The *UNC-112* Gene in *Caenorhabditis elegans* Encodes a Novel Component of Cell–Matrix Adhesion Structures Required for Integrin Localization in the Muscle Cell Membrane

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Abstract. Embryos homozygous for mutations in the *unc-52*, *pat-2*, *pat-3*, and *unc-112* genes of *C. elegans* exhibit a similar Pat phenotype. Myosin and actin are not organized into sarcomeres in the body wall muscle cells of these mutants, and dense body and M-line components fail to assemble. The *unc-52* (perlecan), *pat-2* (α-integrin), and *pat-3* (β-integrin) genes encode ECM or transmembrane proteins found at the cell–matrix adhesion sites of both dense bodies and M-lines. This study describes the identification of the *unc-112* gene product, a novel, membrane-associated, intracellular protein that colocalizes with integrin at cell–matrix adhesion complexes. The 720–amino acid UNC-112 protein is homologous to Mig-2, a human protein of unknown function. These two proteins share a region of homology with talin and members of the FERM superfamily of proteins.

We have determined that a functional UNC-112:: GFP fusion protein colocalizes with PAT-3/β-integrin in both adult and embryonic body wall muscle. We also have determined that UNC-112 is required to organize PAT-3/β-integrin after it is integrated into the basal cell membrane, but is not required to organize UNC-52/ perlecan in the basement membrane, nor for DEB-1/ vinculin to localize with PAT-3/β-integrin. Furthermore, UNC-112 requires the presence of UNC-52/perlecan and PAT-3/β-integrin, but not DEB-1/vinculin to become localized to the muscle cell membrane.

Key words: UNC-112 • integrin • muscle development • adhesion complex • FERM superfamily

Introduction

In the nematode *Caenorhabditis elegans*, a highly ordered series of attachments is required between the myofilament lattice of a body wall muscle cell and the external cuticle for muscle contraction to result in movement. The myofilament lattice is anchored to the muscle cell membrane and adjacent basement membrane by integrin-containing structures called dense bodies and M-lines which, in turn, are linked to intermediate filament arrays that extend across the thin hypodermis and attach to the cuticle (Waterston, 1988; Francis and Waterston, 1991; Moerman and Fire, 1997; Hresko et al., 1999). Dense bodies and M-lines contain many of the components found in focal adhesion plaques (FAs)[1], the cell–extracellular matrix (ECM) contacts of tissue culture cells. Many proteins, including integrins, cytoskeletal proteins, signaling molecules, proteases, protein kinases and phosphatases, as well as proteins of unknown function are associated with FAs (Burridge and Chrzanowska-Wodnicka, 1996). Genetic analysis of focal adhesion analogues in *C. elegans* offers an important opportunity not only to directly test the function of focal adhesion components in vivo, but also to identify new components. Here we describe a novel component of dense bodies and M-lines, and show directly that it plays a critical role in the assembly of these focal adhesion analogues in vivo.

Integrins are a family of adhesion receptors that attach cells to the ECM, or to other cells, and anchor intracellular

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[1]*Abbreviations used in this paper:* aa, amino acids; ECM, extracellular matrix; FAs, focal adhesion plaques; GFP, green fluorescent protein; ORF(s), open reading frame(s); Pat, paralyzed, arrested elongation at twofold.

structural proteins and signal transduction molecules to the plasma membrane (Hynes, 1992). The *C. elegans* genome contains genes encoding β-integrin and α-integrin subunits (Gettner et al., 1995; Baum and Garriga, 1997; *C. elegans* sequencing consortium, 1998; Williams, B., unpublished observations). The α subunit encoded by the *pat-2* gene and the β subunit encoded by the *pat-3* gene are found in most contractile tissues of the worm (Francis and Waterston, 1985; Williams and Waterston, 1994; Gettner et al., 1995; Moerman and Fire, 1997; Williams, B., unpublished observations). In body wall muscle, α-PAT-2/β-PAT-3 heterodimers are transmembrane components of dense bodies and M-lines, the structures that anchor, respectively, the actin and myosin filaments to the cell membrane. Dense bodies are comparable to the Z-lines of vertebrate striated muscle (Waterston, 1988), and include vinculin (Francis and Waterston, 1985; Barstead and Waterston, 1989), α-actinin (Francis and Waterston, 1985; Barstead et al., 1991), talin (Moulder et al., 1996), and the UNC-97/PINCH protein (Hobart et al., 1999), in addition to α-PAT-2/β-PAT-3 integrin heterodimers. With the exception of vinculin and α-actinin, the same proteins are also present in the M-lines. In addition, M-lines contain the *unc-89* gene product (Benian et al., 1996).

Integrins link both the dense body and M-line components to the underlying basement membrane (Francis and Waterston, 1985; Gettner et al., 1995). The *unc-52* gene encodes the nematode homologue of mammalian perlecan (Rogalski et al., 1993), the major heparan sulfate proteoglycan of the extracellular matrix (Noonan et al., 1991; Kallunki and Tryggvason, 1992; Murdock et al., 1992). UNC-52/perlecan is found in the basement membrane between the body wall muscle cells and the hypodermis, and is concentrated at muscle cell dense bodies and M-lines (Francis and Waterston, 1991; Rogalski et al., 1993; Mullen et al., 1999). The interaction of integrin and perlecan, either directly or indirectly, is a key early event in the assembly of these attachment structures (Rogalski et al., 1993; Hresko et al., 1994; Williams and Waterston, 1994; Mullen et al., 1999). This interaction is required for integrin to become localized at the basal membrane of the cell and to assemble into attachment structures.

Null mutations in muscle-affecting genes have been identified and characterized in the nematode (reviewed in Waterston, 1988; Moerman and Fire, 1997). In addition, antibodies to many proteins expressed in muscle have been generated (Miller et al., 1983; Francis and Waterston, 1985, 1991; Moulder et al., 1996; Mullen et al., 1999). Careful analyses of wild-type and mutant animals with these antibodies have revealed that body wall muscle assembly is initiated by events occurring at the muscle cell membrane (Waterston, 1989; Barstead and Waterston, 1991; Rogalski et al., 1993; Williams and Waterston, 1994; Hresko et al., 1994; Moulder et al., 1996). Early in embryonic body wall muscle development, muscle proteins accumulate at membranes where adjacent muscle cells contact each other and the hypodermis (Hresko et al., 1994). This has been termed muscle cell polarization, and at this stage, muscle, basement membrane, and hypodermal components are all colocalized in a continuous linear structure at the site of muscle-hypodermal contact (Hresko et al., 1994). In the later stages of body wall muscle development, perlecan and integrin become organized along the basal cell membrane in structures resembling cell–matrix adhesion complexes. These complexes are not yet organized into the highly ordered arrays that are present in mature muscle. Dense body and M-line components then assemble at the nascent attachment sites. The thick filaments of the lattice assemble with the M-line and the thin filaments with the dense bodies (Waterston, 1989; Barstead and Waterston, 1991; Rogalski et al., 1993; Williams and Waterston, 1994; Hresko et al., 1994; Gettner et al., 1995). Myofilament lattice assembly in the nematode is remarkably similar to the assembly of focal adhesions in mammalian cell culture (Burridge et al., 1988; Moerman and Fire, 1997). In both processes, integrin-ECM interactions are required to initiate assembly and stabilize existing adhesion complexes (Hresko et al., 1994; Yamada and Geiger, 1997).

Many of the muscle-affecting mutations obtained in the nematode are recessive lethal and result in a Pat (paralyzed, arrested elongation at twofold) phenotype, indicating that the missing gene products are essential for the formation of functional embryonic body wall muscle (Waterston, 1989; Williams and Waterston, 1994). Null mutations in several kinds of genes result in a Pat phenotype, including those required for: (a) the assembly of the dense bodies and M-lines, (b) the assembly of thick or thin filaments into the lattice, and (c) the regulation of muscle contraction (see Williams and Waterston, 1994). The most severe Pat phenotype is exhibited by embryos homozygous for mutations in the *unc-52* (perlecan), *pat-2* (α-integrin), *pat-3* (β-integrin), and *unc-112* genes (Williams and Waterston, 1994). Myosin and actin are not organized into sarcomeres in the body wall muscle cells of these mutants, and dense body and M-line components fail to assemble (Rogalski et al., 1993; Williams and Waterston, 1994; Mullen et al., 1999; Williams, B., unpublished observations). The *unc-52*, *pat-2*, and *pat-3* genes encode ECM and membrane proteins found at the cell–matrix adhesion sites of both dense bodies and M-lines (Francis and Waterston, 1985, 1991; Rogalski et al., 1993; Gettner et al., 1995; Williams, B., unpublished observation).

