Cellular and axonal migrations are misguided along both body axes in the maternal-effect mau-2 mutants of Caenorhabditis elegans

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SUMMARY

We have characterized the mau-2 mutants of Caenorhabditis elegans and found that migrating cells and axons are mispositioned along both the antero-posterior and dorso-ventral body axes. This is in contrast to previously characterized guidance mutations in Caenorhabditis and in Drosophila, which have been found to be axis-specific. Two observations suggest that mau-2 acts very early during development: most behavioral phenotypes of mau-2 can be rescued by a maternal effect, and variations in expressivity involve an entire body side at a time. The possibility that mau-2 is involved in the spatial organization of guidance cues encoded by other genes is discussed.

Key words: C. elegans, mau-2, axonal guidance, cell migration, maternal effect

INTRODUCTION

The nematode Caenorhabditis elegans has been used to identify genes involved in the development of the nervous system. Brenner identified a large number of viable behavioral mutants of C. elegans, including many with impaired locomotion (Brenner, 1974). Many more behavioral mutants were later found through screens designed to identify particular classes of mutants (Chalfie and Sulston, 1981; Trent et al., 1983; Park and Horvitz, 1986; Manser and Wood, 1990; Thomas, 1990; Avery, 1993; Hekimi et al., 1995). Although Brenner's screen was not specifically designed to identify genes involved in neural development rather than neural function, a number of the mutants he identified were later found to have profound neuroanatomical defects (Hedgecock et al., 1985, 1987, 1990; McIntire et al., 1992; Hekimi and Kershaw, 1993).

Detailed phenotypic analysis of three genes identified by Brenner, unc-5, unc-6 and unc-40, has provided compelling evidence that they are involved in the guidance of migrating cells and axons (Hedgecock et al., 1990). Mutations in these genes affect almost exclusively circumferential migrations which require guidance along the dorso-ventral axis. Specifically, mutations in unc-6 affect both dorsally and ventrally directed movements, while those in unc-5 affect only dorsally directed and unc-40 only ventrally directed movements. These mutations appear to affect migration along the longitudinal axis in only very minor and probably indirect ways (Hedgecock et al., 1990). These findings clearly indicate that axonal and cellular guidance are organized in axis-specific ways, at least in worms.

The mechanisms of guidance in the longitudinal axis have also been studied, but are less well understood. Manser and Wood (1990) have studied several genes affecting the migration of cells along the longitudinal axis. They found that mutations in some genes can result in excessive rather than incomplete migrations, suggesting that at least some of the genes they studied affect guidance rather than the mechanisms of migration per se. vab-8, one of the genes identified by Manser and Wood, has recently been shown to affect axonal pathfinding of posteriorly directed migrations (Wightman et al., 1996). Our own work indicates that unc-53 is involved in longitudinal guidance and suggests that distinct guidance cues operate in different body regions in contrast to what is observed with circumferential guidance (Hekimi and Kershaw, 1993). unc-53 has also been shown to be involved in the longitudinal guidance of the sex myoblasts’ migration (Chen et al., 1997). Finally, a study of mutants defective in aspects of longitudinal axonal elongation suggests that the control of longitudinal elongation within process bundles is distinct from that along the body wall (McIntire et al., 1992).

The organization of axonal guidance into orientation-specific genetic systems, as observed in Caenorhabditis, could be a general feature of the development of all nervous systems. Two proteins, netrin-1 and -2, both highly similar to UNC-6, have been identified in vertebrates (Serafini et al., 1994). The extraordinary finding is that the netrins also mediate circumferential guidance in the vertebrate neural tube (Kennedy et al., 1994). This suggests that, beyond evolutionary conservation of their structure, the precise topological role of molecules...
involved in the development of the nervous system is conserved. Recently, similar evidence has been obtained for unc-5 (Ackerman et al., 1997; Leonardo et al., 1997) and unc-40 (Keino-Masu et al., 1996; Fazeli et al., 1997), which both have vertebrate homologues involved in nervous system development. Furthermore, the phenotype of mice lacking a functional Dcc gene (the homologue of unc-40) is similar to that of netrin-1-deficient mice (Fazeli et al., 1997), suggesting that here too, the topological function of the gene has been conserved. Finally, a large-scale genetic screen in Drosophila led to the isolation of a number of lethal mutants affecting only longitudinal or only transversal process tracts (Seeger et al., 1993), suggesting that axis-specific genetic systems are also used in flies.

Here, we describe maternal-effect mutations in the gene mau-2 that affect antero-posterior as well as dorso-ventral guidance of cellular and axonal migrations. mau-2 mutations display a maternal effect, in contrast to all previously described mutations affecting pathfinding in Caenorhabditis and Drosophila. This fact and other evidence presented suggest that this gene is used at an earlier time than most known guidance genes, possibly when the distinction between the two axes along which pathfinding later proceeds is being established.

