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The *mec-8* gene of *C. elegans* encodes a protein with two RNA recognition motifs and regulates alternative splicing of *unc-52* transcripts

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SUMMARY

Mutations in the *mec-8* gene of *Caenorhabditis elegans* were previously shown to affect the functions of body wall muscle and mechanosensory and chemosensory neurons. Mutations in *mec-8* also strongly enhance the mutant phenotype of specific mutations in *unc-52*, a gene that encodes, via alternative splicing of pre-mRNA, a set of basement membrane proteins, homologs of perlecan, that are important for body wall muscle assembly and attachment to basement membrane, hypodermis and cuticle. We have cloned *mec-8* and found that it encodes a protein with two RNA recognition motifs, characteristic of RNA binding proteins. We have used reverse transcription-PCR and RNase protection experiments to show that *mec-8* regulates the accumulation of a specific subset of alternatively spliced *unc-52* transcripts. We have also shown with antibodies to UNC-52 that *mec-8* affects the abundance of a subset of UNC-52 isoforms. We propose that *mec-8* encodes a *trans*-acting factor that regulates the alternative splicing of the pre-mRNA of *unc-52* and one or more additional genes that affect mechanosensory and chemosensory neuron function.

Key words: *C. elegans*, *mec-8* gene, RRMs, alternative RNA splicing, *unc-52* gene

INTRODUCTION

For many eukaryotic genes, alternative patterns of splicing or polyadenylation generate alternative mRNAs from identical mRNA precursors (reviewed by McKeown, 1992; Mattox et al., 1992). One function of regulated alternative splicing is to provide developmental on-off switches, as in sex determination in *Drosophila* (Baker, 1989) or meiotic differentiation in yeast (Engebrecht et al., 1991). A second function of regulated alternative splicing is to produce a diversity of proteins or isoforms from a single form of pre-mRNA. Genetic analysis has helped to identify several *trans*-acting factors that regulate alternative splicing involved in on-off developmental switching, but very little is known about *trans*-acting factors that regulate alternative splicing involved in generating diverse isoforms, despite the fact that the transcripts of a large number of genes are known to be subject to alternative splicing of this kind.

A gene in *C. elegans* that produces different protein products through alternative splicing is *unc-52* (Rogalski et al., 1993, 1995). The *unc-52* proteins are homologs of perlecan, the core protein of the mammalian basement membrane heparan sulfate proteoglycan. A subset of UNC-52 isoforms is present in the basement membrane adjoining body wall muscle (Francis and Waterston, 1991; Rogalski et al., 1993; Moerman et al., 1996) and is important both for initiation of myofilament lattice assembly and for maintaining the anchorages of body wall muscle to basement membrane, hypodermis and cuticle. Null mutations in *unc-52* lead to paralysis and arrest at the twofold stage of embryonic elongation (Rogalski et al., 1993; Williams and Waterston, 1994; Hresko et al., 1994); the muscle cells in these mutants have severely disorganized thick and thin filaments, suggesting an early failure in assembly of the myofilament lattice. Viable *unc-52* mutants exhibit normal embryogenesis but suffer a gradual disruption of body wall muscle cells posterior to the pharynx beginning at the third or fourth larval stage (Mackenzie et al., 1978; Waterston et al., 1980; Waterston, 1988; Gilchrist and Moerman, 1992); the muscle disruption correlates with progressive paralysis. Nucleotide sequence alterations responsible for several viable *unc-52* mutations have been shown to affect subsets of the *unc-52*-encoded isoforms generated by alternative RNA splicing (Rogalski et al., 1995; G. P. Mullen and D. G. Moerman, unpublished data).

Mutations in the *C. elegans* gene *mec-8* have pleiotropic effects. They affect mechanosensory (Chalfie and Sulston, 1981; Chalfie and Au, 1989) and chemosensory (Perkins et al., 1986) neuron function. Strong *mec-8* mutants also exhibit an incompletely penetrant, cold-sensitive embryonic and larval arrest, which has been correlated with defects in the attachment of body muscle to adjacent hypodermis and cuticle (Lundquist and Herman, 1994). Finally, *mec-8* mutations interact genetically with viable *unc-52* mutations to confer a synthetic lethal phenotype, which mimics the *unc-52* null phenotype

(Lundquist and Herman, 1994). The *mec-8-unc-52(viable)* interaction is completely penetrant, unconditional and allele-nonspecific. We have cloned *mec-8* and found that it encodes a protein with two RNA recognition motifs, characteristic of RNA binding proteins. We have also found that *mec-8* mutation affects the accumulation of specific alternatively spliced *unc-52* transcripts and a corresponding UNC-52 isoform. We therefore propose that MEC-8 protein acts in *trans* on *unc-52* pre-mRNA to regulate its alternative splicing.

MATERIALS AND METHODS

General genetics and nematode strains

Nematodes were cultured as described by Brenner (1974). The wild-type strain was N2. We have followed standard *C. elegans* genetic nomenclature (Horvitz et al., 1979). Unless otherwise noted, all genetic experiments were performed at 20˚C. Alleles and rearrangements (Hodgkin et al., 1988) were the following. Linkage group (LG) I: *mec-8* (Chalfie and Au, 1989; Lundquist and Herman, 1994), *unc-29(h2), dpy-24(s71)* and *hP6* (Starr et al., 1989). LG II: *mnC1*, *dpy-10(e128), unc-4(e120), unc-52(e444)* (Rogalski et al., 1995).

Genetic and physical mapping of *mec-8*

Unc non-Dpy and Dpy non-Unc recombinants were picked from the self progeny of *unc-29* + *hP6 dpy-24/*+ *mec-8(e398)* + + hermaphrodites. Strains homozygous for each recombinant chromosome were scored for touch insensitivity (Mec phene) and abnormal dye filling of amphid and phasmid neurons (Dyf phene) conferred by *mec-8* mutations; scoring was as described by Lundquist and Herman (1994). The presence or absence of *hP6* was scored by probing Southern blots of *Eco*RI-digested DNA with an 0.8-kb *Eco*RI fragment that hybridized to an 0.8-kb band for strains lacking *hP6* and a 2.4-kb band for strains containing *hP6*, which is a 1.6-kb insert of the transposable element Tc1 (Starr et al., 1989). Among 30 Unc non-Dpy recombinants, 12 contained the recombinant chromosome *unc-29 mec-8* + + and 18 contained an *unc-29* + *hP6* + chromosome. Among 36 Dpy non-Unc recombinants, 20 contained a + *mec-8* + *dpy-24* chromosome, 15 contained a + + *hP6 dpy-24* chromosome and one contained a + *mec-8 hP6 dpy-24* chromosome. We conclude that *mec-8* maps very near and probably to the left of *hP6.*

Germline transformation

Hermaphrodites of genotype *mec-8(u74)* were transformed by the methods of Mello et al. (1991). DNA to be tested was injected at 12.5-25 μg/ml, and plasmid pRF4, which contains the semidominant mutation *rol-6(su1006),* was coinjected at 100 μg/ml. Transgenic roller (Rol) hermaphrodites were picked to identify stably transformed lines, which were scored for Mec and Dyf phenes. To ascertain transformation rescue of the Dyf phene, at least 30 Rol animals were scored. Rescued lines showed greatly increased dye filling of both amphid and phasmid neurons.

