Tackling nervous movements:
Migration of Q neuroblasts and their descendents in *Caenorhabditis elegans*

Master Thesis

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Outline

Abstract

Cell migration is a crucial process in animal development and defective cell migration can lead to developmental abnormalities and disease. *C.elegans* has been widely used as a model organism to study the control of cellular movements in vivo. Particularly, the migration of two neuronal stem cells, the left and right Q neuroblasts, and their descendents has been proven to be an excellent model system to study the developmental control of cell migration. At hatching, the left (QL) and right (QR) Q neuroblasts are located in the lateral row of hypodermal seam cells. In the first larval stage both Q neuroblasts delineate from the row of seam cells, migrate a short distance and then undergo cell division. Q daughter cells continue to migrate along the anteroposterior axis of the animal. At hatching both Q neuroblasts are similar in shape and morphology. Interestingly, the QR neuroblast and its descendents migrate anteriorly while QL and its descendents migrate posteriorly. Extensive genetic analysis revealed many insights into the genetic control of this asymmetric process. A highly conserved canonical Wnt/ß-catenin pathway regulates the posterior migration of QL descendent cells while a non-canonical Wnt signaling pathway controls anterior migration of QR daughter cells. Furthermore, it was shown that *Homeobox* genes were also involved in the control of Q cell migration. For example, the *Antennapedia*-like gene *mab-5* is both necessary and sufficient for posterior migration of QL daughter cells. It is widely accepted that cell migration requires dramatic reorganizations of the actin cytoskeleton. Indeed, many genes that were shown to be involved in regulating actin reorganizations were also implicated in the control of Q cell migration. Finally, in determining the direction of migration, the initial short-range migration of Q neuroblasts was shown to be critical since mutations affecting this process were also shown to result in aberrant migration of Q neuroblast descendents. Here, I will give an overview of the various control mechanisms known to date that are involved in the regulation of Q cell migration.

1. Introduction

Animal development requires the precise coordination of a wide variety of cellular processes. Upon fertilization the zygote starts to proliferate, generating an enormous number of cells. During development these cells undergo a tightly regulated program of events in which the embryo will undergo patterning and cells are instructed to adopt a certain fate. Ultimately, differentiation of cells towards a specialized cell fate will result in the formation of tissues and organs with well-defined functions. Not surprisingly, differentiation of cells should occur in a proper spatiotemporal manner. Therefore, correct positioning of cells is intrinsic to organism development.

1.1 Cell movements

Unlike plant cells that are constricted by a rigid cell wall, animal cells have the ability to dramatically change their morphology and migrate long distances throughout the organism. Several processes in organism development utilize this ability to ensure the correct localization of cells. For example, during gastrulation cells move towards the inner embryo, giving rise to the endodermal and mesodermal germ layers. Migration of neural crest cells is another important event that occurs during vertebrate development. These cells from ectodermal origin detach from the neurectoderm upon closure of the neural tube and migrate towards distant sites in the embryo, giving rise to various cell types like melanocytes and sensory neurons (Sauka-Spengler and Bronner-Fraser 2008). In the adult organism cell migration plays an important role in the immune defense system since leukocytes can migrate over long distances and move across endothelial cells towards the site of inflammation (Madri and Graesser 2000, Muller 2003). Furthermore, the migratory potential of animal cells is used by cancer cells to invade surrounding tissues and metastasize towards distant sites (Kassis et al 2001, Lambrechts et al 2004). Extensive research of cell migration in normal and diseased contexts using various different model organisms revealed many insights into the genetic and molecular circuitry that underlies cell migration.

1.2 Recurring themes of cell migration

Cell motility requires a tight interplay between many cellular and molecular processes. First, a cell must sense an extracellular migration cue and interpret this signal. Extracellular guidance cues can either act as attractants, like IL-6 that indirectly serves as an attractant for leukocytes via specific chemokine expression (Romano et al 1997), or as repellents, like Wnt3a in chick development where it was recently proposed to form a repulsive gradient for the migration of cardiac progenitors (Yue et al 2008). Extracellular guidance cues finally impinge on regulators that control cellular adhesions and the dynamics of the actin cytoskeleton. Upon signaling, epithelial cells can detach from their neighbors by downregulation of adhesion molecules, a process called epithelial to mesenchymal transition (EMT). Loss of specific adhesive cadherins is one of the prerequisites for cell migration and EMT (Yang and Weinberg 2008). For example, neural crest cells downregulate E-cadherin prior to detachment and N-cadherin upon delamination resulting in the loss of affinity for cells in the neurectoderm (Sauka-Spengler and Bronner-Fraser 2008). Furthermore, expression of adhesive junction components like E-cadherin, ß-catenin or p120 is often lost

in invasive carcinomas stressing the fact that cell adhesions are important to constrict cell migrations (Birchmeier et al 1996, Onder et al 2008, Yang and Weinberg 2008).

In addition to downregulation of cellular adhesions, a migrating cell needs to dramatically reorganize its actin cytoskeleton in an asymmetrical fashion. Regulation of actin dynamics during cell migration was shown to be facilitated by the polarized activities of molecules of the Rho/Rac family of GTPases. Rac GTPases promote actin polymerization in the front end while Rho GTPases act at the rear end to mediate formation of contractile actin-myosin filaments. This coordinated action of actin cytoskeleton rearrangements is the driving force of cell migration (Itoh et al 2002, Jaffe and Hall 2005, Kraynov et al 2000, Ridley et al 1992). In vitro, the polarized action of Rho/Rac and thereby cell migration can occur randomly towards any direction (Jaffe and Hall 2005, Pankov et al 2005). In vivo, cells might utilize the ability to migrate randomly in order to explore their environment whereas extracellular guidance molecules may impinge on the Rho/Rac signaling pathway to direct cells towards a specific location (Chung et al 2000, Endo et al 2005, Srinivasan et al 2003, Yue et al 2008).

1.3 Caenorhabditis elegans as a model to study cell migration

Many insights into the molecular processes that control cell migration have been obtained using in vitro and in vivo experimental approaches. Since cell migration is such a common phenomenon it is likely that the genetic circuitry controlling this process has emerged early in evolution. This validates the use of experimental systems like the small nematode *Caenorhabditis elegans* and indeed, many insights into the core regulatory pathways of cell migration have been obtained using *C.elegans* as an experimental model organism (Lundquist 2006, Silhankova and Korswagen 2007). Ever since its introduction as a model organism over 30 years ago (Brenner 1974, Sulston and Horvitz 1977), *C.elegans* has been widely used to address questions of high biological and clinical relevance. Major advantages of *C.elegans* as a model organism are its short life cycle, powerful genetics, transparency of body cells, and the fact that the cell lineage of *C.elegans* is nearly invariant and has been mapped completely. Briefly, the fertilized oocyte undergoes many cleavage cell divisions during embryogenesis. After embryogenesis the larvae hatches and undergoes 4 larval stages, L1-L4, to give rise to the adult animal in which all the organs and tissues are present and fully functional (Suslton et al 1983, Sulston and Horvitz 1977).

During development of the *C.elegans* nervous system, many different cells migrate through the organism. P cells migrate ventrally towards the ventral midline in the first larval stage and will give rise to neurons and vulval tissue. Furthermore, many cells of neuronal origin like HSN, BDU, CAN, ALM and the Q neuroblasts migrate along the anteroposterior axis of the animal. Q neuroblasts are two seemingly similar cells that are both located in the lateral row of hypodermal seam cells; QR on the right side of the animal and QL on the left side. After hatching both Q neuroblasts migrate a short distance, moving out of the lateral row of seam cells. However, QR will migrate anteriorly over the neighboring seam cell V4 while QL will migrate over its posterior neighbor V5 (Honigberg and Kenyon 2000, Sulston and Horvitz 1977). After this initial neuroblast migration both Q cells undergo an identical pattern of cell division in the L1 stage. Each Q neuroblast generates two descendents that go into apoptosis and three daughter cells that will migrate further along the anteroposterior axis (figure 1) and differentiate into sensory neurons (Chalfie and Sulston 1981, Sulston and

Horvitz 1977). Similar to the initial Q cell migration, QL descendents (QL.d) will migrate posteriorly while QR descendents (QR.d) migrate anteriorly. Although obvious landmarks at the final destination of Q.d cells seem to be absent, in wildtype, Q neuroblast descendents are precisely positioned along the anteroposterior axis having their endpoints well-defined. The QR daughter cells SDQR and AVM are positioned near the V1 seam cell and move to dorsal and ventral sites respectively, while AQR migrates further towards the anterior. Both the QL daughter cells SDQL and PVM migrate a short distance and ultimately occupy positions near V5. PQR displays long-range migration towards a site in the tail region (figure 1, Sulston and Horvitz 1977).

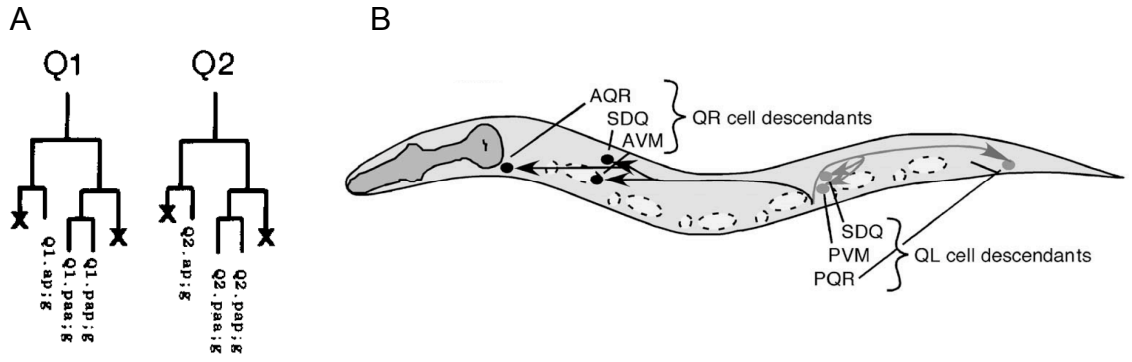

Figure 1. Q neuroblast lineage and descendent migrations. A Q cell lineage. Q1 (QL) and Q2 (QR), born on opposite lateral sides, undergo an identical pattern of cell division and generate 5 daughter cells, two will go into apoptosis (marked "X") and three daughter cells will migrate along the anteroposterior axis (from: Sulston and Horvitz 1977). **B** The QR descendents AQR (QR.ap), SDQR (QR.pap) and AVM (QR.paa) migrate anteriorly while the QL descendents SDQL, PVM and PQR migrate posteriorly. Arrows mark paths of migration. Dashed lines mark hypodermal seam cells. Figure adapted from: Zinovyeva and Forrester 2005.

Because the start and finish location of migrating QR.d and QL.d cells is well-defined and nearly invariant, these migratory cells provide an excellent model system to study cell migration in vivo. Using Q.d cell migration as a read-out to test the role of different genes, many insights into the control of both the directionality of cell movements and cell motility itself have been obtained. In this thesis I will give an overview of the research results that addressed questions like: Which factors are involved in Q cell migration? What determines the directionality of Q.d cell migration? Which factors determine the final positioning of Q neuroblast descendents? Are the control mechanisms of Q cell migration similar to those involved in other cell movements?

2. Q neuroblast descendent migration

2.1 Canonical Wnt signaling determines directionality of QL.d migrations

Genetic analysis pointed out an important role for the highly conserved Wnt signaling pathway in the control of Q cell migrations (Maloof et al 1999, Silhankova and Korswagen 2007, Wangbo and Kenyon 1999). Although elements of Wnt signaling may vary in different contexts, the general signaling framework has been well characterized. In the absence of Wnt ligands ß-catenin is phosphorylated by the destruction complex, consisting of GSK3ß kinase, Axin and the Adenopolyposis coli protein (APC), and subsequently degraded via the proteasomal pathway. Upon Wnt binding to its receptor Frizzled (Fz), the destruction complex is inhibited via the action of Disheveled and by sequestration of Axin to the co-receptor LRP/Arrow (Mao et al 2001, Wodarz and Nusse 1998). As a result, ß-catenin is stabilized, moves to the nucleus and activates target gene transcription together with proteins of the Tcf/LEF-1 transcription factor family. This Wnt/ß-catenin pathway is referred to as canonical Wnt signaling and was shown to be involved in a wide variety of developmental processes (Cadigan and Nusse 1997). In addition, many ß-catenin independent Wnt responsive pathways are being identified and characterized. These are generally referred to as non-canonical Wnt signaling pathways (see below). The *C.elegans* genome encodes five Wnt ligands: *cwn-1, cwn-2, egl-20, lin-44, mom-2*, and four Frizzled receptors: *cfz-2, lin-17, mig-1, mom-5* (Herman et al 1995, Rochelau et al 1997, Ruvkun and Hobert 1998, Sawa et al 1996, Shackleford et al 1993, Thorpe et al 1997).

