Review

Signaling systems, guided cell migration, and organogenesis: insights from genetic studies in Drosophila

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Abstract

During development, cells change their position extensively. Although the basic cellular mechanisms involved in cell locomotion have been studied mostly in cultured cells, genetic and molecular approaches using model organisms are starting to shed light on the complex events influencing cell migration during development. Recent technical advances in following and analyzing migrating cells inside the living embryo offer the possibility of understanding how different signaling systems regulate the fundamental cellular processes underlying guided cell migration in vivo. In Drosophila melanogaster, studies of migrating cells have concentrated mainly on hemocytes, germ cells, border cells, and tracheal cells. Interestingly, most of these cells were recently shown to make different cellular extensions and to use receptor tyrosine kinases to sense the chemoattractive signal. This review describes our current understanding of how different signaling networks control guided migration in these four systems and discusses the impact of novel imaging techniques on the study of guided cell migration during development.

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Introduction

During the development of multicellular organisms, cells change their relative position extensively as organs and tissues take up their final location and function. Over many decades, such cell movements have been analyzed in tissue explants or in vitro using cultured cells, and these studies have provided a wealth of knowledge regarding the intracellular events that occur as a cell moves over a substratum. Both the actin cytoskeleton and the microtubular network have to be reorganized extensively in a migrating cell, ultimately contributing to the forward movement and to the temporal stability of the cell (Lauffenburger and Horwitz, 1996; Sheetz et al., 1999).

More recently, events which focus the migratory forces into a given direction have been investigated in more detail in the context of single cells. Studies using Dictyostelium discoideum and neutrophils have resulted in the description of molecular scenarios that allow a cell to translate a shallow extracellular concentration gradient of a chemoattractant into a migratory response (Chung et al., 2001; Iijima et al., 2002; Sanchez-Madrid and del Pozo, 1999). Upon the activation of a cell surface receptor by binding to its chemoattractive ligand, positive and negative feedback loops along the cell surface enhance the signal intracellularly proximal to the signal and decrease it in more distal regions. These events ultimately lead to a localized signaling center at the front of the cell, characterized in these systems by increased levels of lipid products of phosphoinositide 3-kinase (PI3K) activity. This signal is then thought to be further translated via members of the small GTPase family into appropriate cytoskeletal responses, eventually allowing the cell to displace its body in a coordinated manner (Etienne-Manneville and Hall, 2002). While the detailed molecular mechanisms controlling guided cell migration in multicellular organisms might differ from the ones described in Dictyostelium, the work on this system nicely shows how the use of molecular, cellular, and genetic approaches can lead to a deeper understanding of guided cell migration.
Chemoattraction also plays a crucial role in cell migration during the development of multicellular organisms, but in such an environment, migrating cells are faced with a number of additional constrains. In many cases, cells first have to acquire the capability to detach from surrounding cells and invade other territories, and migration has to be initiated at precise developmental times. Often, cells do not move individually but in groups, and not all cells in such groups perform the same role in the migratory process. In particular cases, it appears that cell migration sculpts the three dimensional appearance of entire organ systems. Ultimately, migrating cells have to stop their movement as they reach their final destination and differentiate into non-motile cells of distinct functions. The necessity of cells to coordinate their movement with their neighborhood in multicellular systems requires that motility as such is regulated by events that control cell adhesion, either between similar and/or different cells and the cellular matrix. In vivo, each cell migration event has its own particularity with regard to the issues just described.

We would like to give a brief overview of the current knowledge gained from the genetic analysis of cell migratory events in Drosophila melanogaster. Since studies regarding cell migration are most advanced in germ cells, border cells, hemocytes, and tracheal cells, we limit our comparison to these systems. We describe a number of signaling pathways that have recently been associated with these cases of directional cell migration, and outline the different cellular contexts that allow migration to shape organs and tissues of completely different architecture (see Fig. 1 for a schematic representation of the systems described in this review).

Migratory systems and their signaling mechanisms

Hemocytes

Hemocytes are the Drosophila blood cells and play a major role in the innate immune response and in the removal of apoptotic cells. Prior to the initiation of their role as blood cells, they are deployed throughout the embryo by a stereotyped, genetically encoded migratory program. Hemocytes originate as a cluster of cells from the head meso-
derm (Fig. 1A). This cluster loosens up and splits into two ill-defined groups of cells (Fig. 1B), one moving to the posterior end of the embryo by crossing the amnioserosa, which at that stage is folded due to germ band extension (Fig. 1C). Both populations then spread toward the middle of the embryo and disperse evenly throughout the embryo as single cells (Fig. 1D; Cho et al., 2002; Tepass et al., 1994).

