Collective cell migration in development

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Summary

Collective cell migration is a key process during the development of most organisms. It can involve either the migration of closely packed mesenchymal cells that make dynamic contacts with frequently changing neighbour cells, or the migration of epithelial sheets that typically display more stable cell-cell interactions and less frequent changes in neighbours. These collective movements can be controlled by short- or long-range dynamic gradients of extracellular signalling molecules, depending on the number of cells involved and their distance of migration. These gradients are sensed by some or all of the migrating cells and translated into directed migration, which in many settings is further modulated by cell-contact-mediated attractive or repulsive interactions that result in contact-following or contact-inhibition of locomotion, respectively. Studies of collective migration of groups of epithelial cells during development indicate that, in some cases, only leader cells sense and migrate up an external signal gradient, and that adjacent cells follow through strong cell-cell contacts. In this Commentary, I review studies of collective cell migration of differently sized cell populations during the development of several model organisms, and discuss our current understanding of the molecular mechanisms that coordinate this migration.

This article is part of a Minifocus on collective cell migration. For further reading, please see related articles: 'Mechanisms of collective cell migration at a glance' by Olga Ilina and Peter Friedl (*J. Cell Sci.* **122**, 3203-3208) and 'Wound repair at a glance' by Tanya Shaw and Paul Martin (*J. Cell Sci.* **122**, 3209-3213).

Key words: Cell-cell signalling, Collective migration, Chemotaxis, Gradient sensing, Motive force

Introduction

Collective cell migration plays a key role in developmental processes that range from gastrulation to organogenesis. In many cases, individual cells migrate actively in large cohorts of closely interacting cells over long distances. However, cells can also migrate as epithelial sheets, in which there is often limited change in neighbour relationships. The mesenchymal migration of *Dictyostelium discoideum* cells, or of neural crest cells and ingressing mesoderm cells during the development of higher organisms, is better understood than the migration of epithelial sheets: although mesenchymal cells are in frequent contact with their neighbours, their migration occurs in a manner similar to that of single cells, about which much is known (Keller, 2005). Migrating mesenchymal cells extend lamellopodia and filopodia at their leading edges in an actin-dependent process, make and break contacts with neighbouring cells and the extracellular matrix, and pull the cell body forward through the action of myosin thick filaments located mainly at the rear of the cell (Affolter and Weijer, 2005; Ridley et al., 2003). During development, mesenchymal cells can move in cohorts in a collective manner to their destination. Their behaviour is often orchestrated by a collective signal, which might require that all cells have access to guidance information and the ability to interpret this information individually. Alternatively, coordination of mesenchymal migration might be achieved by only selected cells that read a signal and then instruct other cells to follow them by relaying the guidance information to follower cells through chemical or mechanical signalling. In some situations, a mixture of these two mechanisms might operate (Garcia and Parent, 2008; Goswami et al., 2005).

It is much more difficult to understand how cells in epithelial sheets move, because these cells have a distinct apical-basal polarity, interact strongly with each other through adherens and tight junctions at their apical side, and interact with a complex basal lamina at their basal side. These interactions restrict the movement of the epithelial cells within the sheet and limit the rearrangements that can occur between them (Zallen and Blankenship, 2008). However, it is known that local cell rearrangements in epithelial sheets do occur, and that when they occur throughout a tissue they cause large-scale tissue deformations and the transport of cells over large distances, as observed during gastrulation (Keller, 2005). It is commonly thought that, in order for epithelial cells to move actively over long distances, they must undergo at least a partial epithelial-to-mesenchymal transition (EMT), which causes to the cells loosen their strong junctional interactions and become individual polarised mesenchymal-like cells (Montell, 2008; Revenu and Gilmour, 2009). This transition allows for greater freedom of movement and even allows neighbouring cells to move to distinct locations.

In this Commentary, I discuss collective cell migration in development using examples from *Dictyostelium*, zebrafish, *Drosophila*, and gastrulation in higher organisms. To set the stage, I begin with a description of the mechanisms that might be involved in instructing cell movement in these different settings.

Instructing collective cell migration

In development, collective cell migration is a process by which groups of cells are transported to new locations, where they are required to perform specific functions. Cells require precise guidance signals to instruct them how to reach their specific destinations. The number of migrating cells is highly variable: it can involve as few as ten cells (as in border-cell migration during *Drosophila* egg chamber development); hundreds of cells [as occurs during the migration of the lateral line primordium (LLP) in zebrafish]; thousands of cells (as in the migration of mesendoderm cells during gastrulation in higher organisms) or as many as several hundred thousand cells (as in *Dictyostelium* slug development). In

addition, the distances that the cells move can vary widely from as few as tens of microns to centimetres. Thus, it is clear that the signals that guide these movements must also vary considerably (Affolter and Weijer, 2005). When cells move over short distances (in the range of tens of cell diameters), diffusion gradients of chemicals are effective guidance signals (Fig. 1A). When the migration distance is greater, 'trails' of guidance substances might operate. Finally, if the migration distance is very great (millimetre to centimetre range), other mechanisms such as signal relay are probably involved to counteract the attenuation of the signal over distance (Fig. 1B). Specific examples of each of these situations are discussed in more detail below.