To test for transformation rescue of *mec-8; unc-52(e444)* synthetic lethality, *unc-52/*+ males were mated with *mec-8(u74); Ex* hermaphrodites, where *Ex* refers to a heritable extrachromosomal array formed after DNA germline transformation that confers a Rol phenotype and presumably also carries copies of the DNA clone whose ability to transform was to be tested. Many Rol non-Mec hermaphrodite progeny were picked, and those of genotype *mec-8/+; Ex; unc-52/*+ were identified. Many of their hermaphrodite Rol non-Mec non-Unc self-progeny were picked. Among these, animals whose non-Rol self progeny were all Mec, and hence were homozygous for *mec-8*, were identified. Two-thirds of these animals would be expected to be *unc-52/*+. The presence of Rol Unc self progeny in some broods indicated rescue of the *mec-8; unc-52(e444)* synthetic lethality by the extrachromosomal array. When Rol Unc animals were present, some were picked and found to segregate arrested embryos and Rol Unc self progeny, as expected. Arrays that were found not to rescue the *mec-8; unc-52* synthetic lethality were tested again to confirm the result. For tests of arrays carrying an *hsp16-2* heat-shock promoter (Stringham et al., 1992), broods of *mec-8; unc-52(e444)/+; Ex* animals and *mec-8(u74)* and *unc-52(e444)* animals as controls were subjected to the heat shock regime described below, and another set of duplicate plates was maintained at 20˚C.

Heat-shock experiments

Twenty gravid hermaphrodites were picked to a plate. These animals and their self progeny were subjected to the following heat shock regime on three successive days: 0.5 hour at 33˚C, 3 hours at 20˚C, 0.5 hour at 33˚C, 3 hours at 20˚C, 0.5 hour at 33˚C, 16.5 hours at 20˚C. The parental hermaphrodites were removed on day 2. Self progeny were scored as adults with respect to Mec and Dyf phenes and synthetic lethality of *mec-8(u74);unc-52(e444).*

Molecular biology

Standard molecular biology techniques (Sambrook et al., 1989) were used. Two *C. elegans* cDNA libraries were used: a λgt10 library provided by S. Kim and a λZAP library provided by R. Barstead and R. Waterston. All plasmid subcloning was done using pBlueScript SK⁻ (Stratagene). DNA was sequenced by the dideoxy chain termination method (Sanger et al., 1977) using Sequenase Version 2.0 (U. S. Biochemicals). Deletion series of clones for sequencing were obtained by timed exonuclease III digestion (Henikoff, 1987). Sequence analysis made use of the GCG sequence analysis package.

RNA isolation

RNA for RNase protection and northern analysis was extracted by suspending 1-5 ml of packed animals in 10 ml extraction solution (6 M guanidine-HCl, 0.2 M sodium acetate, 0.5% Sarkosyl, 1 mM EDTA and 1% β-mercaptoethanol), extruding the suspension through a French press cylinder at 11,000 psi, and extracting the solution 3-5 times with 50:48:2 phenol:chloroform:isoamyl alcohol and once with 24:1 chloroform:isoamyl alcohol. RNA was precipitated by adding 1/2 volume of ethanol.

RT-PCR of *unc-52* transcripts

Nematodes of mixed stages grown at 15˚C were washed from three 50 mm plates, placed in liquid nitrogen for 5 minutes and resuspended in 500 μl of 100 mM Tris, pH 8.5, 1 mM EDTA, 200 mM sodium acetate, 1% SDS and 200 μg/ml proteinase K. After incubation at 65˚C for 1 hour, the samples were extracted 3 times with 50:48:2 phenol:chloroform:isoamyl alcohol and once with 24:1 chloroform:isoamyl alcohol. RNA was precipitated with 1/2 volume of ethanol, resuspended in 50 μl of AMV-RT buffer (Promega), 3 mM $MgCl_2$, 0.2 pM/μl RT primer, 200 μM of each dNTP and 5 units of AMV reverse transcriptase and incubated at 42˚C for 1 hour followed by 5 minutes at 95˚C. The nucleotide sequence of the RT primer, which is in exon 19 (Rogalski et al., 1993), was CCGTTGGAGAAGTCGAGAAT.

For the quantitative PCR experiments described in Fig. 4, 0.5-7.5 μl (corresponding to the range of dilutions shown) from the reverse transcription reaction were added to Taq polymerase buffer (Promega), 1.5 mM $MgCl_2$, 200 μM of each dNTP, forward and reverse primers at 1 pM/μl each and 2.5 units Taq polymerase to a final volume of 50 μl and amplified using a PTC-100 Programmable Thermal Controller (MJ Research) under the following conditions, per cycle: 94˚C for 30 seconds, 55˚C for 1 minute and 72˚C for 1.5 minutes. For the replicated RT-PCR experiments, 5 μl from the reverse transcription reaction were used, and the PCR was run for 35 cycles. The nucleotide sequences of the primers were as follows. Exon 15 forward: ATCCGCTGCTGGGTTCCCGG; exon 16 forward: GACATCCAAGTGTTCAGC; exon 17 forward: CGCATTCAGTG-

TACAGTA; exon 19 reverse: AACTGATGTCGCTCTCCT (nested 5′ of the reverse transcription primer in exon 19). The exon-specific probes referred to in the legend to Fig. 4 were the exon 16 and exon 17 forward primers listed above and the exon 18 probe TCCATC-CTGGTTCACATG.

RNase protection

The method of Haines and Gillespie (1992) was used: 15 μg of total RNA from mixed stages were dissolved in 50 μl of 4 M guanidine thiocyanate, 25 mM sodium citrate, pH 7.0, and 0.5% sarcosyl, heated at 70˚C for 2 minutes and allowed to hybridize with ^{32}P-labeled probe, generated by in vitro transcription using T3 RNA polymerase (Sambrook et al., 1989), for 16 hours at 42˚C, after which 500 μl of 10 mM Tris, pH 7.0, 300 mM NaCl and 5 mM EDTA containing 10 μg RNase A and 500 U RNase T1 were added. The solution was incubated at 4˚C for 40 minutes, and the products were analyzed by electrophoresis through an 8% denaturing polyacrylamide gel. Control samples that were pre-digested with RNase A gave no bands in the RNase protection assay.

Immunofluorescence and confocal microscopy

Immunofluorescence staining and confocal microscopy techniques were as previously described (Rogalski et al., 1995; Moerman et al., 1996). Arrested *mec-8(mn463); mnC1 dpy-10 unc-52(e444)* embryos were obtained as self progeny from a balanced *mec-8; mnC1 dpy-10 unc-52(e444)/unc-4* stock. Embryos from *mnC1 dpy-10 unc-52(e444)/unc-4* or *dpy-10 unc-52(e444)* or *mec-8(mn463)* strains maintained at 20˚C exhibited neither the embryonic lethal phenotype nor defects in UNC-52 expression observed with *mec-8(mn463); mnC1 dpy-10 unc-52(e444)* embryos.

RESULTS

Positional cloning of *mec-8*

We mapped *mec-8* genetically (Fig. 1A) between *unc-29* and *dpy-24* and very near the dimorphism *hP6* (Starr et al., 1989). Both *unc-29* (Lackner et al., 1994) and *hP6* have been placed on the *C. elegans* physical map (Coulson et al., 1991). We used cosmid clones that had been positioned near *hP6* as probes to Southern blots of genomic DNA from wild-type (N2) and *mec-8* mutants (Fig. 1B). Three overlapping cosmids detected genomic alterations in four *mec-8* mutants (among 16 mutants tested). The same 2.4-kb *Hin*dIII fragment was altered in all

