

Review Article

Molecular Structure of Sarcomere-to-Membrane Attachment at M-Lines in *C. elegans* Muscle

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C. elegans is an excellent model for studying nonmuscle cell focal adhesions and the analogous muscle cell attachment structures. In the major striated muscle of this nematode, all of the M-lines and the Z-disk analogs (dense bodies) are attached to the muscle cell membrane and underlying extracellular matrix. Accumulating at these sites are many proteins associated with integrin. We have found that nematode M-lines contain a set of protein complexes that link integrin-associated proteins to myosin thick filaments. We have also obtained evidence for intriguing additional functions for these muscle cell attachment proteins.

1. Structure of *C. elegans* Body Wall Muscle

Sarcomeres, highly ordered assemblages of several hundred proteins, perform the work of muscle contraction. Despite ever increasing knowledge of the components of sarcomeres and their functions, we still do not have a clear picture about how sarcomeres are assembled and maintained in the face of muscle contraction. A number of laboratories are studying the questions of sarcomere assembly and maintenance in the model genetic organism, *C. elegans*. In addition to being an excellent system to carry out mutational analysis in a whole organism, through both forward and reverse genetics, this nematode offers several advantages for studying muscle. These include its optical transparency, which allows evaluation of myofibrillar structure by polarized light, and localization of GFP-tagged proteins. In addition, its usual mode of self-fertilization allows propagation of muscle mutants that would be unable to mate. In the major striated muscle of *C. elegans* which is found in the body wall and used for locomotion (Figure 1), the myofibrils are restricted to a narrow $\sim 1.5 \mu\text{m}$ zone adjacent to the cell membrane along the outer side of the muscle cell [1–3]. The thin filaments are attached to the dense bodies (Z-disk analogs), and the thick filaments are organized around M-lines. Moreover, all the dense bodies and M-lines are anchored to the muscle cell membrane and extracellular matrix (ECM), which is

attached to the hypodermis and cuticle (see Figure 1). This allows the force of muscle contraction to be transmitted directly to the cuticle and allows movement of the whole animal. Thus, worm muscle M-lines and dense bodies serve the function of analogous structures in vertebrate muscle. But, in addition, because of their membrane anchorage and protein composition, they are also similar to focal adhesions of non-muscle cells and costameres of vertebrate muscle [4, 5].

2. Discovery of Muscle Attachment Components through Immunological and Genetic Approaches

First success in identifying muscle attachment components came from an immunological approach. Francis and Waterston [7, 8] used protein fractions enriched in nematode body wall muscle components to generate a battery of monoclonal antibodies and then determined their staining patterns in nematodes and their western blot reactivities. These monoclonal antibodies recognize several muscle or hypodermis attachment structures. Using two of these antibodies that localize to the base of dense bodies to screen an expression library, *C. elegans* vinculin, encoded by the *deb-1* gene, was identified [9]. After placing *deb-1* on the *C. elegans* genetic map, a genetic screen was conducted for loss of function

