mau-2 acts cell-autonomously to guide axonal migrations in *Caenorhabditis elegans*

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Summary

The gene *mau-2* has been found to be required for the guidance of cellular and axonal migrations along both the anteroposterior and the dorsoventral body axes during the development of the nematode *C. elegans*. We show that *mau-2* encodes a novel, previously uncharacterized protein that is highly conserved among animals. Maternal *mau-2* gene expression is sufficient for normal development until the fourth larval stage, and a MAU-2::GFP fusion protein localizes to the cytoplasm of neurones. *mau-2* is ubiquitously expressed in embryos by late gastrulation and becomes predominantly expressed in the nervous system as morphogenesis progresses. Expression of *mau-2* within individual neurones rescues the guidance defects of *mau-2* mutants, indicating that *mau-2* functions cell-autonomously. Altering the activity of both the dorsal repellent *slt-1* and *mau-2* leads to the abnormal dorsal projection of the AVM axon, a phenotype that is novel and specific to the interaction of these two genes, indicating that *mau-2* participates in the guidance of AVM by a *slt-1*-independent mechanism. Taken together, *mau-2* defines a novel guidance factor that might be involved in the intracellular processing of guidance cues encountered by migrating cells and axons during development.

Key words: *mau-2*, Migration, Guidance, *slt-1, slit, Caenorhabditis elegans*, Maternal effect, Cell autonomous

Introduction

Understanding how nervous systems develop is a central issue in biology. Genetic studies using *C. elegans* have yielded remarkable insights into the processes of neuronal differentiation, including how migrations are guided and how synaptic connectivity is established. Although most cells of the worm are born close to their final positions as a result of cell division and differentiation, a number of neurones and their growth cones, as well as mesodermal cells, undergo stereotyped long-range migrations during development (Hedgecock et al., 1987). Neurones migrate between the hypodermal cell membrane and the basement membrane secreted by the hypoderm, and mesodermal cells migrate along the basement membrane on the side facing the pseudocoelomic cavity.

A number of genes that are known to be required for proper migration in *C. elegans* were identified through screens for behavioural mutants (Brenner, 1974), the subsequent characterization of which revealed specific neuroanatomical defects (e.g. Hedgecock et al., 1990; Hedgecock et al., 1985; McIntire et al., 1992). Other screens have used surrogate markers such as transparent larvae or withered tails (Forrester et al., 1998; Wightman et al., 1997). Yet more genes were identified through screens for mutants that affect the position of particular neurones and axons, regardless of their behavioural consequences, such as *sax-3*, which affects the development of the nerve ring (Zallen et al., 1998), and *nid-1*, which affects the position of nerve bundles (Kim and Wadsworth, 2000).

The molecular characterization of some of these genes has revealed that a wide range of proteins contribute to the proper development of the nervous system of the worm (reviewed by Antebi et al., 1997; Blelloch et al., 1999). Some proteins act as guidance cues or as receptors for these guidance cues, other are components of the extracellular matrix or regulate cell adhesion, and yet other proteins participate in the cellular response to guidance cues. A number of proteins required for the guidance of migrations are novel, such as MIG-13 (Sym et al., 1999), CED-12 (Gumienny et al., 2001), UNC-53 (Stringham et al., 2002), VAB-8 (Wolf et al., 1998), and UNC-76 (Bloom and Horvitz, 1997), and their biochemical functions remain unknown.

Most of the genes required for the development of the nervous system identified so far are zygotic. In an attempt to identify genes involved in the earliest stages of the patterning of the nervous system, a genetic screen for maternal-effect behavioural mutants was carried out (Hekimi et al., 1995). Three mutations (*qm4*, *qm5* and *qm40*) identified in this screen define the gene *mau-2 (mau* stands for maternal-effect uncoordinated).

mau-2 mutants are severely uncoordinated and egg-laying defective, and these phenotypes are completely penetrant (Hekimi et al., 1995; Takagi et al., 1997). In addition, approximately one-fifth of the larvae fail to complete development and become filled with fluid (the Lid phenotype). At the anatomical level, *mau-2* mutants display defects in the

positions of cell bodies and processes that belong to the locomotory, egg-laying and osmoregulation systems. In fact, numerous cells that undergo long-range migrations during the development of the worm are misplaced in *mau-2* mutants, including neurones (e.g. AVM, PVM, SDQR/L and HSNR/L), axons (e.g. of AVM, PLM, and CAN), as well as the distal tip cells (DTCs) (Takagi et al., 1997). *mau-2* mutations affect migrations that occur embryonically as well as post-embryonically. For example, the embryonic migration of the cell body of HSN and the extension of the axons of PLM and CAN, are affected in *mau-2* mutants. In addition, the extension of the axon of AVM, the projection of motoneurone axons into the dorsal cord, the migration of the Q5 descendants and of the DTCs, all of which occur during larval development, are abnormal in *mau-2* mutants. In addition, migrations that occur along both the longitudinal and the circumferential body axes of the worm, oriented anteriorly-, posteriorly-, ventrally- or dorsally-, are all affected in *mau-2* mutants. Thus, rather than being involved in a specific subset of migrations, *mau-2* is broadly required for the proper migration of cells and axons.

Importantly, migrating cells frequently undergo excessive migrations in *mau-2* mutants, such that their final position is beyond the target region where the migration should have halted. For example, the SDQR and HSN neurones, which undergo anteriorly directed migrations during development, are frequently placed too anteriorly in *mau-2* mutants (Takagi et al., 1997). In addition, the axon of AVM, which normally projects ventrally, can instead extend posteriorly for long distances in *mau-2* mutants. Thus, mutations in *mau-2* do not impair the motility of the cells per se, but rather affect the proper guidance of these migrations. Here, we report that *mau-2* encodes a novel protein that functions cell autonomously to guide migrations during the development of the nervous system.

Materials and methods

Nematode strains

Worms were grown as described (Brenner, 1974) at 20°C unless otherwise specified. The wild-type strain was N2. *mau-2* alleles (*qm4, qm5, qm40* and *qm160*) were outcrossed at least five times. Strains used were:

che-3(e1124), *dpy-5(e61)*, *fer-1(hc1)*, *lin-10(e1439)*, *unc-13(e51)*, *unc-29(e1072)*; *unc-55(e402)*, *glp-4(bn2ts)*, *fem-2(b245ts)*, *fem-3(q20ts)*, *unc-6(e78)*, *unc-6(ev400)*, *slt-1(eh15)*, *unc-3(e151)*;

zdIs4 IV (an integrant of *mec-4::gfp*, a gift from Dr Clark and Dr Bargmann);

kyIs218 X and *kyIs209 X* [two integrants of *myo-3::slt-1* misexpressing *slt-1* in all body wall muscles (a gift from Dr Bargmann) (Yu et al., 2002)];

mgIs71 (an integrant of *tph-1::gfp*) (Sze et al., 2000);

unc-31::gfp (Takagi et al., 1997);

and see below.

Hermaphrodites were injected with cosmids, deletion constructs and pRF4 (Mello and Fire, 1995) at concentrations of 5, 50 and 100 ng/μl, respectively. Insert orientation in cosmid C09H6 was determined by restriction mapping.

Neuroanatomical observations

The position of neurones, axons and fascicles was examined using the reporter *unc-31::gfp*, which is expressed in virtually all neurones and in vulval muscles (Takagi et al., 1997); the axon of HSN, using *tph-1::gfp* [*mgIs71*; (Sze et al., 2000)]; AVM, using the *mec-4::gfp* reporter. In tissue-specific rescue assays, plasmids for *mec-4::gfp* or *unc-31::gfp* was injected at 100 ng/μl. Examination of maternally rescued animals was carried out using an integrated *unc-31::gfp*. For the analysis of genetic interactions with *unc-40::myr* and *slt-1*, *zdIs4* was used. The morphology of the axon of AVM was scored only when the position of the AVM cell body was normal.

Molecular analysis

General molecular manipulations were carried out as described (Sambrook et al., 1989). *Pfu* polymerase was used for PCR (Stratagene). All cloned inserts were sequenced.

mau-2 transcript

SL1- or SL2-specific primers, combined with primers specific for predicted exons, were used on two independent first-strand mixed-stages cDNA libraries. RT-PCR products and yk462a (gift of Dr Kohara) were sequenced. *mau-2* encodes a single message of 2010 bp (not including SL1, nor the poly A tail). The 5′UTR is SL1 transpliced at the acceptor site CAG, 6 bp upstream of the initiating ATG. The polyadenylation signal is probably AAAAAA, located 211 bp downstream of the TAA Stop codon. The polyadenylation cleavage site is 8 bp downstream of the polyadenylation signal, resulting in a 222 bp long 3′UTR. yk462a lacks the 5′UTR and the first 9 coding base pairs, and part of the 3′UTR.

mau-2 mutant alleles

A library of UV-TMP-mutagenized worms was screened by nested PCR with primers specific for the gene *mau-2* and *qm160* was isolated (Edgley et al., 2002). The primer sequences correspond to the following bases on cosmid C09H6: 27984-28007, 32138-32161, 27507-27530 and 31892-31915. *qm160* fails to genetically complement *qm5*.