Already in 1992 Salser and Kenyon showed that the *Homeobox* gene *mab-5* is expressed in the QL neuroblast and that this expression persists in QL daughter cells. *mab-5* expression in Q neuroblasts is both necessary and sufficient to direct posterior migration of Q cell descendents. Forced expression of *mab-5* in QR causes its descendents to migrate posteriorly while *mab-5* loss of function causes QL neuroblasts to adopt a QR fate, resulting in anterior migration of both QR.d and QL.d. These results already indicated that *mab-5* expression acts as a pivotal switch in determining the direction of Q cell migration (Salser and Kenyon 1992). In the search for upstream signals that regulate *mab-5* it was found that loss of *egl-20/Wnt*, *lin-17/Fz* and *mig-1/Fz* all resulted in the lack of *mab-5* expression and anterior migration of both QL.d and QR.d (Harris et al 1996). Single mutations in any of the other *Wnt* or *Frizzled* homologs does not affect the direction of Q.d migrations, indicating that *egl-20* signaling is crucial for *mab-5* expression in QL (Zinovyeva et al 2008).

Further analysis demonstrated that a canonical Wnt signaling pathway regulates *mab-5* expression in Q neuroblasts. Mutant alleles of *mig-5*/*Disheveled* as well as *mig-5* RNAi (Korswagen et al 2002, Walston et al 2006), loss of function of *bar-1*/*B-catenin* (Maloof et al. 1999) and loss of function of *pop-1/Tcf* (Herman 2001) all affected the ability to express *mab-5* in QL and resulted in anterior migration of both QL.d and QR.d. Furthermore, it was shown that an interaction between *bar-1* and *pop-1* is necessary for *mab-5* expression in QL (Korswagen et al 2000). BAR-1 contains several consensus GSK3ß phosphorylation sites and deletion of these sites results in ectopic Wnt signaling during vulva development (Eisenmann 1998, Gleason et al 2002). In addition, overexpression of the *C.elegans* homolog of GSK3ß, *sgg-1*, resulted in anterior migration of QL.d, consistent with the fact that GSK3ß functions as a negative regulator of canonical Wnt signaling (Korswagen et al 2002).

Based on sequence similarity the *C.elegans* genome does not encode a clear-cut Axin homolog (Ruvkun and Hobert 1998). However, both *pry-1* and *axl-1* were reported to be involved in the repression of *egl-20/Wnt* signaling in the QR neuroblast. Detailed sequence analysis revealed that both *pry-1* and *axl-1* encode highly divergent Axin-like proteins. *pry-1* loss of function resulted in ectopic *mab-5* activation in the QR neuroblast and subsequent posterior migration of QR.d cells while *pry-1* overexpression induced anterior migration of QL.d (Korswagen et al 2002, Maloof et al 1999). Loss of *axl-1* function alone did not show a Q cell migration phenotype but greatly enhanced the *pry-1* phenotype from 35% of posterior QR.d migration to a full penetrance phenotype in which 100% of QR.d cells migrated posteriorly (Oosterveen et al 2007). Furthermore, epistatic analysis placed *pry-1* and *axl-1* downstream of *egl-20* and *mig-5* but upstream of *bar-1* and *mab-5*. Using a yeast-two-hybrid binding assay PRY-1 as well as AXL-1 were shown to physically interact with BAR-1/B-catenin, GSK-3/GSK3B and MIG-5/DSH. PRY-1 also interacted with the *C.elegans* APC homolog APR-1 and surprisingly, *pry-1* expression could rescue the *axin1* phenotype in zebrafish (Korswagen et al 2002, Oosterveen et al 2007). Together, these results suggest that *pry-1* and *axl-1* are indeed functional *Axin* homologs that negatively regulate canonical *egl-20*/Wnt signaling in the QR neuroblast.

A clear role for the *C. elegans* APC homolog, *apr-1*, in Q neuroblast migration was not established. The majority of homozygous *apr-1* mutants are embryonic lethal. Loss of *apr-1* function causes a variety of developmental abnormalities including defects in endoderm induction, hypodermal migration and vulva induction. Surprisingly, together with *sgg-1*, *apr-1* acts as a positive regulator of Wnt signaling in embryonic endoderm induction and tissue specific RNAi of *apr-1* in the developing vulva causes an underinduced phenotype similar to *bar-1* loss of function (Hoier et al 2000, Rochelau et al 1997). However, *apr-1* RNAi could enhance the vulval phenotype of *pry-1* and overexpression of a constitutively active BAR-1 (Gleason et al 2002). Furthermore, the finding that PRY-1 interacts with APR-1 in a yeast two-hybrid assay suggests that *apr-1* can function in canonical Wnt signaling (Korswagen et al 2002). Therefore, it is likely that APR-1 functions in a destruction complex together with PRY-1 and SGG-1 to inhibit BAR-1 accumulation in QR neuroblasts. Additional experiments, like tissue specific RNAi in Q cells or detailed *apr-1* expression and mosaic analysis might provide insights into the role of *apr-1* in Q cell migration.

Figure 2. EGL-20 gradient. EGL-20 (green) is synthesized in the tail region and forms an anteroposterior gradient. The bracket indicates the region of EGL-20 synthesis, arrowhead marks P11/12 and dashed lines mark the range of the EGL-20 gradient. Figure adapted from Coudreuse et al 2006.

Expression analysis revealed that the action of *egl-20* is not cell autonomous. Rather, EGL-20 is expressed in a group of cells in the tail region and exerts its function via long-range signaling. EGL-20 is distributed in an anteroposterior gradient and formation of this gradient is necessary for QL to express *mab-5* (Coudreuse et al 2006, Whangbo and Kenyon 1999). Interestingly, EGL-20 gradient formation requires more than *egl-20* expression alone. It was demonstrated that a specialized machinery composed of the retromer complex and *mig-14/Wls* is required for EGL-20 secretion.

Over a decade ago it was shown that *mig-14* mutants gave a Q cell migration phenotype similar to *egl-20* loss of function (Harris et al 1996). Further analysis using varying model systems revealed that Wls is necessary for Wnt secretion and that MIG-14 is the *C.elegans* Wls homologue (Banziger et al 2006, Bartscherer et al 2006). In addition, Coudreuse et al showed that loss of function of a component of the retromer complex, *vps-35*, also displayed a Q cell phenotype similar to *mig-14* and *egl-20*. This phenotype can be rescued by expression of *vps-35* under the control of an *egl-20* promoter (Coudreuse et al 2006). More recently, several lines of evidence in different experimental systems indicated that the retromer complex prevents lysosomal degradation of Wls by mediating endosome to golgi retrieval (Belenkaya et al 2008, Franch-Marro et al 2008, Pan et al 2008, Port et al 2008, Yang et al 2008). Furthermore, it was shown in C. elegans that *dpy-23/AP-2* loss of function results in accumulation of MIG-14 in the plasma membrane of EGL-20 producing cells (Pan et al 2008). In addition, *vps-35* loss of function resulted in a decrease of MIG-14 levels due to lysosomal degradation (Yang et al 2008). Together, these results led to a model in which EGL-20 is secreted via the action of MIG-14. In turn, MIG-14 is internalized via the clathrin mediated endocytosis pathway and subsequently retrieved from the endosome to lysosome pathway via the retromer complex. This machinery is necessary for the formation of a proper gradient of EGL-20 that regulates *mab-5* expression and thereby the directionality of Q cell migration.

The mechanism by which an anteroposterior EGL-20 gradient can lead to *mab-5* activation in QL but not in QR remains unknown. Because EGL-20 levels do not exhibit left-right asymmetry it is unlikely that EGL-20 levels determine whether *mab-5* will be expressed or not. Furthermore, ubiquitous *egl-20* overexpression in an *egl-20* mutant background activates *mab-5* in QL and, at higher dosage, also in QR, resulting in the posterior migration of both QL.d and QR.d (Whangbo and Kenyon 1999). Together these findings indicate that the difference in *mab-5* expression between QR and QL is mediated via differential EGL-20 sensitivity rather than unequal distribution of EGL-20. In addition, reversal of the EGL-20 gradient partially rescued the QL.d migration defect of *egl-20* mutants indicating that EGL-20 functions as a permissive cue for posteriorly directed migration of QL.d rather than as a molecular attractant (Whangbo and Kenyon 1999). Different levels of regulation might impinge on this asymmetric EGL-20 responsiveness. QR might express higher levels of *pry-1* and *axl-1* or lower levels of positive regulators of canonical Wnt signaling. Another clue may come from the initial Q neuroblast migration, which is already asymmetric and is seemingly *egl-20* independent (see below).

Several lines of evidence indicate that the action of EGL-20/Wnt signaling is inhibited by *cam-1. cam-1* encodes a Ror receptor tyrosine kinase (RTK) and contains an extracellular Ig domain, a cysteine-rich domain (CRD), an intracellular tyrosine kinase domain and a region rich in serine and threonine residues (Forrester 2002, Forrester et al 1999). During embryogenesis, *cam-1* loss of function causes anterior displacement of posteriorly directed migrations of the CAN and ALM neurons. In addition, anteriorly directed HSN and BDU neurons often overshoot their normal migrations (Forrester et al 1999). Furthermore, Q.d cell migrations were also found to be mildly disrupted in *cam-1* mutants (Forrester and Garriga 1997, Kim and Forrester 2003). The migration of QL.d cells was not significantly affected in *cam-1* loss of function mutants. However, *cam-1* overexpression using its endogenous promoter often caused the QL.d cells to reverse the direction of migration. Similarly, overexpression of transgenic *cam-1* encoding only the

transmembrane region and the CRD domain displayed the same QL.d migration phenotype. *cam-1* transgenes that lack the CRD domain, however, do not show this phenotype indicating that membrane attachment and the extracellular CRD domain are necessary to alter the direction of QL.d migration (Kim and Forrester 2003). These results already show that CAM-1 does not act as a canonical receptor tyrosine kinase since deletion of the kinase domain does not affect its ability to redirect migrating QL.d cells.

Further analysis revealed that animals overexpressing *cam-1* displayed lower levels of *mab-5* expression in QL. Again, similar results were obtained when transgenic *cam-1* encoding only the transmembrane region and the CRD domain were overexpressed, but not when a CRD deletion construct was overexpressed. In addition, *mab-5* gain of function could rescue the QL.d defect upon *cam-1* overexpression (Forrester et al 2004). Together these results favor a model in which the action of *cam-1* inhibits *egl-20* signaling resulting in the lack of *mab-5* expression in QL and subsequent anterior migration of QL descendents. Therefore, *cam-1* likely acts as an antagonist of *egl-20* mediated *mab-5* expression in QL.

The molecular mechanism by which *cam-1* antagonizes canonical Wnt signaling remains elusive. Via its cysteine rich domain CAM-1 might antagonize EGL-20 directly by binding and sequestration of extracellular EGL-20. *cam-1* expression was found throughout the animal with high expression levels at the anterior and expression was progressively lowered towards the posterior. This is consistent with a model in which CAM-1 acts in a "source-sink" mechanism where EGL-20 is produced and secreted in the posterior and degraded or sequestered anteriorly by CAM-1. In this way, CAM-1 might also contribute to the maintenance of an anteroposterior EGL-20 gradient. Conversely, since *cam-1* loss of function does not result in a QL.d cell migration phenotype it cannot be excluded that the effect of *cam-1* overexpression on QL.d migration is due to an experimental artifact. In this scenario *cam-1* does not function in the control of endogenous QL.d cell migration. However, considering the significant effect of *cam-1* overexpression, the absence of a clear phenotype might be explained by the existence of other factors that act redundantly with *cam-1*.

