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Genetics of cell and axon migrations in *Caenorhabditis elegans*

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Summary

The *Caenorhabditis elegans* epidermis comprises 78 cells which cover the external surface of the embryo as a single cell layer. These cells secrete the cuticle from their exterior faces and support the body wall muscles and most of the nervous system on their interior faces. The epidermal cells arise by autonomous embryonic cell lineages but show regulative interactions after their assembly into an epithelium. It is believed that the various epidermal cells express different kinds or amounts of surface molecules that govern their mutual assembly and also guide the attachments and migrations of the underlying body muscles and neurones. The first muscles and neurones may in turn express new surface molecules that refine later cell movements. Mutations in some 30 known genes disrupt the movements of cells or axons along the body wall.

Key words: *Caenorhabditis elegans*, neurone, migration, genetics, cell lineage, axon.

Introduction

Cells assemble into organs by selectively attaching to neighbouring cells. In animals, the final neighbours need not be close relatives as cell migrations can separate clonally related cells or bring unrelated cells into contact. Cells can also make remote contacts by extending active processes that migrate to distant sites while their somata remain stationary. In the nervous system, for example, the growth cones on the tips of advancing axons undertake elaborate and precise migrations that establish the synaptic connections. A major goal of developmental biology is to describe the timing and trajectories of cell and process migrations and to determine their mechanisms of locomotion and guidance.

In this paper, we review the cell and axon migrations that occur during development of the nematode *Caenorhabditis elegans* and mutations affecting these migrations.

Embryonic body wall

A single layer of epidermal cells, joined to one another by belt desmosomes, forms the external surface of the nematode. The desmosomes divide the surface of each cell in the epithelium into two topologically and functionally distinct faces. The exterior (apical) face secretes the cuticle, a strong flexible layer of collagen that covers and protects the animal, while the interior (basal) face secretes a basal lamina (Fig. 1). Neurones and their axons develop sandwiched between the cell membrane and basal lamina of the epidermis. Muscle cells also attach to the interior face of the epidermis, but, unlike the nervous system, are topologically distal to the epidermal basal lamina. The epidermal cells and the attached neurones and muscles are collectively termed the nematode body wall.

Epidermis

The epidermis in *Caenorhabditis elegans* (traditionally called the hypodermis) comprises only 78 embryonic cells (Sulston, Schierenberg, White & Thomson, 1983). Many of these are smaller cells in the head, arranged geometrically to provide openings for the buccal passage and cuticular sensory organs, and cells posterior to the rectum that form the tail taper. The 55 larger cells, which form most of the surface, are arranged longitudinally in two dorsal, two lateral and two ventral rows with 17, 20 and 18 cells, respectively. Early in morphogenesis, the dorsal rows interleave to form a single row of dorsal cells, each spanning from side to side (Figs 2, 3). After completing these

movements, the 17 dorsal cells plus 6 of the ventral cells fuse together to form a single large epidermal syncytium called hyp7 (Singh & Sulston, 1978; Priess & Hirsh, 1986). Thus, at hatching, most of the dorsal surface and parts of the ventral surface of the head and the tail are covered by a single large cell with 23 nuclei.

At hatching, the 12 unfused ventral epidermal cells (called P1/2, P3/4,...,P11/12) form two symmetrical rows with each cell confronting its bilateral homologue along the ventral midline (Fig. 2). During the first larval stage, these cells interleave to form a single row of ventral cells spanning from side to side (Sulston & Horvitz, 1977). There is some natural variation between animals in the anterior/posterior cell order that results as left/right pairs interleave, and therefore, the ventral cells are renamed P1–P12 in accordance with their positions in the completed

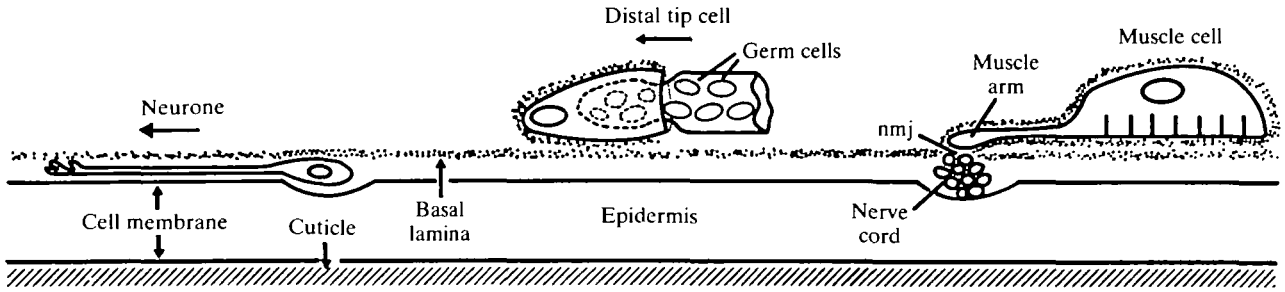

Fig. 1. Topology of body wall. (Left) A neurone positioned between the epidermal cell membrane and basal lamina. The arrow indicates the direction of axon outgrowth. (Middle) A distal tip cell, ensheathing the distal germ cells in the hermaphrodite gonad, migrates along the basal laminae of either body wall muscles (not shown) or epidermal cells. The arrow indicates the direction of gonad elongation. (Right) A body wall muscle attached to the epidermis. A muscle arm extends to a nearby nerve where it forms a neuromuscular junction (nmj) traversing the basal laminae.

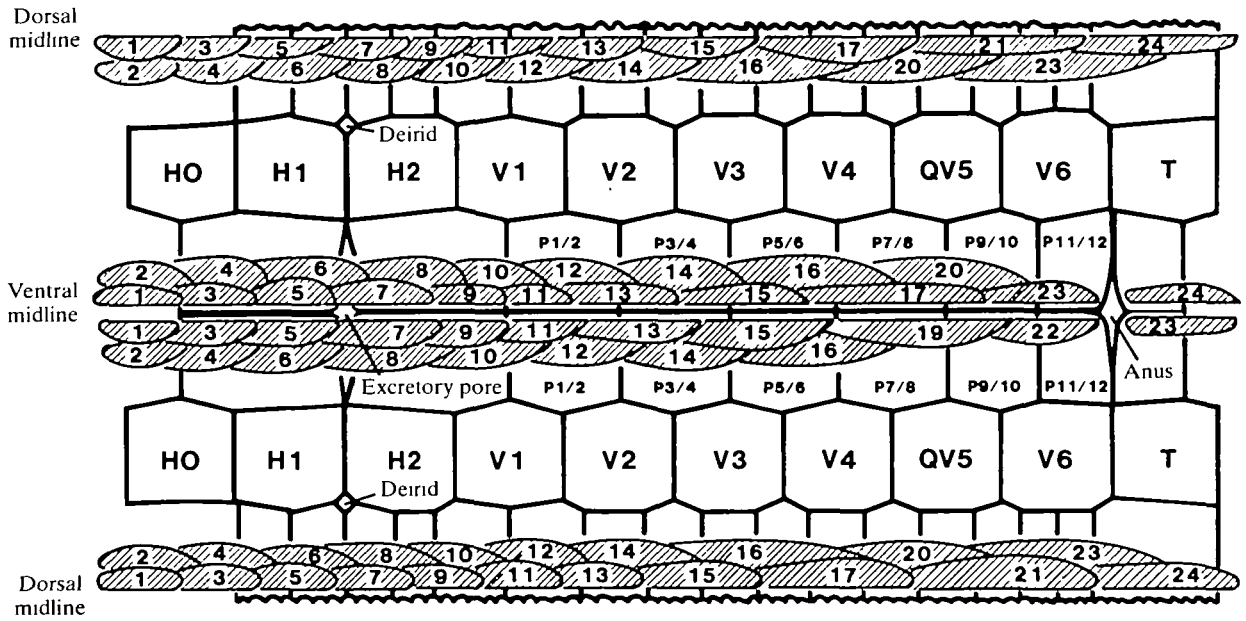

Fig. 2. Cylindrical projection of embryonic body wall cut open along the dorsal midline showing the approximate boundaries of the body muscles and the larger epidermal cells (after Sulston *et al.* 1983; Priess & Hirsh, 1986). Anterior is drawn to the left. The smaller epidermal cells of the head (hyp1–hyp6) and the tail (hyp8–hyp11) and the interfacial cells forming the openings for the deirid sensilla, the excretory pore and the anus are omitted. The lateral and ventral blast cells are labelled according to Sulston *et al.* (1983). The 23 epidermal cells that will form the hyp7 syncytium are unlabelled. At the stage shown, the right and left dorsal epidermal cells have already intercalated to form a single longitudinal row. Ventral epidermal cells P1–P12 undergo a similar intercalation during the first larval stage (Sulston & Horvitz, 1977).

The body muscles (striped) are arranged in four longitudinal quadrants (dorsal left, dorsal right, ventral left, ventral right) along the dorsal and ventral epidermis. The muscles are numbered by quadrant, from anterior to posterior. Gaps in the numbers correspond to positions where additional muscle cells generated in the first larval stage from the M mesoblast may intercalate (Sulston & Horvitz, 1977). It is unknown whether the arrangement of muscle cells following the larval intercalations is invariant between individuals.