In all cases, it remains a major question whether all of the migrating cells sense the guidance signals and derive information from this, or whether only some leader cells read the primary guidance signal and then instruct other cells to follow them via chemical signals or physical cell-cell or cell-matrix interactions. In addition, whether feedback mechanisms that link signalling and movement exist – for instance the migrating cells might modulate the signal – is a question that has yet to be addressed.

Collective cell migration in *Dictyostelium*

The *Dictyostelium* life cycle

In evolutionary terms, *Dictyostelium* is a simple organism, and sits on the threshold between single cell and multicellular life (Baldauf and Doolittle, 1997). It has been used extensively to study the molecular machinery of cellular polarisation and chemotaxis, but also has an interesting multicellular life cycle that is dominated by collective cell migration. Normally, the organism lives as a single amoeba in soil, but in response to nutrient starvation it enters a multicellular developmental cycle in which up to a million amoeboid cells can aggregate to form a motile slug structure. Under appropriate environmental conditions, the slug transforms into a fruiting body, which consists of a stalk that supports a mass of spores (Kessin, 2001; Weijer, 2004). *Dictyostelium* cells are in direct contact during the multicellular stages of development, but stay amoeboid during all stages of this multicellular life cycle until the very last stages of fruiting body formation when the cells terminally differentiate into spores and dead vacuolated stalk cells.

Cell migration during the multicellular stages of *Dictyostelium* slug development is highly similar to the collective migration of mesenchymal cells that occurs in higher organisms. In both settings, the migrating cells are closely packed, make dynamic contacts with surrounding cells and move collectively in response to a dynamic signal. *Dictyostelium* cells get traction from surrounding cells and from a specialised extracellular matrix, the slime sheath, which they secrete and modulate. Important questions that this model has helped to investigate include which signals control collective cell migration, how are these signals detected and interpreted, and what are the mechanisms of collective movement?

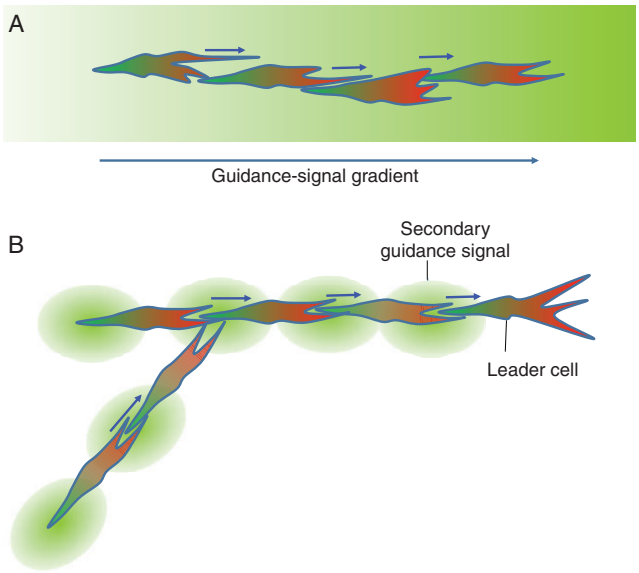

Fig. 1. Different modes of signalling that might operate during collective migration. (A) All cells detect an extracellular gradient of a guidance factor (green) and read a signal, become polarised (indicated by red and green colouring of cells) in response to this signal and move in the direction of the gradient. (B) Only leader cells sense a primary extracellular guidance cue, and then instruct other cells to follow them. This can be achieved either by localised secretion of a secondary guidance signal (green), which is then detected by follower cells and used to polarise their behaviour. Alternatively, this interaction could be mechanical, whereby the leader cells 'pull' on follower cells, resulting in their polarisation and movement in the direction of the leader cell. Finally, it could be that that the cells sense local deformations of the ECM as a result of the remodelling activity of the preceding cells. These mechanical interactions are indicated by the blue arrows.

cAMP signalling centres

Cell movement during the multicellular stages of *Dictyostelium* development is controlled by a series of signalling centres that emit periodic pulses of cyclic AMP (cAMP). The initial aggregation phase has been well investigated: aggregation of the starving cells is mediated by chemotaxis in response to cAMP 'waves' secreted by a group of cells that form a signalling centre (Fig. 2A). Starvation triggers the expression of many genes that are involved in cAMP production and detection and that are responsible for aggregation. Such genes include those that encode cAMP receptors, G-proteins and the aggregation-stage adenylylcyclase (ACA) (Kessin, 2001). cAMP produced by the cells in the signalling centre is degraded continuously by an extracellular phosphodiesterase (PDE). However, a proportion of the secreted cAMP binds to its receptors and, in an autocrine cAMP amplification step, results in the production and secretion of more cAMP in a so-called cAMP relay reaction (Mahadeo and Parent, 2006). Cells that express more ACA, more cAMP receptors and/or less PDE (owing to the stochastic nature of gene expression) secrete more cAMP than their neighbours and thus locally amplify the cAMP signal in an autocrine manner, which then spreads by diffusion to neighbouring cells. Once these neighbouring cells are stimulated with cAMP above a certain threshold concentration, they in turn produce and secrete cAMP, passing the signal on to their neighbours and propagating the cAMP wave from the signalling centre outward. These waves typically propagate as spirals, as is typical for excitable systems (Fig. 2A) (Siegert and Weijer, 1989; Siegert and Weijer, 1991).