Embryonic body wall muscle assembly in *C. elegans* is an excellent in vivo system in which to study the assembly of integrin-containing adhesion structures, and to identify essential components that are involved in this process. This study describes the identification of the *unc-112* gene product, a novel, membrane-associated, intracellular protein that colocalizes with perlecan and integrin at cell–matrix adhesion complexes. The 720–amino acid UNC-112 protein is most similar to a human protein of unknown function called Mig-2 (Wick et al., 1994), but also shares a short, ~200–amino acid region of homology with talin and other members of the FERM superfamily of proteins. The analysis of mutant embryos presented here demonstrates that UNC-112 is required for the proper spatial localization of PAT-3/β-integrin in the basal cell membrane, but is not required to organize UNC-52/perlecan in the basement membrane nor for DEB-1/vinculin to associate with PAT-3/β-integrin. We also show that UNC-112 requires the presence of UNC-52/perlecan and PAT-3/β-integrin, but not DEB-1/vinculin to become localized in the muscle cell membrane.

Materials and Methods

Nematode Strains

The CB0228: *unc-61(e228)*V and TR0466: *unc-112(r367)*V strains were provided by the *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota (Minneapolis, Minnesota). The following transgenic strains were obtained from James Thomas (University of Washington, Seattle, WA; see Birnby et al., 2000): JT8175: +/+; saEx77 [T19G4(V) + T25B1(V) + C29A7(V) + M05D1(V) + pRF#4*{rol-6(su1006dm)}*]; JT8200: +/+; saEx78 [T19G4(V) + T25B1(V) + C29A7(V) + M05D1(V) + pRF#4*{rol-6(su1006dm)}*]; JT8203: +/+; saEx80 [M05D1(V) + K12G18(V) + T10E3(V) + R08A2(V) + C18G4(V) + pRF#4*{rol-6(su1006dm)}*]; JT8208: +/+; saEx86 [R15H1(V) + F54H7(V) + W08A7(V) + ZC457(V) + ZC394(V) + pRF#4*{rol-6(su1006dm)}*]; JT8209: +/+; saEx87 [R15H1(V) + F54H7(V) + W08A7(V) + ZC457(V) + ZC394(V) + pRF#4*{rol-6(su1006dm)}*]; JT8316: +/+; saEx106 [R08A2(V) + pRF#4*{rol-6(su1006dm)}*]; JT8318: +/+; *saEx108* [R08A2(V) + pRF#4*{rol-6(su1006dm)}*]; JT8321: +/+; *saEx111* [T10E3(V) + pRF#4*{rol-6(su1006dm)}*]; JT8323: +/+; *saEx113* [C18G4(V) + pRF#4*{rol-6(su1006dm)}*]; JT8324: +/+; *saEx114* [C18G4(V) + pRF#4*{rol-6(su1006dm)}*]; and JT8327: +/+; *saEx117* [M05D1(V) + pRF#4*{rol-6(su1006dm)}*]. The following strains were provided by Barbara Meyer at the University of California, Berkeley, California (see Klein and Meyer, 1993): TY1312: +/nT1*[let(m435)]*IV; *unc-42(e270)yDf9/nT1[let(m435)]*V; and TY1313: +/nT1*[let(m435)]*IV; *yDf11/nT1[let(m435)]*V. The VC0002: *unc-112(gk1)*/+V strain was obtained from the *C. elegans* Reverse Genetics Core Facility at the University of British Columbia (Vancouver, British Columbia). The DM5002: *unc-76(e911)*V and DM5116: *unc-112(gk1)*+/+unc-39(e257)V strains were constructed for this study. Other strains used in this study include RW3562: + unc-44(e362)deb-1(st555) +/unc-82(e1223) + + unc-24(e318)IV (Barstead and Waterston, 1991); RW3570: *dpy-11(e224)unc-23(e25)* +/+ + *unc-112(st562*)V; RW3600: *pat-3(st564)/qC1[dpy-19(e1259)glp-1(q339)]*III; and RW3609: *unc-112(st581)* +/+ unc-39(e257)V (Williams and Waterston, 1994).

Complementation Tests with Deficiencies

The *unc-112* gene was shown to be deleted by the *yDf9* deficiency in the following manner. The hypomorphic allele of *unc-112* was used for these experiments because animals homozygous for *r367*ts, although paralyzed, are viable and fertile. All matings were done at 20°C, and the F1 progeny were raised at 20°C. Unc-76 hermaphrodites of genotype + *unc-76(e911)/ unc-42(e270) yDf9* were mated with *unc-112(r367)/*+ males and wild (outcross) progeny were selected. Approximately one-fourth of the wild F1 progeny from this cross were expected to have the genotype + *unc-112(r367)/unc-42(e270) yDf9* if the deficiency does not delete the *unc-112* gene, whereas none would have this genotype if the deficiency does delete *unc-112*. The genotype of 20 wild F1 hermaphrodites was determined by progeny testing, and none carried both the *unc-112(r367)* and *unc-42(e270) yDf9* marked chromosomes. The + *unc-112(r367)/unc-42(e270) yDf9* animals were present among the outcross F1 progeny, and they were found to arrest development at various stages from Pat embryos to sterile, paralyzed adults.

A similar strategy was used to show that the *yDf11* deficiency does not delete the *unc-112* gene. In this case, Unc-61 hermaphrodites of genotype *unc-61(e228)/yDf11* were mated with *unc-112(r367)/*+ males. Wild F1 progeny with the genotype *unc-112(r367)/yDf11* were obtained from this cross indicating that the *unc-112* gene is not deleted by the *yDf11* deficiency.

Complementation Tests with unc-112(gk1)

The formaldehyde-induced *gk1* mutation was isolated by the *C. elegans* Reverse Genetics Core Facility in a PCR screen of mutagenized hermaphrodites using *unc-112* primers. Genomic DNA from *gk1* mutants amplified a smaller PCR fragment than expected with these primers indicating the presence of a deletion in the *unc-112* locus. Embryos homozygous for *unc-112*(*gk1)* exhibit a Pat terminal phenotype. This mutation was shown to be an allele of the *unc-112* gene by mating *unc-112(gk1)* +/+ *unc-39(e257)V* hermaphrodites with *unc-112(r367)/*+ males. The matings were done at 20°C, and then the mated hermaphrodites were transferred to 15°C. At 15°C the majority of the *unc-112(gk1)/unc-112(r367)* mutants develop to the L4 or adult stage, but were paralyzed.

Mapping Deficiency Breakpoints by PCR

The following PCR strategy was used to determine if a particular cosmid was deleted by either *yDf9* or *yDf11*. Mutants homozygous for either the *yDf9* deficiency (arrested embryos) or the *yDf11* deficiency (arrested larvae) were lysed with proteinase K, and the DNA obtained was used in PCR reactions as previously described (Barstead et al., 1991; Rogalski et al., 1995). The genomic sequence of the cosmids being tested was obtained from The *Caenorhabditis elegans* Genome Sequencing Consortium (1998) and one set of primers that would amplify a fragment between 0.9 and 1.2 kb in size was used per cosmid. If a fragment of the correct size was amplified using DNA isolated from the arrested mutants, then that region of the cosmid was not deleted by the deficiency. If the correct fragment was not obtained then the region covered by the primers was deleted by the deficiency being tested. Approximately 4–6 arrested *yDf9* or *yDf11* homozygous animals were used per PCR reaction.

Complementation Tests with Transgenic Strains Carrying Cosmid Arrays

To determine whether a particular cosmid array carried a wild-type *unc-112* gene, we mated *unc-112(r367)/*+ males with Rol hermaphrodites carrying the cosmid array (*Ex*) to obtain animals with the genotype *unc-112(r367)/*+; Ex. Since paralyzed Unc-112 animals do not exhibit the Rol phenotype, it was necessary to select several Rol progeny from the above hermaphrodites and determine their genotype by progeny testing. If the cosmid array being tested carried a wild-type copy of the *unc-112* gene then some of these animals would be homozygous for the *unc-112(r367)* allele. Rol hermaphrodites with the genotype *unc-112(r367)/unc-112(r367); Ex* were obtained for the *saEx80* (DM5102), *saEx106* (DM-5103), *saEx108* (DM5104), *saEx111* (DM5105), *raEx10* (DM5112), *raEx11* (DM5113) and *raEx16* (DM5114) cosmid arrays.

The DM5115: *unc-112(st581)/unc-112(st581); raEx16[unc-112*::GFP; *rol-6(su1006)]* strain was obtained in the following manner. Males of genotype *unc-112(st581)/*+ were mated to *unc-112(r367)/unc-112(r367); raEx16[unc-112*::GFP; *rol-6(su1006)]* hermaphrodites, and Rol F1 progeny with the genotype *unc-112(st581)/unc-112(r367); raEx16[unc-112*:: GFP; *rol-6(su1006)]* were identified by progeny testing. Viable Rol hermaphrodites that were homozygous for *unc-112(st581)* were obtained from among the progeny of these animals.

