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REVIEW ARTICLE

The genetics of cell migration in *Drosophila melanogaster* and *Caenorhabditis elegans* development

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SUMMARY

Cell migrations are found throughout the animal kingdom and are among the most dramatic and complex of cellular behaviors. Historically, the mechanics of cell migration have been studied primarily in vitro, where cells can be readily viewed and manipulated. However, genetic approaches in relatively simple model organisms are yielding additional insights into the molecular mechanisms underlying cell movements and their regulation during development. This review will focus on these simple model systems where we understand some of the signaling and receptor molecules that stimulate and guide cell movements. The chemotactic guidance factor encoded by the *Caenorhabditis elegans unc-6* locus, whose mammalian homolog is Netrin, is perhaps the best known of the cell migration guidance factors. In addition, receptor tyrosine kinases (RTKs), and FGF receptors in particular, have emerged as key mediators of cell migration in vivo, confirming the importance of molecules that were initially identified and studied in cell culture. Somewhat surprisingly, screens for mutations that affect primordial germ cell migration in *Drosophila* have revealed that enzymes involved in lipid metabolism play a role in guiding cell migration in vivo, possibly by producing and/or degrading lipid chemoattractants or chemorepellents. Cell adhesion molecules, such as integrins, have been extensively characterized with respect to their contribution to cell migration in vitro and genetic evidence now supports a role for these receptors in certain instances in vivo as well. The role for non-muscle myosin in cell motility was controversial, but has now been demonstrated genetically, at least in some cell types. Currently the best characterized link between membrane receptor signaling and regulation of the actin cytoskeleton is that provided by the Rho family of small GTPases. Members of this family are clearly essential for the migrations of some cells; however, key questions remain concerning how chemoattractant and chemorepellent signals are integrated within the cell and transduced to the cytoskeleton to produce directed cell migration. New types of genetic screens promise to fill in some of these gaps in the near future.

Key words: *Caenorhabditis elegans*, *Drosophila*, Cell migration

INTRODUCTION

Cell migration is a fascinating and dramatic feature of normal animal development, immune system function and wound healing. Cell migration gone awry can lead to birth defects or tumor metastasis. The mystery of cell movement can be divided into several questions. What signals cause cells to initiate movement? How do cells move? What signals guide cells along appropriate pathways? What causes cells to stop migrating when they reach the appropriate destination? Are the varied cell migrations that are observed at different developmental stages, in different tissues and even in different organisms, related mechanistically?

Many studies of cell movement are carried out on cultured cells because the migration can be observed in real time and the culture medium can be altered to examine the factors that affect cell motility. Furthermore, detailed and precise observations can be made of the moving cells. The current view of cell migration is that motile cells extend and retract numerous actin-rich protrusions (broad, flat lamellipodia and/or long, thin filopodia) into the surrounding environment (reviewed in Lauffenburger and Horwitz, 1996). If sufficiently strong adhesion develops between the protrusion and the substratum, then retraction is prevented, stabilizing certain extensions while less-adherent protrusions disappear. Movement ultimately also requires release of the back of the cell, either through loosening of adhesive contacts or an active contraction or both. While it seems that regulated activities of cell adhesion molecules, the actin cytoskeleton and its associated proteins are essential to cell migration, the complete

molecular mechanism remains to be elucidated. One issue that remains to be resolved is determining whether there is a difference in adhesive strength, or some other protein activity, between the front and back of the cell and, if so, understanding how these differences are generated. It remains puzzling that actin can polymerize at the leading edge of the cell while rapidly depolymerizing a few microns away, and it is as yet unclear which of the myriad actin-binding proteins mediate these effects. Furthermore, during embryonic development, cell migration must be regulated temporally and spatially and this behavior must be coordinated with cell fate specification and differentiation of the motile cell.

Model systems for molecular genetic studies of cell migration in *Drosophila melanogaster* and *Caenorhabditis elegans*

In vitro studies can suggest, but cannot establish, the nature of the factors that act to control when cells migrate or what guides migrating cells, in vivo. Furthermore, cellular behavior can depend upon the precise composition of the natural environment. Thus it is important to study cell migration in vivo. However, it is more difficult to manipulate migratory cells and their environment in vivo, consequently a number of investigators have turned to genetically tractable organisms such as the nematode worm, *Caenorhabditis elegans*, and the fruitfly, *Drosophila melanogaster*. In the most optimistic view, saturating genetic screens for mutants displaying migration defects might result in the identification of most of the genes required for cell migration and its developmental regulation. One advantage of a genetic approach is that genes are identified on the basis of their functional significance, in vivo. In the first section of this review, I will briefly describe some of the cells in *C. elegans* and *Drosophila* whose migrations have been studied relatively extensively. In the second section, I will discuss the types of genes that have been identified and what is known about the specific roles their products play in the process of cell migration.

Cell migrations in the worm

During development of the *C. elegans* embryo, most cells move very little; however, twelve cells migrate significant distances from the locations where they are born (Sulston et al., 1983; Table 1). The migrations of the mesoblast (M), its sibling mu int R, and of the somatic gonad precursors are shown schematically in Fig. 1A. Migration of the HSN and CAN neuroblasts are shown schematically in Fig. 1B. During larval stages, additional cell migrations occur. For example, the Q neuroblasts and their descendants undertake baroque trajectories, as diagrammed in Fig. 1C and D (Kenyon, 1986). QL and QR are contralateral homologs (but not siblings), and while QL embarks on a posteriorward migration, QR migrates anteriorly. Each cell stops and divides and the daughter cells continue to migrate. The QR daughters both continue to migrate towards the anterior and then stop to divide again, whereas the QL daughters migrate to the posterior of the embryo. The larval sex myoblasts represent another migratory cell type in the worm. These cells originate in the posterior of the animal and, in the hermaphrodite, they migrate precisely to the center of the developing gonad (Fig. 1E). The distal tip cells initiate gonad formation and their characteristic migration dictates the form of the gonad (Fig. 1F; Kimble and White, 1981)

Numerous genetic screens have been carried out in the worm to detect mutants with defects in cell migration (Garriga and

