REVIEW

Cell migration: from tissue culture to embryos

Germán Reig[1,2], Eduardo Pulgar[1,2] and Miguel L. Concha[1,2,*]

ABSTRACT

Cell migration is a fundamental process that occurs during embryo 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, cell-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, Rac and RhoA, which regulate actin dynamics, adhesion organisation and protrusion formation (Box 1).
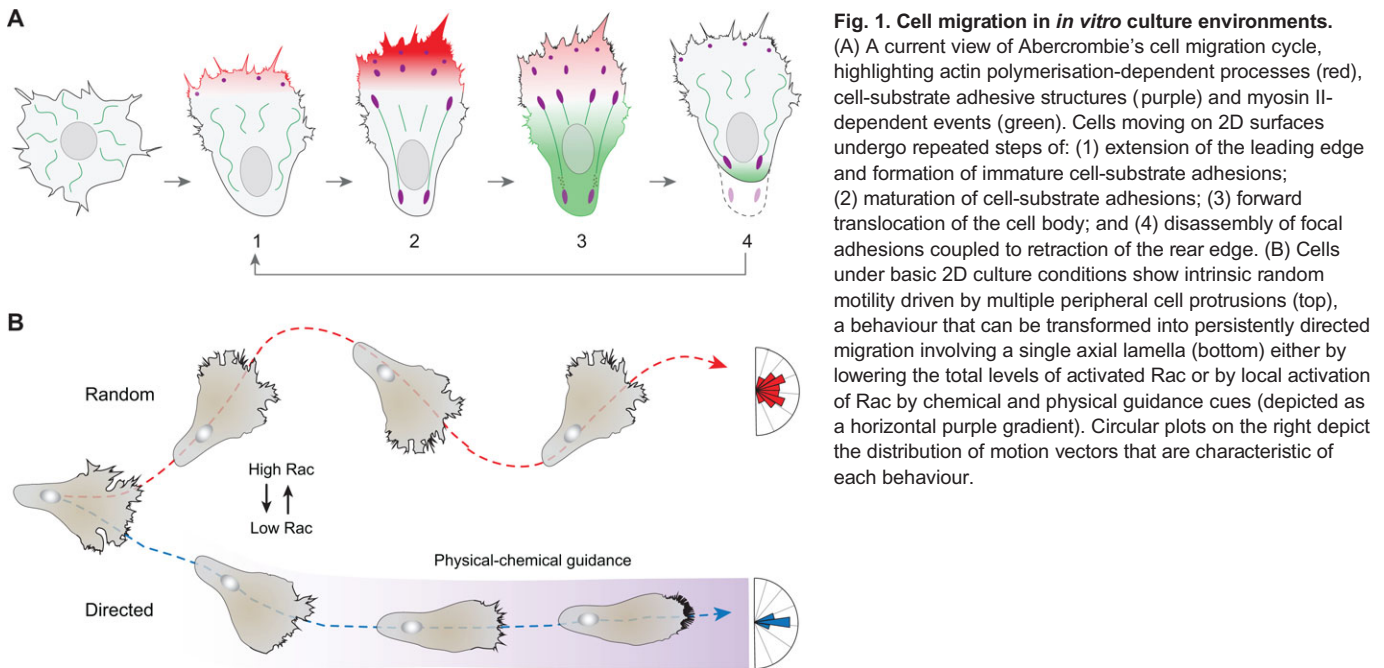

Box 1. Front-to-back polarity in a migrating cell
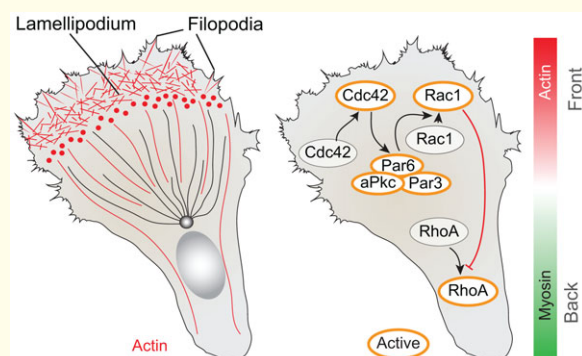
A key morphological readout of front-to-back polarity in cells migrating on 2D surfaces is the emission of membrane protrusions in the form of broad lamellipodia and spike-like filopodia. Lamellipodia contain a highly branched dendritic network of actin filaments (red) whereas filopodia are formed by 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 Ca^{2+}-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 Ca^{2+} 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.)

[1]Anatomy and Developmental Biology Program, Institute of Biomedical Sciences and [2]Biomedical Neuroscience Institute, Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago 8380453, Chile.

*Author for correspondence (mconcha@med.uchile.cl)

Fig. 1. Cell migration in *in vitro* culture environments.
(A) A current view of Abercrombie's cell migration cycle, highlighting actin polymerisation-dependent processes (red), cell-substrate adhesive structures (purple) and myosin II-dependent events (green). Cells moving on 2D surfaces undergo repeated steps of: (1) extension of the leading edge and formation of immature cell-substrate adhesions; (2) maturation of cell-substrate adhesions; (3) forward translocation of the cell body; and (4) disassembly of focal adhesions coupled to retraction of the rear edge. (B) Cells under basic 2D culture conditions show intrinsic random motility driven by multiple peripheral cell protrusions (top), a behaviour that can be transformed into persistently directed migration involving a single axial lamella (bottom) either by lowering the total levels of activated Rac or by local activation of Rac by chemical and physical guidance cues (depicted as a horizontal purple gradient). Circular plots on the right depict the distribution of motion vectors that are characteristic of each behaviour.

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.

Guided *in vitro* cell locomotion
ECM-mediated contact guidance
In early studies, Paul Weiss and co-workers observed that cells and axons seeded on 2D culture dishes elongate and migrate along topographical features of the substrate, such as engraved parallel microgrooves and oriented fibrillar structures (Weiss, 1945, 1959; Weiss and Taylor, 1956). This ability of cells to use the lack of homogeneity of the extracellular matrix (ECM) as cues to adhere, polarise and orient their migration was defined as 'contact guidance' (Fig. 2A) (Carter, 1965; Dunn, 1982; Weiss, 1961). Contact guidance has since been demonstrated *in vitro* for a wide variety of cell types (Dickinson et al., 1994; Dubey et al., 2001; Teixeira et al., 2003; Webb et al., 1995; Wood, 1988). Oriented features of the substrate, such as aligned ECM fibrils, can induce contact-guided behaviours by imposing geometrical constraints on cell-matrix adhesion sites and by providing physical cues to initiate polarisation of cell shape, orientation of cellular organelles and directional cell migration (Doyle et al., 2009; Loesberg et al., 2007; Petrie et al., 2009; Weiss, 1945). In addition, tension imposed on the ECM (e.g. by substrate stretching) can align a random fibrillar meshwork and thus promote directed migration through a contact-guided mechanism (Fig. 2B)

DEVELOPMENT

Box 3. Cell migration in 2D versus 3D culture environments
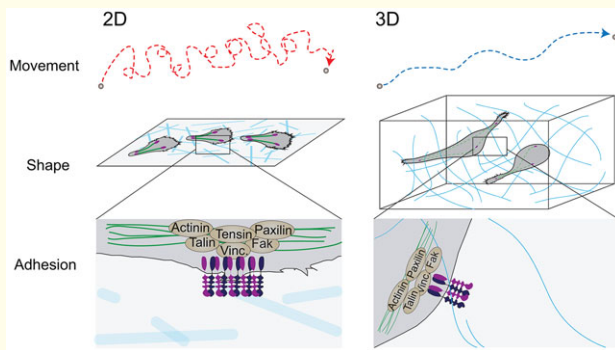
When transferred from 2D (e.g. standard Petri dishes, glass coverslips) to 3D (e.g. gel matrix scaffolds, hanging drops) culture environments, fibroblasts change their shape, cell matrix adhesive structures and migratory behaviour (Bard and Hay, 1975; Elsdale and Bard, 1972; Pankov et al., 2005; Petrie 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 Actinin, Tensin, Paxilin, Talin, Vinculin (Vinc) and Focal adhesion kinase (Fak).

(Nakatsuji and Johnson, 1984; Weiss, 1961). Alternatively, cells may by themselves align a random fibrillar meshwork (Harris et al., 1981; Stopak and Harris, 1982; Tranquillo, 1999) and thus assist their own directed migration along fibrillar structures (Fig. 2B). A clear example of the latter is observed in the case of epithelial tumour cells, which can align collagen fibres at the tumour-stromal interface to facilitate their local metastatic invasion (Provenzano et al., 2006, 2008). ECM-mediated contact guidance works as a mechanism that promotes oriented cell migration, but in principle it does not provide directionality (Fig. 2C) (Weiss, 1961). Additional cues must thus impose the directionality of migration (Fig. 2C). For example, cells showing contact-guided behaviour may direct their locomotion towards regions of increasing ECM deformation (Angelini et al.,

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.