The binding of cAMP also triggers an adaptation process that, after a certain time delay, results in the desensitisation of the cells to cAMP and a cessation of cAMP production (Comer and Parent, 2006). As cAMP is degraded continuously by the extracellular PDE, cAMP concentrations fall, which results in a gradual re-sensitisation of the cells. As result of chemotaxis, the cells that initiated the cAMP wave have at this point attracted more cells, resulting in a cell density increase near the signalling centre. This increases the probability that a new wave will be initiated at this location, establishing these cells as a signalling centre (Dormann et al., 2001; Mahadeo and Parent, 2006). When the wave passes and the cAMP gradient

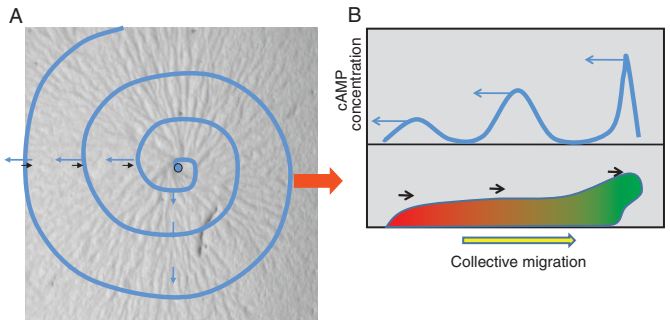

Fig. 2. Collective cell migration in *Dictyostelium* is controlled by cAMP waves. (A) During the development of starving *Dictyostelium* cells, cAMP waves are secreted by cells in the signalling centre and they propagate outward (blue arrows) mainly as spiral waves (blue line). Cells move up the cAMP gradient by chemotaxis (black arrows), resulting in their aggregation at the signalling centre. (B) In the slug stage, cells in the pre-stalk region, cells in the anterior quarter of the slug, and the anterior-like cells (which make up roughly 10% of the cells in the back three-quarters of the slug) express the aggregation stage ACA responsible for cAMP secretion. This results in a gradient in excitability (indicated by a gradient of red to green in the slug, where green indicates the most active region of signalling). Waves are initiated by cells in the tip and relayed by cells in the pre-stalk zone and by anterior-like cells at the rear of the slug. This results in periodic waves of cAMP signalling from the front to the rear of the slug (blue arrows) and the migration of the cells forward (black arrows). The preferential expression of cytoskeletal components such as myosin II in pre-stalk cells provides them with more force than pre-spore cells in response to the cAMP signal and allows them to move towards the front of the slug.

reverses, cells that have been recruited to the signalling centre cannot detect this reverse gradient because they are adapted to peak cAMP levels. This prevents them from turning around and following the wave away from the signalling centre; instead, they stop during the falling phase of the wave and begin to move again when cAMP concentrations rise as a result of the next passing wave. Overall, the chemotaxis of cells towards the cAMP gradient emitted during the rising phase of the wave results in the collective migration of the cells towards the signalling centre.

Coordination of collective movement during *Dictyostelium* aggregation

After a few cAMP waves have passed, cells make contact with each other and form extensive aggregation streams. cAMP waves propagate through the streams outward, directing the movement of the cells towards the signalling centre. Cells in aggregation streams are highly polarised. They are highly elongated and make specific end-to-end contacts through special calcium-independent adhesion molecules, as well as side-to-side contacts through calcium-dependent cadherin-type molecules (Gerisch, 1977; Siu et al., 2004). These cell-cell contacts are presumably important for aggregation; however, deletion of either of the adhesion molecules involved does not result in major defects in stream formation or in multicellular development (Harloff et al., 1989; Wong et al., 2002). Stream formation might involve localised cAMP secretion from the rear of the cell. It has been described that aggregation-stage ACA localises to the rear of aggregating cells and is often associated with small vesicles. It is possible that cAMP is secreted by the fusion of ACA-containing vesicles with plasma membrane via a mechanism that resembles synaptic transmission, whereby one cell directly signals a follower cell (Kriebel et al., 2008; Maeda and Gerisch, 1977). However, earlier experiments showed that the rate of secretion of cAMP was proportional to the internal cAMP concentration, and that there was no appreciable delay between cAMP production and secretion, which supports the idea that cAMP secretion occurs via a pump (Dinauer et al., 1980; Kesbeke and Van Haastert, 1988).

During aggregation of *Dictyostelium*, cells start to differentiate into precursors of the stalk and spore cells. There is no correlation between the tendency of cells to initially differentiate into pre-stalk or pre-spore cells and their position in the forming aggregate; they arrive in the aggregate at different times and form a random distribution of cell types. Once all of the cells have entered the aggregate to form the hemispherical mass of cells known as the 'mound', the pre-stalk cells are sorted from the pre-spore cells to form a distinct morphological structure known as the tip. This cell-sorting process results from the chemotactic aggregation of pre-stalk cells within the mass of pre-spore cells. The mechanism by which this reorganisation occurs is still not completely resolved, but probably involves differential signalling and motility of the pre-stalk and pre-spore cells (Vasiev and Weijer, 2003).

Compared with pre-spore cells, pre-stalk cells express ACA and higher levels of myosin II, and assemble more myosin II thick filaments. The thick filaments provide the cells with the force required to move through the mass of other cells to the cAMP signal coming from the centre of the aggregate (Maeda et al., 2000; Maeda et al., 2003; Verkerke-van Wijk et al., 2001) (Fig. 2B). During slug migration, the cells in the tip periodically produce cAMP signals, which are relayed through the slug by the anterior-like cells that are scattered throughout the body of the slug (Dormann and Weijer, 2001) (Fig. 2B). Tip cells must stay at the front of the slug because they initiate the cAMP signal. Other cells cannot 'overtake' the tip cells unless they acquire the ability to initiate their own cAMP signals and can entrain cAMP secretion by other cells.