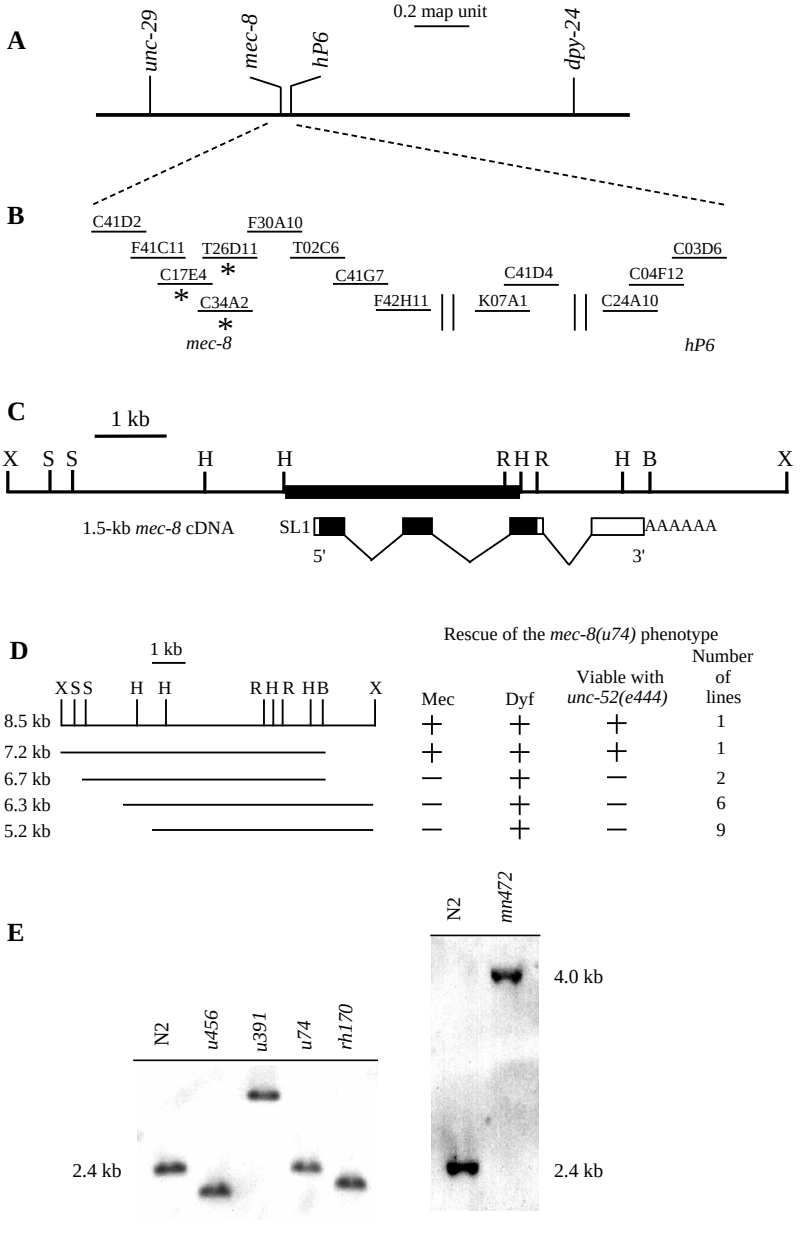

Fig. 1. Cloning of *mec-8*. (A) Genetic map. *mec-8* was positioned between *unc-29* and *dpy-24* very near and slightly to the left of the restriction fragment length dimorphism *hP6*. (B) Relative positions of cosmid clones on the *C. elegans* physical map (Coulson et al., 1991) in the region of *hP6* (Starr et al., 1989), which maps to clone C03D6. All of the cosmids shown were used to probe Southern blots of genomic DNA from *mec-8* mutants. Three cosmids, marked by asterisks, detected alterations in four *mec-8* mutants. (Gaps in cosmid coverage of the physical map, marked by double vertical lines, are spanned by yeast artificial chromosomes.) (C) Restriction map of an 8.5-kb *Xho*I fragment from cosmid C34A2 that contains *mec-8* (X, *Xho*I; S, *Sty*I, H, *Hin*dIII; R, *Eco*RI and B, *Bgl*II). The heavy line marks the 2.4-kb *Hin*dIII fragment that is altered in four different *mec-8* mutants. The 1.5-kb cDNA is shown, with boxes representing exons, lines introns, and filled boxes open reading frame. (D) Rescue of the *mec-8* phenotype by germline transformation with genomic clones. The top line on the left represents the 8.5-kb *Xho*I fragment represented in C; the lower lines represent deleted derivatives of the 8.5-kb *Xho*I fragment. The table to the right gives the abilities of the clones on the left to rescue the Mec and Dyf phenes conferred by *mec-8* mutation and the synthetic lethality of *mec-8; unc-52(e444)*. + indicates rescue, and − indicates lack of rescue. The number of independent roller lines that were scored for each clone is given. Independent lines of the same type gave identical results. (E) Southern blots of DNA from N2 (wild-type) and five *mec-8* mutants. Genomic DNA was digested with *Hin*dIII and probed with the 2.4-kb *Hin*dIII fragment denoted by the heavy line in C. Alterations are apparent for *u456, u391, rh170* and *mn472*. The EMS-induced mutant *mec-8(u74)* is not discernably different from N2.

four mutants. For only one mutant, *mec-8(u391)*, were additional bands altered. The 2.4-kb *Hin*dIII fragment was cloned from cosmid C34A2 and used as a probe to Southern blots of N2 and *mec-8* mutant genomic DNA digested with *Hin*dIII (Fig. 1E). The *u456* mutation is a 200-bp deletion of the 2.4-kb *Hin*dIII fragment; *u391* is a complex rearrangement; *rh170* is a 100-bp deletion; and *mn472* is a 1.6-kb insertion in the 2.4-kb *Hin*dIII fragment. Alteration of the same 2.4-kb *Hin*dIII fragment in four independent *mec-8* mutants suggests that this fragment is part of the *mec-8* locus.

Transformation rescue of the *mec-8* mutant phenotype

Mutations in *mec-8* confer three mutant phenes that we have monitored in germline transformation experiments: insensitivity to light touch, the Mec phene (Chalfie and Sulston, 1981); reduced ability of neurons in the amphid and phasmid chemosensilla of living animals to fill with a fluorescent dye, the Dyf phene (Perkins et al., 1986; Lundquist and Herman, 1994); and the synthetic lethality of *mec-8; unc-52(viable)* double mutants (Lundquist and Herman, 1994). From cosmid C34A2, we cloned an 8.5-kb *Xho*I genomic fragment (Fig. 1C), which contains within it the 2.4-kb *Hin*dIII fragment. As indicated in Figure 1D, *mec-8* animals transformed with the 8.5-kb *Xho*I fragment were rescued with respect to all three *mec-8* mutant phenes. Transformation with a 7.2-kb genomic fragment that lacked 1.3 kb from the right end of the 8.5-kb *Xho*I fragment also rescued all three mutant phenes (Fig. 1D). Removal of 0.5 kb, 2.2 kb or 3.3 kb from the left end of the 8.5-kb *Xho*I fragment, however, resulted in fragments that could rescue the Dyf phene only (Fig. 1D). For each of the three clones that rescued only the Dyf phene, at least two independent lines were tested with respect to all three phenes and gave identical results. The fragments that rescued only the Dyf phene may be missing *mec-8* regulatory sequence needed to rescue the other two phenes or may simply provide a low level of *mec-8* function, which is capable of rescuing only the Dyf phene.

Expression of a 1.5-kb cDNA rescues three *mec-8* mutant phenes

Eleven cDNAs that hybridized to the 8.5-kb *Xho*I fragment were isolated from two independent cDNA libraries and appeared to be variably truncated forms of the longest cDNA, which is 1.5 kb. We determined the nucleotide sequences of both strands of the 1.5-kb cDNA (Fig. 2A). We also determined the nucleotide sequence of the genomic DNA through the region corresponding to the cDNA sequence, including the 2.4-kb *Hin*dIII fragment and extending to the right end of the 8.5-kb *Xho*I fragment. The 5′ end of the 1.5-kb cDNA nucleotide sequence is identical to the sequence of the last nine nucleotides of the trans-spliced leader sequence SL1 (Krause and Hirsh, 1987). The 3′ end has a poly(A) tail preceded by the polyadenylation signal AATAAA. The 1.5-kb cDNA spans 3.2 kb of the genome and is composed of four exons. The 936-bp ORF, beginning with an AUG methionine codon, spans the first three exons. An in-frame stop codon (UAA) is positioned near the end of exon 3 (Figs 1C, 2A). This stop was present in the genomic sequence and in the sequence of two other independently derived cDNAs.