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
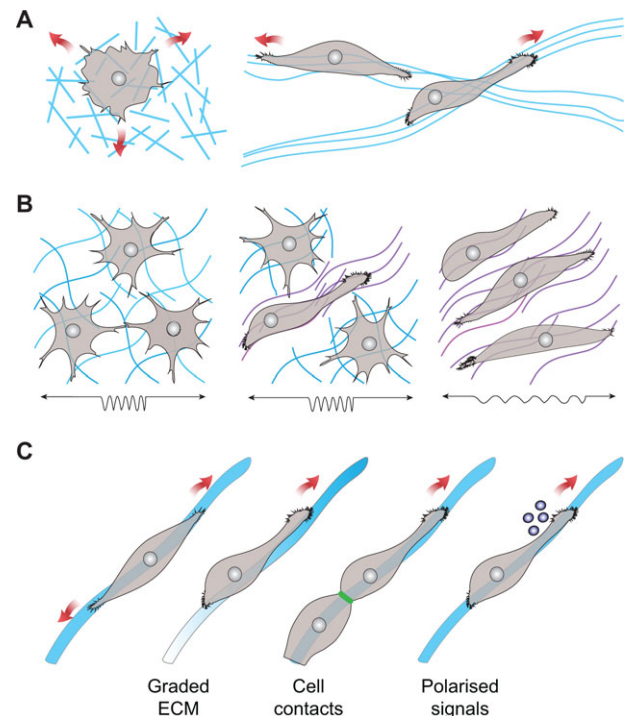

Fig. 2. ECM-mediated contact guidance. (A) Contact guidance is the process by which cells sense and use inhomogeneities of the substrate to adhere, polarise and orient their migration. On a randomly oriented ECM fibrillar meshwork (left), cells adopt a stellar shape and move with no preferred directionality. 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).
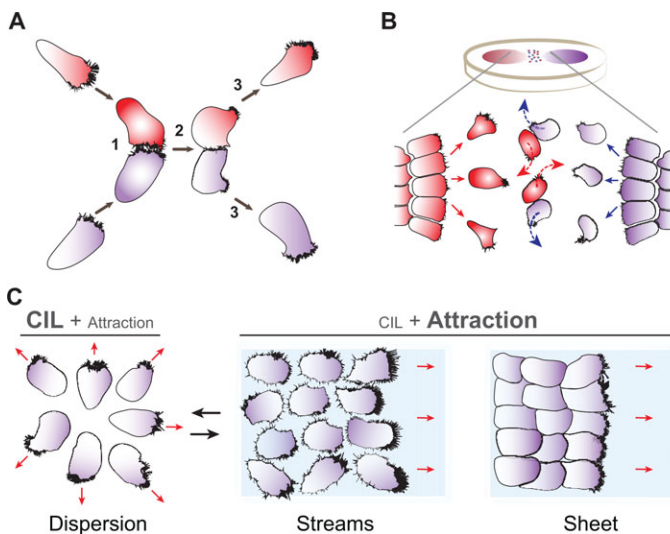
Fig. 3. Contact inhibition of cell locomotion. (A) Contact inhibition of cell locomotion (CIL) is the process by which migrating cells move apart from each other following an event of cell-cell collision. It involves three well-defined steps: (1) adhesion of the cell front to a colliding cell; (2) contraction and paralysis of protrusion and ruffling; and (3) the formation of a new cell front and migration away from the cell-cell contact zone. (B) A classic *in vitro* assay of CIL. When two culture explants (labelled in red and purple) confront each other, CIL drives both the radial cell spreading away from explants (unbroken arrows) and the repolarisation of cell movement following events of collision (dashed arrows). (C) The balance between repulsive (CIL) and attractive (co-attraction, cell adhesion) forces determines whether a group of migrating cells disperse as individuals or move as a collective cell group, forming cellular streams (in the case of mesenchymal cells) or sheets (in the case of epithelial cells). Cell density and spatial constraints imposed by the substrate (depicted as a light blue box) also determine the spatial configuration of CIL events, thus directing the formation of large, well-oriented and stable protrusions at the front of the collective, facing the free space (red arrows).

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 *in vitro*, movements of which were not restricted by contact with normal fibroblasts owing to defective CIL (Abercrombie, 1979; Vesely and Weiss, 1973).

CIL works to re-set the polarity of migrating cells. Cells initially sense contact with other cells either at the lamellipodium (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; Steketee 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 leukocytes, fibroblasts and neurons (Swaney et al., 2010; von Philipsborn and Bastmeyer, 2007; Vorotnikov, 2011). Despite exhibiting different modes of cell locomotion (ameboid in *D. discoideum* and leukocytes; mesenchymal in fibroblasts) and utilising different signal transduction mechanisms [G protein-coupled receptor (GPCR)-dependent in *D. discoideum* and leukocytes, 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 chemoattractant 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 chemoattractant. 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 chemoattractants along the gradient (Insall, 2013; Swaney et al., 2010; Vorotnikov, 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-triphosphate (PIP_3), towards the highest chemoattractant 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 chemoattractants 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). These
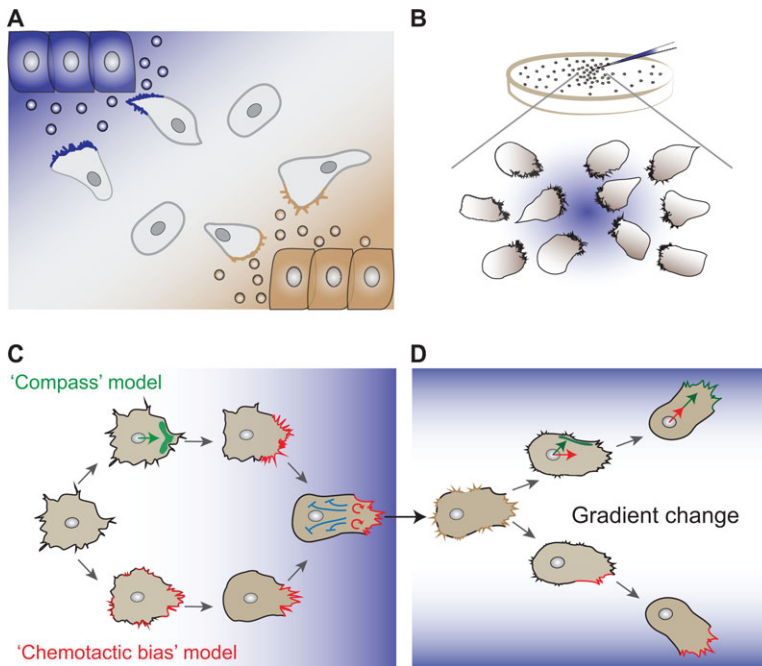
Fig. 4. Chemotaxis and chemotactic gradients. (A) Chemotaxis is the process by which cells undergo directed migration towards higher or lower concentrations of chemical stimuli. In the example shown, cells are attracted by gradients of two different chemicals (depicted in blue and brown). (B) In the chemotactic 'pipette assay', the open-tip of a pipette previously loaded with an attractant [e.g. cAMP for *D. discoideum*, formyl-methionyl-leucyl-phenylalanine (fMLP) for neutrophils, PDGF for fibroblasts] sets up a diffusion gradient in the culture dish, which attracts cells towards the source. (C) Two main models of chemotaxis have been proposed. In the 'compass' model (top), the sensing of a chemical gradient (purple) leads to intracellular accumulation of signalling proteins (green) that function as the arrow of a compass to indicate the presumptive direction of movement (green arrow). The formation of polarised protrusions (red) then follows the direction of the compass. In the 'chemotactic bias' model (bottom), the self-organising, autocatalytic nature of cellular protrusions (red) is constantly biased towards the gradient, without the need of a compass. Once direction is defined, positive-feedback loops at the cell front (red circular arrows) and long-range inhibitory signals (blue lines) restrict protrusion formation towards the gradient in both models. (D) In the 'compass' (top) and 'chemotactic bias' (bottom) models, cells respond to changes in the chemoattractant gradient by correcting the actual movement (red arrow) to the presumptive direction (green arrow) or through the generation of new biased protrusions towards the new signal source, respectively.

events of 'mechanotaxis' 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 dorsal-directed migration and, as a consequence, become mis-localised and in some cases fail to maintain their original fate to become mesoderm (Woo et al., 2012). These observations suggest that, in addition to its role in cell dispersion, random motility might also serve as a control mechanism for the response of the cell to guidance cues, generating a 'noise' that reduces the ability of cells to respond to weak environmental guidance cues that have the potential to interfere with their normal development (Woo et al., 2012).

When random motility occurs in gradients across developing tissues it may also serve to direct collective cell migration. Such a guidance role is observed during posterior elongation of the