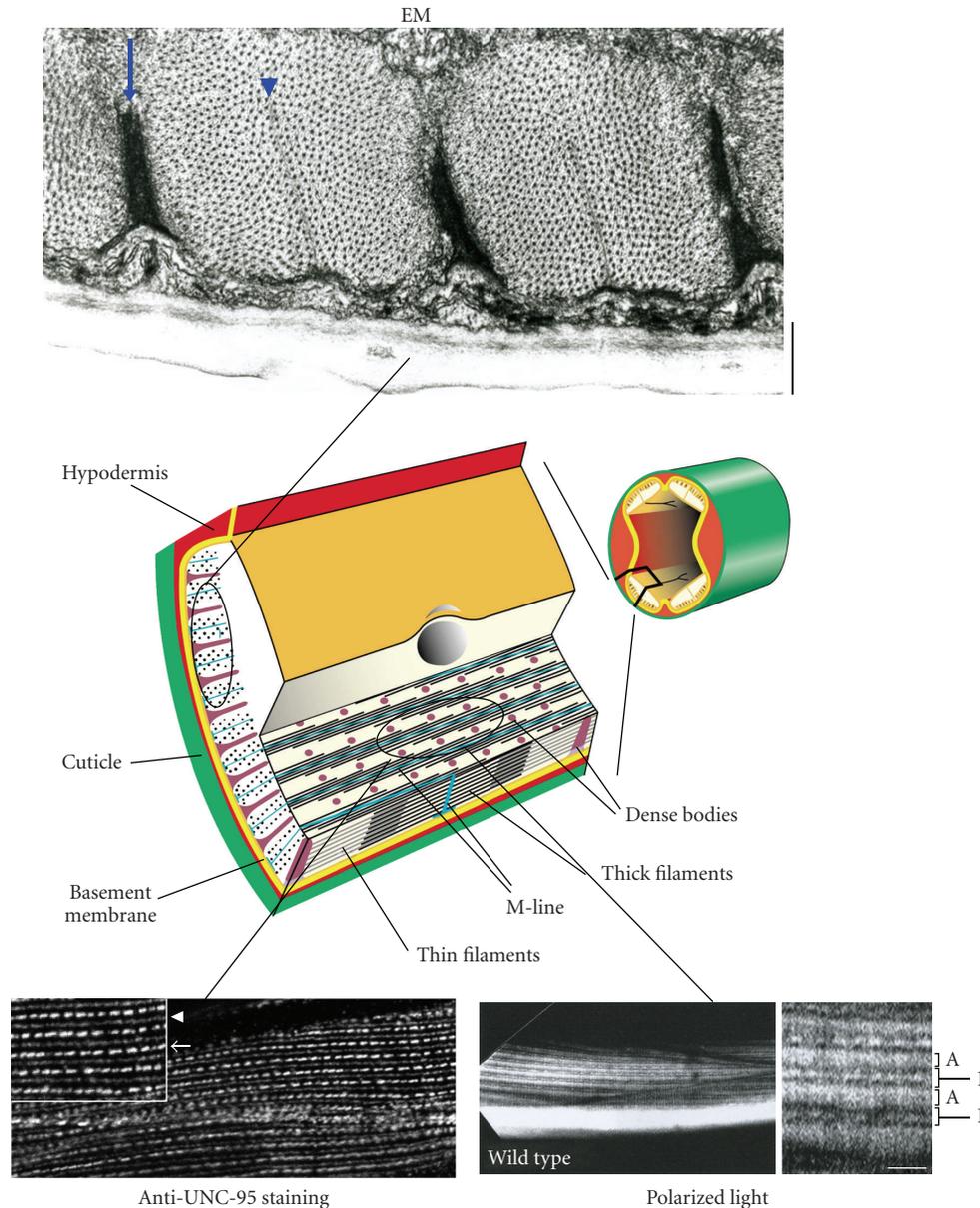


FIGURE 1: The body wall muscle of *C. elegans*. The color drawing on the right depicts a cross-section through an adult nematode emphasizing that the body wall muscle is organized into four quadrants. Each quadrant consists of interlocking pairs of mononuclear spindle-shaped cells (23 or 24 per quadrant). In the enlargement, notice that the myofilament lattice is restricted to one side of the cell, rather than filling the entire cross-sectional area as in a vertebrate striated muscle cell. Several planes of section are depicted; one of which emphasizes that fundamentally this is striated muscle with typical A-bands containing thick filaments organized around M-lines, and overlapping thin filaments attached to the Z-disk analogs called dense bodies. Note the plane parallel to the paper; this is the plane viewed when an animal, lying on a glass slide, is examined by light microscopy. At the bottom right is a typical image obtained with polarizing optics showing that bright A-bands alternate with dark I-bands; in the enlargement (right), the cross-sections of the dense bodies can be seen in the I-bands. At the bottom left is a typical image obtained by immunofluorescence microscopy using an antibody to UNC-95 [6] (enlargement is shown at the top left); notice that UNC-95 localizes to both rows of dense bodies (arrow) and M-lines (arrowhead). In the large drawing, the plane of section shown on the left side is a true cross-section through the nematode and through a body wall muscle cell; at the top of the figure is a typical transmission EM view of two sarcomeres; an arrow marks a dense body and an arrowhead marks an M-line. In the EM, at the bottom is located the thick cuticle and thin hypodermal cell and basement membrane; the cross-sections of thick filaments in the A-bands and the cross sections of the thin filaments in the I-bands (surrounding the dense bodies) can be seen. Notice that in the drawing and in the EM, all of the dense bodies and the M-lines are anchored to the muscle cell membrane. Indeed, the EM reveals that there is electron dense material at the base of each dense body and M-line that is likely to be responsible for this anchorage. This is in contrast to vertebrate striated muscle in which only the peripherally-located sarcomeres are anchored to the muscle cell membrane through costameres at the level of the Z-disks and possibly the M-lines.

