowship from the Howard Hughes Medical Institute, an Advanced Opportunities Fellowship, and a COVID-19 dissertation completion fellowship from the University of Wisconsin-Madison.

REFERENCES


Cryoskeleton 74, 426–442.
Supplementary Materials

*Molecular Biology of the Cell*

Martin et al.
Supplemental Figure 1. Co-immunoprecipitation of FARL-11 with CASH-1, with detailed controls. As described in Materials and Methods, TagRFP-myc-CASH-1 was immunoprecipitated from a lysate of strain SU854, and proteins from consecutive steps of the procedure were separated by SDS-PAGE, blotted and incubated with anti-myc (A) or with anti-FARL-11 (B). From left to right the lanes of each blot contain: (1) total lysate (total), (2) insoluble proteins pelleted from total lysate (pellet), (3) clarified cell lysate (CCL), (4) proteins associated with magnetic agarose control beads (MA beads), (5) proteins that did not bind to anti-RFP magnetic agarose or flow-through (FT), (6) proteins in first wash of anti-RFP magnetic agarose (W1), (7) proteins eluted from anti-RFP magnetic agarose (IP), and (8) molecular weight markers (MW) with sizes indicated in kDa. Arrows indicate the positions of RFP-myc-CASH-1 or FARL-11 in the IP. In (A) note that RFP-myc-CASH-1 is detectable in the worm lysate (CCL lane) and was immunoprecipitated (IP lane). In (B) note that FARL-11 was detectable in the worm lysate (CCL lane) and was co-immunoprecipitated (IP lane).
**Supplemental Figure 2.** Imaging of GB352 which expresses HA-UNC-68 in a *farl-11*(gk437008) mutant background, immunostained with anti-HA and anti-UNC-89, an alternative way to mark M-lines (shown in magenta). Note that in the zoomed-in images there are fewer HA-UNC-68 small puncta surrounding M-lines, as compared to wild type shown in Figure 9A. Scale bar, 10 μm.

**Supplementary Table 1. Penetration of body wall muscle sarcomeric defects in mutants.**

For each of the mutant alleles listed, the organization of each sarcomeric component was observed in 10 animals. The percentages represent the fraction of 10 animals showing the phenotype. Note that the penetration varies from 70 to 100%, even among various alleles of the same gene (e.g. *cash-1*).
<table>
<thead>
<tr>
<th>gene</th>
<th>allele</th>
<th>Disorganization of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UNC-95</td>
</tr>
<tr>
<td>cash-1</td>
<td>gk705529</td>
<td>70%</td>
</tr>
<tr>
<td>cash-1</td>
<td>jc100</td>
<td>70%</td>
</tr>
<tr>
<td>cash-1</td>
<td>syb4646</td>
<td>100%</td>
</tr>
<tr>
<td>farl-11</td>
<td>jc93</td>
<td>100%</td>
</tr>
<tr>
<td>farl-11</td>
<td>jc94</td>
<td>100%</td>
</tr>
<tr>
<td>farl-11</td>
<td>gk437008</td>
<td>100%</td>
</tr>
<tr>
<td>unc-68</td>
<td>e540</td>
<td>70%</td>
</tr>
</tbody>
</table>