Cell migration: from tissue culture to embryos

Germán Reig1,2, Eduardo Pulgar1,2 and Miguel L. Concha1,2,*

ABSTRACT

Cell migration is a fundamental process that occurs during embryonic development. Classic studies using in vitro culture systems have been instrumental in dissecting the principles of cell motility and highlighting how cells make use of topographical features of the substrate, cell-cell contacts, and chemical and physical environmental signals to direct their locomotion. Here, we review the guidance principles of in vitro cell locomotion and examine how they control directed cell migration in vivo during development. We focus on developmental examples in which individual guidance mechanisms have been clearly dissected, and for which the interactions among guidance cues have been explored. We also discuss how the migratory behaviours elicited by guidance mechanisms generate the stereotypical patterns of migration that shape tissues in the developing embryo.

KEY WORDS: Cell migration, Guidance cues, Environmental signals, Cell-cell contact, Tissue culture, Embryo development

Introduction

In the early 1900s, the biologist Ross Harrison developed a method for growing embryonic cells outside the body. He had wanted to observe whether nerve cells were capable of extending axons during development but the opacity of amphibian embryos and the lack of suitable microscopic techniques precluded his examination. To overcome these restrictions, Harrison developed an in vitro assay in which he cultured explants of amphibian nerve tissue on a dish. Using light microscopy, he was then able to reveal that axons were the result of extensions of single nerve cells (Harrison et al., 1907). Since Harrison’s pioneering use of two-dimensional (2D) tissue culture systems, this technique has become fundamental in the study of cell locomotion in real time.

Over time, it became evident that cells in 2D culture, three-dimensional (3D) culture and in vivo contexts show pronounced differences in cell shape, matrix adhesions and migratory behaviour. Nonetheless, studies of in vitro culture systems have served to define major guidance mechanisms of cell migration that rely on intrinsic cell motility, topographical features of the substrate, cell-cell contacts, and chemical and physical environmental cues. In vivo analyses using a variety of vertebrate and invertebrate model organisms that offer amenability for embryo and tissue handling, optical imaging and genetic manipulation have provided insights into how the in vitro guidance mechanisms operate in the context of embryonic development. Here, we review these mechanisms and examine how the migratory behaviours elicited in response to guidance cues work to generate the patterns of individual and collective cell migration that shape tissues in developing embryos. We begin by providing an overview of the types of cell migration that occur in cultured cells in vitro and discussing their underlying principles. We then discuss how these principles apply to the various examples of cell migration that occur in vivo during embryonic development.

Random cell migration in vitro

Much of our insight into cell migration has come from time-lapse studies of fibroblasts cultured in vitro. When plated on 2D surfaces, fibroblasts lose their round morphology and spread to increase their apparent surface area. A few minutes later, they develop multiple peripheral cell protrusions (lamellae) and, as adhesion increases, one lamella becomes dominant and fibroblasts develop a clear front-to-back polarity. This acquisition of cell polarity is fundamental to initiate locomotion and depends on the differential activity of small GTPases such as Cdc42, Rac1 and RhoA, which regulate actin dynamics, adhesion organisation and protrusion formation (Box 1).

Box 1. Front-to-back polarity in a migrating cell

A key morphological readout of front-to-back polarity in cells migrating on 2D surfaces is the emission of membrane protrusions in the form of broad lamellipodia and spike-like filopodia. Lamellipodia contain a highly branched dendritic network of actin filaments (red) whereas filopodia are formed by long parallel actin filament bundles. The acquisition of front-to-back polarity is controlled by small guanosine triphosphate (GTP)-binding proteins (small GTPases), such as Cdc42, Rac1 and RhoA, which regulate actin dynamics, adhesion organisation and the formation of lamellipodia and filopodia. Cdc42 is active towards the front of the cell and both inhibition and global activation of Cdc42 disrupt the directionality of migration. One main output of Cdc42 activity is the local activation of Rac1, and both proteins mediate actin polymerisation in protrusions. Once Rac1 and Cdc42 are active at the cell front, molecular feedback loops and mechanical tensile forces work together to maintain protrusions in the direction of migration. The back of the migrating cell is defined by the activity of Rho, myosin II and Ca2+-activated proteases. Active Rac1 at the cell front suppresses Rho activity whereas Rho is more active at the lateral and rear sides where it suppresses Rac1 activity. RhoA affects actomyosin contractility via Rho kinase (ROCK). In addition, strong adhesions at the cell rear result in increased tension, the opening of stretch-activated Ca2+ channels, and the subsequent activation of proteases that have the potential to cleave focal adhesion proteins. (For reviews on the topic, see Li and Gundersen, 2008; Ridley et al., 2003.)
Once polarised, fibroblasts start moving in one preferred direction by repeated cycles of protrusion, adhesion to the substrate, contraction of the cell body, and rear retraction (Fig. 1A), as identified in the seminal studies by Michael Abercrombie (Box 2). Over time, the position of the dominant lamella changes and fibroblasts turn and move in new directions showing an overall random pattern of cell locomotion (Fig. 1B) (Bard and Hay, 1975; Trinkaus, 1969; Weiss, 1961). This ‘intrinsic’ tendency of the cell to develop multiple peripheral lamellae and exhibit random migration seems to depend on the total level of activated Rac, which translocates to the plasma membrane in its active form to induce actin polymerisation and lamellar extension. Accordingly, when plated on 2D surfaces, cells display relatively high levels of activated Rac, which promotes the formation of multiple peripheral lamellae and random migration (Fig. 1B) (Pankov et al., 2005). To overcome such intrinsic random motility and acquire directional migration, cells need to restrict protrusion formation to a single axial lamella. This can be achieved in 2D systems by lowering the total levels of activated Rac. Alternatively, moving cells from a 2D to a 3D culture environment can alter their intrinsic properties and behaviours (Pankov et al., 2005; Petrie et al., 2009) and can promote directional movements (for more details, see Box 3). However, as we discuss below, environmental guidance cues can also restrict protrusion formation and promote directional cell migration through the local activation of Rac.