It is interesting to note that the cAMP signal is detected through four different receptors that bind to cAMP with different affinities; these receptors are expressed at different stages during the *Dictyostelium* multicellular stage. The high-affinity cAMP receptor, cAR1, is the first receptor to be expressed during *Dictyostelium* aggregation and is responsible for relaying the cAMP signal and for chemotaxis. From the late aggregation stage onwards, a low-affinity cAMP receptor, cAR3, is expressed specifically in pre-spore cells. Finally, there are two lower-affinity receptors, cAR2 and cAR4, that are specifically expressed in pre-stalk cells (Tsujioka et al., 2001). Experiments have shown that cAR1 and cAR3 are required for slug migration (but that slugs can migrate when either one of them is expressed), whereas cAR2 and cAR4 appear to be required for pre-stalk-cell-specific gene expression and are not directly involved in controlling movement (Louis et al., 1994; Saxe et al., 1993) [Dirk Dorman (MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College, London, UK) and C.W., unpublished data].

From the mound stage onwards, the pre-stalk cells secrete a slime sheath, which is an extracellular matrix that keeps the cells together. During slug migration, the slime sheath is synthesised continuously by pre-stalk cells and serves as a substrate for the outer cells of the slug to move on top of and get traction from. The interaction between the slug outer cells and the slime sheath involves specialised cell-matrix-adhesion molecules of the integrin family, and contacts are organised in focal adhesions that contain talin B and paxillin B (Bukharova et al., 2005; Cornillon et al., 2008; Patel et al., 2008). The sheath is stationary with respect to the substrate on which the slug moves and collapses when the slug has moved through, leaving behind a slime trail (Kessin, 2001). The slime sheath keeps the cells

together and even allows the slug to leap through the air between different patches of substrate in its native environment, the upper leaf litter layer of the soil (Sternfeld and O'Mara, 2005).

The slug as a whole can respond to external signals such as light and temperature gradients, but the details of how this occurs are so far unknown. The mechanisms might involve local changes in the speed of cAMP wave propagation, resulting in local changes of cell movement and the steering of the slug towards or away from light and temperature gradients. The photoreceptors and thermoreceptors that might modulate cAMP relay remain to be identified (Fisher et al., 1997; Maree et al., 1999; Miura and Siegert, 2000). Although the study of this simple organism has shed considerable light on the mechanisms that underlie collective cell migration, many detailed questions – especially with respect to the relative importance of signalling pathways and direct cell-cell interactions – remain to be resolved.

Migration of lateral line primordium cells in zebrafish

The development of the lateral line, a mechanosensory organ in fish, involves the directed migration of a cluster of ~100 epithelial-like cells (known as the LLP) over the forming musculature of the developing embryo. The cells in the anterior of the LLP undergo a partial EMT and become more mesenchymal in character, although they remain in close contact with each other and the epithelial-like cells at the rear of the LLP. During this migration process, the LLP drops several mechanosensory organs (known as neuromasts) at regularly spaced intervals during its movement from the anterior head region to the posterior tail region over a period of 2 days. Neuromasts consist of a sensory hair cell innervated by a specialised sensory nerve cell, surrounded by supporting cells. The migration of the LLP appears to follow a line of the chemokine stromal-derived factor 1 (SDF-1), which is expressed by cells of the horizontal myoseptum in the zebrafish embryo, and crucially depends on the expression of an SDF-1 receptor, CXC chemokine receptor 4 (CXCR4), in the cells of the leading edge of the LLP (Haas and Gilmour, 2006) (Fig. 3).

Experiments in which mosaic LLPs (which consist of only a few cells expressing CXCR4 in a background of cells that lack this receptor) were created have shown that only a few CXCR4-expressing cells at the leading edge of the LLP are required to guide its movement. The behaviour of the leader cells is characterised by the extension of protrusions in the direction of the SDF-1 signal; deletion of CXCR4 abolishes the extension of these protrusions and prevents the forward movement of the cells. It has become apparent that the cells in the LLP also express another SDF-1 receptor, CXCR7, which is mainly expressed in the posterior part of the LLP (Valentin et al., 2007). Deletion of this receptor impairs the migration of the LLP, indicating that, in addition to CXCR4, CXCR7 is also important for migration in this setting.

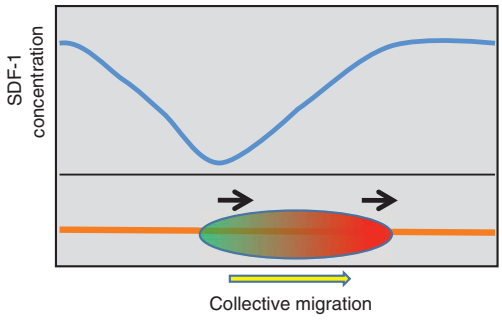

Fig. 3. Proposed mechanism of collective cell migration of the lateral line primordium in zebrafish. Cells in the lateral line primordium (LLP; containing ~100 cells) move in an anterior to posterior direction by following a SDF-1 signal that can be detected as a stripe of *sdf1* RNA expression (orange). Cells in the front of the LLP express the SDF-1 receptor CXCR4 (red), whereas cells at the rear express CXCR7 (green). Internalisation of SDF-1 by cells in the LLP, especially by cells expressing CXCR7 at the rear, result in a gradient of SDF-1 protein (blue concentration profile), which the cells follow.