The characterization of the genes encoding the fly homologue of the PDGF/VEGF receptor (PVR, also named VEGF receptor or Stasis) and its putative ligands (PDGFs/VEGFs; named PVFs in the following) has shown that the PDGF/VEGF signaling pathway controls important aspects of the migratory behavior of the Drosophila hemocytes (Cho et al., 2002). Upon specification, hemocytes start to express PVR (Cho et al., 2002; Heino et al., 2001); developing blood cells lacking PVR differentiate and initiate migration correctly but stall before crossing the amnioserosa and do not disperse uniformly. The PVFs are expressed in cell populations along the migratory route of the hemocytes. Due to apparent functional redundancy, mutants for single Pyf genes show no effect on blood cell migration; however, inactivation of all three Pyf genes by RNAi injection revealed a phenotype similar to the one seen in mutants for the receptor. Strikingly, ectopic expression of a Pyf results in the misrouting of hemocytes, supporting a role of the PDGFR/VEGFR pathway in guided migration of developing blood cells (Cho et al., 2002). However, PVR signaling seems to be mainly required for producing two groups of cells (an anterior and a posterior one) by guiding cells to the posterior end of the embryo, while initiation of migration and dispersal seems to rely on other mechanisms.

**Germ cells**

Similar to hemocytes, germ cells migrate without being firmly attached to each other. However, instead of dispersing throughout the embryo, they converge into the future somatic gonad tissue. The Drosophila pole cells are formed at the posterior pole of the blastoderm embryo (Fig. 1E). As a result of their adhesiveness to the underlying tissue, they are passively carried into the midgut pocket by the subsequent movements of germ band extension and midgut invagination. Subsequently, the germ cells actively invade and cross the endodermal epithelium to reach the overlying mesoderm (Fig. 1F). Germ cells then migrate toward the gonadal mesoderm where they coalesce, giving rise to the compact embryonic gonad (Fig. 1G and H; Starz-Gaiano and Lehmann, 2001).

Despite the fact that extensive loss-of-function genetic screens have been undertaken to elucidate the molecular mechanisms underlying germ cell migration in Drosophila, only a limited number of key components have thus far been identified. These components reveal the existence of attractant and repellant factors that are produced by somatic cells and guide migrating germ cells (Starz-Gaiano and Lehmann, 2001).

In mutants of the genes encoding the Drosophila homologues of the mammalian lipid phosphate phosphatase-1 (wunen and the neighboring wunen-2), germ cells fail to reach the somatic mesoderm; ectopic expression of wunen or wunen-2 throughout the mesoderm has a repellant effect on germ cells. wunen RNA is expressed at the bottom of the posterior midgut, and the corresponding protein thus appears to repel germ cells from this part of the midgut pocket. In analogy to the mammalian lipid phosphate phosphatase-1 protein, the Drosophila Wunen proteins might expose their catalytic site extracellularly and either produce a repellant or destroy a phospholipid acting as an attractant (Starz-Gaiano et al., 2001; Zhang et al., 1996, 1997).

A separate attractive signal is produced by the columbus gene, which encodes the enzyme HMGCoA reductase (HMGCoAR) (Van Doren et al., 1998). HMGCoAR is expressed at high levels in the somatic gonadal precursors. In mutants for columbus, germ cells fail to associate with the somatic mesoderm; ectopic expression of HMGCoAR attracts germ cells to the newly expressing tissue. The cell...
surface molecules, which represent the actual signal produced in the somatic gonad in response to the activity of HMGCoxAR, as well as their presumptive receptors on germ cells, remain elusive.

**Border cells**

Border cells represent one of the best studied systems with regard to cell migration in the fruitfly. Border cells are a group of about eight cells, which originate from the most anterior part of the egg chamber (Fig. 1I) and which after detaching from the monolayered follicular epithelium invade the germ cell cluster (Fig. 1J) and migrate, still as a group, on and in-between the nurse cells in the direction of the oocyte (Fig. 1K). When they reach the oocyte, they migrate dorsally where they come to stop over the oocyte nucleus (Fig. 1L). The border cell cluster ultimately contributes to the formation of the micropyle, the structure on the eggshell that allows the entry of the sperm and therefore fertilization (Montell, 2001; Rorth, 2002).