Table 1. Genes required for cell migration in *C. elegans*

Locus/gene	Cells affected	Type of protein encoded	Reference
ced-5	distal tip cells	MBC/DOCK180 homolog	(Wu and Horvitz, 1998)
cam-1	CAN	not reported	(Forrester and Garriga, 1997)
cam-2	CAN	not reported	(Forrester and Garriga, 1997)
ceh-10	CAN	PAX type homeodomain	(Forrester et al., 1998)
egl-5	HSNs and QL	ABD-B homolog	(Chisholm, 1991)
egl-15	SMs	FGF receptor homolog	(DeVore et al., 1995)
egl-17	SMs	FGF homolog	(Burdine et al., 1997)
egl-20	Q	Wnt homolog	(Maloof et al., 1999)
egl-27	QL	nuclear protein	(Herman et al., 1999)
egl-43	HSN	zinc finger txn factor	(Garriga et al., 1993)
ham-2	HSN	zinc finger txn factor	(Baum et al., 1999)
hch-1	QL	Zn protease/tolloid family	(Hishida et al., 1996)
ina-1	Many*	beta integrin	(Baum and Garriga, 1997)
lin-17	QL	frizzled homolog	(Sawa et al., 1996)
lin-39	QR neuroblast	SCR-like homeodomain	(Clark et al., 1993)
mab-5	QL & male SMs	homeodomain txn factor	(Salser and Kenyon, 1992)
mig-1	QL	not reported	(Harris et al., 1996)
mig-2 l-o-f	Q cells and coelomocytes	Rho family GTPase	(Zipkin et al., 1997)
mig-2 g-o-f	Many‡	activated Rho GTPase	(Zipkin et al., 1997)
mig-10	CAN,ALM,HSN	Grb7/Grb10 adaptor protein	(Manser, 1997)
mig-14	QL	not reported	(Harris et al., 1996)
sem-5	SMs	SH2-SH3 adaptor protein	(Clark et al., 1991)
unc-5	distal tip cells	unc-6 receptor	(Leung-Hagesteijn et al., 1992)
unc-6	distal tip cells, linker cell	Netrin homolog	(Ishi and Hedgecock, 1992)
unc-40	linker cell, QL daughters	unc-6 receptor/DCC homolog	(Chan et al., 1996)
unc-73	HSN,Q,SM, CAN	Trio homolog, activates the Rac GTPase	(Steven et al., 1998)
unc-86	HSN	POU domain txn factor	(Baum et al., 1999)
vab-8	many posterior migrations	novel protein	(Wolf et al., 1998)

*ALM, CAN, coelomocytes, distal tip cells, HSNs, Q cells & decendents.
‡ALM, CAN, coelomocytes, HSN, Q cells & decendents, SMs.

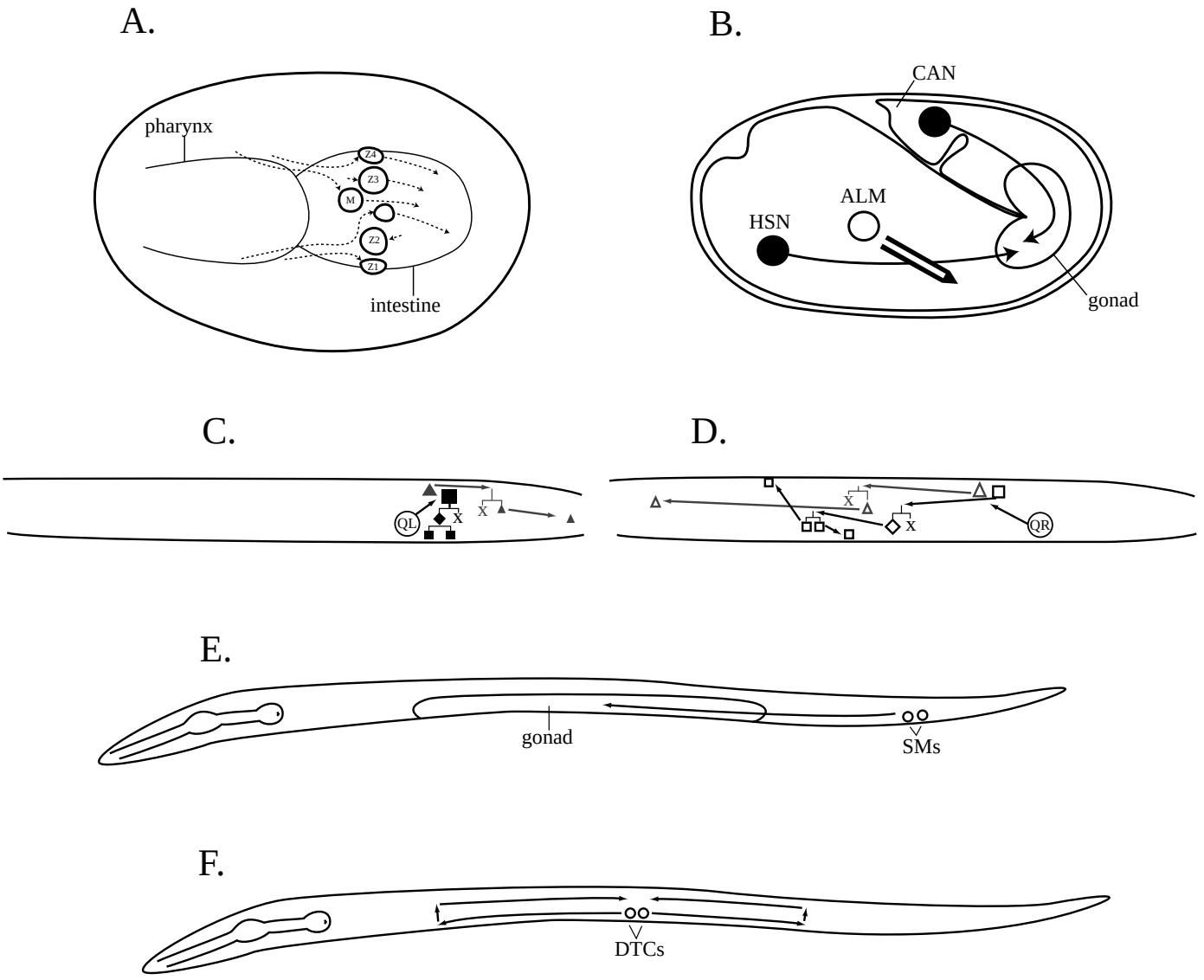

Fig. 1. Cell migrations in the nematode worm. Schematic representations of cell migrations in nematode embryos (A,B) and larvae (C-F). (A) Migrations of six cells are indicated. The M mesoblast, its sibling Mu int R (unlabeled cell) and the gonad precursors Z1-Z4 are shown; drawing adapted from Sulston et al. (1983). (B) Migration paths of the neuroblasts CAN and HSN are indicated, as is that of ALM; drawing taken from (Forrester and Garriga, 1997). (C) Posteriorly directed migration paths of QL and its daughters (indicated by filled shapes). (D) Anteriorly directed migration paths of QR and its daughters (indicated by open shapes). X indicates a cell that will die. The drawings in C and D were taken from (Harris et al., 1996). (E) Migration of the sex myoblasts (SMs) to the gonad is indicated; drawing taken from (Thomas et al., 1990). (F) Migration paths of the distal tip cells dictate that shape of the gonad. In all cases anterior is to the left.

Stern, 1994; Harris et al., 1996; Hedgecock, 1987, 1990). Some mutants exhibit defects only in the migration of a specific cell while others affect multiple migratory cells. Many of the cell migration mutants also display defects in the extension, bundling and/or pathfinding of axons, demonstrating that these processes are related mechanistically. A summary of many of the mutations that affect cell migrations in the worm are listed in Table 1.