**MATERIALS AND METHODS**

**Reporter genes**

To visualize neurons we used three reporter constructs. One construct (els[unc-31::lacZ]) consists of an in-frame fusion of most of the coding sequence of the C. elegans gene unc-31 to lacZ. The construct includes a nuclear localization sequence and 15 kb of 5′ upstream sequence, and is integrated in the genome (Livingstone, 1991). This construct, which is capable of rescuing unc-31 mutants, is expressed in a large fraction of the nervous system, including cell bodies and axons (Livingstone, 1991; D. Livingstone and R. Hoskins, unpublished). Transformation by this construct was initially monitored along which pathfinding later proceeds is being established. This fact and other evidence presented suggest that this gene is used at an earlier time than most known guidance genes, possibly when the distinction between the two axes along which pathfinding later proceeds is being established.

**Lethality and behavioural phenotypes**

20-30 adult hermaphrodites were allowed to lay eggs on a single plate and were then removed. Eggs were transferred to a new plate; unhatched eggs and dead larvae were counted twice (2 and 3 days later). Two classes of dead or dying larvae were distinguished: Lid (living dead) larvae, which have a characteristic stiff and transparent appearance, and non-Lid dead larvae, which generally appeared deformed. Among the worms that did reach adulthood, egg-laying defects (Trent et al., 1983) and uncoordination were scored.

To score the phenotype of maternally rescued mau-2 mutants (mau-2), mau-2 hermaphrodites were outcrossed with N2 males. Individual F2 progeny were transferred to fresh plates at the L3 to L4 stage and their phenotype (locomotion and egg-laying) recorded up to late adulthood. Each F2 was observed at least three times. The genotype of F2s was inferred from their F1 self-progeny. No dead larvae, Lid or non-Lid, were found in the F2 generation after careful inspection of broods from ten F1 mothers. To study the phenotype of mau-2 in trans to a deficiency, we used the deficiency nDf24, which removes mau-2 (Hekimi et al., 1995). dpy-5(e61) mau-2/+ + males were mated to unc-13(e1091) lin-11(n566) nDf24 hermaphrodites. For all three alleles of mau-2, the resulting hermaphrodites of genotype dpy-5 mau-2/nDf24 are similar (Egl but not Unc; dpy-5 does not rescue dpy-5) and do not display a mutant phenotype before reaching adulthood, when hermaphrodites become Egl; this is expected as the unc-13 lin-11 chromosome presumably carries a wild-type allele of mau-2. These dpy-5 mau-2/nDf24 animals were allowed to self-fertilize and the resultant Dpy progeny were scored for the Lid phenotype (Table 2D).

**Neuroanatomical observations**

All systematic observations were done using the qm# allele of mau-2. Lid larvae were examined by DIC microscopy. Cells were identified by a number of criteria, including the size and shape of the cells and their nuclei, the morphology of their processes, and their absolute and relative positions. Formally, there is no control for these criteria as Lid larvae are unique to mau-2 mutants. However, the position and morphologies of 50% or more of the cells observed in Lid larvae strikingly resemble those of the wild type when they have been visualized with other methods, including DIC on normal living larvae and adults, EM reconstruction (White et al., 1986), antibody staining (Hekimi, 1990) and expression of reporter genes (e.g. Livingstone, 1991). Furthermore, some non-migrating (BDU) and migrating (CAN) cells were always found at their known wild-type positions. This suggests that the normality or abnormality of the placement of a cell can be reliably scored in Lid larvae. We scored cell positions in relation to landmarks such as the pharynx, the vulva, the muscle boundaries and the alae, as well as relative to each other. For example, the normal position of the ALMR is dorsal and relatively much closer to the vulva than to the pharynx. Therefore, when a cell and its process had the morphology of an ALM and was close to the dorsal muscle boundary, but placed much closer to the pharynx than to the vulva, including being anterior to a cell with a morphology and the placement of AVM, and when no other cell resembling an ALM was found posterior to it, we concluded that this cell was an abnormally placed ALM. The boxes in Fig. 3B reflect this manner of scoring. For example, the box in which most abnormally positioned ALMs have been placed corresponds to the approximate position of SDQR in the wild type; the box below corresponds to the approximate antero-posterior position of SDQR in the wild type, but more ventral, at the dorso-ventral level of the alae. This way of identifying and scoring the positions of cells was difficult to apply to small groups of cell normally found together, such as the cells of the post-deirids for which we can only give an informal description (see Results).