We placed the expression of the cDNA under the control of

A

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AAGTTTGAGCCATAACGATTGAAAACAGCCGCAAACGGAAGATGGACGGCCCGAAGCCGA   60
                                              M  D  G  P  K  P  N    7

ACCTGGCGTCGGCCGCTTCGATGGAGTCGTTGAACAGTGTTTCTTCTGAAGCAACGAATC  120
  L  A  S  A  A  S  M  E  S  L  N  S  V  S  S  E  A  T  N  P   27

CATCACAGGTCCGCACCTTGTTTGTTTCGGGTCTTCCAATGGATGCTAAGCCGCGTGAGC  180
  S  Q  V  R  T  L  F  V  S  G  L  P  M  D  A  K  P  R  E  L   47

TTTATCTTCTGTTCCGTGGATGTCGTGGTTATGAGGGAGCTCTTTTGAAAATGACATCGA  240
  Y  L  L  F  R  G  C  R  G  Y  E  G  A  L  L  K  M  T  S  K   67

AGAATGGAAAACCAACGTCTCCAGTCGGATTTGTCACCTTTCTTTCGCAACAAGATGCGC  300
  N  G  K  P  T  S  P  V  G  F  V  T  F  L  S  Q  Q  D  A  Q   87
                          ↓
AGGACGCCAGAAAAATGTTGCAAGGTGTCCGATTCGATCCGGAATGTGCACAGGTACTTC  360
  D  A  R  K  M  L  Q  G  V  R  F  D  P  E  C  A  Q  V  L  R  107

GACTAGAACTTGCAAAATCGAACACAAAAGTAGCTCGACCTAAACAATCTCCACCACCAC  420
  L  E  L  A  K  S  N  T  K  V  A  R  P  K  Q  S  P  P  P  P  127

CACAACATCGGGCACTATCAGCCGCTGCAGCCGGAGTCCCGCAGTTTTTGGCACCAATGC  480
  Q  H  R  A  L  S  A  A  A  A  G  V  P  Q  F  L  A  P  M  Q  147

AACACGATCTTCTACTAGATCCTCAATCAGCAGCTCTTTTCAATGAGCAACAACTATTGG  540
  H  D  L  L  L  D  P  Q  S  A  A  L  F  N  E  Q  Q  L  L  A  167

CTCTTTCACTTCCACATTTACATGCTGCACAGGCACTTCAAGCAGCCTATATGCCAGCTT  600
  L  S  L  P  H  L  H  A  A  Q  A  L  Q  A  A  Y  M  P  A  S  187

CTGCTCTACAACAATACAGTCAGAATCAATTGTTTGCAGCTGCTCAAATGCACCCAGCAG  660
  A  L  Q  Q  Y  S  Q  N  Q  L  F  A  A  A  Q  M  H  P  A  A  207
             ↓
CCGCTGCAGCAGCCAGCCTCCAACATTCTCAACAAGCATCTCAAGCTTCCACCTCTGCGT  720
  A  A  A  A  S  L  Q  H  S  Q  Q  A  S  Q  A  S  T  S  A  C  227

GCTCCACTCTTTTCGTCGCCAATCTATCGGCTGAAGTGAATGAAGATACTCTTCGGGGTG  780
  S  T  L  F  V  A  N  L  S  A  E  V  N  E  D  T  L  R  G  V  247

TATTCAAAGCATTCTCTGGTTTCACACGTCTACGACTGCACAACAAGAATGGATCATGTG  840
  F  K  A  F  S  G  F  T  R  L  R  L  H  N  K  N  G  S  C  V  267

TGGCGTTTGTTGAATATTCGGATCTTCAGAAAGCAACTCAAGCGATGATATCACTACAAG  900
  A  F  V  E  Y  S  D  L  Q  K  A  T  Q  A  M  I  S  L  Q  G  287

GATTCCAGATAACAGCCAACGATCGAGGTGGTCTTCGTATTGAATATGCTCGGAACAAGA  960
  F  Q  I  T  A  N  D  R  G  G  L  R  I  E  Y  A  R  N  K  M  307
                                                 ↓
TGGCTGATGTGAATGGATAAGAAGAGAAGCAAGAATTCTTTGCAGTTTTGTTTTGGGAAG 1020
  A  D  V  N  G  *                                             312
CGCACCACCATCACCAAATGCACAATCATCACTCAGCAACTACTACTTCTACTTCTTCTT 1080
CTTCTTCCTGTGCACAACCACCACCACCTCCACCAACGTACACCCGCTCTCATTACACTT 1140
AGTGCATTTTCGTCCTTTTTTCATTTTACTCTCCCAAAAAATCACCAAAAATCCTTTCGG 1200
ATCTCTTTTTATTCGCATTTTATTTTCCTTTCTCTTAATTTTTACAAAATTCGAAGTGTT 1260
TGTAAGCACCCTACAGGAACTTCTATTGTCTTGACCGTAGAGCTCCTTTGCATCATATCT 1320
TTTCTATTTCTGATTTACTCTCTGTAAATATATATCACGAGAGCTTATCCACCTGTCTGT 1380
CTGTCCGAACCTGAAATTTTCTGTGATTTGTTCTAATTTTTCATAAGATCCTTTTTCCCC 1440
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GTAATAAATTTATACACTTTAAAAAAA                                   1527
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B

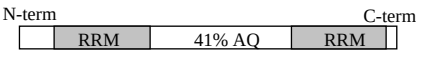

Fig. 2. Analysis of 1.5-kb *mec-8* cDNA (EMBL accession number X95608; X95609 for genomic sequence). (A) The nucleotide sequence of the 1.5-kb *mec-8* cDNA. The italicized bases at the 5′ end are the last nine bases of the 22-nucleotide *trans*-spliced leader sequence SL1, which is presumed to be at the 5′ end of the corresponding *mec-8* mRNA. The arrows above the cDNA sequence mark the positions of the three *mec-8* introns, which are 529, 762 and 424 nucleotides long, respectively. The putative polyadenylation signal is underlined near the 3′ end of the cDNA sequence. Below the cDNA sequence is the amino acid sequence of the putative *mec-8* polypeptide encoded by the 936-bp open reading frame. Underlined in the amino acid sequence are the two putative *mec-8* RNA recognition motifs (RRMs). (B) Schematic diagram of the putative *mec-8* polypeptide showing the relative positions of the two *mec-8* RRMs (stippled boxes) and the intervening AQ-rich region.

the heat-inducible *hsp16-2* promoter (Stringham et al., 1992). After a series of heat shocks, *mec-8* animals transformed with the *hsp16-2::mec-8* cDNA fusion plasmid were rescued for the Mec and Dyf phenes and the synthetic lethality of *mec-8; unc-52(e444)*. None of the three *mec-8* phenes was rescued in the absence of heat shock, and heat-shocked *mec-8* animals were not rescued in any respect. We repeated these experiments with an independently derived *hsp16-2::mec-8* cDNA-bearing line and obtained the same results. We conclude that the 1.5-kb cDNA encodes a *mec-8* protein capable of rescuing all three *mec-8* mutant phenes.

A northern blot of poly(A)-enriched RNA isolated from wild-type animals and probed with the 1.5-kb *mec-8* cDNA showed four bands – corresponding to 1.5, 1.8, 2.4 and 3.2 kb (data not shown). We have not fully characterized the compositions of the four bands, but it appears that the 2.4-kb and 3.2-kb transcripts contain unspliced intron 3 sequence, that the 1.8-kb and 2.4-kb transcripts contain additional genomic sequence distal to the 3′ end of the 1.5-kb *mec-8* cDNA sequence, and that none of the transcripts contains sequence derived from upstream of the 5′ end of the cDNA within the 8.5-kb *Xho*I genomic fragment (data not shown).

mec-8 encodes a protein with two RNA recognition motifs

The 1.5-kb *mec-8* cDNA can encode a polypeptide of 312 amino acid residues (Fig. 2A). A search of protein databases revealed that the predicted *mec-8* protein is similar to a wide variety of proteins implicated in RNA binding and processing, such as RNA splicing factors and hnRNPs. The regions of similarity reside primarily in two copies of an RNA recognition motif (RRM), also referred to as RNP motif, RNP consensus sequence (RNP-CS) and consensus sequence RNA-binding domain (CS-RBD) (Query et al., 1989; Kenan et al., 1991; Birney et al., 1993; Burd and Dreyfuss, 1994). Each RRM is composed of about 80 amino acid residues (underlined in Fig. 2A). The region between the two *mec-8* RRMs, a segment of approximately 116 amino acid residues, is 41% alanine and glutamine (Fig. 2B). Regions of high alanine and glutamine (AQ) composition are present in the products of the *Drosophila* genes *couch potato* (Bellen et al., 1992), *elav* (Robinow et al., 1988) and *musashi* (Nakamura et al., 1994). All of these proteins contain RRMs and affect neural development. The function, if any, of these AQ-rich regions is not known.

