Development 129, 4999-5008 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
DEV8945

MEC-8 regulates alternative splicing of *unc-52* transcripts in *C. elegans* hypodermal cells

Caroline A. Spike, Andrew G. Davies, Jocelyn E. Shaw and Robert K. Herman*

Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN 55455, USA
*Author for correspondence (e-mail: bob-h@umn.edu)

Accepted 2 August 2002

SUMMARY

Previous work has shown that *C. elegans* MEC-8 is a putative RNA-binding protein that promotes specific alternative splices of *unc-52* transcripts. *unc-52* encodes homologs of mammalian perlecan that are located extracellularly between muscle and hypodermis and are essential for muscle development in both embryos and larvae. We show that MEC-8 is a nuclear protein found in hypodermis at most stages of development and not in most late embryonic or larval body-wall muscle. We have also found that overexpression of MEC-8 in hypodermis but not muscle can suppress certain *unc-52* mutant phenotypes. These are unexpected results because it has been proposed that UNC-52 is produced exclusively by muscle. We have constructed various tissue-specific *unc-52* minigenes fused to a gene for green fluorescent protein that have allowed us to monitor tissue-specific *mec-8*-dependent alternative splicing; we show that *mec-8* must be expressed in the same cell type as the *unc-52* minigene in order to regulate its expression, supporting the view that MEC-8 acts directly on *unc-52* transcripts and that UNC-52 must be synthesized primarily by the hypodermis. Indeed, our analysis of *unc-52* genetic mosaics has shown that the focus of *unc-52* action is not in body-wall muscle but most likely is in hypodermis.

Key words: *mec-8*, *unc-52*, Perlecan, Alternative splicing, RRM

INTRODUCTION

Much remains to be learned about the regulation of alternative splicing of pre-mRNA and the important role alternative splicing plays in eukaryotic development (Lopez, 1998). The *mec-8* gene encodes a protein, MEC-8, that regulates certain alternative splices in *Caenorhabditis elegans*. MEC-8 has two RNA-recognition motifs (RRMs) spaced by a region that is rich in alanine and glutamine (Lundquist et al., 1996), and loss-of function *mec-8* mutations reduce the levels of two alternatively spliced mRNAs produced by the *unc-52* gene (Lundquist et al., 1996), suggesting that MEC-8 regulates the alternative splicing of *unc-52* mRNA directly. MEC-8 may also regulate the processing of other gene transcripts: loss-of-function mutations in *mec-8* lead to a partially penetrant cold-sensitive embryonic lethality and to chemosensory and mechanosensory defects in larvae and adults (Lundquist and Herman, 1994) that are likely to be independent of *unc-52* function (Lundquist et al., 1996; Mullen et al., 1999).

UNC-52 plays an essential role in muscle development: *unc-52* null mutations cause severe defects in myofilament lattice assembly in body-wall muscle and lead to arrest and paralysis at the twofold stage of embryonic morphogenesis (Hresko et al., 1994; Williams and Waterston, 1994). UNC-52 is concentrated under the muscle dense bodies and M lines in the basement membrane between muscle and hypodermis (Francis and Waterston, 1991; Hresko et al., 1994; Mullen et al., 1999). UNC-52 begins to accumulate in the basement membrane during early embryonic morphogenesis (Hresko et al., 1994), when it is faintly detected within muscle cells (Mullen et al., 1999), suggesting that it is produced by muscle. Basement membranes around the pharynx, gonad and the anal depressor and sex muscles also contain UNC-52 at various stages (Francis and Waterston, 1991; Mullen et al., 1999). UNC-52 proteins are homologs of mammalian perlecan (Rogalski et al., 1993), an extracellular matrix protein found in all basement membranes and synthesized by many vertebrate cell types (Noonan and Hassell, 1993). Mice and humans that lack perlecan have abnormal cartilage development and defects in certain basement membranes (Arikawa-Hirasawa et al., 1999; Arikawa-Hirasawa et al., 2001; Costell et al., 1999; Nicole et al., 2000).

unc-52 generates several different mRNA and protein isoforms. Transcripts with alternative 3′ ends generate short (S), medium (M) and long (L) UNC-52 isoforms; mutational analysis has shown that only M isoforms are required for proper embryonic and larval development (Mullen et al., 1999). Antibodies that detect M and L isoforms stain the body-wall muscle basement membrane in embryos (Mullen et al., 1999). Exons 16, 17 and 18 of *unc-52* are alternatively spliced to generate M and L isoforms with varying numbers of copies of a motif found in neural cell adhesion molecules (11-14 copies of the motif for M isoforms), which appear to be largely functionally redundant (Mullen et al., 1999). Weak alleles of *unc-52* that cause progressive muscle disruption and late larval and adult paralysis cluster in this alternatively spliced region

(Rogalski et al., 1995). Defects in these *unc-52(viable)* animals appear to be caused by reduced levels of UNC-52 in larvae (Mullen et al., 1999). Some *unc-52(viable)* mutations generate nonsense codons in exon 17 (*e669* and *e1012*) or exon 18 (*e444* and *e998*). Animals homozygous for any of these alleles seem to be normal during embryogenesis and early larval development.

Loss-of function mutations in *mec-8* enhance *unc-52(viable)* alleles: *mec-8; unc-52(viable)* embryos resemble *unc-52(null)* embryos (Lundquist and Herman, 1994) and have severely reduced levels of UNC-52 (Lundquist et al., 1996; Mullen et al., 1999). *mec-8* is required to generate *unc-52* transcripts that have either exon 15 spliced directly to exon 19 or exon 16 spliced directly to exon 19 (Lundquist et al., 1996). These mRNAs skip *unc-52(viable)* mutations and provide enough UNC-52 for normal embryonic and early larval development. Other *unc-52* mRNA isoforms that lack either exon 17 or exon 18 have been identified (Rogalski et al., 1995), but anti-UNC-52 staining of *mec-8; unc-52(viable)* embryos suggests that these *mec-8*-independent mRNA isoforms are spatially restricted or are present at low levels during embryogenesis (Mullen et al., 1999).

We show that MEC-8 is a nuclear protein and is expressed primarily in hypodermal cells when *mec-8*-dependent UNC-52 isoforms begin to accumulate. We have found that transgenic expression of MEC-8 in hypodermis (but not in muscle) can suppress both embryonic and postembryonic phenotypes caused by *unc-52* mutations. We have constructed tissue-specific *unc-52* minigenes whose patterns of expression are *mec-8*-dependent when *mec-8* is expressed in the same tissue. Finally, we have used mosaic analysis to show that UNC-52 is not a cell-autonomous product of muscle, as suggested previously (Moerman et al., 1996), but is probably produced by the hypodermis. We propose that MEC-8 regulates the alternative splicing of *unc-52* pre-mRNA directly and that the regulation occurs primarily in the hypodermis.

MATERIALS AND METHODS

Strains and culture

Nematodes were cultured as described by Brenner (Brenner, 1974). Alleles other than *unc-36(e251) III* and *him-5(e1490) V* (Hodgkin, 1997) are specified in the text.

Molecular biology and germline transformation

Standard molecular biology techniques were used (Sambrook et al., 1989). PCRs were performed as recommended using either Vent (Promega) or Pfu (Stratagene) thermostable DNA polymerase. Plasmids pPD52.99, pPD93.97 and pPD95.75 (www.ciwemb.edu) were generated by A. Fire, S. Xu, J. Ahn and G. Seydoux. Constructs were injected at 20 ng/µl along with 100 ng/µl plasmid pRF4 containing *rol-6(su1006*sd*)* (Mello and Fire, 1995), 50 ng/µl R1p16 containing *unc-36(+)* (obtained from L. Lobel) or 100 ng/µl pTG96 containing *sur-5::gfp* (Yochem et al., 1998). Chromosomal integration of arrays was induced by γ-irradiation (Mello and Fire, 1995).

mec-8::gfp

A 6 kb *Apa*I-*Pvu*I fragment from a previously-described 8.5-kb *Xho*I *mec-8* genomic subclone (Lundquist et al., 1996) was cloned into the *Sma*I site of pPD95.75. The MEC-8::GFP fusion protein made by this construct is predicted to contain all but the last 18 amino acids of MEC-8; its expression rescued the dye-filling defect of *mec-8* animals (Lundquist and Herman, 1994) but failed to rescue other *mec-8* phenotypes.