Similar criteria to those given above were used for cell identification in animals carrying the unc-31::lacZ reporter gene. In addition, these animals were stained with DAPI (Sigma) to be able to visualize nuclear morphologies, which are typical and recognizable for each of the cell types examined. The positions of the cells on the animals’ lateral sides were measured in the following way. The outline of the animals and the positions of the stained...
cells were drawn on paper with a camera lucida. The outlines were drawn while focusing the microscope so as to maximize the apparent width of the animal, and therefore presumably focusing on the center of the body. For the longitudinal position, the distance from the tip of the head of the animal to the middle of the cell body of a given cell was measured, and reported as a percentage of the distance from the tip of the head to the vulva of the animal, so that cells anterior to the vulva have positions <100% and cells posterior to the vulva have positions >100%. For the dorso-ventral position, the distance from the ventral edge to the cell was divided by the total width of the animal at that antero-posterior level.

The amphid and phasmid neurons were tested for their ability to take up dye (DiI) as described by Starich et al. (1995).

The gonad shapes in adults were scored in animals that had maturing oocytes and less than three eggs per gonad arm.

Descriptive statistics
A graphical representation of the location of migrating cells is shown in Fig. 5. We calculated the means and standard deviations of the positions of the different cell types along each axis (Table 1). We carried out statistical analysis of the validity of the features that are discernible on the graphical representation (see main text). We tested normality of the samples by the Kolmogorov-Smirnov test and estimated the similarity of the variances using the F-test. We compared the means using the Student’s t-test when the variances were unequal (significance level at P=0.05). The distribution was not normal for only one measure, the antero-posterior position of HSN, for which we used the non-parametric Mann-Whitney test to compare the wild type and the mutants.

RESULTS
Isolation
In an attempt to identify genes involved in the earliest stages of the patterning of the nervous system, we carried out a genetic screen for maternal-effect behavioural mutants. Three recessive non-complementing mutations (qm4, qm5 and qm40) identified in this screen define the gene mau-2 (mau stands for maternal-effect uncoordinated) on chromosome I (Hekimi et al., 1995). Many of the defects produced by these mutations are phenotypically rescued by the presence of a wild-type allele in the mother of a homozygous mutant (see below). No strict maternal-effect was detected, i.e. an animal carrying a wild-type allele of the gene is phenotypically wild type even when derived from a homozygous mutant mother (Hekimi et al., 1995).

Behavioral defects
Mutants from all three mau-2 alleles are uncoordinated in an identical way. Forward movement is sluggish but not strongly abnormal. When attempting backward movement, mutants frequently kink or coil up in a tight spiral instead of propagating sinusoidal waves (Fig. 1B). The head bends sharply ventrally or dorsally and remains bent even during further backward movement (Fig. 1B). In addition, mau-2 mutants are egg-laying defective (Egl) and retain their eggs inside their bodies (Fig. 1B). Strongly Egl mau-2 animals frequently die as a result of internal hatching of the eggs (the bag-of-worms phenotype). We have quantified these phenotypes for all three alleles (Table 2A), which by these criteria appear to be very similar to each other.

Lethality and the living dead (Lid) phenotype
Approximately half of mau-2 mutants never reach adulthood. We have quantified embryonic and larval lethality for all three alleles (Table 2B). A significant proportion of the larvae arrest development and take on a specific appearance: they are immobile, transparent and appear to be dead. Examination at high magnification shows that these larvae are still capable of sporadic pumping and defecation movements, sometimes for several days, and we have therefore called this phenotype the living dead (Lid) phenotype (Fig. 1B). The hypodermal cells

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**Table 1. Statistical description of the location of cells in animals carrying the reporter gene unc-31::lacZ**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AVM n</th>
<th>antero-posterior position</th>
<th>dorso-ventral position</th>
<th>SDQR n</th>
<th>antero-posterior position</th>
<th>dorso-ventral position</th>
<th>HSN n</th>
<th>antero-posterior position</th>
<th>dorso-ventral position</th>
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<tbody>
<tr>
<td>N2</td>
<td>41</td>
<td>62.8±4.0</td>
<td>32.8±6.9</td>
<td>40</td>
<td>54.8±5.7</td>
<td>65.8±7.5</td>
<td>69</td>
<td>3.3±1.8</td>
<td>30.5±5.8</td>
</tr>
<tr>
<td>mau-2</td>
<td>47</td>
<td>60.8±4.8</td>
<td>34.5±9.6</td>
<td>47</td>
<td>44.7±6.1</td>
<td>60.1±13.8</td>
<td>83</td>
<td>3.0±3.2</td>
<td>35.2±12.3</td>
</tr>
</tbody>
</table>

n, total number of cells examined. Values are mean and s.d. for each cell, and are shown in bold if different from wild type by the statistical tests described.
of such animals appear to be filled with fluid, while their gut cells are shrunk around the lining of the gut lumen. Such an appearance suggests ionic or water balance defects and may be due to defects in the excretory system (Nelson and Riddle, 1984). Indeed, the examination of Lid animals shows that the excretory canal is invariably short, terminating in an abnormal, highly convoluted structure (Fig. 2). Other elements of the excretory system might be affected as well. A high proportion of Lid animals arrests at the second and third larval stages, although some animals arrest at the fourth larval stage. A tem-
perature-sensitive mutation in another gene, \textit{clr-1}, also produces a transparent phenotype (Hedgecock et al., 1990). However, \textit{clr-1} mutants appear different from Lid animals. In particular, \textit{clr-1} mutants do not have a shrunken gut and are capable of movement, even at the restrictive temperature.