All together, these results demonstrate that a highly conserved canonical Wnt signaling pathway is responsible for instructing QL.d cells to migrate posteriorly. The critical effector for posterior migration downstream of canonical *egl-20/Wnt* signaling is the *Antennapedia*-like *mab-5* acting in a switch-like manner. However, for none of the canonical Wnt pathway components shown to function in regulating posterior QL.d migration it was determined whether they function cell autonomously. Although, based on gene expression patterns it is likely that canonical Wnt signaling occurs in QL itself, a non-autonomous role cannot be excluded. Up till now the molecular mechanism by which *mab-5* governs directionality of QL.d movements remains elusive. Given the fact that *mab-5* encodes a Homeobox transcription factor it would be interesting to determine which genes it affects. Because EGL-20 is thought to function as a permissive cue for posteriorly directed migration, it is unlikely that *mab-5* target genes mediate the cellular affinity for *egl-20*. However, it is possible that *mab-5* expression in Q cells sensitizes for another chemoattractant or repellent of unknown origin.

2.2 Non-canonical Wnt signaling directs Q.d migrations

The role of *egl-20* in Q cell migration is not restricted to the activation of *mab-5* in the QL neuroblast. In a vast majority of *egl-20* mutants the final position of QR.d cells is shifted posteriorly. This QR.d migration phenotype was shown to be *bar-1* independent indicating that this function of *egl-20* is mediated via non-canonical Wnt signaling (Whangbo and Kenyon 1999). Since EGL-20 is synthesized in the tail region and forms an anteroposterior gradient, it is tempting to speculate that EGL-20 acts as a repellent for QR.d cells. However, whereas high levels of EGL-20 will trigger *mab-5* activation, ectopic pharyngeal *egl-20* expression partially rescues the *egl-20* QR.d migration phenotype (Whangbo and Kenyon 1999). This suggests that *egl-20* does not act as a molecular repellent but rather activates a response pathway that promotes anterior migration. These results favor a model in which the two Q neuroblasts are already programmed to respond differently to EGL-20. The QL neuroblast has a low threshold for canonical Wnt signaling by *egl-20* and will activate *mab-5* followed by posterior migration of its descendents. The QR neuroblast has a high threshold for EGL-20 mediated *mab-5* activation but responds to endogenous EGL-20 levels by an alternative response pathway that mediates anterior migration.

It is unlikely that EGL-20 functions as a repellent per se. However, it has been shown that Wnt proteins can act as molecular repellents (Harris and Beckendorf 2007, Yue et al 2008). Therefore, EGL-20 signaling might sensitize QR.d cells for other Wnt homologs that do act as molecular repellents. Two other Wnt homologs, *lin-44* and *cwn-1*, are also expressed in the tail region during late embryogenesis and early L1 larvae but only *cwn-1* mutants displayed a QR.d phenotype similar to *egl-20* loss of function (Gleason et al 2006, Herman et al 1995). Furthermore, loss of *cwn-2* function, which is thought to be expressed more ubiquitously along the anteroposterior axis, resulted in a more subtle but significant percentage of posteriorly shifted positions of QR.d (Gleason et al 2006, Zinovyeva et al 2008, Zinovyeva and Forrester 2005). *cwn-2* enhances the *cwn-1* but not the *egl-20* phenotype while *cwn-1*; *egl-20* double mutants show an increased posterior displacement of QR.d (Zinovyeva et al 2008). These results might point towards a model in which *cwn-1* instructively acts as a repellent while *cwn-2* and *egl-20* act as a permissive factors for anterior QR.d cell migration. This can be tested by determining the rescuing ability of ubiquitously or anteriorly overexpressed *cwn-1* and *cwn-2*. In addition, the precise *cwn-1* and *cwn-2* expression patterns during the early L1 stage might shine a different light on the molecular function of these genes in directing QR.d migration.

Interestingly, in *cwn-1*; *egl-20 cwn-2* triple mutants the final position of QR cell descendents in each animal varies greatly. In all cases QR.d cells are posteriorly displaced with a small percentage of QR.d cells that were found posterior from their starting points indicating that migration was slightly randomized in the absence of *cwn-1 egl-20* and *cwn-2*. In *Wnt* quadruple and quintuple mutants this effect was slightly more pronounced (Zinovyeva et al 2008). Surprisingly, *lin-44* loss of function partially suppressed the *egl-20* QR.d phenotype suggesting an antagonistic relation. However, *lin-44* single mutants did not display a phenotype nor could *cwn-1* or *cwn-2* single or double mutant phenotypes be rescued by *lin-44*. Although a subtle role cannot be excluded, *lin-44* is not crucial for QR.d migration. *mom-2* mutants did not give a QR.d phenotype and modification of the phenotype associated with any of the other Wnt homologs was not observed either (Zinovyeva et al 2008).

As expected, *C. elegans* Frizzleds are also involved in QR.d migration. However, when single mutants were examined only *mom-5* displayed a posterior shift in the final QR.d position. Additional *Frizzled* mutations enhanced the *mom-5* phenotypes with *lin-17* as being the most potent enhancer. Double or triple mutant combinations of *cfz-2*, *mig-1* and *lin-17* did not show a significant QR.d phenotype. These results already demonstrated that *mom-5* is crucial for proper QR.d migration while the other *Frizzled* homologs are dispensable in the presence of *mom-5*. The other *Frizzled* homologs function redundantly in the absence of *mom-5*. *Frizzled* quadruple mutants (in which all *Frizzled* homologs are mutated) show a QR.d migration phenotype comparable to *Wnt* quintuple mutants validating the idea that *C.elegans* Wnts indeed exert their action via Frizzled receptors (Zinovyeva et al 2008). In order to determine whether the control of Q cell migration by non-canonical Wnt signaling occurs cell autonomously it would be interesting to establish which cells require functional Frizzled receptors. For example, in the L1 stage *cfz-2* is expressed in cells in the head and in a pair of cells in the tail region and functions non-autonomously in positioning the ALM neuron (Zinovyeva and Forrester 2005).

Except for *egl-20*, single mutants of any of the other *C.elegans Wnt* homologs do not display a QL.d migration phenotype. However, both *cwn-1* and *cwn-2* suppress the *egl-20* phenotype. In *egl-20 cwn-2* or *cwn-1*; *egl-20* double mutants QL.d cells migrate anteriorly but on average the final positions are more posterior than in *egl-20* single mutants (Zinovyeva et al 2008). This can be explained by the fact that QL.d cells adopt a QR.d cell fate (Harris et al 1996) resulting in anterior migration that is subjected to guidance by the action of *cwn-1* and *cwn-2*. It would be interesting to determine at what molecular level the dual function of *egl-20* as a promoter of posterior migration via *mab-5* expression and as a cue for anterior migration of QR.d diverges. Because ectopic expression of *egl-20* results in *mab-5* expression and posterior migration of both QR.d and QL.d it was established that a difference in *egl-20* sensitivity is the first line of diversification. Consistent with this hypothesis was the finding that low levels of EGL-20 can rescue the *egl-20* QR.d migration phenotype (Whangbo and Kenyon 1999). However, the underlying mechanism of this differential sensitivity remains elusive. Furthermore, molecular details about the way in which *mab-5* expression in Q neuroblasts results in desensitization for the postulated action of *cwn-1* and *cwn-2* are also lacking.

Interestingly, in *cwn-1*; *egl-20 cwn-2* triple mutants a small but substantial percentage of animals displays posterior migration of QL.d cells to a final position that is located more posterior than in wild type animals. Like in QR.d migrations, additional knockdown of *lin-44* and *mom-2* further enhances this effect, rendering the direction of QL neuroblast migration random (Zinovyeva et al 2008). This is remarkable since, due to the absence of canonical *egl-20* signaling, it is unlikely that QL neuroblasts in these triple mutants express *mab-5*. An explanation might be that the absence of all potent regulators of long range Q.d cell migration is responsible for stochastic determination of the direction of movement.

For the Frizzled homologs, *mig-1* and *lin-17* but not *cfz-2* or *mom-5* single mutations result in anterior QL.d migrations. In *mig-1* or *lin-17* some QL.d cells migrate as far as QR.d cells, consistent with the idea that *mig-1* and *lin-17* are necessary to adopt the QL fate (Harris et al 1996, Zinovyeva et al 2008). The observation that QL often fails to express *mab-5* in *mig-1* or *lin-17* mutants and that the absence of *mab-5* expression correlates with anterior displacements further support this hypothesis (Harris et al 1996, Salser and Kenyon

1992). Loss of function of *cfz-2* or *mom-5* alone or in combination does not show a QL.d migration defect. However, in the absence of *mig-1* or *lin-17 mom-5* and *cfz-2* slightly enhance the QL.d migration phenotype. Again, when all *Frizzleds* are mutated the final distribution of QL.d cells appears to be randomized with some QL.d cells migrating posteriorly while others do not migrate or move anteriorly (Zinovyeva et al 2008).

The randomized positioning phenotype of QR.d and QL.d was observed in mutant combinations of multiple *Wnts* and *Frizzleds*. Although more dramatic, similar phenotypes were found in mutants that affect the initial polarization and short-range migration of Q neuroblasts. Because the direction of initial short range Q migration appears to correlate with the direction of further long-range migration, it was postulated that the initial polarization determines the later direction of long-range Q daughter migration. Despite the fact that the initial polarization and migration were shown to be *egl-20* independent, it would be interesting to see whether animals mutant for other *Wnts* or *Frizzleds* affect this process (see below).

Extensive mutational analysis of Wnt pathway components provided evidence for a complex regulatory network of canonical and non-canonical Wnt signaling that controls the positioning of Q.d cells along the anteroposterior axis. However, detailed expression and mosaic analysis is needed to determine in which cells Wnt pathway components act to control the migration of Q daughter cells. Considering the genetic data, it is tempting to speculate that MIG-1 and LIN-17 both act as receptors for EGL-20 and are present on QL leading to activation of the canonical Wnt signaling pathway in QL. Examination of the *mig-1* and *lin-17* expression patterns might reveal that these Frizzled homologs are expressed in QL but not in QR. This might be a mechanism to control the differential sensitivity of the QL neuroblast to the EGL-20 signal. Similarly, *mom-5* might be expressed in QR but not in QL to regulate anterior migration of QR.d.

2.3 Involvement of homeotic genes in Q neuroblast migrations

In *C.elegans*, morphogenesis of many tissues involves the cooperate action of different cells that are not directly related by cell lineage (Sulston et al 1983, Sulston and Horvitz 1977). Therefore, external positioning cues are required to correctly pattern individual cells for cooperate morphogenesis. Like in other organisms, positional information in *C.elegans* is provided by homeotic genes. The *C.elegans* genome encodes six well-characterized *Homeobox* (*Hox*) genes that, like their metazoan and mammalian counterparts, are known to be involved in anteroposterior patterning. During *C.elegans* embryonic development the *Labial*-like *Hox* gene *ceh-13* is required for anterior specification while the *AbdB*-like *Hox* genes *nop-1* and *php-3* specify posterior compartments (Brunschwig et al 1999, Van Auken et al 2000). Three other *Hox* genes, *lin-39*, *mab-5* and *egl-5* were shown to be required for postembryonic patterning. Similar to *Hox* genes in other species, *lin-39*, *mab-5* and *egl-5* are located in an organized cluster and their expression pattern corresponds to their genomic localization: *lin-39* is located most upstream in the cluster and is expressed in the midbody region whereas *mab-5* and *egl-5* are located downstream of *lin-39* and function more posteriorly and in the tail region, respectively (Chisholm 1991, Clark et al 1993, Wang et al 1993).