Antibodies

A 0.93-kb *Eag*I-*Eco*RI *mec-8* cDNA fragment (Lundquist et al., 1996) was cloned into the *Sma*I site of pGEX-2T (Amrad). GST::MEC-8 fusion protein was purified by SDS-polyacrylamide gel electrophoresis. Rabbits were immunized four times in 10 months with 400 µg GST::MEC-8 and 1 ml Ribi Adjuvant System (Sigma). Serum collected after the third immunization was affinity purified (Bar-Peled and Raikhel, 1996). Antibody staining was performed as described (Bowerman et al., 1993; Finney and Ruvkun, 1990). Antibody dilutions were: 1:500-2000 anti-MEC-8 serum; 1:100 affinity-purified anti-MEC-8; 1:500 anti-LIN-26 (Labouesse et al., 1996) and anti-β-galactosidase (β-gal; Promega); 1:1000 DM5.6 (Miller et al., 1983) and MH2 (Francis and Waterston, 1991); and 1:500 goat anti-rabbit or anti-mouse 2° antibodies conjugated to FITC, rhodamine (Cappel) or Cy3 (Jackson ImmunoResearch).

mec-8 tissue-specific expression constructs

Phlh-1::mec-8(+) was created by inserting *mec-8* cDNA sequence into pPD52.99 using the restriction enzymes *Nhe*I and *Nco*I. *mec-8* cDNA sequence was PCR amplified using primers GAGCTAG-CGAAGTTTGAGCCATAACGATTG and CTCCATGGTCAAGAC-AATAGAAGTTCC. *Pdpy-7::mec-8(+)* was created by replacing the *Hin*dIII-*Nhe*I fragment containing *Phlh-1* with a *Hin*dIII-*Xba*I fragment containing *Pdpy-7* (Gilleard et al., 1997). *Pdpy-7* was PCR-amplified from cosmid C38F3 (provided by A. Coulson) using primers CAAAGCTTCTCCGGTAGCGGCGG and CTTCTAGATT-TATCTGGAACAAAATGTAAG.

Suppression of *unc-52* and rescue of *mec-8; unc-52* synthetic lethality

Animals of general genotype *unc-52; unc-36; mnEx[mec-8(+) unc-36(+)]* were generated by crossing *unc-36; mnEx* males with *unc-52; unc-36* hermaphrodites, picking array-bearing (non-Unc-36) cross progeny, and picking many of their progeny to establish *unc-52; unc-36; mnEx* lines, in which all Unc-36 animals were also Unc-52. *unc-52(su250e669*ts*)* was not suppressed in seven lines generated by injection of *Phlh-1::mec-8(+)* and R1p16 into *unc-52(su250e669); unc-36* hermaphrodites.

Animals of general genotype *mec-8; unc-52; unc-36; mnEx[mec-8(+) unc-36(+)]* were generated by crossing *unc-36; him-5; mnEx* males to *unc-52; unc-36* hermaphrodites; non-Unc-36 male progeny were then crossed to *mec-8; unc-36* hermaphrodites, and non-Unc-36 hermaphrodite progeny were picked and allowed to self-fertilize. Finally, many non-Unc-36 progeny were picked from broods that contained Unc-52 segregants and were progeny tested.

unc-52::gfp minigene constructs

unc-52 exons 17-19 were PCR amplified from wild-type or *unc-52(e444)* genomic DNA using primers GCGAGCTCAACACA-GACAATCCCTGAAGG and GAGAGCTCTTTGGCTCAAGCGG-TGTAAC and cloned into the *Sac*I site of pPD93.97. *unc-52* exons 15-17 were PCR-amplified from wild-type or *unc-52(e669)* genomic DNA using primers GCTCTAGATGCATCCAAACATCCAACT-CCAG and GCTCTAGAAAGGCAAACCAGGTGTGAC, and cloned into vectors containing exons 17-19 using *Xba*I and *Sal*I. The *Hin*dIII-*Xba*I fragment containing *Pmyo-3* was replaced with a *Hin*dIII-*Xba*I fragment containing *Pdpy-7* for expression in hypodermal cells. Constructs were co-injected with either R1p16 or pRF4.

Males carrying integrated minigenes were crossed to *mec-8(u74)* or *mec-8(u74); unc-36* hermaphrodites. Array-bearing Mec F_2 progeny were picked. Plates with all roller or all non-Unc-36 progeny were retained; the embryos of subsequent generations were examined for GFP. At least two independent integrated lines were tested for each construct. *mec-8; unc-36; mnIs25[Pmyo-3::unc-52::gfp rol-*

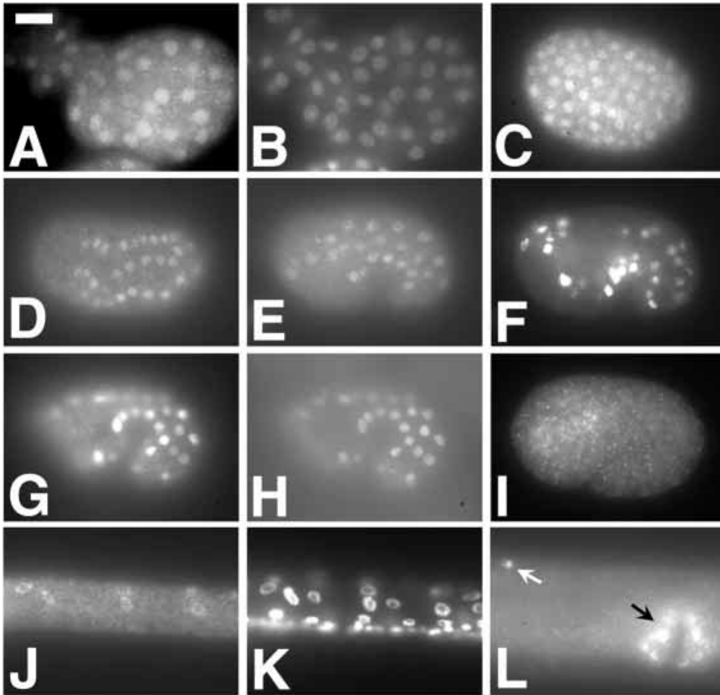

Fig. 1. MEC-8 expression pattern. (A,C) Early embryos with anti-MEC-8 staining in all nuclei. (B) DAPI staining of the embryo shown in A. (D,E) Older embryos with anti-MEC-8 staining in hypodermal nuclei. (F) A lower focal plane of the same embryo as in E. This embryo was transgenic for pPD37.48, an *hlh-1::lacZ* fusion construct (Krause et al., 1994); muscle cells stained with anti-β-galactosidase and not with anti-MEC-8. (G) MEC-8::GFP in a 1.5-fold embryo. (H) Anti-LIN-26 hypodermal staining of the embryo shown in G, showing overlapping staining with anti-MEC-8. (I) A *mec-8(u391)* embryo lacking anti-MEC-8 nuclear stain. (J) The mid-body region of an L1 larva in which hyp7 nuclei were stained with anti-MEC-8. (K) DAPI stain of the larva shown in J. (L) The mid-body region of an adult stained with anti-MEC-8. The white arrow indicates ALML (touch neuron), and the black arrow indicates the vulva. Scale bar: 10 μm.

6(su1006)] strains carrying extrachromosomal arrays with tissue-specific *mec-8(+)* expression constructs were generated by crossing *unc-36; him-5; mnEx113[Phlh-1::mec-8(+) unc-36(+)]* or *unc-36; mnEx136[Pdpy-7::mec-8(+) unc-36(+)]* males to *mec-8; unc-36; mnIs25* hermaphrodites. Non-Unc-36 roller F_2 progeny were picked and progeny tested.

unc-52 mosaic analysis

Extrachromosomal arrays *mnEx126* and *mnEx133*, each carrying *unc-52(+) unc-36(+) sur-5::gfp*, were generated by injecting overlapping cosmids ZC101 and C3836 (5 ng/μl each) along with R1p16 and pTG96 into *unc-36; him-5* hermaphrodites. Non-Unc-36 males were used to transfer the arrays into different genetic backgrounds. Potential mosaics were scored for cell-autonomous expression of GFP as described by Yochem et al. (Yochem et al., 1998). For example, C(–) mosaics lacked GFP in hyp11 and the DVC neuron, which descend from the two immediate daughters of C, respectively, and lacked GFP in C-derived body wall muscles.