mutations [10]. Two mutants in *deb-1* were shown to be embryonic lethal and displayed the same phenotype that was first identified for loss of function mutations in the *myo-3* gene [11]. *myo-3* encodes one of two myosin heavy chains, called MHC A, that is expressed in nematode body wall muscle and is localized to the middle of thick filaments [12]. Soon after the discovery of the *myo-3* phenotype, it was shown that strong loss of function alleles for the *unc-45* gene display a similar embryonic lethal phenotype [13]. (*unc-45* was later shown to encode a highly conserved myosin head chaperone [14, 15]). The phenotype of loss of function alleles consistent with a null state for *myo-3*, *unc-45*, and *deb-1* is “Pat”, which is an abbreviation for paralyzed and arrested at two-fold embryonic stage. During normal nematode embryonic development there are both an expansion of cell number and morphogenesis in which the initially football-shaped embryo elongates 4-fold, going through stages that are named according to length, 1.5-fold, 2-fold, and 3-fold, before hatching from the eggshell. Pat embryos do not move in the eggshell at the 1.5-fold stage and arrest development at the 2-fold stage. Other aspects of development continue and the embryo hatches as an abnormal L1 larva and dies. Encouraged by finding the Pat phenotype for *myo-3*, *deb-1*, and *unc-45*, Williams and Waterston [16] conducted a genome-wide screen and identified 13 additional genes with a Pat phenotype, and new alleles of *deb-1* and *myo-3*. From the precise characterization of the Pat mutant embryos, the Pat genes were classified into five classes. The other major phenotypic class of muscle affecting mutant genes is the “Unc” (uncoordinated) class, which result in slow moving or paralyzed adult worms [17, 18]. This class includes about 40 genes. However, the severity of the Pat phenotype suggests that genes of this class are crucial for the initial assembly of myofibrils. Interestingly, for a number of genes (*unc-45*, *unc-52*, *unc-97*, and *unc-112*), the phenotype of hypomorphic alleles is Unc, and the phenotype of alleles consistent with a null state is Pat. Later molecular cloning of the Pat genes revealed that classes I and II both have severe defects in myosin and actin organization, and encode many components of muscle attachment structures: *unc-52* encodes the ECM protein, perlecan [19], *pat-3* encodes β -integrin [20], *pat-2* encodes α -integrin (16; B. Williams, pers. commun.), *unc-112* encodes the nematode ortholog of mammalian Kindlin [21], *pat-4* encodes integrin-linked kinase, ILK [22], and *pat-6* encodes the nematode ortholog of mammalian actopaxin [23]. In addition to these cloned Pat genes, molecular analysis of *unc-97* revealed that *unc-97* encodes the *C. elegans* ortholog of mammalian PINCH [24], and the null state for *unc-97* is also Pat [25]. All of the abovementioned *pat* and *unc* gene products, except for *unc-45*, have been localized to both dense bodies and M-lines by GFP fusions (original papers cited above), and also in some cases by specific antibodies [6, 26]. Yeast two-hybrid assays using cloned fragments fused to yeast two-hybrid vectors and binding experiments using purified proteins have demonstrated that these class I and II Pat gene products interact with each other (see Figure 2). Based on what is known about its mammalian counterparts, it is likely that UNC-52 (perlecan) associates with PAT-2

(α -integrin) and PAT-3 (β -integrin) at the outside of muscle cells. Inside the muscle cell, it has been demonstrated that the cytoplasmic tail of the PAT-3 associates with a four-protein complex consisting of UNC-112 (Kindlin)/PAT-4 (ILK)/UNC-97 (PINCH)/PAT-6 (actopaxin) (22, 23, 25; H. Qadota, D. G. Moerman, G. M. Benian, submitted). As indicated in Figure 2, UNC-52 (perlecan), integrins (PAT-2/PAT-3), and the four-protein complex are found at the base of both M-lines and dense bodies. At the dense bodies, there are additional dense body-specific proteins such as DEB-1 (vinculin) [9], ATN-1 (α -actinin) [27], UIG-1 (Cdc42 GEF) [28], ALP-1 (ALP/Enigma) [29], DYC-1 [30], and ELP-1 (EMAP-like protein) [31]. The only known nematode M-line-specific protein is UNC-89 (obscurin) [32, 33]; as mentioned below, UNC-98 and UNC-96 are most prominent at M-lines. In this review, we will focus on the M-line and its molecular components. For the structure and molecular components of dense bodies see the recent review by Lecroisey et al. [34].