Because Q neuroblasts and their descendents migrate along the anteroposterior axis it is not surprising that *Hox* genes are involved in the control of these cell migrations. As

described in the previous chapter, the posteriorly expressed *Hox* gene *mab-5* is a critical regulator of posterior QL.d migration (Salser and Kenyon 1992, see above). Interestingly, the proper migration of Q daughter cells also requires the action of *lin-39*. During L1 larval development *lin-39* is expressed in the midbody region in ventral cord neurons, vulval precursor cells and in Q neuroblasts and their descendents. Loss of function of *lin-39* results in homeotic transformations in the midbody region leaving other regions largely unaffected. In *lin-39* mutants all Pn.p cells fuse with the hypodermal syncytium while all Pn.aap cells undergo apoptosis, which are cell fates normally observed only in non-vulval Pn.p and Pn.a cells (Clark et al 1993, Wang et al 1993).

Furthermore, as implied by the *lin-39* expression pattern, Q.d migrations are also affected in *lin-39* mutants. The anterior migration of QR.d cells is aborted prematurely in *lin-39* mutants with QR.paa and QR.pap migrating near V3-V4 instead of V1. Mosaic analysis showed that *lin-39* functions cell autonomously in this process. In most *lin-39* mutants the QL.d migrations were unaffected, with only minor initial QL neuroblast migration defects. Because QL.d cells adopt a QR.d-like fate in *mab-5* mutants one would expect QL.d cell migration in *lin-39 mab-5* double mutants to be similar to QR.d cell migration in *lin-39* single mutants. Indeed this was found but surprisingly, the initial QL neuroblast migration was found to be altered in *lin-39 mab-5* double mutants. In these mutants QL often migrated too far posteriorly and turned around again before division, a phenotype never observed in *mab-5* or *lin-39* single mutants suggesting that *mab-5* and *lin-39* might function redundantly in terminating initial QL migration (Clark et al 1993, Wang et al 1993). Interestingly, in *lin-39* single mutants the direction of QR.d migration is not reversed like QL.d migration in *mab-5* mutants indicating that, unlike *mab-5* in QL, *lin-39* does not act as a pivotal switch in cell fate determination. Rather, together with other factors *lin-39* contributes to the anterior migration cell fate of QR.d cells.

Given the fact that *lin-39* functions cell autonomously, it would be interesting to determine which upstream signals regulate *lin-39* expression in QR and its descendents. As in other organisms positioning cues are likely to be provided by extracellular morphogens, like one of the Wnt homologs. However, the premature stop of QR.d in *egl-20* and *lin-39* single mutants is enhanced in *lin-39* ; *egl-20* double mutants indicating that these two genes function in parallel pathways (Harris et al 1996, Yang et al 2005). Other candidates would be *cwn-1* and *cwn-2*, since their QR.d migration phenotypes are also similar to that observed in *lin-39* mutants. In contrast, some controversial evidence suggests that *Hox* genes in *C.elegans* can be regulated by cell intrinsic mechanisms independent of their position. During embryogenesis the M cell migrates from an anterior position to a posterior compartment where it starts to express *mab-5*. However when migration is blocked, *mab-5* is still expressed in the anteriorly located M cell. Similarly, in V6 cells that were anteriorly mispositioned by laser ablation of P2 and EMS in early embryogenesis *mab-5* expression was still observed (Cowing and Kenyon 1996). These results indicated that expression of *mab-5* during embryogenesis might be partially regulated by cell intrinsic factors independent of cell position rather than extracellular morphogens. A similar cell intrinsic mechanism might control the expression of *lin-39* in QR.d cells. However, for *mab-5* expression in the QL neuroblast this is not the case since this was shown to be regulated by the long-range action of EGL-20 (Maloof et al 1999). Further analysis is needed to determine whether *lin-39* is regulated via cell intrinsic or extrinsic mechanisms.

More recently it was demonstrated that two Hox cofactors also play a role in Q cell migration. Hox cofactors were originally identified as genes that, like *Hox* genes, caused homeotic transformations (Rauskolb et al 1993, Rauskolb and Wieschhaus 1994). Further analysis revealed that roughly two classes of Hox cofactors exist: the PBC class and the MEIS class of cofactors. The PBC class includes Extradenticle in flies, CEH-20 in *C.elegans* and Pbx1-4 in vertebrates. PBC-like cofactors bind to Hox proteins and thereby modulate the specificity and often the affinity of these molecules for certain DNA sequences. Proteins of the MEIS class of cofactors include Homothorax in flies, UNC-62 in *C.elegans* and Meis and Prep in vertebrates. This cofactor class regulates Hox activity of the DNA-bound Hox protein complex but can also affect Hox proteins indirectly by modulating the activity of PBC class proteins (Moens and Selleri 2006).

In *C.elegans*, mutations in the Hox cofactors *ceh-20*/*Extradenticle* and *unc-62*/*Homothorax* resulted in vulval abnormalities and QR.d cells often aborted their anterior migrations. Furthermore, the QL.ap cell was often positioned posterior to its wildtype position in the tail region. Expression analysis of a *ceh-20*::*gfp* translational fusion gene revealed that *ceh-20* is expressed in QR, QL and their descendents. Expression was also observed in P and V cells possibly reflecting the role of *ceh-20* in vulval development and V cell patterning respectively. Furthermore, in all ventral cord neurons, several anterior neurons, body wall muscle cells and in migratory cells like M, BDU, ALM and HSN *ceh-20::gfp* expression was observed (Yang et al 2005). Despite the finding that *ceh-20* is expressed in Q cells and their descendents, functional mosaic analysis is still required to determine whether *unc-62* and *ceh-20* function cell autonomously in the positioning of Q cell descendents.

Interestingly, in *ceh-20* mutants neuronal cells were found in the midbody on both the left and right lateral sides. Detailed analysis revealed that these cells result from inappropriate cell survival and neuronal differentiation. In wildtype animals Q.aa and Q.pp cells die by apoptosis (Figure 1). However, in *ceh-20* mutants, surviving Q.pp cells were observed in about 50% of all mutant animals. Furthermore, occasional ectopic cell division of both Q.pp and Q.ap was observed. These results indicated that, in addition to regulating proper cell migration, the Hox cofactor *ceh-20* functions in the coordination of Q cell divisions (Yang et al 2005).

Although more severe, the QR.d migration phenotype of *unc-62* and *ceh-20* mutants displays a striking similarity with *lin-39* loss of function, suggesting that *unc-62* and *ceh-20* might function as cofactors for the *Hox* gene *lin-39*. However, *unc-62* as well as *ceh-20* enhanced the QR.d migration phenotype of *lin-39* indicating that *unc-62* and *ceh-20* function, at least partially, independently of *lin-39*. Further analysis revealed that in *lin-39* mutants QR.d cells are still susceptible to regulation by MIG-13 while in *ceh-20* and *unc-62* mutants they are not (Sym et al 1999, Yang et al 2005). These results suggest that *ceh-20* and *unc-62* function is necessary for QR.d cells to respond to the action of *mig-13*.

mig-13 was identified as a regulator of anteriorly directed QR.d cell migration (Harris et al 1996, Sym et al 1999). In *mig-13* mutants QR.d cells are posteriorly displaced. Furthermore, *mig-13* can dramatically enhance posterior QR.d cell displacement of *egl-20* mutants. In *egl-20* single mutants QR.pax cell positions are shifted slightly posterior, while in *mig-13*; *egl-20* double mutants most QR.d cells are located posterior from their starting positions. In addition, *mig-13* was also shown to control migrations of other neuronal cells like BDU, ALM, CAN and HSN. These results indicated that *mig-13* is necessary for the

positioning of migrating cells along the anteroposterior axis rather than for migration per se. Mosaic expression analysis revealed that *mig-13* functions non-autonomously in the control of QR.d cell positioning. Except for the posterior neuron DA9, *mig-13* is predominantly expressed in the anterior half of the animal in pharyngeal-intestinal valve cells, and in neurons of the retrovesicular ganglion and the ventral cord. In posterior compartments *mig-13* expression is inhibited by the action of *mab-5*. Interestingly, ubiquitous overexpression of *mig-13* using a heat shock promoter revealed that *mig-13* promotes anterior migration in a dose dependent manner, with QR.pax being anteriorly displaced upon a prolonged heat shock treatment (Sym et al 1999). Since *mig-13* functions non-autonomously and the expression pattern overlaps with that of *ceh-20* in ventral cord neurons, *ceh-20* and *unc-62* might function in *mig-13* expressing cells downstream of *mig-13* and exert a non autonomous effect on QR.d migration. Conversely, *ceh-20* and *unc-62* might function autonomously in QR.d and respond to *mig-13* mediated cell non-autonomous signaling. Mosaic analysis will reveal in which cells *unc-62* and *ceh-20* exert their actions.

mig-13 encodes a single-pass transmembrane protein with no clear homologs. Expression analysis showed that indeed, *mig-13* is enriched on the cell periphery. The presence of an N-terminal signal sequence suggests that its N terminus points towards the extracellular milieu. The predicted extracellular region has two conserved CUB domains, predicted to be involved in protein-protein interactions, and a single LDL receptor repeat (Bork and Beckmann 1993, Sym et al 1999). Interestingly, the migration phenotype of *mig-13* shows similarities with *cwn-1* and *cwn-2* loss of function. Mutations in *mig-13* as well as *cwn-1* and *cwn-2* result in aborted anterior migrations of QR.d but also of BDU, which is another neuron that migrates anteriorly. Furthermore, *mig-13*, *cwn-1* and *cwn-2* all enhance the *egl-20* migration phenotype (Sym et al 1999, Zinovyeva et al 2008). Double or triple mutants for these genes might reveal whether *mig-13* functions in a pathway similar to *cwn-1* or *cwn-2*. Wnt molecules are highly hydrophobic but can form long-range gradients nevertheless (Coudreuse et al 2006, Neumann et al 1997). In *Drosophila*, Wnts were shown to colocalize and copurify with lipophorin suggesting that Wnt is transported in lipoprotein particles (Panakova et al 2005). Therefore, it would be interesting to determine which of the extracellular domains of *mig-13* are necessary for its function. The LDL receptor repeat in *mig-13* might function as a docking site for putative *cwn-1* or *cwn-2* containing lipoprotein particles. However, unambiguous evidence for the existence of these particles in *C.elegans* is still lacking. Many hypotheses can be made that place *mig-13* upstream or downstream in a non-canonical Wnt signaling pathway. However, first, genetic analysis must point out whether there is a functional link between *mig-13* and non-canonical Wnt signaling.

All together, it is now well established that *Hox* genes play a major role in the positioning of Q neuroblast daughter cells. *mab-5* functions as a pivotal switch in posterior QL.d migration whereas *lin-39*, together with other factors, regulates anteriorly directed migration of QR.d cells (Clark et al 1993, Salser and Kenyon 1992, Wang et al 1993). Neither *egl-5* nor *ceh-13*, *nop-1* or *php-3* were shown to participate in the control of Q cell migrations. A role for the latter three genes cannot be excluded. However, it is difficult to examine Q cell migrations in these mutants since they display lethal phenotypes in which the entire body plan is dramatically altered during embryogenesis (Brunschwig et al 1999, Van Auken et al 2000). The fact that hypomorphic alleles of these genes have not been found in genetic screens for Q cell migration defects argues against a role in the control of Q cell migration. Surprisingly, the Hox cofactors *ceh-20*/*Extradenticle* and *unc-*

62/*Homothorax* were shown to act at least partially independent of *lin-39* and *mab-5* and seem to act downstream of *mig-13* (Yang et al 2005). Future experiments will be needed to see whether Wnt homologs also act in this novel *mig-13* pathway.