The region of *mau-2* was sequenced on two independent PCR products amplified from genomic DNA of N2, *qm4*, *qm5*, *qm40* and *qm160*.

Northern blot analysis

Worm populations were synchronized at different developmental stages as described (Wood, 1988). Total RNA was isolated using Trizol (Gibco). A *mau-2*-specific probe made by ^{32}P-radiolabelling (Ready-to-go kit, Pharmacia) using the entire insert of yk462a was used for northern blotting.

Anti-MAU-2 polyclonal antibodies and western analyses

A BSA-conjugated peptide located (ASRRMLSVENLTPL-VANMPASK, residues 552-573 near the C terminus of MAU-2) was synthesized (Sheldon Center, McGill University) and injected into two rabbits (Animal Resources Center, McGill University). Polyclonal antibodies were affinity purified from crude serum on an Affigel column (BioRad) (Harlow and Lane, 1999). The purified antibodies detect the conjugated antigen of an expected size of 70 kDa, free peptide at ~2.5 kDa, a band of the expected size of MAU-2 (~68 kDa) in N2 but not in *mau-2(qm160)* extracts, and a band at ~68 kDa in *mau-2(qm160)* mutants carrying wild-type copies of *mau-2* on an extrachromosomal array. Few other non-specific bands were detected.

Total protein extracts were prepared by grinding. Affinity purified anti-MAU-2 antibody (see below) was used at a 1:5 dilution, and donkey anti-rabbit IgG antibody (Jackson Laboratories) at 1:1000, followed by detection with ECL (Amersham). Analysis MAU-2 in maternally rescued animals: 400 Dpy worms for each genotype and stage were picked, and denatured in sample buffer.

Reporter constructs for *mau-2*

For translational fusions between *mau-2* and *gfp*, synthetic *Pst*I and *Bam*HI sites on primers allowed directional cloning of bases 27054-32154, 24119-32154, or 22063-32154 of C09H6 into pPD95.77.

Constructs for tissue-specific expression of *mau-2*

Extrachromosomal arrays carrying *mau-2(+)* do not give maternal rescue (non-transgenic worms derived from a transgenic *mau-2* hermaphrodite are invariably Mau). In addition, a promoterless transgene containing the entire *mau-2* cDNA (pCB42) cannot rescue the *mau-2* mutants.

pCB42 contains the entire *mau-2* cDNA from the ATG to the last codon, excluding the Stop, fused in frame to *gfp*. yk462a was the template, and the missing 5′UTR, along with a *Pst*I site was added on the 5′ primer. The 3′ primer contained a synthetic *Bam*HI site for cloning into pPD95.77.

Plasmids were obtained by cloning promoters in front of *mau-2* in pCB42**.** $P_{dpy\text{-}7}$*::mau-2::gfp* contains a 0.9 kb fragment corresponding to the promoter of the gene *dpy-7* (Gilleard et al., 1997); $P_{myo\text{-}3}$*::mau-2::gfp,* a 2.5 kb fragment for the *myo-3* promoter (Okkema et al., 1993); $P_{unc\text{-}119}$*::mau-2::gfp,* a 1.2 kb region for the *unc-119* promoter (Maduro and Pilgrim, 1995); $P_{mec\text{-}7}$*::mau-2::gfp*, a contains a 0.8 kb fragment for the *mec-7* promoter (Hamelin et al., 1992).

These constructs were injected into N2 and *qm160* at 10 ng/μl, along with pRF4 (100 ng/μl) and *mec-4::gfp* or *unc-31::gfp* at 100 ng/μl.

Results

Larval and locomotory defects but not egg-laying defects of *mau-2* mutants can be maternally rescued

mau-2 mutants are severely uncoordinated and egg-laying defective, and these phenotypes are completely penetrant (Hekimi et al., 1995; Takagi et al., 1997). In addition, approximately one-fifth of the larvae fail to complete development and become filled with fluid (the Lid phenotype). *mau-2* mutants were recovered in a screen for mutations that displayed a maternal rescue effect (Hekimi et al., 1995), i.e. homozygous mutant animals issued from a heterozygous mother appear phenotypically wild type (maternally rescued animals are denoted as m^+z^- throughout the paper). The maternal effect is not strict: heterozygous animals derived by mating from a homozygous mother are fully wild type. Several aspects of the phenotype of *mau-2* mutants can be completely rescued maternally, while others aspects are not. For example, maternally rescued *mau-2* animals complete larval development normally and have a wild-type locomotion, but they remain severely egg-laying defective (Takagi et al., 1997). This failure of maternal rescue of the egg-laying defect points to a role of *mau-2* in the development of the relevant structures. As the egg-laying system develops relatively late (L3 and L4 larval stages), it is conceivable that the maternally supplied product has run out by that time and that these structures develop abnormally. Alternatively, *mau-2* could be required for the function of the neurones and muscles required for egg laying in adults, rather than for the development of these structures per se.

To gain insight into the function of *mau-2*, we determined to what extent the development of m^+z^- animals proceeds normally. We examined *mau-2(qm5)* maternally rescued animals at the anatomical level using the reporter *unc-31::gfp*, which allows the visualization of the nervous system and the vulval muscles. In contrast to *mau-2(qm5)* mutants, the nervous system of m^+z^- is virtually indistinguishable from that of the wild type: the ventral nerve cord, the commissures and laterals are normally organized, the dorsal cord is always fasciculated, and the cell body of migratory neurones such as AVM, SDQR and HSN, as well as of the DTCs, are normally positioned (Fig. 1). The defect of the axon of AVM is also completely maternally rescued. Thus, the parts of the nervous system that underlie locomotion develop normally in maternally rescued animals. As the excretory and locomotory systems are laid down largely during embryogenesis or shortly after hatching (L1 and early L2 larval stages), it appears that sufficient wild-type *mau-2* product can be supplied by the mother for the early development of its progeny to occur normally. However, maternally rescued *mau-2* animals display anatomical defects in their egg-laying system, consistent with their abnormal egg laying. In particular, at least one out of the four vm1 vulval muscles is abnormally placed in 24% of the maternally rescued animals (Fig. 1). In addition, the axon of one of the two HSN neurones projects abnormally in 21% of maternally rescued animals, often extending laterally from the cell body first, and projecting ventrally at a position anterior to the vulva, instead of posterior to it. Other aspects of the egg-laying system, such as the placement of the vm2 vulval muscles and uterine muscles, which are more difficult to examine, might also be affected in *mau-2* m^+z^- animals, possibly accounting for the completely penetrant egg-laying defect.

Positional cloning of *mau-2*

mau-2 was previously mapped between *dpy-14* and *unc-29* on linkage group I, outside the chromosomal deficiency *nDf23*, but inside *nDf24* (Hekimi et al., 1995). We mapped *mau-2* to the right of *unc-55* (2.35 cM), tightly linked to *lin-10* (2.56 cM) and *che-3* (2.52 cM) (Fig. 2A, see Table S1 in the supplementary material). Physical mapping of the left end of the chromosomal deficiency *nDf23* (Whitfield et al., 1999), which genetically does not delete *mau-2*, indicated that *mau-2* lies to the left of *ceh-8*/T01G9.2. Cosmids covering the physical region spanning from *unc-55* to *ceh-8* were assayed for transformation rescue of *mau-2* mutants (Fig. 2B). Cosmid C09H6 and the deletion construct C09H6Δ*Nar*I fully rescued *mau-2* animals for larval development, locomotion and egg laying. A PCR fragment containing the only predicted gene on C09H6Δ*Nar*I fully rescued *mau-2* mutants. The gene C09H6.3, currently annotated in WormBase, corresponds to *mau-2* and encodes a predicted protein of 593 amino acids.

mau-2 mutations lead to a complete loss of function

We identified the molecular lesions that underlie the *mau-2* mutants. Allele *qm5* carries a nonsense mutation at codon 20, *qm40* has a nonsense mutation at codon 279, and *qm4* is a missense allele substituting a Gly to an Arg at codon 53 (Fig. 2C). To unequivocally determine the null phenotype of *mau-2*, we isolated a knockout allele in the gene *mau-2*, from a PCR-based screen for the isolation of deletions. Allele *qm160* deletes 1922 bp of the gene (bases 28330 to 30252 on cosmid C09H6). In *qm160*, codon 66 in exon 1 is joined to the last 54 bp of intron 5, introducing two in-frame Stop codons. A translated product, if any, would be at most 68 amino acids long. It is worth pointing out that a construct coding for the first 84 amino acids does not rescue any of the *mau-2* mutants (data not shown), making it unlikely that a translated product encoded by *mau-2(qm160)* would be functional. Therefore, *mau-2(qm160)* is a molecular null allele.