A number of elegant studies have contributed to our current understanding of border cell specification and the subsequent events culminating in guided migration. Border cells are thought to be specified within the follicular epithelium by the JAK-STAT pathway leading to the expression of the transcription factor Slow border cells (Slbo), which controls the expression of most genes required for migration (Beccari et al., 2002; Montell et al., 1992; Silver and Montell, 2001). Specification is followed by the detachment of the border cell cluster from the follicular epithelium and the invasion of the space in between the nurse cells. Border cells delaminate from the follicle cell epithelium in a process reminiscent of an epithelium-to-mesenchyme transition. This is of special interest as this process is reminiscent of the situation encountered in many human metastatic tumors (Thiery, 2002). Somewhat surprisingly, the classical DE-cadherin is required both in the migrating border cells and in their substratum for the migration to occur. This observation suggests that homophilic interaction between DE-cadherin in the two cell types provide the adhesion and/or traction required for migration (Niewiadowska et al., 1999).

Migration in between the nurse cells towards the oocyte is controlled by two receptor tyrosine kinase (RTK) signaling pathways, centered around the PVR (Duchek et al., 2001) and the epidermal growth factor receptor (EGFR) (Duchek and Rorth, 2001). Migration of the border cell cluster is primarily guided toward the oocyte by the PVR ligand PVF1. The small GTPase Rac is very likely to mediate this guidance effect. The Drosophila EGFR has a largely redundant role in this migration process so that cells can find their way using either PVR or EGFR. When border cells meet the oocyte, EGFR has also a second guidance function; it is required for dorsally directed migration in response to the ligand Gurken, which is concentrated in the dorsal aspect of the oocyte membrane.

Still little is known about the cellular events induced by the guidance receptors and the intracellular signal relay. Chemotaxis in Dictyostelium and neutrophils suggests that PI3K provides the localized intracellular signal. Experiments in which PI3 levels were manipulated by using overexpression of an activated form of PI3K suggest that PI3K does not accumulate preferentially at the leading edge of the border cell cluster (Fulga and Rorth, 2002), but further investigations are necessary to clarify this important issue.

It has recently been shown that an early consequence of signaling via the guidance receptors at the cellular level is the formation of a single long cellular extension (LCE) by one cell of the border cell cluster (Fig. 2A). Formation of these LCEs requires a functional DE-cadherin gene, but again, PI3K signaling does not appear to control LCE formation. Interestingly the interaction between DE-Cadherin and Myosin VI seems to be required for proper border cell migration by linking this adhesive complex to the cytoskeleton. It has been suggested that Myosin VI promotes LCE formation and the LCE itself may function as a “pathfinder” and grapple, helping the border cell cluster to be pulled forward toward the oocyte by Myosin II mediated contraction of the LCE (Fulga and Rorth, 2002; Geisbrecht and Montell, 2002; Schober and Perrimon, 2002).

**Tracheal cells**

Tracheal development represents a very interesting case in which cell migration not only leads to the repositioning of cells within the organism but sculpts the three-dimensional appearance of the entire organ (for general reviews on tubulogenesis, see Affolter et al., 2003; Hogan and Kolodziej, 2002; Lubarsky and Krasnow, 2003). Again, only a limited number of cells display a migratory behavior. The exceptional features of the developing tracheal system with regard to cell movement relies in the fact that cells migrating in different directions remain firmly connected with each other throughout the migratory process, ultimately giving rise to a branched tubular network.

The respiratory system of Drosophila develops from ten clusters of ectodermal cells, the tracheal placodes that invaginate to form the tracheal pits on both sides of the embryo, each containing approximately 80 cells (Fig. 1M). Each sac is expanded without further cell division in 6 directions by stereotypical, directed migration and cell shape changes (Fig. 1N). Subsequent fusion events lead to the interconnection of the individual metaneres (Fig. 1O and P; reviewed in Affolter et al., 2003; Hogan and Kolodziej, 2002; Lubarsky and Krasnow, 1999; Petit et al., 2002; Samakovlis et al., 1996).

Similar to border cells, the JAK/STAT pathway is crucial in specifying the tracheal cell fate. In this particular case, JAK/STAT signaling induces the expression of the transcription factor Tracheless (Trh) in the tracheal placodes (Brown et al., 2001; Chen et al., 2002). Trh is essential for making the cells competent for further migration events by...
known chemoattractant in the tracheal system, the
colony stimulating factor (CSF), confers tracheal cells with the ability to respond to the only
chemoattractant that has been identified, Branchless (Bnl) (Englund et al., 2002). This
chemoattractant results in the rerouting of tracheal cells, confirming that Bnl is indeed acting as a chemoattractant for
tracheal cells (Sutherland et al., 1996).