RESULTS

MEC-8 is present in embryonic hypodermal nuclei

The distribution of MEC-8 protein was analyzed by two methods: immunolocalization using polyclonal anti-MEC-8 serum produced in rabbits and expression of green fluorescent protein (GFP) from a *mec-8::gfp* fusion transgene that partially rescued the *mec-8* mutant phenotype. Anti-MEC-8 serum recognized a nuclear antigen in wild-type *C. elegans* embryos (Fig. 1A-C). The youngest embryos to exhibit immunostaining contained about 50 cells, all of which showed nuclear staining. All nuclei showed staining in embryos containing up to hundreds of nuclei. Two *mec-8* mutants, *mec-8(u391)* (Fig. 1I) and *mec-8(u314)*, failed to show any trace of nuclear staining at any stage of development, from which we conclude that our anti-MEC-8 serum is specific for MEC-8. The *mec-8(u391)* mutation is associated with a complex rearrangement (Lundquist et al., 1996), and *mec-8(u314)* is a nonsense mutation in the first RNA-recognition motif (RRM) (Davies et al., 1999). During the late proliferative phase of embryogenesis, prior to the onset of morphogenesis, MEC-8 staining was confined largely to hypodermal nuclei (Fig. 1D,E). Prior to this shift, MEC-8 was found in most nuclei, including nuclei that were also marked with an *hlh-1::lacZ* reporter, which is expressed in early blastomeres that subsequently produce only body wall muscle cells (Krause et al., 1990); but MEC-8 was not detectable in body muscles after the onset of morphogenesis (Fig. 1E,F).

The pattern of GFP expression by transgenic embryos carrying *mec-8::gfp* was very similar to the pattern of MEC-8 expression seen by immunolocalization. GFP was seen in most nuclei at about the 50-cell stage. Just prior to morphogenesis, GFP became brighter in hypodermal nuclei and faded in the nuclei of other cells. During embryonic elongation, hypodermal nuclei exhibited bright GFP fluorescence while other nuclei fluoresced faintly or not at all (Fig. 1G,H). The nuclei of hypodermal cells and their precursor cells were marked by staining with anti-LIN-26 (Labouesse et al., 1996). The only difference between the GFP expression and the anti-MEC-8 staining was that the faint expression seen in non-hypodermal nuclei carrying *mec-8::gfp* was not detected with anti-MEC-8 serum. This difference could have been caused by overexpression or perdurance of the MEC-8::GFP fusion protein or by poor antibody sensitivity to low levels of MEC-8.

MEC-8 is expressed in many different tissues in larvae

In L1-L4 larvae, MEC-8 was detected by anti-MEC-8 serum in the nuclei of the large hypodermal syncytium, hyp7, that covers most of the worm (Fig. 1J,K). This staining was fainter than the staining of the embryonic hypodermal nuclei, became even fainter during later larval development and was undetectable in adults. The nuclei of head hypodermal cells not fused with hyp7 (hyp4 and hyp5 nuclei in particular) stained well with anti-MEC-8 in all larval stages and in adults. Anti-MEC-8 also stained the nuclei of many neurons in the head (probably including chemosensory neurons); a few neurons in the central body region [including the ALM (Fig. 1L) and AVM touch neurons, and neurons in the post-deirid]; vulval nuclei in L4 and adult stage hermaphrodites (Fig. 1L); anterior- and posterior-most intestinal nuclei; and other unidentified nuclei

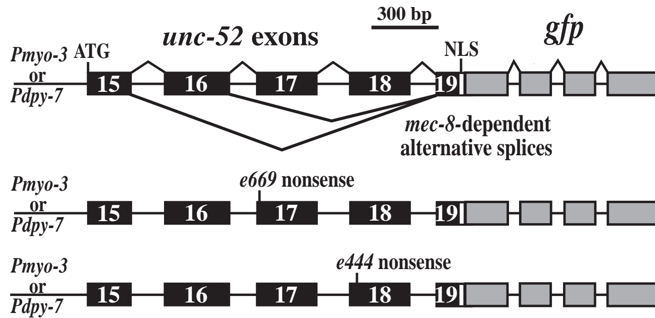

Fig. 2. Exon-intron structures of six *unc-52* minigenes. Exons are represented by boxes, introns by horizontal lines and splices by angled lines that join exons together. *unc-52* exons are filled with black; the position of a nuclear localization signal (NLS) is marked by a white line. The positions of nonsense codons found in *unc-52(e669)* and *unc-52(e444)* are marked. Known *mec-8*-dependent splices of transcripts from the complete *unc-52* gene (Lundquist et al., 1996) are shown below the *unc-52(+)* minigene; an expected *mec-8*-independent splice form (Rogalski et al., 1995) that includes all of the minigene exons is shown above the *unc-52(+)* minigene.

in the head and tail. The anterior-most muscle nuclei in the heads of larvae had low but detectable levels of MEC-8, but none of the muscle cells in the main body appeared to stain with anti-MEC-8.

This pattern of MEC-8 expression was largely confirmed using the *mec-8::gfp* reporter construct. For example, GFP was detected in larval hyp7 nuclei at levels reduced from those seen in embryonic hypodermis and was not detected in larval body muscle cells. There were some differences between the antibody and GFP results: first, we were unable to detect GFP reliably in the nuclei of ALM and AVM; and second, the nuclei of ventral hypodermal cells had detectable levels of GFP in young (L1-L2 stage) larvae but did not appear to stain with anti-MEC-8 antibodies.

mec-8 can regulate expression of *unc-52* minigenes expressed in embryonic muscle or hypodermis

We constructed three pairs of *unc-52* minigenes to monitor cell-specific *mec-8*-dependent alternative splicing in living embryos. All six minigenes contain a region of the *unc-52* gene extending from within exon 15 into the beginning of exon 19 (Fig. 2) and are fused at their 3′ ends to a nuclear localization signal and a gene for green fluorescent protein (*gfp*). For the first pair of minigenes, the *unc-52* sequence is wild type. The second pair contain the nonsense mutation *e669* in exon 17, and the third pair contain the nonsense mutation *e444* in exon 18. Each member of a minigene pair is driven either by the *myo-3* promoter, which drives expression in body wall muscle, or by the *dpy-7* promoter, which drives expression in hypodermis, from just prior to embryonic elongation until the end of the fourth larval stage (Gilleard et al., 1997). All six constructs were integrated into chromosomes, made homozygous and analyzed in at least two independent lines. The cell-specific promoters led to the expected cell-specific expression of GFP; thus, the *Pdpy-7::unc-52(+)::gfp* construct gave strong GFP expression specifically in hypodermis, and the *Pmyo-3::unc-52(+)::gfp* construct gave strong GFP expression specifically in body muscle (Fig. 3A-D). In both cases, GFP expression was unaltered by making the animals homozygous for *mec-8(u74)*.

In *mec-8(+)* embryos containing either of the nonsense mutant *unc-52* minigenes driven by the hypodermal-specific promoter, *Pdpy-7::unc-52(e669)::gfp* or *Pdpy-7::unc-52(e444)::gfp*, we saw very high hypodermal GFP expression, comparable with that seen from the wild-type minigene constructs. By contrast, GFP expression from these constructs was virtually abolished in *mec-8* mutant embryos (Fig. 3E,F and data not shown). We presume that the *mec-8(+)*-dependent GFP expression of these constructs requires the skipping of exon 17 or exon 18 of the minigene and that such skipping requires *mec-8(+)* function, as it does for the endogenous *unc-52* gene (Lundquist et al., 1996). We performed a reverse-transcription (RT) PCR experiment using forward and reverse primers in *unc-52* exon 16 and *gfp*, respectively, to determine whether the *unc-52* exon 16-19 splice form made by the *Pdpy-7::unc-52(+)::gfp* minigene on *mnIs61* was *mec-8*-dependent.