The mutant phenotype of all four *mau-2* alleles is identical

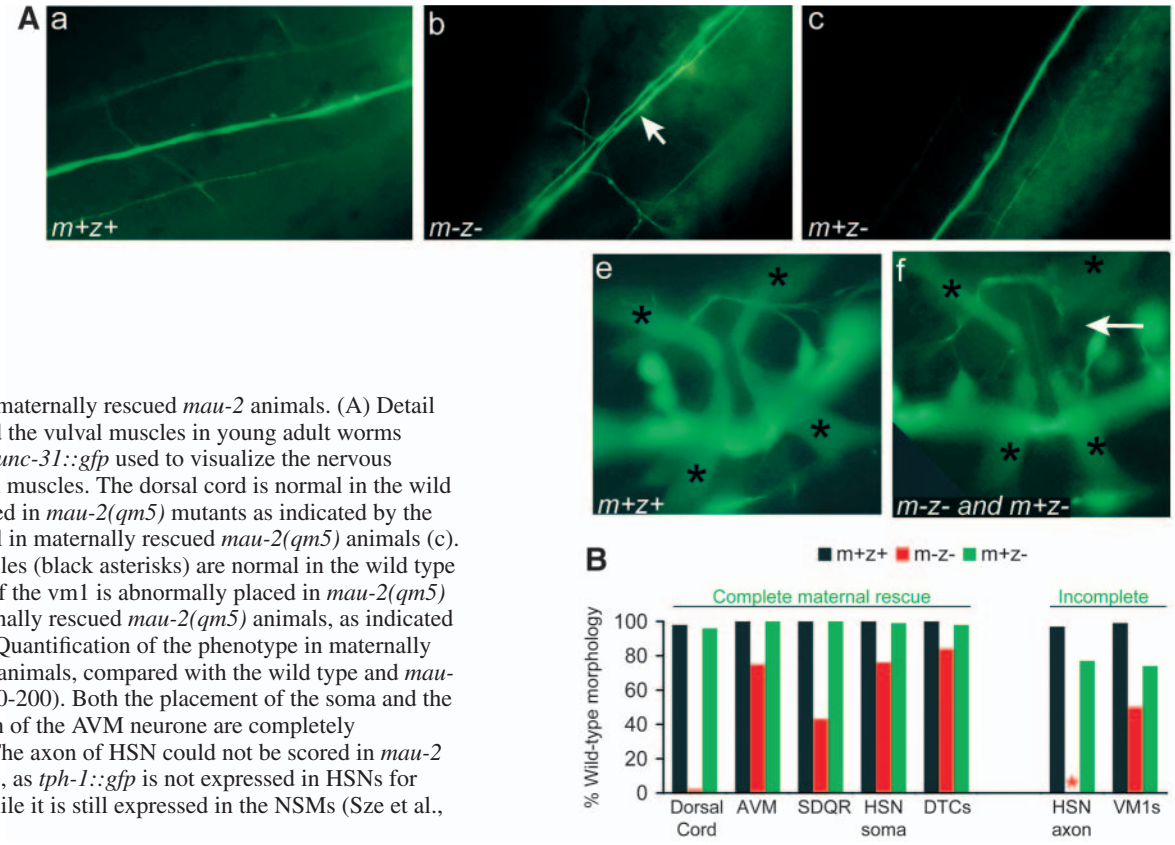


Fig. 1. Phenotype of maternally rescued *mau-2* animals. (A) Detail of the dorsal cord and the vulval muscles in young adult worms carrying the reporter *unc-31::gfp* used to visualize the nervous system and the vulval muscles. The dorsal cord is normal in the wild type (a), defasciculated in *mau-2(qm5)* mutants as indicated by the arrow (b), and normal in maternally rescued *mau-2(qm5)* animals (c). The vm1 vulval muscles (black asterisks) are normal in the wild type (e), and at least one of the vm1 is abnormally placed in *mau-2(qm5)* mutants and in maternally rescued *mau-2(qm5)* animals, as indicated by the arrow (f). (B) Quantification of the phenotype in maternally rescued *mau-2(qm5)* animals, compared with the wild type and *mau-2(qm5)* mutants (n=50-200). Both the placement of the soma and the projection of the axon of the AVM neurone are completely maternally rescued. The axon of HSN could not be scored in *mau-2* mutants (red asterisk), as *tph-1::gfp* is not expressed in HSNs for unknown reasons, while it is still expressed in the NSMs (Sze et al., 2000).

for phenotype penetrance and expressivity, at both the dissecting microscope and neuroanatomical levels. We have previously described the phenotype of *qm4*, *qm5* and *qm40* (Takagi et al., 1997), and now describe *qm160*. Like the other alleles, all *mau-2(qm160)* animals are uncoordinated and egg-laying defective (41% of the Egl animals actually become bags of worms, n=359), and 17% of the larvae acquire a straight and translucent appearance and die – the Lid phenotype [n=200 larvae (Takagi et al., 1997)]. In addition, the dorsal cord defasciculation defect is similarly affected in *qm160* compared with the other *mau-2* alleles and the penetrance of the defect of the guidance of the AVM axon is very similar to that of *qm5* and *qm4* [see below (Takagi et al., 1997)]. As all four *mau-2* alleles have identical phenotypes, it appears that they all result in a complete loss of the gene function.

We examined protein levels in *mau-2* mutants compared to the wild type (Fig. 3A). We developed affinity-purified polyclonal antibodies directed against MAU-2 and carried out immunoblot analysis on worm extracts. We believe that a band of ~68 kDa, the expected molecular weight for MAU-2, corresponds to the full-length MAU-2 protein as it is detected in wild-type worms, absent in the deletion allele *mau-2(qm160)*, and detected in *mau-2* mutants that carry wild-type copies of *mau-2*. MAU-2 is undetectable in the nonsense alleles *mau-2(qm5)* and *mau-2(qm40)*, indicating that they are likely molecular nulls, although we cannot exclude that undetected truncated proteins may be produced in these two nonsense mutants. Finally, the level of MAU-2 is strongly reduced in the mis-sense allele *mau-2(qm4)*, indicating that the *qm4* mutation severely affects the stability of MAU-2, and probably also the function of the remaining mutant protein, as this allele is as severe as the other *mau-2* mutants. Interestingly, the Gly residue that is mutated in *qm4* is conserved through evolution in all animals.

mau-2 encodes a novel highly conserved protein

Database searches for proteins homologous to *C. elegans* MAU-2 revealed that it is similar to predicted polypeptides across metazoans (Fig. 2D). Thorough database searches have uncovered only one protein homologous to MAU-2 in each species for which a complete genome sequence is available, suggesting that the genes encoding proteins of the MAU-2 family are orthologues. *C. elegans* MAU-2 and the related vertebrate proteins are 25% identical. MAU-2 is extremely well conserved among vertebrate species: the MAU-2 proteins are 95% identical between the fish *Fugu rubripes* and humans, and are more than 99% identical among mammals. Comparisons between the proteomes of *F. rubripes* and *H. sapiens* reveal that only 5% of the vertebrate proteins display more than 95% amino acid similarity (Aparicio et al., 2002), indicating that MAU-2 is among the best conserved proteins through vertebrate evolution.

Extensive searches using prediction programs detect no functional domains, conserved motifs or subcellular localization signals within *C. elegans* MAU-2, other than a tetratricopeptide-like domain (IPR008941, residues 485-495). A tetratricopeptide (TRP) repeat is detected in vertebrate MAU-2 proteins (IPR001440, residues 443-476 in *F. rubripes* and 459-492 in *H. sapiens*). TRP repeats occur in diverse proteins and are believed to mediate protein-protein

interactions and the assembly of multiprotein complexes. However, common features in the interaction partners remain undefined (D'Andrea and Regan, 2003; Lamb et al., 1995), providing no clue to what the biochemical function of MAU-2 might be. All four *mau-2* mutant alleles can be rescued with a transgene that carries the first five exons of *mau-2*, which code for a truncated protein from residues 1 to 371 of MAU-2. Thus, at least when overexpressed from an extrachromosomal array, this N-terminal region of MAU-2 is sufficient to carry out fully the identified functions of *mau-2*, suggesting that it is functionally the most important.