Construction of Genomic Clones for Microinjection

C47E8 cosmid DNA was prepared using a modified alkaline lysis/PEG precipitation procedure and then cut with the XhoI restriction enzyme. A 7.5-kb XhoI fragment containing the complete *unc-112* gene plus ~2.5 kb of 5′ sequence was gel purified using Low Melting Point agarose, subcloned into the XhoI site of the bluescript plasmid and then transformed into *E. coli* XL1 blue cells. The ends of the insert were sequenced to confirm that the correct fragment had been cloned and to determine its orientation. Two clones, pDM#208 and pDM#209, carrying the 7.5-kb XhoI fragment in opposite orientations were obtained.

The four GFP encoding exons from the plasmid pPD107.48 were inserted into the above 7.5-kb fragment in the following manner. First, pDM#208 DNA was isolated and then digested with XbaI to remove a 150-bp fragment containing the HindIII site in the polylinker and ~120 bp of DNA at the 5′ end of the genomic insert. This new clone, pDM#210 contains only two HindIII restriction sites located adjacent to each other in the first intron of the *unc-112* gene. A 1.1-kb HindIII DNA fragment containing the complete coding sequence of the Green Fluorescent Protein was excised from the plasmid pPD107.48 (kindly provided by A. Fire, S. Xu, J. Ahnn, and G. Seydoux, Carnegie Institute, Baltimore, MD) and cloned into the HindIII site in pDM#210. The plasmid carrying the GFP exons inserted into the *unc-112* gene was transformed into *E. coli* XL1 blue cells to generate the pDM#211 strain. This construct allows the four GFP exons to be incorporated into the reading frame of the *unc-112* mRNA, and results in the production of an UNC-112 protein with GFP inserted between aa 28 and 29.

Microinjection of C. elegans

Microinjections of N2 hermaphrodites were performed as described by Mello and Fire (1995). The pRF4 plasmid DNA was prepared using a standard alkaline lysis protocol and then purified over a CsCl gradient. The pDM#208, pDM#209, and pDM#211 plasmid DNAs were prepared using either a modified alkaline lysis/PEG precipitation procedure or a

QIAprep Spin miniprep kit (catalog no. 27106). The pDM#208, pDM#209 and pDM#211 plasmid DNAs (~1 μg/ml) were coinjected with the pRF4 *rol-6(su1006dm)* plasmid (~86 μg/ml) into the gonad syncytium of wild-type hermaphrodites as described by Mello and Fire (1995). F1 Rol hermaphrodites were selected and any that produced Rol progeny were maintained as transgenic strains. The following strains were obtained: DM7010: +/+; raEx10 [pRF4*{rol-6(su1006dm)}* + pDM#208*{unc-112(+)}* + pDM#209*{unc-112(+)}*]; DM7011: +/+; *raEx11*[pRF4 *{rol-6(su1006dm)}* + pDM#208*{unc-112(+)}*]; and DM7016: +/+; *raEx16* [pRF4*{rol-6(su1006dm)}* + pDM#211*{unc-112*::*GFP}*].

Sequence Analysis

All sequencing was done by the Nucleic Acid/Protein Service (NAPS) unit at the University of British Columbia. The YK12c6 cDNA was obtained as a lambda ZapII clone from the *C. elegans* cDNA project (kindly provided by Y. Kohara; data are available from GenBank/EMBL/DDBJ under accession numbers D34763 and D27524). The bluescript plasmid containing the cDNA insert was excised following the protocol provided by Stratagene and transformed into *E. coli* XL1 blue cells. This strain was named pDM#205. Plasmid DNA was prepared for sequencing using a modified alkaline lysis/PEG precipitation procedure. The entire cDNA insert was sequenced on one strand to confirm the intron/exon boundaries predicted by the Genefinder program. The nucleotide and protein sequence have been submitted to GenBank/EMBL/DDBJ under accession number AF217185.

Three overlapping DNA fragments were amplified from *st562/st562* and *st581/st581* arrested embryos and *r367/r367* adult hermaphrodites using the polymerase chain reaction as described by Barstead et al. (1991) and Rogalski et al. (1995). The 3 primer sets used cover a 4.65-kb region of the *unc-112* gene from ~160 bp upstream of the start codon to the middle of exon 6. An additional DNA fragment including exon 6 and part of the 3′ untranslated region was amplified from *r367/r367* animals. One DNA fragment was amplified from the *gk1/gk1* deletion mutant using a forward primer in the second intron and a reverse primer in exon 6. All PCR products were prepared for sequencing using a QIAquick PCR Purification Kit (catalog no. 28104). DNA fragments containing the *r367*, *st562*, and *st581* mutations were independently amplified and sequenced three times.

UNC-112 homologous proteins were identified using the BLAST search algorithm (Altschul et al., 1990). Sequence alignments were constructed using the Clustal W program in the Mac Vector software package (Oxford Molecular Group).

Construction of Strains Carrying the unc-112::GFP Transgenic Array

The *raEx16[unc-112::GFP; rol-6(su1006)]* array was crossed into strains carrying null mutations in the *deb-1*, *unc-52*, or *pat-3* genes. In the case of *unc-52*, males of genotype *unc-52(ra401)/+* were mated to *+/+; raEx16[unc-112::GFP; rol-6(su1006)]* hermaphrodites. Rol progeny with the genotype *unc-52(ra401)/+; raEx16[unc-112::GFP; rol-6(su1006)]* were identified by progeny testing, and a strain, DM5118, was established. Embryos homozygous for *unc-52(ra401)* and carrying the *unc-112*::GFP array were obtained as segregants from these hermaphrodites. The transgenic strains DM5119: *pat-3(st564)/+; raEx16[unc-112::GFP; rol-6(su1006)]*, and DM5120: *unc-44(e362)deb-1(st555)/+ +; raEx16[unc-112::GFP; rol-6(su1006)]* were obtained in a similar manner.

Immunofluorescence Staining

For most experiments (Figs. 5, B–G, 6, and 7), embryos and adult hermaphrodites were stained using a freeze-fracture procedure adapted from Albertson (1984). The worms were fixed in −20°C acetone for 4 min, and then rehydrated through a graded (75, 50, and 25%) acetone series. Incubation times were overnight at 20°C with the primary antibodies, and 2.5–3.0 h at room temperature with the secondary antibodies. To observe GFP fluorescence in the absence of antibody staining, hermaphrodites (see Fig. 5 A) were fixed in 1% formaldehyde for 10 min, washed with TBS and then resuspended in mounting media (2.5% wt/vol; DABCO; 90% glycerol in TBS).

For immunofluorescence staining, the mouse monoclonal antibodies MH2, MH24, MH25 (Francis and Waterston, 1985, 1991), and DM5.6 (Miller et al., 1983) were diluted 1:100, 1:100, 1:50, and 1:50, respectively. The secondary antibody, TRSC-labeled donkey anti–mouse IgG $F(ab')_2$ (Jackson ImmunoResearch Laboratories) was diluted 1:200.

Worms from the following strains were used in these experiments. DM5115 for Figs. 5 A and 6, B, D, F, and H; DM7016 for Fig. 5, B–G; N2 for Fig. 6, A, C, E, and G; DM5118 for Fig. 7, A, B, E, and F; DM5120 for Fig. 7, C and D; and DM5119 for Fig. 7, G and H.

Microscopy

Confocal images were collected using the MRC 600 system (Bio-Rad Laboratories) attached to a Nikon Optiphot-2 compound microscope. Optical sections were collected at 0.2-μm intervals and combined using the maximum projection function. For publication, confocal images were transferred to a Macintosh computer, and arranged and annotated using Adobe Photoshop 4.0. Final images were printed on a Codonics NP-1600 printer.

Results

The unc-112 Gene Encodes a Novel 720–Amino Acid Protein

Embryos homozygous for null mutations in the *unc-112* gene exhibit a Pat terminal phenotype. They arrest at the twofold stage of embryogenesis and have severely disorganized body wall muscle (Williams and Waterston, 1994). In contrast, animals homozygous for the *r367* allele of *unc-112* are viable, although they do have disorganized body wall muscle and are paralyzed as adults (Bejsovec et al., 1984). The *unc-112(r367)* phenotype is affected by temperature, being somewhat less severe in animals raised at 15°C, compared with animals raised at 20°C.

The *unc-112* gene maps to the left of *unc-76* on LG V in the interval between the left breakpoints of the deficiencies, *yDf9* and *yDf11*. We correlated the physical and genetic maps for this region and localized the *unc-112* gene to the interval between the cosmids R02D5 and C48G7 (Fig. 1). Our results reveal that the left breakpoint of *yDf9* lies between cosmids R02D5 and R11H6, and the left breakpoint of *yDf11* is located between C47E8 and C48G7. Genetic crosses using the *unc-112(r367)* allele and transgenic strains carrying cosmids from this region of the genome (Birnby et al., 2000) revealed that *unc-112* resides on the cosmid T10E3/C47E8.