**Maternal rescue**

We have quantified the degree to which the various defects described above are rescued when homozygous mutants derive from mothers carrying a wild-type allele of the gene (Table 2C). The larval lethality is fully rescued. The Unc phenotype is almost fully rescued. The egg-laying defect, however, does not appear to be rescued at all. Thus, a homozygous \textit{mau-2} mutant originating from a mother carrying a wild-type allele of the gene displays a fully wild-type locomotion but is severely Egl (Fig. 1B). As most of the egg-laying system develops post-embryonically, the absence of maternal rescue for the Egl phenotype suggests that \textit{mau-2} is also required post-embryonically, and that the maternal \textit{mau-2} contribution is not sufficient to rescue developmental events in later developmental stages. Maternal rescue of the dye-uptake phenotype (see below) was also scored and found to be only partially rescued (Table 2E).

**The null phenotype**

To investigate whether the \textit{mau-2} mutations result in a complete loss of function of the gene, we have examined the phenotype of animals carrying a mutant allele of the gene and a deficiency (\textit{nDf24}) that removes \textit{mau-2}. Animals genotypically \textit{mau-2}/\textit{nDf24} derived from mothers of the same genotype, but not \textit{+}/\textit{nDf24} animals, appear very sluggish and have a strongly reduced brood size compared to \textit{mau-2} homozygotes. Also, among homozygous \textit{mau-2} mutants derived from \textit{mau-2}/\textit{nDf24} mothers, the frequency of Lid death is strongly increased as compared to controls (Table 2D). These observations suggest that none of the three \textit{mau-2} mutations result in a full loss of function of the gene. It remains possible, however, that the observed phenotypes and the enhancement of the Lid larval lethality result from a maternal-effect haplo-insufficiency of other loci removed by \textit{nDf24}.

In these experiments, the frequency of Lid death is higher than previously measured, including in the controls (compare to Table 2B). This probably results from the fact that, in order to ascertain the genotypes of mothers and their offspring in these experiments, the chromosomes carrying the \textit{mau-2} mutations were marked with a \textit{dpy-5} mutation (\textit{dpy-5} is neither maternally rescued nor removed by \textit{nDf24}). It appears therefore that \textit{dpy-5} enhances the penetrance of the Lid phenotype.

**Defects in the guidance of cellular migrations**

A number of cells in \textit{C. elegans} undergo long-distance migrations during embryonic or post-embryonic development (Sulston and Horvitz 1977; Sulston et al., 1983; for review see Hedgecock et al., 1987), and their final positions in the wild type have been well documented. We have used several techniques to observe the positions of these cells in \textit{mau-2} mutants.

**Abnormal neuronal cell migrations visualized in Lid worms**

We have taken advantage of the fact that using DIC microscopy, one can visualize single cells with particular clarity in animals with the Lid phenotype (Fig. 2). In these animals we have scored the positions of a number of neurons...
that reach their final positions as a result of embryonic or post-embryonic migrations (Fig. 3). Although no formal control is available for Lid larvae that are unique to mau-2, the following reasons suggest that we could reliably score abnormal positions. (1) The wild-type placement of the cells we scored are well known by DIC observation of living animals (Sulston and Horvitz, 1977; Sulston et al., 1983) and by electron microscopic reconstruction (White et al., 1986). (2) We have examined their positions by scoring them in relation to defined anatomical landmarks (see Materials and methods for details). (3) When we examined these cells by different techniques in non-Lid mau-2 animals, similar misplacements were observed (see below). (4) Two cell types we scored, CAN, which is migratory, and BDU, are never misplaced, indicating that misplacement of cells is not a result of the general condition of the Lid larvae.

The ALMs (anterior lateral microtubule cells) are a pair of bilaterally symmetrical cells that migrate posteriorly during embryogenesis. The point of departure of this migration can be inferred from the position of the BDU (not an acronym) neurons, which are the non-migrating sister cells of the ALMs. The BDUs are positioned anteriorly, on the body midline, in contact with the excretory canal. The positions of both the left and right ALMs, but not of the BDUs, are frequently abnormal in Lid animals. Most frequently the ALMs are positioned too anteriorly and, more rarely, too ventrally compared to the wild type (Fig. 3). We have not observed ALMs in positions more posterior than the wild-type positions. We have also scored the position of 90 BDUs, which all are within the wild-type range (not shown).