Cell migrations in the fly

The greater number of cells and more complex body plan of *Drosophila* are reflected in the increased complexity of cell migration patterns in the embryo. The first embryonic migration is that of the primordial germ cells (PGCs), which are also known as pole cells. The early *Drosophila* embryo develops as a collection of syncitial nuclei embedded in a common cytoplasm. The PGCs are the first cells to pinch off from the syncitial blastoderm. At the time of gastrulation, the PGCs rest within the pocket of the posterior midgut invagination. As gastrulation proceeds, *Drosophila* embryos undergo a dramatic morphogenetic movement known as germband extension, in which the embryo lengthens substantially along the anteroposterior axis and folds over in order to remain within the eggshell (Fig. 2). As the entire germband elongates, the pole cells are carried passively, within the posterior midgut invagination, along the dorsal side of the embryo, and ultimately both the posterior midgut invagination and the pole cells that it

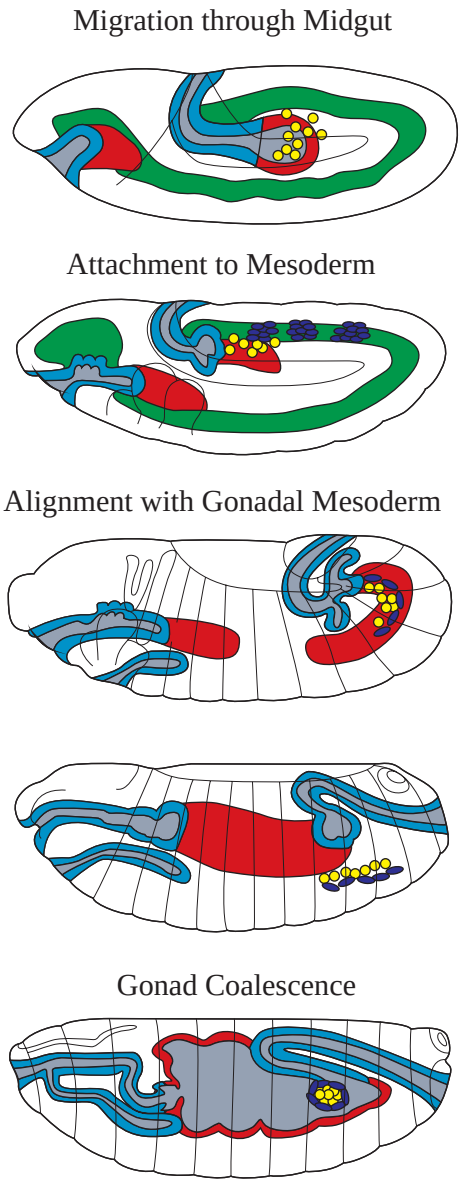

Fig. 2. Primordial germ cell migration in the fruitfly embryo. The various stages of PGC (yellow circles) migration are shown. The posterior midgut pocket is indicated by the red horseshoe. Somatic mesoderm is indicated in green except for the somatic gonadal precursors which are indicated in purple. Endoderm is indicated in blue. The first stage shown is a germband-extended embryo (see text for description of germband extension). The PGCs exit the posterior midgut pocket via the dorsal surface of the epithelium. During the remaining stages, germband retraction occurs, resulting in an unfolding of the embryo (see text for details). The PGCs find and align with the somatic gonadal precursor cells; then the two cell types condense into a gonad with the germ cells interior to the somatic cells. Anterior is to the left and dorsal is up. This figure was modified from (Moore et al., 1998).

contains are carried inward towards the center of the embryo. It is at this point that the pole cells become actively motile (Howard et al., 1993; Warrior, 1994). Their entire migratory pathway consists of only three or four cell diameters, yet this is clearly an active process. The cells first traverse in between the cells of the posterior midgut epithelium to contact the mesoderm. Then the PGCs squeeze through the mesoderm and associate with specific somatic cells, the gonadal precursors. At this point, the embryo begins germband retraction, a process that reverses germband extension. During germband retraction, the PGCs and specific somatic cells condense to form a gonad on either side of the embryo (Fig. 2).

Another set of cells to migrate actively in the *Drosophila* embryo are the cells of the tracheal system. In the germband-extended embryo, placodes of approximately 40 cells per hemisegment differentiate within the surface ectoderm (Fig. 3, top). The cells invaginate and undergo one final round of cell division, forming a series of pouches along the length of the embryo, which are known as tracheal pits. Subsequently these cells migrate into a complex array, while simultaneously patching together to form multicellular tubes that are known as the primary tracheal branches (Fig. 3, bottom). As tracheal development proceeds, the cells elongate and selected cells differentiate into so-called secondary branching cells, which extend cellular processes in stereotypical patterns. Some of the secondary branches fuse with their contralateral homologs or with branches from an adjacent segment, ultimately interlocking all of the branches. Finer branches, called tertiary or terminal branches, form from most of the secondary branch-forming cells. These long, thin cytoplasmic extensions hollow out to form a lumen that is continuous with that of the secondary branches. Whereas primary and secondary branches form in stereotypic patterns, terminal branches do not, possibly forming in response to oxygen deprivation, rather than according to a fixed developmental program (Samakovlis et al., 1996).

Additional migratory cells in the *Drosophila* embryo include hemocytes, the fly's phagocytic cells, which migrate initially along a stereotyped pathway; later the hemocytes travel throughout the hemolymph (extracellular fluid) and respond to infection and/or signals from dying cells (Franc et al., 1996; Niewiadomska et al., 1999). A variety of neuronal and glial cells undertake rather limited cell migrations, which are nonetheless quite important for establishing correct axon pathways within the nervous system (Klambt et al., 1991). Only one cell migration has been described during larval development, that of the retinal glial cells, which migrate along the optic stalk and into the eye imaginal disc during the third larval instar (Choi and Benzer, 1994). However, these migrations have not been submitted to extensive genetic analyses.

The complexity of cell migration makes simple examples of this phenomenon attractive to study. One of the simplest examples of developmentally regulated cell migration is that of the border cells, which migrate during *Drosophila* oogenesis. The *Drosophila* ovary consists of egg chambers, each of which is composed of 16 central germline cells surrounded by a monolayer epithelium of 1100 somatic follicle cells. The follicle cells are initially a uniform cuboidal monolayer. However, during stage 9 of oogenesis, these cells reorganize so that >90% of the cells change into a columnar shape and move into the posterior half of the egg chamber, in contact with the oocyte. The remaining follicle cells become thin and flat and stretch to cover the nurse cells, with the exception of six to ten cells that remain rounded at the anterior tip of the egg chamber and subsequently migrate through the middle of the nurse cell cluster (Fig. 4A). These cells stop at the border between the nurse cells and oocyte and have consequently been named border cells (King, 1970).

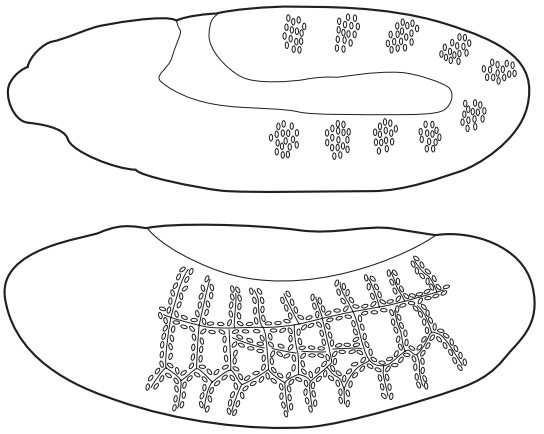

Fig. 3. Tracheal cell rearrangement in the fruitfly embryo. (Top) Differentiation of tracheal placodes within the surface ectoderm. (Bottom) The cells migrate, elongate and meet up with cells from adjacent segments in order to form approximately the pattern indicated. Then they patch together to form tubules with a lumen (indicated by the solid lines). Germband retraction occurs in between the two stages shown. Anterior is to the left and dorsal is up.