3. UNC-98, UNC-96, and UNC-95

Our interest in the molecular mechanisms by which sarcomeres are attached to the muscle cell membrane at the M-line began with our molecular cloning of two muscle Unc genes, *unc-98* and *unc-96*, that have similar mutant phenotypes. These two genes were first identified by Zengel and Epstein [18] from their genetic screen for mutants that are defective in muscle function and structure, which involved mutagenesis followed by enrichment for mutants with slow motility, and then examination of myofilament lattice structure by polarized light microscopy. *unc-98* and *unc-96* mutants are slower moving than wild type, and by polarized light microscopy display a moderately disorganized myofilament lattice and birefringent “needle-like” structures at the ends of their body wall muscle cells. These “needles” correspond to accumulations of proteins that contain paramyosin, but not actin, myosin, UNC-89, or α -actinin [35–37]. By electron microscopy, both *unc-98* and *unc-96* mutants display indistinct A and I bands, and short and irregularly shaped dense bodies, and short or even absent M-lines. *unc-98* encodes a 310 residue polypeptide containing 4 C2H2 Zn finger domains and several predicted nuclear localization and nuclear export signal sequences [35]. Antibodies to UNC-98 localize to M-lines. However, in transgenic animals, UNC-98::GFP localizes to M-lines, dense bodies, and muscle cell nuclei. In addition, *unc-98* mutant animals, when rescued with a wild-type copy of the gene, show localization of anti-UNC-98 antibodies to M-lines, dense bodies and nuclei. Our interpretation is that UNC-98 is mainly localized to M-lines, but also exists normally at low levels at dense bodies and nuclei, but below the level of detection by antibody staining. Deletion derivatives of UNC-98::GFP in transgenic worms demonstrates that the N-terminal 110 residues of UNC-98 are necessary and sufficient for nuclear localization, and that all four Zn fingers are sufficient for localization to M-lines and dense bodies. Using an UNC-98 bait to screen a collection of 2-hybrid clones representing 16 known M-line and dense body proteins, interaction with UNC-97 (PINCH)