It has been shown that two signalling pathways control the expression of CXCR4 and CXCR7 and other crucial cell behaviours in the LLP. Wnt signalling appears to be active in the cells of the leading zone of the LLP, whereas fibroblast growth factor (FGF) initiates a signalling cascade in the trailing zone. Wnt signalling represses CXCR7 expression in the leading zone, and FGF signalling might play a role in suppressing CXCR4 expression in the trailing zone (Aman and Piotrowski, 2008). Furthermore, FGF signalling appears to control the formation of rosettes at the rear of the LLP that are associated with the formation of neuromasts by controlling and stabilising the epithelial fate of the cells (Lecaudey et al., 2008; Nechiporuk and Raible, 2008). Inhibition of FGF signalling results in a failure of rosette formation and indirectly inhibits cell migration, possibly by allowing CXCR4 expression throughout the LLP while simultaneously inhibiting CXCR7 expression (Aman and Piotrowski, 2008). However, this remains to be tested in more detail.

Although it has been argued that the SDF-1 signal does not provide directional information for the LLP to follow, evidence from studies of zebrafish support the idea that the regulated expression of CXCR4 and CXCR7 is involved in translating the continuous SDF-1 signal into a graded signal across the LLP, which the migrating cells of the primordium might be able to sense and respond to. It was first suggested that CXCR4 and CXCR7 read and shape an SDF-1 gradient in studies of the migration of primordial germ cells (PCGs) in zebrafish. PCGs cells move as individuals in a coordinated manner in response to a dynamic expression pattern of SDF-1, which is also directed by CXCR4 in this setting (Stebler et al., 2004). Surprisingly, it was recently found that cells in the tissue through which the PGCs migrate abundantly express CXCR7. It was proposed that CXCR7 plays an essential role in controlling the local dynamics of the SDF-1 signal by sequestering and possibly internalising the ligand so that it becomes unavailable for PCG guidance via CXCR4 (Boldajipour et al., 2008; Raz and Mahabaleshwar, 2009). Assuming that a similar mechanism controls the migration of cells in the LLP, CXCR4 expressed by leading edge cells might be responsible for sensing the SDF-1 gradient, and CXCR7 might be involved mainly in shaping the gradient across the LLP through receptor-mediated downregulation (Fig. 3). However, confirmation that there is an SDF-1 gradient across the LLP awaits measurements of in vivo SDF-1 protein concentrations. In addition, the exact mechanisms by which cells might follow such a gradient remain to be resolved. Although direct data are not yet available, it is reasonable to speculate that all cells in the LLP are moving actively. In the simplest case, all cells might read the SDF-1 gradient and contribute to directing cell movement. Alternatively, it might be that only some leader cells read the gradient and signal to follower cells by other chemical and/or mechanical signals.

Collective migration of border cells in *Drosophila*

Another fascinating and well-investigated example of collective cell migration is the migration of border cells in *Drosophila*. These are

cells that derive from the follicular epithelium of the egg chamber and, after undergoing a partial EMT, migrate initially towards the oocyte and then upward to form the micropyle, a structure important for sperm entry. The small cluster of six to eight border cells surrounding two central cells moves between the nurse cells and towards the developing oocyte (Montell, 2003) (Fig. 4). The movement of these cells is guided by signals from the oocyte, in particular epidermal growth factor (EGF) and PVF (PDGF- and PDGF/VEGF-related factor) (Duchek and Rorth, 2001; Rorth, 2002) (Fig. 4). The border cells are epithelial-like in character and, similarly to the cells in the LLP, have an apical-basal polarity and make contact with a basal lamina. It is evident that cell-cell adhesion needs to be dynamically regulated during the migration of border cells, and that the control of cadherin-mediated adhesion is also crucial (Geisbrecht and Montell, 2002; Melani et al., 2008).

Two different phases of border-cell migration have been distinguished (Bianco et al., 2007; Prasad and Montell, 2007; Tekotte et al., 2007). Initially, migration depends on cell polarisation and the extension of long protrusions, and involves clear leader cells. During this initial phase of migration, the cells move in response to gradients of EGF and PVF in a chemotactic process as they polarise and extend long protrusions in the direction of migration towards the oocyte; these are used to pull the cell body forward (Rorth, 2003). In the second phase of migration, the cells extend shorter protrusions and lamellipodia. Interestingly, it was recently described that the same cells are not always found at the front of the migrating cluster; particularly during the second phase of migration, there appears to be a continuous exchange of leader cells (Bianco et al., 2007; Prasad and Montell, 2007). Thus, being a leader cell is a temporary role in this setting.