The border cells appear to undergo a fairly typical epithelial-to-mesenchymal transition, superficially similar to that of neural crest cells exiting the neural tube. The border cells extend actin-filled cytoplasmic processes in between two nurse cells (Fig. 4B). The border cells remain clustered and retain epithelial-like adherens junctions with each other throughout their migration.

Systematic genetic screens for mutations affecting PGC migration (Moore et al., 1998), tracheal migration (Samakovlis et al., 1996) and border cell migration (Liu and Montell, 1999; Montell et al., 1992) have been conducted though, as yet, they have not been as exhaustive as those conducted in worms. Many of the genes that have been shown to be required for cell migration in *Drosophila* are listed in Table 2.

Unc-6/Netrins and their receptors guide cell migration and axon pathfinding

Among the ~30 loci *C. elegans* known to be required for normal cell migration, *unc-5*, *unc-6* and *unc-40* became the focus of intense interest because they seemed to function in a common pathway to control cell migration and axon pathfinding along the dorsoventral axis in the developing worm (Hedgecock et al., 1990). *unc-5* mutants exhibit defects in numerous dorsal migrations whereas, in *unc-40* mutants, ventralward migrations are prevented. In *unc-6* mutants, both classes of defects are apparent. Genetic analysis alone, that is careful analysis of the cells and axons that are affected in both single and double mutants, suggested that these gene products might affect a common pathway. The prediction was even made that *unc-6* might encode a ligand and *unc-5* and *unc-40* subunits of a receptor for the ligand. Subsequent cloning and sequencing of the genes as well as analysis of the expression patterns confirmed these predictions. The *unc-6* gene encodes a probable extracellular matrix protein with substantial homology to the B2 subunit of laminin (Ishi and Hedgecock, 1992), whereas *unc-5* and *unc-40* encode transmembrane receptors. The UNC-5 protein is composed of thrombospondin and immunoglobulin repeats in the extracellular domain (Leung-Hagesteijn et al., 1992). The intracellular domain is largely unrelated to other proteins with the exception of a src homology 3 (SH3) domain. The extracellular domain of the UNC-40 protein is composed of immunoglobulin and fibronectin type III repeats and is highly related to a human protein known as DCC (deleted in colorectal cancer; Chan et al., 1996). Genetic and biochemical evidence suggests that UNC-5 and UNC-40 are receptors for UNC-6 and that cells expressing UNC-5 alone migrate toward increasing concentrations of UNC-6 whereas cells expressing UNC-40 and UNC-5 migrate away from UNC-6.

The relevance of these observations to mammalian development became clear with the isolation of Netrin, a protein purified from developing brain based on its ability to attract spinal cord axons (Serafini et al., 1994). Netrin is a protein homologous, in both sequence and domain structure, to UNC-6. Even more strikingly, Netrin is expressed in the ventral portion of the developing neural tube, known as the floorplate (Serafini et al., 1994), just as UNC-6 is expressed at highest levels on the ventral side of the worm (Wadsworth et al., 1996). Furthermore, in vitro, Netrin appears to attract axons that will cross the midline at the floorplate, but repel other axons such as those of trochlear motor neurons. The importance of Netrin for axon pathfinding was demonstrated conclusively by analysis of mouse mutants, which displayed significant defects in commissural pathfinding (Serafini et al., 1996).

Mammalian homologs of UNC-40 and UNC-5 have also been identified, based on sequence homology with the worm proteins, and also appear to act as Netrin receptors (Keino-

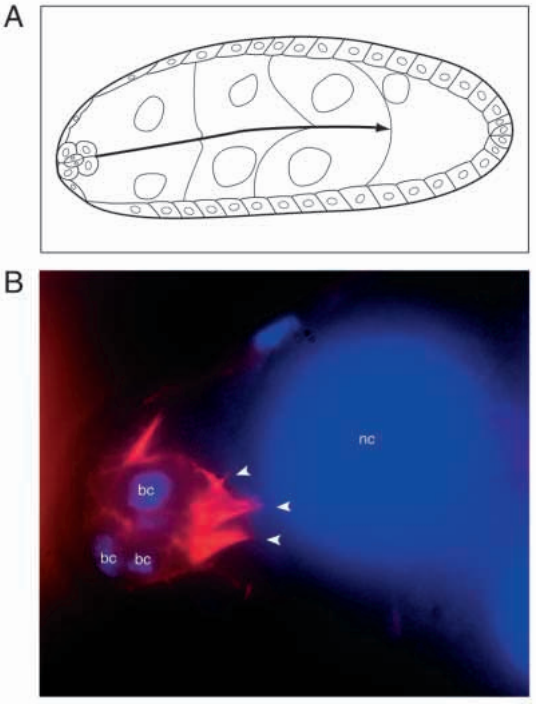

Fig. 4. Border cell migration in the fruitfly ovary. (A) Schematic diagram of the border cell migration path in between the nurse cells to the border between nurse cells and oocyte. (B) Fluorescence micrograph of border cells (bc) initiating migration through the nurse cell cluster (nc). F-actin is visualized in red and nuclear staining is in blue. The arrowheads indicate actin-rich filopodia emanating from the border cells.

Table 2. Genes required for cell migration in *Drosophila melanogaster*

Locus/gene	Cells affected	Type of protein encoded	Reference
branchless	MGCs*/tracheal cells	FGF-like protein	(Sutherland et al., 1996)
breathless	MGCs*/tracheal cells	FGF receptor homolog	(Klambt et al., 1992)
columbus	PGCs	HMG CoA reductase	(Van Doren et al., 1998)
DRac1	border cells	Rho family GTPase	(Murphy and Montell, 1996)
dpp	subset of tracheal cells	TGFbeta family member	(Vincent et al., 1998)
drifter	tracheal cells	POU domain txn factor	(Anderson et al., 1995)
torpedo/DER	subset of tracheal cells	EGF receptor homolog	(Wappner et al., 1997)
heartbroken/dof	mesoderm and tracheal cells	novel protein	(Michelson et al., 1998; Vincent et al., 1998)
pointed	MGCs*/tracheal cells	ETS domain txn factor	(Klambt, 1993)
shotgun	border cells	E-cadherin homolog	(Niewiadomska et al., 1999)
slow border cells	border cells	C/EBP homolog	(Montell et al., 1992)
spaghetti squash	border cells	myosin light chain	(Edwards and Kiehart, 1996)
thick veins	subset of tracheal cells	dpp receptor	(Vincent et al., 1997)
ventral veinless	tracheal cells	POU domain txn factor	(Llimargas and Casanova, 1997)
wunen	PGCs	lipid phosphatase homolog	(Zhang et al., 1997)

*Midline glial cells.