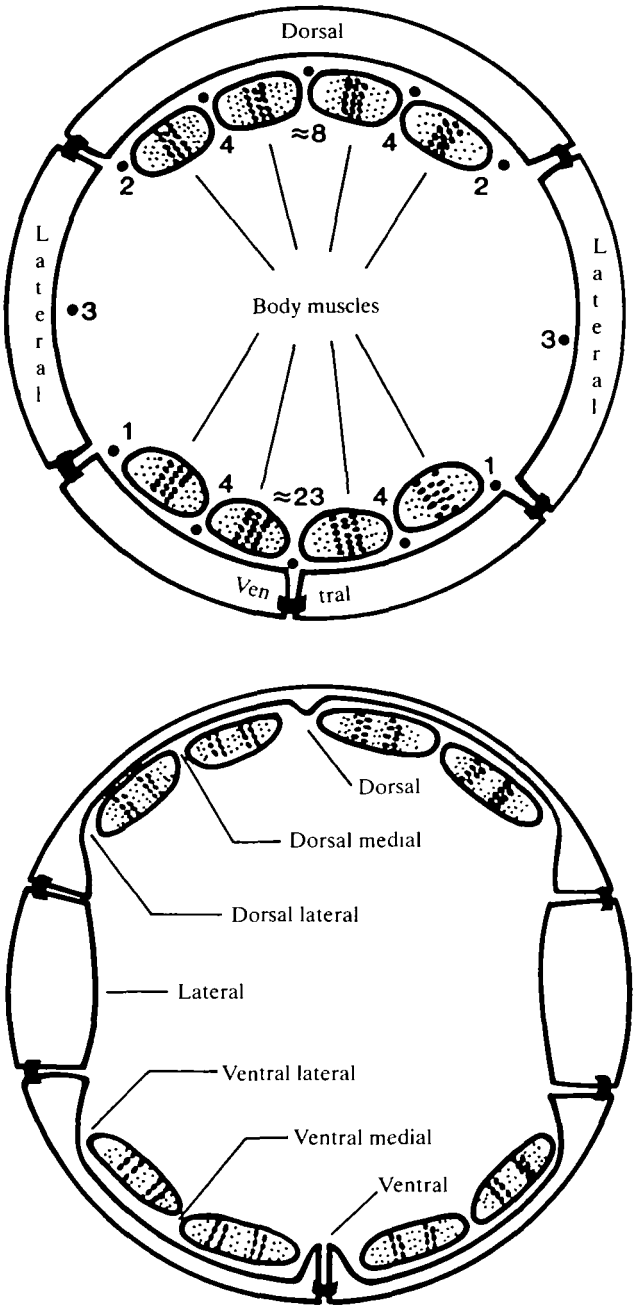

Fig. 3. Transverse section of embryonic body wall (after Sulston *et al.* 1983; White *et al.* 1986). (Upper) Five epidermal cells, joined to each other by desmosomes, complete the circumference. Body muscles, typically four abreast, are attached to the dorsal and ventral epidermal cells. The various longitudinal nerves are positioned against the interior face of the epidermal cells (solid dots). The numbers of axons in these nerves are indicated. These numbers are representative for sections at the level of the V1 cells, excepting that the ventral lateral PLM axons terminate more posteriorly (see Figs 2, 4). The axon counts for the dorsal and ventral nerves vary somewhat as local motor axons either begin or terminate within a sampled region. (Lower) At hatching, the epidermal nuclei and the bulk of the cytoplasm are confined to four ridges complementary to the four muscle quadrants.

queue. These ventral cell movements closely resemble the embryonic movements of the dorsal cells. Both intercalations are disrupted in *unc-83* and *unc-84* mutants (Sulston & Horvitz, 1981). The ventral cells P1–P12 divide soon after intercalating. After the divisions, the belt desmosomes of the anterior daughters constrict apically and these cells detach from the epithelium to become neuroblasts (Sulston & Horvitz, 1977; C. Kenyon, personal communication). The posterior daughters remain in the epithelium at the position of the mother cell. The subsequent fates of both the neuroblasts and the epidermal daughters vary by position along the ventral queue and also by the sex of the animal. The lateral epidermal cells, excepting H0 (see Fig. 2), also divide during larval development, producing additional epidermal cells and some neuroblasts. Again, these lineages vary by cell position and by the sex of the animal.

As outlined above, the epidermal cells have diversified fates which accord with their final positions in the epithelium. Two extreme developmental mechanisms could explain this precise relation between cell position and cell fate. First, the various epidermal cells could be equivalent cells which adopt different fates according to cell interactions that occur as they assemble into an epithelium. Alternatively, differences between cells could be established before the epidermal cells assemble together. In this latter case, the precise positioning of the cells in the epithelium could be assisted by differences in the surface molecules expressed by the committed cell types. Both the cell lineages that generate the embryonic epidermis and the final positions of these cells are invariant between individual embryos, i.e. each cell position in the epithelium correlates to a fixed cell ancestry (Sulston *et al.* 1983). This does not itself imply that all differences between individual epidermal cells are predetermined by cell ancestry but it does indicate that wherever cell interactions modify intermediate cell lineages or terminal cell fates then these signals or contacts must also be reproducible between individuals.

To test for possible cell interactions, individual epidermal precursors have been experimentally ablated during embryogenesis with a laser microbeam (Sulston *et al.* 1983). For some precursors, the treated embryos generally rupture during elongation, apparently because the missing epidermal cells leave a gap in the epithelium through which cells are extruded by internal pressure (see Priess & Hirsh, 1986). For other ablations, the remaining epidermal cells are usually able to complete the epithelium by joining to unusual neighbours. These embryos elongate and hatch normally and the resulting larvae have been examined in some detail. With specific exceptions,

the cell lineages of the surviving precursors, and the fates of their epidermal descendants, appear unaltered in these experimental animals. In particular, the embryonic epidermis is unable to recruit replacements for the descendants of an ablated cell from other lineages. These experiments suggest that, during normal embryogenesis, the epidermal precursors proceed autonomously in generating precise numbers of committed epidermal cells, appropriately placed for their subsequent assembly into an epithelium.

After the embryonic epidermal cells are assembled into an epithelium, cell interactions further modify certain cell fates, including the postembryonic (larval) behaviour of the lateral and ventral blast cells (Sulston & White, 1980). For example, when the lateral epidermal cell V6 is ablated in young male larvae, descendants of V5 can shift posteriorly and generate some of the sensory neurones normally made by V6. Related experiments have demonstrated that V4 and even V3 can imperfectly substitute for V6 when all intervening cells are removed. Similar substitutions have been observed following ablations in the queue of ventral epidermal cells.

Cells that can replace another, either perfectly or imperfectly, are said to constitute an equivalence group (Sulston & White, 1980). There is always a directionality to these experimental replacements. For example, V5 descendants can be recruited to replace a missing V6 cell but not conversely. In general, there appears to be an ordering of cell positions or fates for each equivalence group such that cells adopt a preferred or primary fate when available and adopt a secondary or tertiary alternative only when other cells within the equivalence group have pre-empted more favoured choices. These experiments suggest that cell interactions, either directly between members of an equivalence group or with an asymmetrically positioned inductor cell near to the group, govern the normal selection of alternative fates (see Sternberg & Horvitz, 1986).

There are few genes presently known that affect the cell lineages that generate the embryonic epidermal cells or their assembly into an epithelium. As mentioned above, embryos with missing or unassembled epidermal cells may be expected to arrest during elongation or earlier. In contrast, there are many viable mutants that have an essentially normal embryonic epidermis but abnormal postembryonic fates of the lateral and ventral epidermal blast cells (Sternberg & Horvitz, 1984). For example, *lin-20* and *lin-22* mutations transform the larval lineages of certain anterior and midbody epidermal cells, respectively, into patterns normally generated by more posterior cells (Hedgecock, 1985; W. Fixsen, personal communication; Horvitz *et al.* 1983). Conversely, *mab-5* mutations transform the larval lineages of certain posterior epidermal cells into patterns normally generated by more anterior cells (Kenyon, 1986). The *lin-12* mutations transform cell fates in various tissues including ventral and interfacial epidermal cells, i.e. cells that connect openings in the epidermis to internal epithelia. In each case, homologous cells, which normally adopt different fates, instead adopt the same fate (Greenwald, Sternberg & Horvitz, 1983). The *lin-12* gene product has recently been shown to be related to epidermal growth factor, supporting the view that cell interactions govern the selection of alternative fates in these instances (Greenwald, 1985).

Muscles

Locomotion is mediated by 95 muscle cells attached to the dorsal and the ventral epidermis in two wide, longitudinal bands, each roughly four cells abreast (Figs 2, 3). The cells in each band are separated into quadrants (left and right dorsal, left and right ventral) by the dorsal and ventral nerves, respectively (described below). In the head, the muscle cells are further subdivided functionally into rows of single cells reflecting differences in the innervation of medial and lateral cells within each quadrant (White, Southgate, Thomson & Brenner, 1986). The muscle cells compress the epidermal cytoplasm to a thin sheet where they attach and induce a transepidermal anchorage to the cuticle. As a result, the epidermal nuclei and the bulk of the cytoplasm are confined to four prominent epidermal ridges (dorsal, left and right lateral, ventral) complementary to the muscle quadrants in the completed body wall (Fig. 3).

81 of the muscle cells are formed during embryogenesis while an additional 14 are made during the first larval stage from the M mesoblast cell and then intercalate with the embryonic muscle cells (Sulston *et al.* 1983; Sulston & Horvitz, 1977). The cell lineages that generate the embryonic muscle cells and the final positions of these cells in the quadrants are invariant between individuals (Fig. 2). The M mesoblast lineage and, possibly, the positions at which the larval muscle cells intercalate with the embryonic muscle cells are also invariant (Fig. 10).

The individual muscle cells are nearly identical in structure except for their positions along the body wall and their modes of innervation (described below). The reproducible positioning of the muscle cells, including both their anterior/posterior order and their segregation to either the dorsal or ventral epidermis, could be an accident of their birth positions or could also reflect cell intrinsic differences in epidermal preference. There are four cases in the

embryonic muscle lineages where sister muscle cells attach to opposite, dorsal and ventral, quadrants, apparently by active movements. In laser ablation experiments, killing an individual precursor of eight or ten body muscle cells, appeared to cause an equal reduction in the total of surviving muscle cells and to leave gaps in the corresponding quadrants (Sulston *et al.* 1983). The inability of surviving muscles to stretch over these gaps may reflect a mechanical limit to their cell length or indicate that the survivors lack surface adhesion molecules appropriate to the denuded regions.

In *unc-23* mutants, the anterior muscle cells in all four quadrants selectively detach from the epidermis during larval growth (Waterston, Thomson & Brenner, 1980). The embryonic arrangement of the muscles in these mutants is apparently normal. Recently, we have identified five *mup* genes with embryonic defects in *mu*scle *p*ositioning on the body wall (unpublished data). These include mutants in which some individual muscle cells are simultaneously attached to both the dorsal and ventral epidermis and mutants in which the cells are organized into quadrants but the quadrants are improperly attached.