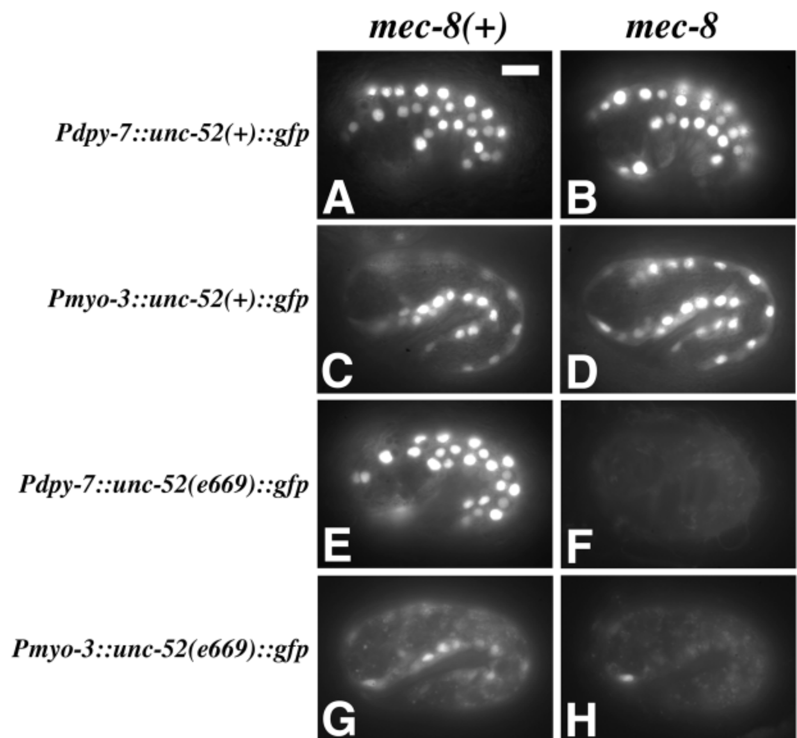

Fig. 3. Embryos expressing *unc-52::gfp* minigenes driven by hypodermis-specific or by muscle-specific promoters. (A,B) 0.5 second exposures of wild-type (A) and *mec-8* (B) embryos carrying the hypodermis-expressing *unc-52(+)::gfp* minigene on *mnIs61*. (C,D) 2 second exposures of wild-type (C) and *mec-8* embryos (D) carrying the muscle-expressing *unc-52(+)::gfp* minigene on *mnIs64*. (E) 0.75 second exposure of a wild-type embryo carrying the hypodermis-expressing *unc-52(e669)::gfp* mini-gene on integrated array *mnIs63*. (F) 6 second exposure of a *mec-8* embryo carrying *mnIs63*. (G,H) 6 second exposures of wild-type (G) and *mec-8* (H) embryos carrying the muscle-specific *unc-52(e669)::gfp* mini-gene on *mnIs27*. Scale bar: 10 μm.

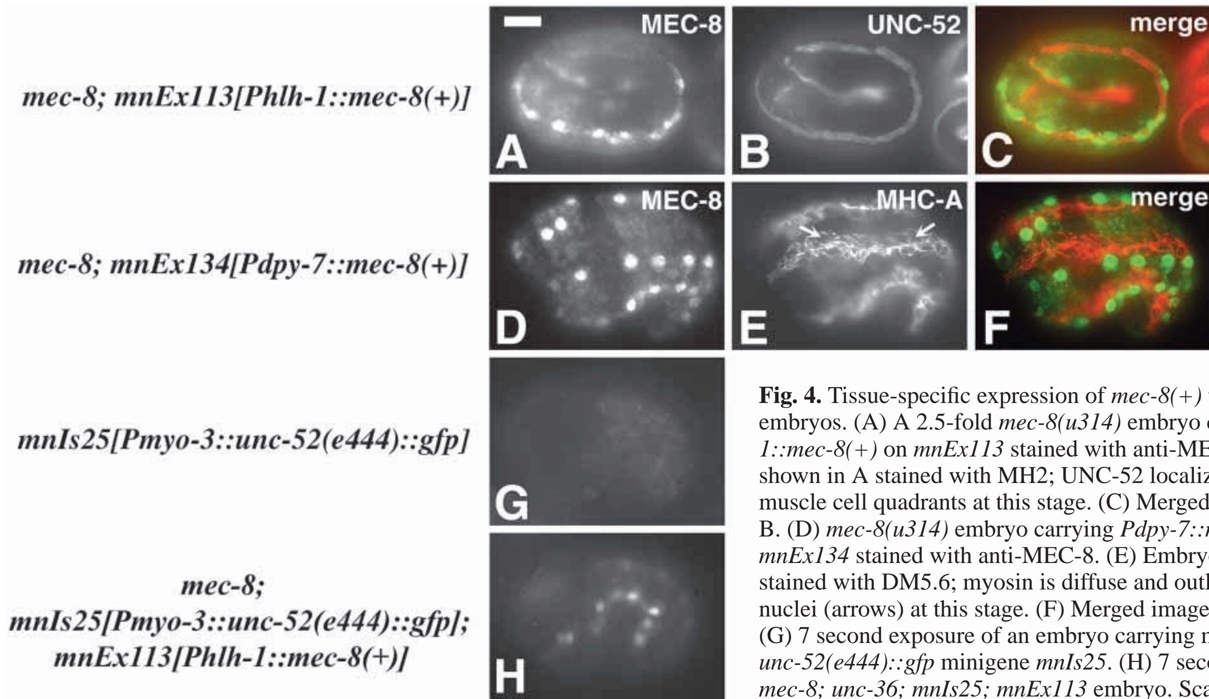

Fig. 4. Tissue-specific expression of *mec-8(+)* transgenes in embryos. (A) A 2.5-fold *mec-8(u314)* embryo carrying *Phlh-1::mec-8(+)* on *mnEx113* stained with anti-MEC-8. (B) Embryo shown in A stained with MH2; UNC-52 localizes in stripes over muscle cell quadrants at this stage. (C) Merged images of A and B. (D) *mec-8(u314)* embryo carrying *Pdpy-7::mec-8(+)* on *mnEx134* stained with anti-MEC-8. (E) Embryo shown in D stained with DM5.6; myosin is diffuse and outlines muscle cell nuclei (arrows) at this stage. (F) Merged images of D and E. (G) 7 second exposure of an embryo carrying muscle-specific *unc-52(e444)::gfp* minigene *mnIs25*. (H) 7 second exposure of a *mec-8; unc-36; mnIs25; mnEx113* embryo. Scale bar: 10 μm.

RT-PCR on a population of wild-type embryos carrying *mnIs61* amplified primarily a product that was the expected size for the 16-19 splice form (data not shown). The same RT-PCR experiment on a population of *mec-8(u74)* embryos carrying *mnIs61* amplified primarily a product that was the expected size of the 16-17-18-19 isoform; only low levels of the 16-19 isoform were seen (data not shown). These results suggest that the splicing of the *Pdpy-7::unc-52::gfp* minigene transcripts accurately mimicked *mec-8*-dependent splicing of *unc*-52 transcripts.

Larvae carrying either of the hypodermally driven mutant *unc-52* minigenes expressed hypodermal GFP, but the levels of expression were lower than that seen from the wild-type *unc-52* minigene. The larval expression was reduced further in a *mec-8* background.

Both of the nonsense-bearing minigenes driven by the muscle-specific promoter, *Pmyo-3::unc-52(e669)::gfp* and *Pmyo-3::unc-52(e444)::gfp*, showed rather weak embryonic expression (Fig. 3G and data not shown). This expression was *mec-8* dependent (Fig. 3H) until late embryogenesis, but not in subsequent stages of development, as if a factor other than MEC-8 were able to promote exon skipping in muscle at the later stages.

We detected additional differences among the minigene constructs in their expression patterns. For example, *mec-8* embryos carrying the *Pmyo-3::unc-52(e669)::gfp* construct expressed GFP in one to two cells at the anterior tip of each body-wall muscle quadrant (Fig. 3H). This was not seen in *mec-8* embryos carrying the equivalent *e444* minigene (data not shown). We also observed that the *mec-8(+)* larvae carrying the *e444* or *e669* minigenes driven by *dpy-7* had higher levels of GFP in some head hypodermal cells than in hyp7, whereas larvae carrying the equivalent *unc-52(+)* minigene had comparable levels of expression in these cells. We suggest that these differences may be due to complex developmental regulation of *unc-52* alternative splicing (see Discussion).