Masu et al., 1996; Leonardo et al., 1997). Mouse mutants deficient in DCC or UNC-5 have also been identified. DCC mutant mice exhibit axon pathfinding defects that are similar to netrin mutants (Fazeli et al., 1997). Intriguingly, an UNC-5-related gene appears to be mutated in a strain of mice that exhibits profound defects neuronal cell migration. Specifically the migrations of cells that form the laminar structure typical of the cerebellar cortex are affected (Ackerman et al., 1997).

Given the conservation between worms and mice, it is not surprising that fly homologs of *unc-6* and *unc-40* have also been identified (Harris et al., 1996; Kolodziej et al., 1996; Mitchell et al., 1996). The two *unc-6* related genes are expressed at high levels at the midline of the developing *Drosophila* nervous system and deletion mutations that remove both genes cause defects in pathfinding at the midline (Mitchell et al., 1996). Thus the UNC-6, UNC-5 and UNC-40 story demonstrates a striking degree of conservation in the mechanisms controlling cell migration and axon pathfinding from worms to flies to mice.

Basic fibroblast growth factor-like proteins and their receptors guide cell migration

Unc-6/Netrins are not the only type of secreted factors that can guide cell migration. Mutations in *Drosophila* and *C. elegans* genes coding for proteins similar to basic fibroblast growth factor (bFGF) and FGF receptors demonstrate a requirement for these proteins in sex myoblast migration in the worm and tracheal cell migration in the fly. In *Drosophila*, two genes that encode proteins similar to mammalian FGF receptors were identified by low stringency hybridization (Glazer and Shilo, 1991; Shishido et al., 1993). One of these, *breathless* (*btl*), was found to be expressed in the cells of the developing tracheal system, which is the respiratory system of the fly. Loss-of-function mutations in *btl* were identified and found to exhibit severe tracheal system defects (Glazer and Shilo, 1991; Klambt et al., 1992). Although the correct numbers of tracheal cells appear to form in *btl* mutants and they express a number of markers typical of differentiated tracheal cells, the cells fail to undergo the morphogenetic movements necessary to produce the normal tracheal tree. The *btl* mutant embryos die, presumably due to oxygen deprivation. Subsequent analyses demonstrated that *btl* function is required for proper formation of all three types of tracheal branches, primary, secondary and terminal (Reichman-Fried and Shilo, 1995). Another locus, *branchless* (*bnl*) exhibits a mutant phenotype identical to *btl* (Samakovlis et al., 1996). Unlike *btl*, which is expressed in the tracheal cells, *bnl* is expressed in cells surrounding the tracheal system, in a dynamic pattern that prefigures the tracheal branching pattern. Cloning and sequencing of the *bnl* locus revealed the satisfying result that *bnl* encodes a protein with a domain similar to mammalian basic FGF (Sutherland et al., 1996). Thus it appears that signaling from *bnl* to *btl* is required for migration of tracheal cells and, in fact, for all morphogenesis of the tracheal tree.

In the worm, it is known that precise positioning of the SMs requires the presence of the gonad. If the gonad is ablated, the SMs end up dispersed and, if the gonad is displaced, for example to a dorsal position, the SMs migrate to the ectopic gonad (Thomas et al., 1990). Mutations in two loci produce strong defects in the final position of the SMs: *egl-15* and *egl-17* (Stern and Horvitz, 1991). Strikingly, these mutations cause a more severe defect in SM position than gonad ablation. Cloning and sequencing of these loci revealed that *egl-15* encodes a protein homologous to FGF receptors (DeVore et al., 1995) and *egl-17* encodes a protein related to FGF (Burdine et al., 1997).

While these results demonstrated that FGF signaling is essential for these migrations, it was not clear whether the role of FGF signaling was a direct one, i.e. chemotactic guidance, or an indirect role, for example, in cell differentiation. In the latter case, the migratory cells might require stimulation of the FGF receptor tyrosine kinase in order for activation of the Ras/Raf/MAP kinase cascade, which would in turn alter expression of additional genes that could play a more direct role in guiding the migration. Alternatively, localized expression of the FGF-like ligand could lead to asymmetric activation of the receptor within the migrating cell, providing information as to which direction to move in. In the *Drosophila* tracheal system, there is evidence to support both types of roles for the signal.

Several lines of evidence support the model that signaling through the FGF receptor plays an instructive role in guiding tracheal cell rearrangement in *Drosophila*. For example, the BNL ligand is expressed in a dynamic pattern that prefigures the pattern of tracheal cell migration (Sutherland et al., 1996). This suggested that BNL signaling through BTL might guide tracheal cell movements. To test this hypothesis, Lee et al. (1996) made a constitutively active form of the BTL receptor and expressed it in embryos during tracheal development. The

rationale was that a chemotactic guidance receptor must, by definition, be activated asymmetrically; therefore, a constitutively active receptor would be expected to fail to rescue a *btl* mutant and possibly to interfere in a dominant way with tracheal cell migration. On the contrary, a constitutively active receptor might function quite well in stimulating cell differentiation and changes in gene expression, as is the case for example with the Sevenless receptor (Dickson et al., 1992).

The constitutively active form of BTL fails to rescue the migration defects of *btl* mutant embryos, whereas expression of the wild-type receptor rescues fully (Lee et al., 1996). Furthermore, the constitutively active receptor interferes with tracheal cell migration in a dominant fashion, consistent with a chemotactic guidance role for BTL. In support of this conclusion, Sutherland et al. (1996) showed that, if the BNL ligand is expressed ectopically, it causes migration of tracheal cells to the new location resulting in formation of a new tracheal branch. Thus it appears the BNL signaling through the BTL receptor can guide tracheal cells to the appropriate location.

However, this is not the only role for BNL/BTL signaling in tracheal development. As mentioned previously, BTL function is also required for development of both secondary and terminal tracheal branches (Reichman-Fried and Shilo, 1995). Cells that form terminal tracheal branches require the expression of a transcription factor, SRF, in order to differentiate and extend processes (Guillemin et al., 1996). Expression of high levels of constitutively active BTL can lead to the expression of SRF in many of the tracheal cells resulting in extra branches (Lee et al., 1996). Overexpression of BNL can produce an even more dramatic overproduction of branches (Sutherland et al., 1996). Thus BNL/BTL signaling is also required for differentiation of terminal branching cells.

Yet a third role for BNL/BTL signaling in tracheal development is in defining the direction of extension of secondary branches. The evidence for this is that expression of the constitutively active form of BTL in tracheal cells, at the time of secondary branch formation, can cause the branches to extend in a circular pattern, rather than in a single direction (Lee et al., 1996). It appears, then, that asymmetric signaling through BTL is necessary to produce the correct pattern of primary branches, and that the cells that receive the highest levels of signal differentiate into secondary branch forming cells, a step that requires changes in gene expression. Finally the secondary branches extend in a directed manner that again requires asymmetric signaling through the BTL receptor.