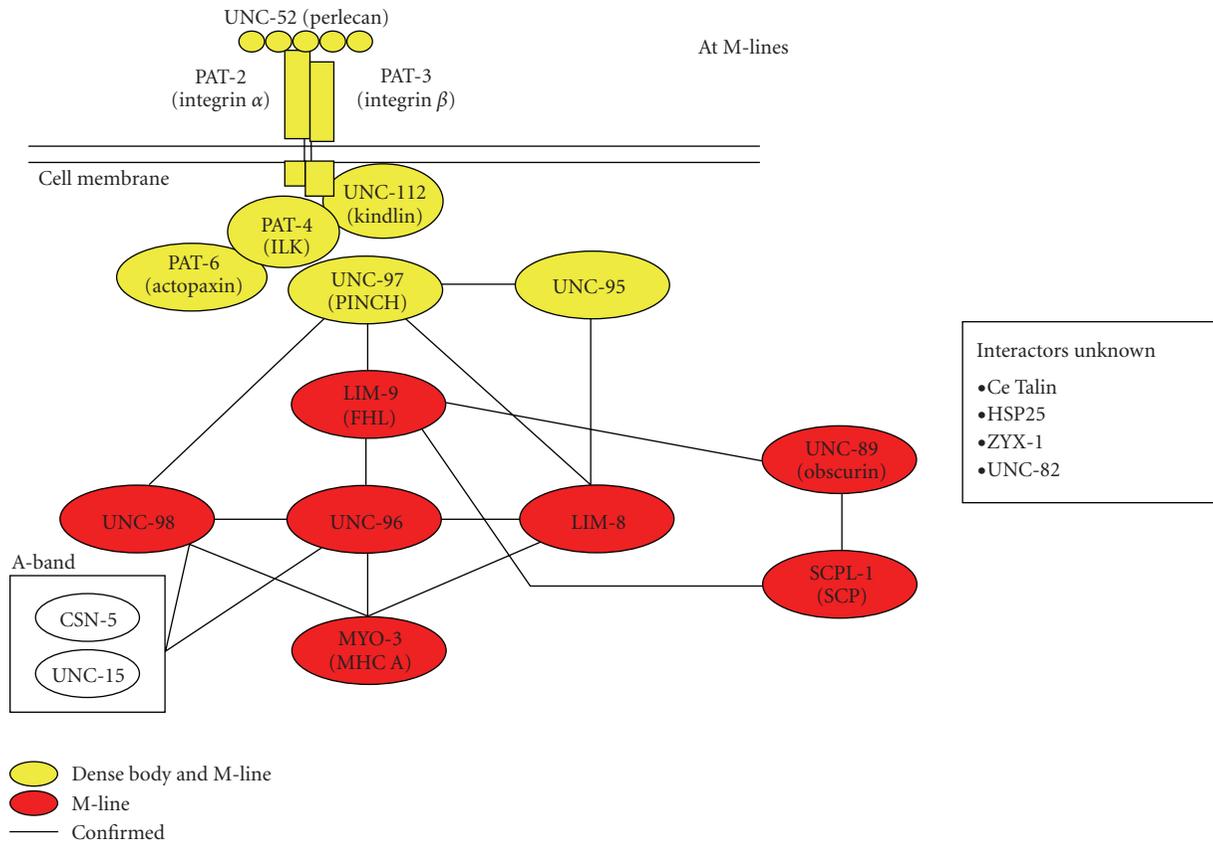


FIGURE 2: A protein interaction matrix for M-lines in *C. elegans* striated muscle. The myofilament lattice is located close to the surface and anchored by M-lines and dense bodies to the muscle cell membrane. At these attachment structures, UNC-52 (perlecan) is located in the ECM, and by homology is likely to interact with an integrin $\alpha\beta$ heterodimer (PAT-2 and PAT-3). Inside the muscle cell, the cytoplasmic tail of PAT-3 (β integrin) is associated with a complex of four conserved proteins [UNC-112 (Kindlin), PAT-4 (ILK), PAT-6 (actopaxin), UNC-97 (PINCH)]. UNC-95 is also found at both M-lines and dense bodies. At M-lines, specific proteins link UNC-97 (PINCH) to myosin heavy chains (MHC A). These are UNC-98, LIM-9 (FHL) and UNC-96, LIM-8, and UNC-95 and LIM-8. UNC-98, UNC-96 and LIM-8 have all been shown to interact with portions of the rod domain of MHC A. LIM-9 links to the giant protein UNC-89 (obscurin) and to the phosphatase SCPL-1 (SCP). Additionally, UNC-98 and UNC-96 interact with UNC-15 (paramyosin) and to CSN-5 (COP9 signalosome component), but these interactions occur outside the M-line. The box on the right indicates that there are four more proteins that have been reported to reside at the M-line (Ce Talin, HSP25, ZYX-1 (zyxin), and UNC-82 (ARK5/SNARK)), but these have not yet been connected with components of the interaction matrix. Lines indicate interactions that were identified by 2-hybrid screens and later shown to occur with purified proteins in vitro. All proteins indicated have been localized to M-lines by specific antibodies and/or GFP fusions. References for all the interactions have been cited in the text except for the LIM-8/UNC-95 interaction (A. Simionescu, H. Qadota, and G. Benian, unpub. data).

was revealed, thus showing a connection to the conserved four-protein complex associated with integrin. The UNC-98/UNC-97 interaction was confirmed by in vitro binding using purified proteins. Moreover, binding requires the first two LIM domains of the 5 LIM domain protein UNC-97, and all 4 C2H2 Zn fingers of UNC-98.

We later showed that *unc-96* encodes 408 and 418 residue polypeptides by alternative splicing, and these proteins lack recognizable domains [36]. Nonetheless, UNC-96 may consist of two domains separated by a flexible loop or linker [37]. Antibodies to UNC-96 localize the protein to muscle M-lines, although UNC-96::GFP localizes to both M-lines and dense bodies and not to nuclei [36]. A role for UNC-96 in embryonic muscle development is uncertain: although UNC-96 is detectable by the 1.5-fold stage, its

expression decreases with time and by the 3-fold stage is nearly undetectable. The strongest mutant allele of *unc-96*, *sf18*, is not Pat embryonic lethal, and yet is presumably a null mutant, as it is a nonsense mutation, and no protein can be detected by western blot. Intriguingly, either a decreased (by loss of function mutation) or an increased level (by a heat-shock promoter in adult muscle) of UNC-96 results in disorganization of thick filaments [6]. Thus, the level of UNC-96 must be precisely controlled in order to obtain proper organization of thick filaments. By both genetic and biochemical criteria, UNC-98 and UNC-96 interact with each other. Although *unc-98; unc-96* double mutants do not show an enhanced or suppressed phenotype, when either UNC-98 is overexpressed in an *unc-96* mutant background, or UNC-96 is overexpressed in an *unc-98* mutant background

there is enhancement; by polarized light microscopy, there is a greater degree of myofibril disorganization and larger and brighter needles. Moreover, protein accumulations at the ends of the muscle cells contain UNC-98 protein in *unc-96* mutants, and contain UNC-96 protein in *unc-98* mutants. UNC-96 was shown to interact with UNC-98 by both a yeast 2-hybrid assay and a far Western assay using purified proteins. This interaction requires the C-terminal half of UNC-96 and the C-terminal three C2H2 domain of UNC-98. Although obvious homologs of either UNC-98 or UNC-96 cannot be found in vertebrate proteomes, it is possible that structural or functional homologs do exist.

unc-95 was also first isolated from the genetic screen reported by Zengel and Epstein [18], and then molecularly cloned by Broday and colleagues [38]. *unc-95* mutants are slow moving and have disorganized muscle structure. Immunostaining with various antibodies shows that thick and thin filaments and dense bodies are disorganized [38]. Myofibrillar disorganization is also obvious by EM; moreover, dense bodies are short and irregular and M-lines are missing. *unc-95* encodes a 350-residue polypeptide with a single LIM domain near its C-terminus, and a region predicted to have coiled-coil structure and a predicted NLS sequence [38]. Although the UNC-95 LIM domain was reported to be most homologous to LIM domains in fly and vertebrate paxillin [38], more recently, a true ortholog of vertebrate paxillin has been found in *C. elegans*, PXL-1 (A. Warner and D. G. Moerman, pers. commun.). An UNC-95::GFP translational fusion localizes to M-lines, dense bodies, muscle cell-cell boundaries, and nuclei in adult body wall muscle [38]. UNC-95::GFP is clearly also expressed in embryonic muscle, and by the 3-fold stage is localized to muscle attachment sites and nuclei. Antibodies that we later developed to UNC-95 clearly label the M-lines, dense bodies, and cell-cell boundaries, but not nuclei in adult body wall muscle [6].