The C47E8 cosmid has been sequenced by the *C. elegans* genome consortium (1998; data are available from GenBank/EMBL/DDBJ under accession number Z75530) and eight open reading frames (ORFs) have been identified by the Genefinder program. Birnby et al. (2000) has shown that the *daf-21* gene corresponds to the C47E8.5 ORF, and that the mutant phenotype of *daf-21(p673)* is not rescued by cosmid R08A2 which overlaps with the right end of C47E8. We crossed *unc-112(r367)* into a transgenic strain carrying R08A2 and found that homozygous mutant hermaphrodites were rescued by the R08A2 cosmid array. This result allowed us to position the *unc-112* gene to the right of *daf-21*, and narrowed down the candidate ORFs to either C47E8.6, C47E8.7 or C47E8.8. A 7.5-kb DNA fragment containing the complete C47E8.7 ORF and 2.5 kb of upstream sequence (Fig. 2) was subcloned into pBluescript and coinjected into wild-type hermaphrodites with the roller plasmid pRF4. The transgenic array obtained rescues the paralyzed phenotype of *unc-112(r367)* homozygous animals, allowing us to tentatively identify the C47E8.7 ORF as *unc-112*. To confirm that the C47E8.7 ORF is in fact the *unc-112* gene, we identified the

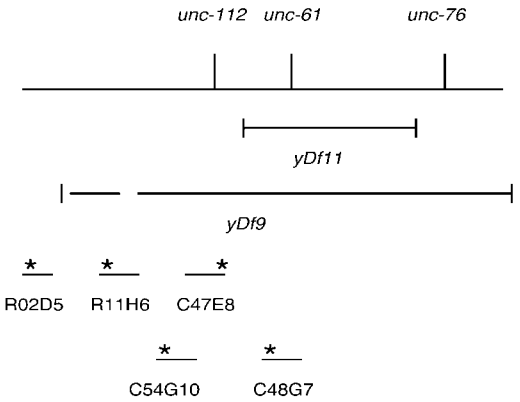

Figure 1. Diagram showing the correlation of the genetic and physical maps in the *unc-112* region of LG V. The top region of the figure is a partial genetic map showing the relative positions of the three genes and two deficiencies used in this study. The location of sequenced cosmids in this region of the genome relative to the two deficiency breakpoints is shown below. Asterisks indicate the approximate regions of the various cosmids that were tested for amplification by PCR. The *unc-112* gene is located on cosmid C47E8.

sequence alterations corresponding to three putative null alleles (Fig. 2; see below).

The C47E8.7 ORF was identified using the Genefinder program (Eeckman and Durbin, 1995), which predicted the intron/exon structure shown in Fig. 2. Several cDNA clones corresponding to this ORF were isolated and partially sequenced as part of the *C. elegans* cDNA project (Kohara, Y., personal communication). We have completely sequenced one of these clones, YK12c6, a 2.769-kb

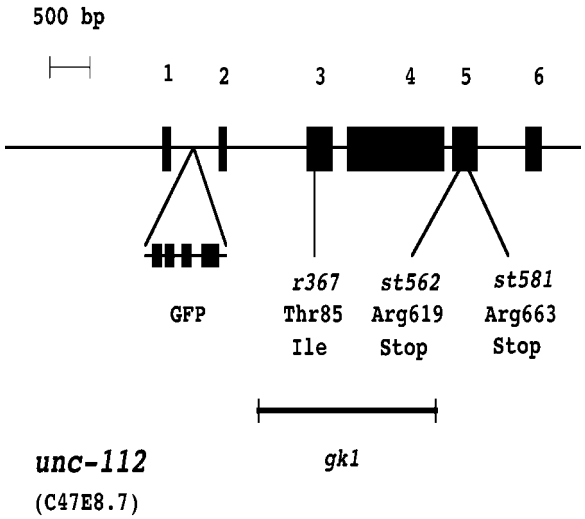

Figure 2. Diagram of a 7.5-kb genomic DNA fragment containing the C47E8.7/*unc-112* ORF. The six exons are represented as boxes. The intron/exon boundaries shown here have been confirmed by sequencing the full-length YK12c6 cDNA clone (data are available from GenBank/EMBL/DDBJ under accession number 217185). The sequence alterations corresponding to four *unc-112* mutations are also indicated. Four exons encoding the GFP protein were introduced into the first intron of the *unc-112* ORF to obtain the pDM#211 construct.

cDNA which contains the complete ORF that was predicted by Genefinder plus 288 bp of 5′ untranslated sequence and 303 bp of 3′ untranslated sequence (data are available from GenBank/EMBL/DDBJ under accession number AF217185). A conventional polyadenylation signal is located 13 bp upstream from the poly A tail. The predicted 720–amino acid UNC-112 protein does not appear to have a signal sequence nor a transmembrane domain.

A search of the database identified three proteins that exhibit significant similarity to UNC-112 (Fig. 3); a human protein encoded by a gene called *mig*-2 (for mitogen inducible gene; Wick et al., 1994), and the CG7729 and CG14991 gene products from *Drosophila melanogaster* (Adams et al., 2000). The nematode, fly, and human proteins are ~60% similar (~41% identical) over their entire length, and share a short, ~200–amino acid region of homology with talin, band 4.1, and ezrin (Fig. 4). This conserved sequence is found in members of the FERM protein superfamily, and may be important for attachment to the plasma membrane (Chishti et al., 1998). Fig. 4 A shows the alignment of the conserved amino acid regions of UNC-112 and talin, and Fig. 4 B shows the alignment of this same region in UNC-112, band 4.1, and ezrin. The UNC-112 sequence is more similar to talin (~53% homology) than to band 4.1, and ezrin (~33% homology). Other, short regions of the UNC-112 protein sequence are similar to the golgin-97 protein (amino acids [aa] 159–199), human PACE 4 proteases (aa 185–238), and mammalian oxysterol-binding protein (aa 422–464 and 487–528).

The sequence alterations corresponding to the putative null alleles, *unc-112(st562*), *unc-112(st581)* and *unc-112(gk1),* have been identified (Fig. 2). The EMS-induced *st562* and *st581* mutations (Williams and Waterston, 1994) are single nucleotide alterations that introduce stop codons into the *unc-112* coding sequence. For these two alleles, we began sequencing ~160 bp upstream of the start codon, and continued downstream until a nucleotide alteration was identified. Both mutations are C to T transitions that change arginine codons (cga) to stop codons (tga), and both are located in exon 5. The *st562* mutation alters the Arg619 codon and the *st581* mutation alters the Arg663 codon. The formaldehyde-induced *gk1* allele of *unc-112* (see Materials and Methods) is a 2.18-kb deletion. Sequence analysis identified the deletion breakpoints which are located in the second intron and close to the 3′ end of exon 4. We have also identified the sequence alteration corresponding to the hypomorphic *r367* mutation, which is a C to T transition changing the Thr85 codon (aca) to an Ile codon (ata). To identify this allele, we sequenced the entire coding region of the *unc-112* gene from *r367* mutant hermaphrodites. This was the only nucleotide change found after comparing the sequence of the mutant locus to that of the wild-type gene.

The UNC-112 Protein Colocalizes with PAT-3/β-Integrin in Body Wall Muscle

Several groups have shown that the green fluorescent protein (GFP) can be fused to some nematode proteins without affecting their function in living animals, thus allowing the expression pattern of these proteins to be detected