Some growth factors and their receptors can play a role in tracheal cell migration that is distinct from chemotactic guidance. For example, the EGF receptor homolog and its ligand are specifically required for tracheal cells to populate the branches that extend anterior and posterior to the tracheal pits (Wappner et al., 1997). If the ligand or receptor is mutant, the tracheal system develops only branches extending along the dorsoventral axis. On the contrary, if the ligand is ectopically expressed or if the receptor is constitutively active, many extra cells can be diverted into the anterior and posterior branches. Conversely, the secreted TGF-β-like protein encoded by the *dpp* gene, and its receptor, are specifically required for the migration of cells into the branches that extend along the D/V axis (Vincent et al., 1997; Wappner et al., 1997). Ectopic expression of this ligand or constitutive activation of the receptor leads to excessive population of the D/V branches at the expense of the A/P branches. However, the effect does not appear to be direct chemoattraction in either case. First of all there are transcription factors whose activities are required downstream of either the EGF receptor or the DPP receptor in order for these factors to exert their effects (Chen et al., 1998; Kuhnlein and Schuh, 1996). Secondly, constitutive activation is sufficient for the receptors to produce their effects (Chen et al., 1998). As described above, this is not consistent with a chemotactic guidance role.

The concept that emerges from these studies is that cells can be guided by gradients of secreted chemoattractants in vivo. These studies confirm cell culture studies that indicate that numerous soluble growth factors can stimulate directed motility of various cell types. However, in vivo, growth factors can also affect migratory behavior indirectly by regulating the differentiation of the migratory cells.

Lipids may play a role in guiding cell migration in vivo

One of the advantages of a genetic approach is that one need not have a preconceived idea of the nature of the products involved in cell migration in order to identify them and, therefore, the opportunity exists to learn something completely unexpected. Recent screens for mutations that disrupt gonad formation in *Drosophila* embryos have turned up a big surprise, namely that enzymes involved in lipid metabolism appear to play a key role in directing the migration of these cells. Specifically, mutations in a gene named *wunen* (pronounced oonen, abbreviated *wun*) cause PGCs to migrate into areas of the embryo from which they are normally excluded (Zhang et al., 1997). The observation that the *WUN* mRNA is expressed in these very regions, indicates that this product is required to repel PGCs. Furthermore, misexpression of WUN in the mesoderm is sufficient to prevent PGCs from migrating there (Zhang et al., 1997).

Cloning and sequencing of the *wun* gene revealed the gene product to be a protein highly related to lipid phosphatase 2A (Zhang et al., 1997). The enzyme is predicted to reside in the plasma membrane with its catalytic activity outside of the cell. This finding was interesting but stood alone until the recent report that another gene required for normal PGC migration also encodes an enzyme involved in lipid metabolism. This gene, named *columbus* (*clb)*, encodes the *Drosophila* HMG CoA reductase, an enzyme which, in mammals, is rate-limiting for cholesterol biosynthesis (Van Doren et al., 1998). Since fruitflies are reported to be cholesterol auxotrophs, the role of HMG CoA reductase is more likely to be in production of some other type of lipid product.

In *clb* mutants, few PGCs migrate to reach the mesoderm (Moore et al., 1998). This phenotype, combined with the observation that *CLB* mRNA is expressed at higher levels in the mesoderm than in surrounding tissues, suggest that *clb* function plays a role in attracting PGCs to the mesoderm (Van Doren et al., 1998). Furthermore, mis-expression of *clb* in other tissues is sufficient to cause PGCs to migrate into those tissues. Thus one simple idea is that a lipid chemoattractant is synthesized in the mesodermal cells and is required for PGCs to migrate into that tissue. Meanwhile a lipid chemorepellent, produced by WUN, might actively prevent PGCs from straying into non-target tissues. Alternatively CLB could be involved in producing a lipid chemoattractant while WUN might degrade it. However, it is also possible that the lipid products play much less direct roles in guiding PGC migration. For example HMG CoA reductase function is necessary to produce the dolichol moiety upon which

N-linked sugars are built prior to their transfer to glycosylated proteins and also for production of the geranyl and farnesyl groups that are required for membrane localization of proteins such as Ras. Identification and further characterization of the relevant lipid product and/or additional genes that function in this pathway will be necessary to determine whether lipids have a relatively direct effect on PGC cell motility.

Cell-cell and cell-matrix adhesion molecules

Studies of cells in culture have strongly implicated integrin receptors, which bind to extracellular matrix (ECM) proteins such as laminin or fibronectin, in mediating cell migration. However, until relatively recently, genetic evidence was lacking. In fact mouse knockouts lacking specific integrin receptors have exhibited either no defect in cell migration or such severe embryologic defects that it has been difficult to definitively answer the question of what role these receptors play in cell migration in vivo (Hynes, 1996). Functional overlap between related integrins may also complicate the genetic analysis of integrin function in mammals since there were, at last count, 18 alpha and 8 beta subunits, capable of forming at least 24 different integrin receptors.

However, genetic studies in *C. elegans* have demonstrated that there are defects in cell migration, and surprisingly axon fasciculation, in mutants that lack INA-1, one of the two integrin alpha chains (Baum and Garriga, 1997). INA-1 is most highly related to integrin alpha chains that are known to bind laminin, therefore it seems likely that INA-1 is also a laminin receptor. There was little evidence from previous studies to suggest a role for integrins in axon fasciculation, though there was a wealth of data suggesting that laminin stimulates neurite outgrowth in cell culture (Hammarback et al., 1985). Studies in *Drosophila* had previously demonstrated that laminin is present on axon fascicles and the glial cells that ensheath them (Montell and Goodman, 1989). Furthermore mutations in the gene coding for the A chain of laminin exhibit defects in pathfinding of specific axons in the fly visual system (Garcia-Alonso et al., 1996). These results once again highlight the power of genetics in relatively simple organisms to both confirm results expected from tissue culture experiments and to provide some surprises.

A role for the homophilic cell-cell adhesion molecule E-cadherin, perhaps surprisingly, has been identified in regulating the migration of the *Drosophila* border cells (Niewiadomska et al., 1999). Though current dogma would suggest that cell-cell adhesion must be downregulated in order for cells to migrate, this is apparently not true in every case. The *Drosophila* border cells exhibit increased expression of E-cadherin during their migration. Border cells lacking E-cadherin, due to a mutation in the gene, fail to squeeze in between the nurse cells. Furthermore, if the nurse cells are mutant for E-cadherin, the border cells either fail to migrate or they migrate around the nurse cells. It may be that cells that migrate through ECM-rich environments depend upon adhesion mediated by ECM molecules and integrin receptors whereas cells, such as the border cells, that migrate through a tissue that is densely packed with other cells, utilize cell-cell adhesion for their migration.