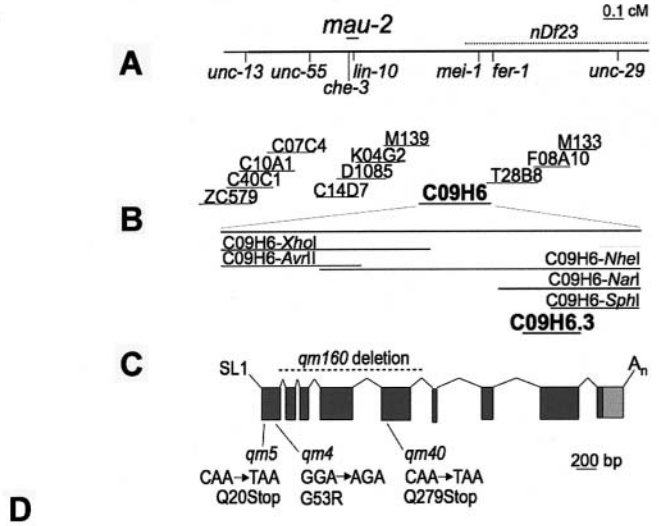
Abundant *mau-2* transcripts accumulate in the oocytes

We examined the abundance of the *mau-2* transcript throughout development in wild-type worm populations by northern blot analysis. A single band of 2 kb, which is the expected size for the *mau-2* mRNA, is detected at all stages (Fig. 3B). The *mau-2* transcript is abundant in the embryonic and young adulthood stages, whereas it is present in low amounts throughout the larval stages.

Given the maternal contribution of *mau-2* to development and the high transcript level in young adults, we investigated the *mau-2* mRNA levels in the germline by comparing wild-type young adults with adults carrying mutations that affect the germline (Fig. 3B). The *mau-2* transcript level is extremely reduced in *glp-4(bn2ts)* mutants, which fail to develop a germline at 25°C (Beanan and Strome, 1992), indicating that most of the *mau-2* mRNA in young adults is in the germline. Furthermore, the level of the *mau-2* transcript is very high in *fem-2(b245ts)* mutants that make only oocytes at 25°C, indicating that the oocytes are enriched with *mau-2* transcript. However, the *mau-2* transcript is very low in *fem-3(q20ts)* adults that make sperm only at 25°C, indicating that the *mau-2* transcript is not enriched in sperm. Taken together, these results indicate that oocytes accumulate high levels of *mau-2* mRNA, presumably as a store to be used by the embryo.

Consistent with our observations, in situ hybridization data reported in the Nematode Expression Pattern Database (http://nematode.lab.nig.ac.jp/) for clone yk462a, which corresponds to *mau-2*, show an intense signal in the germline of adults, particularly in the pachytene region, which is a major site of transcription in the germline (Lerner and Goldstein, 1988). In addition, a strong level of *mau-2* mRNA is detected at different embryonic stages, including as early as the two-cell stage.

We further characterized the expression pattern of *mau-2* by



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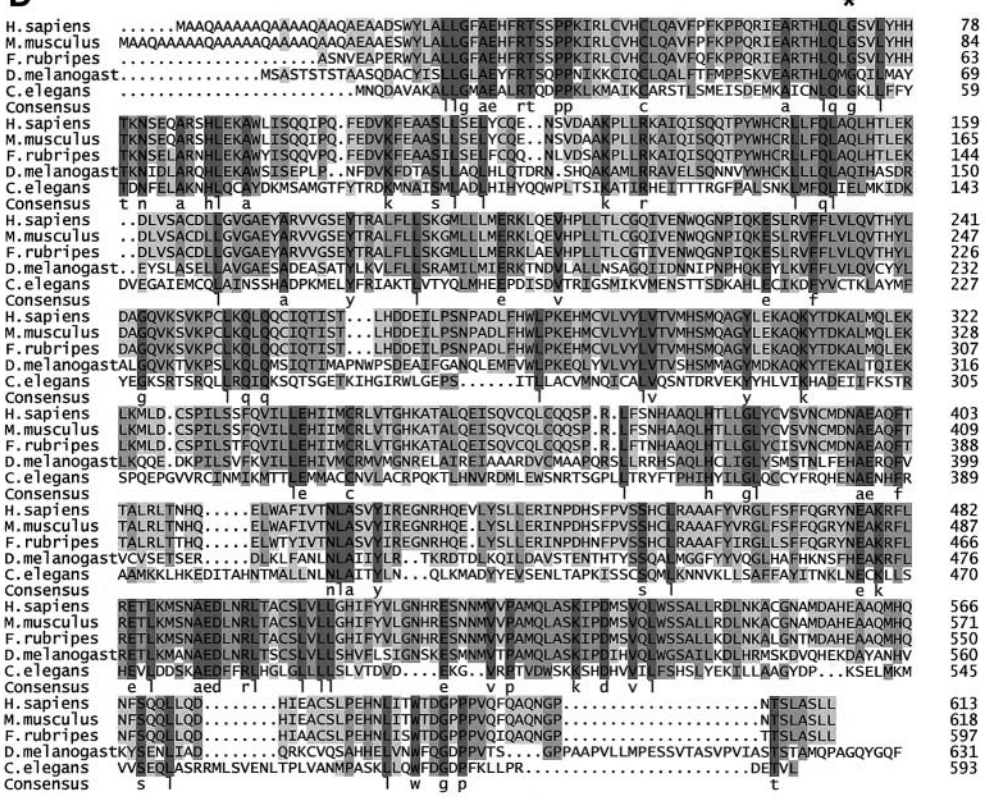

Fig. 2. Positional cloning of *mau-2*. (A) Genetic markers used to map *mau-2*. (B) Cosmids and subclones of C09H6 used for rescue assays (rescuing clones in bold). (C) Genomic structure of *mau-2* and the lesions of the four *mau-2* alleles. (D) An alignment of the predicted amino acid sequence of *C. elegans* MAU-2 with its homologues from *D. melanogaster* (CG4203-PA), *F. rubripes* (SINFRUP00000081396), *M. musculus* (NP_083269) and *H. sapiens* (KIAA0892). Identical residues in all five species are shown in dark grey; identical residues in three or four species are shown in light grey. The *mau-2(qm4)* mutation affects the conserved Gly residue (indicated by an asterisk). The *C. elegans* protein is about 25% identical to the vertebrate and the fly proteins. The sequence in the fish is 95% identical to human MAU-2, and the mouse sequence is more than 99% identical to human MAU-2.

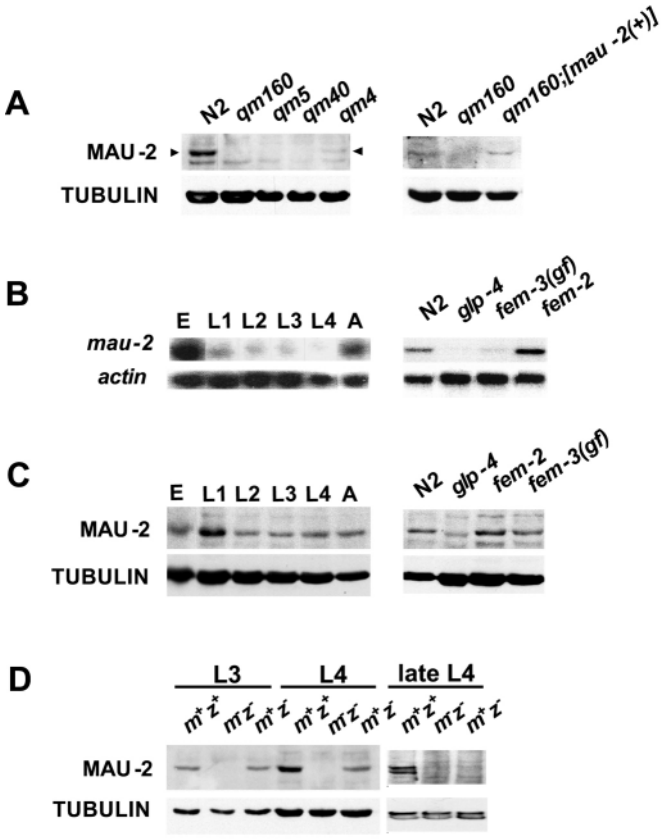

Fig. 3. The expression pattern of *mau-2*. (A) MAU-2 protein level in the wild type (N2) and in the four *mau-2* mutant alleles. No MAU-2 protein is detected in the deletion and nonsense alleles, but a reduced level is detected in the mis-sense allele *qm4* (arrowhead). A band is detected in transgenic worms carrying wild-type copies of the gene *mau-2* [*mau-2(qm160); qmEx[mau-2(+)]*]. Total protein extract loaded per lane is 50 μg. (B) Northern analysis of *mau-2* at all developmental stages (E, embryos; L1-L4, larval stages; A, young adults) and at the adult stage in mutant backgrounds. Total RNA extract loaded per lane is 10 μg. (C) Western analysis of MAU-2 at all developmental stages and at the adult stage in mutant backgrounds. Total protein extract loaded per lane is. (D) Western analysis of MAU-2 at three larval stages, comparing the MAU-2 level in the wild-type and maternally rescued worms. m^+z^+ worms were of the genotype *dpy-5(e61) mau-2(+)*; m^-z^-, *dpy-5(e61) mau-2(qm5)* derived from a *dpy-5(e61) mau-2(qm5)* mutant hermaphrodites; and m^+z^-, *dpy-5(e61) mau-2(qm5)* derived from *dpy-5(e61) mau-2(qm5)/++* hermaphrodites. In each lane, 400 worms loaded. For the late L4 stage worms, the double band both for MAU-2 and tubulin is a migration artefact.

determining the levels of protein throughout development by immunoblot analysis (Fig. 3C). Overall, the content of MAU-2 protein is similar through all developmental stages, suggesting a requirement for MAU-2 at numerous stages of development, including at times of cellular and axonal migrations. Upon repeated immunoblot analysis, there appeared to be a slight enrichment in the embryos and L1 larvae compared with other larval stages, which could reflect a higher need for MAU-2 during the time when numerous cellular migrations occur.