**Box 2. Seminal contribution of Michael Abercrombie to the ‘cell migration cycle’**

In the early 1970s, Abercrombie, Heaysman and Pegrum performed a series of experiments that provided a basic framework for the study of cell migration. Fibroblasts were carefully examined as they migrated away from the edge of chick- and mouse-derived tissue explants onto a 2D glass surface, uncovering the presence of repeated cycles of membrane protrusion and withdrawal at the leading edge of migrating cells (Abercrombie et al., 1970a). Fluctuations in the position of these mobile sheet-like membrane projections, defined as lamellipodia, resulted in membrane ruffles that appeared primarily at the transitions between withdrawal and protrusion events, moving away from the leading edge towards the cell body (Abercrombie et al., 1970b). Although the rates of cell protrusion and withdrawal were similar, it appeared that the net forward movement resulted from the greater time that cells spent protruding (Abercrombie et al., 1970a). Furthermore, ultrastructural analyses revealed that lamellipodia exhibit discrete accumulations of dense material at sites of contact with the substrate, as well as intracellular longitudinal filaments that resembled actin cables (Abercrombie et al., 1971). This systematic description of the leading edge of a migrating cell raised the idea that substrate adhesion can provide a means of traction, which, together with contractile fibrils, allows the cell to pull itself forward. Together, these findings led to the proposal that cell migration is a cyclic process of protrusion, adhesion to the substrate, contraction of the cell body, and rear retraction (Abercrombie et al., 1971). During this cycle, cells require rapid insertion of new material at the leading edge (Abercrombie et al., 1970c, 1972), which allows the formation of new adhesions to the substrate and causes the excess to move backwards, giving rise to membrane ruffles.
Cell migration in 2D versus 3D culture environments

When transferred from 2D (e.g. standard Petri dishes, glass coverslips) to 3D (e.g. gel matrix scaffolds, hanging drops) culture environments, fibroblasts change their shape, cell matrix adhesive structures and migratory behaviour (Bard and Hay, 1975; Elsdale and Bard, 1972; Pankov et al., 2005; Petrie et al., 2009). Cells moving in 3D matrices become elongated and display more directional movements than those migrating on 2D surfaces. Such changes reflect distinct means by which migrating cells interact with the substrate and sense their physical properties (Garber, 1953; Weiss and Garber, 1952). Fibroblasts on 2D surfaces develop prominent and stable elongated focal adhesions, which associate with stress fibres over the broad lamellipodial region (Geiger and Yamada, 2011; Parsons et al., 2010). By contrast, the presence of focal adhesions in 3D environments is still a matter of debate (Fraley et al., 2010, 2011; Geraldo et al., 2012; Harunaga and Yamada, 2011; Kubow and Horwitz, 2011). An interesting observation is that adhesions similar to those found in 2D can be observed at the edge (but not at the centre) of the 3D culture dish, where the anchorage of collagen bundles to the culture dish increases the rigidity of the matrix and thus the tension experienced by cells (Fraley et al., 2011). Accordingly, cells plated on soft 2D substrates show irregular and unstable focal adhesions (Pelham and Wang, 1997) whereas adhesions similar in structure and molecular composition to those found in 2D can be detected in vivo in cells submitted to high tensile forces (Bokstad et al., 2012; Ralphs et al., 2002). These observations suggest that the formation and maturation of focal adhesions is sensitive to cellular tension and substrate stiffness (Kuo, 2013; Parsons et al., 2010). Cells migrating on 2D surfaces thus appear to form exaggerated versions of the adhesive structures found in vivo in cells submitted to elevated mechanical stress, whereas less rigid 3D matrices favour more discrete adhesions that seem to resemble those in most in vivo migrating cells. In the figure, labeled structures correspond to ECM fibrils (blue lines), stress fibers (green lines), focal adhesions (purple dots) and integrin heterodimers (pink/blue structures). A selected group of proteins forming the adhesion complex include Actin, Tensin, Paxillin, Talin, Vinculin (Vinc) and Focal adhesion kinase (Fak).

Box 3. Cell migration in 2D versus 3D culture environments

Guidance by cell-cell contact

Cell-cell contact plays an instructive role in directing cell migration. This feature was noticed many years ago by Abercrombie and Heaysman when examining the behaviour of fibroblasts as they spread radially from culture explants and collided with fibroblasts that were moving in opposite directions from a confronted culture explant (Abercrombie and Heaysman, 1954). In events of cell-cell collision, fibroblasts exhibited a stereotypical behaviour whereby the cell front adhered to the colliding cell and experienced contraction and paralysis of protrusion and ruffling (Fig. 3A,B). Soon after, a new cell front was established away from the cell-cell contact zone and the collided cells

2010; Reinhart-King et al., 2005) and rigidity (Lo et al., 2000). Similarly, gradients of surface-bound ligands can direct cell migration through a process of ‘haptotaxis’, which was initially described for cultured fibroblasts (Carter, 1965, 1967) and later extended to the oriented growth of axons on patterned collagen matrices (Letourneau, 1975) and of tumour cells along gradients of laminin (McCarthy et al., 1983). Finally, cell-cell contacts and polarised chemical signals (Fig. 2C) may direct contact-guided cell locomotion, and are discussed in turn below.

Fig. 2. ECM-mediated contact guidance. (A) Contact guidance is the process by which cells sense and use inhomogeneities of the substrate to adhere, polarise and orient their migration. On a randomly oriented ECM fibrillar meshwork (left), cells adopt a stellar shape and move with no preferred directionality. By contrast, they elongate and orient their migration when cultured on aligned fibrils (right). Fibrils are depicted as light blue lines; red arrows indicate the directionality of movement. (B) A randomly organised fibrillar meshwork (left, blue lines) can become aligned in a defined direction (middle, purple lines) through local ECM remodelling mediated by the same migrating cell. Alternatively, the application of external forces on the substrate (right, depicted as spring stretching) can also align a fibrillar network and, consequently, orient cell movements. (C) Contact guidance provides orientation but not directionality of migration (left). However, cells can adopt a preferred direction in response to gradients in ECM adhesiveness (haptotaxis), rigidity (durotaxis) and substrate deformation (depicted as a blue gradient); anisotropies in cell-cell contacts (green); and polarised chemical signals (purple circles).
moved apart (Fig. 3A, B) (Abercrombie and Ambrose, 1958). Such ‘contact inhibition of locomotion’ (CIL) (Abercrombie, 1970) was then reported in other in vitro contexts, such as when two epithelial sheets meet (Abercrombie and Middleton, 1968) and in the process of radial extension of nerve fibres (Dunn, 1971). In addition, CIL was observed during the migration of corneal fibroblasts in their natural stroma and in artificial 3D collagen lattices (Bard and Hay, 1975). Furthermore, CIL was proposed as an explanation for wound healing in epithelia (Abercrombie, 1970; Farooqui and Fenteany, 2005) and for the invasive properties of certain malignant cells (Abercrombie, 1970; Abercrombie and Heaysman, 1954) or at longer distances through filopodial extensions (Carmona-Fontaine et al., 2008; Davis et al., 2012; Heckman, 2009; Lesseps et al., 1975; Stekete and Tosney, 1999; Teddy and Kulesa, 2004). Sensing is mediated by cell surface molecules, often cell-cell adhesion proteins of the cadherin family (Mayor and Carmona-Fontaine, 2010), which are proposed to change the balance in small GTPases, with local activation of RhoA at the cell-cell contact and suppression of Rac1 and repolarisation of the colliding cell (Nelson et al., 2004; Theveneau et al., 2010). When the sensing of cell-cell contact is perturbed, cells lose their ability to reset polarity and they produce numerous protrusions that are able to extend on top of neighbouring cells (Abraham et al., 2009; Theveneau et al., 2010; Villar-Cervino et al., 2013). Furthermore, the balance between CIL and attractive forces, which we discuss later, determines whether a group of cells disperses as individuals or as a collective group (Fig. 3C).