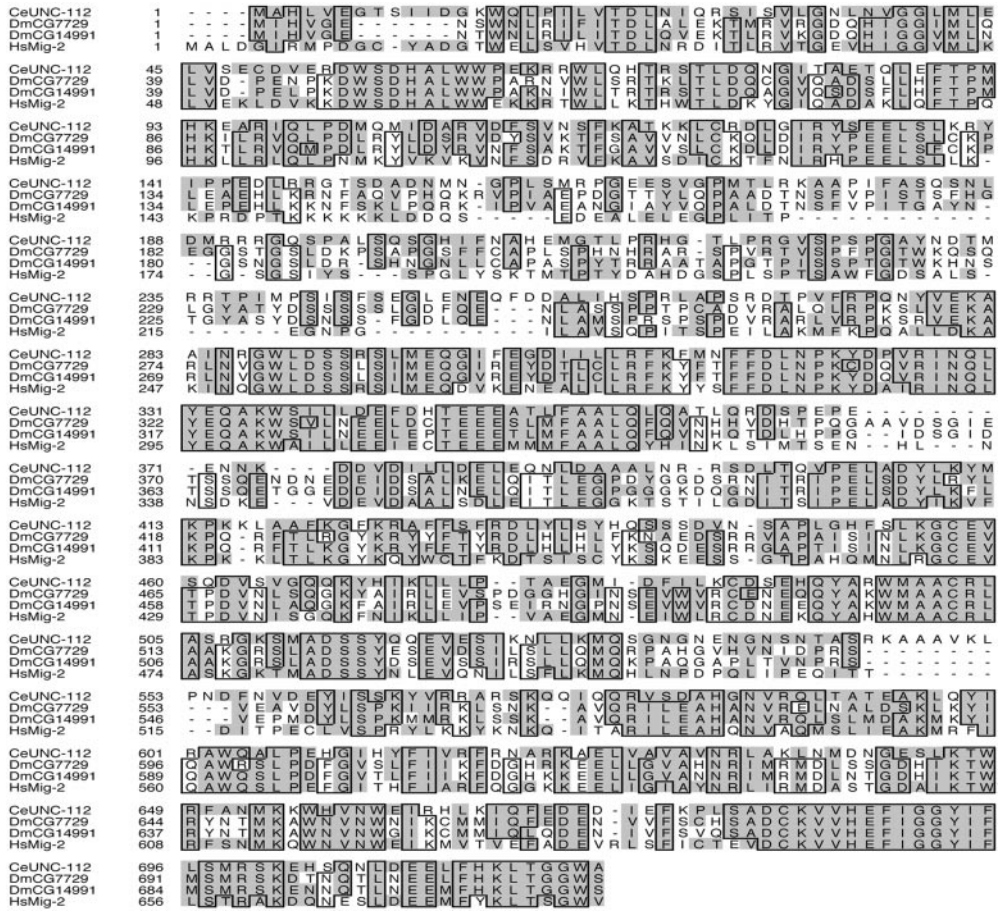

Figure 3. Comparison of the predicted amino acid sequences of UNC-112, the human Mig-2 protein and the CG7729 and CG14991 proteins in *D. melanogaster*. The nematode, fly, and human proteins are ~60% similar (~41% identical) over their entire length, and share a short region of homology with talin and other members of the FERM superfamily (aa 288–488 of UNC-112). The amino acid sequences were aligned using the Clustal W program. Identical amino acids are shaded, and similar amino acids are boxed. The functional UNC-112::GFP protein has the GFP inserted between Arg28 and Ser29 of the UNC-112 amino acid sequence.

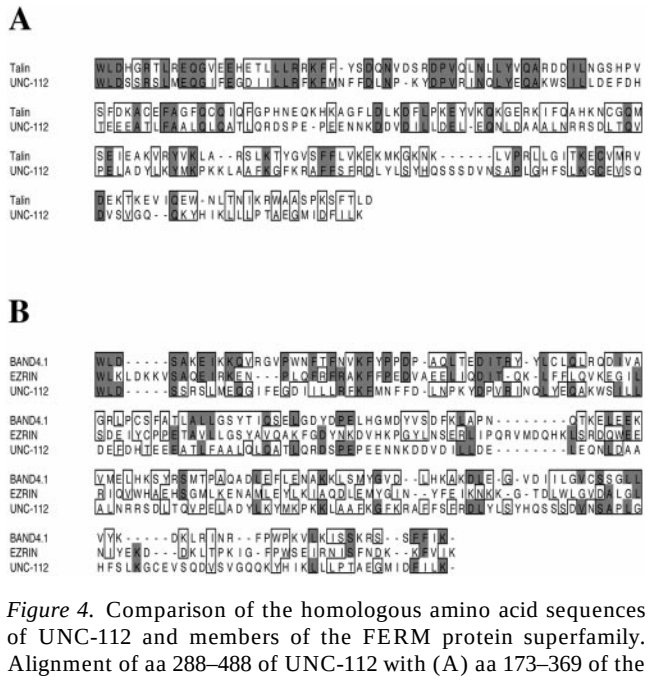

Figure 4. Comparison of the homologous amino acid sequences of UNC-112 and members of the FERM protein superfamily. Alignment of aa 288–488 of UNC-112 with (A) aa 173–369 of the human talin sequence, and (B) aa 107–293 of the mouse band 4.1 sequence and aa 58–258 of the chicken ezrin sequence. The UNC-112 sequence is more similar to talin (~53% homology) than to band 4.1 and ezrin (~33% homology). Note the very high homology between the first 52 amino acids of the alignment of UNC-112 and talin (50% identity; 65% similarity). The sequences were aligned using the Clustal W program. Identical amino acids are shaded, and similar amino acids are boxed.

simply by observing GFP fluorescence (Chalfie et al., 1994; see for example Hobart et al., 1999). Using this approach, we have determined that the UNC-112 protein is a component of dense bodies and M-lines, the structures which attach the myofilament lattice to the muscle cell membrane. The UNC-112::GFP fusion protein is able to rescue the embryonic lethal phenotype of *unc-112(st581)* homozygous animals when expressed from a transgenic array. Rescued hermaphrodites with the genotype *unc-112(st581); raEx16*[*unc-112*::GFP; *rol-6(su1006)*] move well, and their body wall muscle structure appears wild-type when observed using polarized light (data not shown) or GFP fluorescence (Fig. 5 A). These results indicate that the UNC-112::GFP fusion protein retains normal or near normal function, and that its localization should reflect a substantial portion of the range of expression of the endogenous UNC-112 protein.

GFP fluorescence in wild-type and homozygous *st581* adult hermaphrodites carrying the *raEx16*[*unc-112*::GFP; *rol-6(su1006)*] array is found in the body wall, vulval, spermathecal, uterine, and anal sphincter/depressor muscles. In the body wall muscle, UNC-112::GFP is localized to muscle cell boundaries in regions of contact with adjacent muscle cells, and to the dense bodies and M-lines (Fig. 5 A) in a pattern identical to that observed for UNC-52/perlecan (Francis and Waterston, 1991; Rogalski et al., 1993; Mullen et al., 1999), PAT-3/β-integrin (Francis and Waterston, 1985; Gettner et al., 1995), and PAT-2/α-integrin (Williams, B., unpublished observations). GFP fluorescence appears to localize near the membrane and does not