The HSNs (hermaphrodite specific neurons) are born posterior to the anus and migrate anteriorly during embryogenesis to a sub-ventral position slightly posterior to the vulva. In mau-2 Lid animals, nearly half of the HSNs are found in abnormal positions (Fig. 3), including too posterior, too anterior and too dorsal (Fig. 2A).

The CANs (canal associated neurons) are bilaterally symmetric neurons that are born in the head and undergo a long posterior migration during embryogenesis. We have scored the position of the cell bodies of 91 CANs in Lid worms and all are found within the range of their normal wild-type position, just above or slightly anterior to the vulva (Fig. 2B).

AQR, the descendant of the QR neuroblast that has the longest migration path, migrates up to the deirid ganglion in the head. Because of the difficulty of ascertaining its presence within the deirid, we did not attempt to score its position in our mutants. However, a supernumerary cell body was sometimes

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**Fig. 3.** Schematic summary of cell positions and excretory canal length scored in mau-2(qm4) Lid larvae. (A) Relative cell positions in wild-type worms. The wild-type position of each cell body is indicated by a dark disk. SDQR and AVM are found only on the right side of the body; the other cells are bilaterally symmetric. The thick lines correspond to the normal placement of selected neuronal processes: the line contacting SDQR and ALM represents the placement of the process of ALM; the line contacting BDU and CAN represents the placement of the processes of CAN. The normal placement of the excretory canals is also at the midline in contact with the processes of CAN. (B) Boxes correspond to positions defined by various landmarks (see main text). An independent schema is given for every cell. The name of each cell is placed in the box corresponding to its normal position, and the percentage given corresponds to the fraction of cells that appeared to be in their wild-type positions. Abnormal cell positions are indicated by asterisks in the boxes (each asterisk represents one cell), corresponding to the approximate position in which they were found. The lowest panel represents where the excretory canal stops rather than the position of any cell body. For bilateral cells, both left and right cells appear in the scores.
We have examined living adult adults Abnormal neuronal cell migrations visualized in PQR. In addition, PVM is often observed too ventrally but to a lesser degree than found in a much too anterior position (Fig. 2B). PVM is also frequently misplaced. In particular, SDQL is frequently too anteriorly and posteriorly as in the wild type (Fig. 2C). Most identifiable axons, in particular all commissures, appear to be always normally placed, while the two QL descendants are frequently misplaced. In particular, SDQL is frequently found in a much too anterior position (Fig. 2B). PVM is also frequently observed too anteriorly but to a lesser degree than SDQL. In addition, PVM is often observed in too ventral a position, in contact with the axon of PLML. Finally, a large additional cell was sometimes observed posterior to the V5 descendants, and we speculate that it could be an unmigrated PQR.

Abnormal neuronal cell migrations visualized in adults

We have examined living adult mau-2 mutants by DIC microscopy and observed that, as in Lid animals, the cell bodies of migrating neurons are often in abnormal positions (data not shown). To investigate the phenotype of adult animals in more detail and to obtain a precise quantification of defects, we have used an $unc-31$::lacZ reporter gene to visualize neurons migrating on the lateral hypodermis (Fig. 4; see Materials and methods).

In general, the cellular positions observed in adults using this reporter are similar to those observed in Lid worms, as well as to those observed in living animals. However, the cell positions are less extremely abnormal than in Lid worms. Possibly, extremely abnormal cell positions are a hallmark of particularly severely affected mutants, which are less likely to develop to adulthood. In any case, the overall similarity of the cell positions scored in different ways indicates that the $unc-31$::lacZ reporter gene does not affect cell positions in mau-2; $unc-31$::lacZ animals, and that abnormal cell positions are not specific to animals with a Lid phenotype. Fig. 4 illustrates the misplacement of AVM, SDQR and ALMR in mau-2 mutants in comparison to the wild type.

We focused on four cells: AVM, SDQR and the two HSNs. The $unc-31$::lacZ gene is capable of rescuing the $unc-31$ phenotype and was therefore introduced without a dominant rol-6 transformation marker (see Materials and methods). The animals carrying the transgene are therefore not Rol, allowing us to measure precisely the positions of cells in relation to both body axes (see Materials and methods). When these positions are plotted (Fig. 5), the positions of the cells in different genetic backgrounds can be compared accurately (see Materials and methods and Table 1 for a statistical description).

In mau-2 animals, on the right anterior part of the body wall, the position of AVM is slightly anterior on average, as well as more spread out in both axes compared to the wild type; overall, AVM is not strongly affected, as we had already observed in the Lid animals (Fig. 3B). SDQR, however, is more strongly mispositioned: a proportion of the cells are placed much too ventrally, and, as a group, are significantly too anterior. Both HSNs are frequently misplaced, including dorsal, posterior and anterior misplacements. Not many HSNs are placed in abnormal ventral positions (Fig. 5). However, extreme ventral displacement would result in association of the cell with the heavily stained ventral cord and would likely have been missed. Alternatively, there might be non-specific mechanical barriers, such as the ventral muscles, which prevent more extreme ventral migrations.