The N-terminal RRM of *mec-8* is 72% identical to the RRM found in the cpo61.1 protein from the *Drosophila couch potato* locus (Bellen et al., 1992); the C-terminal *mec-8* RRM is 38% identical to the RRM found in the human U2 snRNA-associated B″ antigen (Habets et al., 1987); and the two *mec-8* RRMs are 32% identical. Fig. 3 shows an alignment of the two *mec-8* RRMs with the others just mentioned. Also included in Fig. 3 is the amino terminal RRM of the human U1 snRNA-associated A protein (Sillekens et al., 1987), an RRM whose tertiary structure has been solved by X-ray crystallography (Nagai et al., 1990). The RRMs in Fig. 3 have been aligned with a consensus RRM structural core sequence proposed by Birney et al. (1993). Both *mec-8* RRMs match fairly well the consensus, which includes two short submotifs, RNP-1 (an octamer) and RNP-2 (a hexamer).

mec-8 affects the accumulation of a subset of alternatively spliced transcripts of *unc-52*

We tested the idea that *mec-8* regulates the alternative splicing of *unc-52* transcripts by assaying the patterns of alternative splicing of *unc-52* exons 15 through 19 using reverse transcription-PCR (RT-PCR) on RNA from N2 and *mec-8* animals. Previous work showed that alternative splicing generates a minimum of six different *unc-52* mRNAs, with different combinations of exons 16, 17 and 18 present in the mature transcripts. Each of the exons 16, 17 and 18 encodes one immunoglobulin repeat, and the nucleotide sequences of the exons are such that each of the alternatively spliced transcripts maintains an open reading frame through the region (Rogalski et al., 1995).

In one set of experiments, we used primers specific for exons 15 and 19 for PCR amplification of *unc-52* cDNA. Fig. 4A shows a Southern blot of the PCR products of this reaction hybridized to a probe of *unc-52* genomic sequence that extends from within exon 15 into exon 19. A band corresponding to the 284-bp fragment expected for a 15-19 splice product was apparent for N2 (wild type), but was greatly reduced or absent in *mec-8* animals. This result was highly reproducible. We performed ten additional experiments with the same primers, each starting with independent RNA preparations and using 35 cycles of PCR amplification. In every case, N2 showed a band corresponding to the 15-19 splice product and *mec-8* showed either no band or a much weaker band. Both N2 and *mec-8* gave a band corresponding in size to about 570 bp. Radioactively labeled oligonucleotides specific to exons 16 and 18 both hybridized to this band in N2 (Fig. 4B), indicating that the N2 band is composed of 15-16-19 (581 bp) and 15-18-19 (563 bp) splice products. The corresponding *mec-8* band hybridized to

```
          β1    loop1     α1     loop2  β2     loop3     β3 loop4   α2       loop5    β4
        ------         ---------      ------          -------  -----------         ------
        1        11         21        31        41       51      61          71        81
m8rrm1  TLFVSGLPM....DAKPRELYLLFRGCRGYEGALLKMTSKNGKPTSPVGFVTFLSQQDAQDARKMLQGVRFDPECAQVLRLELA
  cpo   TLFVSGLPM....DAKPRELYLLFRAYEGYEGSLLKVTSKNGKTASPVGFVTFHTRAGAEAAKQDLQGVRFDPDMPQTIRLEFA
 u2b2   ILFLNNLPE....ETNEMMLSMLFNQ.FPGFKEVRLV.....PGRHDIAFVEFENDGQAGAARDALQGFKITPSHAMKITYAKK
m8rrm2  TLFVANLSA....EVNEDTLRGVFKA.FSGFTRLRLH....NKNGSCVAFVEYSDLQKATQAMISLQGFQITANDRGGLRIEYA
 u1a1   TIYINNLNEKIKKDELKKSLYAIFSQ.FGQILDILVS...RSLKMRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRIQYAKTD
 core    UxUxxLxx....xZxxxxLxxxFxx.xGxUxxZxxx.....xxxxxxUxVxFxxxxxZxxA
        ------                                          --------
         RNP-2                                           RNP-1
```

Fig. 3. Alignment of the two *mec-8* RRMs with other RRM family members. m8rrm1 is the N-terminal *mec-8* RRM; cpo is the *couch potato* cpo61.1 RRM (GenBank accession no. Z14974); u2b2 is the RRM found in the human U2 snRNA-associated B″ antigen (GenBank accession no. M15841); m8rrm2 is the C-terminal *mec-8* RRM; and u1a1 is the N-terminal RRM of the human U1 snRNA-associated A protein (GenBank accession no. X06347). For the core consensus sequence proposed for RRMs (Birney et al., 1993), x, any residue; U, uncharged residues: L, I, V, A, G, F, W, Y, C, M; Z, U + S, T. The consensus was based in part on the known tertiary structure of u1a1, and the segments of β-sheet and α–helix secondary structural elements and intervening loops (for u1a1) are indicated. Numbering is relative to position in the RRM, not to total protein sequence.