Directed cell migration via chemotaxis

The ability of cells to undergo directed locomotion along a chemical gradient, a process known as chemotaxis (Fig. 4A, B), was first described in bacteria by Pfeffer (Pfeffer, 1884) and later in phagocytic leucocytes by Metchnikoff (Metchnikoff, 1893). Since then, chemotaxis has been a subject of intense research in both prokaryotic (Hazelbauer, 2012) and eukaryotic cells, including the free-living amoeba Dictyostelium discoideum, mammalian leucocytes, fibroblasts and neurons (Swaney et al., 2010; von Philippsborn and Bastmeyer, 2007; Vorontnikov, 2011). Despite exhibiting different modes of cell locomotion (ameboid in D. discoideum and leucocytes; mesenchymal in fibroblasts) and utilising different signal transduction mechanisms [G protein-coupled receptor (GPCR)-dependent in D. discoideum and leucocytes, and receptor tyrosine kinase (RTK)-dependent in fibroblasts], most in vitro models of eukaryotic chemotaxis share three general principles. First, cells often exhibit intrinsic random motility. Second, exposure to a chemooattractant gradient leads to small spatial or temporal differences in receptor activation that are amplified within the cell to induce Rac-mediated actin polymerisation and protrusion formation on the side of the cell facing the highest concentration of chemooattractant. Third, cell polarity is stabilised by positive-feedback loops (at the cell front) combined with long-range inhibitory signals (in the rest of the cell) that restrict protrusion formation towards the cell front and increase its sensitivity to chemooattractants along the gradient (Insall, 2013; Swaney et al., 2010; Vorontnikov, 2011; Wang et al., 2011). Under these general principles, there is major discussion concerning the mechanism by which receptor activation leads to the formation of polarised protrusions. The ‘chemotactic compass’ model (Fig. 4C, top) proposes that sensing of the gradient results in local accumulation of intracellular signalling molecules, such as phosphatidylinositol-3,4,5-trisphosphate (PIP3), towards the highest chemooattractant concentration. Such localised signalling functions upstream of activated Rac and is used as a ‘compass’ to adjust the actual cell polarity by turning the cell front towards the gradient (Bourne and Weiner, 2002; Rickert et al., 2000; Swaney et al., 2010; Wang, 2009). The alternative ‘chemotactic bias’ model (Fig. 4C, bottom) proposes that chemooattractants simply bias the dynamic and self-organising autocatalytic nature of protrusions towards the gradient without the need of a compass (Arrieumerlou and Meyer, 2005; Insall, 2010). As such, this model seems to better integrate recent data showing that membrane tension resulting from actin polymerisation acts as a long-range physical signal that inhibits protrusion formation in regions other than the cell front (Batchelder et al., 2011; Houk et al., 2012). Although the compass and chemotactic bias models may differ in some aspects, it should be noted that they are not mutually exclusive and thus both probably operate in most eukaryotic cells (Insall, 2013).

Mechanical guidance of cell locomotion

In addition to gradients in ECM rigidity, which are known to direct cell migration from soft to stiff substrates (Lo et al., 2000), it has recently been shown that physical forces applied at the cell-matrix and at cell-cell interfaces can also direct cell migration. For instance, cells migrating on soft ECM matrices may produce patterns of substrate deformation that serve as a cue to attract the migration of adjacent and distant cells (Angelini et al., 2010; Reinhart-King et al., 2005). In addition, cells forming a cohesive group can generate and sense gradients of intercellular tension to coordinate the direction of their collective migration (Tambe et al., 2011; Weber et al., 2012).
events of ‘mechano taxis’ require strategies to sense physical forces and transduce them into polarised chemical cues and directed cell locomotion, which are still poorly understood. Recent work has shown that such mechanotransduction may rely on force-induced conformational changes to scaffolding proteins, such as filamin and talin, that ultimately modulate the activation of small GTPases, such as Rac (del Rio et al., 2009; Ehrlicher et al., 2011). In addition, cells seem able to measure the force required to obtain a given substrate deformation (Ghassemi et al., 2012; Ghibaudo et al., 2008) and compute differences in intercellular tension among neighbours (Tambe et al., 2011; Weber et al., 2012). Once force sensing and polarity is established, mechanisms similar to those used during chemotaxis appear to operate to reinforce polarity and direct cell migration (Roca-Cusachs et al., 2013).

Mechanisms of cell migration during development

Embryo development is a highly dynamic process during which cells and their environment are constantly changing and communicating with each other. In contrast to in vitro culture systems, in which external variables can be easily manipulated, it is not easy to control the chemical and physical guidance cues that affect cell migration in vivo, which are often diverse in nature and overlapping in function. In addition, embryonic cells can use either the ECM or other cells as a substrate for migration, in contrast to the more typical ECM-mediated mode of cell locomotion observed in vitro. Furthermore, although cells can migrate as individuals they often move as collective groups during development, which imposes an additional challenge: to resolve whether guidance cues work at the individual or the collective group level. In this context, the complexity of developing systems challenges the ability to study guidance mechanisms as isolated entities and to address how different guidance strategies cooperate to generate in vivo patterns of cell migration. In this section, we examine how the aforementioned guidance principles of in vitro cell locomotion have been used to explain a number of in vivo events of directed cell migration during development (summarised in Table 1). We focus on developmental examples in which individual guidance mechanisms and cues have been dissected and explored to some extent. We also discuss the insights that emerge from these analyses, which provide us with clues as to how the migratory behaviours elicited by guidance mechanisms generate the stereotypical patterns of migration that shape developing tissues.