We examined the level of MAU-2 in the germline mutants *glp-4(bn2ts)*, *fem-3(q20ts)* and *fem-2(b245ts)* compared with the wild type (Fig. 3C). The level of MAU-2 in germline mutants is similar to the wild type, indicating that MAU-2 is not particularly enriched in the germline. Thus, the maternal *mau-2* product that is provided to the zygote via the oocyte appears to be principally RNA. Maternally contributed *mau-2* mRNA is probably responsible for the maternal rescue effect observed with *mau-2* mutations.

Abundant MAU-2 protein is detected in maternally rescued animals up to the L3 larval stage

We examined the level of MAU-2 protein in *mau-2(qm5)* maternally rescued animals at the L3 and L4 stages compared with the wild type (Fig. 3D). Given that no MAU-2 protein is detected in *mau-2(qm5)*, any MAU-2 protein detected in m^+z^- animals derived from a *qm5/+* mother must result from a maternal contribution. MAU-2 protein is detected in maternally rescued animals, and up to the third larval stage, the level of MAU-2 in maternally rescued animals is very similar to that of the wild type. This indicates that maternally contributed *mau-2* is sufficient to sustain a wild-type protein level up to the L3 stage. By the L4 larval stage, the level of MAU-2 is reduced in m^+z^- animals compared with the wild type: a low level is present in early- to mid-L4 larvae, and it is no longer detected in late-L4 larvae, while it is detected in the wild type. Thus, the maternal contribution of *mau-2* product runs out at the L4 stage in maternally rescued worms. In addition, these results indicate that new MAU-2 protein is translated at the L4 stage in wild-type worms, as the level of MAU-2 is higher in L4 than L3 in wild-type larvae (400 worms were loaded in each lane). Thus, *mau-2* is re-expressed at the time of the development of most of the egg-laying system.

A functional *mau-2::gfp* transgene is expressed ubiquitously by mid-embryogenesis and subsequently becomes restricted to the nervous system

To gain more insight into the expression pattern of *mau-2*, we generated transgenes containing the entire genomic region of *mau-2*, including a region of upstream sequence, as well as all exons and introns, fused to the green fluorescent protein (*gfp*) gene (Fig. 4A). In three different constructs, *mau-2::gfp* was driven by 1 kb, 4 kb and 6 kb of the putative *mau-2* promoter region. All three translational fusions expressed from extrachromosomal arrays completely rescued the defective locomotion, egg laying and larval development of all four *mau-2* mutant alleles (but did not recapitulate the maternal rescue effect) and produced an identical expression pattern. The expression level of *mau-2::gfp* is ~10 fold stronger when driven by 4 or 6 kb of putative *mau-2* promoter, suggesting that regulatory elements for the transcription of *mau-2* reside more than 1 kb upstream of the gene.

The *mau-2::gfp* transgenes display a dynamic expression pattern (Fig. 4B). *mau-2::gfp* expression is first observed by mid-embryogenesis, at 200-300 minutes of development, where it is ubiquitous. A broad expression is also detected during the comma stage of embryogenesis. By the end of the twofold stage and during the threefold stage of embryogenesis, *mau-2::gfp* expression is largely restricted to the nervous system. Throughout the larval stages and adulthood, *mau-2::gfp* is expressed in virtually all neurones, including in the

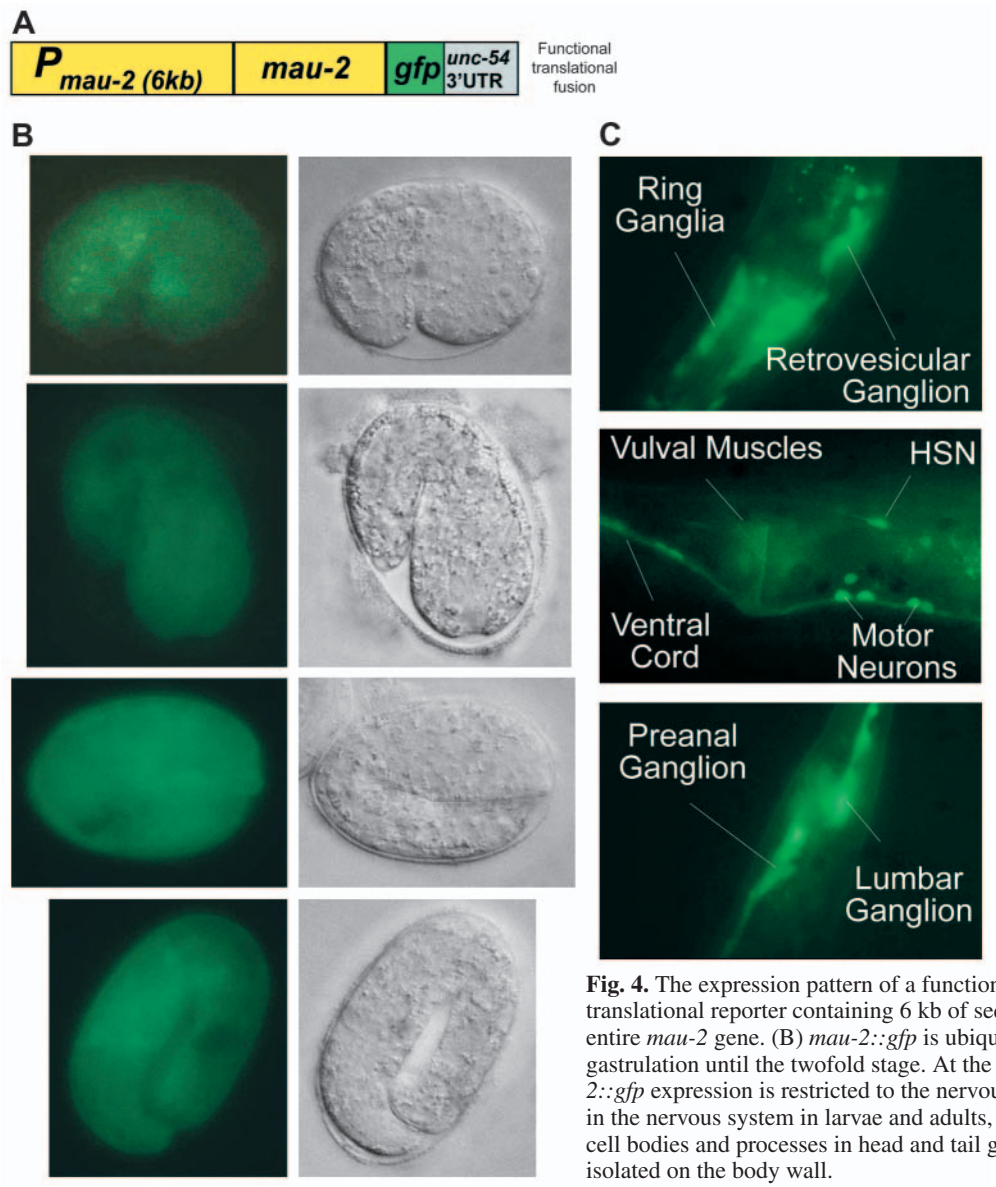

Fig. 4. The expression pattern of a functional *mau-2::gfp* reporter. (A) A translational reporter containing 6 kb of sequence upstream of *mau-2* and the entire *mau-2* gene. (B) *mau-2::gfp* is ubiquitously expressed in embryos from late gastrulation until the twofold stage. At the threefold stage of embryogenesis, *mau-2::gfp* expression is restricted to the nervous system. (C) *mau-2::gfp* is expressed in the nervous system in larvae and adults, and is visible in virtually all neuronal cell bodies and processes in head and tail ganglia, as well as in neurones that are isolated on the body wall.

neurone AVM, although the expression level decreases in adults. Occasionally, *mau-2::gfp* expression is transiently observed in hypodermal cells of the developing vulva of L3 and L4 larvae, and at a very low levels in the excretory canal, body wall and vulval muscles.