2.4 Rho/Rac signaling required to execute Q cell migrations

Upon migration a cell dramatically changes its morphology, which is mainly facilitated by dynamic reorganizations of the actin cytoskeleton. Extensive research, primarily using in vitro experimental systems, provided a comprehensive understanding of the molecular mechanisms that control cytoskeletal dynamics during cell migration. First, a cell polarizes and sends a membrane protrusion towards the future direction of migration. Membrane protrusions were shown to depend on actin polymerization where nucleating actin filaments provide a mechanical force that pushes the cell membrane forward. Furthermore, for successful movements the formation of a contractile actomyosin-based ring is needed for the rear end to follow the protruding front (Welch and Mullins 2002). Proteins of the Rho (Ras homology) family of small GTPases were shown to be critical regulators of actin dynamics and thereby cell migration. When bound to GDP, Rho GTPases are conformationally inactive while upon GTP binding Rho GTPases become active and interact with downstream effector proteins. Rho GTPases are activated by guanine nucleotide exchange factors (GEFs) that promote the exchange of GTP for GDP. Inactivation occurs by GTPase activating proteins (GAPs) that activate the intrinsic GTPase activity of Rho family molecules. Alternatively, guanine nucleotide dissociation inhibitors (GDIs) may inhibit the dissociation of either GDP or GTP (Figure 3A, Etienne-Manneville and Hall 2002). Like Ras, Rho GTPases are prenylated and localize to the cell membrane, which is necessary for their function (Etienne-Manneville and Hall 2002, Zhang and Casey 1996). Three Rho GTPases were shown to be involved in the regulation of membrane protrusions: Rac, Cdc42 and

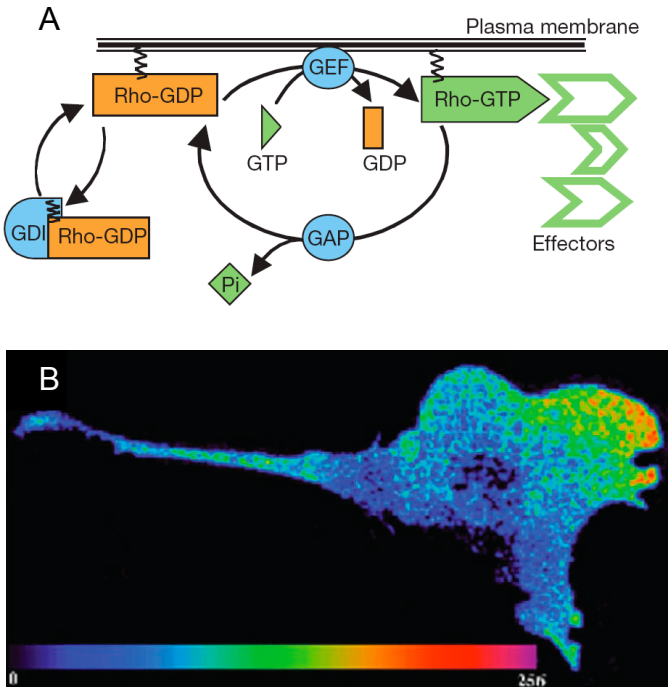

Figure 3. The Rho GTPase cycle. A Rho GTPases are anchored to the plasma membrane and are activated by GEF mediated GTP binding. Upon GTP binding Rho GTPases bind and activate effector proteins. Inactivation occurs via the action of GAPs that promote the intrinsic GTPase activity or by GDIs that sequester Rho GTPases. Figure adopted from Etienne-Manneville and Hall 2002. **B** Activity of Rho and Rac GTPases is polarized with GTP bound Rac GTPases acting in the front end of a migrating cell. Activated Rac in migrating 3T3 cells is visualized here by FRET analysis of Rac and a Rac effector. Red and blue indicate high and low intensities of FRET. Figure adopted from Ridley et al 2003.

RhoG. Both Rac and Cdc42 can lead to activation of WASP/WAVE family proteins that, in turn, stimulate the Arp2/3 complex to promote actin polymerization (Ridley et al 2003). In addition, RhoG is thought to facilitate actin polymerization indirectly by activating a Rac-GEF complex (Katoh and Negishi 2003).

In a migrating cell the activity of different Rho GTPases is polarized. Generally, Cdc42 and Rac act in the front end of a migrating cell where they mediate the formation of membrane protrusions (Figure 3B). Persistence of their activity ensures that migrating cells keep on sending protrusions towards the direction of migration. In the rear, Rho mediates the formation of an actomyosin-based contractile ring. Consistent with the model in which the formation of a contractile ring is necessary for the rear end to follow, inhibition of Rho function often leads to an extended tail. The regulation of polarized GTPase activity and its maintenance during cell movements involves a highly complex network of many regulators and feedback interactions. For example, Rho and Rac might function mutually antagonistic, inhibiting each other's activity (Evers et al 2000). Furthermore, the localization of Rac activity might be regulated by PI3K effector-mediated recruitment of specific Rac GEFs to the front end. PI3K becomes localized at the front end of a migrating cell in response to extracellular cues and downstream PI3K products were shown to bind and activate several potent Rac GEFs (Ridley et al 2003).

The *C.elegans* genome encodes three predicted *Rac*-like genes: *ced-10*, *rac-2* and *mig-2.* Both CED-10 and RAC-2 share 83% aminoacid sequence similarity with human Rac1, whereas *mig-2* encodes a more divergent homolog that shares about 65% of aminoacid sequence identity with Rac1 (Lundquist et al 2001, Zipkin et al 1997). Genetic analysis revealed that these three GTPases play a profound role in cell migration. A *mig-2* gain of function allele encoding constitutively active MIG-2 results in migration defects of nearly all the migrating cells including QR.d and QL.d. In these mutants Q.d cells terminate their movements prematurely without changing the direction of migration. Time-lapse analysis revealed that in *mig-2* mutants the onset and stop of Q.d cell migration as well as the timing of cell division is unaffected. However, the over all kinetics of migrating Q neuroblasts and their descendents are slower than in wildtype. *mig-2* loss of function results in similar but more subtle migration defects of Q.d cells and coelomocytes leaving other cell migrations largely unaffected (Zipkin et al 1997). These results indicate that (1) *mig-2* is required for the proper execution of Q.d migrations and (2) *mig-2* gain of function alleles act in a dominant negative fashion.

The *mig-2* gain of function alleles that were examined harbor mutations that are likely to result in constitutive GTP binding. Constitutively active Rho GTPases might sequester upstream GAP regulators leading to a dominant negative effect. In addition, the dominant negative effect of these mutations is in agreement with a model in which the activity of specific Rho GTPases needs to be constricted to one side of a migrating cell in order to execute migration properly. In vitro, mutations that result in constitutively active Rho GTPases often lead to migration phenotypes that resemble those of GTPase inactivating mutations (Ridley et al 2003, Yang et al 2006). Therefore, GDP/GTP cycling is thought to be as important for their function as the presence of active Rho GTPases per se. Examination of the intracellular distribution of a MIG-2::GFP fusion protein revealed that MIG-2 is highly enriched at the cell periphery, presumably in the plasma membrane via prenylation, but is not asymmetrically localized (Zhang and Casey 1996, Zipkin et al 1997). This indicates that MIG-2 activity rather than its intracellular localization is asymmetrically

distributed. Strong *mig-2* expression was observed in Q neuroblasts and their descendents, consistent with the idea that MIG-2 functions autonomously to regulate the migration of these cells (Zipkin et al 1997).

Although significant, the effect of *mig-2* mutations on Q cell migration is subtle. Similarly, *ced-10* mutants and *rac-2* RNAi displayed minor defects in Q.d cell migrations. However, in *ced-10* ; *mig-2* double mutants the effect of either single mutant was greatly enhanced. This enhancement was also observed when *ced-10* or *mig-2* loss of function mutations were combined with *rac-2* RNAi. The expression pattern of *ced-10* overlapped greatly with that of *mig-2* and is expressed broadly. Furthermore, like MIG-2, CED-10 localizes to the periphery but is not asymmetrically distributed (Lundquist et al 2001). Also, both *ced-10* loss and gain of function yielded similar defects in Q.d cell migration (Shakir et al 2006). These findings demonstrate that the three *C.elegans Rac1* homologs function partially redundant in the control of Q.d cell migration and furthermore these results strongly indicate that, like in other experimental systems, Rac GTPases function as core regulators of cell migration. However, even when *mig-2* and *ced-10* null alleles are combined migrating cells are still observed. This might be due to the presence of *rac-2* that functions partially redundant or to perdurance of maternal contribution. Alternatively, assuming that rearrangements of the actin cytoskeleton are a prerequisite for cell migration, other currently unidentified regulators of actin dynamics might still be active.

Interestingly, the guanine nucleotide exchange factor (GEF) UNC-73/Trio has also been implicated in the control of Q.d migration. *unc-73* mutants display cell migration phenotypes of many different cells. Similar to loss of function of the *Rac1* homologs, the final positions of Q.d cells are shifted towards their starting points while the direction of Q.d cell migration is not affected (Forrester and Garriga 1997, Hedgecock et al 1987, Honigberg and Kenyon 2000, Shakir et al 2006). *unc-73* is indeed expressed in the migrating Q neuroblasts and their descendents from the moment of initial neuroblast migration onwards. *unc-73* encodes a protein with two Dbl homology domains (DH-1 and DH-2) that are separated by a pleckstrin homology domain (PH-1), an SH3 domain and a putative PEST sequence. Furthermore, another PH domain (PH-2), an Ig-like domain, and a fibronectin typeIII domain are located downstream of the DH-2 domain. However, an alternative splicing transcript, *unc-73B*, encoding only the DH-1, PH-1 and SH3 domains has rescuing activity, indicating that these domains are sufficient for UNC-73 function (Steven et al 1998). Dbl homology domains are known to possess GEF activity (Hall 1998). In vitro analysis demonstrated that recombinant UNC-73 proteins containing the DH-1 and PH-1 domains, either with or without the downstream SH3 domain, have GEF activity for MIG-2 and CED-10 but not for RHO-1 and CDC-42 (Kubiseski et al 2003, Wu et al 2002). Similarly, the UNC-73 C-terminal region comprising the DH-1, PH-1 and SH3 domains can promote GDP/GTP exchange on human Rac1 but not on human Cdc42 or Rho, indicating that *unc-73* encodes a well conserved GEF that activates Rac GTPases specifically (Steven et al 1998).

These results have led to a model in which UNC-73 acts as a GEF for CED-10, MIG-2 and RAC-2. For Q.d cell migration the hierarchy of these genes has not been determined. However, in varying contexts *unc-73* loss of function causes defects similar to loss of function of the *Rac*-like genes (Kishore and Sundaram 2002, Lundquist et al 2001, Wu et al 2002). Together with the in vitro GDP/GTP exchange assays this suggests that *unc-73* functions upstream of *mig-2*, *ced-10* and *rac-2*. Unfortunately, epistasis analysis using

alleles of *unc-73* and the three *Rac1* homologs is difficult for several reasons: *rac-2* functions redundant but *rac-2* alleles have not been well characterized, gain of function alleles of *mig-2* and *ced-10* have dominant negative effects and, moreover, display defects similar to loss of function alleles. When the genetic and biochemical data are combined, it is likely that *unc-73* functions upstream but solid conclusions about the genetic wiring of these genes in Q.d cell migrations cannot be drawn. In addition, the Q.d migration phenotype of an *unc-73* null allele is not as severe as that of *ced-10 ; mig-2* double loss of function mutants suggesting that other Rac GEFs might also impinge on *C.elegans* Rac GTPases (Shakir et al 2006).

Conversely, despite the fact that *unc-73B* can rescue *unc-73* loss of function phenotypes, the second Dml homology domain together with PH-2 has in vitro GEF activity for mammalian RhoA but not for Rac1. RhoA has a high degree of homology with *C.elegans* RHO-1 and, in addition, both *rho-1* and *unc-73* mutations lead to similar defects in P cell migrations (Spencer et al 2001). These results possibly suggest that *unc-73* might also act as a GEF for RHO-1 during P cell migration via its DH-2 domain. Therefore, it would be interesting to uncouple the roles of DH-1 and DH-2 in different cellular and developmental contexts. By analyzing the exact rescuing capability of transgenic *unc-73* constructs comprising these domains separately, insights might be obtained about the activity of both Dml homology domains in vivo. Up till now, *rho-1* has not been implicated in the control of Q neuroblast migration. Analogous to other experimental systems *rho-1* might exert its activity in the rear end of migrating cells, including Q neuroblasts and their descendents. *rho-1* mutants have severe defects in embryogenesis leading to embryonic lethality. However, in order to determine its role in P cell migration Spencer et al used a tissue specific promoter to drive expression of dominant negative *rho-1* in P cells (Spencer et al 2001). Using this approach, a role for *rho-1* in Q cell migration might be established by using a Q cell specific promoter. A pitfall of this technique can be that the effect that will be observed is ectopic and does not reflect a role for endogenous *rho-1*.