Random cell motility during tissue morphogenesis

Embryonic cells can activate random motility using various genetic signals, and this seems to be a prerequisite for initiation of directional migration in response to external guidance cues (Aman and Piotrowski, 2010). Random motility results in random patterns of cell migration, which per se can impact cell and tissue morphogenesis. In the context of individual cell migration, random motility has a dispersive and exploratory effect that prompts cells to colonise new territories within the embryo. Such an effect is characteristic of the early migratory phase of the zebrafish endoderm. During gastrulation, zebrafish endodermal cells disperse from the margin towards the animal pole following an intrinsic cell-autonomous ‘random walk’, which is characterised by the formation of short-lived small protrusions on cells in almost all directions (Fig. 5A, top) (Pezeron et al., 2008; Woo et al., 2012). This random migratory behaviour later switches to become persistently directed as endodermal cells start converging towards the midline in response to chemotactic cues (discussed below), while forming broader and more stable dorsally directed protrusions (Fig. 5A, bottom) (Pezeron et al., 2008; Woo et al., 2012). Random walking is induced by Nodal signalling at least in part through the expression of the Rac activator Prex1 (Woo et al., 2012): when Rac1 activity is disrupted, endodermal cells anticipate their route towards the new signal source, respectively.
<table>
<thead>
<tr>
<th>Mechanism/cell type</th>
<th>Model</th>
<th>Migration event</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Random motility</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cajal-Retzius cells</td>
<td>Mouse</td>
<td>Tangential spreading in the neocortex</td>
<td>Villar-Cervino et al., 2013</td>
</tr>
<tr>
<td>Endoderm</td>
<td>Zebrafish</td>
<td>Random dispersion from the margin to the animal pole in the gastrula</td>
<td>Pezeron et al., 2008; Woo et al., 2012</td>
</tr>
<tr>
<td>Deep cells</td>
<td>Annual fish</td>
<td>Scattered cell dispersion on the early embryo</td>
<td>Lesseps et al., 1979</td>
</tr>
<tr>
<td>Presomitic mesoderm</td>
<td>Chick</td>
<td>Posterior elongation of the embryo</td>
<td>Benazeraf et al., 2010</td>
</tr>
<tr>
<td><strong>ECM and cell-cell mediated contact guidance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior mesoderm</td>
<td>Urodeles,</td>
<td>Extension of the anterior mesoderm following aligned ECM fibrils in the BCR</td>
<td>Boucaut et al., 1990; Dambere and Schwarzbauer, 2000; Nakatsuji et al., 1982; Nakatsuji and Johnson, 1984; Winklbauer et al., 1996</td>
</tr>
<tr>
<td>Neural crest cells</td>
<td>Axolotl, chick</td>
<td>Early migration from the dorsal neural tube</td>
<td>Lofberg et al., 1980; Newgreen and Thiry, 1980</td>
</tr>
<tr>
<td>Primordial germ cells</td>
<td>Mouse,</td>
<td>Migration from the developing gut to the site of the future gonad</td>
<td>Garcia-Castro et al., 1997; Heasman et al., 1981</td>
</tr>
<tr>
<td>Primordial germ cells</td>
<td>Drosophila,</td>
<td>E-cadherin-mediated hitchhiking by the moving endoderm</td>
<td>Chihara and Nance, 2012; DeGennaro et al., 2011</td>
</tr>
<tr>
<td>Mesenchymal cells</td>
<td>Killifish</td>
<td>Migration along actinotrichia during fin morphogenesis</td>
<td>Wood and Thorogood, 1984</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>Rat</td>
<td>Capillary sprouting along a scaffold of ECM</td>
<td>Anderson et al., 2004</td>
</tr>
<tr>
<td>Gonadotropin-releasing hormone (GnRH) neurons</td>
<td>Mouse</td>
<td>'Neurophilic/axonophilic' cell migration along vorneronasal axons</td>
<td>Carboni et al., 2007</td>
</tr>
<tr>
<td>Pyramidal neurons</td>
<td>Mouse</td>
<td>Gliophilic cell migration along radial glial fibres in the developing cortex</td>
<td>Rakic, 1971</td>
</tr>
<tr>
<td>Neuroblasts</td>
<td>Mouse</td>
<td>Vasophilic cell migration along the rostral migratory stream of the forebrain</td>
<td>Saghatelyan, 2009</td>
</tr>
<tr>
<td><strong>Contact inhibition of cell locomotion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemocytes</td>
<td>Drosophila</td>
<td>Individual cell dispersal on the ventral side of embryo</td>
<td>Davis et al., 2012; Stramer et al., 2010</td>
</tr>
<tr>
<td>Cajal-Retzius cells</td>
<td>Mouse</td>
<td>Individual tangential cell spreading along the cortical marginal zone</td>
<td>Borrell and Marin, 2006; Villar-Cervino et al., 2013</td>
</tr>
<tr>
<td>Deep cells</td>
<td>Annual fish</td>
<td>Individual cell dispersal on the embryo surface</td>
<td>Lesseps et al., 1979; 1975</td>
</tr>
<tr>
<td>Primitive myeloid cells</td>
<td>Xenopus</td>
<td>Individual cell scattering from the anterior ventral blood islands</td>
<td>Costa et al., 2008</td>
</tr>
<tr>
<td>Anterior visceral endoderm</td>
<td>Mouse</td>
<td>Individual distal-to-proximal cell dispersion on the epiblast surface</td>
<td>Pezeron et al., 2008</td>
</tr>
<tr>
<td>Epibranchial placodal precursors</td>
<td>Xenopus</td>
<td>Epithelial cell migration away from chasing neural crest cells</td>
<td>Theveneau et al., 2013</td>
</tr>
<tr>
<td>Neural crest cells</td>
<td>Xenopus</td>
<td>Collective mesenchymal cell migration away from the dorsal neural tube</td>
<td>Carmona-Fontaine et al., 2008; 2011</td>
</tr>
<tr>
<td><strong>Chemotaxis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primordial germ cells</td>
<td>Zebrafish</td>
<td>Individual cell migration towards the future gonad, mediated by Cxcl12a-Cxcr4b</td>
<td>Blaser et al., 2006; Boldajipour et al., 2008; Reichman-Fried et al., 2004</td>
</tr>
<tr>
<td>Border cells</td>
<td>Drosophila</td>
<td>Epithelial cluster migration towards the oocyte, mediated by PVR-PVF1 and EGFR-Gurken</td>
<td>Bianco et al., 2007; Duchek and Rørth, 2001; Duchek et al., 2001</td>
</tr>
<tr>
<td>Posterior lateral line primordium</td>
<td>Zebrafish</td>
<td>Epithelial cluster migration along the horizontal myoseptum, mediated by Cxcl12a-Cxcr4b</td>
<td>Dona et al., 2013; Haas and Gilmour, 2006; Venkiteswaran et al., 2013</td>
</tr>
<tr>
<td>Tracheal cells</td>
<td>Drosophila</td>
<td>Leading cell migration during branch formation, mediated by Fgf-Branchless</td>
<td>Sutherland et al., 1996</td>
</tr>
<tr>
<td>Neural crest cells</td>
<td>Xenopus</td>
<td>Mesenchymal collective cell migration to latero-ventral regions, mediated by Cxcl12-Cxcr4</td>
<td>Theveneau et al., 2010; 2013</td>
</tr>
<tr>
<td>Anterior mesoderm</td>
<td>Xenopus</td>
<td>Mesenchymal collective cell migration to the BCR, mediated by Cxcl12-Cxcr4 and PDGFA-PDGFRα</td>
<td>Fukui et al., 2007; Nagel et al., 2004</td>
</tr>
<tr>
<td><strong>Large-scale ECM flow</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epiblast</td>
<td>Chick</td>
<td>Composite movement of epiblast and sub-epiblastic ECM during gastrulation</td>
<td>Zamir et al., 2008</td>
</tr>
<tr>
<td>Mesoderm</td>
<td>Chick</td>
<td>Composite movement of the mesoderm and ECM during gastrulation</td>
<td>Cziro et al., 2004; Filla et al., 2004; Zamir et al., 2008; Aufschnaiter et al., 2011</td>
</tr>
<tr>
<td>Ectoderm, mesoderm</td>
<td>Hydra</td>
<td>Composite movement of the two epithelia and intervening mesoglea during bud outgrowth</td>
<td>Zamir et al., 2008</td>
</tr>
</tbody>
</table>
amniote embryo, during which cells of the presomitic mesoderm undergo directed posterior movements as a consequence of a gradient of random cell motility that decreases in the posterior-to-anterior direction and is controlled by a similar gradient of fibroblast growth factor (FGF)/mitogen-activated protein kinase (MAPK) signalling (green gradient), and involves the formation of broader and more stable dorsally directed membrane protrusions coupled to directed cell displacements (blue arrows). Circular plots on the right depict the distribution of motion vectors that are characteristic of each behaviour. (B) Posterior elongation of the chick embryo is mediated by a gradient of random motility. During embryo elongation, the presomitic mesoderm moves directionally towards the posterior (red arrows). Mathematical subtraction of ECM motion (green arrows) from global cell displacement (purple arrows) reveals a cell-autonomous random motility gradient that decreases from posterior to anterior (blue stars), which coincides with a posterior-to-anterior gradient of FGF/MAPK signalling (purple). The inverse anterior-to-posterior gradient in cell density is probably a result of the random motility gradient.