Roles of Rho-like GTPases in cell migration and axon pathfinding

If gradients of chemoattractants and chemorepellents guide motile cells and growth cones, what happens inside the cell or growth cone to convert asymmetric receptor activity into directional movement? The mechanism is still not well understood; however, it appears that at least part of the answer, in many migrating cells, is that members of the Rho family of GTPases are activated by various types of receptors and, in turn, affect organization of the actin cytoskeleton. The Rho family GTPases are members of the Ras superfamily of 21 kDa proteins. These proteins are inactive when bound to GDP. When an accessory protein known as an exchange factor, replaces the GDP with GTP, the Ras or Rho protein becomes activated and can bind to a number of so-called effector proteins. The intrinsic GTPase activity of Ras and Rho proteins ultimately converts the GTP to GDP, automatically quenching the signal. The Rho subfamily contains several family members, the best-studied of which are Rho, Rac and Cdc42. Each of these proteins is about 30% identical to Ras and 50-60% identical to each other. Within an organism, there are often several, highly related Rac genes but generally only one Cdc42 gene. The Rho proteins of flies, worms and humans are nearly 90% identical to each other, as are the Rac homologs and Cdc42 homologs. Thus this family is highly conserved across phylogeny. In addition, there are some divergent members of the family that appear to be species-specific (Murphy and Montell, 1996; Zipkin et al., 1997).

Cell culture studies in the early 1990s indicated that the Rho proteins are capable of causing dramatic reorganization of the actin cytoskeleton, in response to a variety of secreted factors (Ridley and Hall, 1992; Ridley et al., 1992). These observations made the Rho proteins excellent candidates for playing a role in cell migration in vivo. To test this hypothesis, Murphy and Montell (1996) expressed dominant-negative forms of each of the proteins, specifically in the *Drosophila* border cells. Dominant-negative Rac caused a strong inhibition of border cell migration, whereas dominant negative Cdc42 had no effect. Subsequently, dominant-negative RhoA has been shown to have no effect on border cell migration. In *C. elegans*, a locus called *mig-2* was identified, mutations in which cause migration defects in a number of different cells and axons. Cloning of this gene revealed that it encodes a Rho family GTPase (Zipkin et al., 1997), related to Rac and Cdc42 in sequence.

If, as suggested, these proteins relay chemotactic guidance signals from the membrane to the cytoskeleton, then we would expect constitutive activation of the proteins to disrupt directional movement, as was observed for the Btl receptor. Indeed, mutations in the *C. elegans mig-2* gene that cause amino acid substitutions that are predicted to cause constitutive activation of the protein, cause even more severe defects in cell migration and axon pathfinding than the loss-of-function mutations (Zipkin et al., 1997). The most-likely explanation for the increase in severity is that loss of a single Rho protein may not be detrimental to the motility of all cells because there are multiple family members, providing some redundancy of function. However, constitutive activation disrupts the signaling pattern in every cell that expresses the gene. Recently, constitutively active Rac has also been found to disrupt border cell migration in *Drosophila* (Hu and Montell, unpublished observation). These results provide additional support for a model that Rho proteins transmit chemotactic guidance signals to the actin cytoskeleton.

What controls the activation of Rho proteins in migratory cells? The *C. elegans* mutant *ced-5* exhibits defects in migration of the distal tip cells. This gene encodes a large protein, which is predicted to be cytoplasmic, and which contains an SH3

domain near the carboxy terminus (Wu and Horvitz, 1998). There are human and *Drosophila* homologs of this protein known as DOCK-180 and Myoblast City, respectively. There is evidence to suggest that, at least in some cells, DOCK-180 is an activator of Rac (Kiyokawa et al., 1998), and, while DOCK-180 is normally cytoplasmic, forcing it to the membrane can cause cells to change shape (Hasegawa et al., 1996). Therefore one possibility for the function of this protein is that when receptor molecules become activated in response to a guidance molecule, DOCK-180 is recruited to the plasma membrane where it can locally activate Rac and stimulate cytoskeletal rearrangements. However it is not yet known if Rac activity is in fact required for distal tip cell migration. Furthermore, since *ced-5* function is only required in the distal tip cells and not in other migratory cells in the worm, there may be other proteins that are responsible for activation of Rac in other migratory cells.

One such protein appears to be the *C. elegans* UNC-73 gene product, whose mammalian homolog is known as Trio. The UNC-73 protein is composed of a multitude of protein domains, including a so-called Dbl homology (DH) domain, an SH3 domain, an Ig domain and a fibronectin type III repeat. Furthermore, UNC-73 has been shown to activate Rac directly (Steven et al., 1998). Mutants lacking *unc-73* function exhibit numerous cell migration and axon pathfinding defects, including defects in HSN, CAN, Q and SM migrations (Desai et al., 1988; Hedgecock, 1987).

What is still quite mysterious is the molecular mechanism by which Rho protein activity affects the organization of actin. Many putative effector proteins have been identified on the basis of their ability to bind one or more of the Rho family members, specifically in the GTP bound state (Burbelo et al., 1995). Some of these effectors are kinases, such as PAK and misshapen. However it is as yet quite unclear which of these effectors is/are important for cell migration in vivo.

Non-muscle myosin II

Because of the firmly established role of the actin cytoskeleton in cell motility, and a clear need for the generation of forces to propel migrating cells, it was postulated that myosin driven contraction of the actin cytoskeleton would contribute in an important way to cell motility. Consequently it was a surprise when *Dictyostelium* ameboe genetically engineered to lack the major conventional myosin heavy chain gene, were initially found to exhibit relatively normal chemotaxis (Manstein et al., 1989). These assays were carried out on glass coverslips, a relatively poorly adhesive surface. As it turns out, ameboe that lack myosin migrate much slower than wild-type ameboe when they are tested on a highly adhesive surface (Jay et al., 1995). These observations lend support to the proposal that migration speed is related to the relative balance between adhesive force and contractile force (Lauffenburger and Horwitz, 1996). In other words, there should be sufficient adhesiveness to generate traction but not so much as to prevent forward movement.

Further support for a more general requirement for conventional, non-muscle myosin in cell motility, comes from studies of the *Drosophila* border cells. Mutations in both the heavy and light chains of *Drosophila* non-muscle myosin had been identified (Karess et al., 1991; Young et al., 1993). However, since mutation of either gene causes lethality, Edwards and Kiehart (1996) made transgenic flies expressing the myosin light chain under the control of a heat-inducible promoter. The lethality of the light chain mutant was rescued by providing mutant embryos that carried the transgene with daily heat pulses. When these animals reached adulthood, the investigators discontinued the heat treatment and myosin light chain protein levels decayed over a period of several days. The lack of light chain resulted in aggregation, and presumably loss-of-function, of the myosin heavy chain. One of the prominent phenotypic consequences was failure of border cell migration (Edwards and Kiehart, 1996).

Transcription factors control many aspects of cell migration

Regulation of gene expression at the transcriptional level controls virtually every aspect of biology and cell migration is no exception. Many of the mutations that cause cell migration defects have been found to affect transcription factors. Transcription factors can regulate the specification of migratory cell fate, the differentiation of migratory cells, the timing of migration and even pathway selection. Thus this is a key, albeit indirect, mechanism by which cells regulate migratory behavior and coordinate this behavior with other developmental events. However, in contrast to factors that are fundamental to the migration mechanism and therefore are required in many different migratory cells, the transcription factors that have been identified seem to be expressed in a cell-type-specific manner.