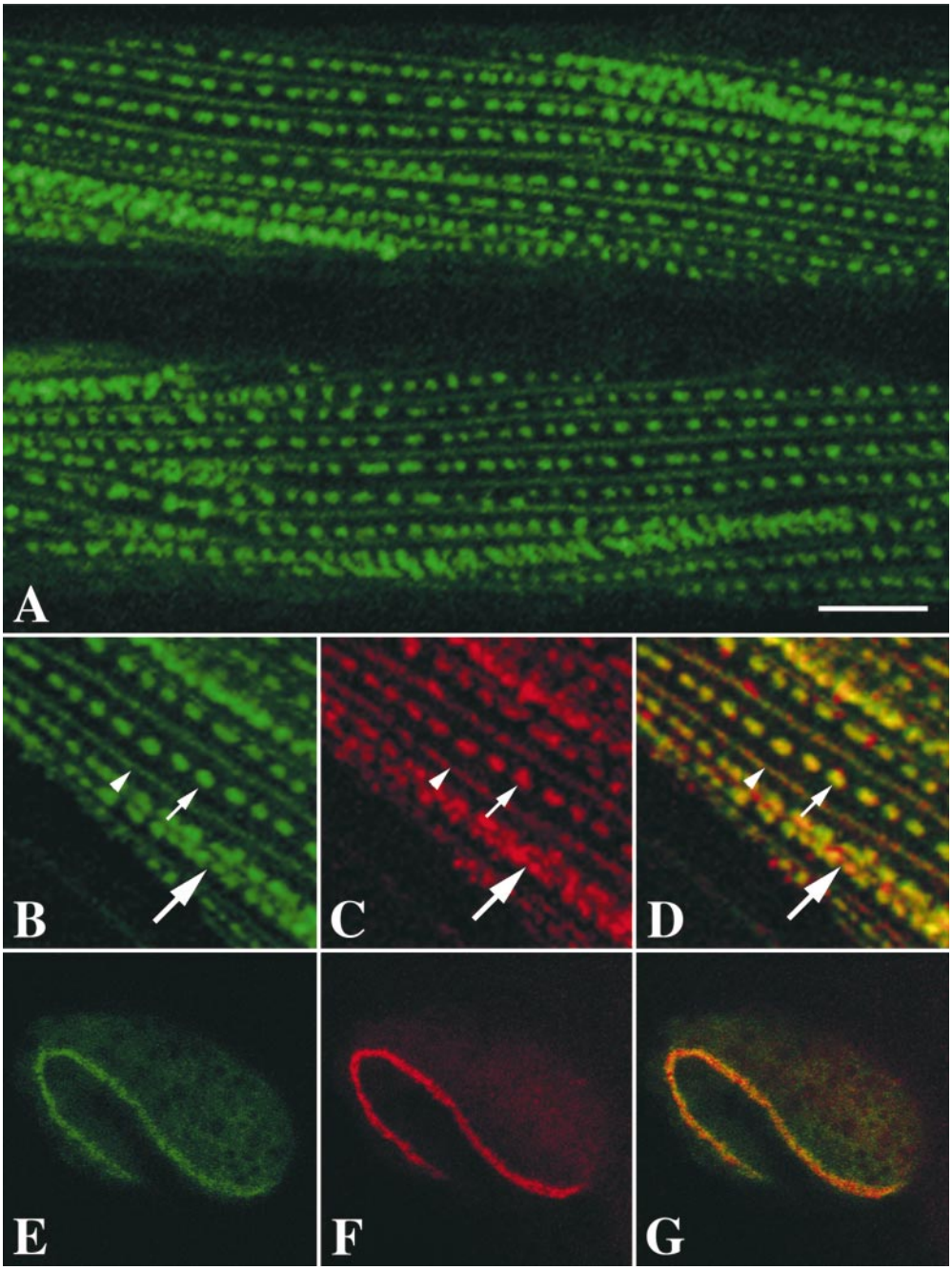

Figure 5. Localization of UNC-112::GFP fluorescence and MH25 (PAT-3/β-integrin) immunofluorescence in adults and embryos. (A) UNC-112::GFP fluorescence in adult body wall muscle of a rescued *unc-112(st581); raEx16[unc-112*::GFP; *rol-6(su1006)]* hermaphrodite. (B-D) colocalization of UNC-112::GFP (green) with PAT-3/integrin (red) in the same body wall muscle cell of a +/+; *raEx16[unc-112*::GFP; *rol-6(su1006)]* hermaphrodite; (B) UNC-112::GFP fluorescence, (C) MH25 immunofluorescence, (D) both GFP and MH25. Arrowheads indicate the M-line; small arrows indicate dense bodies; and large arrows indicate adhesion plaques between muscle cells. (E-G) colocalization of UNC-112::GFP (green) with PAT-3/integrin (red) in the body wall muscle of a 1.5-fold +/+; *raEx16[unc-112*::GFP; *rol-6(su1006)]* embryo. (E) UNC-112::GFP fluorescence, (F) MH25 immunofluorescence, (G) both GFP and MH25. All images are projected to show a single muscle quadrant. Bar, 10 μm.

extend very deeply into the muscle cell. A few unidentified cells also exhibit GFP expression, suggesting that UNC-112 may not be limited to contractile tissues.

The UNC-112::GFP protein colocalizes with PAT-3/β-integrin in adult and embryonic body wall muscle. Fig. 5, B–G show the results obtained when +/+; raEx16[unc-112::GFP; *rol-6(su1006)*] adults and embryos were stained with MH25, a mAb that recognizes PAT-3/β-integrin (Francis and Waterston, 1985; Gettner et al., 1995). In adult body wall muscle, both UNC-112::GFP and PAT-3/β-integrin localize to dense bodies, M-lines, and muscle cell boundaries in regions of contact with adjacent muscle cells (Fig. 5, B–D). The regions of contact between adjacent muscle cells are the adhesion plaques described by Francis and Waterston (1985). The body wall muscle cells in adults and embryos are arranged in four longitudinal stripes or quadrants, each consisting of a double row of unfused muscle cells. In embryos, the UNC-112::GFP protein first localizes to regions of cell–cell contact between adjacent muscle cells, and then spreads over the muscle cell basal surface as the embryo elongates. In a 1.5-fold embryo, UNC-112::GFP appears as a single thin line in each quadrant (Fig. 5 E), corresponding to the line of contact formed between the two rows of muscle cells. PAT-3/β-integrin has a similar distribution pattern during embryogenesis, as do perlecan and vinculin (Hresko et al., 1994). Fig. 5, E–G, show the colocalization of GFP fluorescence and MH25 immunofluorescence in a 1.5-fold embryo.

The UNC-112 Protein Is Required for the Spatial Organization of Integrin within the Muscle Cell Membrane

Williams and Waterston (1994) examined the organization of myosin heavy chain A (mhcA) and actin in the body wall muscle of *unc-112(st562)* mutant embryos using monoclonal antibodies. They found that both mAbs showed a disorganized staining pattern in the mutants when compared with the pattern observed in wild-type embryos. We extend these earlier observations by examining the distribution of UNC-52/perlecan, PAT-3/β-integrin and DEB-1/vinculin in wild-type and *unc-112* mutant embryos (Fig. 6). Our data reveal that the earliest steps in PAT-3/β-integrin localization occur normally, but that later steps are disrupted, leaving PAT-3/β-integrin in a severely abnormal distribution within the cell. In contrast, the distribution of UNC-52/perlecan in the basement membrane appears largely unaffected by the absence of UNC-112, and DEB-1/vinculin still localizes, presumably with integrin, at the basal membrane.

Fig. 6 shows wild-type and *unc-112(st581)* embryos stained with mAbs that recognize PAT-3/β-integrin, DEB-1/vinculin (Francis and Waterston, 1985; Barstead and Waterston, 1989), and the M and L isoforms of UNC-52/perlecan (Francis and Waterston, 1991; Rogalski et al., 1993; Mullen et al., 1999). Although elongation arrests at the twofold stage in *unc-112(st581)* embryos, development continues as indicated by cuticle formation, the development of a well formed pharynx, and the ability to hatch (Williams and Waterston, 1994). Thus, the mutant embryos are comparable in age to threefold, wild-type embryos.

In the threefold, wild-type embryo in Fig. 6, A and A′, and also the mutant Pat embryo in Fig. 6, B and B′, the UNC-52/perlecan mAb, MH2, stains basement membranes associated with the body wall muscles. Although misshapen, the arrested *unc-112(st581)* embryo shows regions where UNC-52/perlecan is properly localized. It should be noted that the structural organization of UNC-52/perlecan may not be entirely normal in the *unc-112* mutant embryo, as suggested by the absence of the fine, regular granularity that is observed in the wild-type embryo (compare Fig. 6, A′ with B′). This feature may be a secondary effect of the failure of the *unc-112* embryos to elongate properly.

The MH25 (PAT-3/β-integrin) staining patterns in wild-type and *unc-112(st581)* mutant embryos at ~350 min after the first cell division are shown in Fig. 6, C, D, C′, and D′. At this stage of development, PAT-3/β-integrin is localized at regions of muscle/muscle cell contact in both embryos. In wild-type embryos just before this stage,