**ECM- and cell contact-guided migration during development**

Contact-mediated guidance can orient cell migration in a wide variety of developmental contexts (Table 1). Key examples include the migration of mesenchymal cells along collagenous unsegmented fibrils known as actinotrichia in the developing teleost fin (Fig. 6A) (Wood and Thorogood, 1984), the migration of neuronal precursors along pre-existing neuronal/axonal and glial scaffolds in the brain (Fig. 6B), and the extension of anterior mesodermal cells along ECM fibrils on the blastocoeol roof (BCR) of the amphibian gastrula (Fig. 6C,D). Studies of urodele and anuran embryos have been especially illuminating as they have combined in vitro observations with in vitro analyses of tissue explants and dissociated cells, and with molecular manipulations of cell-matrix interactions (Boucaut et al., 1991; DeSimone and Johnson, 1991). During gastrulation in amphibians, the prospective head mesoderm migrates towards the animal pole using the inner surface of the epithelial ectodermal BCR as a substrate. Just before the onset of mesoderm migration, a fibrillar network of ECM fibrils forms on the BCR and becomes aligned along the vegetal-animal pole axis (Fig. 6C,D) (Nakatsuji et al., 1982; Nakatsuji and Johnson, 1983a). It was shown that dissociated mesodermal cells seeded on a plastic substrate that contains the ectodermal-derived fibrillar ECM network (obtained by previous conditioning with ectoderm explants) are able to attach and orient their migration along fibrils, as they do in vivo (Fig. 6E) (Nakatsuji and Johnson, 1983b). Importantly, if the ectodermal layer is artificially realigned by exerting mechanical tension in vivo (e.g. by tilting the substrate), the mesodermal cells also reorient their movements (Fig. 6F) (Nakatsuji and Johnson, 1984). Therefore, aligned ECM fibrils appear to orient mesodermal cell migration on the BCR through a contact guidance mechanism (Nakatsuji and Johnson, 1984). This mechanism appears to involve fibronectin (FN), which is a major component of the BCR fibrillar network (Boucaut and Darrabere, 1983; Davidson et al., 2004; Johnson et al., 1992); blockage of FN synthesis, function or fibrillar assembly, as well as abrogation of the integrin α5β1 receptor, leads to abnormal adhesion, spreading and migration of mesodermal cells (Boucaut et al., 1990; Darrabere and Schwarzbauer, 2000; Davidson et al., 2002; Nagel and Winklbauer, 1999; Rozario et al., 2009). Upon contact with FN, mesodermal cells realign their intrinsic protrusive activity along the BCR surface and replace filopodia with lamelliform protrusions (Winklbauer and Keller, 1996; Winklbauer and Nagel, 1991; Winklbauer and Selchow, 1992). These results suggest that the FN-fibrillar network not only provides oriented adhesiveness and resistance to mesoderm cell traction but also regulates the protrusive activity of migrating cells. As described for in vitro ECM-mediated contact guidance, the oriented migration of anterior mesodermal cells requires additional cues and factors to direct locomotion towards the animal pole, and these include CIL amongst mesodermal cells (Johnson et al., 1992; Winklbauer and Selchow, 1992) and attractive chemical signals produced by the BCR, such as the chemokine Cxcl12a (Fukui et al., 2007) and the ECM-bound platelet-derived growth factor A (PDGFA) (Nagel et al., 2004).

In addition to features of the ECM, cells can orient their migration by interacting with a cellular substrate. Such cell-cell mediated contact guidance is observed during the migration of neuronal precursors along pre-existing neuronal/axonal and glial scaffolds in the developing and adult brain, in processes referred to as ‘neurophilic/axonophilic’ and ‘gliophilic’ cell migration, respectively (Table 1). For example, gonadotropin-releasing hormone (GnRH) neurons migrate from the nasal placode towards the olfactory bulb region (Fig. 6B). The GnRH neurons follow vomeronasal axons and this event is mediated by cell-cell interactions involving integrin β1 (expressed in GnRH neurons) and the glycoprophatidylinositol (GPI)-linked semaphorin 7A (expressed in axons) (Carbini et al., 2007). However, contact with the vomeronasal axons only provides orientation, and the nose-to-brain directionality of GnRH neuronal migration depends on a chemoattractive gradient of semaphorin 4D (Fig. 6B) (Messina and Giacobini, 2013).