Another gene shown to regulate Q.d cell migration is *unc-34/Enabled*. *unc-34* encodes the *C.elegans* homolog of Enabled/VASP family of actin binding proteins (Withee et al 2004, Yu et al 2002). In many migrating cells enabled/VASP proteins are highly enriched at filopodial tips and antagonize actin capping, a process that terminates actin filament nucleation (Welch and Mullins 2002). UNC-34 contains an EVH1 domain that recognizes proline motifs on target proteins, a proline-rich domain known to bind SH3 domains, and an EVH2 domain thought to be necessary for tetramerization and proper actin binding (Bachmann et al 1999, Withee et al 2004, Zimmermann et al 2002). Truncation of a large part of the EVH2 domain yields a hypomorphic allele indicating that UNC-34 tetramerizes and is involved in the regulation of actin dynamics. *unc-34* loss of function results in subtle migration defects of Q.d cells in which Q daughter cells were found along their normal route of migration. Again the direction of Q descendent migration was unaffected in *unc-34* mutants. Furthermore, *unc-34* loss of function enhances the Q.d cell migration phenotype of *rac-2* RNAi and both *ced-10* and *mig-2* loss of function mutations indicating that these genes both function in Q.d migration (Shakir et al 2006). Although *unc-34* enhances the Q.d migration phenotype of the *Rac1*-like genes, due to redundancy between the *Rac1*-like genes and possibly maternal product contribution it can not be concluded whether *unc-34* functions in a similar or parallel pathway.

As stated above, Rac and Cdc42 can trigger actin polymerization and nucleation via activation of proteins of the WASP/WAVE family (Ridley et al 2003). In *C.elegans*, *wsp-1* encodes an N-WASP homolog that, like UNC-34, contains an EVH1 domain. Interestingly, *wsp-1* synergizes with *unc-34* in embryonic morphogenesis. *wsp-1* single mutants display embryonic lethality due to hypodermal migration and hypodermal enclosure defects. While *unc-34* single mutants do not display an embryonic phenotype, *unc-34* loss of function greatly enhances the gastrulation phenotype of *wsp-1* mutants. Conversely, unlike *unc-34*, neither *wsp-1* mutants nor *wsp-1* RNAi displayed neuronal migration phenotypes. However, *wsp-1* RNAi on *unc-34* partial loss of function mutants significantly enhanced the *unc-34* migration phenotype of the CAN neuron. In addition, RNAi of the *C.elegans* WAVE homolog *wve-1* was shown to be synthetic lethal with both *unc-34* and *wsp-1*. Further analysis of these animals revealed that the hypodermal cells failed to migrate and enclose properly, a defect similar to that observed in *unc-34 ; wsp-1* double mutants. With respect to neuronal migration, *wve-1* RNAi did not enhance the *unc-34* phenotype but surprisingly, *wve-1* RNAi on wildtype animals resulted in a slightly more severe neuronal migration phenotype (Withee et al 2004). Although the effects of *wve-1* and *wsp-1* loss of function on migration of Q neuroblast daughter cells were not determined, it is likely that *wsp-1*, *wve-1* and *unc-34* function partially redundant in core cell migration regulatory pathways in a wide variety of migrating cells, possibly including Q.d cells.

Proteins of both the WASP/WAVE and Enabled/VASP family have been implemented in Rac GTPase mediated actin reorganization and cell migration (Han et al 2008, Shakir et al 2008, Welch and Mullins 2002). Therefore, it would be interesting to determine the hierarchy of these genes in the control of *C.elegans* cell migration and Q cell migration specifically. In order to dissect the genetic and molecular circuitry that regulates actin dynamics during Q cell migration, lessons might be learnt from studies on axon guidance and growth cone migrations. Recently, Shakir et al showed that *wve-1* and *wsp-1* act in parallel to control PDE axon guidance. Genetic analysis suggests that *wve-1* and *ced-10* act in the same pathway as do *wsp-1* and *mig-2*. These two pathways seem to function in parallel to each other and to *unc-34* in the regulation of PDE axon guidance (Shakir et al 2008). Similar mechanisms might be active to control Q.d cell migration. However, based on the problems described above, solid epistatic analysis is difficult to perform in this system.

To conclude, migration of Q descendents clearly depends on genes whose function in the control of actin dynamics and cell migration has been well characterized in other experimental systems. It is tempting to assemble a model in which UNC-73 functions as a GEF for the Rac-like GTPases that, in turn, mediate actin nucleation and membrane protrusion via the WASP/WAVE like proteins WSP-1 and WVE-1 and the Enabled homolog UNC-34. However, further analysis at the genetic as well as the molecular level is needed to verify or alter this model. However, since all migrating cells encounter the same fundamental challenges upon moving from A to B, it would not be surprising if a core molecular mechanism similar to that found in other systems is active in the regulation of movement of Q neuroblasts and their descendents.

One of the main open questions remaining is: What determines the direction of migrating Q neuroblasts and their descendents? Mutants described in this section all display Q.d migration defects but none of them seems to alter the direction of cell migration. This indicates that these genes are part of the core regulatory pathway that is needed for cell

movements per se. However, molecular signaling provided by canonical and non-canonical Wnt signaling must ultimately impinge on this pathway to instruct cells to move towards a certain direction (see above). One of the key factors in providing directionality to a cell that becomes instructed to migrate towards a certain direction is Cdc42. For example, when Cdc42 is inhibited a macrophage converts its cell movement from a directed migration to a random crawl (Etienne-Manneville and Hall 2002).

A role for *C.elegans cdc-42* in cell polarity has been well established. Upon fertilization the *C.elegans* zygote becomes polarized with an anterior protein complex composed of PAR-3, PAR-6 and PKC-3 and a posterior complex containing PAR-1 and PAR-2. These protein complexes specify anterior and posterior compartments ultimately resulting in an asymmetric cell division. Localization of the two PAR complexes is regulated by actomyosin contractility. Although the exact mechanism is still unclear *cdc-42* plays a major role in establishing anteroposterior polarity in the early embryo (Cowan and Hyman 2007). Interestingly, Welchman et al recently demonstrated a role for *cdc-42* and *par-3* in Q.d cell migration. Upon *cdc-42* or *par-3* RNAi treatment the QR descendent AVM was found to be posteriorly displaced in 21% and 5% respectively (Welchman et al 2007). However, due to embryonic lethality only partial knockdown by RNAi resulted in these phenotypes. Therefore, it cannot be excluded that low levels of these genes still regulate polarity and that *cdc-42* and *par-3* function in the control of directional migration of Q.d cells. Tissue specific RNAi or expression of dominant negative variants might reveal a role for these genes in the establishment of polarity and directional migration of Q.d cells.

As described in the first sections, both canonical and non-canonical Wnt signaling pathways as well as *Hox* gene activities ensure that Q.d cells migrate in the proper direction. However, there is a major gap of knowledge between these signaling pathways and the cell migration effectors that must provide polarity cues and act on the core cell migration machinery. Whangbo and Kenyon demonstrated that high levels of EGL-20 can promote posterior migration of both QL.d and QR.d cells (Whangbo and Kenyon 1999). Yet, endogenous levels of EGL-20 only activate *mab-5* in QL resulting in posterior migration of its daughter cells. These findings show that both QL.d and QR.d are susceptible to *egl-20/Wnt* signaling but cells of the QL lineage are more sensitive to EGL-20 signaling. This asymmetry determines the end positions of Q descendents and is therefore crucial to ensure the proper migration of Q descendents. Already during the start of Q neuroblast migration there is asymmetry between QL and QR since QL migrates posteriorly while QR migrates anteriorly. Therefore, dissecting the genetic wiring that regulates this initial migration might provide molecular insights into the control of differential QR.d and QL.d migration. In the next section factors involved in the regulation of this initial migration are discussed.

3. Initial polarization and migration of Q neuroblasts

As described in the previous sections, many factors affect the migration of cells in the Q lineage. In most studies the final positions of Q cell descendents were used as a read-out to monitor Q migration defects. However, initial polarization and short-range migration of the Q neuroblasts precedes the long-range migration of Q descendents and these processes can be considered as separate events. At hatching both Q neuroblasts are similar in morphology and are located in the lateral row of hypodermal seam cells. Shortly after hatching the Q neuroblasts polarize and send a membrane protrusion towards the anterior (QR) or posterior (QL). Two hours after hatching migration starts and after 3.5 hours QR and QL cells occupy positions dorsal to V4 and V5 respectively. Once the initial migration is finished the Q neuroblast divides giving rise to Q.a and Q.p that continue migration along the anteroposterior axis (Figure 4, Honigberg and Kenyon 2000, Sulston and Horvitz 1977). Several genes have been implicated in the control of Q neuroblast polarization and short-range migration. Moreover, studies that examined the first steps of Q cell migration provide evidence that Q neuroblast migration is intimately linked with the subsequent long-range migration of Q descendents.

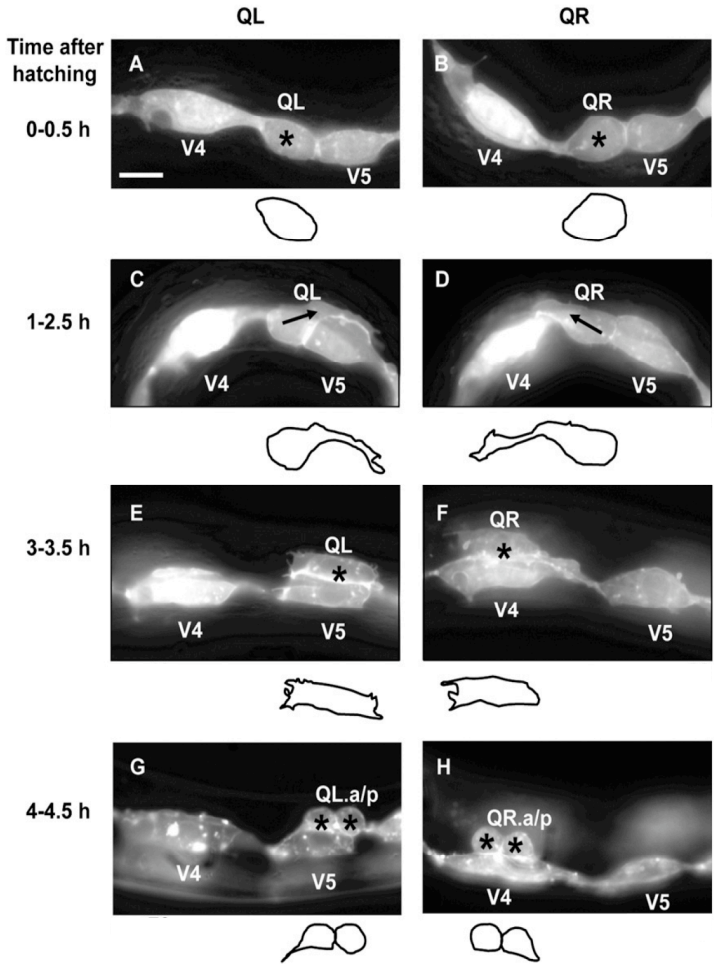

Figure 4. Polarization and migration of Q neuroblasts. Initial polarization and migration of QL (**A**, **C**, **E**, **G**) and QR (**B**, **D**, **F**, **H**) followed in time. Time frames indicate the time after hatching. The left side is anterior and the upper side is dorsal. Cells are visualized by a membrane anchored GFP marker expressed specifically in Q neuroblasts and the lateral row of seam cells. The neighboring seam cells V4 and V5 are indicated. The neuroblast cell outline is depicted below each panel. An asterix marks Q neuroblasts **A-B**, **E-F** and their descendents Q.a and Q.p **G-H**. Q neuroblasts are unpolarized upon hatching **A-B**, polarize after 1-2.5 hours and send a posterior (QL) or anterior (QR) protrusion **C-D**. After 3-3.5 hours Q cells migrate towards positions dorsal to their neighboring seam cells **E-F** where they divide after 4-4.5 hours **G-H**. Figure derived from: Chapman et al 2008