One of the most fascinating effects of a transcription factor on cell migration is that of the *mab-5* homeobox protein whose expression specifies the direction of Q cell migration (Salser and Kenyon, 1992). As described previously, the QL neuroblast normally migrates anteriorly and QR normally migrates to the posterior. In *mab-5* mutants both cells migrate to the anterior. Conversely, transient ectopic expression of *mab-5* in QL can direct a transient reversal in its direction of migration. The mechanism by which *mab-5* does this is quite mysterious. Presumably one or more genes whose expression is regulated by *mab-5*, is responsible for sensing the cell's position and directing the migration accordingly. However, while numerous loci have been identified which are required upstream of *mab-5* (Table 1) (Harris et al., 1996; Maloof et al., 1999), there is as yet no candidate downstream gene.

A series of transcription factors are required for HSN cell differentiation and migration (Baum et al., 1999). The homeodomain protein encoded by the *egl-5* locus is necessary to activate expression of two zinc-finger motif transcription factors, HAM-2 and EGL-43, both of which are necessary for the HSN cell to migrate. However, here again, the downstream targets for these transcription factors that are required for the cell to migrate are not known.

In the case of border cell migration, a transcription factor encoded by the *slow border cells* (*slbo*) locus is required (Montell et al., 1992). This product belongs to the basic region/leucine zipper class of transcription factors and is the *Drosophila* homolog of C/EBP (Rørth and Montell, 1992). Two genes have been reported to be downstream targets of SLBO. The *btl* gene, which was discussed previously with regard to its role in guiding tracheal migration, appears to be expressed in border cells under SLBO control. Furthermore, expression of *btl* using a heat-inducible promoter restores migration to a significant percentage of *slbo* mutant egg chambers (Murphy et al., 1995). A second downstream target for *slbo* appears to be DE-cadherin which, as described previously, also plays an important role in border cell migration (Niewiadomska et al., 1999).

VAB-8, a novel protein required for cell and axon migrations

Some genes have been identified whose precise role in cell migration is difficult to pinpoint. For example, mutations at the *vab-8* locus disrupt numerous cell migrations along the anteroposterior axis in the worm (Wightman et al., 1996), just as all dorsoventral cell migrations are disrupted to one extent or another in *unc-6* mutants. And like *unc-6*, *vab-8* also affects axon outgrowth and pathfinding, in addition to cell migration. However, *vab-8* does not encode a secreted factor, as does *unc-6*. Rather *vab-8* encodes two cytoplasmic protein isoforms. The isoform expressed in migrating cells is a novel protein, while the isoform expressed in neurons contains an additional domain that is distantly related to kinesin motor proteins (Wolf et al., 1998). It has been suggested that this kinesin-like domain may be required to target the protein to neuronal growth cones, by association with the microtubule core of the axon. However, it is unclear what function the novel protein domain performs, and there are at present no homologous protein sequences in other organisms.

Summary and future directions

Taken together, the studies described here suggest an outline of a model for the regulation of cell migration during development. First of all the fate of the migratory cell must be specified and, generally, this occurs through the action of one or more cell-type-specific transcription factors. Then, the migratory cells begins to differentiate, another step controlled at the level of gene expression. The timing of this differentiation step coordinates migratory behavior with other developmental events, preventing precocious or delayed migration. Expression of various growth factor receptors, chemoattractant receptors and/or appropriate cell adhesion molecules is then modulated so that the motile cell can respond to signals in the environment, which could include lipid chemoattractants and/or chemorepellents, in addition to the well-characterized protein factors, such as FGF or Netrin. The cells then migrate, their direction being specified by the integration of attractive and inhibitory cues. Activation of chemoattractant receptors in one part of a cell may lead to local signaling events, including recruitment of CED-5 (DOCK-180, MBC) or UNC-73 (Trio) to that region of the membrane and local activation of one or more Rho family GTPase. This activity might then stimulate actin reorganization or stabilize the actin cytoskeleton preferentially on that side of the cell. For movement to take place, the rear of the cell must release, either by downregulating cell adhesion or by myosin-driven contraction, or both. Many questions remain, firstly, with respect to the pathway leading from receptor activation to activation of Rho family GTPases and, secondly, with respect to the signaling pathway from Rho GTPase to the cytoskeleton. In addition, it is not clear in which part of the cell myosin plays a role.

Many proteins, such as focal adhesion kinase, have been reported to affect cell motility in cultured cells, but have not yet turned up in the genetic screens for migration mutants. This could be because these factors are not essential in vivo, or it could be because they are proteins that are not employed by simpler organisms. However, it is also likely that such factors have not been identified because mutation of the genes would cause many different problems and therefore the phenotype would not be perceived as being specific to cell migration even though the proteins may be critical elements in the migration machinery. To identify this class of migration genes, that is, genes that encode proteins with multiple essential functions including cell migration, it would be useful to be able to make small patches of homozygous mutant cells in an otherwise heterozygous (and therefore phenotypically wild-type) organism. In animals where the homozygous mutant cells are the migratory cells, one would be able to analyze the effect of a particular mutation on cell motility, without concerns about the various other defects that the mutation might be capable of causing. Genetically this can be done in a process called mosaic analysis and technical advances in *Drosophila* over the past 10 years have made it possible to carry out forward genetic screens and analyze the phenotypes produced by newly induced mutations in mosaic clones. This approach has recently been taken to identify 20 loci required for border cell migration (Liu and Montell, 1999). In addition to confirming a role in vivo for proteins expected to play a role, this type of screen has the potential to identify new, and possibly surprising, components of the migration machinery.

Finally there may be components of the migration machinery that cannot be identified by loss-of-function genetics because they play a subtle modulatory role in the process, or because of substantial functional redundancy with other proteins. To address this type of limitation, Rørth et al. (1998), developed an overexpression/misexpression screen. Using this technique, random loci in the genome can be overexpressed or misexpressed in a cell type of interest and the phenotypic consequences analyzed. These investigators screened 2,300 loci for their ability to restore border cell migration in a weak *slbo* mutant background. 60 loci were found to have this effect, including the gene encoding the *Drosophila* Abelson tyrosine kinase. The mechanism by which migration is restored in these mutants is not yet clear. However it seems likely that this type of approach will be able to identify loci that would escape detection using other methods.

Genetic approaches in relatively simple organisms such as worms and flies have been useful both in confirming ideas originally put forward based on cell culture studies of cell motility, and in providing new insights into the molecular mechanisms controlling cell migration. While knockout technology has opened the door to genetic analysis of cell migration in mouse development, the ability to carry out many different types of forward genetic screens remains a significant advantage of the simpler organisms. A further advantage is the single cell resolution with which phenotypic analysis can be carried out. Many of the cell migration defects described in the *C. elegans* mutants are incompletely penetrant, and therefore might be difficult to detect if one were to examine a large population of migratory cells, since some proportion of the cells would be able to migrate in most individuals. The observation that nearly all of gene products that have been identified to be important for invertebrate cell migration are highly homologous to vertebrate proteins confirms the value of these model systems for understanding gene function in more complex organisms. Finally new forward genetic screening strategies hold the promise that model organisms such as flies and worms will continue to provide new insights into the mechanisms controlling cell migration.

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