Neurones

The nematode nervous system is composed of two nearly independent circuits, a somatic circuit comprising neurones associated with the body wall that control locomotion and a smaller circuit contained within the pharynx that controls feeding. In newly hatched *Caenorhabditis elegans* hermaphrodites, the somatic nervous system comprises 202 neurones which have been placed into 82 classes on the basis of their synaptic connections (Sulston *et al.* 1983; White *et al.* 1986). The cell bodies of most of these neurones are clustered in ganglia in the head or tail. Seven additional classes of embryonic neurones are positioned along the body itself (Fig. 4). Three classes of motorneurones (DA, DB, DD) controlling forward and backward movement are arranged single file along the ventral epidermal ridge while four pairs of neurones (ALM, BDU, CAN, HSN) are positioned along the lateral epidermal ridges. The ALM, CAN and HSN neurones reach their midbody positions by

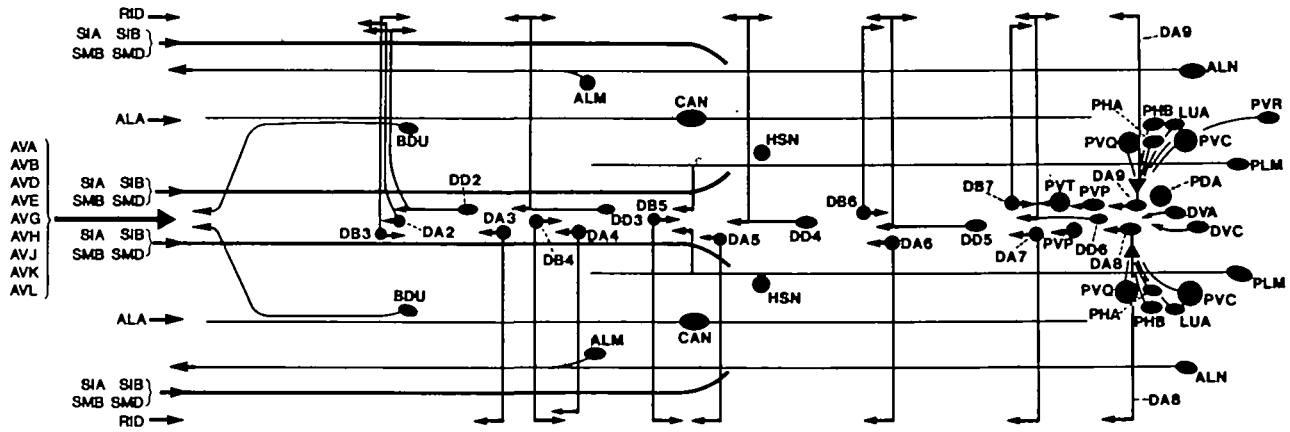

Fig. 4. Cylindrical projection of the larval body wall cut open along the dorsal midline showing the nervous system (after Sulston *et al.* 1983; White *et al.* 1986; Hall & Russell, 1987). Anterior is drawn to the left. The cell bodies and primary axons of all embryonic neurones posterior to the H2 cells (see Fig. 2) are shown. Arrowheads indicate that the axons extend further in that direction. Axons from embryonic cells in the head extend posteriorly along the ventral (AVA, AVB, AVD, AVE, AVG, AVH, AVJ, AVK, AVL), sublateral (SIA, SIB, SMB, SMD), lateral (ALA) and dorsal (RID) nerves. (The sublateral nerves occupy the positions labelled DORSAL MEDIAL and VENTRAL MEDIAL in Fig. 3). The cell bodies of three classes of motorneurones (DA, DB, DD) are arranged single file along the ventral epidermal ridge. In this diagram, they are staggered for clarity. Axons from these motorneurones extend longitudinally along the ventral nerve and circumferentially to the dorsal midline where they form the dorsal nerve. Four classes of cells (ALM, BDU, CAN, HSN) have cell bodies at isolated positions along the lateral epidermal ridge. The ALM axons extend along the lateral margins of the dorsal body wall muscles (positions labelled DORSAL LATERAL in Fig. 3). The BDU and CAN processes fasciculate with ALA, forming the lateral nerves. The HSN axons are not developed at this stage. The preanal ganglion, at the posterior terminus of the ventral nerve, comprises three embryonic interneurones (PVPL, PVPR, PVT) plus three motorneurones (DA8, DA9, DD6). PDA is an interfacial epidermal cell in newly hatched larvae which redifferentiates later as a motorneurone. The lumbar ganglia, comprising neurones adjacent to the lateral epidermal ridges, include sensory neurones (PHA, PHB, PVR) and interneurones (LUA, PVC, PVQ) which extend axons into the ventral nerve *via* a pair of circumferential nerves called the lumbar commissures. The PVR neurone is present only in the right lumbar ganglion. Two additional classes of lumbar neurones (ALN, PLM) extend axons along the lateral margins of the dorsal and ventral body wall muscles (positions labelled DORSAL LATERAL and VENTRAL LATERAL in Fig. 3, respectively).

active embryonic migrations (described below). The pharyngeal nervous system comprises 20 neurones which have been placed into 14 classes (Albertson & Thomson, 1976). The pharynx is a single layer of muscle and structural cells joined by belt desmosomes to form a cylindrical epithelium. The anterior and posterior boundaries of the cylinder are continuous with the epidermal and intestinal epithelia, respectively. Like the epidermis, the pharynx secretes a cuticle from its exterior (apical) surface. The neurones develop on the interior (basal) surface, between the cell membranes and the basal lamina of the epithelium.

The axons of the somatic nervous system form a circumpharyngeal nerve ring plus longitudinal nerves attached to the body wall and joined by various circumferential nerves called commissures (Fig. 4). The nerve ring is a U-shaped nerve comprising some 100 parallel axons in a typical cross section. The nerve ring encircles the pharynx dorsally. The legs of the 'U' coalesce ventrally, forming a ventral nerve which extends posteriorly along the epidermal ridge to the rectum. In addition to axons from interneurones in the nerve ring, this ventral nerve contains axons projecting anteriorly from sensory neurones and interneurones in the tail ganglia and local axons from the motorneurones positioned along the ventral epidermal ridge. In newly hatched larvae, the ventral nerve contains fewer than 30 axons in a typical cross section. Nearly all synapses in the somatic nervous system, excepting neuromuscular junctions (described below), are confined to the nerve ring and ventral nerve. Synapses are made *en passant* between adjacent axons which may be continuously apposed for considerable distances (White, Southgate, Thomson & Brenner, 1983). Axons can change positions, and neighbours, within an axon bundle, usually at nerve junctions where many axons reassort.

The axons from the DA, DB and DD motorneurones grow circumferentially to the dorsal epidermal ridge where they form a longitudinal nerve (Fig. 4). The dorsal and ventral body wall muscles receive innervation by extending processes, called arms, to the dorsal and ventral nerves, respectively, where they form neuromuscular junctions with these neurones across the epidermal basal lamina (Figs 1, 5). The anterior eight muscle cells in each quadrant are exceptional in that they either receive innervation by sending arms to the nerve ring (cells 1–4 in Fig. 2) or by sending arms both to the nerve ring and to the dorsal or the ventral nerve (cells 5–8 in Fig. 2).

The individual neurones in *Caenorhabditis elegans* have simple, invariant geometries (Albertson & Thomson, 1976; Sulston, Albertson & Thomson, 1980; White, Southgate, Thomson & Brenner, 1976; White *et al.* 1986). Most of the cells have only a single,

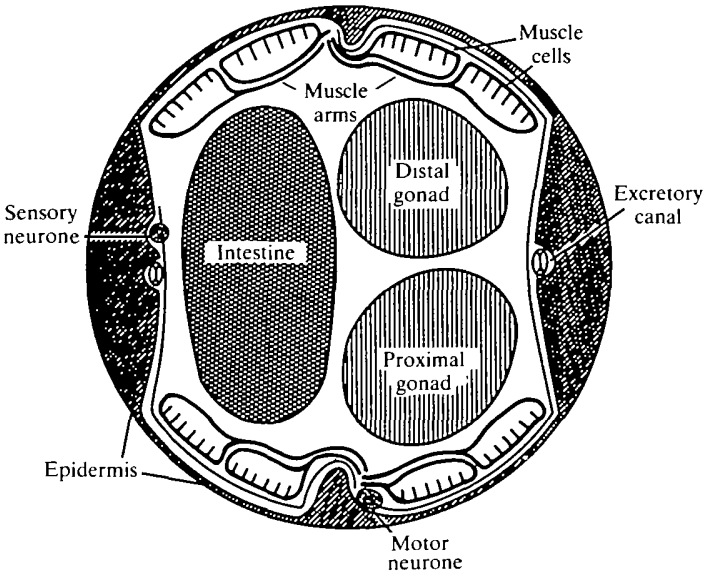

Fig. 5. Transverse section of the adult hermaphrodite body wall (after White *et al.* 1986). A motorneurone with cell body adjacent to the ventral epidermal ridge extends a circumferential axon to the dorsal nerve. A sensory neurone with cell body adjacent to the lateral epidermal ridge extends a circumferential axon to the ventral nerve. The dorsal and ventral body wall muscles extend arms to the adjacent dorsal and ventral nerves, respectively, to form neuromuscular junctions. The excretory canals, seen in cross section, extend longitudinally along the lateral epidermal ridge. The gonad, seen as two parts in transverse section, and the intestine fill the body cavity. The epidermal basal lamina which separates the neurones and excretory cell from the body cavity is not shown.

unbranched axon. Most of the remainder have either two unbranched axons or a single axon with one branch point. Branch points occur at invariant locations. There are four circumstances, two homophilic and two heterophilic, in which branching occurs. First, when axons join midway along an existing nerve, they may bifurcate to grow in the equivalent neighbourhoods of both directions. Interestingly, not all classes of neurones bifurcate in this circumstance, suggesting that entering axons can sense the polarity of the nerve. Second, when an axon confronts two equivalent neighbourhoods, only slightly separated, it may bifurcate to grow in both. Most commonly, this happens when a midline axon confronts bilaterally symmetrical regions of the nerve ring. It may also occur when one member of a symmetrical pair of neurones fails to innervate an ipsilateral neighbourhood and the contralateral neurone branches abnormally to innervate both sides (Albertson & Thomson, 1976). More generally, axons may bifurcate to innervate two nearby, non-equivalent neighbourhoods. Finally, the axons in certain longitudinal nerves bifurcate to extend a

circumferential branch across the epidermis to reach another nerve while a longitudinal branch continues in the original nerve. Embryonic neurones cultured *in vitro* on an adhesive substratum generally have a single, unbranched axon (H. Bhatt and E. Hedgecock, unpublished data).