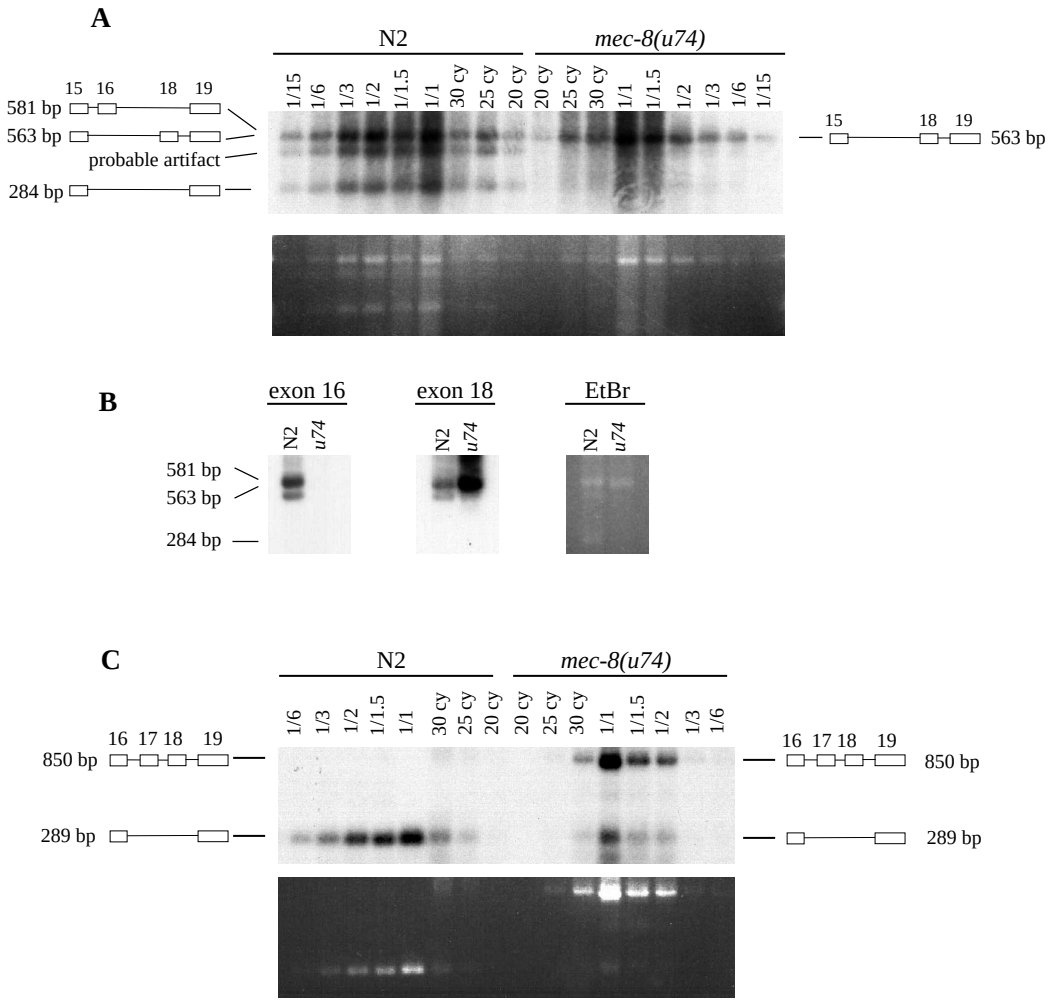

Fig. 4. RT-PCR analysis of alternatively spliced *unc-52* transcripts. (A) RT-PCR experiment using a 15/19 primer pair. The upper panel is an autoradiogram of a Southern blot of cDNAs produced from RT-PCR, using a 15/19 primer pair. The cDNAs were hybridized to a radioactively labeled genomic fragment that extends from exon 15 through exon 19. Various dilutions of the RT reaction (relative dilutions from 1 to 1/15) were used for the PCR. Aliquots of the PCR reaction from the least-diluted sample of the RT were removed at 20, 25, 30 and 35 cycles of amplification. All other samples were amplified for 35 cycles. Diagrams to the sides of the blot represent the compositions and sizes of the amplified alternatively spliced products (boxes are exons included in the cDNA). The lower panel is a photograph of the ethidium bromide-stained agarose gel used to produce the Southern blot. (B) Autoradiograms of a Southern blot of the 15/19 RT-PCR products probed with radioactively labeled oligonucleotides specific to exons 16 and 18. None of these bands hybridized to a probe specific to exon 17 (data not shown). A photograph of the ethidium bromide-stained agarose gel that was used to produce the Southern blot is presented at the right. (C) RT-PCR experiment using a 16/19 primer pair. The upper panel is an autoradiogram of a Southern blot of cDNAs produced from RT-PCR with the 16/19 primer pair hybridized to a radioactively labeled probe of the exon 15-18 genomic region. Dilutions and numbers of cycles of amplification were the same as described for A. The lower panel is a photograph of the ethidium bromide-stained agarose gel used to produce the Southern blot.

the exon 18-specific probe only (Fig. 4B), indicating that the *mec-8* band is largely if not exclusively composed of the 15-18-19 splice product, i. e., the 16-19 splice product, along with the 15-19 product, appears to be greatly reduced in the *mec-8* mutant. An exon 17 probe, which should have recognized a 15-17-19 product (566 bp), hybridized to neither the N2 nor the *mec-8* bands. A third N2-specific band, at about the 500-bp position, was an artifact of PCR amplification, since it was amplified in N2 when the exon 15 primer alone was used.

In a second set of RT-PCR experiments, a set of primers specific for exons 16 and 19 was used. Consistent with the results for the 15/19 primer pair, the 16-19 splice product was reduced in *mec-8* animals compared to N2 (Fig. 4C). This result was also highly reproducible. We performed 12 additional experiments with the same primers, each starting with independent RNA preparations and using 35 cycles of PCR amplification. In every case, N2 showed a strong band corresponding to the 16-19 splice product and *mec-8* showed either no band or a weak band. The results for the larger PCR products were less reproducible. A 16-17-18-19 product (apparent in Fig. 4C) was often found for *mec-8* and sometimes appeared, generally as a weak band, in the case of N2. The 15-16-17-18-19 product was generally not detected with the 15/19 primer pair for either N2 or *mec-8*, probably because it was too large to amplify under our PCR conditions. Finally, PCR with a 17/19 primer pair yielded for both N2 and *mec-8* a band corresponding to a 17-18-19 splice product (569 bp) and a very weak band corresponding to a 17-19 product (290 bp) (data not shown).

In summary, our RT-PCR experiments showed that the 15-19 and 16-19 splice products were reduced in abundance in *mec-8* animals compared to wild-type animals. To confirm these results, we have used RNase protection to monitor splices involving exon 19. An RNA probe complementary to the last 29 nucleotides of exon 18 and the first 200 nucleotides of exon 19 was used. A 229-nt fragment of this probe should be protected by an 18-19 splice product, and a 200-nt fragment should be protected by a transcript containing exon 19 not

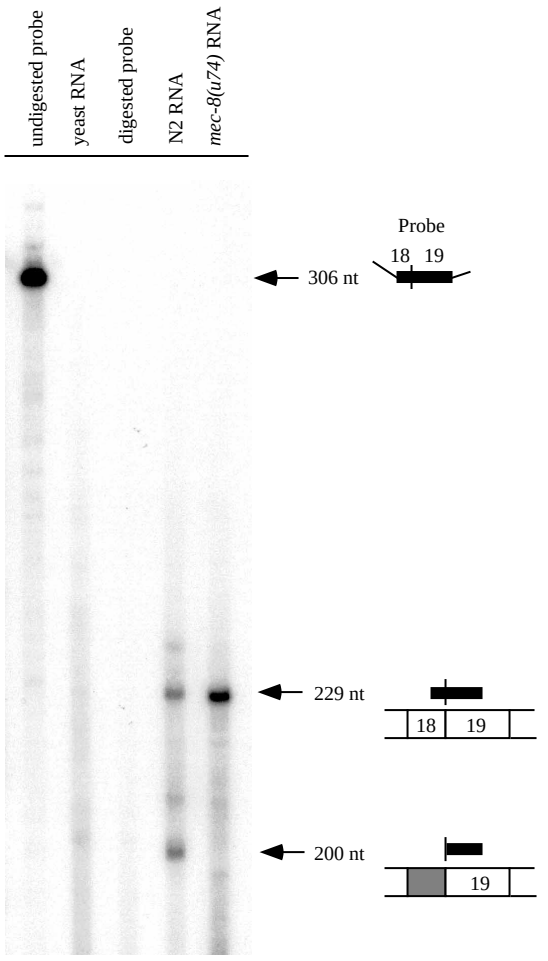

Fig. 5. RNase protection assay of alternatively spliced *unc-52* transcripts. The RNA probe was generated from a *Bam*HI/*Sac*I restriction fragment of an *unc-52* cDNA containing exons 18 and 19. The probe contained 79 nt of pBluescript SK$^-$ vector sequence (indicated by thin lines in the probe diagram), 29 nt of antisense to exon 18 and 200 nt of antisense to exon 19. Sizes of fragments on the gel were determined by reference to a set of sequencing reactions run in parallel. Boxes represent *unc-52* exons; the shaded box represents an exon other than exon 18 spliced to exon 19. Heavy lines above the diagrammes of the exons represent protected fragments of probe.

spliced to exon 18. The prediction from the RT-PCR experiments was that *mec-8* animals should exhibit less of the 200-nt band than wild-type, owing to reduced 15-19 and 16-19 splicing. The results agreed with this prediction (Fig. 5). This experiment was performed with four independent preparations of both N2 and *mec-8* mixed stage RNA, with essentially the same results. We conclude that the sum of the levels of 15-19, 16-19 and 17-19 splice products relative to 18-19 splice product is much lower in *mec-8* animals than in N2.