Expression of MEC-8 in embryonic muscle cells but not in hypodermis stimulates alternative splicing of transcripts from a muscle-specific *unc-52* minigene

To test the idea that MEC-8 promotes alternative splicing of *unc-52* transcripts cell autonomously, we put extrachromosomal arrays containing tissue-specific *mec-8(+)* expression constructs into strains homozygous both for a *mec-8* mutation and an integrated array, *mnIs25*, that carries the muscle-specific minigene *Pmyo-3::unc-52(e444)::gfp* (on its own, the particular array *mnIs25* gave very low GFP expression until close to hatching even in a *mec-8(+)* background). The *hlh-1* promoter was used to produce full-length MEC-8 in muscle cell precursors and in differentiated muscle cells throughout development and into adulthood (Krause et al., 1990; Krause et al., 1994), and the *dpy-7* promoter was used to produce MEC-8 in hypodermis. Each extrachromosomal array also carried *unc-36(+)*, and the animals were otherwise homozygous mutant for *unc-36*. Antibody staining confirmed that MEC-8 was expressed appropriately by the tissue-specific *mec-8(+)* expression constructs. *mec-8(u314)* embryos carrying either *Pdpy-7::mec-8(+)* or *Phlh-1::mec-8(+)* in a transgenic array were stained with anti-MEC-8 serum and either with DM5.6, a monoclonal antibody that recognizes the body-wall muscle myosin heavy chain A (MHC-A) protein (Miller et al., 1983; Miller et al., 1986), or with MH2, a monoclonal antibody that recognizes UNC-52 isoforms found between muscle cells and the hypodermis (Francis and Waterston, 1991; Rogalski et al., 1993). MEC-8 was detected in muscle cells but not hypodermis of embryos carrying *Phlh-1::mec-8(+)* (Fig. 4A-C) and in hypodermal cells but not muscle cells of embryos carrying *Pdpy-7::mec-8(+)* (Fig. 4D-F). Staining was

Table 1. Phenotypes conferred by *mec-8(+)* transgenes

Relevant genotype*	Phenotype
unc-52(e669)	Onset of paralysis at L4 stage
unc-52(e669); mnEx113[Phlh-1::mec-8(+)]	Onset of paralysis at L4 stage
unc-52(e669); mnEx136[Pdpy-7::mec-8(+)]	No paralysis; adult rollers
unc-52(e669); mnEx137[Pdpy-7::mec-8(+)]	No paralysis; adult rollers
unc-52(e669); mnEx138[Pdpy-7::mec-8(+)]	No paralysis; adult rollers
unc-52(e669); mnEx52[mec-8(+)]	Onset of paralysis as adults
unc-52(e444)	Onset of paralysis at L4 stage
unc-52(e444); mnEx136[Pdpy-7::mec-8(+)]	No paralysis; adult rollers
mec-8; unc-52(e669)	Embryonic arrest
mec-8; unc-52(e669); mnEx113[Phlh-1::mec-8(+)]	Embryonic arrest
mec-8; unc-52(e669); mnEx136[Pdpy-7::mec-8(+)]	Viable; fertile; no paralysis; adult rollers
mec-8; unc-52(e669); mnEx137[Pdpy-7::mec-8(+)]	Viable; fertile; no paralysis; adult rollers
mec-8; unc-52(e669); mnEx138[Pdpy-7::mec-8(+)]	Viable; fertile; no paralysis; adult rollers

*All animals were also homozygous for *unc-36*, which had no effect on the indicated phenotypes. All of the arrays listed in this table also carried *unc-36(+)*.

predominantly nuclear in both tissues, although weaker cytoplasmic staining was often seen in cells with intense nuclear staining. The progeny of parents carrying these constructs as well as *mnIs25[Pmyo-3::unc-52(e444)::gfp]* were examined for GFP expression as morphogenesis-stage embryos (comma to 2.5-fold elongation). Hermaphrodites carrying the muscle-specific construct *Phlh-1::mec-8(+)* on an extrachromosomal array segregated many embryos with clear expression of GFP in muscle (compare Fig. 4H with 4G). The proportion of GFP-expressing embryos (0.41; *n*=118) was comparable with the proportion of embryos that inherited the extrachromosomal array (0.44; *n*=433), as ascertained by counting non-Unc-36 animals segregated by the same strain. However, hermaphrodites carrying the hypodermis-specific construct *Pdpy-7::mec-8(+)* on an extrachromosomal array did not segregate any GFP-expressing embryos (*n*=123); the ability of the hypodermis-specific construct to function will be demonstrated in the next section. These data indicate that MEC-8 produced by embryonic muscle but not by embryonic hypodermis can regulate alternative splicing of *unc-52* minigene transcripts produced by embryonic muscle.

Expression of MEC-8 in hypodermis but not in muscle suppresses *mec-8; unc-52(viable)* synthetic lethality

mec-8; unc-52(e669) embryos arrest morphogenesis at the twofold stage of elongation and have diminished levels of UNC-52 (Lundquist and Herman, 1994; Mullen et al., 1999). These observations indicate that MEC-8 regulates alternative splicing of *unc-52* transcripts prior to the twofold stage. To determine whether MEC-8 is required in embryonic muscle or hypodermis, we tested the ability of the tissue-specific *mec-8* expression constructs described in the previous section to rescue *mec-8; unc-52(e669)* synthetic lethality. We were unable to recover viable *mec-8; unc-52(e669)* larvae carrying the muscle-specific construct *Phlh-1::mec-8(+)*, as segregants from *mec-8; unc-52(e669)/+; mnEx113* hermaphrodite parents (Table 1, and Materials and Methods), but *mec-8; unc-52(e669)* larvae carrying the hypodermis-specific construct *Pdpy-7::mec-8(+)* were viable and fertile (Table 1). These results suggest that MEC-8 functions in the hypodermis to regulate alternative splicing of *unc-52* in embryos.

Overexpression of MEC-8 in hypodermis but not muscle suppresses *unc-52* uncoordination

mec-8 function is required to generate *unc-52* transcripts that lack exons 17 and 18 (the exon 15-19 and 16-19 splice forms) (Lundquist et al., 1996). We hypothesized that higher-than-wild-type levels of MEC-8 might increase the levels of these splice forms and thereby increase the amount of full-length UNC-52 protein in animals carrying nonsense mutations in exon 17 or exon 18 of *unc-52*. An increase in full-length UNC-52 protein should delay or suppress the late-larval onset of paralysis exhibited by these *unc-52(viable)* animals. We found that an extrachromosomal array (*mnEx52*) containing multiple copies of an 8.5 kb genomic clone that rescues all *mec-8* phenotypes (Lundquist et al., 1996) suppressed the paralysis conferred by *unc-52(e669)* (Table 1). *unc-52(e669); mnEx52* egg-laying adults were only weakly paralyzed compared with *unc-52(e669)* animals, which become paralyzed prior to the adult stage (Gilchrist and Moerman, 1992).

We tested whether or not MEC-8 overexpression in either muscle or hypodermis would suppress the late-onset paralysis conferred by *unc-52(e669)*. Extrachromosomal arrays carrying *Phlh-1::mec-8(+)* had no effect on the phenotypes of *unc-52(e669)* or *unc-52(su250e669*ts*)* animals (Table 1 and Materials and Methods). The latter allele was tested because it is more sensitive to weak suppression (Spike et al., 2001). By contrast, hypodermal expression of MEC-8 strongly suppressed the paralysis caused by *unc-52(e669)*. All three extrachromosomal arrays containing the *Pdpy-7::mec-8(+)* construct completely suppressed *unc-52(e669)* (Table 1). One of the arrays, *mnEx136*, was tested for its ability to suppress *unc-52(e444)* and was also found to be a good suppressor of this allele. Mullen et al. (Mullen et al., 1999) showed that the *unc-52(e444)* mutation leads to a great reduction after the L4 stage in the UNC-52 protein associated with body wall muscles. We have confirmed this using the UNC-52 antibody MH2, and we have shown that *mnEx136[Pdpy-7::mec-8(+) unc-36(+)]* is an excellent suppressor of this phenotype: hermaphrodites of genotype *unc-52(e444); unc-36; mnEx136[Pdpy-7::mec-8(+) unc-36(+)]* segregated adult Unc-52 Unc-36 progeny that gave very little staining of UNC-52 in the matrix between hypodermis and body wall muscle, and also segregated wild-type progeny that stained well for UNC-52 (data not shown). The MH2 antibody recognizes UNC-52 isoforms that carry an

exon 19-encoded epitope (Rogalski et al., 1993). These UNC-52 proteins can only be generated in *unc-52(e444)* animals by *unc-52* mRNA isoforms that skip exon 18. These results therefore support the idea that MEC-8 overexpression in larval hypodermis leads to an increase in UNC-52 protein isoforms generated by alternative splicing.

Many animals carrying *Pdpy-7::mec-8(+)* were left-handed rollers as adults. Some animals carrying this construct in the arrays *mnEx137* or *mnEx138* became rollers even earlier during development, at the L4 stage; animals containing the *mnEx136* array did not roll until adulthood. We suggest that this novel roller phenotype, like the suppression of *unc-52(e669)* and *unc-52(e444)* late-onset paralysis, is caused by high levels of MEC-8 in hypodermis. Our *Pdpy-7::unc-52(+)::gfp* minigene experiments reported above indicated that *Pdpy-7* promoted strong hypodermal GFP expression in both embryos and L1-L4 larvae.

unc-52(+) is not required in larval or adult muscle cells for wild-type development

The *mec-8* overexpression experiments suggest that most, if not all, *unc-52* pre-mRNAs capable of undergoing *mec-8*-dependent alternative splicing are produced by the hypodermis in both embryos and larvae. We therefore expected that the focus of *unc-52* action for muscle development in both embryos and larvae would be in hypodermis, not muscle; that is, *unc-52* should affect muscle development and function cell non-autonomously. To test this prediction, we analyzed *unc-52* genetic mosaics. Our first set of mosaics made use of the viable mutation *unc-52(e669)*, which causes the onset of muscle paralysis in L4 larvae.