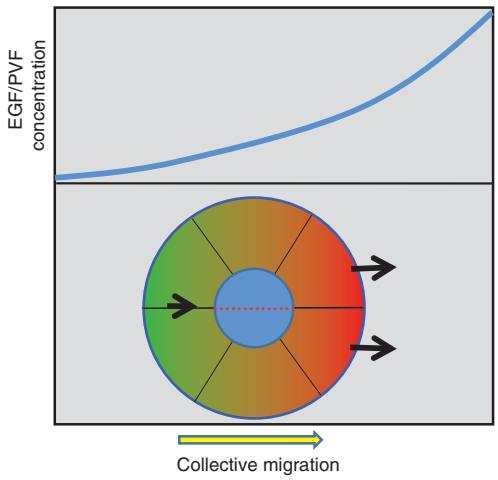

Fig. 4. Proposed mechanism for collective migration of border cells in *Drosophila*. Border cells form a cluster of eight to ten cells. Shown in the diagram are two central cells (blue) surrounded by six peripheral cells, which form a quasi-epithelial structure in which the cells are connected through adherens junctions. This cluster of border cells moves in between nurse cells towards the oocyte (not shown) in response to gradients of EGF and PVF secreted by the oocyte (as depicted in the graph). Detection of the PVF and EGF signal gradients results in MAP kinase activation: cells at the front of the cluster show the highest level of activation (red), and cells at the back show lower levels of activation (green). This difference translates to a motive force that results in movement of the tightly connected cell cluster towards the source of the signal, without the need for specialised permanent leader cells in the cluster.

A key outstanding question is how the cells maintain directionality of movement when the leader cells are continuously changing. Analysis of MAP kinase phosphorylation in fixed egg chambers has shown that there is a gradient of signalling across the cluster of border cells, with highest level of phosphorylation in the most anterior cells of the cluster, without clear differences across individual cells (Bianco et al., 2007). It has been suggested that the cluster of border cells as a whole can sense a gradient along the length of the cell cluster, and that there is no specific requirement for specific cells to be at the front of the cluster for the duration of migration. Rather, the cells at the front might generate a stronger (more polarised) motile response than the cells at the rear of the cluster, and the cells closer to the signal determine the direction migration of the cluster (Rorth, 2007). This migration depends heavily on strong cell-cell contacts. To fully understand the mechanisms at work in this system, it will be necessary to find ways to measure the individual cellular responses to guidance signals in terms of their adhesive interactions and the motive forces that are generated during collective migration.

Collective cell migration during gastrulation

Gastrulation is a period during the development of higher organisms when cells move extensively in a collective manner. During gastrulation, the endoderm and mesoderm (both derived from the mesendoderm) move into the embryo to take up their characteristic positions. The endoderm, which gives rise to the epithelial lining of the digestive tract and associated glands, is surrounded by the mesoderm, which gives rise to the muscles, skeleton and vasculature. The mesoderm is surrounded by the outermost ectoderm, which gives rise to the epidermis and the nervous system. During gastrulation, the epithelial precursors of the mesendoderm undergo a partial (*Xenopus*, fish) or complete (chick, mouse) EMT as they ingress through the blastopore or the primitive streak, respectively (Keller, 2005; Shook and Keller, 2003). In amniotes such as the chick, gastrulation follows with the induction of the mesendoderm at the posterior interface between the extra-embryonic region (area opaca) and the embryonic region (area pellucida) in the epiblast. This begins with the movement of these cells into the midline of the embryo to form the primitive streak, followed by EMT and ingression of the mesendoderm cells. [For details on the similarities and differences in gastrulation in various organisms, see the book by Stern (Stern, 2004).]

Mesenchymal migration

Several competing hypotheses have been proposed to explain the guiding mechanisms that underlie the movement of mesodermal cells during gastrulation. In most species, the cells of the leading edge mesoderm – especially of the prechordal plate mesoderm – are among the first mesendoderm cells to invaginate. These cells undergo a near-complete EMT and migrate towards the anterior part of the embryo essentially as mesenchymal cells. In frogs and fish, it has been shown that cells of the leading edge mesoderm migrate to the anterior in response to graded PDGF signals produced by the overlying ectoderm. During their migration, these cells show extensive protrusive activity of their leading edges in the direction of the PDGF gradient in a phosphoinositide-3-kinase-dependent manner (Montero et al., 2003; Nagel et al., 2004). In addition, there is evidence that this response to PDGF is modulated by sphingosine-1-phosphate (S1P) signalling through the Edg5 receptor expressed by mesoderm cells (Kai et al., 2008).

In contrast to what is known about the leading edge mesoderm, the mechanism of migration of cells in the paraxial and axial mesoderm is less clear. During gastrulation in *Xenopus*, cells of the paraxial mesoderm move from lateral positions towards medial positions in a process known as medio-lateral intercalation, which drives the elongation of the embryo (Keller, 2005). The process starts when the cells become polarised as a result of the planar cell polarity (PCP) signalling pathway, which stimulates cells to acquire polarity in the plane of the cell sheet (Keller, 2002; Klein and Mlodzik, 2005). The cells elongate and restrict their actin-driven protrusive activity to their ends, resulting in bipolar protrusive activity. The cells that are in close contact push and pull on each other and on the extracellular matrix aligning their long axes, and start to move in between each other in the process of medio-lateral intercalation. The mechanisms by which this cellular polarisation is achieved are still unresolved, but there is some evidence that it might depend on signalling through the non-canonical Wnt signalling pathway (Wallingford et al., 2002). The tissue-wide coordination of cell intercalation is probably under the control of the anterior-posterior patterning system; however, the details remain to be established (Ninomiya et al., 2004).