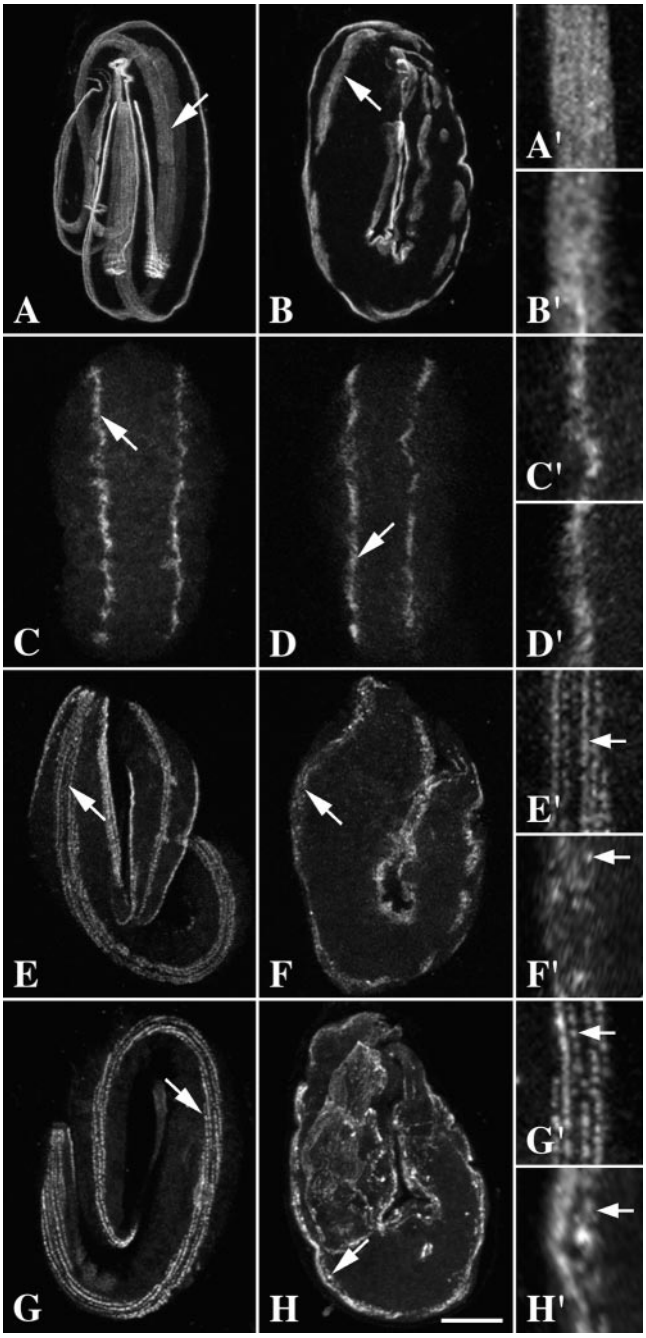

Figure 6. Perlecan, integrin, and vinculin localization in wild-type and *unc-112(st581)* embryos. Wild-type (N2) embryos (A, C, E, and G) and *unc-112(st581)* embryos (B, D, F, and H) stained with the MH2 mAb which recognizes UNC-52/perlecan, the MH25 mAb which recognizes PAT-3/β-integrin, and the MH24 mAB which recognizes DEB-1/vinculin. A and B show staining with MH2; C, D, E, and F show staining with MH25; and G and H show staining with MH24. A′–H′ are magnifications of A–H, respectively. The wild-type embryos in A, E, and G are at the threefold stage of embryonic development. The mutant embryos in B, F, and H have arrested at the twofold stage of embryogenesis, but are comparable in age to the threefold wild-type embryos. The two embryos in C and D were stained at an earlier stage (~350 min after the first cell division), before the mutant embryo has arrested development. The *unc-112(st581)* mutant embryo can be identified at this early stage by a gap in the ventral quadrant which is detectable by ~300 min after the first cell division. All of the mutant embryos were obtained as segregants from *unc-112(st581); raEx16*[*unc-112*::GFP; *rol-6(su1006)*] hermaphrodites. All of the panels show lateral views of the embryos except C and D which show dorsal views. Note the disorganized staining in the mutant embryos in F and H. In all cases, images have been projected from a full Z-series to show two muscle quadrants. The arrows in A–H indicate the regions of each embryo that have been magnified in A′–H′. The arrows in E′–H′ indicate adhesion structures. Bar, 10 μm.

PAT-3/β-integrin is diffusely expressed in the muscle cells, and only assumes this polarized localization once the muscle cells have migrated from the lateral hypodermis to the dorsal or ventral hypodermis (Hresko et al., 1994). The nearly identical PAT-3/β-integrin staining pattern of the ~350-min wild-type and *unc-112* mutant embryos lead us to conclude that the initial expression and polarization of PAT-3/β-integrin occurs normally in the absence of functional UNC-112 protein. MH25 immunofluorescence in a threefold, wild-type embryo and an arrested *unc-112 (st581*) Pat embryo are shown in Fig. 6, E, F, E′, and F′. In the wild-type embryo in Fig. 6, E and E′, PAT-3/β-integrin is organized into distinct adhesion structures that are distributed in a recognizable pattern along the length of each muscle quadrant. Some integrin foci are present in the basal membrane in the *st581* mutant embryo shown in Fig. 6, F and F′ (arrows), but they are highly disorganized compared with the orderly arrays of adhesion structures observed in the wild-type embryo. Hresko et al. (1994) have shown that the organization of PAT-3/β-integrin at the base of the M-line and dense bodies is not affected in embryos homozygous for either *deb-1(st555)* or *myo-3(st386)*, both of which arrest elongation at the same stage as *unc-112(st581)* embryos. The MH25 staining pattern seen in arrested *deb-1(st555)* embryos is not as well organized as in threefold, wild-type embryos, but distinct lines and rows of dots that run obliquely to the long axis of the worm are detected and appear to be spaced as in wild-type (Hresko et al., 1994). Thus, the disorganization of PAT-3/β-integrin observed in the *unc-112* mutant embryo is not the result of developmental arrest, but must be due to the absence of UNC-112. This result leads us to conclude that the UNC-112 protein is required for the proper spatial organization of PAT-3/β-integrin clusters in the muscle cell membrane.

The MH24 (DEB-1/vinculin) staining patterns observed in the wild-type embryo in Fig. 6, G and G′, and the mutant embryo in Fig. 6, H and H′, are very similar to those seen with the MH25 mAb. In wild-type, DEB-1/vinculin colocalizes with PAT-3/β-integrin at the base of the dense bodies (Francis and Waterston, 1985). Although MH24 immunofluorescence is highly disorganized in the arrested *unc-112(st581)* mutant embryo when compared with the wild-type, threefold embryo, it is still associated with the membrane. The similarity in the distribution patterns of DEB-1/vinculin and PAT-3/β-integrin in the mutant embryos leads us to conclude that DEB-1/vinculin does not require the UNC-112 protein to localize with PAT-3/β-integrin in the basal muscle cell membrane.

Localization of the UNC-112 Protein Is Disrupted in unc-52 and pat-3 Mutant Embryos

We examined the distribution of the UNC-112::GFP protein in embryos homozygous for null mutations in the *unc-52*, *pat-3*, or *deb-1* genes to determine the affect of the missing gene products on UNC-112 localization. Hresko et al. (1994) had previously shown that the absence of DEB-1/vinculin does not dramatically affect the organization of PAT-3/β-integrin at the base of the dense body, whereas the absence of UNC-52/perlecan results in the complete loss of PAT-3/β-integrin adhesion complexes. Our data (Fig. 7) demonstrate that the distribution of UNC-112, like

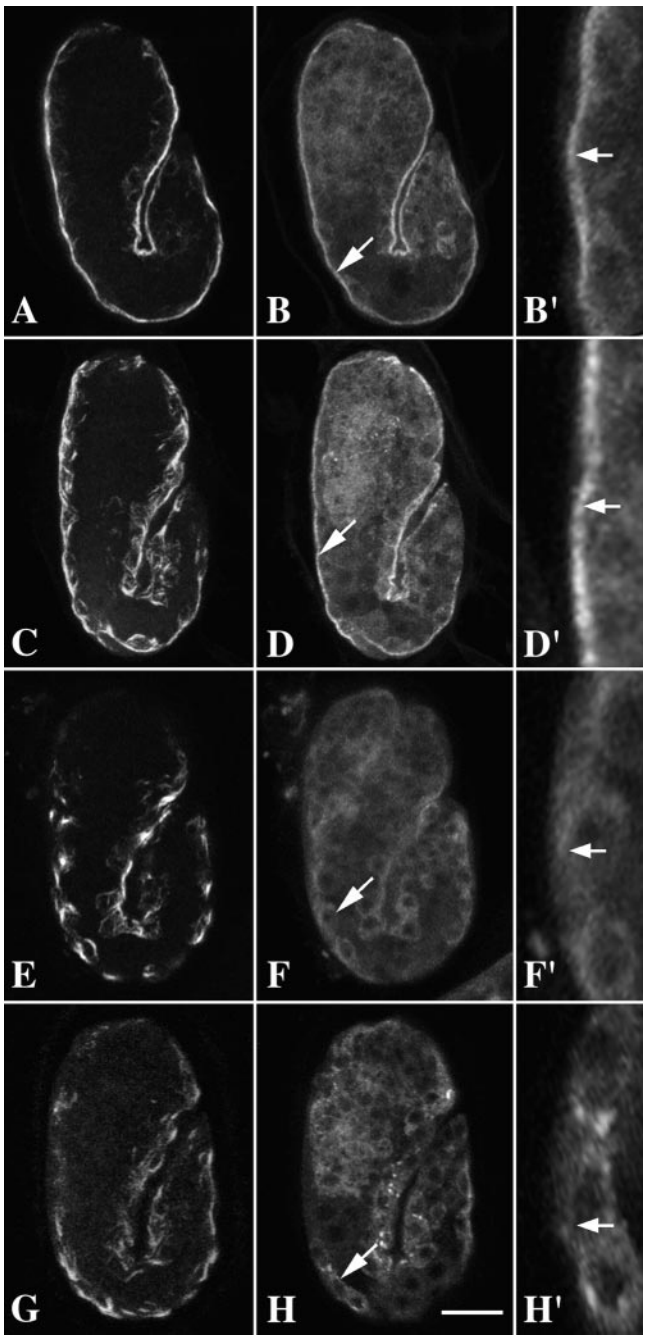

Figure 7. UNC-112 localization in mutant embryos. DM5.6 immunofluorescence and UNC-112::GFP fluorescence in wild-type (A and B), *unc-44(e362)deb-1(st555)* IV (C and D), *unc-52(ra401)* II (E and F) and *pat-3(st564)* III (G and H) embryos. A, C, E, and G show staining with the DM5.6 mAb. B, D, F, and H show GFP fluorescence. B′, D′, F′, and H′ are magnifications of B, D, F, and H, respectively. All of the embryos are at the ~1.5-fold stage of embryonic development. The mutant embryos were obtained as segregants from heterozygous parents carrying the *raEx16[unc-112*::GFP; *rol-6(su1006)]* transgenic array. The genotype of the wild embryo in A and B is either *unc-52(ra401)/* +; raEx16 or +/+; raEx16. All panels show lateral views of the embryos. The images have been projected from a full Z-series to show one dorsal and one ventral muscle quadrant. The arrows in B, B′, D, D′ F, F′ G, and G′ indicate UNC-112::GFP fluorescence. Note the absence of any organized myosin staining in C, E and G, or GFP fluorescence in F, F′, H, and H′. Bar, 10 μm.

that of PAT-3/β-integrin, is not adversely affected in the *deb-1(st555)* mutant, but is severely affected in the *unc-52(ra401)* mutant. In addition, our data demonstrate that PAT-3/β-integrin is required for the proper spatial distribution of UNC-112::GFP in the basal membrane.