Table 1. Summary of the guidance mechanisms of cell migration occurring during development

| Mechanism/cell type	Model	Migration event	References
Random motility			
Cajal-Retzius cells	Mouse	Tangential spreading in the neocortex	Villar-Cervino et al., 2013
Endoderm	Zebrafish	Random dispersion from the margin to the animal pole in the gastrula	Pezeron et al., 2008; Woo et al., 2012
Deep cells	Annual fish	Scattered cell dispersion on the early embryo	Lesseps et al., 1979
Presomitic mesoderm	Chick	Posterior elongation of the embryo	Benazeraf et al., 2010
ECM and cell-cell mediated contact guidance			
Anterior mesoderm	Urodeles, *Xenopus*	Extension of the anterior mesoderm following aligned ECM fibrils in the BCR	Boucaut et al., 1990; Darribere and Schwarzbauer, 2000; Nakatsuji et al., 1982; Nakatsuji and Johnson, 1984; Winklbauer et al., 1996
Neural crest cells	Axolotl, chick	Early migration from the dorsal neural tube	Lofberg et al., 1980; Newgreen and Thiery, 1980
Primordial germ cells	Mouse, *Xenopus*	Migration from the developing gut to the site of the future gonad	Garcia-Castro et al., 1997; Heasman et al., 1981
Primordial germ cells	*Drosophila*, *C. elegans*	E-cadherin-mediated hitchhiking by the moving endoderm	Chihara and Nance, 2012; DeGennaro et al., 2011
Mesenchymal cells	Killifish	Migration along actinotrichia during fin morphogenesis	Wood and Thorogood, 1984
Endothelial cells	Rat	Capillary sprouting along a scaffold of ECM	Anderson et al., 2004
Gonadotropin-releasing hormone (GnRH) neurons	Mouse	'Neurophilic/axonophilic' cell migration along vomeronasal axons	Cariboni et al., 2007
Pyramidal neurons	Mouse	'Gliophilic' cell migration along radial glial fibres in the developing cortex	Rakic, 1971
Neuroblasts	Mouse	'Vasophilic' cell migration along the rostral migratory stream of the forebrain	Saghatelyan, 2009
Contact inhibition of cell locomotion			
Haemocytes	*Drosophila*	Individual cell dispersal on the ventral side of embryo	Davis et al., 2012; Stramer et al., 2010
Cajal-Retzius cells	Mouse	Individual tangential cell spreading along the cortical marginal zone	Borrell and Marin, 2006; Villar-Cervino et al., 2013
Deep cells	Annual fish	Individual cell dispersal on the embryo surface during epiboly	Lesseps et al., 1979, 1975
Primitive myeloid cells	*Xenopus*	Individual cell scattering from the anterior ventral blood islands	Costa et al., 2008
Anterior visceral endoderm	Mouse	Individual distal-to-proximal cell dispersion on the epiblast surface	Pezeron et al., 2008
Epibranchial placodal precursors	*Xenopus*	Epithelial cell migration away from chasing neural crest cells	Theveneau et al., 2013
Neural crest cells	*Xenopus*	Collective mesenchymal cell migration away from the dorsal neural tube	Carmona-Fontaine et al., 2008, 2011
Chemotaxis			
Primordial germ cells	Zebrafish	Individual cell migration towards the future gonad, mediated by Cxcl12a-Cxcr4b	Blaser et al., 2006; Boldajipour et al., 2008; Kardash et al., 2010; Reichman-Fried et al., 2004
Border cells	*Drosophila*	Epithelial cluster migration towards the oocyte, mediated by PVR-PVF1 and EGFR-Gurken	Bianco et al., 2007; Duchek and Rørth, 2001; Duchek et al., 2001
Posterior lateral line primordium	Zebrafish	Epithelial cluster migration along the horizontal myoseptum, mediated by Cxcl12a-Cxcr4b	Dona et al., 2013; Haas and Gilmour, 2006; Venkiteswaran et al., 2013
Tracheal cells	*Drosophila*	Leading cell migration during branch formation, mediated by Fgf-Branchless	Sutherland et al., 1996
Neural crest cells	*Xenopus*	Mesenchymal collective cell migration to latero-ventral regions, mediated by Cxcl12-Cxcr4	Theveneau et al., 2010, 2013
Anterior mesoderm	*Xenopus*	Mesenchymal collective cell migration to the BCR, mediated by Cxcl12-Cxcr4 and PDGFA-PDGFRα	Fukui et al., 2007; Nagel et al., 2004
Large-scale ECM flow			
Epiblast	Chick	Composite movement of epiblast and sub-epiblastic ECM during gastrulation	Zamir et al., 2008
Mesoderm	Chick	Composite movement of the mesoderm and ECM during gastrulation	Czirok et al., 2004; Filla et al., 2004; Zamir et al., 2006
Ectoderm, mesoderm	Hydra	Composite movement of the two epithelia and intervening mesoglea during bud outgrowth	Aufschnaiter et al., 2011

DEVELOPMENT
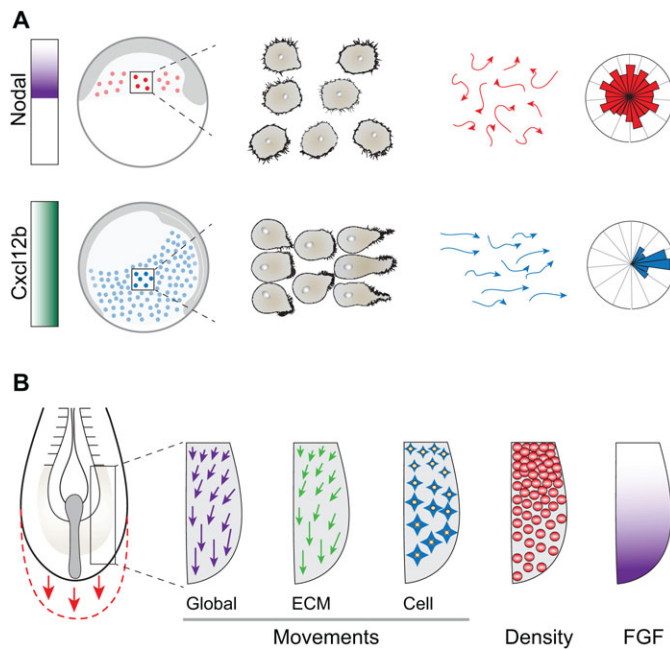
Fig. 5. Random motility in morphogenesis. (A) Zebrafish endodermal cells undergo a switch from random (top) to persistently directed (bottom) migration during gastrulation. The early phase of 'random walk' is induced by Nodal signalling at the blastoderm margin (purple gradient) and is characterised by cells forming non-oriented and unstable membrane protrusions coupled to random cell translocations (red arrows). The late phase of directionally persistent endodermal migration is a response to Cxcl12b/Cxcr4a 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.

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 (Fig. 5B). Accordingly, genetic and chemical disruption of the random motility gradient impairs embryo elongation, and computer simulations that mimic a random motility gradient can induce directional movements in a virtual cell population (Benazeraf et al., 2010).

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 blastocoel roof (BCR) of the amphibian gastrula (Fig. 6C,D). Studies of urodele and anuran embryos have been especially illuminating as they have combined *in vivo* 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 vitro* (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 Darribere, 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; Darribere and Schwarzbauer, 2000; Davidson et al., 2002; Nagel and Winklbauer, 1999; Rozario et al., 2009). Upon contact with FN, mesodermal cells relocalise 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 among 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 glycophosphatidylinositol (GPI)-linked semaphorin 7A (expressed in axons) (Cariboni 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 chemotactic 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
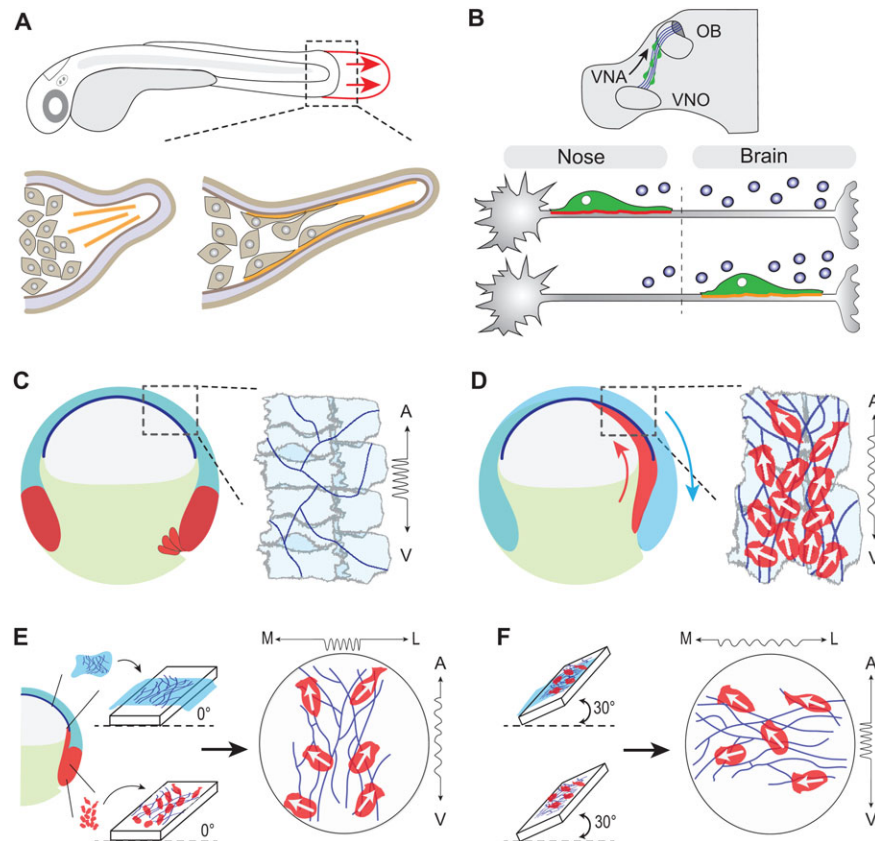
Fig. 6. ECM- and cell-mediated contact guidance during development. (A) During teleost fin morphogenesis, mesenchymal cells migrate along an array of banded collagen fibrils or actinotrichia (orange lines) aligned along the proximal-distal axis of the teleost fin bud. (B) In the developing mouse brain, 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 glycophosphatidylinositol (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).