Axonal guidance defects

In Lid worms, individual axons could frequently be observed (Fig. 2). Most identifiable axons, in particular all commissures, always appear to follow normal trajectories. However, the trajectory of axons of migrating neurons, including the CANs, frequently appeared severely abnormal. Although no specific pattern of defect emerged, the most striking defects included axons following trajectories along the body axis orthogonal to that of their normal trajectory. For example, CAN neurons were observed to send axons ventrally and dorsally, rather than anteriorly and posteriorly as in the wild type (Fig. 2C).

To further observe and quantify defects in axons of migrating neurons in living mau-2 animals we have used a $mec-7$::lacZ reporter gene, with which the seven microtubule cells can be visualized (Hamelin et al., 1992). With this
technique, we concentrated on the two bilaterally symmetric Posterior Lateral Microtubule cells (PLMs) because they were the most strongly and reliably stained by this technique. The axons of the PLMs normally run on the two ventro-lateral body sides. In *mau-2* mutants, we found that approximately half of the PLM axons were misplaced to the ventral cord (Table 3 and Fig. 6A). In general, it appears that the axons of misplaced PLMs directly entered the ventral cord in the tail. More rarely, the process runs first at its normal location and then is deflected to the ventral cord. The PLMs appear therefore to be defective in dorso-ventral guidance.

We have used a reporter gene expressing Green Fluorescent Protein (GFP) under the control of the *unc-31* promoter to visualize the axons of the AVMs and the ALMs as well as the morphology of the dorsal cord. In the wild type, the cell body of AVMs is placed on the right lateral side, and its axon is directed ventrally into the ventral cord where it turns and runs anteriorly. In *mau-2*, we found that the axons of one third of the AVMs are not directed ventrally but run on the lateral body side. Approximately one third of these misplaced axons run anteriorly, and two thirds posteriorly, therefore displaying simultaneously dorso-ventral and antero-posterior guidance defects (Table 3; Fig. 6B,C).

The axons of the ALMs are very rarely affected (Table 3). In all cases, axonal placement defects are seen in conjunction with extremely misplaced cell bodies, and are therefore difficult to interpret in terms of a particular guidance defect.

In addition, the ventral and dorsal cord frequently present an abnormal morphology, including loose fasciculation and splitting into several bundles. The abnormal morphology of the dorsal cord is the easiest to observe (Fig. 6D) and highly penetrant (Table 3).

**Other neuronal defects: abnormal dye-uptake**

A subset of sensory neurons in *C. elegans* can be stained with the dye DiI, and when the observed pattern differs from the wild type, the animals are said to show a Dyf (dye-filling defective) phenotype (Starich et al., 1995). In the wild type, DiI stains six neurons on each side of the head (the amphid neurons) and two

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Wild type PLM axon placement (%)</th>
<th>PLM axon misplaced to the ventral cord (%)</th>
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<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>mau-2</em> (+); mec-7::lacZ</td>
<td>70</td>
<td>96</td>
<td>4</td>
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<tr>
<td><em>mau-2</em> (qm4); mec-7::lacZ</td>
<td>101</td>
<td>58</td>
<td>42</td>
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</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Wild type AVM axon placement (%)</th>
<th>AVM axon does not reach the ventral cord (%)</th>
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</thead>
<tbody>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>mau-2</em> (+); <em>unc-31</em>::gfp</td>
<td>49</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td><em>mau-2</em> (qm4); <em>unc-31</em>::gfp</td>
<td>98</td>
<td>67</td>
<td>33 (23+10)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Wild type ALM axon placement (%)</th>
<th>Abnormal ALM axon placement (%)</th>
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<tbody>
<tr>
<td>C</td>
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<td></td>
<td></td>
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<tr>
<td><em>mau-2</em> (+); <em>unc-31</em>::gfp</td>
<td>98</td>
<td>100</td>
<td>0</td>
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<td><em>mau-2</em> (qm4); <em>unc-31</em>::gfp</td>
<td>185</td>
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<table>
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<tr>
<th>Genotype</th>
<th>n</th>
<th>Wild type Dorsal Cord morphology (%)</th>
<th>Abnormal Dorsal Cord morphology (%)</th>
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<tr>
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<td>96</td>
<td>16</td>
<td>84</td>
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</table>