Excretory cell

The excretory cell, a large ectodermal cell in the head, resembles the somatic neurones in that it extends long, active processes between the cell membrane and basal lamina of the epidermis (Figs 5, 6). These processes, called canals, are larger than nerve axons and have lumens which open externally *via* the excretory duct. A small lateral nerve containing the axons of three embryonic neurones (ALA, BDU, CAN) runs in close association with the posterior excretory canals (Fig. 4).

The excretory cell, and its associated duct and pore cells, appear to function in osmoregulation (Singh & Sulston, 1978; Nelson & Riddle, 1984). When any of these cells are killed, the animals fill with fluid and die within a few days, often without progeny. The canal-associated neurones (CAN), although nominally embryonic neurones, make no known synapses and enlarge to a non-neuronal morphology in older larvae. Interestingly, when the CAN cells are killed in newly hatched larvae, the animals take on a pale, starved appearance and die before becoming adults (J. Sulston, personal communication). Conceivably, the CAN cells have a role in regulating the adjacent excretory canals.

Axon migrations

To understand how the nervous system is assembled, it is important to know the temporal order of cell births and axon outgrowth. The neuronal cell lineages of *Caenorhabditis elegans*, including cell birth times, migrations and programmed deaths, have been determined by light microscopic observations of living embryos (Sulston *et al.* 1983) and larvae (Sulston & Horvitz, 1977; Sulston *et al.* 1980). More recently, serial electron microscopy has been used to establish the order of axon outgrowth in some of the embryonic nerves and to identify embryonic cells that line the paths of axon migration (R. Durbin, personal communication). The earliest axons, called pioneers, which grow posteriorly along the ventral nerve from the head and anteriorly from the tail have been identified. Similarly, the sequence of outgrowth of the axons from the DA, DB and DD motorneurones has been examined. Such descriptive studies can suggest positions where cell interactions may guide growing axons. Laser ablation of individual cells can then be used to explore the role of substratum cells in guiding pioneer axons and the role of pioneer axons in guiding later arrivals (R. Durbin; W. Walthall and M. Chalfie; personal communications).

A second experimental approach involves identifying and interpreting mutants with altered neurone morphologies. From a larger collection of behavioural mutants (Brenner, 1974; Swanson, Edgeley & Riddle, 1984), over twenty genes have been identified that affect the outgrowth of sensory, motor or interneurones in *Caenorhabditis elegans*. A partial list of these genes and their mutant phenotypes is given in Table 1. This list is necessarily incomplete in that it omits less characterized mutants and, more seriously, the full scope of defects is unknown for many of the genes. An important class of mutants not included in Table 1 is those with apparently normal axon placement but abnormal synapse formation. Two mutants

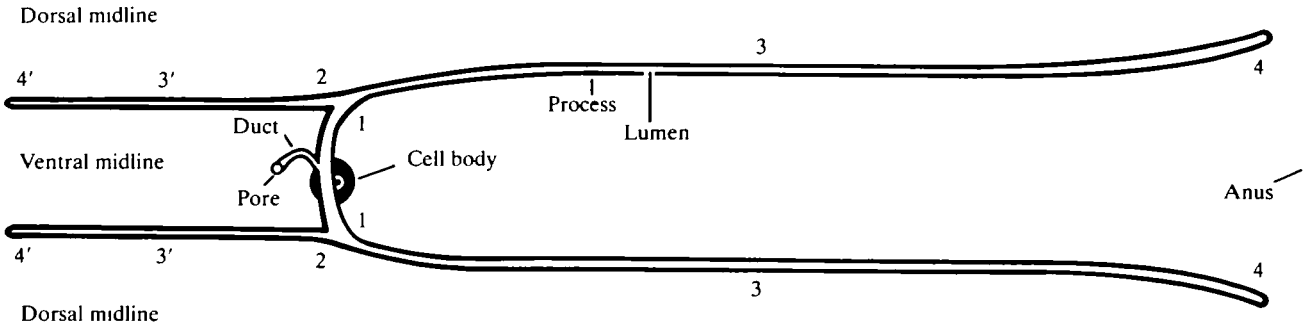

Fig. 6. Cylindrical projection of the larval body wall cut open along the dorsal midline showing the excretory cell (Nelson, Albert & Riddle, 1983; Sulston *et al.* 1983; White *et al.* 1986). The excretory cell body is adjacent to the ventral epidermal ridge. A bipolar, circumferential process extends dorsally in both directions (POSITIONS 1). These processes split at the lateral epidermal ridges (POSITIONS 2) to form longitudinal canals extending both anteriorly and posteriorly. The anterior canals run near the ventral margins of the lateral epidermal ridges (POSITIONS 3'). The posterior canals run near the middle of the lateral epidermal ridges (POSITIONS 3) and shift to the dorsal margins where they terminate near the boundaries of the V6 and T cells (POSITIONS 4). The continuous, hollow lumen of the H-shaped canal system is connected to the exterior by a duct and a pore cell whose bodies are not shown.

in this category, *unc-4* and *unc-55*, have been reported. The class VA motorneurones in *unc-4* mutants receive inappropriate input from the ventral nerve interneurones (J. White, E. Southgate and N. Thomson, personal communication). The class VD motorneurones in *unc-55* mutants make neuromuscular junctions with dorsal, rather than ventral, body wall muscles (L. Nawrocki and N. Thomson, personal communication).

The axons that pioneer the somatic nerves are presumably guided by adhesive interactions with the underlying epidermal cells and, perhaps, the overlying muscle cells which form the body wall. The various longitudinal nerves are positioned near the centres or margins of these cells (Fig. 3). This suggests, for example, that lateral axons are guided primarily by interactions with the row of lateral epidermal cells while ventral lateral axons may have an affinity for both the ventral and lateral epidermal cells. A simple prediction is that, in the absence of the adhesive interaction supplied by the lateral epidermal cells, a normally lateral axon might simply fail to grow while a ventral lateral axon might grow near the centre of the ventral epidermal cells rather than near their lateral margins. Indeed, in both *unc-53* and *unc-73* mutants, the lateral excretory canals terminate in midbody and the ventral lateral axons of the PLM neurones veer abnormally into the ventral nerve (Tables 1, 2). Both phenes might be explained if adhesive interactions with the lateral epidermal cells V4 or QV5 were reduced in these mutants.

Gradients or discontinuities in epidermal adhesiveness might help orient growing axons or delimit their extents. Longitudinal gradients must be established in fairly coarse steps, perhaps with subsequent smoothing, as there are so few epidermal cells spanning the length of the animal. For example, the anteriorly growing PLM axons normally terminate at about the V2/V3 boundary (Fig. 4). This is also about the posterior limit of the ALM cell migration

Table 1. *Genes affecting axon growth*

Gene	Reference allele	Linkage group	Number of isolates	Principal defects
unc-3	(*e151*)	X	4	All classes of motor axons mispositioned within ventral nerve; ventral nerve interneurones normal. [13]
unc-5	(*e53*)	IV	11	General failure of dorsal axon and cell migrations. [1,2,4,5,9,12,14]
unc-6	(*ev400*)	X	22	General failure of both dorsal and ventral axons and cell migrations. [1,2,4,7,8,9,10,12,15]
unc-30	(*e191*)	IV	8	Class DD and VD motor axons mispositioned within ventral nerve; GABA specifically absent from DD and VD neurones. [5,12,13]
unc-33	(*e204*)	IV	9	Many classes of neurones have abnormal axons; abnormal sensory cilia; abnormal microtubules in sensory dendrites. [7,8,10]
unc-34	(*e315hs*)	V	3	[1,6,7,12]
unc-40	(*e271*)	I	7	General failure of ventral axon and cell migrations; milder disruption of dorsal axon and cell migrations. [3,4,7,8,9,12]
unc-44	(*e362*)	IV	14	Many classes of neurones have abnormal axons; abnormal sensory cilia. [7,8,10]
unc-51	(*e369*)	V	8	Many classes of neurones have abnormal axons; some axons have abnormal varicosities. [7,8,10,12]
unc-53	(*e404*)	II	6	Defects in the posterior body. [1,6,8,11]
unc-62	(*e644*)	V	1	[6,12]
unc-71	(*e541*)	III	1	[10,12]
unc-73	(*e936*)	I	3	Defects in the posterior body. [1,6,8,10,11,12]
unc-76	(*e911*)	V	1	Many classes of neurones have abnormal axons. [7,8,12]

1) Abnormal excretory canals, see Table 2.
2) Abnormal head mesodermal cell migrations, see Table 3.
3) Abnormal Q neuroblast migrations, see Table 4.
4) Abnormal distal tip cell migrations, see Table 4.
5) Brenner (1973).
6) Abnormal male copulatory structures, Hodgkin (1983).
7) Abnormal PHA, PHB chemosensory axons, Hedgecock *et al.* (1985); unpublished data.
8) Abnormal PDE mechanosensory axons, Hedgecock *et al.* (1985); unpublished data.
9) Hall *et al.* (1986); Hedgecock *et al.* (1987).
10) Abnormal PHC, PVN axons, Siddiqui & Culotti (1987).
11) Abnormal PLM mechanosensory axons, S. S. Siddiqui, personal communication; unpublished data.
12) Abnormal DD, VD GABAergic motor axons, S. McIntire & H. Horvitz, personal communication.
13) J. White, E. Southgate & N. Thomson, personal communication.
14) R. Wyman & N. Thomson, personal communication.
15) L. Nawrocki & N. Thomson, personal communication.