Cell-mediated contact guidance is also observed in Caenorhabditis elegans and Drosophila primordial germ cells. These cells are quiescent and establish E-cadherin-mediated adhesive interactions with the endoderm, and as the endoderm moves they are carried along...
by a ‘hitchhiking’ mechanism (Chihara and Nance, 2012; DeGennaro et al., 2011). Importantly, contact-guided cell migration can be regulated by switching the adhesion efficiency and/or by changing the way that cells interpret the landscape along their route of migration. For example, distal tip cells in the C. elegans gonad show an initial phase of directional migration that is driven by the integrin INA-1 α subunit which is followed by an arrest in cell migration after switching integrin receptor expression from INA-1 α to PAT-2 α (Meighan and Schwarzbauer, 2007, 2008). Similarly, GnRH neurons transform their early directional migration along vomeronasal axons into a state of decreased attachment and spreading by switching integrin β1 to plexin C1 after crossing the nasal-forebrain border (Fig. 6B) (Messina and Giacobini, 2013).

**Self-generated patterns of migration by contact inhibition of locomotion**
Analogous to its role in the radial dispersion of fibroblasts in culture explants, CIL appears to be a driving force for cell dispersion during development, in events when cells spread and colonise expanded territories starting from confined regions of the embryo (Table 1). For example, Drosophila haemocytes, attracted by a source of the Drosophila platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF) ligand PVF1, migrate from the head mesoderm to form a linear cellular array at the ventral midline (Cho et al., 2002; Wood et al., 2006), and from this position they disperse laterally to form a ‘three-lined’ organisation pattern as a result of CIL (Fig. 7A) (Davis et al., 2012; Stramer et al., 2010). As haemocytes contact each other, a stable arm-like microtubule bundle that extends into the lamellae collapses, enabling cell repulsion, turning and dispersion (Stramer et al., 2010). In other *in vivo* contexts, cells undergoing CIL do not disperse but instead organise as a cohesive cluster owing to additional forces that restrict cell dispersal. For instance, cell-cell adhesive interactions keep epithelial cells together during collective migration driven by CIL, as observed in the movement of Xenopus pre-placodal cells (Theveneau et al., 2013). By contrast, mesenchymal cells moving by CIL organise in collective migratory streams owing to attractant cues that the cells themselves produce and sense. For example, the cohesive migration

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**Fig. 6. ECM- and cell-mediated contact guidance during development.** (A) During teleost fin morphogenesis, mesenchymal cells migrate along an array of banded collagen fibrils or actinotrichia (orange lines) aligned along the proximal-distal axis of the teleost fin bud. (B) In the developing mouse brain, GnRH neurons migrate from the vomeronasal organ (VNO) towards the region of the olfactory bulb (OB) following the route of vomeronasal axons (VNA, arrow in top diagram). The bottom panel shows that the early steps of migration within the nose are highly directional and depend on interactions between integrin β1 (in GnRH neurons, red line) and the glycosphatidylinositol (GPI)-linked semaphorin 7A (expressed by vomeronasal axons) coupled to sensing of a chemoattractant gradient of semaphorin 4D (purple circles). In the transition from nose to brain migration, GnRH neurons arrest their migration by switching to expression of plexin C1 receptors (orange line). (C,D) During gastrulation in urodele amphibians, mesodermal cells (red) move from the blastopore to the animal pole (red arrow) using the ectodermal blastocoel roof (BCR, dark blue line) as a substrate, while the ectoderm (blue) undergoes epibolic expansion (blue arrow). Just before the onset of mesoderm migration (C), the BCR forms a network of ECM fibrils (C, right panel, blue lines) that later becomes oriented along the animal-vegetal (A-V) axis (D, right panel), probably influenced by the epibolic expansion of the ectoderm (indicated by the stretched spring), which serves as a guidance cue for oriented mesodermal cell migration (white arrows). (E) The BCR fibrillar network (blue) can be experimentally transferred to the surface of a coverslip by preconditioning with late-gastrula ectodermal explants. Under such *in vitro* conditions, dissociated mesodermal cells (red) use the aligned fibrillar array to direct their migration (white arrows). (F) Mechanical tension imposed on the ectodermal explant by tilting the coverslip by 30° can re-align the BCR fibrillar network along the medio-lateral (M-L) axis, and also re-orients mesodermal cell movements along the axis of tension (stretched spring).
of *Xenopus* neural crest cells (NCCs) (Fig. 7B) is favoured by the C3a complement immune-derived component, which is produced by NCCs and works as an attractant cue for the same cells (Carmona-Fontaine et al., 2011). When NCCs contact each other within the cluster, cell-cell contact mediated by N-cadherin triggers a repulsive response by activating RhoA at the contact site, leading to cell repolarisation and promotion of cell dispersal (Carmona-Fontaine et al., 2008). Therefore, NCCs within the collective migratory group are not static but they constantly split, collide and reassemble, showing cryptic protrusions when in inner positions but a strong front-to-back polarity if placed at the free edge of the cluster (Carmona-Fontaine et al., 2008, 2011; Thenevau et al., 2010). Importantly, cells that in the embryo normally migrate as a cohesive group (e.g. NCCs) can disperse as individuals if the forces of attraction are inhibited, whereas the induction of attraction among embryonic cells that normally disperse (e.g. myeloid cells) results in the formation of cohesive migratory streams (Carmona-Fontaine et al., 2011). These findings indicate that cells undergoing CIL are able to switch between individual and collective migratory behaviours according to the balance of attractive and repulsive interactions that they experience (Fig. 3C). When the balance favours repulsive interactions, CIL leads to cell dispersal. By contrast, migratory cells organise as a cohesive group when attractive forces become dominant.

Cell-cell contact repulsive interactions triggered by CIL seem sufficient to generate self-organised patterns of individual and collective cell migration, and the random versus directional nature of this migration is determined, at least in part, by factors that influence the rate and spatial organisation of cell-cell collision events, such as cell density, cell shape and the geometry of the substrate (Abercrombie and Heaysman, 1954; Bindschadler and McGrath, 2007; Carmona-Fontaine et al., 2011; Coburn et al., 2013; Costa et al., 2008; Davis et al., 2012; Lesseps et al., 1979; Villar-Cervino et al., 2013). In addition, intrinsic random motility may favour stochastic cell dispersal triggered by CIL, as observed in the ‘random walk’ of zebrafish endodermal cells (Fig. 5A) (Pezeron et al., 2008) and mouse Cajal-Retzius cells (Fig. 7C) (Villar-Cervino et al., 2013). Finally, external guidance cues such as the chemokine Cxcl12 may be integrated into the CIL process to either restrict CIL events within defined spatial domains (Borrell and Marín, 2006) or promote directed cell migration by enhancing the polarity induced by CIL (see next section).