n, total number of axons or of dorsal cords examined. (A) Phenotype of the axon of PLM. (B) Phenotype of the axon of AVM; in the case of *mau-2* mutants, 23% run posteriorly and 10% anteriorly (in parentheses). (C) Phenotype of the axon of ALM. (D) Phenotype of the dorsal cord. See main text for abbreviations and reporter gene descriptions.
neurons on each side in the tail (the phasmid neurons). In *mau-2* mutants, the Dil staining pattern is frequently abnormal, with fewer than the normal complement of neurons stained (Table 2E). In particular, although the amphid neurons stain frequently, in two *mau-2* alleles (*qm4* and *qm5*) the phasmid neurons stain rarely. In the third *mau-2* allele (*qm40*), only very rarely can any cell be stained (Table 2E). This was surprising since *qm40* does not appear to be more severe than the two other alleles by other phenotypic criteria. *mau-2* maps genetically in the vicinity of the gene *che-3*, in which mutations totally abolish Dil staining. We therefore tested *qm40* for complementation of *che-3(e1124)* and found that the chromosome carrying this *mau-2* allele fails to complement *che-3*. However, *mau-2* and *che-3* are almost certainly not allelic, for several reasons: (1) we found the other two *mau-2* alleles (*qm4* and *qm5*) to complement *che-3(e1124)*; (2) no other *che-3* allele is uncoordinated (Lewis and Hodgkin, 1977); and (3) we have shown that *mau-2* and *che-3* are physically distinct (C. Bénard and S. Hekimi, unpublished results).

**Defects in non-neuronal cells**

We have found defects in two types of mesodermal cells, the excretory cell and the distal tip cell (DTC). The four lateral projections of the excretory cell (two posterior and two anterior excretory canals) are short and generally terminate in an abnormal knobby structure where the canal winds several times onto itself (Fig. 2D). The posterior canal normally ends dorsally above the anus. We have systematically scored the length of the posterior canals in Lid worms (Fig. 3B). It appears that abnormally short canals do not stop preferentially at any given location, but can stop randomly at any position along their normal trajectory. Short canals are also observed in living adults by DIC microscopy.

The shape of the gonads of *mau-2* mutants is frequently abnormal. The most common defect is that, in adults, the anterior arm reflects backwards close to the vulva, the bend no longer occurring between the ovary and the oviduct but at the level of mature oocytes, with maturing oocytes situated dorsally (Fig. 7A). In these animals, the DTC is too posterior to the vulva and appears detached from the basal lamina. Of the 104 anterior gonad arms examined, 17% showed such a defect (1% out of 86 anterior gonad arms examined in the wild type). In the case of the posterior gonad arm, 12% of the 106 posterior gonad arms scored were abnormal (2% out of 92 posterior gonad arms examined in the wild type). In some animals a defect similar to that of the anterior arm is found, but most often the reverse phenotype is observed. The bend occurs at the level of the oviduct and syncytial nuclei can be found ventrally. In these adults, the DTC also appears detached and is posterior to the vulva. These phenotypes are probably not the result of an abnormal migration path of the DTC, but of its detachment from the basal lamina of the body wall. Indeed, observed gonad shapes in adults were sometimes extremely abnormal (e.g. winding around the gut), and are likely to have resulted from random motions imposed on a detached gonad by the movements of the animal.

We have also examined gonads in L4 larvae, where the...
DTCs have not finished their migration and appear normally attached to the basal lamina. Different defects in the gonad shapes from the ones described above are found. For example, the distal part of the arm can be, partly or entirely, placed ventrally instead of dorsally. Sometimes, the anterior distal arm is anterior to the loop, with the DTC near the pharynx (Fig. 7B). Thus, in addition to detaching from the basal lamina in adults, the migration path of the DTC is sometimes altered in mau-2 mutants both longitudinally and circumferentially.

Correlation between anterior and posterior dye uptake defects
As described above, we have observed effects of mau-2 mutations on a number of diverse cells, which migrate at different times during development, and in several distinct regions of the body. Such pleiotropic effects could be due to either an early requirement for mau-2 in a process organizing the spatial cues encoded by other genes, or a ubiquitous local use of the mau-2 gene product by migrating cells in all regions of the body.

We reasoned that a correlation between the severity of the phenotype of cells in non-adjacent regions of the body would tend to favour a requirement for mau-2 in a process organizing spatial cues. To try to establish such a correlation, we have focused on the Dyf defect of the amphid and phasmid neurons. These neurons are functionally related cells that exist in four sets, found at the four corners of the worm, left and right at both the anterior and the posterior. There is no immediate common lineal ancestry between these sets. Fully mutant animals cannot be used to establish such a correlation, since the phasmid defect is completely penetrant (Table 2E). We therefore examined the Dyf phenotype in maternally rescued mutant animals, qm4(m+z−). We stained animals and identified those in which the phasmid and the amphid neurons were stained on only one side of the animal, and scored whether the two groups of stained neurons were on the same or on different sides of the animal. We found that the groups of stainable neurons were almost always on the same side (Table 2F), without preference for the left or right side. As a control, the same experiment was performed using mec-1(e1066) mutants that have been reported to show variable staining (Starich et al., 1995), and no correlation between stainable sides was found with these mutants (Table 2F). These results suggest that the processes requiring mau-2(+) acts on a whole side of the embryo at a time, rather than mau-2(+) being required locally.