3.1 Factors controlling initial polarization and migration

Unlike the asymmetry in QL.d and QR.d migrations, initial Q cell polarization and migration does not depend on the canonical *egl-20/Wnt* pathway or *mab-5* expression since loss of function mutations in *egl-20, lin-17, bar-1* or *mab-5* do not affect this process (Chapman et al 2008, Harris et al 1996, Salser and Kenyon 1991). In *mab-5* loss of function mutants the initial posterior migration of QL is normal but Q descendents change direction afterwards (Chalfie et al 1983). Almost a decade ago Honigberg and Kenyon described three mutants that display aberrant Q neuroblast polarization and migration phenotypes, namely *unc-40*, *dpy-19* and *unc-73* (Honigberg and Kenyon 2000). Out of these, *unc-40* and *unc-73* (see above) were previously shown to display migration phenotypes in other cells. *unc-40* had already been demonstrated to be involved in guiding dorsoventral cell and axon migrations where it acts as a receptor for the well conserved UNC-6/Netrin guidance cue. *unc-6/Netrin* is expressed in the ventral midline and attracts cells and migrating axons via UNC-40 or serves as a repellent via the coordinate action of UNC-40 and UNC-5 (Chan et al 1996, Hamelin et al 1993, Wadsworth et al 1996).

unc-40 also controls the direction of Q neuroblast polarization and migration. In *unc-40* mutants the initial polarization is randomized with some Q cells that do not polarize at all while others were observed that even polarize in the wrong direction. Time-lapse analysis revealed that Q cells in *unc-40* mutants are able to polarize in multiple directions in time indicating that this process is variable in *unc-40* mutants (Honigberg and Kenyon 2000). *unc-40* has been shown to act autonomously in axon outgrowth (Chan et al 1996). Analysis of an UNC-40::GFP reporter gene revealed that *unc-40* is indeed strongly expressed in Q neuroblasts suggesting that it might also function cell autonomously in the regulation of Q neuroblast migration. Interestingly, unlike its well-characterized role in dorsoventral axon guidance, the action of *unc-40* does not depend on *unc-6/Netrin* or the putative coreceptor encoded by *unc-5* (Honigberg and Kenyon 2000). Therefore, since *unc-40* encodes a transmembrane protein it might act as a receptor of a currently unidentified ligand in the control of Q neuroblast migration.

Furthermore, loss of function of *dpy-19* was shown to display a similar Q neuroblast polarization phenotype as *unc-40* mutants. *dpy-19* encodes a predicted transmembrane protein with 13 hydrophobic regions and shows homology with a human cDNA. Intracellular analysis of a GFP fusion protein revealed that it is located to the cell periphery presumably in the plasma membrane. While *unc-40* mutants have a variety of defects in other cell and axon migrations, *dpy-19* only shows a Q neuroblast migration phenotype leaving other cell or axon migrations unaffected. Like in *unc-40* mutants, the direction of initial Q neuroblast polarization is randomized and is variable in time. *dpy-19* is expressed faintly in Q neuroblasts but more strongly in the surrounding hypodermal cells. Therefore, mosaic analysis is still required to point out whether *dpy-19* functions cell autonomously in Q cells (Honigberg and Kenyon 2000). In addition to its defects in long-range migration and axon outgrowth, *unc-73* also affects initial polarization and migration of Q neuroblasts. However the phenotype displayed by *unc-73* loss of function was qualitatively different from those observed in *dpy-19* and *unc-40* mutants. Mutations in *unc-73* affected the ability of Q neuroblasts to polarize and migrate but migration in the wrong direction was not observed. Rather, Q cells were often found unpolarized and short-range migration was blocked (Honigberg and Kenyon 2000).

More recently, Chapman et al described the Q cell polarization and migration defects displayed by *mig-15* mutants. In *mig-15* loss of function mutants the Q cell polarization and migration phenotype strongly resembled that observed in *unc-40* and *dpy-19* mutants since both polarization and migration were often reversed (Chapman et al 2008). In addition, other migratory processes like axon polarity were also found to be compromised in *mig-15* mutants (Poinat et al 2002, Shakir et al 2006). *mig-15* encodes a homolog of the vertebrate Nck-interacting kinase (NIK) and *Drosophila* Misshapen and both were implicated in the control of cell migration and act with the JNK/MAPK pathway (Poinat et al 2002, Xue et al 2001, Su et al 1998). Furthermore, tissue culture experiments have shown that NIK-mediated ERM phosphorylation is responsible for lamellipodium formation via actin reorganization (Baumgartner et al 2006). MIG-15 contains a STE20-like serine/threonine protein kinase domain, a proline-rich domain and a Citron-NIK homology (CNH) domain. Interestingly, Citron proteins interact with Rho GTPases and are thought to function as Rho effectors during cytokinesis (Madaule et al 2000). Two *mig-15* loss of function alleles were examined: one weak allele having a missense mutation in the ATP binding pocket of the STE20-like kinase domain, and the other allele is a stronger loss of function allele and has a premature stop after the kinase domain. Both alleles display migration phenotypes of Q neuroblasts and their descendents (see below, Chapman et al 2008, Shakir et al 2006). These results indicate that the kinase domain, as well as sequences downstream of this domain, are necessary for MIG-15 to exert its function.

Examination of the Q descendent cell migrations in *unc-40*, *mig-15* and *dpy-19* mutants revealed that the direction of Q.d migration was affected similar to their ancestral neuroblasts. In these mutants Q.d cells were found along the anteroposterior axis having migrated in a random direction where the most severe defects were found in QL.d cells that normally migrate posteriorly. In *unc-40* and *dpy-19* mutants Q.d cells still had the ability to migrate long distances. Furthermore, in *mab-5 dpy-19* and *unc-40 ; mab-5* double mutants most Q.d cells migrate long distances (see below). Therefore, it is unlikely that these mutants affect the migratory potential of Q.d cells. However, as described in the previous sections, in *unc-73* mutants the direction of Q descendent migration is unaffected but Q.d cells were found at locations along their normal route of migration (Forrester and Garriga 1997, Hedgecock et al 1987, Honigberg and Kenyon 2000, Shakir et al 2006,). These results indicate that, unlike *unc-40* and *dpy-19*, *unc-73* affects both the Q neuroblast polarization process as well as the migratory potential of Q descendents. Since UNC-73 has in vitro GEF activity for both Rho and Rac-like GTPases it is tempting to speculate that *C.elegans* Rho and Rac GTPases control initial Q neuroblast polarization as effectors of UNC-73 (Kubiseski et al 2003, Spencer et al 2001, Steven et al 1998, Wu et al 2002). Interestingly, *mig-2* gain or loss of function was shown to result in defects in the initial Q neuroblast migration. In *mig-2* gain of function mutants about 17% of QR cells migrated posteriorly as opposed to 4% of QL cells that migrated anteriorly. In *mig-2* loss of function these numbers shifted to 7% and 1% respectively. (Levy-Strumpf and Culotti 2007). These results suggest that *mig-2* might be involved in providing polarization cues possibly together or in parallel with *unc-73*. Surprisingly, these neuroblast migration defects in *mig-2* mutants were not reflected in the migration of Q descendents since the direction of Q.d migration is unaffected in *mig-2* mutants (Zipkin et al 1997).

In *mig-15* mutants the direction of Q.d migration is often reversed, with the Q descendents AQR and PQR migrating in random directions. Again, this is equivalent to the

short-range migration defects of Q neuroblasts in these mutants. Mosaic analysis revealed that *mig-15* acts autonomously in AQR and PQR. However, it would be interesting to determine whether the directionality of migration depends solely on the action of *mig-15* in the Q neuroblast or whether sustained *mig-15* activity is also required in Q daughter cells (Chapman et al 2008). Mosaic analysis can assess this by analyzing animals in which the rescuing array is present in Q but is lost in Q.a or in Q.ap. This, however, is difficult due to the low frequency by which transgenic arrays are lost.

In addition, the ability of Q.d cells to exert migration seems to be affected in *mig-15* mutants since Q.d cells were found to move shorter distances than in wildtype (Chapman et al 2008). This potentially reflects defects in the core migration machinery. Interestingly, complete *mig-2* loss of function greatly enhanced the AQR/PQR direction of migration phenotype found in weak alleles of *mig-15*. The phenotype of these *mig-15 mig-2* double mutants resembles that of stronger *mig-15* alleles possibly suggesting that *mig-2* and *mig-15* function in the same pathway to control migration of cells in the Q lineage (Shakir et al 2006). As stated above, in *mig-2* single mutants the direction of Q.d cell migration is not affected while the kinetics of cell migration are much slower than in wildtype (Zipkin et al 1997). However, despite the fact that these data do not correspond to the Q.d defects seen in *mig-2* mutants, defects in the initial migration of Q cells in *mig-2* mutants were reported (Levy-Strumpf and Culotti 2007). Therefore, a *mig-2/mig-15* pathway might provide directionality to the polarization and migration events whereas a *mig-2/unc-73* pathway is required for polarization and execution of cell movements per se. The finding that UNC-73 has in vitro GEF activity for MIG-2 and that MIG-15 contains a CNH domain that putatively interacts with Rho/Rac GTPases might link these proteins together in a regulatory network that controls Q cell migrations.

Other studies pointed out a role for *mig-2* together with another gene, *vab-8*, in providing directionality to the Q.d cell migration system. *vab-8* mutants display defects in axon guidance and, moreover, posteriorly directed cell migrations were found to be affected. ALM, CAN, the coelomocytes ccmR and ccmL and the QL descendent PQR were all found anterior to their normal positions upon *vab-8* loss of function (Wightman et al 1996, Wolf et al 1998). Furthermore, in *vab-8* mutants AQR occasionally overshoots its normal migration end point, occupying a position anterior to that in wildtype (Wightman et al 1996). *vab-8* encodes a protein that has an N-terminal kinesin-like motor domain. This domain is necessary for axon guidance and growth cone migrations but was shown to be dispensable for migration of the CAN neuron, and possibly other posteriorly directed migrations. Although *vab-8* seems to be acting cell autonomously in other contexts, this was not determined for migrations of cells in the Q lineage (Wolf et al 1998).

The direction of migrating Q.d cells is not affected in *vab-8* single mutants. In contrast, the Q neuroblast migration defect of *mig-2* gain of function mutants, in which QR migrates posteriorly in 17% of cases, is shifted in *vab-8 mig-2* double mutants resulting in a Q neuroblast migration phenotype that resembles that of *unc-40* mutants (Levy-Strumpf and Culotti 2007). It would be interesting to determine whether the Q.d migrations in *vab-8 mig-2* double mutants are also affected in a manner similar to those in *unc-40* single mutants. Furthermore, a detailed analysis of the initial Q cell migration in *mig-2* and *vab-8* mutants might provide insights into their function in the control of Q neuroblast migration.

Interestingly, yeast two-hybrid analysis revealed that UNC-73B, the shorter splicing variant of UNC-73 with rescuing activity, interacts with VAB-8 and also with the

cytoplasmic domain of UNC-40. Further analysis revealed that the region on UNC-73 necessary for this interaction mapped to the spectrin-like repeats located upstream of the C terminal Dbl homology domain (Watari-Goshima et al 2007). Although these in vitro data might not be representative for the in vivo situation, they do provide potential molecular links in the regulatory network that controls Q cell migration. Consistent with the yeast two-hybrid data are results obtained in studies on axon guidance and growth cone migration. Two studies, published at the same time, revealed a hierarchical regulatory network composed of *vab-8*, *unc-73*, *mig-2* and *unc-40* that controls proper axon guidance and growth cone migration. It was suggested that *vab-8* functions through *unc-73* and *mig-2* to control axon polarity and growth cone migrations via the action of *unc-40* (Levy-Strumpf and Culotti 2007, Watari-Goshima et al 2007). Although it is difficult to draw linear parallels between different processes like cell migration and axon guidance, the fact that these processes share key regulators might suggest that a similar or comparable pathway controls Q neuroblast migrations.