The first *C. elegans* embryonic division generates the daughter cells AB and P1 (Sulston et al., 1983). All but one of the 95 body-wall muscle cells descend from P1; cells contributing to the hypodermis descend from both P1 and AB. These and other relevant details of the cell lineage are shown in Fig. 5. To determine the phenotype of animals lacking *unc-52(+)* in 94 of 95 muscle cells, we looked among the progeny of *unc-52(e669); unc-36; mnEx126[unc-52(+) unc-36(+) sur-5::gfp]* hermaphrodites for animals in which *mnEx126* was absent in all P1-derived cells. The inclusion of *sur-5::gfp* in the array provided a useful cell autonomous marker for tracking cell-by-cell inheritance of the array (Yochem et al., 1998). We found that six out of seven animals with array loss at P1 did not become paralyzed either as larvae or as adults (Fig. 5). We suspect that the one exceptional animal either had suffered an additional loss of the array or was defective for *unc-52(+)* expression in the AB lineage. We occasionally found apparently non-mosaic animals that were Unc-52. However, animals that failed to inherit the array were invariably Unc-52. Animals with losses by the cell EMS were also non-Unc-52. One of these animals had a slow-moving Unc-36-like phenotype but no muscle paralysis. We found that this animal had a second loss in cells derived from the AB blastomere (Fig. 5), consistent with the observation that *unc-36(+)* is required in the neurons that descend from ABp (Kenyon, 1986). We looked for other Unc-36 non-Unc-52 animals and found one with a loss at AB and four with losses at ABp (Fig. 5). We conclude that *unc-52(+)* is not required in muscle cells to prevent the larval paralysis caused by *unc-52(e669)* and that the most likely focus of action is in the hypodermis, as *unc-52(+)* expression by either AB or

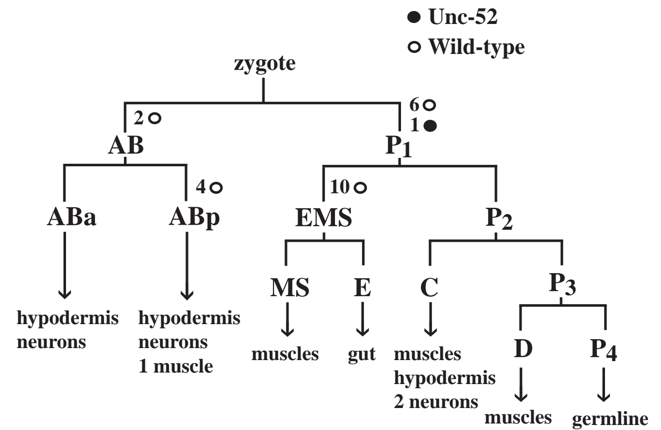

Fig. 5. Genetic mosaics in which an *unc-52(+)*-bearing extrachromosomal array was lost somatically in animals that were otherwise homozygous for *unc-52(e669)*. The early *C. elegans* cell lineage and the cell types generated by each founder cell are indicated. Points in the lineage at which array losses were identified are shown. One mosaic animal lost the array at both AB and EMS. All other mosaic animals had single array losses in this part of the lineage. White and black circles represent non-Unc-52 and Unc-52 animals, respectively; thus, one P1(–) mosaic was Unc-52 and six were wild type.

P1 descendants is sufficient to prevent the onset of the uncoordination conferred by *unc-52(e669)*.

unc-52(+) is not required in body-wall muscles for embryonic viability

To examine where *unc-52* function is required in embryos, we performed mosaic analysis using the null allele *unc-52(st549)*. Embryos homozygous for *unc-52(st549)* arrest at the twofold stage of elongation with paralyzed body wall muscles lacking a myofilament lattice (Williams and Waterston, 1994). We screened the progeny of *unc-52(st549); mnEx133[unc-52(+) sur-5::gfp]* hermaphrodites for genetic mosaics, again using the cell autonomous GFP expression conferred by *sur-5::gfp* to track array loss in the cell lineage. We found eight viable but abnormal animals with losses at P1 [referred to as P1(–) mosaics] and seven wild-type animals with losses at EMS (Fig. 6). The P1(–) mosaics were small and dumpy, tended to roll or twist while moving and had a dorsal bump opposite the vulva. Adult P1(–) mosaic animals were fertile, although their progeny were all arrested embryos, as expected, as the germline descends from P1. The *unc-52(st549); mnEx133* P1(–) mosaics, which lack *unc-52(+)* in 94 of 95 body wall muscle cells, were not paralyzed. We stained two adult P1(–) mosaics with the myosin heavy chain A antibody (Miller et al., 1983; Miller et al., 1986). Muscle cells throughout the bodies of both animals had formed myofilament lattices. We conclude that *unc-52(+)* is not required in body-wall muscles for embryo viability or myofilament lattice assembly.

unc-52(st549); mnEx133 animals that resembled mosaics with losses at P1 were found that had extrachromosomal array losses at P2 and C (Fig. 6). These mosaics suggest that the body shape defects seen in P1(–) mosaics were caused by a partial requirement for *unc-52(+)* function in C-derived hypodermis during embryogenesis (see Discussion). Additional

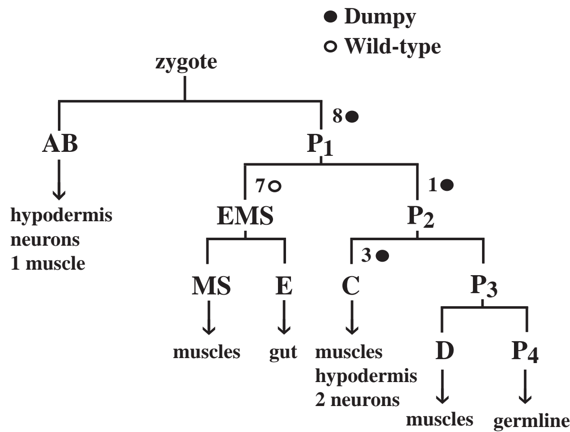

Fig. 6. Genetic mosaics in which an *unc-52(+)*-bearing extrachromosomal array was lost somatically in animals that were otherwise homozygous for *unc-52(st549)*. A wild-type mosaic is represented by an white circle. Black circles represent viable and fertile dumpy rollers. One of the C(–) mosaics also suffered array loss at EMS, and the P2(–) mosaic also suffered array loss at E. The other mosaic animals had single array losses in this part of the lineage.

abnormalities were also observed in specific *unc-52(st549); mnEx133* mosaic animals. Four P1(–) mosaics were allowed to develop into older egg-laying adults; two of these animals were bloated with arrested embryos, and the other two discharged gonadal and intestinal cells through the vulva. Differential interference contrast microscopy also suggested that mosaics with losses at P1 or C had misplaced seam cells. Seam cells in larvae are found in two lateral rows, one row per side. Just before the adult stage, neighboring seam cells fuse and form longitudinal cuticular structures called alae (Singh and Sulston, 1978). Alae were branched in the mid-body region of the adult P1(–) and C(–) mosaics, but not the EMS(–) mosaics.

DISCUSSION

We conclude that MEC-8 regulates the accumulation of *mec-8*-dependent *unc-52* mRNA isoforms in the hypodermis of embryos and larvae. The idea that UNC-52 is produced by the hypodermis is surprising, because it was previously concluded that UNC-52 is produced exclusively by muscle (Moerman et al., 1996; Mullen et al., 1999). However, Kondo et al. (Kondo et al., 1990) suggested several years ago that *unc-52* might be expressed in hypodermis and not muscle. This suggestion was based on the abilities of eight different tRNA amber suppressors to suppress amber mutations in genes with different tissue-specific patterns of expression. The amber suppressors *sup-21* and *sup-28*, for example, seemed to be effective in suppressing hypodermal-specific but not muscle-specific mutations, and both were effective suppressors of *unc-52(e669)*. We discuss our evidence on this issue first and then return to the regulation of *unc-52* alternative splicing by MEC-8.