mec-8 regulates the accumulation of an UNC-52 isoform

The DNA sequence alterations responsible for six *unc-52(viable)* mutations have been determined (Rogalski et al., 1995): all are situated in the interval containing the three alternatively spliced exons 16, 17 and 18. Two are nonsense mutations in exon 17, and two are nonsense mutations in exon 18, and for each of these classes of mutant, an exon 19-encoded epitope accumulates in the basement membrane adjacent to body wall muscle in embryos (Rogalski et al., 1995). We conclude that some exon 19-containing mRNA that is translated into UNC-52 in these animals lacks exons 17 and 18 as a consequence of alternative splicing. From the results of our RT-PCR and RNase protection experiments, we predict that the accumulation of at least some of this mRNA, and hence its protein product, is regulated by MEC-8. We tested this prediction by staining *mec-8; unc-52(e444)* embryos with a monoclonal antibody, MH2/3 (Francis and Waterston, 1991), that recognizes an UNC-52 epitope encoded by exon 19 (Rogalski et al., 1993). MH2/3 staining in wild-type embryos is detected prior to the 1.5-fold stage of elongation and thereafter (Hresko et al., 1994; Moerman et al., 1996). The *unc-52(e444)* allele is a nonsense mutation in exon 18 and alone has little effect on MH2/3 staining in embryos (Rogalski et al., 1995; Fig. 6L). Mutation in *mec-8* by itself also has no apparent effect on MH2/3 staining (Lundquist and Herman, 1994), as expected, since exon 19-containing products are formed in *mec-8* animals. The *mec-8; unc-52(e444)* double mutant, however, which arrests embryonic elongation at the twofold stage, exhibits no detectable MH2/3 staining (Fig. 6K), presumably because exon 18 is not removed by alternative splicing in the absence of MEC-8, and so the nonsense mutation in exon 18 terminates translation of most exon 19-containing transcripts. The *mec-8; unc-52(e444)* embryos are similar to *unc-52* null embryos both in their arrest phenotype and in their inability to organize body muscle (Rogalski et al., 1993; Williams and Waterston, 1994; Lundquist and Herman, 1994), as shown by staining with antibody to myosin heavy chain A (Fig. 6D,F). Unlike *unc-52* null mutant embryos, however, *mec-8; unc-52(e444)* embryos do express at least one UNC-52 isoform. The polyclonal antibody GM1 recognizes a region of UNC-52 that is common to all isoforms and in wild-type animals stains the basement membranes associated with the pharynx and anal muscles as well as the body wall muscles (Moerman et al., 1996; Fig. 6B). The *unc-52(st549)* null mutant embryos show no staining with GM1 (Moerman et al., 1996); *mec-8; unc-52(e444)* embryos show normal pharyngeal and anal muscle staining but greatly reduced body wall staining with GM1 (Fig. 6D,E,J). We conclude that *mec-8* regulates the accumulation of a specific subset of one or more UNC-52 isoforms. These isoforms contain an epitope encoded by exon 19, lack the exon 18-encoded immunoglobulin repeat and are localized to basement membrane adjacent to body wall muscle.

DISCUSSION

The putative *mec-8* protein contains two copies of an RNA recognition motif (RRM) that corresponds to a well-characterized RNA-binding domain (Kenan et al., 1991; Birney et al., 1993; Burd and Dreyfuss, 1994). Many RNA-binding proteins contain one or more RRMs. These include proteins that bind pre-mRNA, poly(A), hnRNA, pre-ribosomal RNA and small nuclear RNAs (snRNA). Some RRM-containing proteins have been shown to play roles in pre-mRNA processing. Two that have been shown to bind pre-mRNA and affect specific patterns of alternative splicing are the products of the

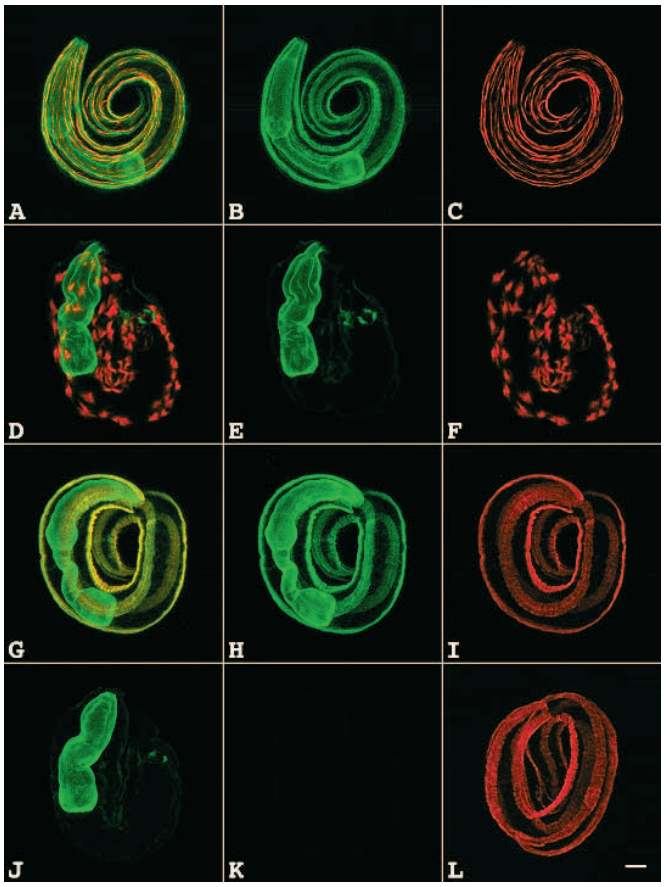

Fig. 6. mec-8 controls the accumulation of an UNC-52 isoform. Embryos were visualized by confocal immunofluorescence microscopy. Embryos in A-F were double labeled with antibodies GM1 (green, FITC), which recognizes a region common to all UNC-52 isoforms, and DM5.6 (red, TRSC), which recognizes the body wall myosin heavy chain A. A and D show both channels simultaneously; B,C,E and F show single channel images. (A-C) A wild-type embryo; (D-F) An arrested *mec-8(mn463); mnC1 dpy-10 unc-52(e444)* embryo. Embryos in G-K were double labeled with GM1 (green, FITC) and MH2/3 (red, TRSC), which recognizes an epitope encoded by exon 19 of *unc-52*. G shows both channels simultaneously; H-K show single channel images, with the channel in K for MH2/3 staining. (G-I) A wild-type embryo; (J-K) An arrested *mec-8(mn463); mnC1 dpy-10 unc-52(e444)* embryo. (L) A *dpy-10 unc-52(e444)* embryo stained with MH2/3. While the *mec-8; unc-52* embryos are arrested at the twofold stage of elongation, they are comparable in age to the three-fold embryos of other genotypes. Wild-type, *mec-8(mn463)* and *unc-52(e444)* embryos exhibited staining with MH2/3 before the 1.5-fold stage of elongation, but the *mec-8; unc-52(e444)* double mutants showed no significant staining. Scale bar, 10 μm.

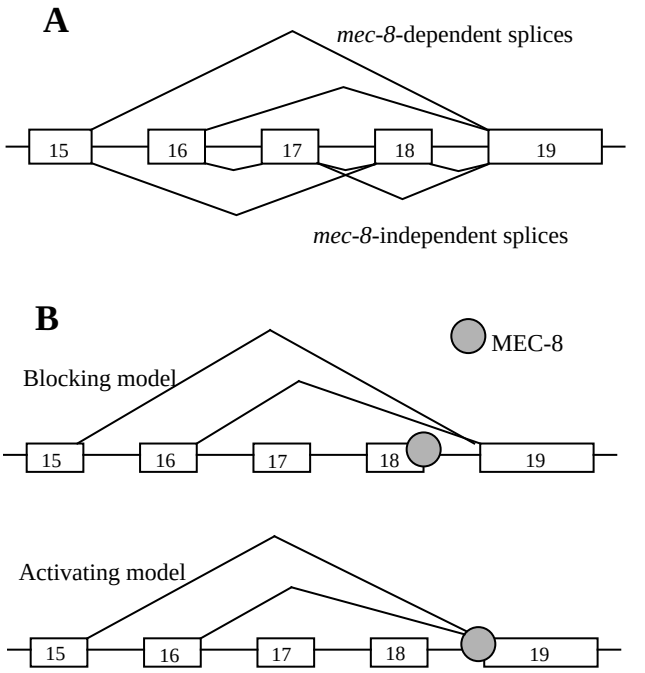

Fig. 7. Action of *mec-8* on alternative splicing of *unc-52* transcripts. (A) The wild-type accumulation of products in which 15-19 and 16-19 splices occurred (diagrammed above the row of exons) is dependent on *mec-8(+)*, whereas the splices diagrammed below the exons appear to be independent of *mec-8(+)* function. We have not assayed for all possible splices in this region; in earlier work, 15-16, 15-17 and 16-18 splices were detected in wild-type animals (Rogalski et al., 1995). (B) MEC-8 might act by partially blocking the 18-19 splice, which could indirectly increase the incidence of 15-19 and 16-19 splices. Alternatively, MEC-8 could actively promote the 15-19 and 16-19 splices.