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
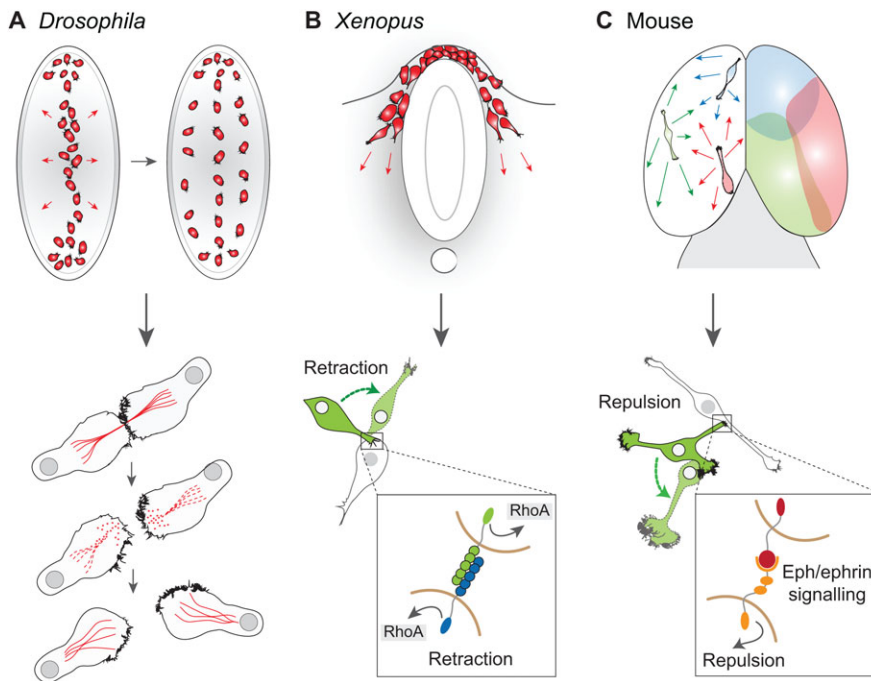
Fig. 7. Contact inhibition of cell locomotion during development. (A) Haemocytes use contact inhibition of locomotion (CIL) to disperse from the ventral midline to form a 'three-lined' organisation in the *Drosophila* embryo. (B) Cranial neural crest cells in *Xenopus* migrate as cellular streams away from the dorsal side of the neural tube towards both sides of the embryo. (C) Cajal-Retzius cells in the E12.5 mouse telencephalon spread tangentially from the cortical hem (red), pallial septum (blue) and ventral pallium (green) to distribute along the marginal zone of the developing neocortex. The proposed mechanisms of CIL are depicted below each example. In haemocytes, CIL involves a collision of lamellae containing conspicuous arms of microtubule bundles (red lines) that disassemble upon cell-cell contact to enable cell repulsion and turning. Neural crest and Cajal-Retzius cells form highly dynamic membrane processes that retract or are repulsed upon cell-cell contact, after which cells repolarise and move away from the cell-cell contact zone. Cell retraction/repulsion is mediated by N-cadherin adhesive interactions and downstream RhoA activation in neural crest cells, and through Ephrin/Eph bidirectional signalling in Cajal-Retzius cells.

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; Theveneau 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 Marin, 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; Tarbashevich and Raz, 2010; Theveneau 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
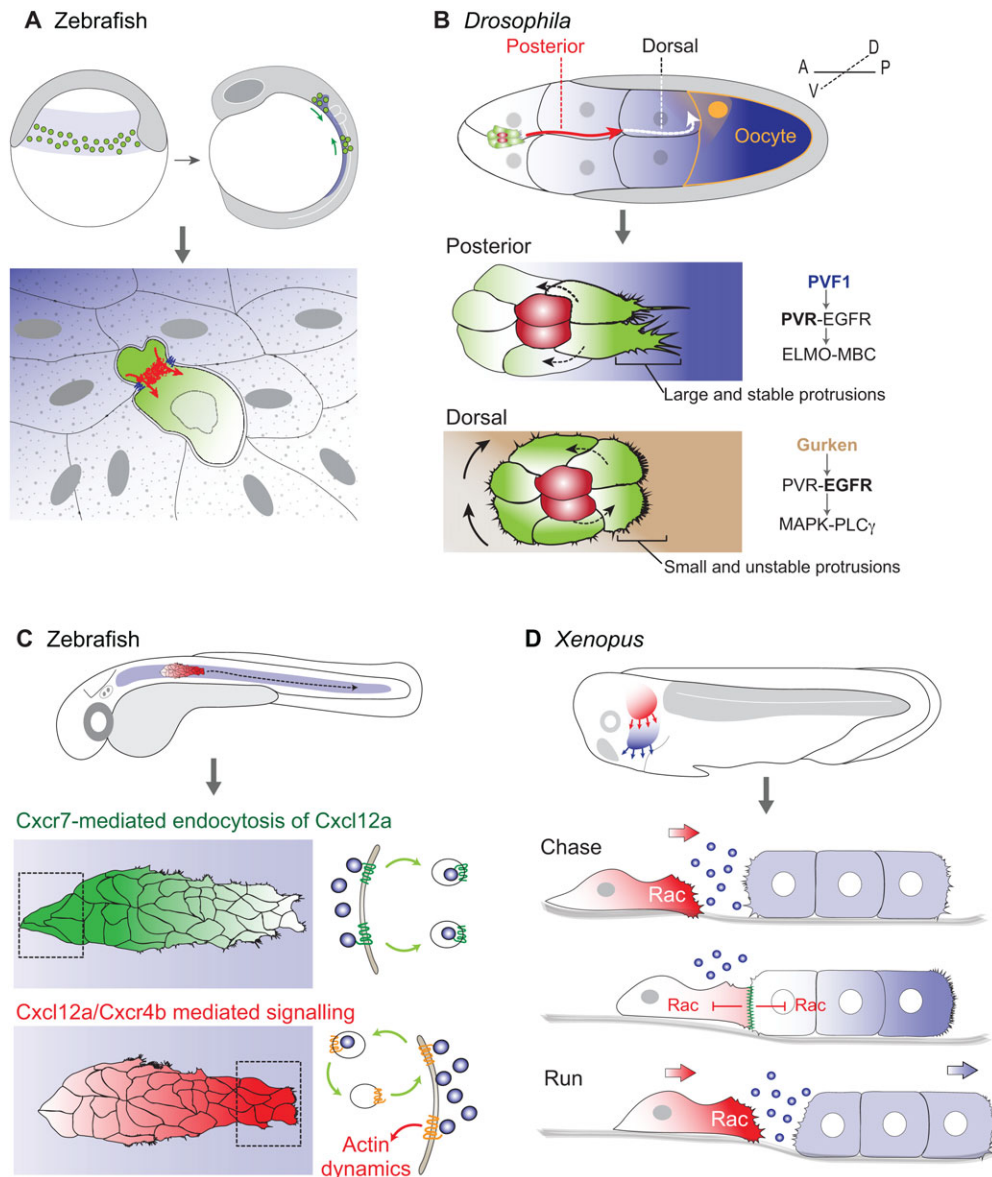
Fig. 8. Chemotaxis and chemotactic gradients during development. (A) Top: Zebrafish primordial germ cells (PGCs, green) begin migrating (green arrows) soon after their specification and cluster in the region of the future gonad, guided by Cxcl12a (purple). Bottom: PGCs move by linking retrograde cortical actin flow (red arrows) to E-cadherin-mediated adhesions (blue) with surrounding somatic cells. A functional chemoattractant gradient (purple) is shaped by somatic cells through Cxcr7-mediated endocytosis of Cxcl12a. (B) Top: *Drosophila* border cells (green/red) migrate in between and along large nurse cells from the anterior pole of the egg chamber, moving in two distinct phases: early posterior (red arrow) and late dorsal (white arrow). Bottom: Border cells migrate as a compact cluster containing two inner polar cells (red) and four to eight migratory cells (green). The PDGF/VEGF-related receptor (PVR) and its ligand PVF1 have a dominant role in posterior migration, when the cluster moves by cells at its front forming long and stable cellular extensions. PVR activates the atypical Rac exchange factor MBC and its co-factor ELMO. Signalling via the EGF receptor (EGFR) and its ligand Gurken, which activates MAPK or phospholipase C γ (PLCγ), is required for dorsal migration, which is characterised by cells forming short, unstable extensions with a front-bias, often exchanging positions (dashed arrows) and sometimes showing rotation of the entire cluster (solid arrows). (C) Top: The zebrafish posterior lateral line primordium (red) forms a cluster of >100 cells that migrates from the anterior postotic area to the tail following a stripe of Cxcl12a (purple). Bottom: During migration, the cluster transforms a uniform stripe of extracellular Cxcl12a (purple) into a gradient of Cxcl12a/Cxcr4b signalling (red) across the primordium. Such a self-generated gradient is achieved by restricting Cxcr7-mediated endocytosis (green) to the rear of the cluster (middle panel, dashed box). Cxcl12a/Cxcr4b signalling directs cell migration by enhancing actin dynamics at the front of the cluster (lower panel, dashed box), where Cxcr4b shows constant turnover involving endocytosis/degradation and delivery of new receptor to the plasma membrane. Right-hand panels depict the process of internalization and turnover of chemokine receptors (Cxcr7 in green, Cxcr4b in orange) in response to the ligand Cxcl12a (purple circles). (D) Top: *Xenopus* cranial neural crest cells (red) move laterally, guided by a moving source of Cxcl12 produced by pre-placodal cells (purple). Bottom: Neural crest and pre-placodal cells coordinate their migration by combining chemotaxis with CIL, engaging in repeating 'chase-and-run' cycles: neural crest cells, which are polarised and activate Rac at their cell front, migrate towards ('chase') pre-placodal cells, attracted by Cxcl12 (purple circles). Upon cell-cell contact (green) CIL induces cell repolarisation through Rac inhibition and pre-placodal cells move away from neural crest cells ('run').