Finally, it was demonstrated that the Fat-like cadherin CDH-4 also controls the direction of Q daughter cell migration (Schmitz et al 2008). Fat-like cadherns are characterized by a large number of extracellular cadherin repeats often accompanied by other sequences motifs like EGF repeats (Tanoue and Takeichi 2005). In *Drosophila* Fat-like cadherins are involved in a variety of processes including cell proliferation and planar cell polarity whereas in mice *mFAT1* mutations were shown to result in a range of defects that are likely due to the loss of glomerular slit junctions (Bryant et al 1988, Ciani et al 2003, Matakatsu and Blair 2004). *cdh-4* in *C.elegans* encodes a Fat-like cadherin with 32 N-terminal cadherin domains and four EGF repeats that are separated by a single laminin G domain close to the membrane (Schmitz et al 2008).

cdh-4 loss of function mutations result in a variety of defects including compromised axon guidance, aberrant hypodermal and pharyngeal development and Q.d cell migration defects. In these mutants QR.d cells often migrated posteriorly. Similarly, QL.d cells were also found to migrate in the wrong direction. Analysis of a *cdh-4::gfp* reporter gene revealed that *cdh-4* is expressed in most affected tissues but expression was not observed in Q neuroblasts (Schmitz et al 2008). This might indicate that expression is below the detection limit or, alternatively, that *cdh-4* is not required in cells of the Q lineage. Although not examined, given the fact that the Q.d migration phenotype of *cdh-4* strongly resembled that of *mig-15*, *unc-40* and *dpy-19* mutants it is likely that initial Q neuroblast polarization and migration was also affected in *cdh-4* mutants.

The mechanism by which *cdh-4* regulates Q.d cell migration is unknown. Since CDH-4 contains 32 cadherin repeats it might be involved in cellular adhesion. However it is difficult to envision how loss of cellular adhesion results in altered direction of Q.d cell migration. Another possibility is that *cdh-4* is involved in the control of cellular polarity like *unc-40*, *dpy-19* and *mig-15*. In *Drosophila* Fat-like cadherins were shown to regulate planar cell polarity and act upstream of the Wnt receptor Frizzled (Strutt and Strutt 2005). In Q.d migration *cdh-4* seems to act upstream of the canonical *egl-20/Wnt* signaling pathway in QL (see below). Therefore a mechanism similar to the control of planar cell polarity in *Drosophila* might regulate Q.d cell migration.

3.2 mab-5 functions downstream of genes involved in Q neuroblast polarization

unc-40, *dpy-19* and *mig-15* mutants were all shown to affect the direction of migration of Q daughter cells in a manner that correlates with the initial polarization and migration of the Q neuroblasts. Since *mab-5* was shown to act as a critical switch in the determination of Q.d migration the hypothesis emerged that polarization of Q cells towards the posterior results in *mab-5* expression. Indeed, in *unc-40* and *dpy-19* mutants the degree of posterior Q cell polarization correlated with the intensity of *mab-5* expression which, in turn, correlated with the percentage of Q.d cells migrating posteriorly. Detailed analysis demonstrated that the overall time a Q neuroblast spends being polarized towards the posterior determines whether it will express *mab-5* or not. As expected, in *unc-73* mutants the pattern of *mab-5* expression was only slightly altered (Honigberg and Kenyon 2000). In addition, *mab-5* loss of function combined with mutations in *mig-15*, *unc-40*, *dpy-19* or *cdh-4* all phenocopied *mab-5* single mutants with respect to Q.d cell migration (Chapman et al 2008, Honigberg and Kenyon 2000, Schmitz et al 2008). In the wildtype situation canonical *egl-20/Wnt* signaling results in *mab-5* expression in QL and subsequent posterior migration of its descendents. When *unc-40*, *dpy-19* or *unc-73* mutations were combined with *egl-20* loss of function no *mab-5* expression was observed in any of the Q cells (Honigberg and Kenyon 2000).

Together these results indicate that factors controlling the *egl-20* independent polarization and migration of Q neuroblasts sensitize QL but not QR for the *egl-20/Wnt* signal. These findings favor a model in which posterior polarization results in *mab-5* expression via *egl-20* mediated Wnt signaling whereas anterior polarization results in the inability to activate canonical Wnt signaling. *egl-20* is expressed in the posterior and forms an anteroposterior gradient. This led to the hypothesis that posterior polarization of Q neuroblasts results in higher EGL-20 levels in their local environment. However, the finding that pharyngeal expression of *egl-20* can partially rescue the *egl-20* mutant phenotype argues against this hypothesis (Whangbo and Kenyon 1999).

3.3 Models for the control of left/right asymmetry in Q neuroblasts

Over the last couple of years many insights have been obtained into the control of Q neuroblast migration. Analysis of the initial polarization and migration of Q neuroblasts showed that they polarize differently despite their analogous position along the anteroposterior axis. Several studies reported a hand full of genes involved in this initial polarization and migration process. However, how these genes act together is still unknown. Future genetic analysis in which multiple mutants are examined together might provide insights into the signaling pathways that regulate Q neuroblast polarization. However, as for now the underlying molecular control mechanism remains elusive.

As described in the first sections mutations in *C.elegans* Wnts and Frizzleds result in defective Q.d cell migrations. Interestingly, if multiple *Wnts* or *Frizzleds* are mutated simultaneously the direction of Q.d cell migration is randomized similar as in mutants that affect the initial polarization process. This was observed for both QR.d and QL.d in *cwn-1*; *egl-20 cwn-2* triple mutants where the randomization was subtle. Additional mutations in *lin-44* and *mom-2* further enhance this randomization process. Comparable results were obtained in *Frizzled* quadruple mutants (Zinovyeva et al 2008). These results strongly suggest that the initial Q cell polarization depends on Wnt signaling. However this has not been demonstrated. Since *mig-14* is involved in the secretion of Wnt molecules examination

of the Q cell polarization in *mig-14* loss of function mutants might reveal whether this hypothesis is true or false (Banziger et al 2006, Bartscherer et al 2006, Harris et al 1996, Yang et al 2008).

Even if Wnt signaling is important in the control of Q cell polarity the underlying mechanisms still remain open to speculation. Several scenarios might be true. For example, extracellular signaling molecules might have left/right asymmetry causing Q cells to polarize accordingly. Furthermore, Q neuroblasts might respond differently to extracellular molecules, similar to the difference in *egl-20* responsiveness after the initial migration process. In this model, extracellular Wnt proteins might provide this signaling. However in this model, the left/right asymmetry must already be present at hatching. Although QR and QL occupy equivalent positions in the lateral row of seam cells along the anteroposterior axis, a molecular difference at hatching might not be surprising since both neuroblasts have different ancestry (Sulston et al 1983, Sulston and Horvitz 1977). In this model a difference already present at hatching mediates differential sensitivity of Q neuroblasts to extracellular cues. Subsequently, during the first hours of larval development this molecular asymmetry translates to differential polarization mediated by factors like *dpy-19*, *unc-40*, *mig-15* and *cdh-4*. Alternatively, it cannot be excluded that one or more of these factors actually act earlier to provide asymmetry cues. Further genetic and molecular analysis is needed to discriminate between various models.

4. Conclusion and future perspectives

The developmental control of migration of Q neuroblasts and their descendents has been extensively studied. Many different genes have been shown to affect this process. It is now evident that a canonical Wnt/ß-catenin signaling pathway specifies the fate of QL and controls the posterior migration of its descendents. Epistatic analysis assembled a pathway in which Wnt signaling components are placed in a hierarchical order similar to that in other experimental systems (Eisenmann 1998, Gleason et al 2002, Herman 2001, Korswagen et al 2000 and 2002, Maloof et al. 1999, Oosterveen et al 2007, Walston et al 2006). This Wnt signaling pathway ultimately triggers posterior migration via the expression of an *Antennapedia*-like *Hox* gene, *mab-5* (Maloof et al 1999, Salser and Kenyon 1992). Less is known about the role of Wnt signaling in the control of anteriorly directed QR.d migrations. Wnt and Frizzled homologs have been implicated in this process in a highly redundant manner but the downstream genetic wiring remains elusive (Zinovyeva et al 2008). Furthermore, although many genes have been shown to be involved, at the molecular level many open questions still remain. For example, what is the mechanism by which *mab-5* expression leads to posterior migration? Somehow, the action of *mab-5* provides directionality to QL.d cells. Directional cell migration requires the polarized activity of Rho/Rac GTPases and, therefore, *mab-5* effectors must ultimately impinge on this polarization mechanism.

In addition, the left-right asymmetry of Q neuroblasts remains an intriguing aspect of this cell migration system. Studies described in the previous section showed that the initial direction of Q neuroblast polarization is intimately linked with *mab-5* expression and the subsequent direction in which Q descendents migrate. Several mutants have been identified that randomize the initial polarization and these mutants also show random *mab-5* expression in Q cells accompanied by randomization of the Q.d migration direction (Chapman et al 2008, Honigberg and Kenyon 2000). The current model states that the initial polarization event sensitizes QL for *egl-20* mediated *mab-5* expression by means of posterior polarization. In this model, initial Q cell polarization is the first event that occurs asymmetrically. However, left-right asymmetry in *C.elegans* is already present at the six cell stage and, furthermore, QL and QR neuroblasts are derived from a different lineage (Hutter and Schnabel 1995, Sulston et al 1983, Wood 1991). Therefore, Q neuroblasts might already be programmed asymmetrically prior to hatching. In this scenario, a single symmetric signaling mechanism can instruct QR to polarize anteriorly and QL posteriorly. In this model two separate mechanisms control the direction of Q cell migration. The first mechanism polarizes the Q cells according to their lineally programmed susceptibility, which is then followed by *egl-20/Wnt* signaling to activate *mab-5* expression. Alternatively, Q cells might be equally sensitive to the factors that control initial polarization but this polarization system displays left-right asymmetry. In this model Q cells are not lineally asymmetric but are instructed via a mechanism that acts right after hatching. In the latter model one polarization system results in differential sensitivity to the egl-20/Wnt signal. One could argue that this would be more advantageous since a single system is less error prone than a more complicated dual specification system. Conversely, if cells are programmed to respond differently to similar signals the organism could use the same system in different cellular contexts.

Future studies might provide molecular details of the Q migration control

mechanisms. Detailed expression and mosaic analysis of the key players will demonstrate in which cells individual gene activities are required. As experimental techniques evolve, it will get less difficult to obtain cell biological and molecular data from an in vivo context. For example, one of the major caveats of conventional expression analysis in *C.elegans* is that the gene of interest is overexpressed; either via an extrachromosomal array or via transgenic lines in which multiple gene copies are integrated into the genome. Therefore, conclusions based on examination of these types of transgenic animals might turn out to be false. Techniques in which single transgenic gene copies are integrated into the genome in a site-specific manner are now being developed (Robert and Bessereau 2007). Furthermore, a sensitive fluorescence in situ hybridization (FISH) technique to visualize single mRNA molecules has recently been developed and this technique was demonstrated to be effective, not only in tissue culture but also in multilayered organisms like *C.elegans* (Raj et al 2008). Using these techniques, information about the true endogenous gene expression patterns can be obtained which circumvents problems that arise due to overexpression.

Furthermore, cell biological experiments in which the subcellular localization of factors involved in Q cell migrations are examined might reveal molecular insights into this process. It would be interesting to examine the intracellular localization of factors like PAR proteins or Rho/Rac GTPases that are expected to be asymmetrically localized or activated during cell migration. These subcellular localization patterns can then be used as a read-out to determine the roles of different genes in the intracellular distribution of regulatory proteins.

All together, the migration of Q neuroblasts and their descendents provides an excellent model system to examine the developmental control of cell migration. Insights obtained in Q cell migration might reveal novel genetic circuitries that regulate cell migration in vivo. Therefore, these findings might be translated across species and result in a more comprehensive understanding of cell migration in healthy and diseased contexts. Considering the fact that deregulated cell migration is one of the major hallmarks of metastatic cancer, insights into the fundamental processes that control cell migration is of high therapeutic relevance.

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