We initially attempted to observe GFP fluorescence in arrested *unc-52(ra401)*, *pat-3(st564)*, and *unc-44(e362)deb-1(st555)* Pat embryos. However, the continuous accumulation of UNC-112::GFP in these mutants made interpretation of the images obtained difficult. To overcome this problem, we stained embryos with DM5.6, a mAb that recognizes the minor body wall myosin, mhcA (Miller et al., 1983). This allowed us to identify the mutant embryos before developmental arrest by looking for disorganized myosin staining (compare Fig. 7 A with C, E, and G; see also Williams and Waterston, 1994). All of the embryos shown are at the 1.5-fold stage of development. In a +/+; *raEx16[unc-112*::GFP; *rol-6(su1006*)] embryo at this stage, mhcA is organized into recognizable myofilaments (Fig. 7 A) and UNC-112::GFP is distributed over the basal face of the body wall muscle cells, appearing in this figure as a line near the margin of the embryo due to the orientation of the dorsal muscle quadrant in the plane of focus (Fig. 7, B and B′). The pattern of GFP fluorescence observed in the *unc-44(e362)deb-1(st555); raEx16[unc-112*::GFP; *rol-6 (su1006)]* embryo (Fig. 7, D and D′) is identical to that seen in the wild-type embryo. Thus, it appears that DEB-1/vinculin is not required for the polarization of UNC-112 to the basal membrane. However, in both the *unc-52 (ra401*); *raEx16[unc-112*::GFP; *rol-6(su1006)]* and *pat-3 (st564); raEx16[unc-112*::GFP; *rol-6(su1006)]* mutant embryos (Fig. 7, F, F′, H, and H′), very little, if any UNC-112:: GFP protein appears to be associated with the basal membrane. Instead of the continuous pattern of fluorescence that is observed in the wild-type and *deb-1(st555)* embryos, GFP fluorescence in these mutants appears as small disorganized dots. The presence of GFP fluorescence in the cytoplasm of most muscle cells in these mutant embryos confirms that they carry the *unc-112*::GFP transgenic array. These results lead us to conclude that the presence of UNC-52/perlecan and PAT-3/β-integrin are necessary for the proper spatial localization of UNC-112 in the muscle cell membrane.

Discussion

Our analysis of the *unc-112* gene in *C. elegans* has identified a new component of cell–matrix adhesion structures, the ~80-kD UNC-112 protein. UNC-112 is a nematode homologue of the human Mig-2 protein, which was identified by sequencing a cDNA clone obtained after induction of WI-38 fibroblast cells with fetal calf serum (Wick et al., 1994). A recent search of the database identified two additional human cDNA clones that exhibit significant similarity to the COOH-terminal 300 aa of the UNC-112 protein sequence. Thus, it likely will be necessary to wait for the completion of the human genome sequence to determine whether UNC-112 and Mig-2 are orthologs. The *D. melanogaster* genome contains two homologues of UNC-112. These proteins were identified by sequencing the fly genome and have not been correlated with any known genes. Neither the function nor localization of the human or fly proteins is known. A short, ~200–amino acid region of UNC-112 shows homology to a membrane attachment sequence found in talin and other members of the FERM superfamily of proteins (Chishti et al., 1998). This homology, together with the absence of a signal peptide or transmembrane domain led us to suspect that the *unc-112* gene product may be intracellular and associated with the plasma membrane. The fact that the UNC-112::GFP protein is associated with dense bodies and M-lines at the muscle cell membrane confirmed these speculations, and also revealed that this protein is a component of cell–matrix adhesion structures.

The UNC-112::GFP fusion protein used in this study rescues the severe Pat phenotype exhibited by *unc-112 (st581)* embryos and fully restores functional body wall muscle. This is strong evidence that the UNC-112::GFP localization described here accurately reflects the range and expression of the endogenous UNC-112 protein. In addition, the subcellular localization of UNC-112::GFP to the dense bodies and M-lines is consistent with the mutant phenotype which suggests that UNC-112 is required for attachment of the myofilament lattice to the basal cell membrane (Williams and Waterston, 1994).

Tissue culture focal adhesions are considered to be reasonable models of mammalian in vivo adhesion complexes. Many components common to vertebrate FAs are present in dense bodies and M-lines in the body wall muscle of *C. elegans*. These include α-/β-integrin, talin, and UNC-97/PINCH. In addition, the dense bodies which anchor actin filaments also contain vinculin and α-actinin. It was originally thought that dense bodies and M-lines were structurally very different. However, as more components of these structures are identified, it appears that many of the same proteins are present in both, at least where they are anchored to the muscle cell membrane. Another protein known to interact with integrin is integrin-linked kinase or ILK (Hannigan et al., 1996). The nematode ortholog of ILK is encoded by the *pat-4* gene, and this protein has been localized to dense bodies and M-lines in the body wall muscle (Williams, B., unpublished observations). Nematode orthologs of other vertebrate proteins found in focal adhesions have been identified recently by searching the completed genomic sequence of *C. elegans*. These include tenascin, paxillin and zyxin. The analysis of these proteins in the nematode should help to elucidate their function in cell–matrix adhesion sites.

The severe Pat phenotype exhibited by embryos homozygous for the s*t562*, *st581*, and *gk1* alleles suggests that these mutations completely eliminate *unc-112* gene function. The sequence alterations that we identified are consistent with this hypothesis. The formaldehyde-induced *gk1* mutation which deletes almost one half of the *unc-112* gene is certainly a null allele. The other two mutations, which introduce stop codons into the open reading frame of this gene, are also likely to be null mutations. The sequence alteration responsible for the hypomorphic phenotype of *r367* homozygous hermaphrodites is a missense mutation in the NH_2-terminal region of the UNC-112 protein. The Thr85-Ile missense mutation results in a milder, temperature-sensitive phenotype, suggesting the presence of a protein product with reduced or altered function.

Hresko et al. (1994) has shown that the basement membrane proteoglycan UNC-52/perlecan is necessary for the proper localization of PAT-3/β-integrin to the muscle cell membrane, and that PAT-3/β-integrin, in turn, is required for DEB-1/vinculin to localize to the base of the dense bodies. It appears from the mutant analysis described here that the UNC-112 protein plays a role downstream from UNC-52/perlecan, and that PAT-3/β-integrin and UNC-112 are mutually required for proper localization in the muscle cell membrane. Our results show that UNC-112 is not required for the initial polarization of integrin in the muscle cell, nor for its clustering into nascent attachments, events which are blocked in *unc-52* null mutants. Instead, UNC-112 is needed for the subsequent localization of the nascent attachments into an ordered array within the muscle cell membrane. Whether UNC-112 interacts directly with PAT-3/β-integrin or through an additional protein or proteins is not known. Our results also show that UNC-112 is not required for DEB-1/vinculin to assemble at nascent dense bodies, and conversely, that DEB-1/vinculin is not required for UNC-112 to localize properly in the muscle cell membrane.

In the absence of the UNC-112 protein, actin and myosin filaments do not attach to the muscle cell membrane (Williams and Waterston, 1994). Perhaps UNC-112 is needed for the assembly of additional, membrane-distal components of the dense body and M-line, and these in turn mediate the attachment of the thick and thin filaments. In this model, failure of the nascent attachments to become properly arranged in a striated array within the basal membrane would be a direct result of these lost connections and the corresponding loss of tension that the myofilament lattice would normally exert on the nascent attachment sites. Conversely, the disorganized nature of the integrin adhesion complexes may be a direct effect of the absence of UNC-112, perhaps by blocking integrin's association with other elements that normally position the nascent dense bodies and M-lines in the muscle cell basal membrane. In this model, the failure of thin and thick filaments to associate with the integrin adhesion complexes might be caused by the failure of these structures to localize properly in the membrane.

At present, UNC-52/perlecan and UNC-112 are the only proteins identified in *C. elegans* that affect integrin organization. Although there are mammalian orthologs for both polypeptides, neither of these proteins were previously known to be involved in this process in vertebrate systems. Perlecan is found in all mammalian basement membranes, so it is not unreasonable to assume that it will be involved in the assembly of adhesion complexes in at least some vertebrate tissues in vivo. A recent study of a mouse perlecan knockout mutant describes defects in heart muscle and other tissues that are compatible with this hypothesis (Costell et al., 1999). The distribution and function of the mammalian homologue of UNC-112 has yet to be determined. Our data point to a probable role for the Mig-2 protein, or possibly another homologous protein, in integrin localization at adhesion complexes.

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