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

DEVELOPMENT

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 (Fig. 8A, bottom) (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 to 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) (Fig. 8B, top) (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 chemoattractants, 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 rescue 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 chemoattractant, 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 the ECM (Theveneau 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 towards the free edge of the collective cell group (Carmona-Fontaine et al., 2008, 2011). As NNCs migrate, they also sense a source of the chemoattractant Cxcl12, which is produced by epithelial epibranchial placodal precursors (EPPs), and they respond to this cue by enhancing the polarity induced by CIL (Theveneau 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) (Theveneau et al., 2013).

Shaping chemoattractant gradients *in vivo*

As highlighted above, cell migration during development is often dependent on gradients of chemoattractants, 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 Cxcr7-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 Cxcl12a 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 chemoattractant 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 chemoattractant 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.

References

Abercrombie, M. (1970). Contact inhibition in tissue culture. *In Vitro* **6**, 128-142.

Abercrombie, M. (1979). Contact inhibition and malignancy. *Nature* **281**, 259-262.

Abercrombie, M. and Ambrose, E. J. (1958). Interference microscope studies of cell contacts in tissue culture. *Exp. Cell Res.* **15**, 332-345.

Abercrombie, M. and Heaysman, J. E. M. (1954). Observations on the social behaviour of cells in tissue culture: II. Monolayering of fibroblasts. *Exp. Cell Res.* **6**, 293-306.

Abercrombie, M. and Middleton, C. A. (1968). Epithelial-mesenchymal interactions affecting locomotion of cells in culture. In *Epithelial-Mesenchymal Interactions* (ed. R. Fleischmajer and R. E. Billingham), p. 56. Baltimore: Williams and Wilkins.

Abercrombie, M., Heaysman, J. E. M. and Pegrum, S. M. (1970a). The locomotion of fibroblasts in culture. I. Movements of the leading edge. *Exp. Cell Res.* **59**, 393-398.

Abercrombie, M., Heaysman, J. E. M. and Pegrum, S. M. (1970b). The locomotion of fibroblasts in culture: II. "Ruffling". *Exp. Cell Res.* **60**, 437-444.

Abercrombie, M., Heaysman, J. E. M. and Pegrum, S. M. (1970c). The locomotion of fibroblasts in culture: III. Movements of particles on the dorsal surface of the leading lamella. *Exp. Cell Res.* **62**, 389-398.

Abercrombie, M., Heaysman, J. E. M. and Pegrum, S. M. (1971). The locomotion of fibroblasts in culture: IV. Electron microscopy of the leading lamella. *Exp. Cell Res.* **67**, 359-367.

Abercrombie, M., Heaysman, J. E. M. and Pegrum, S. M. (1972). Locomotion of fibroblasts in culture: V. Surface marking with concanavalin A. *Exp. Cell Res.* **73**, 536-539.

Abraham, S., Yeo, M., Montero-Balaguer, M., Paterson, H., Dejana, E., Marshall, C. J. and Mavria, G. (2009). VE-Cadherin-mediated cell-cell interaction suppresses sprouting via signaling to MLC2 phosphorylation. *Curr. Biol.* **19**, 668-674.

Aman, A. and Piotrowski, T. (2010). Cell migration during morphogenesis. *Dev. Biol.* **341**, 20-33.

Anderson, C. R., Ponce, A. M. and Price, R. J. (2004). Immunohistochemical identification of an extracellular matrix scaffold that microguides capillary sprouting in vivo. *J. Histochem. Cytochem.* **52**, 1063-1072.

Angelini, T. E., Hannezo, E., Trepat, X., Fredberg, J. J. and Weitz, D. A. (2010). Cell migration driven by cooperative substrate deformation patterns. *Phys. Rev. Lett.* **104**, 168104.

Arrieumerlou, C. and Meyer, T. (2005). A local coupling model and compass parameter for eukaryotic chemotaxis. *Dev. Cell* **8**, 215-227.

Assaker, G., Ramel, D., Wculek, S. K., Gonzalez-Gaitan, M. and Emery, G. (2010). Spatial restriction of receptor tyrosine kinase activity through a polarized endocytic cycle controls border cell migration. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 22558-22563.

Aufschnaiter, R., Zamir, E. A., Little, C. D., Ozbek, S., Munder, S., David, C. N., Li, L., Sarras, M. P., Jr and Zhang, X. (2011). In vivo imaging of basement membrane movement: ECM patterning shapes Hydra polyps. *J. Cell Sci.* **124**, 4027-4038.

Bard, J. B. and Hay, E. D. (1975). The behavior of fibroblasts from the developing avian cornea. Morphology and movement in situ and in vitro. *J. Cell Biol.* **67**, 400-418.

Batchelder, E. L., Hollopeter, G., Campillo, C., Mezanges, X., Jorgensen, E. M., Nassoy, P., Sens, P. and Plastino, J. (2011). Membrane tension regulates motility by controlling lamellipodium organization. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11429-11434.

Bénazéraf, B., Francois, P., Baker, R. E., Denans, N., Little, C. D. and Pourquié, O. (2010). A random cell motility gradient downstream of FGF controls elongation of an amniote embryo. *Nature* **466**, 248-252.

Bianco, A., Poukkula, M., Cliffe, A., Mathieu, J., Luque, C. M., Fulga, T. A. and Rørth, P. (2007). Two distinct modes of guidance signalling during collective migration of border cells. *Nature* **448**, 362-365.

Bindschadler, M. and McGrath, J. L. (2007). Sheet migration by wounded monolayers as an emergent property of single-cell dynamics. *J. Cell Sci.* **120**, 876-884.

Blaser, H., Reichman-Fried, M., Castanon, I., Dumstrei, K., Marlow, F. L., Kawakami, K., Solnica-Krezel, L., Heisenberg, C.-P. and Raz, E. (2006). Migration of zebrafish primordial germ cells: a role for myosin contraction and cytoplasmic flow. *Dev. Cell* **11**, 613-627.

Bokstad, M., Sabanay, H., Dahan, I., Geiger, B. and Medalia, O. (2012). Reconstructing adhesion structures in tissues by cryo-electron tomography of vitrified frozen sections. *J. Struct. Biol.* **178**, 76-83.

Boldajipour, B., Mahabaleshwar, H., Kardash, E., Reichman-Fried, M., Blaser, H., Minina, S., Wilson, D., Xu, Q. and Raz, E. (2008). Control of chemokine-guided cell migration by ligand sequestration. *Cell* **132**, 463-473.

Borrell, V. and Marín, O. (2006). Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. *Nat. Neurosci.* **9**, 1284-1293.

Boucaut, J.-C. and Darribere, T. (1983). Fibronectin in early amphibian embryos. Migrating mesodermal cells contact fibronectin established prior to gastrulation. *Cell Tissue Res.* **234**, 135-145.

Boucaut, J. C., Johnson, K. E., Darribere, T., Shi, D. L., Riou, J. F., Bache, H. B. and Delarue, M. (1990). Fibronectin-rich fibrillar extracellular matrix controls cell migration during amphibian gastrulation. *Int. J. Dev. Biol.* **34**, 139-147.

Boucaut, J. C., Darribere, T., Shi, D. L., Riou, J. F., Delarue, M. and Johnson, K. E. (1991). The amphibian embryo: an experimental model for the in vivo analysis of interactions between embryonic cells and extracellular matrix molecules. *In vivo* **5**, 473-481.

Bourne, H. R. and Weiner, O. (2002). Cell polarity: a chemical compass. *Nature* **419**, 21.

Cariboni, A., Maggi, R. and Parnavelas, J. G. (2007). From nose to fertility: the long migratory journey of gonadotropin-releasing hormone neurons. *Trends Neurosci.* **30**, 638-644.

Carmona-Fontaine, C., Matthews, H. K., Kuriyama, S., Moreno, M., Dunn, G. A., Parsons, M., Stern, C. D. and Mayor, R. (2008). Contact inhibition of locomotion in vivo controls neural crest directional migration. *Nature* **456**, 957-961.

Carmona-Fontaine, C., Theveneau, E., Tzekou, A., Tada, M., Woods, M., Page, K. M., Parsons, M., Lambris, J. D. and Mayor, R. (2011). Complement fragment C3a controls mutual cell attraction during collective cell migration. *Dev. Cell* **21**, 1026-1037.

Carter, S. B. (1965). Principles of cell motility: the direction of cell movement and cancer invasion. *Nature* **208**, 1183-1187.

Carter, S. B. (1967). Haptotaxis and the mechanism of cell motility. *Nature* **213**, 256-260.

Chihara, D. and Nance, J. (2012). An E-cadherin-mediated hitchhiking mechanism for C. elegans germ cell internalization during gastrulation. *Development* **139**, 2547-2556.

Cho, N. K., Keyes, L., Johnson, E., Heller, J., Ryner, L., Karim, F. and Krasnow, M. A. (2002). Developmental control of blood cell migration by the Drosophila VEGF pathway. *Cell* **108**, 865-876.

Coburn, L., Cerone, L., Torney, C., Couzin, I. D. and Neufeld, Z. (2013). Tactile interactions lead to coherent motion and enhanced chemotaxis of migrating cells. *Phys. Biol.* **10**, 046002.

Costa, R. M. B., Soto, X., Chen, Y., Zorn, A. M. and Amaya, E. (2008). spib is required for primitive myeloid development in Xenopus. *Blood* **112**, 2287-2296.

Crick, F. (1970). Diffusion in embryogenesis. *Nature* **225**, 420-422.

Czirók, A., Rongish, B. J. and Little, C. D. (2004). Extracellular matrix dynamics during vertebrate axis formation. *Dev. Biol.* **268**, 111-122.