4. Multiple Protein Complexes Link Integrin to Myosin in Thick Filaments at Nematode M-Lines

During the past 7 years, we have discovered proteins that interact with UNC-97 (PINCH) at nematode M-lines. The first of these was the M-line protein UNC-98 [35], as mentioned above: the first two of the five total LIM domains of UNC-97 interact with the 4 C2H2 Zn fingers of UNC-98. In Miller et al. [26] we showed that the N-terminal 110 residues of UNC-98 interact with the C-terminal portion of a myosin heavy chain, MHC A, which resides in the middle of thick filaments in the proximity of M-lines. A combination of genetic, cell biologic and biochemical evidence supports a model in which UNC-98 links integrin-associated proteins to myosin in thick filaments at M-lines. Although vertebrate costameres are usually regarded to reside at the level of Z-disks, some components of focal adhesions, including α integrin, have also been found at M-lines [39]. Thus, our results for *C. elegans* muscle suggest the possibility of a similar mechanism of linkage between integrins and myosin

thick filaments at the M-lines of peripheral myofibrils of vertebrate muscle.

In Qadota et al. [6] we demonstrated additional mechanisms by which this linkage from muscle cell membrane to myosin occurs. To identify additional proteins that interact with UNC-97, we screened a collection of known and candidate components of M-lines and/or dense bodies, UNC-112 interactors, and UIG-1 interactors, by the 2-hybrid method. Three new UNC-97 (PINCH) interactors were identified: LIM-8, LIM-9, and UNC-95. It was shown that these proteins are involved in three additional mechanisms by which linkage from UNC-97 to myosin occurs: from UNC-97 through LIM-8 to myosin, or from UNC-97 through LIM-9/UNC-96 to myosin, or from UNC-97 through UNC-95/LIM-8 to myosin. LIM-8 is a novel LIM domain-containing protein. LIM-9 is the nematode homolog of mammalian FHL (four-and-a-half LIM domain protein). All three new UNC-97 interactors contain LIM domains that are required for binding. Among the three interactors, LIM-8 and LIM-9 also bind to UNC-96. UNC-96 and LIM-8 also bind to the C-terminal portion of MHC A (to a slightly different portion of MHC A that binds to UNC-98). All interactions were first identified by yeast 2-hybrid and then confirmed by in vitro binding assays using purified proteins. All three novel UNC-97 interactors are expressed in body wall muscle and by antibodies localize at least partially to M-lines. LIM-8 and LIM-9 localize also to I-bands, around and between dense bodies.

Like UNC-98 and UNC-96, UNC-89 (mammalian homolog is called "obscurin" [40–42]) is also an M-line protein in *C. elegans* muscle. UNC-89 is a giant (up to 900 kDa) multidomain protein consisting primarily of Ig domains, SH3, DH and PH domains, and two protein kinase domains. We have shown recently that a portion of UNC-89 (containing the kinase domains) interacts with a member of the UNC-98/UNC-96 complex, namely, LIM-9 (FHL) [43]. Moreover, LIM-9 also interacts with an additional M-line protein that was first identified as an UNC-89 interactor, namely, SCPL-1, a CTD-type protein phosphatase [44]. This class of phosphatases was known to be involved in the regulation of transcription either through dephosphorylation of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II, or in dephosphorylating Smad transcription factors. However, our results implicate new functions for this class of protein phosphatases, namely, in the assembly or maintenance of the M-line, and/or giant kinase signaling. We can now incorporate the majority of the known *C. elegans* M-line proteins into an M-line protein interaction matrix; the interactions were first identified through 2-hybrid analysis and later confirmed by both in vitro binding using purified proteins and immunolocalization to M-lines (Figure 2).

5. Additional Functions for M-Line Attachment Proteins

In addition to a structural and signaling role for UNC-96 and UNC-98 at the M-line, we have shown that these proteins interact with paramyosin to promote paramyosin's