The existence of an overlap in the molecular mechanisms used in axonal pathfinding in the central nervous system and those used in guided tracheal migration has also been suggested (Enlund et al., 2002). The authors of the study propose that certain tracheal branches respond in an attractive and repulsive manner to Slit signaling and that these effects are mediated by different combinations of the Slit receptors Roundabout (Robo) and Roundabout2 (Robo2). The effects seen in mutants for Slit signaling are less penetrant than the ones seen in mutants for FGFR signaling and the exact role of these molecules in tracheal morphogenesis awaits further clarification.

Recently, in vivo confocal microscopy has provided compelling evidences that the FGFR pathway controls motility by inducing dynamic filopodia exclusively in the cells at the tip of the embryonic tracheal branches (Ribeiro et al., 2002; Sato and Kornberg, 2002) (Fig. 2B). The motile force produced by the cells at the tip of the branches seems to be used to drag along the passive distal cells eventually leading to the formation of an elongated, branch-like structure. A structure comparable to the LCE in border cells though has not been observed emanating from the tracheal cells indicating different strategies in generating the tractive force.

Strikingly, FGFR signaling mediated chemoattraction is not sufficient for successful outgrowth of tracheal branches. As shown in detail for the embryonic dorsal branch, further signaling systems [in this case the BMP-like Decapentaplegic (DPP) signaling cascade] are needed to integrate the motility program into the branching morphogenesis program thereby allowing the productive and correct morphogenesis of individual branches (Ribeiro et al., 2002; Vincent et al., 1997). DPP is thought to control cell rearrangements (e.g., intercalation), cell shape changes or adhesive properties of the cells specific to the dorsal branch. The molecular mechanisms, which mediate the signaling information of the FGFR to the cytoskeletal motility machinery and integrate the effects of the additional signaling systems acting in trachea, remain to be elucidated.

The small GTPase Rac has been suggested to play a major role in controlling chemoattractant-induced actin dynamics and cell adhesion (Etienne-Manneville and Hall, 2002). In the context of the tracheal system, one of the major roles of Rac seems to be the tight regulation of epithelial cadherin activity. Strikingly, no effect on filopodial activity was observed. While the precise effect of Rac loss- and gain-of-function in tracheal cells remains to be worked out in detail, Rac seems to act mainly by controlling

inducing the expression of the FGF receptor (FGFR) Breathless (Btl) and the intracellular FGF signaling component Downstream of FGFR (Dof)/Stumps (Boube et al., 2000; Isaac and Andrew, 1996; Wilk et al., 1996). This confers tracheal cells with the ability to respond to the only known chemoattractant in the tracheal system, the Drosophila FGF-like protein Branchless (Bnl). bnl is expressed dynamically in cells surrounding the invaginated tracheal placodes, prefiguring the direction of outgrowth of the six primary branches (Sutherland et al., 1996). In the absence of either Bnl/FGF, Btl/FGFR (Glazer and Shilo, 1991; Klambt et al., 1992; Reichman-Fried and Shilo, 1995), or Dof/ Stumps (Imam et al., 1999; Michelson et al., 1998; Vincent et al., 1998), cell migration and subsequent events in tracheal development fail to occur. Additionally, ectopic expression of bnl results in the rerouting of tracheal cells, confirming that Bnl is indeed acting as a chemoattractant for tracheal cells (Sutherland et al., 1996).

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the precise balance between assembly and disassembly of cadherin at the junctions, thereby regulating the remodeling capacities of epithelial structures (Chihara et al., 2003).

Progress has recently been made in understanding the molecular interactions between tracheal cells and their surrounding substrata. Surface receptors of the integrin family have been implicated in promoting the spreading of the visceral branch over the visceral mesoderm (Boube et al., 2001). The αPS1 and the αPS2 integrins of *Drosophila* are specifically expressed on the surface of the cells of the tracheal visceral branch and the visceral mesoderm, respectively. Specific interactions of both integrin receptors with the extracellular matrix allow the visceral branch to move over the visceral mesoderm; in mutants for the αPS1 integrin, the visceral branch stalls after the first contact with its future substratum. A similar, although molecularly less well understood, mechanism is used by the cells of the dorsal trunk. These cells use a specific mesodermal cell, the so-called bridge cell, as a substratum to cross the gap separating adjacent metameres (Wolf and Schuh, 2000). 

**Comparison between the different systems**

Genetic analysis of cell migration in *Drosophila* has recently led to the identification of a number of chemottractants and their receptor systems. Somewhat strikingly, all of the identified receptors are transmembrane tyrosine kinases (PVR, EGFR, and FGFR). The same receptors might also be required for cell migrations at other developmental stages. For example, FGF signaling was shown to be required in the pupa for the formation of the air sac by promoting filopodia based cell motility and cell proliferation (Sato and Kornberg, 2002), for the recruitment of mesodermal cells to the male genital imaginal disc (Ahmad and Baker, 2002), for the migration of midline glia cells (Klambt et al., 1992), and for the spreading of mesodermal cells along the dorsalventral axis of the early *Drosophila* embryo (Beiman et al., 1996; Gisselbrecht et al., 1996; Michelson et al., 1998). Clearly, long-range cell movements during *Drosophila* development appear to be controlled to a large extent by RTKs.