Dambly-Chaudière, C., Cubedo, N. and Ghysen, A. (2007). Control of cell migration in the development of the posterior lateral line: antagonistic interactions between the chemokine receptors CXCR4 and CXCR7/RDC1. *BMC Dev. Biol.* **7**, 23.

Darribère, T. and Schwarzbauer, J. E. (2000). Fibronectin matrix composition and organization can regulate cell migration during amphibian development. *Mech. Dev.* **92**, 239-250.

Davidson, L. A., Hoffstrom, B. G., Keller, R. and DeSimone, D. W. (2002). Mesendoderm extension and mantle closure in Xenopus laevis gastrulation: combined roles for integrin alpha(5)beta(1), fibronectin, and tissue geometry. *Dev. Biol.* **242**, 109-129.

Davidson, L. A., Keller, R. and DeSimone, D. W. (2004). Assembly and remodeling of the fibrillar fibronectin extracellular matrix during gastrulation and neurulation in Xenopus laevis. *Dev. Dyn.* **231**, 888-895.

Davis, J. R., Huang, C.-Y., Zanet, J., Harrison, S., Rosten, E., Cox, S., Soong, D. Y., Dunn, G. A. and Stramer, B. M. (2012). Emergence of embryonic pattern through contact inhibition of locomotion. *Development* **139**, 4555-4560.

DeGennaro, M., Hurd, T. R., Siekhaus, D. E., Biteau, B., Jasper, H. and Lehmann, R. (2011). Peroxiredoxin stabilization of DE-cadherin promotes primordial germ cell adhesion. *Dev. Cell* **20**, 233-243.

del Rio, A., Perez-Jimenez, R., Liu, R., Roca-Cusachs, P., Fernandez, J. M. and Sheetz, M. P. (2009). Stretching single talin rod molecules activates vinculin binding. *Science* **323**, 638-641.

DeSimone, D. W. and Johnson, K. E. (1991). The Xenopus embryo as a model system for the study of cell-extracellular matrix interactions. *Methods Cell Biol.* **36**, 527-539.

Dickinson, R. B., Guido, S. and Tranquillo, R. T. (1994). Biased cell migration of fibroblasts exhibiting contact guidance in oriented collagen gels. *Ann. Biomed. Eng.* **22**, 342-356.

Dona, E., Barry, J. D., Valentin, G., Quirin, C., Khmelinskii, A., Kunze, A., Durdu, S., Newton, L. R., Fernandez-Minan, A., Huber, W. et al. (2013). Directional tissue migration through a self-generated chemokine gradient. *Nature* **503**, 285-289.

Doyle, A. D., Wang, F. W., Matsumoto, K. and Yamada, K. M. (2009). One-dimensional topography underlies three-dimensional fibrillar cell migration. *J. Cell Biol.* **184**, 481-490.

Dubey, N., Letourneau, P. C. and Tranquillo, R. T. (2001). Neuronal contact guidance in magnetically aligned fibrin gels: effect of variation in gel mechano-structural properties. *Biomaterials* **22**, 1065-1075.

Duchek, P. and Rørth, P. (2001). Guidance of cell migration by EGF receptor signaling during Drosophila oogenesis. *Science* **291**, 131-133.

Duchek, P., Somogyi, K., Jékely, G., Beccari, S. and Rørth, P. (2001). Guidance of cell migration by the Drosophila PDGF/VEGF receptor. *Cell* **107**, 17-26.

Dunn, G. A. (1971). Mutual contact inhibition of extension of chick sensory nerve fibres in vitro. *J. Comp. Neurol.* **143**, 491-507.

Dunn, G. A. (1982). Contact guidance of cultured tissue cells: a survey of potentially relevant properties of the substratum. In *Cell Behaviour* (ed. R. Bellairs, A. S. G. Curtis and G. A. Dunn), pp. 247-280. Cambridge, UK: Cambridge University Press.

Ehrlicher, A. J., Nakamura, F., Hartwig, J. H., Weitz, D. A. and Stossel, T. P. (2011). Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. *Nature* **478**, 260-263.

Elsdale, T. and Bard, J. (1972). Collagen substrata for studies on cell behavior. *J. Cell Biol.* **54**, 626-637.

Farooqui, R. and Fenteany, G. (2005). Multiple rows of cells behind an epithelial wound edge extend cryptic lamellipodia to collectively drive cell-sheet movement. *J. Cell Sci.* **118**, 51-63.

Filla, M. B., Czirok, A., Zamir, E. A., Little, C. D., Cheuvront, T. J. and Rongish, B. J. (2004). Dynamic imaging of cell, extracellular matrix, and tissue movements during avian vertebral axis patterning. *Birth Defects Res. C Embryo Today* **72**, 267-276.

Fraley, S. I., Feng, Y., Krishnamurthy, R., Kim, D.-H., Celedon, A., Longmore, G. D. and Wirtz, D. (2010). A distinctive role for focal adhesion proteins in three-dimensional cell motility. *Nat. Cell Biol.* **12**, 598-604.

Fraley, S. I., Feng, Y., Wirtz, D. and Longmore, G. D. (2011). Reply: reducing background fluorescence reveals adhesions in 3D matrices. *Nat. Cell Biol.* **13**, 5-7.

Fukui, A., Goto, T., Kitamoto, J., Homma, M. and Asashima, M. (2007). SDF-1 alpha regulates mesendodermal cell migration during frog gastrulation. *Biochem. Biophys. Res. Commun.* **354**, 472-477.

Fulga, T. A. and Rørth, P. (2002). Invasive cell migration is initiated by guided growth of long cellular extensions. *Nat. Cell Biol.* **4**, 715-719.

Garber, B. (1953). Quantitative studies on the dependence of cell morphology and motility upon the fine structure of the medium in tissue culture. *Exp. Cell Res.* **5**, 132-146.

Garcia-Castro, M. I., Anderson, R., Heasman, J. and Wylie, C. (1997). Interactions between germ cells and extracellular matrix glycoproteins during migration and gonad assembly in the mouse embryo. *J. Cell Biol.* **138**, 471-480.

Geiger, B. and Yamada, K. M. (2011). Molecular architecture and function of matrix adhesions. *Cold Spring Harb. Perspect. Biol.* **3**, a005033.

Geraldo, S., Simon, A., Elkhatib, N., Louvard, D., Fetler, L. and Vignjevic, D. M. (2012). Do cancer cells have distinct adhesions in 3D collagen matrices and in vivo? *Eur. J. Cell Biol.* **91**, 930-937.

Ghassemi, S., Meacci, G., Liu, S., Gondarenko, A. A., Mathur, A., Roca-Cusachs, P., Sheetz, M. P. and Hone, J. (2012). Cells test substrate rigidity by local contractions on submicrometer pillars. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 5328-5333.

Ghibaudo, M., Saez, A., Trichet, L., Xayaphoummine, A., Browaeys, J., Silberzan, P., Buguin, A. and Ladoux, B. (2008). Traction forces and rigidity sensing regulate cell functions. *Soft Matter* **4**, 1836-1843.

Haas, P. and Gilmour, D. (2006). Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Dev. Cell* **10**, 673-680.

Harris, A. K., Stopak, D. and Wild, P. (1981). Fibroblast traction as a mechanism for collagen morphogenesis. *Nature* **290**, 249-251.

Harrison, R. G., Greenman, M. J., Mall, F. P. and Jackson, C. M. (1907). Observations of the living developing nerve fiber. *Anat. Rec.* **1**, 116-128.

Harunaga, J. S. and Yamada, K. M. (2011). Cell-matrix adhesions in 3D. *Matrix Biol.* **30**, 363-368.

Hazelbauer, G. L. (2012). Bacterial chemotaxis: the early years of molecular studies. *Ann. Rev. Microbiol.* **66**, 285-303.

Heasman, J., Hynes, R. O., Swan, A. P., Thomas, V. and Wylie, C. C. (1981). Primordial germ cells of Xenopus embryos: the role of fibronectin in their adhesion during migration. *Cell* **27**, 437-447.

Heckman, C. A. (2009). Contact inhibition revisited. *J. Cell. Physiol.* **220**, 574-575.

Houk, A. R., Jilkine, A., Mejean, C. O., Boltyanskiy, R., Dufresne, E. R., Angenent, S. B., Altschuler, S. J., Wu, L. F. and Weiner, O. D. (2012). Membrane tension maintains cell polarity by confining signals to the leading edge during neutrophil migration. *Cell* 148, 175-188.

Insall, R. H. (2010). Understanding eukaryotic chemotaxis: a pseudopod-centred view. *Nat. Rev. Mol. Cell Biol.* 11, 453-458.

Insall, R. (2013). The interaction between pseudopods and extracellular signalling during chemotaxis and directed migration. *Curr. Opin. Cell Biol.* 25, 526-531.

Janssens, K., Sung, H.-H. and Rorth, P. (2010). Direct detection of guidance receptor activity during border cell migration. *Proc. Natl. Acad. Sci. U.S.A.* 107, 7323-7328.

Jékely, G., Sung, H.-H., Luque, C. M. and Rørth, P. (2005). Regulators of endocytosis maintain localized receptor tyrosine kinase signaling in guided migration. *Dev. Cell* 9, 197-207.

Johnson, K. E., Darribère, T. and Boucaut, J.-C. (1992). Ambystoma maculatum gastrulae have an oriented, fibronectin-containing extracellular matrix. *J. Exp. Zool.* 261, 458-471.