incorporation into thick filaments [36, 37]. Paramyosin is an invertebrate-specific protein that is primarily an α -helical coiled-coil rod and is ~40% identical in amino acid sequence to the rod domains of myosin heavy chains. In *C. elegans* body wall muscle, the myosins and a portion of paramyosin are organized around a tubular core consisting of paramyosin and filagenins in a specific geometry [45–47]. The birefringent needles in *unc-96* and *unc-98* mutants contain paramyosin located outside the thick filaments. By genetic and biochemical criteria, paramyosin interacts with UNC-98 and UNC-96. By both yeast 2-hybrid analysis and ELISAs using purified proteins, UNC-98 interacts with paramyosin residues 31–693, whereas UNC-96 interacts with a separate region of paramyosin, residues 699–798. Although UNC-98 and UNC-96 affect, at least partially, the localization of paramyosin (some in accumulations, some in its normal A-band location), they do not affect the total level of paramyosin: paramyosin levels do not change in either *unc-96* or *unc-98* loss of function mutants. Nevertheless, the state of paramyosin does affect the localization and total amount of UNC-98 and possibly UNC-96: UNC-98 and UNC-96 colocalize with paramyosin aggregates of *unc-15* missense mutants, and when paramyosin is undetectable (*unc-15* nonsense mutant), UNC-98 and UNC-96 are diffusely localized. Indeed, by quantitative immunoblot, the levels of UNC-98 follow the paramyosin state: when paramyosin is undetectable (in the *unc-15* nonsense mutant *e1214*), the level of UNC-98 is diminished, and in paramyosin missense mutants (*e1215* and *e73*, which form paramyosin aggregates), the level of UNC-98 is increased. The dependence of UNC-98, and possibly UNC-96, levels on the state of paramyosin might be due to a chaperone function for UNC-98 and UNC-96 to prevent aggregation of paramyosin. An additional piece of data supports the idea that UNC-96 could act as a chaperone for paramyosin: both *unc-96* mutant alleles are suppressed at lower temperature; that is, when grown at 15°C, rather than the usual 20°C, the paramyosin “needles” (containing aggregated paramyosin) are not seen. One *unc-96* mutant allele is a stop codon, the other is a splicing site mutation, types of mutations not usually associated with temperature sensitivity. Alternative possibilities are that (1) the formation of paramyosin paracrystals is temperature dependent, and thus reduced at cold temperatures, and/or (2) the competing formation of proper thick filaments is enhanced at lower temperatures. However, the temperature effect seems to be specific to *unc-96*: neither *unc-98* nor *unc-15* is suppressed at lower temperature [36]. Therefore, these alternative possibilities do not seem to be supported. Perhaps the lower temperature might even reduce the rate of thick filament assembly or turnover so that the UNC-96 chaperone is no longer required to prevent aggregation.

Most recently, we have demonstrated that both UNC-98 and UNC-96 interact with CSN-5 [48]. Interactions were identified by a yeast 2-hybrid screen and confirmed by biochemical methods. CSN-5 is a member of the highly conserved “COP9 signalosome complex” which has been found in multiple organisms to regulate protein stability, usually through SCF ubiquitin ligases [49, 50]. Anti-CSN-5 antibody colocalizes with paramyosin at A-bands in wild

type, and co-localizes with abnormal accumulations of paramyosin found in *unc-98*, *unc-96*, and *unc-15* mutants. Knockdown of *csn-5* results in an increase in the level of UNC-98 protein and a slight reduction in the level of UNC-96 protein, suggesting that normally CSN-5 promotes the degradation of UNC-98 and that CSN-5 stabilizes UNC-96. In *unc-15* and *unc-96* mutants, CSN-5 protein is reduced, implying the existence of feedback regulation from myofibril proteins to CSN-5 protein levels. This is the first report to implicate CSN-5 or the COP9 signalosome in myofibrillar organization or function. Nevertheless, the report is consistent with the growing recognition that the ubiquitin proteasome system is required for muscle protein turnover in vertebrate muscle, and mediated by the muscle-specific ubiquitin ligase Atrogin-1, and the MuRF family (Muscle-specific RING Finger proteins) [51, 52]. Indeed, for *C. elegans* muscle, the RING finger protein, RNF-5 is localized to dense bodies and regulates the levels of UNC-95 [38]: RNF-5 and UNC-95 interact by yeast 2-hybrid [53]; heat shock induced overexpression of RNF-5 results in a reduction in UNC-95::GFP, and this reduction depends on the presence of an active RING finger domain in RNF-5; in contrast, RNAi knockdown of *rnf-5* results in an increase in UNC-95::GFP [38].