In addition, the MAU-2::GFP fusion appears to entirely and uniformly fill the cytoplasm of all neurones, including along their axonal projections, and but excluding the nucleus. The human counterpart of MAU-2 (KIAA0892), expressed as a GFP fusion under the control of the Pcmv promoter in transiently transfected COS7 cells, also appeared cytoplasmic, and excluded from the nucleus (data not shown).

Neuronally expressed *mau-2* is necessary and sufficient to rescue the *mau-2* mutants

mau-2 is required for the guidance of cellular and axonal migrations during the development of the worm, and rescuing *mau-2::gfp* transgenes are expressed, among other cells, in cells that migrate during development. It is possible therefore that *mau-2* could function inside the migratory cells to participate in their guidance. To directly determine where *mau-2* is required during development, we expressed *mau-2(+)* in distinct tissues of the worm and assessed where the activity of *mau-2* is required for normal development. We generated constructs that drive the expression of *mau-2(+)* in the hypodermis, the body wall muscles, the entire nervous system, or in six mechanosensory neurones, by placing the *mau-2* cDNA fused to the *gfp* gene under the control of the promoters of the genes *dpy-7*, *myo-3*, *unc-119* and *mec-7*, respectively. We verified the expression pattern of these constructs carried on extrachromosomal arrays and found that they were identical in both the wild-type and *mau-2(qm160)* backgrounds (Fig. 5A). $P_{dpy\text{-}7}$*::mau-2::gfp* was expressed in hypodermal cells from the early comma stage in embryogenesis onwards, $P_{myo\text{-}3}$*::mau-2::gfp* was expressed in the body wall muscles from the comma stage onwards, $P_{unc\text{-}119}$*::mau-2::gfp* was

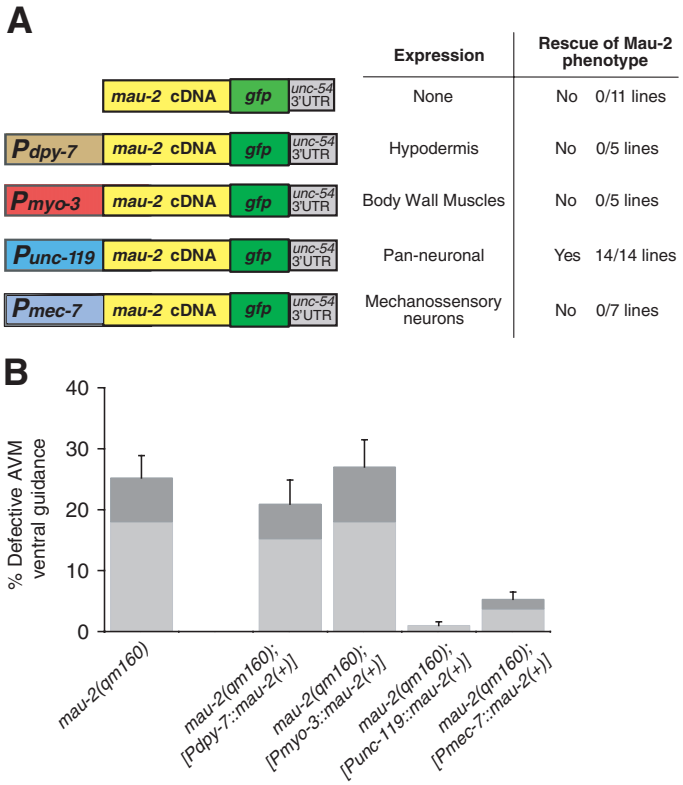

Fig. 5. *mau-2* functions cell autonomously for the guidance of AVM. (A) Constructs used to express *mau-2(+)* in different tissues. The expression of each transgene was monitored with *gfp*. Rescue of the locomotion, egg laying and larval development are indicated. (B) Phenotype of the axon of AVM in *mau-2(qm160)*-expressing *mau-2(+)* in different tissues. AVM guidance was examined with *mec-4::gfp*. Four independent transgenic lines were scored (n>200 for each construct). Light grey indicates that the axon projected anteriorly; dark grey, posteriorly. The AVM guidance defect observed in *mau-2* is rescued by expression of *mau-2(+)* in the nervous system and in the mechanosensory neurones alone. Error bars indicate standard error of the proportion. The effect of expressing *mau-2(+)* under these different promoters was also examined in the *mau-2(+)* background. The transgenic animals were completely wild type for locomotion and egg-laying, as well as for the guidance of the AVM axon (n>137 transgenic animals from three lines scored for each construct, data not shown).

expressed throughout the nervous system from the comma stage onwards (as well as in vulval muscles in late larvae and adults), and, finally, $P_{mec\text{-}7}$*::mau-2::gfp* was expressed in the mechanosensory neurones (AVM, ALMs, PVM, PLMs) from late embryogenesis onwards.

Transgenic *mau-2(qm160)* animals carrying $P_{unc\text{-}119}$*::mau-2(cDNA)::gfp* in over 25 lines were completely rescued for the uncoordination, egg laying and larval development (Fig. 5A). By contrast, none of the transgenic *mau-2(qm160)* animals carrying any of the other constructs was rescued for locomotion, egg-laying and larval development. We examined transgenic *mau-2(qm160)* animals carrying $P_{unc\text{-}119}$*::mau-2(cDNA)::gfp* from three independent lines in detail, and found that the architecture of their nervous system was the same as that of the wild type, including the organization of the major bundles and the position of neurones and axons. For example, the dorsal cord was normally fasciculated (n=180, 94% normal) and the soma of the HSN neurones was normally placed (n=126, 100% normal). It is worth noting that the complete rescue exhibited by these transgenic animals does not result from a maternal rescue effect of the transgene, as the non-transgenic animals (non Rol) from these lines were completely Mau-2. Thus, zygotic expression of *mau-2(+)* in the nervous system is necessary and sufficient for normal development.

Expression of *mau-2* in AVM can rescue the ventral guidance of the AVM axon

To distinguish between a non-autonomous role of *mau-2* between cells within the nervous system and an autonomous role of *mau-2* within the migrating cell themselves, we assessed whether expression of *mau-2(+)* in the neurone AVM could rescue the axonal guidance defect that is displayed by *mau-2* mutants. The neurone AVM is born and its axon migrates during the L1 larval stage (Sulston and Horvitz, 1977; Hedgecock et al., 1987). In the wild-type L1 larva, the axon of AVM pioneers its ventral migration along the body wall, from the lateral aspect of the body towards the ventral nerve cord. The axon then turns anteriorly towards the nerve ring (Hedgecock et al., 1987; White et al., 1986) (Fig. 6A). In ~30% of the *mau-2* mutants, this axon fails to migrate ventrally and instead runs anteriorly or posteriorly along the body wall (Takagi et al., 1997) (Fig. 6A). We expressed *mau-2(+)* exclusively in AVM and five other mechanosensory neurones under the control of the promoter of the gene *mec-7* ($P_{mec\text{-}7}$*::mau-2::gfp*). This expression of *mau-2(+)* strongly rescued the projection of the AVM axon, of both posteriorly and anteriorly misguided AVM axons in three independent transgenic lines (Fig. 5B). Thus, *mau-2* functions within the AVM neurone to guide its axon ventrally. As expected, the narrow expression of $P_{mec\text{-}7}$*::mau-2::gfp* did not rescue the locomotion, egg laying and larval development of the *mau-2* mutants. Expression of *mau-2(+)* under the *mec-7* promoter also rescued the abnormal projection of the PLM axons (data not shown), which instead of projecting laterally, as in the wild type, run ventrally in 40% of the *mau-2(qm4)* mutants (Takagi et al., 1997). This suggests that *mau-2* functions cell autonomously in other cells as well. In addition, consistent with a cell-autonomous role for *mau-2*, overexpression of *mau-2(+)* in muscles, hypodermis or the nervous system in a wild-type *mau-2(+)* background leaves the guidance of AVM entirely unaffected.

mau-2 acts independently of slit/*slt-1* to guide AVM ventrally

Previous work has established that two guidance cues, netrin/*unc-6* and slit, and their receptors, *unc-40* and *sax-3*, respectively, are required for the ventral guidance of the axon of AVM (Chan et al., 1996; Hao et al., 2001; Hedgecock et al., 1990; Ishii et al., 1992; Zallen et al., 1998). In fact, this axon relies heavily on these two cues for its ventral migration, as evidenced by the fact that it fails to migrate ventrally in 95% of the cases, and instead extends laterally, in the double mutants *unc-6 slt-1* and *unc-40;slt-1* (Hao et al., 2001). Given that *mau-2* functions cell autonomously in AVM and that MAU-2::GFP is cytoplasmic, it is possible that *mau-2* may function as a cytoplasmic effector of the netrin and/or slit cues.

To address this issue, we determined whether *mau-2* is required for the signalling of netrin and slit in the ventral guidance of AVM.