DISCUSSION
The phenotype of mau-2 mutants suggests that this gene is involved in guidance of several types of cellular migrations, including those of neurons and their axons. We observed that the final positions of migrating cells in mau-2 are frequently abnormal. It included positions implying excessive migration, suggesting that the ability to migrate per se is not altered in these cells. We observed defects in the morphology of axonal tracts such as the ventral and dorsal cords, as well as in the guidance of individual axons such as those of the PLMs and AVM. These are cells whose axons pioneer at least part of their trajectories. Indeed, the process of AVM is associated with no other process until it reaches the ventral cord, and the only axon loosely associated on the lateral body wall with PLM is that of PLN, a neuron born during postembryonic development (Sulston and Horvitz, 1977), and which therefore cannot contribute to the development of the embryonic PLMs. Thus, the defects observed in the dorsal and ventral cord could be due to the inappropriate behaviour of the axons which pioneer these process tracts.

As a guidance gene, mau-2 displays a number of novel properties. (1) It is the first pathfinding gene to be described that is
defined by mutations displaying a maternal effect. (2) mau-2 appears to be required for cellular and axonal guidance along both main body axes, dorso-ventral and antero-posterior, while previously identified genes appear to have effects that are mostly confined to a single axis, in Caenorhabditis (Wightman et al., 1995; Hedgecock et al., 1990; Hekimi and Kershaw, 1993), Drosophila (Seeger et al., 1993) and even vertebrates (Kennedy et al., 1994; Colamarino and Tessier-Lavigne, 1995).

(3) In mau-2 mutants, the expression of defects in anterior and posterior cells is correlated within the left or right sides of the body. We are not aware of a similar phenomenon in other mutants, although such a correlation may not have been looked for.

What do these properties tell us about the action of mau-2? When we say that guidance genes have previously been found to be axis-specific, we are referring to the already established body axes of the embryo. The establishment of the primary embryonic axes is being intensely studied in several model systems. In Drosophila and in C. elegans, genes which appear to be involved in main body axes specification all appear to be defined by strict maternal-effect mutations and act during oogenesis or very early in development. By contrast, the genes which appear to encode spatial guidance cues for migrating cells or axons, including those required for direction-specific guidance, are all encoded by genes which appear to have no maternal component (Brenner, 1974; Manser and Wood, 1990), suggesting that their expression is probably only required during later differentiation. For mau-2, however, and a number of other genes affecting the development of the nervous system in C. elegans (Hekimi et al., 1995), their expression in either the mother or the zygote is sufficient for a wild-type phenotype, in animals where the mother or the zygote is sufficient for a wild-type phenotype, suggesting that their expression is probably only required during later differentiation. For mau-2, however, and a number of other genes affecting the development of the nervous system in C. elegans (Hekimi et al., 1995), their expression in either the mother or the zygote is sufficient for a wild-type phenotype, suggesting that their expression is probably only required during later differentiation. The observation that mau-2 appears to act on a whole side of the animal at a time supports this interpretation, as it suggests that mau-2 acts at the time when positional information is still being globally organized.

What could the function of mau-2 be? We suggest that mau-2 might be involved in setting up pattern of guidance cues along the primary axes. Such guidance cues could be encoded by genes such as unc-5 or unc-6 (Ishii et al., 1992; Leung-Hagesteijn et al., 1992). For example, one type of complex pattern of expression that has often been proposed for guidance cues is diffusible or substrate-bound gradients. Indeed, the vertebrate netrins, and therefore UNC-6, are molecules that very likely act in a gradient (Kennedy et al., 1994). mau-2 could be one of the genes required to produce a gradient of UNC-6 or some other guidance cue. On the other hand, it has been shown that unc-6 is expressed in a very dynamic pattern of cellular expression during development, likely creating a hierarchy of guidance cues (Wadsworth et al., 1996; Wadsworth and Hedgecock, 1996). For this type of complexity too, upstream-acting gene products, for example transcription factors, will be necessary to produce the observed pattern.

We thank Jonathan Ewbank, Bernard Lakowski, and in particular Tom Barnes for helpful discussion and for critically reading the manuscript. We thank Eric Aamodt for sending Jels11[mech-7::lacZ]. Some nematode strains used in this work were provided by the Caenorhabditis Genetic Center, which is funded by the NIH National Center for Research Resources (NCRR). This work was supported by a National Science and Engineering Research Council (NSERC) grant to S.H., and by the Yamada Foundation for Promotion of Science and the grants from the Ministry of Education, Science and Culture of Japan to S.T.

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(Accepted 3 October 1997)