Evidence that *unc-52(+)* is required in hypodermal cells

Our mosaic analysis has shown that *unc-52* function is not required in muscle cells for embryo viability or wild-type larval development and suggests that hypodermis is the focus of *unc-52* function in both embryos and larvae. Hypodermis is the only tissue with substantial contributions from both AB and P1, and the defects seen in *unc-52(e669)* larvae were rescued by *unc-52(+)* expression in either AB or P1 descendants. Although *unc-52* function is not required in the descendants of P1 for embryonic viability or myofilament lattice assembly, *unc-52(st549)* larvae lacking *unc-52(+)* in all descendants of P1 were abnormal: they were dumpy and twisted with branched alae. If these abnormalities were caused by a partial requirement for *unc-52(+)* in body-wall muscle cells, we would have expected the phenotypes of EMS(–) and C(–) mosaics to be similar to each other and less severe than the phenotypes of P1(–) mosaics (EMS, C and P1 generate 42, 32 and 94 body wall muscle cells, respectively), but we found that C(–) mosaics were just as abnormal as P1(–) mosaics, and EMS(–) mosaics were wild type. These observations are consistent with a partial requirement for *unc-52(+)* in C-derived hypodermis; C is the only founder cell descended from P1 that contributes to hypodermis.

UNC-52 accumulation in the basement membrane between muscle and hypodermis has been first visualized at the beginning of morphogenesis (Hresko et al., 1994). The C-derived hypodermal cells form the posterior half of the dorsal hypodermis in pre-morphogenesis stage embryos (Sulston et al., 1983). At about the 1.5-fold stage of embryonic elongation, the C-derived and AB-derived hypodermal cells fuse to form the large hypodermal syncytium hyp7 (Podbilewicz and White, 1994). Thus, after hypodermal fusion, hyp7 in P1(–) and C(–) mosaics will have *unc-52(+)* function contributed by the AB lineage. This may explain why myofilament lattice formation seems to be relatively unaffected in these mosaics. However, the stage prior to fusion, when C-derived hypodermal cells fail to produce UNC-52, may be crucial for proper positioning of hypodermal seam cells and elongation of hypodermis.

Body-wall muscles may recruit UNC-52

Previous experiments, in which UNC-52 could be visualized faintly in muscle cells but not in hypodermal cells of early elongation-stage embryos by several UNC-52-specific antibodies (Mullen et al., 1999), suggested that UNC-52 found in embryonic basement membranes between body-wall muscle and hypodermis was produced exclusively by muscle cells (Moerman et al., 1996; Mullen et al., 1999), but our experiments indicate that if UNC-52 is produced by body muscle, it is not crucial for embryonic development. Why was UNC-52 not detected in hypodermal cells? Possibly UNC-52 produced in hypodermis is exported more rapidly or is less accessible to antibodies than UNC-52 produced in muscle cells. Alternatively, it is possible that muscle cells produce little if any UNC-52 but accumulate it by endocytosis, which could be part of a process of UNC-52 signal reception by muscle. There is growing evidence that muscle and hypodermis communicate during myofilament lattice assembly and elongation (Chin-Sang and Chisholm, 2000). Laser ablation of muscle cell precursors caused gaps in the distribution of extracellular UNC-52 in the regions corresponding to the missing muscles (Moerman et al., 1996). Assuming that much of the missing UNC-52 would normally have been produced by hypodermis, we suggest that the muscle is needed to bind and concentrate UNC-52 produced by adjacent hypodermis.

Similar cell ablation experiments have indicated that myotactin, another *C. elegans* protein produced by the hypodermis, is recruited to the hypodermal membrane near muscle cells by the adjacent muscle cells (Hresko et al., 1999). Myotactin is a transmembrane protein with a large extracellular domain and has a localization pattern similar to that of UNC-52 at certain stages of embryonic development (Hresko et al., 1994).

Spatial regulation of *unc-52* alternative splicing

Antibodies specific for an UNC-52 epitope encoded by exon 19 (Rogalski et al., 1993) did not stain *mec-8; unc-52(e444)* embryos (Lundquist et al., 1996) but did stain a region between the anterior-most body-wall muscle cells and hypodermis of *mec-8; unc-52(e669)* embryos (Mullen et al., 1999). These results suggest that certain anterior-most embryonic cells produce a *mec-8*-independent *unc-52* transcript that skips exon 17 (and hence *e669*) but not exon 18 (and *e444*). We found that *mec-8* embryos carrying a muscle-specific *unc-52(e669)* minigene but not a muscle-specific *unc-52(e444)* minigene accumulated GFP in the nuclei of the one or two anterior-most muscle cells per quadrant (Fig. 3H). These cells could be the source of UNC-52 in *mec-8; unc-52(e669)* embryos. UNC-52-specific antibodies have also been shown to stain *unc-52(e444)* and *unc-52(e669)* adults (Mullen et al., 1999) in the head but not in the main body region. The pattern of GFP accumulation we observed in wild-type animals carrying hypodermally expressed *unc-52(e444)* and *unc-52(e669)* minigenes suggests that UNC-52 in these animals could come from head hypodermal cells.

MEC-8 regulates *unc-52* alternative splicing primarily in embryos

RT-PCR experiments have indicated that the *unc-52* mRNA isoform containing exons 16-17-18-19 is more abundant in larvae than the *mec-8*-dependent 16-19 isoform (Spike et al., 2001) (and data not shown). Similar experiments have indicated that the 16-19 isoform is most abundant in embryos (G. Mullen, personal communication; C. Spike, data not shown), suggesting that endogenous MEC-8 may promote *unc-52* alternative splicing primarily in embryos. This is consistent with the developmental expression pattern of MEC-8 in hypodermal and muscle cells, and with the reduction of GFP in hyp7 after embryogenesis in animals carrying hypodermal *unc-52(e444)* or *unc-52(e669)* minigenes. It seems likely that GFP levels decrease, at least in part, because there are reduced levels of MEC-8 in the main hypodermal syncytium of larvae.

let-2, which encodes a type IV collagen, and *nid-1*, which encodes nidogen, also produce different protein isoforms in embryos and larvae (Kang and Kramer, 2000; Sibley et al., 1993). These proteins (along with UNC-52) are components of basement membranes in *C. elegans*, including the basement membrane between muscle and hypodermis (Graham et al., 1997; Kang and Kramer, 2000). *C. elegans* larvae and embryos are subject to different mechanical stresses and may therefore require substantially different basement membranes.

MEC-8 regulates *unc-52* alternative splicing primarily in the hypodermis

The properties of our hypodermis-expressing *mec-8(+)* constructs, as well as the embryonic MEC-8 expression pattern, suggest that MEC-8 regulates the alternative splicing of *unc-52* transcripts in the hypodermis. We did see that muscle-expressing *unc-52(e444)* and *unc-52(e669)* minigenes exhibited *mec-8*-dependent GFP accumulation in early morphogenesis-stage embryos, but GFP expression was very low and was increased by enhancing expression of MEC-8 in muscle; embryos carrying the wild-type versions of these minigenes expressed GFP abundantly at the same stage. We suggest that MEC-8 is present at low levels in embryonic muscle cells and that only a fraction of the *unc-52* minigene pre-mRNAs underwent *mec-8*-dependent alternative splicing. By contrast, the amount of embryonic GFP produced by the hypodermis-expressing *unc-52(e444)* and *unc-52(e669)* minigenes was comparable with that expressed by the wild-type versions of these minigenes. Consistent with the larval expression pattern of MEC-8, *unc-52(e444)* and *unc-52(e669)* minigenes expressed in hypodermis, but not muscle, were *mec-8*-dependent in larvae.

We thank J. Yochem for invaluable assistance with mosaic analysis and comments on the manuscript; G. Mullen and D. Moerman for sharing unpublished results; and A. Coulson, A. Fire, M. Labouesse, L. Lobel, D. Miller and J. Yochem for reagents. This work was supported by NIH research grants GM56367 (J. E. S.) and GM22387 (R. K. H.). Some nematode strains were supplied by the Caenorhabditis Genetics Center, which is supported by a contract between the NIH National Center for Research Resources and the University of Minnesota.

REFERENCES

Arikawa-Hirasawa, E., Watanabe, H., Takami, H., Hassell, J. R. and Yamada, Y. (1999). Perlecan is essential for cartilage and cephalic development. *Nat. Genet.* **23**, 354-358.

Arikawa-Hirasawa, E., Wilcox, W. R., Le, A. H., Silverman, N., Govindraj, P., Hassell, J. R. and Yamada, Y. (2001). Dyssegmental dysplasia, Silverman-Handmaker type, is caused by functional null mutations of the perlecan gene. *Nat. Genet.* **27**, 431-434.