**Guidance by chemotactic signals in developing systems**

Chemotaxis works as a guidance mechanism for individual and collective cell migration in a variety of developmental contexts (Table 1). Among the best-studied examples are the individual cell-based migration of zebrafish primordial germ cells, the epithelial cluster-based migration of *Drosophila* border cells and of the zebrafish posterior lateral line primordium, and the mesenchymal collective-based migration of *Xenopus* cephalic neural crest cells (Fig. 8) (Aman and Piotrowski, 2010; Montell et al., 2012; Rørth, 2002; Tarasheevich and Raz, 2010; Thenevau and Mayor, 2012). These *in vivo* examples share two main principles of chemotaxis. First, intrinsic random cell motility is present before exposure to a chemoattractant gradient. Indeed, genetic pathways that are independent of chemotaxis trigger random cell motility, and this appears to be a pre-requisite for initiating directional migration in response to chemoattractants. Second, exposure to a chemoattractant gradient activates receptor-mediated signalling that biases the intrinsic random cell motility towards the gradient by directing front-to-back polarity, stabilisation of cellular protrusions and cell translocation. Consistent with these observations, both the abrogation of receptor-mediated signalling and the exposure to homogeneous chemoattractant concentrations impair directional cell migration in the same way, resulting in random polarity and protrusion formation without affecting motility per se. Below, we discuss each of these developmental examples of guidance by chemotactic signals in turn.

Zebrafish primordial germ cells (z-PGCs) migrate as individuals from their site of specification towards the future gonad (Fig. 8A, top) displaying a unique mode of intrinsic locomotion that is characterised by alternating phases of persistently directed migration (‘run’) and pauses (‘tumbling’) (Reichman-Fried et al., 2004), which resemble the biphasic chemotactic behaviour of nerve growth cones (Ming et al., 2002). During ‘runs’, z-PGCs form spherical...
blebs that are propelled by RhoA-mediated myosin II cortical contraction and hydrostatic pressure, which result in a retrograde flow of cortical actin. This event is modulated by chemokine signalling, specifically Cxcr4b receptor activation by a gradient of Cxcl12a, which induces local amplification of intracellular Ca\(^{2+}\) influx at the cell front resulting in enhancement of RhoA-mediated
myosin II contraction, stabilisation of cell protrusions and increase in
the time spent in ‘runs’ (Blaser et al., 2006; Kardash et al., 2010;
Reichman-Fried et al., 2004). Traction forces for cell translocation
are generated by linking the retrograde actin flow to E-cadherin (also
known as Cadherin 1)-mediated adhesions with somatic cells
(Blaser et al., 2006; Kardash et al., 2010). Such behaviour differs from the most common mode of locomotion in
which cells move by forming protrusions through Rac-mediated
actin polymerisation at the front (Box 1).

Drosophila border cells (d-BCs) are organised as a cluster of six
eight epithelial cells that migrates from the anterior follicular
epithelium towards the oocyte in two distinct phases that require
attachment to a cellular substrate through E-cadherin (also known as
Shotgun) (Niewiadomska et al., 1999; Pacquelet and Rørth, 2005).
In the early phase (Fig. 8B, middle), d-BCs emit long and
stable extensions that firmly attach to nurse cells and allow the
cluster to be pulled forward in a highly directional manner through
actomyosin contraction (Bianco et al., 2007; Fulga and Rørth, 2002;
Poukkula et al., 2011). In the late phase (Fig. 8B, bottom), the BC
cluster adopts a round shape and moves with less directionality, and
cells exchange positions and form short and unstable extensions
with a front bias (Bianco et al., 2007; Poukkula et al., 2011). During
these events, d-BCs use two RTKs to sense chemotactants,
although the PVF1 receptor PVR has a dominant role in the early
migratory phase whereas EGFR (and its ligand Gurken) takes over
at later stages to allow the final dorsal migration (Duchek and Rørth,
2001; Duchek et al., 2001; Poukkula et al., 2011; Prasad and
Montell, 2007). Both receptors utilise different effector proteins
and guidance strategies, which are based on the subcellular
localisation of signalling within the leading cell (in the case of
PVR) and differences in signal levels among the constituents of the
cluster (in the case of EGFR) (Assaker et al., 2010; Bianco et al.,
2007; Janssens et al., 2010; Jekely et al., 2005; Ramel et al., 2013).
However, signalling seems to converge on Rac activation, as focal
photoactivation of Rac is sufficient to direct protrusion formation
and rescue directional migration of the entire cluster when PVR and
EGFR function are disrupted (Wang et al., 2010).

The zebrafish posterior lateral line primordium (z-pLLP) is
organised as a cohesive cluster of >100 cells that migrate from the
otic placode towards the tail following the horizontal myoseptum
(Fig. 8C, top). The z-pLLP shows clear group polarity, displaying
more extensive protrusions at the front than at the back of the cluster
(Haas and Gilmour, 2006). Mosaic genetic analysis revealed that
individual cells containing a functional chemosensing receptor adopt
a front position within a cluster lacking the receptor, and are able to
escape migration of the entire primordium (Haas and Gilmour, 2006;
Xu et al., 2014). This is similar to the behaviour observed in the early
phase of d-BC migration (Bianco et al., 2007), indicating that in both
developmental contexts the activity of front cells is fundamental for
cluster migration. Chemokine signalling is also involved in z-pLLP
migration; Cxcl12 is a major chemotactant, and graded activation
of Cxcl12a/Cxcr4b signalling, which decreases from front to back,
results in enhanced actin dynamics and the stabilisation of protrusions
towards the front of the cluster (Fig. 8C, bottom) (Dona et al., 2013;
Venkiteswaran et al., 2013; Xu et al., 2014).