Table 2. *Genes affecting excretory canals*[1]

Gene	Reference allele	Linkage group	Number of isolates	Principal defects
emb	(*rh54*)	III	1	Embryonic lethal; no canal outgrowth in viable somatic mosaics when excretory cell is mutant; mesoderm abnormal.
lin-17	(*n671*)	I	8	Posterior canals extend past V6/T boundary at POSITIONS 4. [3]
unc-5	(*e53*)	IV	11	Defective dorsal growth at POSITIONS 1; canals sometimes extend along ventral epidermal ridge. [2]
unc-6	(*ev400*)	X	22	Similar to *unc-5*. [2]
unc-34	(*e315hs*)	V	3	Cells mispositioned; canals sometimes fail to reach POSITIONS 2; posterior canals (POSITIONS 3) may terminate prematurely.
unc-53	(*e404*)	II	6	Posterior canals (POSITIONS 3) terminate prematurely.
unc-73	(*e936*)	I	3	Similar to *unc-53*.

1) Canal POSITIONS refer to Fig. 6.
2) Hall *et al.* (1986); Hedgecock *et al.* (1987).
3) Sternberg & Horvitz (1987); unpublished observations.

(described below). The result is that the receptive fields of the ALM and PLM neurones, which, respectively, mediate reverse and forward escape responses to mechanical stimulation anywhere along their axons, are flush but do not overlap (Chalfie & Sulston, 1981).

Homeotic genes, such as *lin-20*, *lin-22* and *mab-5* (described above), which establish the spatial fates of the epidermal cells as judged by their larval cell lineages, may also specify the spatial roles of these cells in guiding cell and axon migrations. For example, *lin-17* mutations transform the larval lineage of the T cell to a pattern reminiscent of more anterior lateral epidermal cells (Sternberg & Horvitz, 1987; unpublished data). Interestingly, the posterior excretory canals, which normally stop at about the V6/T boundary, grow over the T cell as well in these mutants. Similarly, *mab-5* mutations, which transform the larval lineages of posterior epidermal cells into more anterior patterns, also disrupt certain posterior cell migrations over these cells (Kenyon, 1986).

Circumferential gradients, if they exist, must be even coarser than longitudinal gradients as there are only three epidermal cells in a hemicircumference from ventral to dorsal. The *unc-5*, *unc-6* and *unc-40* genes affect the circumferential migrations of both cells and axons in the body (Hall, Hedgecock & Culotti, 1986; Hedgecock, Culotti & Hall, 1987). Mutations in the *unc-5* and *unc-6* genes disrupt the dorsal, circumferential growth of the motor axons (Table 1) and the excretory canals (Table 2) as well as the dorsal migrations of certain mesodermal cells (Tables 3, 4). Mutations in *unc-40*, as well as *unc-6*, disrupt the ventral, circumferential growth of sensory axons (Table 1) and the ventral migrations of certain mesodermal cells. One possible model is that the *unc-5*, *unc-6* and *unc-40* genes encode adhesive molecules expressed by the dorsal, lateral and ventral epidermal cells, respectively.

Herman (1984) has introduced a technique for generating clones of genotypically mutant cells in otherwise normal individuals. Using such mosaic individuals, he has shown that the normal site of *unc-3* (Table 1) expression is probably the ventral nerve motorneurones themselves rather than the adjacent epidermal cells (R. K. Herman, personal communication). More generally, genetically mosaic animals can be used to determine whether gene products important in cell–cell interactions, such as axon guidance, are expressed by the signalling cells or the responding cells or both.

Embryonic cell migrations

During embryogenesis, many cells move short distances relative to their neighbours, presumably by interchanging cell contacts (Sulston *et al.* 1983). During gastrulation, for example, precursors to the intestine, the germline, the pharynx and the body muscles, are drawn into the interior of the embryo from the ventral surface, leaving epidermal cells covering the dorsal and lateral surfaces and mainly neuroblasts exposed on the ventral surface. As the neuroblasts complete their divisions, the epidermal cells spread over the ventral surface and meet up along the ventral midline to complete the body wall epithelium.

A few cells undergo long, longitudinal migrations in the embryo (Sulston *et al.* 1983). These differ from the shorter movements described above in that the migrating cells must repeatedly form new attachments and then abandon them before reaching their final positions. The cells that migrate farthest are the neurones ALM, CAN and HSN, the mesoblasts M,

Z1 and Z4, the right intestinal muscle and the coelomocyte mother cells. All of these longitudinal migrations are posteriorward, except for HSN (Fig. 7).

As described earlier, some body wall muscle cells migrate circumferentially as they sort into dorsal or ventral bands. The head mesodermal cell (hmc) and its contralateral homologue also migrate circumferentially in the embryo to the dorsal midline (Sulston *et al.* 1983). The hmc homologue dies later in embryogenesis. The function of the surviving head mesodermal cell is not known (Sulston & Horvitz, 1977). It appears to be noncontractile but it makes extensive gap junctions with the dorsal and ventral body muscles (Fig. 8).

Mutations affecting each of the major embryonic cell migrations are known (Table 3). Many of these genes affect more than one class of cell migration. For example, *unc-5* and *unc-6* mutations disrupt both the dorsal embryonic migration of the head mesodermal cell and a dorsal larval migration of the distal tip cells of the hermaphrodite gonad described below (Table 4). Some of the mutations listed in Tables 3 and 4 are impenetrant or unexpressive, i.e. the cells migrate, perhaps fully, in some individuals or migrate partially in all individuals.

The phenotypes of *mig*ration mutants generally reflect the normal functions of the migrating cells. Defective ALM migrations should leave only the small region between the ALM cell bodies and the ends of the PLM axon without mechanosensory innervation. Defective CAN migrations in *vab-8* mutants result in a dramatic withering of the posterior body (J. Sulston and J. Hodgkin, personal communication). This phenotype resembles that of mutants with shortened excretory canals but is more severe. The hermaphrodite-specific neurones (HSN) innervate the vulval muscles and control egg laying (Trent, Tsung & Horvitz, 1983; White *et al.* 1986). Defective HSN migrations in *egl-43* mutants result in animals filled with undeposited eggs (C. Desai, G. Garriga, S. McIntire and H. Horvitz, personal communication). Defective M mesoblast migrations in *unc-39* embryos, or defective sex myoblast migrations in *egl-15* larvae, cause mispositioning of the vulval and uterine muscles and prevent egg laying (unpublished data; Trent *et al.* 1983; M. Stern, P. Sternberg, W. Champness and R. Horvitz, personal communication). Defective migration of the right intestinal muscle in *unc-39* mutants causes no obvious phenotype, perhaps because the left intestinal muscle alone is sufficient for defaecation.

The embryonic gonad comprises two germ-line cells (Z2, Z3) plus two mesoblasts (Z1, Z4) which generate the somatic structures of the gonad (Kimble & Hirsh, 1979). The germ cells and somatic cells have spatially separate embryonic origins (Sulston *et al.* 1983). The two somatic cells are generated near the head and then migrate posteriorly to attach to the germ cells. The four cells are initially arranged transversely but shift obliquely as the embryo elongates. The final arrangement, which is invariant in the wild type, is with the right-hand somatic cell

Table 3. *Genes affecting embryonic cell migrations*

Gene	Reference allele	Linkage group	Number of isolates	Principal defects
egl-5	(*n486*)	III	6	HSN migration defective, serotonin absent in HSN; uncoordinated. [1,3]
egl-27	(*n170*)	II	1	HSN, ALM, Q migrations defective; abnormal posterior larval lineages. [1,3]
egl-43	(*n997*)	II	2	HSN migration defective. [3]
lin-32	(*e1926*)	X	2	ALM migration defective. [4]
mig-2	(*rh17*)	X	2	ALM, CAN, HSN, cc, PQR migrations defective; uncoordinated.
mig-4	(*rh51*)	III	2	Z1, Z4 migrations defective; gonad primordium displaced dorsally. [5]
unc-5	(*e53*)	IV	11	Head mesodermal cell migration defective. [2]
unc-6	(*ev400*)	X	22	Similar to *unc-5*. [2]
unc-39	(*rh72*)	V	2	CAN, M, mu int R migrations defective; extra somatic gonad precursor; uncoordinated.
vab-8	(*e1017*)	V	2	CAN migration defective; uncoordinated. [6]

1) Trent *et al.* (1983).
2) Hall *et al.* (1986); Hedgecock *et al.* (1987).
3) C. Desai, J. Garriga, S. McIntire & H. Horvitz, personal communication.
4) M. Chalfie, personal communication; C. Kenyon & E. Hedgecock, unpublished data.
5) J. Thomas (personal communication) has identified an additional gene affecting the Z1 and Z4 migrations.
6) J. Sulston & J. Hodgkin, personal communication; J. Manser (personal communication) has identified additional genes affecting the CAN migrations.

(Z1) displaced anterior to the left-hand somatic cell (Z4) and with the two germ cells in between them (Fig. 11). The completed organ primordium is positioned against the ventral body wall muscles at about the boundary of ventral epidermal cells P5/6 and P7/8 (Fig. 2).

The somatic gonad precursors (Z1, Z4) are essential for the proliferation and maturation of the germ cells (described below). A complete defect in their posterior migrations, such that they fail to envelop the germ cells, is expected to result in sterility. Mutants with partial defects in these migrations, and slightly mispositioned gonad primordia, have been identified (Table 3). Interestingly, the somatic structures can develop normally at ectopic sites and in the absence of germ cells (Sulston *et al.* 1983; unpublished data).