Kardash, E., Reichman-Fried, M., Maître, J.-L., Boldajipour, B., Papusheva, E., Messerschmidt, E.-M., Heisenberg, C.-P. and Raz, E. (2010). A role for Rho GTPases and cell-cell adhesion in single-cell motility in vivo. *Nat. Cell Biol.* 12, 47-53.

Kubow, K. E. and Horwitz, A. R. (2011). Reducing background fluorescence reveals adhesions in 3D matrices. *Nat. Cell Biol.* 13, 3-5.

Kuo, J. C. (2013). Mechanotransduction at focal adhesions: integrating cytoskeletal mechanics in migrating cells. *J. Cell. Mol. Med.* 17, 704-712.

Lesseps, R. J., van Kessel, A. H. M. G. and Denucé, J. M. (1975). Cell patterns and cell movements during early development of an annual fish, Nothobranchius neumanni. *J. Exp. Zool.* 193, 137-146.

Lesseps, R. J., Hall, M. and Murnane, M. B. (1979). Contact inhibition of cell movement in living embryos of an annual fish, Nothobranchius korthausae: its role in the switch from persistent to random cell movement. *J. Exp. Zool.* 207, 459-470.

Letourneau, P. C. (1975). Cell-to-substratum adhesion and guidance of axonal elongation. *Dev. Biol.* 44, 92-101.

Li, R. and Gundersen, G. G. (2008). Beyond polymer polarity: how the cytoskeleton builds a polarized cell. *Nat. Rev. Mol. Cell Biol.* 9, 860-873.

Lo, C.-M., Wang, H.-B., Dembo, M. and Wang, Y.-l. (2000). Cell movement is guided by the rigidity of the substrate. *Biophys. J.* 79, 144-152.

Loesberg, W. A., te Riet, J., van Delft, F. C. M. J. M., Schön, P., Figdor, C. G., Speller, S., van Loon, J. J. W. A., Walboomers, X. F. and Jansen, J. A. (2007). The threshold at which substrate nanogroove dimensions may influence fibroblast alignment and adhesion. *Biomaterials* 28, 3944-3951.

Löfberg, J., Ahlfors, K. and Fällström, C. (1980). Neural crest cell migration in relation to extracellular matrix organization in the embryonic axolotl trunk. *Dev. Biol.* 75, 148-167.

Mayor, R. and Carmona-Fontaine, C. (2010). Keeping in touch with contact inhibition of locomotion. *Trends Cell Biol.* 20, 319-328.

McCarthy, J. B., Palm, S. L. and Furcht, L. T. (1983). Migration by haptotaxis of a Schwann cell tumor line to the basement membrane glycoprotein laminin. *J. Cell Biol.* 97, 772-777.

Meighan, C. M. and Schwarzbauer, J. E. (2007). Control of C. elegans hermaphrodite gonad size and shape by vab-3/Pax6-mediated regulation of integrin receptors. *Genes Dev.* 21, 1615-1620.

Meighan, C. M. and Schwarzbauer, J. E. (2008). Temporal and spatial regulation of integrins during development. *Curr. Opin. Cell Biol.* 20, 520-524.

Messina, A. and Giacobini, P. (2013). Semaphorin signaling in the development and function of the gonadotropin hormone-releasing hormone system. *Front. Endocrinol.* 4, 133.

Metchnikoff, E. (1893). *Lectures on the Comparative Pathology of Inflammation.* London: Kegan Paul, Trench, Trubner & Co. Ltd.

Ming, G.-l., Wong, S. T., Henley, J., Yuan, X.-B., Song, H.-j., Spitzer, N. C. and Poo, M.-m. (2002). Adaptation in the chemotactic guidance of nerve growth cones. *Nature* 417, 411-418.

Montell, D. J., Yoon, W. H. and Starz-Gaiano, M. (2012). Group choreography: mechanisms orchestrating the collective movement of border cells. *Nat. Rev. Mol. Cell Biol.* 13, 631-645.

Nagel, M. and Winklbauer, R. (1999). Establishment of substratum polarity in the blastocoel roof of the Xenopus embryo. *Development* 126, 1975-1984.

Nagel, M., Tahinci, E., Symes, K. and Winklbauer, R. (2004). Guidance of mesoderm cell migration in the Xenopus gastrula requires PDGF signaling. *Development* 131, 2727-2736.

Nakatsuji, N. and Johnson, K. E. (1983a). Comparative study of extracellular fibrils on the ectodermal layer in gastrulae of five amphibian species. *J. Cell Sci.* 59, 61-70.

Nakatsuji, N. and Johnson, K. E. (1983b). Conditioning of a culture substratum by the ectodermal layer promotes attachment and oriented locomotion by amphibian gastrula mesodermal cells. *J. Cell Sci.* 59, 43-60.

Nakatsuji, N. and Johnson, K. E. (1984). Experimental manipulation of a contact guidance system in amphibian gastrulation by mechanical tension. *Nature* 307, 453-455.

Nakatsuji, N., Gould, A. C. and Johnson, K. E. (1982). Movement and guidance of migrating mesodermal cells in Ambystoma maculatum gastrulae. *J. Cell Sci.* 56, 207-222.

Nelson, C. M., Pirone, D. M., Tan, J. L. and Chen, C. S. (2004). Vascular endothelial-cadherin regulates cytoskeletal tension, cell spreading, and focal adhesions by stimulating RhoA. *Mol. Biol. Cell* 15, 2943-2953.

Newgreen, D. and Thiery, J.-P. (1980). Fibronectin in early avian embryos: synthesis and distribution along the migration pathways of neural crest cells. *Cell Tissue Res.* 211, 269-291.

Niewiadomska, P., Godt, D. and Tepass, U. (1999). DE-Cadherin is required for intercellular motility during Drosophila oogenesis. *J. Cell Biol.* 144, 533-547.

Pacquelet, A. and Rørth, P. (2005). Regulatory mechanisms required for DE-cadherin function in cell migration and other types of adhesion. *J. Cell Biol.* 170, 803-812.

Pankov, R., Endo, Y., Even-Ram, S., Araki, M., Clark, K., Cukierman, E., Matsumoto, K. and Yamada, K. M. (2005). A Rac switch regulates random versus directionally persistent cell migration. *J. Cell Biol.* 170, 793-802.

Parsons, J. T., Horwitz, A. R. and Schwartz, M. A. (2010). Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat. Rev. Mol. Cell Biol.* 11, 633-643.

Pelham, R. J., Jr and Wang, Y.-l. (1997). Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl. Acad. Sci. U.S.A.* 94, 13661-13665.

Petrie, R. J., Doyle, A. D. and Yamada, K. M. (2009). Random versus directionally persistent cell migration. *Nat. Rev. Mol. Cell Biol.* 10, 538-549.

Pézeron, G., Mourrain, P., Courty, S., Ghislain, J., Becker, T. S., Rosa, F. M. and David, N. B. (2008). Live analysis of endodermal layer formation identifies random walk as a novel gastrulation movement. *Curr. Biol.* 18, 276-281.

Pfeffer, W. (1884). Locomotorische Richtungsbewegungen durch chemische Reise. *Untersuch. Bot. Inst. Tübingen* 1, 363-482.

Poukkula, M., Cliffe, A., Changede, R. and Rorth, P. (2011). Cell behaviors regulated by guidance cues in collective migration of border cells. *J. Cell Biol.* 192, 513-524.

Prasad, M. and Montell, D. J. (2007). Cellular and molecular mechanisms of border cell migration analyzed using time-lapse live-cell imaging. *Dev. Cell* 12, 997-1005.

Provenzano, P. P., Eliceiri, K. W., Campbell, J. M., Inman, D. R., White, J. G. and Keely, P. J. (2006). Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC Med.* 4, 38.

Provenzano, P. P., Inman, D. R., Eliceiri, K. W., Trier, S. M. and Keely, P. J. (2008). Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. *Biophys. J.* 95, 5374-5384.

Rakic, P. (1971). Guidance of neurons migrating to the fetal monkey neocortex. *Brain Res.* 33, 471-476.

Ralphs, J. R., Waggett, A. D. and Benjamin, M. (2002). Actin stress fibres and cell-cell adhesion molecules in tendons: organisation in vivo and response to mechanical loading of tendon cells in vitro. *Matrix Biol.* 21, 67-74.

Ramel, D., Wang, X., Laflamme, C., Montell, D. J. and Emery, G. (2013). Rab11 regulates cell-cell communication during collective cell movements. *Nat. Cell Biol.* 15, 317-324.

Reichman-Fried, M., Minina, S. and Raz, E. (2004). Autonomous modes of behavior in primordial germ cell migration. *Dev. Cell* 6, 589-596.

Reinhart-King, C. A., Dembo, M. and Hammer, D. A. (2005). The dynamics and mechanics of endothelial cell spreading. *Biophys. J.* 89, 676-689.

Rickert, P., Weiner, O. D., Wang, F., Bourne, H. R. and Servant, G. (2000). Leukocytes navigate by compass: roles of PI3Kgamma and its lipid products. *Trends Cell Biol.* 10, 466-473.

Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T. and Horwitz, A. R. (2003). Cell migration: integrating signals from front to back. *Science* 302, 1704-1709.

Roca-Cusachs, P., Sunyer, R. and Trepat, X. (2013). Mechanical guidance of cell migration: lessons from chemotaxis. *Curr. Opin. Cell Biol.* 25, 543-549.

Rørth, P. (2002). Initiating and guiding migration: lessons from border cells. *Trends Cell Biol.* 12, 325-331.