Bar-Peled, M. and Raikhel, N. V. (1996). A method for isolation and purification of specific antibodies to a protein fused to the GST. *Anal. Biochem.* **241**, 140-142.

Bowerman, B., Draper, B. W., Mello, C. C. and Priess, J. R. (1993). The maternal gene *skn-1* encodes a protein that is distributed unequally in early *C. elegans* embryos. *Cell* **74**, 443-452.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

Chin-Sang, I. D. and Chisholm, A. D. (2000). Form of the worm: genetics of epidermal morphogenesis in *C. elegans*. *Trends Genet.* **16**, 544-551.

Costell, M., Gustafsson, E., Aszodi, A., Morgelin, M., Bloch, W., Hunziker, E., Addicks, K., Timpl, R. and Fassler, R. (1999). Perlecan maintains the integrity of cartilage and some basement membranes. *J. Cell Biol.* **147**, 1109-1122.

Davies, A. G., Spike, C. A., Shaw, J. E. and Herman, R. K. (1999). Functional overlap between the *mec-8* gene and five *sym* genes in *Caenorhabditis elegans*. *Genetics* **153**, 117-134.

Finney, M. and Ruvkun, G. (1990). The *unc-86* gene product couples cell lineage and cell identity in *C. elegans*. *Cell* **63**, 895-905.

Francis, R. and Waterston, R. H. (1991). Muscle cell attachment in *Caenorhabditis elegans*. *J. Cell Biol.* **114**, 465-479.

Gilchrist, E. J. and Moerman, D. G. (1992). Mutations in the *sup-38* gene of *Caenorhabditis elegans* suppress muscle-attachment defects in *unc-52* mutants. *Genetics* **132**, 431-442.

Gilleard, J. S., Barry, J. D. and Johnstone, I. L. (1997). *cis* regulatory requirements for hypodermal cell-specific expression of the *Caenorhabditis elegans* cuticle collagen gene *dpy-7*. *Mol. Cell Biol.* **17**, 2301-2311.

Graham, P. L., Johnson, J. J., Wang, S., Sibley, M. H., Gupta, M. C. and

Kramer, J. M. (1997). Type IV collagen is detectable in most, but not all, basement membranes of *Caenorhabditis elegans* and assembles on tissues that do not express it. *J. Cell Biol.* **137**, 1171-1183.

Hodgkin, J. (1997). Genetics. In *C. elegans II* (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 881-1047. Plainview, NY: Cold Spring Harbor Laboratory Press.

Hresko, M. C., Williams, B. D. and Waterston, R. H. (1994). Assembly of body wall muscle and muscle cell attachment structures in *Caenorhabditis elegans*. *J. Cell Biol.* **124**, 491-506.

Hresko, M. C., Schriefer, L. A., Shrimankar, P. and Waterston, R. H. (1999). Myotactin, a novel hypodermal protein involved in muscle-cell adhesion in Caenorhabditis elegans. *J. Cell Biol.* **146**, 659-672.

Kang, S. H. and Kramer, J. M. (2000). Nidogen is nonessential and not required for normal type IV collagen localization in *Caenorhabditis elegans*. *Mol. Biol. Cell* **11**, 3911-3923.

Kenyon, C. (1986). A gene involved in the development of the posterior body region of *C. elegans*. *Cell* **46**, 477-487.

Kondo, K., Makovec, B., Waterston, R. H. and Hodgkin, J. (1990). Genetic and molecular analysis of eight tRNA^{TRP} amber suppressors in *Caenorhabditis elegans. J. Mol. Biol.* **215**, 7-19.

Krause, M., Fire, A., Harrison, S. W., Priess, J. and Weintraub, H. (1990). CeMyoD accumulation defines the body wall muscle cell fate during C. elegans embryogenesis. *Cell* **63**, 907-919.

Krause, M., Harrison, S. W., Xu, S. Q., Chen, L. and Fire, A. (1994). Elements regulating cell- and stage-specific expression of the *C. elegans* MyoD family homolog *hlh-1*. *Dev. Biol.* **166**, 133-148.

Labouesse, M., Hartwieg, E. and Horvitz, H. R. (1996). The *Caenorhabditis elegans* LIN-26 protein is required to specify and/or maintain all non-neuronal ectodermal cell fates. *Development* **122**, 2579-2588.

Lopez, A. J. (1998). Alternative splicing of pre-mRNA: developmental consequences and mechanisms of regulation. *Annu. Rev. Genet.* **32**, 279-305.

Lundquist, E. A. and Herman, R. K. (1994). The *mec-8* gene of *Caenorhabditis elegans* affects muscle and sensory neuron function and interacts with three other genes: *unc-52*, *smu-1* and *smu-2*. *Genetics* **138**, 83-101.

Lundquist, E. A., Herman, R. K., Rogalski, T. M., Mullen, G. P., Moerman, D. G. and Shaw, J. E. (1996). The *mec-8* gene of *C. elegans* encodes a protein with two RNA recognition motifs and regulates alternative splicing of *unc-52* transcripts. *Development* **122**, 1601-1610.

Mello, C. and Fire, A. (1995). DNA transformation. In *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, Vol. 48 (ed. H. F. Epstein and D. C. Shakes), pp. 451-482. San Diego, CA: Academic Press.

Miller, D. M., 3rd, Ortiz, I., Berliner, G. C. and Epstein, H. F. (1983). Differential localization of two myosins within nematode thick filaments. *Cell* **34**, 477-490.

Miller, D. M., Stockdale, F. E. and Karn, J. (1986). Immunological identification of the genes encoding the four myosin heavy chain isoforms of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **83**, 2305-2309.

Moerman, D. G., Hutter, H., Mullen, G. P. and Schnabel, R. (1996). Cell autonomous expression of perlecan and plasticity of cell shape in embryonic muscle of *Caenorhabditis elegans*. *Dev. Biol.* **173**, 228-242.

Mullen, G. P., Rogalski, T. M., Bush, J. A., Gorji, P. R. and Moerman, D. G. (1999). Complex patterns of alternative splicing mediate the spatial and temporal distribution of perlecan/UNC-52 in *Caenorhabditis elegans*. *Mol. Biol. Cell* **10**, 3205-3221.

Nicole, S., Davoine, C. S., Topaloglu, H., Cattolico, L., Barral, D., Beighton, P., Hamida, C. B., Hammouda, H., Cruaud, C., White, P. S. et al. (2000). Perlecan, the major proteoglycan of basement membranes, is altered in patients with Schwartz-Jampel syndrome (chondrodystrophic myotonia). *Nat. Genet.* **26**, 480-483.

Noonan, D. M. and Hassell, J. R. (1993). Perlecan, the large low-density proteoglycan of basement membranes: structure and variant forms. *Kidney Int.* **43**, 53-60.

Podbilewicz, B. and White, J. G. (1994). Cell fusions in the developing epithelia of *C. elegans*. *Dev. Biol.* **161**, 408-424.

Rogalski, T. M., Williams, B. D., Mullen, G. P. and Moerman, D. G. (1993). Products of the *unc-52* gene in *Caenorhabditis elegans* are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan. *Genes Dev.* **7**, 1471-1484.

Rogalski, T. M., Gilchrist, E. J., Mullen, G. P. and Moerman, D. G. (1995). Mutations in the *unc-52* gene responsible for body wall muscle defects in adult *Caenorhabditis elegans* are located in alternatively spliced exons. *Genetics* **139**, 159-169.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Sibley, M. H., Johnson, J. J., Mello, C. C. and Kramer, J. M. (1993). Genetic identification, sequence, and alternative splicing of the *Caenorhabditis elegans* alpha 2(IV) collagen gene. *J. Cell Biol.* **123**, 255-264.

Singh, R. N. and Sulston, J. E. (1978). Some observations on moulting in *Caenorhabditis elegans*. *Nematologica* **24**, 63-71.

Spike, C. A., Shaw, J. E. and Herman, R. K. (2001). Analysis of *smu-1*, a gene that regulates the alternative splicing of *unc-52* pre-mRNA in *Caenorhabditis elegans*. *Mol. Cell Biol.* **21**, 4985-4995.

Sulston, J. E., Schierenberg, E., White, J. G. and Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64-119.

Williams, B. D. and Waterston, R. H. (1994). Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. *J. Cell Biol.* **124**, 475-490.

Yochem, J., Gu, T. and Han, M. (1998). A new marker for mosaic analysis in *Caenorhabditis elegans* indicates a fusion between hyp6 and hyp7, two major components of the hypodermis. *Genetics* **149**, 1323-1334.