6. Nuclear Function for Muscle Focal Adhesion Proteins?

In recent years, there has been growing recognition that in mammalian striated muscle, a number of Z-disk and M-line proteins translocate to the nucleus in response to mechanical stimuli or extracellular signals, and once inside the nucleus, influence gene transcription [54, 55]. A similar situation appears to exist for nematode muscle, but at this time, the mechanisms of nuclear translocation and the functional significance of M-line and dense body proteins in the nucleus are less understood than they are for mammalian muscle. But given the power of worm genetics and its advantages for imaging live muscle cells, this area holds much promise for future insights. Here is what we know for proteins that are at least partly localized to M-lines: In transgenic worms, translational GFP fusions of full-length UNC-97 [24], UNC-98 [35], UNC-95 [38], and ZYX-1 (zyxin) [30] show localization to M-lines, dense bodies, and nuclei. However, antibodies that we have developed to UNC-98 [35], to UNC-97 [26], and to UNC-95 [6], when used in immunofluorescent experiments, failed to localize to nuclei under normal conditions. There are no reports of antibodies having been generated or localized for ZYX-1. Nevertheless, anti-UNC-98 reacted to nuclei, when a nonstandard fixation method was used on wild-type nematodes, or when UNC-98 was overexpressed [35]. Additional support that endogenous UNC-98 and UNC-97 reside in nuclei was obtained during our purification of native thick filaments reported in Miller et al. [26]: nuclear-enriched fractions from wild-type worms contain western blot detectable UNC-98 and UNC-97. In the 2-hybrid system, when either UNC-98 [35] or UNC-97 (H. Qadota, K. Norman, and D. Moerman, unpub. data) is fused to the GAL4 DNA-binding domain, they can activate

transcription, suggesting that UNC-98 and UNC-97 may activate transcription *in vivo*. By testing deletion derivatives of UNC-98::GFP, we have shown that the N-terminal 110 residues of UNC-98 are sufficient for nuclear localization [35]. A similar approach by Norman et al. [25] indicates that LIM2 and LIM3 are required for nuclear localization of UNC-97 (PINCH).

In addition to nuclear localization of proteins that are localized to M-lines and dense bodies, nuclear localization has also been found for the dense-body-specific protein ALP-1 [29]. The *alp-1* gene encodes 4 different isoforms; one is ALP-like (ALP-1A), and three are Enigma-like (ALP-1B, -1C, -1D). Use of GFP translational fusions demonstrates a complex pattern of expression of these proteins in embryos and adults, and localization to muscle cell dense bodies, and nuclei of muscle and hypodermal (epithelial) cells. In fact, ALP-1 is one of the few muscle focal adhesion proteins showing strong localization to embryonic muscle (ALP-1A) and hypodermal cell (ALP-1B,C,D) nuclei.

7. Future Directions

Three additional proteins have been localized at both M-lines and dense bodies; these are ZYX-1 (zyxin) [30] (as noted above), HSP25 [56], and Ce Talin [57]. However, the mutant or RNAi phenotypes and molecular interactions of these proteins have not yet been reported. Although ZYX-1 has been demonstrated to interact with the dense-body-specific protein DYC-1 [30], its interactions with proteins at the M-line have not been determined. The most recently discovered M-line component is UNC-82, a 1600-residue polypeptide with a serine/threonine kinase domain (orthologous to human ARK5 or SNARK) near its N-terminus [58]. It is required for maintaining proper organization of thick filaments and the M-line during growth of muscle cells. It will be interesting to determine how UNC-82, HSP25, Ce Talin, and ZYX-1 fit into the growing M-line protein interaction matrix (Figure 2).

So far, there are no reports on the molecular cloning of one muscle Unc gene, *unc-100*, and 3 Pat genes (*pat-9*, *pat-11*, and *pat-12*). It will be interesting to learn the molecular nature of the encoded proteins for these genes, and whether the proteins are localized to M-line, dense bodies, or both. Additionally, we will also be able to determine with which existing proteins of the M-line and dense body interacting network these proteins interact. As mentioned above, we have found that two M-line proteins, LIM-9 and SCPL-1, interact with the C-terminal portion of the giant protein UNC-89 (obscurin). Efforts are underway to search for additional binding partners for this giant, and this is likely to identify new M-line proteins. Finally, it will be interesting to determine how many of the 108 new genes identified by Meissner et al. [59] encode proteins localized to M-lines and/or dense bodies. It will then be interesting to determine how they interact with previously described and new components of these structures, to obtain a more complete picture of sarcomere assembly.

Intragenic deletions (consistent with a null state) of the genes encoding many of the proteins that have been iden-

tified recently as components of M-lines and dense bodies have either weak defects or no obvious defects in myofibril assembly or motility (e.g., UIG-1, ATN-1, LIM-9, LIM-8, SCPL-1, ZYX-1). One explanation is that these proteins have noncrucial functions, or are redundant. Redundancy would be a reasonable explanation if such proteins were members of closely-related multigene families (e.g., the actin gene family). However, this does not appear to be the case. Another possibility is that our ability to discern a phenotype depends on the assay employed. Indeed, we can hypothesize that under the usual laboratory growth conditions and motility assays worm muscle operates far below its maximal capacity. Therefore, assays for motility usually employed may not be sufficient to reveal the requirement of many of these individual components. Perhaps more sophisticated assays, in which, for example, worms are required to “work harder” will reveal phenotypes. Indeed, we would expect that M-line and dense body components should function in the transmission of force from the thick and thin filaments through the muscle cell membrane, ECM, and ultimately to the cuticle.

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