The PCP signalling pathway – possibly through its control of cell polarity – also controls the remodelling and alignment of the extracellular matrix, which might also regulate collective cell migration (Davidson et al., 2006; Goto et al., 2005). Alternatively, it has been proposed that the direction of medial migration of mesoderm cells is regulated by E-cadherin-mediated adhesive gradients, because cells in medial areas are more adhesive than those in lateral positions. The underlying hypothesis is that cells move towards regions of greater adhesion where they can get more traction. These adhesivity gradients are probably controlled by signalling pathways initiated by bone morphogenetic protein (BMP). BMP expression is high in lateral regions of the embryo and low in medial regions, and has been shown to inhibit cell-cell adhesion through an unknown mechanism (von der Hardt et al., 2007).

Finally, it has been proposed that, for fish and amniotes, in which the paraxial and lateral plate mesoderm precursors undergo a complete EMT during their ingression in the primitive streak, mesoderm cells move in response to chemotactic signals. Several potential chemoattractants have been identified. It has been shown in zebrafish that apelin, which is expressed in the midline of the embryo and is detected by the G-protein-coupled serpentine transmembrane receptor Agtrl1b, directs migration of lateral plate mesoderm cells towards the heart field (Zeng et al., 2007). In addition, studies carried out with frog, zebrafish and amniotes have provided evidence that growth factors of the FGF, PDGF and VEGF families direct the migration of axial and paraxial mesodermal cells (Dormann and Weijer, 2006; Solnica-Krezel, 2005). In chick embryos, the combined availability of chemoattractants and chemorepellents steers the paraxial and lateral plate mesoderm cells in specific directions after their ingression through the streak (Dormann and Weijer, 2006) (Fig. 5). Another study of chick embryos showed that FGF8 produced in the primitive streak acts as a repellent that causes cells to move away, whereas FGF4 produced in the midline by the axial mesoderm (headprocesss and notochord) acts as an attractant (Yang et al., 2002). These findings are in agreement with studies carried out in mice showing that the deletion of *fgf8* results in the accumulation of mesodermal cells in the primitive streak (Sun et al., 1999). In addition, cells ingressing through the posterior streak were found to move in response to VEGF signalling triggered by activation of the receptor VEGFR2. These cells were shown to be in direct contact, and it was proposed that leader and follower cells could be distinguished on the basis of differential VEGFR2 internalisation [Xuesong Yang (Key Laboratory for Regenerative Medicine of the Ministry of Education, Medical College, Jinan University, Guangzhou, China), Manli Chuai (Division of Cell and Developmental Biology, College of Life Sciences, University of Dundee, Dundee, UK) and C.W., unpublished observations].

It has been shown that PDGFA secreted by cells in the epiblast and detected by migrating paraxial mesodermal cells through the

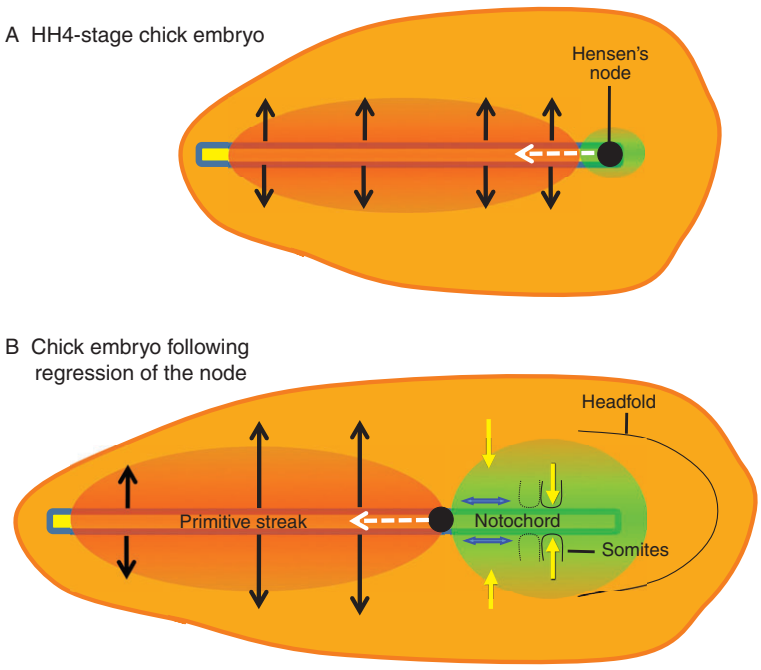

Fig. 5. Collective cell migration of mesoderm cells during the early streak regression stages of chick gastrulation. Shown are two early chick embryos at successive stages of gastrulation. (A) Embryo at stage HH4, the fully extended streak stage. The tip of the primitive streak forms Hensen's node (black dot), which starts to regress in the posterior direction (dashed white arrow). Embryonic regions are shown by orange shading. Black arrows indicate the direction of cell movement. (B) Embryo after regression of the node. Here, the streak has started to regress; the first outline of the head becomes visible and the first somites (the precursors of the muscles and skeleton) are formed. During gastrulation, mesendoderm cells undergo EMT in the primitive streak; they then ingress and move away in all directions as individual mesenchymal cells (black arrows). The cells move in response to signalling pathways triggered by FGF8 and Wnt3a in a process that might involve chemorepulsion. mRNAs encoding FGF8 and Wnt3a show high levels of expression in the primitive streak, which decay rapidly towards peripheral embryonic regions (dark orange shading). The forming notochord secretes FGFs such as FGF4 (in the area shaded in green), which is a strong chemoattractant for mesoderm cells and promotes their movement back towards the midline (yellow arrows) to form paraxial mesoderm once the node has regressed. This movement back towards the midline results in cell intercalation and convergent extension (double blue arrow) of the presomitic mesoderm, which later assembles into somites. Cells that migrate out of the middle and posterior streak migrate for longer periods of time, before the node regresses past them. As a consequence, these cells end up in more lateral positions, and migrate back to form the lateral somitic tissue and lateral plate mesoderm.