Larval cell migrations

During larval development, certain ectodermal and mesodermal cells migrate actively along the body wall. Neuroblasts derived from the QV5 and T epidermal cells (see Fig. 2) migrate longitudinally in the first larval stage (Sulston & Horvitz, 1977). Descendants of the M mesoblast migrate circumferentially and longitudinally, contributing additional body wall muscles used for locomotion, and, later, sex-specific muscles used for egg laying in the hermaphrodite and mating in the male, respectively (Sulston & Horvitz, 1977; Sulston *et al.* 1980). Descendants of the Z1 and Z4 mesoblasts migrate longitudinally and circumferentially, creating the sex-specific shapes of the adult gonads (Kimble & Hirsh, 1979). Finally, the hermaphrodite and the male body

Table 4. *Genes affecting larval cell migrations*

Gene	Reference allele	Linkage group	Number of isolates	Principal defects
(A) *Mutations affecting the Q neuroblast descendants*				
egl-27	(*n170*)	II	1	Q, HSN, ALM migrations defective; abnormal posterior larval lineages. [3]
emb	(*e1933*)	III	1	QL migration reversed in viable larvae from heterozygous mothers; maternal contribution sufficient for embryogenesis.
hch-1	(*e1734*)	X	2	QL migration reversed; embryos unable to digest outer eggshell, rescued by wild-type hatching fluid or trypsin.
lin-21	(*e1751*sd)	III	1	QR migration reversed; mildly uncoordinated.
mab-5	(*e1239*)	III	4	QL migration reversed; abnormal posterior larval lineages. [4,6]
mig-1	(*e1787*)	I	1	QL migration reversed; HSN migration defective.
mig-5	(*rh94*)	II	1	QL migration reversed; distal tip cell often absent; maternal contribution partially sufficient.
unc-11	(*e47*)	I	3	Terminal cells (AQR, AVM, SDQR, PQR) fail to migrate; uncoordinated.
unc-40	(*e271*)	I	7	QL migration reversed; P1/2 intercalations fail; uncoordinated. [7]
unc-73	(*e936*)	I	3	Q migrations reduced in extent; uncoordinated.
(B) *Mutations affecting the sex myoblast migrations*				
egl-15	(*n484*)	X	1	Hermaphrodite SM migration abnormal; males normal. [3,8]
mab-5	(*e1239*)	III	4	Male sex mesoblasts migrate anteriorly; QL migration reversed; abnormal posterior larval lineages. [4,6]
(C) *Mutations affecting the distal tip cell migrations*[1]				
dpy-24	(*s71*)	I	2	Distal tip cells turn centrifugally at POSITIONS 4. [2,9]
lin-20	(*e1796*)	X	1	Distal tip cells turn centrifugally near POSITIONS 5. [5]
mig-6	(*e1931*)	V	1	Distal tip cells fail to migrate from POSITIONS 1; sterile.
mig-7	(*rh84*)	X	2	Distal tip cells fail to reflex at POSITIONS 2.
unc-5	(*e53*)	IV	11	Distal tip cells fail to migrate dorsally at POSITIONS 3, instead reflex ventrally. [7]
unc-6	(*ev400*)	X	22	Similar to *unc-5*. [7]
unc-40	(*e271*)	I	7	Similar to *unc-5*. [7]

1) Distal tip cell POSITIONS refer to Fig. 11.
2) Rose & Baillie (1980).
3) Trent *et al.* (1983).
4) Chalfie *et al.* (1983).
5) Hedgecock *et al.* (1985).
6) Kenyon (1986).
7) Hall *et al.* (1986); Hedgecock *et al.* (1987).
8) J. Thomas & H. Horvitz, personal communication.
9) T. Schedl, personal communication.

walls undergo major cellular rearrangements during the morphogenesis of the vulva and the male copulatory structures, respectively (Sulston & Horvitz, 1977; Sulston *et al.* 1980).

Q neuroblasts

The neuroblasts QR and QL are bilateral homologues which undergo identical divisions during the first larval stage, each generating three differentiated neurones (AQR/PQR, AVM/PVM and SDQR/SDQL) and two programmed cell deaths (Sulston & Horvitz, 1977; White *et al.* 1986). These cell lineages are remarkable in that many of the intermediate cells actively migrate and, also, because the direction of migration is anterior for the QR descendants but posterior for the QL descendants (Fig. 9). These asymmetric cell migrations effectively convert right/left homologues into anterior/posterior homologues. Presumably, this provides a better spatial deployment of the sensory neurones AQR/PQR and AVM/PVM (see Chalfie & Sulston, 1981; White *et al.* 1986). This mechanism might not have evolved in a less cell-parsimonious organism which could employ four neuroblasts, two anterior and two posterior, to achieve the same sensory innervation.

Mutations in several genes reduce the extents or reverse the direction of migration of the Q descendants (Table 4). Of the latter, *mab-5* and at least six other genes are required for the normal posterior migration of the QL descendants. The PQR, PVM and SDQL cells in these mutants are often found at the same anterior positions as their right homologues. The QL divisions and migrations have been followed in detail in *mab-5* mutants where it was observed that QL itself migrates posteriorly, but after division, its daughter cells resume migration in the incorrect, anterior direction (Chalfie, Thomson & Sulston, 1983). This suggests that the migrations of the various Q intermediates may be under somewhat different controls. The single known mutation, *lin-21* (*e1751*), which reverses the direction of the QR descendants is semidominant and apparently a neomorph, i.e. a gene expressing an altered product which causes a phenotype different from that of simply underexpressing the normal product (unpublished data). Taken together, these mutants suggest that the normal, asymmetric migration of the Q neuroblasts is achieved by modifying the QL behaviour from a developmental 'ground state' in which both QR and QL descendants migrate anteriorly. Interestingly, it was recently proposed that the normal *mab-5* product

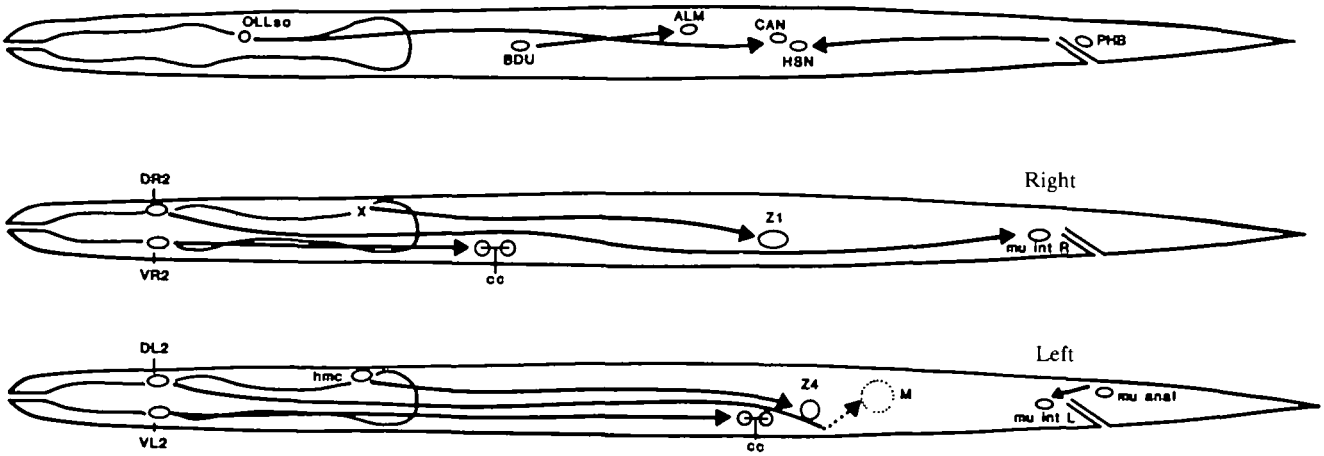

Fig. 7. Projection of embryonic cell migrations onto the larval body wall (after Sulston *et al.* 1983). The final, larval positions of migrating cells and their closest lineal relatives are shown, connected by arrows. The migrations are completed before the general elongation of the embryo (see Preiss & Hirsh, 1986). Hence, the actual paths of migration are much shorter than the final separations of cells shown here. (Upper) Left lateral aspect showing the ALM, CAN, HSN neurones and their embryonic sisters, OLLso, BDU and PHB, respectively. The right lateral homologues of these neurones (not shown) undergo symmetrical migrations. (Middle and lower) Right and left lateral aspects, respectively, showing positions of coelomocytes (cc), mesoblasts (M, Z1, Z4), and intestinal muscles (mu int). The sisters of the left and right coelomocyte mother cells are ventral body muscles VL2 and VR2, respectively. The sisters of the somatic gonad precursors, Z4 and Z1, are the head mesodermal cell (hmc) and its nonsurviving homologue (X). These latter two cells, themselves, have undergone a dorsal migration to reach the positions shown. The right intestinal muscle (mu int R) and its lineal homologue, mesoblast M, are the surviving cousins of dorsal body muscles DR2 and DL2, respectively. During embryogenesis, both cells migrate posteriorly in single file along the ventral midline (Sulston *et al.* 1983). The leading cell, mu int R, reaches the posterior end of the intestine where it differentiates as an intestinal muscle. The trailing cell, M, shifts to the right of the intestine and attaches to the body wall over epidermal cell QV5. Interestingly, a second intestinal muscle (mu int L), the functional homologue of mu int R, originates as sister of the anal depressor muscle (mu anal) in the tail.

may function in detecting an informational gradient along the posterior body wall that is used both for regulating the larval lineages of the lateral and ventral epidermal cells (described above) and for guiding certain posterior cell migrations (Kenyon, 1986).

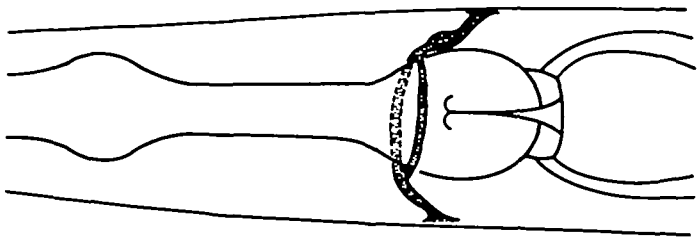

Fig. 8. Lateral aspect of head mesodermal cell in adult (J. White, personal communication). A short, broad dorsal process makes gap junctions at the dorsal midline with arms from dorsal body wall muscles. A similar ventral process splits into two branches which run around either side of the pharynx and then fuse together ventrally. The fused process makes gap junctions at the ventral midline with arms from ventral body wall muscles.