We first asked whether *mau-2* is required for signalling through the netrin receptor UNC-40. Expression of a hyperactive form of the netrin receptor UNC-40::MYR in mechanosensory neurones leads to profound defects in AVM guidance, and also results in highly abnormal cell-body shapes and branching patterns of AVM and PLM (Gitai et al., 2003). We found that complete loss of *mau-2* function does not suppress the effects caused by expression of UNC-40::MYR (in a strain carrying the extrachromosomal array *kyEx456*). In *mau-2(qm160)*; *kyEx456*, the severity and penetrance of the defects was similar to those observed in a wild-type background [83% abnormal in 108 *mau-2(qm160)* young adults, and 88% abnormal in 92 *mau-2(+)* young adults]. Thus, the function of *mau-2* is not absolutely required for the signalling downstream of the *unc-40* receptor. Consistent with this, we found that a *mau-2(qm4); unc-6(e78)* double mutant, where *e78* is a results in a partial loss of function of *unc-6* (Lim and Wadsworth, 2002), displays migration defects that are more severe than either of the single mutants (data not shown), indicating that *mau-2* probably acts in parallel to *unc-6* in the guidance of at least some cells and axons during their migration. We could not, however, determine the phenotypic consequences of the complete loss of function for both genes [*ev400* is a null allele of *unc-6* (Wadsworth et al., 1996)], as the *mau-2(qm160);unc-6(ev400)* double mutant is unviable.

Next, we asked whether *mau-2* is required for the signalling of *slt-1*. SLT-1 is secreted from the dorsal muscles and functions to repel the AVM axon, thus contributing to its ventral migration (Hao et al., 2001). Misexpression of *slt-1* in all of the body wall muscles of the worm, i.e. in ventral as well as dorsal muscles, results in the lateral projection of the AVM axon in about 30% of the animals (Hao et al., 2001) (Fig. 6). We asked whether the complete loss of function of *mau-2* could suppress the effect of misexpressing *slt-1*. We found that *mau-2(qm160)* does not suppress the defects caused by misexpression of *slt-1*, indicating that *mau-2* is not absolutely required for the signalling of *slt-1*. By contrast, *mau-2(qm160)* enhances the AVM axon guidance defects generated by *slt-1* misexpression (Fig. 6A,B). In *mau-2(qm160)* that misexpress *slt-1*, the defect of the AVM axon is profoundly enhanced

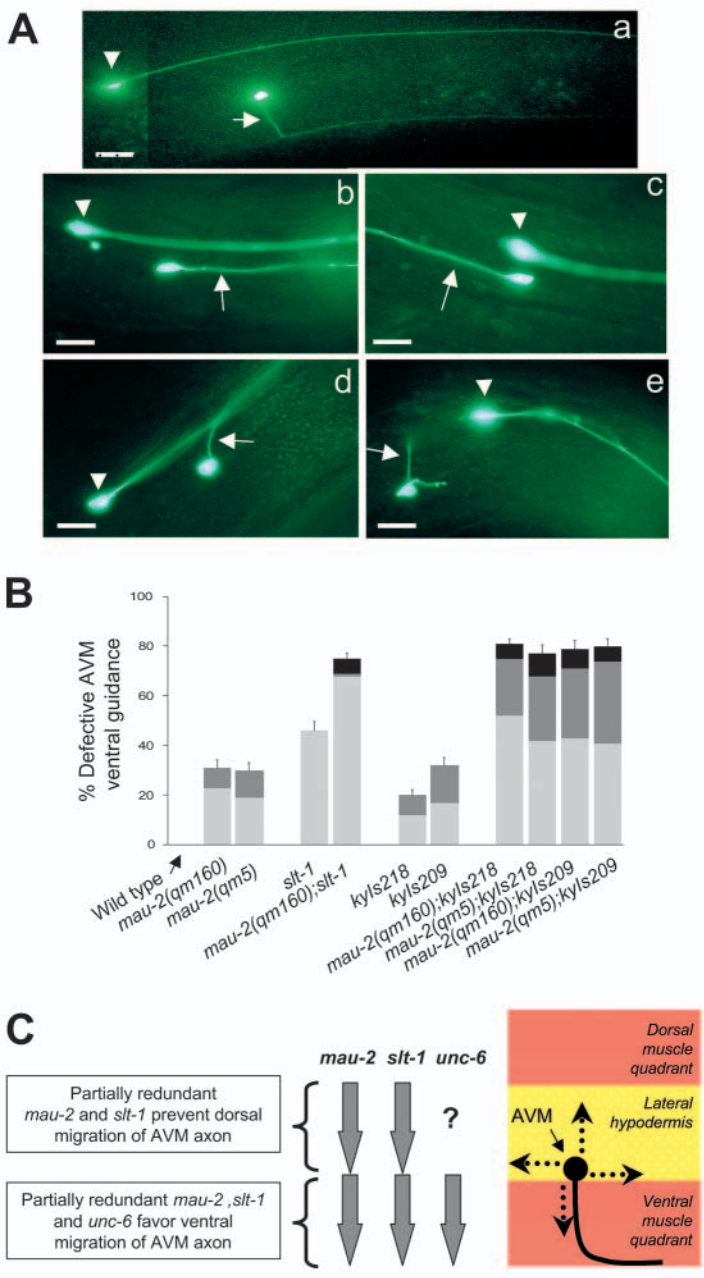

Fig. 6. The action of *mau-2* is independent of that of *slt-1* to guide AVM ventrally. (A) AVM was visualized with *mec-4::gfp*. Anterior is towards the right, ventral at the bottom. (a) A wild-type AVM axon in which the axon first projects ventrally and then turns anteriorly. The axon of AVM fails to migrate ventrally (b-d): the axon of AVM projects anteriorly in *mau-2* and *slt-1* single mutants (b), or posteriorly in *mau-2* single mutant (c), and dorsally in *mau-2;slt-1* double mutants and in *mau-2(qm160)* misexpressing SLT-1 under the *myo-3* promoter (d,e). (e) The axon of AVM projects dorsally (past the dorsoventral position of the neurone ALM) and, although not in the plane of focus here, then turns anteriorly, migrating alone for a distance and finally joining the axon of ALM. A short anterior branch is also present. Arrows and arrowheads in a-e indicate AVM and ALM, respectively. (B) Phenotype of the axon of AVM in *mau-2;slt-1* double mutants and in *mau-2(qm160)* animals misexpressing SLT-1 (*kyIs218* and *kyIs209* are integrated *myo-3::slt-1* transgenes). Light grey indicates that the AVM axon projected anteriorly; dark grey, posteriorly; black, dorsally. The *mau-2;slt-1* double mutants exhibit an enhancement of the ventral guidance defect compared with the single mutants, and display a novel phenotype (dorsal projection; n=170-300). Error bars indicate standard error of the proportion. (C) The function of *mau-2* appears to be acting in parallel to those of *slt-1* and *unc-6* to guide the axon of AVM. The AVM neurone is shown in the right-hand panel. Its cell body rests at the boundary between the ventral muscle quadrant and the lateral hypodermis. The axon of AVM normally projects ventrally (towards the bottom), along the ventral muscle quadrant, and then turns anteriorly (towards the right). The broken arrows indicate that the axon of AVM can project abnormally (anteriorly, posteriorly or dorsally) in some mutant situations. Loss of function of *unc-6*, *slt-1* or *mau-2* results in abnormal anteriorly projecting AVM axons; loss of function of *unc-6* or *mau-2* can also result in posteriorly oriented axons. When the activity of both *mau-2* and *slt-1* are altered, the axon of AVM can project dorsally. It appears that three mechanisms (*unc-6*-mediated attraction, *slt-1*-mediated repulsion and that involving *mau-2*) are partially redundant to guide AVM ventrally. Scale bars: 5 μm.

(~80% abnormal) compared with the *mau-2* mutants (~30%) or to transgenic animals misexpressing *slt-1* (~30%). In addition to this quantitative difference, the phenotype of *mau-2(qm160); myo-3::slt-1* is qualitatively different from that of the *mau-2* mutants or of the animals misexpressing *slt-1*: in 7% of the animals, the axon of AVM actually projects dorsally. These dorsally projecting axons of AVM reach the level of the axon of ALMR or the dorsal sublateral. They then turn anteriorly, with the axon frequently changing its dorsoventral position, so that they do not extend in a straight line towards the head of the worm. This dorsal projection of AVM is a novel mutant phenotype, specific to the interaction of these two genes. These results indicate that *mau-2(+)* could act to reduce the signalling of *slt-1* or in a pathway parallel to *slt-1*.