Rozario, T., Dzamba, B., Weber, G. F., Davidson, L. A. and DeSimone, D. W. (2009). The physical state of fibronectin matrix differentially regulates morphogenetic movements in vivo. *Dev. Biol.* 327, 386-398.

Saghatelyan, A. (2009). Role of blood vessels in the neuronal migration. *Semin. Cell Dev. Biol.* 20, 744-750.

Scherber, C., Aranyosi, A. J., Kulemann, B., Thayer, S. P., Toner, M., Iliopoulos, O. and Irimia, D. (2012). Epithelial cell guidance by self-generated EGF gradients. *Integr. Biol.* 4, 259-269.

Shields, J. D., Fleury, M. E., Yong, C., Tomei, A. A., Randolph, G. J. and Swartz, M. A. (2007). Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling. *Cancer Cell* 11, 526-538.

Steketee, M. B. and Tosney, K. W. (1999). Contact with isolated sclerotome cells steers sensory growth cones by altering distinct elements of extension. *J. Neurosci.* 19, 3495-3506.

Stopak, D. and Harris, A. K. (1982). Connective tissue morphogenesis by fibroblast traction: I. Tissue culture observations. *Dev. Biol.* 90, 383-398.

Stramer, B., Moreira, S., Millard, T., Evans, I., Huang, C.-Y., Sabet, O., Milner, M., Dunn, G., Martin, P. and Wood, W. (2010). Clasp-mediated microtubule bundling regulates persistent motility and contact repulsion in Drosophila macrophages in vivo. *J. Cell Biol.* 189, 681-689.

Sutherland, D., Samakovlis, C. and Krasnow, M. A. (1996). branchless encodes a Drosophila FGF homolog that controls tracheal cell migration and the pattern of branching. *Cell* **87**, 1091-1101.

Swaney, K. F., Huang, C.-H. and Devreotes, P. N. (2010). Eukaryotic chemotaxis: a network of signaling pathways controls motility, directional sensing, and polarity. *Ann. Rev. Biophys.* **39**, 265-289.

Tambe, D. T., Hardin, C. C., Angelini, T. E., Rajendran, K., Park, C. Y., Serra-Picamal, X., Zhou, E. H., Zaman, M. H., Butler, J. P., Weitz, D. A. et al. (2011). Collective cell guidance by cooperative intercellular forces. *Nat. Materials* **10**, 469-475.

Tarbashevich, K. and Raz, E. (2010). The nuts and bolts of germ-cell migration. *Curr. Opin. Cell Biol.* **22**, 715-721.

Teddy, J. M. and Kulesa, P. M. (2004). In vivo evidence for short- and long-range cell communication in cranial neural crest cells. *Development* **131**, 6141-6151.

Teixeira, A. I., Abrams, G. A., Bertics, P. J., Murphy, C. J. and Nealey, P. F. (2003). Epithelial contact guidance on well-defined micro- and nanostructured substrates. *J. Cell Sci.* **116**, 1881-1892.

Theveneau, E. and Mayor, R. (2012). Neural crest delamination and migration: from epithelium-to-mesenchyme transition to collective cell migration. *Dev. Biol.* **366**, 34-54.

Theveneau, E., Marchant, L., Kuriyama, S., Gull, M., Moepps, B., Parsons, M. and Mayor, R. (2010). Collective chemotaxis requires contact-dependent cell polarity. *Dev. Cell* **19**, 39-53.

Theveneau, E., Steventon, B., Scarpa, E., Garcia, S., Trepat, X., Streit, A. and Mayor, R. (2013). Chase-and-run between adjacent cell populations promotes directional collective migration. *Nat. Cell Biol.* **15**, 763-772.

Tranquillo, R. T. (1999). Self-organization of tissue-equivalents: the nature and role of contact guidance. *Biochem. Soc. Symp.* **65**, 27-42.

Trinkaus, J. P. (1969). *Cells into Organs: The Forces that Shape the Embryo*. Englewood Cliffs, NJ: Prentice-Hall.

Valentin, G., Haas, P. and Gilmour, D. (2007). The chemokine SDF1a coordinates tissue migration through the spatially restricted activation of Cxcr7 and Cxcr4b. *Curr. Biol.* **17**, 1026-1031.

Venkiteswaran, G., Lewellis, S. W., Wang, J., Reynolds, E., Nicholson, C. and Knaut, H. (2013). Generation and dynamics of an endogenous, self-generated signaling gradient across a migrating tissue. *Cell* **155**, 674-687.

Veselý, P. and Weiss, R. A. (1973). Cell locomotion and contact inhibition of normal and neoplastic rat cells. *Int. J. Cancer* **11**, 64-76.

Villar-Cerviño, V., Molano-Mazón, M., Catchpole, T., Valdeolmillos, M., Henkemeyer, M., Martínez, L. M., Borrell, V. and Marín, O. (2013). Contact repulsion controls the dispersion and final distribution of Cajal-Retzius cells. *Neuron* **77**, 457-471.

von Philipsborn, A. and Bastmeyer, M. (2007). Mechanisms of gradient detection: a comparison of axon pathfinding with eukaryotic cell migration. *Int. Rev. Cytol.* **263**, 1-62.

Vorotnikov, A. V. (2011). Chemotaxis: movement, direction, control. *Biochemistry* (*Mosc.*) **76**, 1528-1555.

Wang, F. (2009). The signaling mechanisms underlying cell polarity and chemotaxis. *Cold Spring Harb. Perspect. Biol.* **1**, a002980.

Wang, X., He, L., Wu, Y. I., Hahn, K. M. and Montell, D. J. (2010). Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo. *Nat. Cell Biol.* **12**, 591-597.

Wang, Y., Chen, C.-L. and Iijima, M. (2011). Signaling mechanisms for chemotaxis. *Dev. Growth Diff.* **53**, 495-502.

Webb, A., Clark, P., Skepper, J., Compston, A. and Wood, A. (1995). Guidance of oligodendrocytes and their progenitors by substratum topography. *J. Cell Sci.* **108**, 2747-2760.

Weber, G. F., Bjerke, M. A. and DeSimone, D. W. (2012). A mechanoresponsive cadherin-keratin complex directs polarized protrusive behavior and collective cell migration. *Dev. Cell* **22**, 104-115.

Weber, M., Hauschild, R., Schwarz, J., Moussion, C., de Vries, I., Legler, D. F., Luther, S. A., Bollenbach, T. and Sixt, M. (2013). Interstitial dendritic cell guidance by haptotactic chemokine gradients. *Science* **339**, 328-332.

Weiss, P. (1945). Experiments on cell and axon orientation in vitro: the role of colloidal exudates in tissue organization. *J. Exp. Zool.* **100**, 353-386.

Weiss, P. (1959). Cellular dynamics. *Rev. Mod. Phys.* **31**, 11-20.

Weiss, P. (1961). Guiding principles of cell locomotion and cell aggregation. *Exp. Cell Res.* **8** Suppl., 260-281.

Weiss, P. and Garber, B. (1952). Shape and movement of mesenchyme cells as functions of the physical structure of the medium: contributions to a quantitative morphology. *Proc. Natl. Acad. Sci. U.S.A.* **38**, 264-280.

Weiss, P. and Taylor, A. C. (1956). Fish scales as a substratum for uniform orientation of cells in vitro. *Anat. Rec.* **124**, 381.

Winklbauer, R. and Keller, R. E. (1996). Fibronectin, mesoderm migration, and gastrulation in Xenopus. *Dev. Biol.* **177**, 413-426.

Winklbauer, R. and Nagel, M. (1991). Directional mesoderm cell migration in the Xenopus gastrula. *Dev. Biol.* **148**, 573-589.

Winklbauer, R. and Selchow, A. (1992). Motile behavior and protrusive activity of migratory mesoderm cells from the Xenopus gastrula. *Dev. Biol.* **150**, 335-351.

Winklbauer, R., Nagel, M., Selchow, A. and Wacker, S. (1996). Mesoderm migration in the Xenopus gastrula. *Int. J. Dev. Biol.* **40**, 305-311.

Woo, S., Housley, M. P., Weiner, O. D. and Stainier, D. Y. R. (2012). Nodal signaling regulates endodermal cell motility and actin dynamics via Rac1 and Prex1. *J. Cell Biol.* **198**, 941-952.

Wood, A. (1988). Contact guidance on microfabricated substrata: the response of teleost fin mesenchyme cells to repeating topographical patterns. *J. Cell Sci.* **90**, 667-681.

Wood, A. and Thorogood, P. (1984). An analysis of in vivo cell migration during teleost fin morphogenesis. *J. Cell Sci.* **66**, 205-222.

Wood, W., Faria, C. and Jacinto, A. (2006). Distinct mechanisms regulate hemocyte chemotaxis during development and wound healing in Drosophila melanogaster. *J. Cell Biol.* **173**, 405-416.

Xu, H., Ye, D., Behra, M., Burgess, S., Chen, S. and Lin, F. (2014). Gß1 controls collective cell migration by regulating the protrusive activity of leader cells in the posterior lateral line primordium. *Dev. Biol.* **385**, 316-327.

Zamir, E. A., Czirok, A., Cui, C., Little, C. D. and Rongish, B. J. (2006). Mesodermal cell displacements during avian gastrulation are due to both individual cell-autonomous and convective tissue movements. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 19806-19811.

Zamir, E. A., Rongish, B. J. and Little, C. D. (2008). The ECM moves during primitive streak formation-computation of ECM versus cellular motion. *PLoS Biol.* **6**, e247.