M mesoblast

At the completion of its embryonic migration (described above), the single M mesoblast is positioned on the right-hand side of the body, roughly over the QV5 epidermal cell, where it spans the body wall from ventral to dorsal. When it divides during the first larval stage, the first and second division axes are dorsal/ventral and left/right, respectively. The initial four descendants are thus symmetrically positioned over each of the muscle quadrants (Fig. 10). All subsequent divisions are anterior/posterior along the body wall. In hermaphrodites, the M cell lineage generates a total of 14 body wall muscles, 2 coelomocytes, and 2 sex myoblasts (SM) by the end of the first larval stage (Sulston & Horvitz, 1977). The sex myoblasts migrate anteriorly along the ventral body wall during the second larval stage to reach a position midway along the developing gonad. The sex myoblasts divide during the third larval stage to produce 16 vulval and uterine muscles involved in egg laying.

In males, the M cell undergoes an identical pattern of early divisions to generate 14 body wall muscles

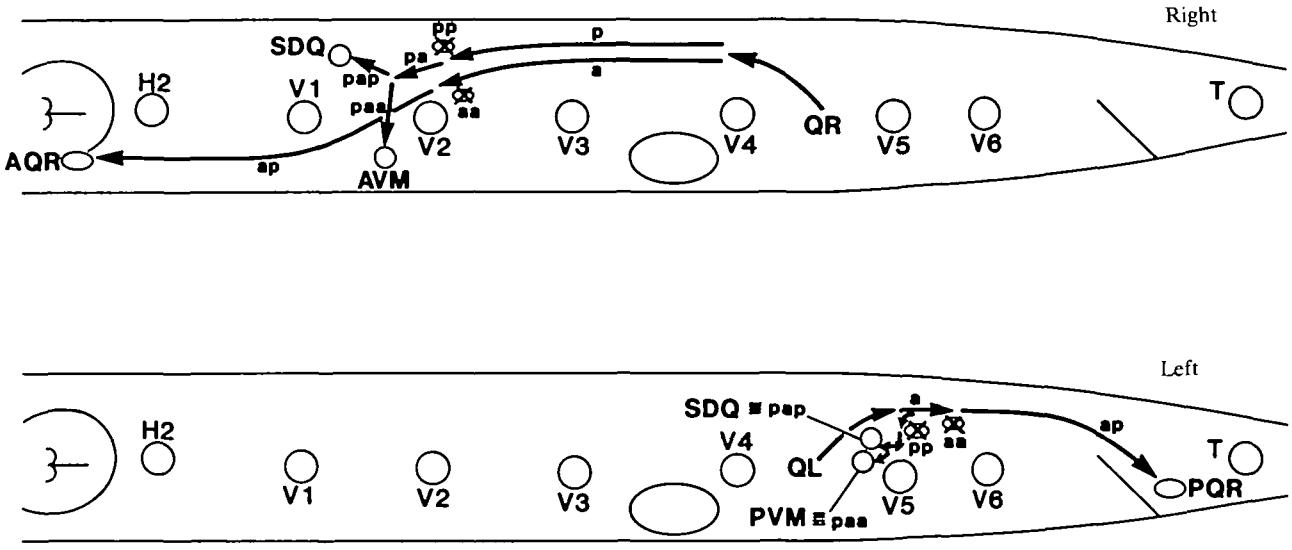

Fig. 9. Migrations of the QR (upper) and QL (lower) neuroblasts and their descendants (after Sulston & Horvitz, 1977). Lateral aspects of newly hatched larvae with anterior at the left. The positions of the pharynx, gonad primordium, rectum and lateral epidermal nuclei (H2, V1–V6, T) are shown as landmarks. Arrows indicate paths of cell migrations and their heads indicate positions of cell divisions or terminal cells. Cells are named according to the binary nomenclature of Sulston & Horvitz (1977) in which, for example, QR.ap is the name for the posterior daughter of the anterior daughter of QR. Cells that undergo programmed cell death are crossed out.

The Q neuroblasts, which are the anterior daughters of the lateral epidermal cells QV5 (see Fig. 2), are born at symmetrical right and left positions in the lateral epidermis about 1 h before hatching. Shortly after hatching, QR begins moving anteriorly, stopping to divide at a position just dorsal to cell V4. The QR daughter cells, QR.a and QR.p, continue anterior migration to about the position of cell V2 where they divide again, generating two cell deaths (QR.aa and QR.pp) and two surviving cells (QR.ap and QR.pa). The cell QR.ap continues anterior migration to the level of cell H2 where it differentiates into the ciliated mechanosensory neurone AQR. The cell QR.pa divides again to generate the microtubule-filled mechanosensory neurone AVM = QR.paa and an interneurone of unknown function SDQ = QR.pap.

QL undergoes a pattern of cell divisions bilaterally symmetrical to QR but the corresponding cell migrations are reversed in direction and generally shorter in extent. The three surviving cells, PQR = QL.ap, PVM = QL.paa and SDQ = QL.pap, differentiate into neurone types equivalent to their QR homologues (White *et al.* 1986).

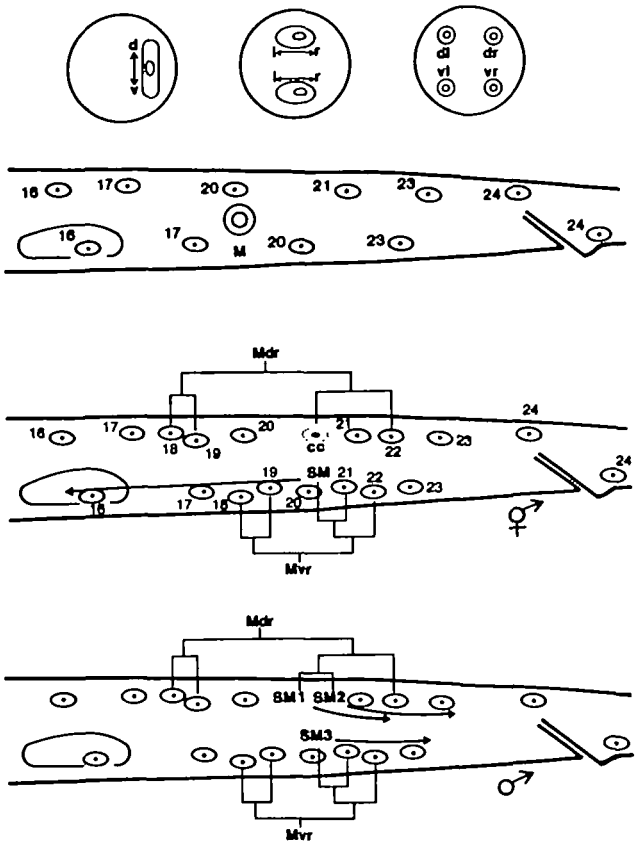

Fig. 10. Cell lineage of the M mesoblast and the migrations of its descendants (after Sulston & Horvitz, 1977; Sulston *et al.* 1980). (Upper) Right lateral aspect of newly hatched larva showing the position of the M mesoblast and the embryonic body wall muscles (see Fig. 2 for explanation of muscle numbering). The three transverse sections at top depict the first two divisions of M. These result in four blast cells (M.dl, M.dr, M.vl, M.vr) positioned over the dorsal left, dorsal right, ventral left and ventral right muscle quadrants, respectively. (Middle) The longitudinal divisions of the M.dr and M.vr cells in hermaphrodites generate seven body wall muscles, one coelomocyte (cc), and one sex myoblast (SM). The M.dl and M.vl cells undergo symmetrical lineages on the left side of the animal (not shown). It is unknown whether the final arrangement of body wall muscles following these larval additions is invariant between individuals. The sex myoblasts migrate anteriorly (arrow) during the second larval stage. Their later divisions are described in Sulston & Horvitz (1977). (Lower) The longitudinal divisions of the M.dr and M.vr cells in males generate seven body wall muscles and three sex mesoblasts (SM1, SM2, SM3). The M.dl and M.vl cells undergo symmetrical lineages on the left side of the animal (not shown). The sex mesoblasts migrate posteriorly (arrows) during the third larval stage. Their later divisions are described in Sulston *et al.* (1980).

and two ventral sex myoblasts (SM3). The cells homologous to the hermaphrodite coelomocytes divide in males to produce two pairs of dorsal sex mesoblasts (SM1 and SM2) not present in hermaphrodites (Fig. 10). During the third larval stage, all three pairs of mesoblasts (SM1, SM2, SM3) migrate posteriorly and divide to generate 41 male sex muscles and 1 coelomocyte (Sulston *et al.* 1980). These late lineages are notable both because they involve extensive cell movements and because certain lineal homologues adopt different fates on the left and the right sides of the body.

The M lineages are abnormal in *mab-5* mutants (Hodgkin, 1983; Kenyon, 1986). Both males and hermaphrodites produce supernumerary sex mesoblasts. These migrate anteriorly in both sexes rather than posteriorly in males. This reversal of direction is reminiscent of the abnormal, anterior migration of the QL descendants in *mab-5* mutants (described above).

Gonad (Z1 and Z4 mesoblasts)

The characteristic, reflexed shapes of the adult gonads are determined by the migrations of the linker cell in the male and the two distal tip cells in the hermaphrodite (Kimble & Hirsh, 1979). In addition, the anchor cell in the hermaphrodite undergoes a short ventral movement to attach the uterus to the developing vulva. These migratory cells are generated by divisions of the somatic gonad precursors, Z1 and Z4, during the first larval stage.

In hermaphrodites, Z1 and Z4 undergo equivalent early lineages to generate a total of 12 cells. Two of these cells, Z1.aa and Z4.pp, are distal tip cells responsible for the elongation and reflexion of the gonad and for maintaining the mitotic proliferation of nearby germ-line cells (Kimble & White, 1981). Two other cells, Z1.ppp and Z4.aaa, comprise an equivalence group (Kimble, 1981). Each cell has the potential of becoming the unique anchor cell (primary fate) or a uterine precursor (secondary fate). This selection, which varies randomly between individuals, is made near the end of the second larval stage. Shortly thereafter, the committed anchor cell migrates to the ventral midline of the gonad primordium. Neither the distal tip cells nor the anchor cell divide again. The remaining somatic cells are blast cells which divide extensively in the third and fourth larval stages, raising the number of somatic cells to 143 (Kimble & Hirsh, 1979).