We also examined the *mau-2(qm160);slt-1(ev15)* double mutants [*ev15* is a null allele of *slt-1* (Hao et al., 2001)]. This double mutant displays an enhanced phenotype for the guidance of AVM. The overall penetrance of the AVM axon defect appears to be the sum of the effect of the two single mutations; however, the phenotype of the double mutant is qualitatively different from that of either of the single mutants. In *mau-2* mutants, one-third of the abnormal axons project posteriorly. This appears to be suppressed in the double mutant with *slt-1*. Moreover, 7% of the AVM axons project dorsally in the double mutant. Again, this phenotype has not previously been observed to be a consequence of the loss of function of genes implicated in the guidance of AVM. Together, our results indicate that *mau-2* and *slt-1* act independently to guide the AVM axon ventrally. Although *slt-1*, *unc-6* and *mau-2* are all necessary to ensure that AVM migrates ventrally, the combined action of *mau-2* and *slt-1* is required to prevent AVM from projecting dorsally (Fig. 6C).

Discussion

The role of *mau-2* in the guidance of migrations is cell autonomous

The function of *mau-2* is broadly required for the proper guidance of cells and axons that undergo long-range migrations during the development of the worm. In fact, *mau-2* mutants display defects in migrations that occur both embryonically and post-embryonically, and that occur along both the longitudinal and the circumferential axes of the body of the worm. The protein encoded by *mau-2* is novel, yet highly conserved evolutionarily, and has not been previously studied in any organism. Although the biochemical role of the protein MAU-2 remains unknown, MAU-2 appears to function within the migrating cell and to localize to the cytoplasm, where it may participate in the intracellular interpretation of guidance cues encountered by a cell or axon during its migration.

Our results demonstrate that *mau-2* functions cell-autonomously to guide the axon of the AVM neurone. First, *mau-2* mutations impair the proper axonal guidance of AVM. Second, a functional *mau-2*::*gfp* transgene is expressed in the AVM neurone, including at the time of its axonal migration. Third, expression of *mau-2(+)* in AVM, in otherwise *mau-2* null mutant animals, is sufficient to rescue the axonal guidance defect of AVM. Similar observations were made for the axon of the PLM neurone.

Several lines of evidence suggest that *mau-2* may function within other migrating cells to participate in their guidance. *mau-2* mutants display defects in the guidance of a number of cellular migrations, including numerous neurones and their axons, and *mau-2* is expressed in the affected cells at the time of their migration. Neuronal migration and axon outgrowth start from around 370 minutes and the major nerve tracts form from around 450 minutes. Expression from the functional *mau-2::gfp* transgene is visible in the embryo, including in neuroblasts and neurones, starting about 300 minutes after the first cleavage. Functional *mau-2::gfp* keeps being expressed in the nervous system, from the threefold embryonic stage (after 500 minutes) onwards, coincident with the occurrence of additional neural migrations, including the integration of new axons in the dorsal cord at the L1 stage. For example, the positions of the embryonically migrating cell body of HSN and the embryonically extending PLM and CAN axons, is abnormal in *mau-2* mutants, and *mau-2::gfp* is expressed in these cells, including at the time when their migration is occurring in the embryo. Importantly, pan-neuronal expression of *mau-2(+)* under the *unc-119* promoter rescues the *mau-2* mutant defects (e.g. locomotion, egg-laying, Lid larval lethality, nervous system organization), indicating that *mau-2* functions in other neurones as well. Taken together, these observations suggest that *mau-2* may function cell autonomously in other migrating cells as well. Consistent with this, nonautonomous activity of *mau-2* is unlikely, as the *mau-2* mutant defects are not rescued by expression of *mau-2(+)* in the mechanosensory neurones, in the hypodermis or in the body wall muscles.

Furthermore, *mau-2* may function cell autonomously in cells that develop later, such as those constituting the egg-laying system. In *mau-2* mutants, the vulva is frequently asymmetrical and the vulval muscles are often misplaced. Interestingly, our analysis of wild-type and maternally rescued animals revealed that new MAU-2 protein is also produced at the L4 larval stage, and de novo *mau-2::gfp* expression was observed in the muscle cells, and occasionally in hypodermal cells, of the developing vulva in L3 and L4 larvae. Expression of *mau-2* at the L4 stage appears to be necessary for the normal development of the vulva, as vulval muscles attach abnormally in maternally rescued animals.

mau-2 acts in parallel to *slt-1* to prevent AVM from projecting dorsally

Simultaneous perturbation of the activities of *mau-2* and *slt-1* profoundly affects the guidance of the axon of AVM. As many as ~80% of the double null mutants display guidance defects, which is twice as many as in each of the single mutants, indicating that *mau-2* and *slt-1* function in parallel pathways. Importantly, the AVM axon projects dorsally in a significant number of animals in which both *mau-2* and *slt-1* activities are perturbed. This was observed in the *mau-2; slt-1* double mutants, as well as in *mau-2* mutants misexpressing *slt-1* in all body wall muscles [*slt-1* is normally secreted only from dorsal muscles to repel the AVM axon (Hao et al., 2001)]. This indicates that *slt-1* and *mau*-2 act redundantly to prevent the dorsal projection of AVM.

The dorsal projection of AVM is a guidance defect that has not previously been reported for any single or double mutant that affects the guidance of the AVM axon. In fact, mutations in the genes *unc-6*, *unc-40*, *slt-1*, *sax-3*, *unc-34* and *mau-2* that affect the guidance of AVM, result in the lateral projection of

AVM in ~25-40% of the cases (Hedgecock et al., 1990; Takagi et al., 1997; Yu et al., 2002). In these mutants, when the axon of AVM fails to project ventrally, it instead extends anteriorly. The AVM axon can project posteriorly as well in the single mutants *mau-2* and *unc-6* [in ~7-10% of the animals (Takagi et al., 1997; Yu et al., 2002)]. In addition, the axon of AVM can extend posteriorly in animals that misexpress *slt-1* in all body wall muscles (Hao et al., 2001). The axon of AVM has been observed to migrate dorsally and reach the dorsal cord, and then turn anteriorly along the dorsal cord in animals that misexpress UNC-5 in AVM under the control of a heterologous promoter (Hamelin et al., 1993). UNC-5 is a netrin receptor that mediates the repulsion of a growth cone of migrating motor axons away from the netrin source, but is not expressed in AVM (Killeen et al., 2002; Su et al., 2000) and does not appear to normally participate in the guidance of the AVM axon (Leung-Hagesteijn et al., 1992). Thus, our analysis of the interactions between *mau-2* and *slt-1* reveals that the AVM axon is capable of migrating dorsally as a result of the loss of function of genes implicated in its guidance. Our results also indicate that the ventral guidance of AVM does not only rely on *unc-6* and *slt-1*, as previously reported (Yu et al., 2002).

Our results furthermore indicate the existence of a new mechanism, involving *mau-2*, that functions in parallel to *slt-1* to repel AVM from the dorsal area of the body wall (Fig. 6C). The effects of perturbing both *mau-2* and *slt-1* gene activities are observed even in the presence of *unc-6(+)* activity, indicating that the *unc-6* mediated attraction of AVM is insufficient to prevent dorsal migration by itself. Although we could not fully characterize the interactions between *mau-2* and *unc-6*, it appears that, *unc-6*-mediated attraction, *slt-1*-mediated repulsion and a mechanism involving *mau-2,* are all partially redundant in the ventral guidance of AVM. It is also possible that the mechanism involving *mau-2* may impinge upon both the *unc-6* pathway (for example, upstream of *unc-40*) and the *slt-1* pathways, rather than being a fully independent pathway.

It will be interesting to find out how *mau-2* participates in the dorsoventral guidance of the AVM axon and, specifically, to determine if it is involved in a guidance mechanism entirely distinct from those of netrin and slit. For example, one question is whether *mau-2* might interact with *clr-1,* a receptor tyrosine phosphatase that has been recently implicated in the regulation of netrin signalling (Chang et al., 2004).

Maternal expression of *mau-2*

The zygotic activity of *mau-2* is sufficient for the normal development of the worm. Indeed, heterozygous animals produced by a homozygous mutant mother are completely wild type. In addition, functional *mau-2::gfp* transgene expression is first detected as late as by mid-embryogenesis, which is well passed the time at which the zygotic genome is turned on [at the onset of gastrulation (Edgar et al., 1994 ; Powell-Coffman et al., 1996; Kaltenbach et al., 2000)]. However, *mau-2* mutants can be profoundly maternally rescued, and high levels of *mau-2* transcript are loaded into the oocytes and still detectable in the very early embryo. Why is *mau-2* maternally contributed at all?

It is possible that *mau-2* might play role in very early development that may be masked by the function of redundant, yet non-homologous, genes. It is also conceivable that the maternal contribution of *mau-2* serves to ensure a sufficient level of *mau-2* expression at the time of the start of *mau-2* expression in the zygote. The effect of such a stabilization might not easily be detected in the experimental setting, but might be of sufficient magnitude to have been evolutionarily favoured.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/131/23/5947/DC1

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