Xenopus neural crest cells (x-NCCs) also migrate as a collective
group, from the dorsal neural tube to lateral and ventral regions of
the embryo (Fig. 8D, top). The traction forces for NCC locomotion
are generated by cell protrusions that adhere to ECM (Theneveau
et al., 2010). Furthermore, x-NCCs coordinate their directed
movements by combining CIL with chemotaxis. As previously
described, the collective movement of NCCs away from the dorsal
neural tube is directed by both CIL and ‘co-attraction’ among
NCCs, which induces cell polarisation and protrusion formation
through the free edge of the collective cell group (Carmona-
Fontaine et al., 2008, 2011). As NNCs migrate, they also sense a
source of the chemotactant Cxcl12, which is produced by
epithelial epibranchial placodal precursors (EPPs), and they respond
to this cue by enhancing the polarity induced by CIL (Theneveau
et al., 2010). Bidirectional interactions between NCCs and EPPs
direct the coordinated migration of both cell populations towards
lateral and ventral regions (Fig. 8D). NCCs are attracted by Cxcl12
produced by EPPs (‘chase’) and, as both cell types make contact,
EPPs move away from NCCs through CIL (‘run’), leading to
forward displacement of the Cxcl12 source and the engagement of
both NCCs and EPPs in repeating cycles of ‘chase-and-run’
behaviour (Fig. 8D, bottom) (Theneveau et al., 2013).

**Shaping chemoattractant gradients in vivo**

As highlighted above, cell migration during development is often
dependent on gradients of chemotactants, and studies have shown that embryonic cells use different strategies to shape these
chemoattractant gradients in vivo. Chemoattractants may be produced in discrete spatial domains and from there diffuse and exert their
graded effect over a long distance, as seen in the Drosophila egg
chamber where PVF1 is produced at the posterior pole and attracts
d-BCs located at the anterior pole (Fig. 8B) (Duchek et al., 2001). It is
also possible that a stable gradient of immobilised chemoattractant
is formed after diffusion, as observed for the mouse chemokine
CCL21, which is released by lymphatic vessels and attracts dendritic
cells through a decay-mediated gradient of CCL21 bound to heparan
sulfates (Weber et al., 2013). In addition, chemoattractants may work
over a short-distance. For instance, the Drosophila FGF Branchless
is expressed in focal domains surrounding the developing tracheal
system and exerts a local guidance effect that directs branch formation
(Sutherland et al., 1996). Similarly, x-NCCs exhibit short-range
attraction to a moving source of Cxcl12 produced by pre-placodal
cells, from which they never seem to distance themselves due to a
‘chase-and-run’ behaviour (Fig. 8D). However, embryonic cells may
also move directionally along stripes of homogeneously produced
chemoattractants, and for this to occur they require an active way to
shape the gradient. The principle of such a mechanism resembles the
source-sink model (Crick, 1970) and relies on the asymmetric removal
of chemoattractants from the extracellular space by receptor-mediated
endocytosis, which can be performed either by cells forming the
substrate of migration or by the migrating cells themselves. An
example of the former is observed during the guidance of z-PGCs
when a functional Cxcl12a gradient is shaped by somatic cells through
Cxc7-mediated endocytosis (Boldajipour et al., 2008). However,
embryonic cells may by themselves generate a chemoattractant
gradient as they migrate, as demonstrated in vitro for cancer cells in 3D
matrices (Shields et al., 2007) and for epithelial cells using microscale
engineering techniques (Scherber et al., 2012). Cells in the z-pLLP
also promote their own directional migration along the horizontal
myoseptum through polarised Cxcr7-mediated endocytosis of
Cxc7 at the back of the cluster, which generates a functional
linear gradient of Cxcl12a/Cxcr4b signalling across the migrating
primordium that increases towards the front (Fig. 8C) (Dambly-
Chaudiere et al., 2007; Dona et al., 2013; Valentin et al., 2007;
Venkiteswaran et al., 2013). Such a self-generated strategy for guiding
cell migration differs from classical chemotaxis as it predicts that, once
formed, the gradient will continually move with cells and will lock
them in a persistent migratory phenotype until they encounter a stop
signal (or lose polarity).
Directed cell migration by long-range tissue deformation

Recent *in vivo* studies have shown that the ECM itself displays tissue-level movements that have been suggested to direct cell and tissue locomotion (Aufschnaiter et al., 2011; Benazeraf et al., 2010; Czirok et al., 2004; Filla et al., 2004; Zamir et al., 2006, 2008). For example, during avian gastrulation the epiblast and the underlying sub-epiblastic ECM move together as a tissue composite (Zamir et al., 2008) and similar large-scale coordinated movements are observed among mesodermal cells and their surrounding ECM (Czirok et al., 2004; Zamir et al., 2006). In both cases, the directed composite movements are proposed to be driven by both long-range mechanical forces, which are transmitted from global embryo morphogenesis into large-scale ECM flows, and by cell-autonomous migratory activities (Benazeraf et al., 2010; Zamir et al., 2008). Although the mechanism of force transmission is yet to be elucidated, these findings challenge our view of how guidance mechanisms based on chemotactrant gradients and cell-cell contacts operate in developmental contexts in which cells and the ECM move together as a composite.

Conclusions

Here, we have reviewed how the main guidance principles of *in vitro* cell locomotion apply to defined developmental contexts in a variety of vertebrate and invertebrate model organisms. Embryonic cells transit between mesenchymal and epithelial states, and in both conditions they must trigger motility to initiate random or directional migration. Intrinsic random motility is observed in the absence of guidance cues, when cells are unable to sense these signals, and in the presence of homogeneous (non-graded) levels of guidance cues. This random cell motility has a morphogenetic role per se by promoting cell dispersion or, if spatially organised, by coordinating the migration of collective cell groups. However, migrating cells can also follow topographical features of the substrate through contact guidance and, in these situations, the directionality of movements is provided by additional chemical or physical cues. Such guidance cues normally bias random cell motility towards a polarised source of the signal or, in the case of gradients, towards the highest or lowest concentration of the signal. Motility bias relies on the induction of front-to-back cell polarity, which involves actin polymerisation at the cell front and the stabilisation of protrusion formation towards the signal. These events are frequently mediated by signal transduction events downstream of receptor activation that modulate the activity of small GTPases and actin dynamics. The transmission of membrane tension across the migrating cell also plays an instructive role in directing polarised motility. All the aforementioned processes show tight genetic regulation during development with respect to both the timing of cellular events and the spatial configurations of environmental signals. Such genetic control pre-configures the substrate landscape and determines the possible response mechanisms that cells can activate to direct their migration. However, migrating cells can also self-organise the chemical and physical substrate to which they will respond (e.g. by self-generating a chemotactrant gradient). In addition, cells can generate patterns of migration through interactions with other migrating cells (e.g. by means of contact inhibition of locomotion). The latter migratory events are not pre-configured and thus represent emerging properties of the system. Further studies exploiting the advantages offered by model organisms for embryo and tissue handling, optical imaging and genetic manipulation, will provide novel insights into how chemical and physical signals are integrated with intrinsic cellular properties to produce stereotypical and robust migratory responses. This task is especially crucial for the guidance of collective cell groups, which is the most common way of locomotion during critical stages of development.

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