In males, Z1 and Z4 undergo equivalent early lineages to generate a total of 10 cells. Two of these cells, Z1.a and Z4.p, are distal tip cells. Unlike the hermaphrodite cells, the male distal tip cells do not actively migrate but they are required for maintaining the mitotic proliferation of nearby germ cells (Kimble

& White, 1981). Two other cells, Z1.paa and Z4.aaa, comprise an equivalence group (Kimble, 1981). Each cell has the potential of becoming the unique linker cell (primary fate) or a vas deferens precursor (secondary fate). This selection, which varies randomly between individuals, is made soon after the cells are born at the end of the first larval stage. Neither the distal tip cells nor the linker cell divide again. The remaining somatic cells are blast cells which divide extensively in the third and fourth larval stages, raising the number of somatic cells to 55 (Kimble & Hirsh, 1979).

The trajectories of the hermaphrodite distal tip cells and the male linker cell are shown in Fig. 11. In hermaphrodites, the somatic precursor cells remain stationary while the two distal tip cells, followed by proliferating germ cells, migrate centrifugally and later centripetally. The result is that the principal somatic structures, i.e. uterus and spermathecae, are positioned centrally over the developing vulva while the germ cells fill the two reflexed, distal arms. In males, by contrast, the distal tip cells and the proliferating germ cells remain stationary while the somatic precursor cells, led by the linker cell, migrate anteriorly and later posteriorly. The result is that the principal somatic structures, i.e. vas deferens and seminal vesicle, are joined to the developing cloaca in the male tail.

Mitotic proliferation and meiotic maturation of the germline cells occur in a spatial and temporal gradient along the distal to proximal axes of the gonad. This orderly progression is established by the distal tip cells which maintain the mitotic proliferation of the germ cells nearest them (Kimble & White, 1981). In *glp-1* mutants, the distal tip cells are present and migrate normally in hermaphrodites, but germline proliferation fails after only one or two cell divisions (J. Kimble, personal communication). Conversely, in *mig-6* hermaphrodites (Table 4), the distal tip cells have largely lost their ability to migrate but still

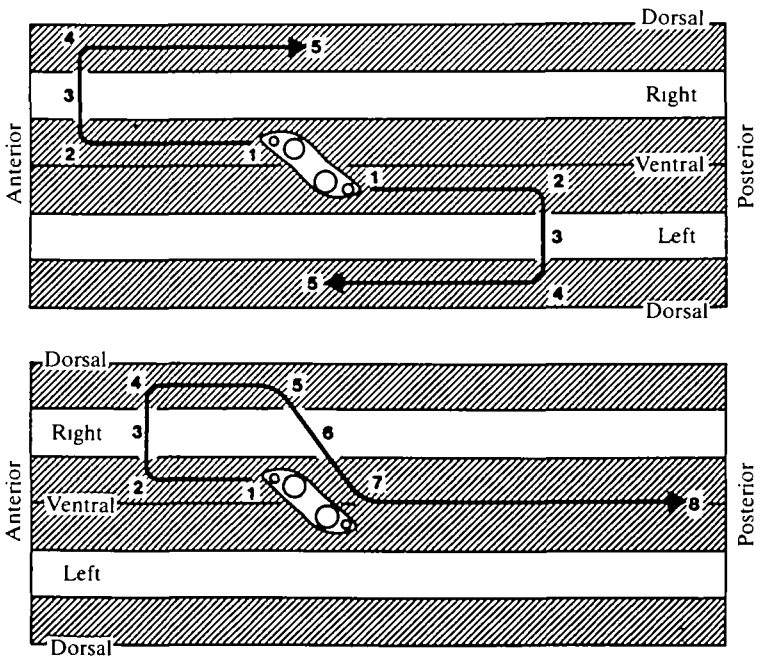

Fig. 11. (Upper) Distal tip cell migrations in the hermaphrodite. The two distal tip cells are generated late in the first larval stage at POSITIONS 1 (over body wall muscles VR15 and VL16). Early in the second larval stage, they begin migrating anteriorly and posteriorly, respectively. Late in the third larval stage, they reach POSITIONS 2 (over muscles VR12 and VL19) where they pause and turn dorsalward, crossing the lateral epidermis at POSITIONS 3. Shortly before the third stage moult, they reach the dorsal muscles (POSITIONS 4) and turn retrograde. They continue along the dorsal muscles throughout the fourth larval stage, reaching POSITIONS 5 (over muscles DR16 and DL17) shortly before the fourth stage moult. The distal tip cells detach from the body wall and cease active migration in the adult. (Lower) Linker cell migration in the male. The linker cell is generated late in the first larval stage at POSITION 1 (over body wall muscle VR15). Early in the second larval stage, it begins migrating anteriorly. Late in the second larval stage, it reaches POSITION 2 (over muscle VR13) where it turns dorsalward, crossing the lateral epidermis at POSITION 3. Shortly before the second stage moult, it reaches the dorsal body muscles at POSITION 4 and turns retrograde. It continues along the dorsal muscles, reaching POSITION 5 (over muscle DR16) late in the third larval stage. There it turns obliquely, crossing the lateral epidermis at POSITION 6 and reaching POSITION 7 (over muscle VR17) shortly before the third stage moult. The linker cell continues posteriorly along the ventral epidermis until reaching the cloaca (POSITION 8) late in the fourth larval stage. There the linker cell is engulfed and destroyed by specific killer cells (Sulston *et al.* 1980).

support normal proliferation of neighbouring germ cells. These animals have bulb-shaped ovotestes and are usually sterile, apparently because the orderly maturation of the gametes requires a cylindrical geometry. This suggests that the evolutionary purpose of the gonad reflexions is to increase the length of gonad available for gametogenesis within the fixed volume of the animal.

The positions and the timing of the turns in the hermaphrodite distal tip cell and male linker cell migrations are reproducible in wild-type animals (Fig. 11). Simple extrinsic cues might govern these decisions. For example, the hermaphrodite trajectory could be largely explained if the distal tip cells have a hierarchy of affinities for the substratum cells with midbody muscles > lateral epidermis > head or tail muscles. At POSITIONS 2 in Fig. 11, the distal tip cells would migrate over the lateral epidermis in preference to advancing over the head or tail muscles. At POSITIONS 4, the distal tip cells would turn retrograde, preferring the midbody muscles to the head or tail muscles. Alternatively, the affinities of the migrating cells may change during migration either in accordance to some developmental clock (see Ambros & Horvitz, 1984) or by sequential titration of strong receptors, say for the muscles, allowing weak receptors, say for the epidermis, to steer the cell.

Mutations affecting each decision point in the hermaphrodite distal tip cell migrations have been identified (Table 4). In *mig-7* mutants, the distal tip cells fail to turn at POSITIONS 2 and, instead, continue along the ventral body wall muscles into the extreme head or tail. This might be explained, for example, if the surfaces of the body muscles in the head and tail are normally masked to render them nonadhesive for the distal tip cells. In *unc-5*, *unc-6* and *unc-40* mutants, the distal tip cells invariably stop at POSITIONS 2 but often fail to turn dorsally across the epidermis, returning retrograde along the ventral rather than the dorsal body wall muscles. Interestingly, each of these genes also affects the dorsal migrations of motor axons (Table 1). The gonad primordium is sometimes positioned over the dorsal rather than the ventral body wall muscles in *mig-4* mutants (Table 3). When this occurs, the hermaphrodite distal tip cells migrate outward along the dorsal, rather than the ventral, muscles and return, after stopping at the dorsal equivalents of POSITIONS 2, along the dorsal muscles rather than turning ventrally across the epidermis. It may be interesting to examine these migrations in double mutants with *mig-7* or *unc-5*.

Some of the genes listed in Table 4 also affect the migration of the male linker cell. A specific defect in the linker cell migration has been observed in *him-4* mutants (Hodgkin, Horvitz & Brenner, 1979).

Prospectives

Migrating cells and axons are believed to be guided by the shape and adhesiveness of the substratum and, in some circumstances, by diffusible attractants (Bray, 1982; Trinkaus, 1984). For a full understanding of directed migration, we need to (1) identify the cells that line the path of migration, (2) identify the adhesive ligands or other signals made by these cells and establish both their gross spatial distribution and their ultrastructural localization (cell surface or extracellular matrix), (3) identify complementary receptors on the migrating cells or growth cones, determine their subcellular distribution and learn their restrictions to specific cell types and (4) determine how the contractile machinery of the cytoskeleton uses receptor/ligand binding or other cues to steer the cell.

A combination of *in vivo* and *in vitro* approaches have been used to answer some of the above questions. Molecules promoting cell adhesion and their complementary receptors have been identified using *in vitro* assays of cell aggregation, attachment, migration or neurite extension. Antibodies to these molecules have revealed their spatial distribution *in vivo* and, in a few cases, been used to disrupt cell movements.

We have illustrated here how molecules that guide cell and axon migrations, and molecules essential for cell motility, can be identified indirectly by selecting mutants with abnormal cell positions or axon geometries. Unlike *in vitro* methods, this genetic approach makes no prior assumptions about the molecular or cellular mechanisms of guidance and, in principle, can reveal both minor components and those for which no suitable *in vitro* assay is available (Brenner, 1973). As mutants offer a rigorous test of biological function *in vivo*, even guidance molecules discovered by *in vitro* assays of cell adhesion may require some genetic verification.

Using the techniques of molecular genetics, it is now possible to isolate genes defined only by mutation, to characterize their RNA and protein products, and to manipulate and reintroduce them into *C. elegans* (see Fire, 1986). Soon, it may be possible, by altering the cell-specific expression of guidance cues or their receptors, to redirect the migrations of cells or axons in this simple animal.

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