Drosophila genes *Sex-lethal* (*Sxl*) (Inoue et al. 1990; Valcárcel et al., 1993; Horabin and Schedl, 1993; Wang and Bell, 1994) and *transformer-2* (*tra-2*) (Hedley and Maniatis, 1991; Tian and Maniatis, 1992, 1993; Inoue et al., 1992; Amrein et al., 1994). The *snf* gene of *Drosophila* encodes an RRM protein that regulates *Sxl* splicing (Flickinger and Salz, 1994). Additional RRM-containing *Drosophila* proteins, including the proteins encoded by the genes *embryonic lethal abnormal visual system* (*elav*) (Robinow et al., 1988), *no-on-transient-A* (Rendahl et al., 1992), *couch potato* (Bellen et al., 1992) and *musashi* (Nakamura et al., 1994), have been shown, by mutant analysis, to affect nervous system development. It has been proposed that these proteins affect pre-mRNA splicing, but their targets have not been identified. In addition to containing RRMs, the *elav*, *couch potato* and *musashi* proteins share with MEC-8 regions of very high alanine and glutamine composition.

mec-8 regulates the alternative splicing of *unc-52* pre-mRNA

We have shown that a *mec-8* mutation reduces the abundance of two *unc-52* alternatively spliced transcripts: those involving splicing of exon 15 to 19 and of exon 16 to 19 (Fig. 7A). Given the sequence of *mec-8*, we favor the idea that MEC-8 protein binds to *unc-52* pre-mRNA and promotes 15-19 and 16-19 splicing. The accumulation of other alternatively spliced *unc-52* transcripts appears to be *mec-8*-independent (Fig. 7A).

Studies of the regulation of alternative splicing in the *Drosophila* sex determination pathway have shown that *trans*-acting splicing factors can either block a splice site donor or acceptor or can activate an otherwise disfavored splice site. Thus, the *Sxl* gene product blocks the use of a splice donor site in its own pre-mRNA (Horabin and Schedl, 1993; Wang and Bell, 1994) and blocks a splice acceptor site in *transformer*

(*tra*) pre-mRNA (Sosnowski et al., 1989; Inoue et al., 1990; Valcárcel et al., 1993), and *tra* and *tra-2* gene products together activate the use of a suboptimal splice site acceptor of a *doublesex* transcript (Ryner and Baker, 1991; Hedley and Maniatis, 1991; Inoue et al., 1992; Tian and Maniatis, 1992, 1993). In all of these examples, alternative splicing provides a switching mechanism in which a functional product either is or is not produced.

We suggest that MEC-8 may block the splice site donor immediately downstream of exon 18, with the result that the exon 19 splice site acceptor would then favor splicing to exon 15 and 16 donors (Fig. 7B). An alternative model would involve MEC-8 activating 15-19 and 16-19 splicing by, for example, making the exon 19 acceptor more attractive to the exon 15 and 16 donors (Fig. 7B). In either of these models, the *mec-8*-independent splices, including the 18-19 splice, must also occur. It is possible that the *mec-8*-dependent and independent splices are temporally restricted; it seems more likely, however, that the alternative splices occur simultaneously in the same cells, such that a set of different functional products are simultaneously produced. This might be achieved by careful regulation of the concentration of MEC-8. Another possibility is that some *unc-52* transcripts may be inaccessible to MEC-8.

Effects of *mec-8* and *unc-52* mutations on body wall muscle function

Null mutations in *unc-52* result in unorganized sarcomere structure, paralysis and arrest of embryonic elongation at the twofold stage (Rogalski et al., 1993). The defect in sarcomere organization is consistent with a model in which UNC-52 is required to initiate myofilament lattice assembly at the muscle membrane (Hresko et al., 1994). The paralysis and embryonic arrest are characteristic of many mutants that lack body wall muscle function (Williams and Waterston, 1994).

The late-appearing body muscle defects in *unc-52(viable)* animals, which lack only a subset of UNC-52 isoforms (those translated from messages containing exons 16, 17 or 18), could be due either to the absence of specific UNC-52 isoforms or simply to the reduction in total functional UNC-52 in the basement membrane adjoining body muscle (Rogalski et al., 1995); as animals increase in size during later larval growth, the overall level of UNC-52 protein may become critical, either for providing new sites for initiation of muscle assembly and attachment or for increasing the strength of pre-existing muscle attachments.

In *unc-52(+)* animals, the MEC-8-dependent *unc-52* splice products are not absolutely essential for embryogenesis, since *mec-8* mutants are capable of normal muscle development and embryogenesis. At low temperature (16˚C), however, about 30% of *mec-8* null mutants arrest as embryos or larvae with muscle attachment defects (Lundquist and Herman, 1994). The muscle defects are distinct from those of *unc-52(viable)* animals in that they are apparent during embryogenesis and do not appear to increase in severity during late larval development. We suggest that specific *mec-8*-dependent UNC-52 isoforms, such as those generated from messages carrying 15-19 and 16-19 splices, are required to facilitate embryonic muscle development at low temperature. Another possibility is that the cold-sensitive muscle defects in *mec-8* animals are due to the effects of *mec-8* on the splicing of a gene other than *unc-52*.

To explain the *mec-8; unc-52(viable)* synthetic lethal phenotype, which is very similar to the phenotype of *unc-52* null mutants, we propose that *unc-52(viable)* mutations result in an unconditional embryonic requirement for *unc-52* mRNA lacking exons 16, 17 or 18, and we propose that MEC-8, which promotes 15-19 and 16-19 splicing of *unc-52* transcripts, is essential for formation of sufficient levels of these mRNAs. According to this view, the UNC-52 isoforms dependent on MEC-8 function and the UNC-52 isoforms affected by *unc-52(viable)* mutations provide partially overlapping functions: the absence of either leads to somewhat different body muscle defects, but the absence of both is unconditionally lethal. In agreement with this view, both *mec-8* and *unc-52(e444)* mutants accumulate UNC-52 isoforms containing exon 19-encoded epitope beginning prior to the 1.5-fold stage of embryonic elongation, but the *mec-8; unc-52(e444)* arrested embryos accumulate very little if any such isoform, although they do accumulate at least one other UNC-52 isoform.

Role of *mec-8* in processing transcripts of other genes

In addition to their effects on body wall muscle, mutations in *mec-8* result in defects in the function of mechanosensory (Chalfie and Sulston, 1981; Chalfie and Au, 1989) and chemosensory neurons (Perkins et al., 1986). These defects appear to be unrelated to *unc-52* function because viable *unc-52* mutants exhibit a wild-type response to light touch response and show normal dye filling of chemosensory neurons (unpublished data). In addition, staining of wild-type animals with the GM1 antibody has provided no evidence that any UNC-52 isoform is localized near the implicated mechanosensory or chemosensory neurons (G. P. Mullen and D. G. Moerman, unpublished data; Fig. 6). We thus propose that *mec-8* regulates the processing of transcripts from one or more additional genes, which play roles in mechanosensation and chemosensation. Identification of additional substrates of *mec-8* action would allow us to address the interesting question of how a *trans*-acting regulator of alternative RNA processing regulates the splicing of different target pre-mRNAs.

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