What are the cellular targets of these RTK signaling systems? Although some of these pathways, in particular the EGFR pathway, have been extensively studied at the genetic level with regard to their gene regulatory effects, little is known about the requirements of downstream signaling components concerning the regulation of migration. Cellular analysis has established that in many cases these signaling systems regulate the formation of cellular extensions linked to cell migration (e.g., LCE in border cells and filopodia in trachea; Fig. 2; Fulga and Rorth, 2002; Jaglarz and Howard, 1995; Ribeiro et al., 2002; Tepass et al., 1994) and it is thought that these receptor systems directly regulate cytoskeletal dynamics rather than transcription. It is likely that actin polymerization is one of the intracellular events regulated by these receptors, but whether the information is transmitted via the local accumulation of specific lipid products (in analogy to the accumulation of PIP3 in neutrophils and *Dictyostelium*) has been questioned.

Quite obviously, migration is crucial for the development of organs and tissues of rather different final shape and structure (Fig. 3, bottom; and Fig. 1D, H, L, and P). The different migratory strategies of the systems discussed here closely reflect the different purposes for which migration is used and the final role the migrating cells will perform at their site of arrival. In one extreme case, hemocytes are dispersed as uniformly as possible throughout the embryo (Fig. 1D); quite in contrast, tracheal cells remain tightly attached to each other in an epithelial tubular structure while subpopulations of cells migrate in different directions, thereby sculpting the three-dimensional appearance of the entire organ system (Fig. 1P). These examples illustrate the requirement for a complex interplay between the cell motility machinery and organ-specific programs that specify functional requirement. It is questionable, for example, whether tracheal cells have to retract their rear end actively, or whether this step is unnecessary due to their migration as an epithelial sheet. Migrating cells also do interact quite differently with cells in their immediate environment, and little is known at what level such interactions (via cadherins and integrins) intersect with the motility machinery.

A very interesting question to address in the future is how a cell achieves the competence to migrate as a result of the activation of a particular signaling system during development. In several cases, transcription of the gene encoding the chemotactant receptor is specifically activated in cells prior to migration (PVR in hemocytes, FGFR in tracheal, glial, and muscle cells; Fig. 3, top right). Although this might suggest that particular receptors are migration-inducers, the same receptors induce migration only in a specific time window and regulate other events in the same cells. This is very striking in the case of the EGFR, a receptor which is widely distributed (most or all cells in *Drosophila* express it), and yet only subgroups of cells respond in a particular time window with guided migration to receptor activation as in the case of border cells. Much has been learned in the last few years about the specific interpretation of widely used signaling systems with regard to nuclear gene-regulatory events, implying an integration of nuclear selector proteins and signaling mediators on specific enhancer elements (Affolter and Mann, 2001; Curtiss et al., 2002). Little or close to nothing is known about the molecular mechanisms involved in the generation of specific cytoplasmatic responses of different cells to widely used signaling systems.

The recent development of in vivo live visualization methods and their combination with the powerful genetic and reverse genetic approaches available in several animal model systems will be a great help in integrating cell movement into tissue and organ development in the near future (Lichtman and Fraser, 2001). Most dynamic cellular struc-
tures (such as filopodia) are lost upon fixation. Motility being per definition a dynamic process, it is understood that live visualization is the method of choice for studying this complex cellular behavior, as nicely exemplified in Dictyostelium (Melki et al., 1999; Parent et al., 1998; Ueda et al., 2001). In vivo multiple color labeling will help to understand how migrating cells interact with their surrounding tissue and how cellular structures (e.g., actin and microtubuli filaments) influence each other to mediate guidance. More sophisticated imaging approaches even open the possibility to analyze protein dynamics in the living organism (Lippincott-Schwartz et al., 2001). Using fluorescence recovery after photobleaching (FRAP) one can assess the mobility of fluorescently labeled proteins inside the cell and determine how different signaling events control the remodeling capacities of tissues. Fluorescence resonance energy transfer (FRET) finally allows the visualization of the subcellular localization and dynamics of protein interactions, opening a window on the largely unknown contribution of signaling localization inside of the cell in directed movement. These studies should reveal to what extent common themes prevail and to what extent migrating cells use different molecular routes to help them find their way.

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