receptor PDGFR-α modulates the expression of N-cadherin in mesoderm cells. N-cadherin is typically enriched at points of cell contact and probably influences the amount of traction that these cells get when they move on and between other cells. Therefore, N-cadherin might be a crucial determinant for collective cell migration (Yang et al., 2008).

Finally, there is evidence in chick that Wnt3a, which is expressed in the primitive streak, acts as a repellent for cardiac mesoderm cells that are leaving the streak. There are also strong indications that Wnt5 and Wnt11b, also expressed in the streak, are involved in promoting the migration of posterior mesoderm cells away from the streak (Hardy et al., 2008; Sweetman et al., 2008; Yue et al., 2008). It is still unresolved whether Wnt5 and Wnt11b signaling controls cell movement, EMT and/or adhesion through regulation of E-cadherin expression in amniotes**,** as has been proposed in studies of zebrafish (von der Hardt et al., 2007). It is likely that many more guidance factors that control the collective migration of mesendoderm cells remain to be identified.

After gastrulation, EMT continues during many stages of development. A well-studied example is the formation of crest cells that delaminate from the closing neural tube and migrate to form pigment cells, parts of the head skeleton and several types of ganglia along the body axis. Recently, it has been proposed that contact inhibition of locomotion could result in directed cell migration and the ability of neural crest cells to invade other tissues. It was shown that neural crest cells show contact inhibition of locomotion when they meet one another, retracting their processes and setting off in another direction. Surprisingly, when these cells contact other types of cell, they do not show this behaviour and can therefore invade new tissues. It was shown that contact inhibition depends on signalling through the non-canonical Wnt signalling pathway and might impinge on ephrin signalling (Carmona-Fontaine et al., 2008). Other mechanisms that control the collective migration of neural crest cells have been reviewed in detail recently and will not be discussed further here (Sauka-Spengler and Bronner-Fraser, 2008).

Migration of epithelial sheets

In addition to the collective migration of individual cells that have undergone EMT, gastrulation also involves massive tissue deformations in epithelial sheets, beginning with the outer epithelial layer that eventually surrounds the entire embryo. Cells of the outer epithelial layer move towards the site of ingression – the blastopore in fish and amphibians, and the primitive streak in amniotes. This involves extensive changes in the local organisation of epithelial cells, which occurs through mechanisms that are not yet fully understood. On the basis of studies carried out in *Drosophila* and frogs, it has been proposed that small local rearrangements (such as cell-cell intercalation that occurs through junctional remodelling) could result in large-scale tissue deformations. Evidence that supports how this process might occur has mainly been obtained from studies of germband elongation in *Drosophila*. It has been proposed that epithelial cells polarise their actin-myosin cytoskeleton (which is under the control of the anterior-posterior patterning system) such that myosin II localises mainly in cell-cell junctions formed between anterior and posterior cells, and other molecules such as Bazooka (Par-3 in mammals) localise mainly in junctions between dorsal and ventral cells (Bertet et al., 2004; Zallen and Wieschaus, 2004). The actin-myosin-rich junctions then contract to form rosette-like structures, which then relax along the anterior-posterior axis, resulting in a change in tissue shape and an elongation of the embryo along this axis (Blankenship et al., 2006; Zallen and Blankenship, 2008). Similar intercalation mechanisms might underlie the formation and extension of the primitive streak in mouse and chick, which involves large-scale flows of cells in the epiblast (Chuai et al., 2006; Cui et al., 2005; Voiculescu et al., 2007). However, it is possible that other mechanisms, such as the movement of epithelial sheets in response to chemotactic signals embedded in the basal lamina, are also involved (Chuai and Weijer, 2007). Interestingly, it was recently reported that fibronectin, a component of the basal lamina, shows little movement relative to the migrating cells, possibly suggesting that the migrating cells take extracellular matrix with them when they move, or even that the cells do not move actively but are transported passively by deformations of the extracellular matrix (Czirok et al., 2006; Zamir et al., 2008) [see Chuai and Weijer (Chuai and Weijer, 2009) for a commentary on this idea]. Based on the studies discussed here, it is clear that our understanding of the mechanisms by which epithelial sheets move, and the signals that influence this movement, is still in its infancy, and that these are certainly exciting areas for future investigations.

Conclusions and outlook

Study of both the mechanisms that guide collective cell migration and the mechanisms that execute it is of crucial importance for understanding many essential steps in the development of higher organisms. There is substantial evidence that collective cell migration depends on gradients of chemoattractants that instruct cells where to move. As reviewed here, however, it is not yet clear in many settings whether all cells receive and interpret a guidance signal or whether only some cells receive and read the signal and therefore other factors (such as cell-cell contact) determine whether the migration of cells in a population occurs in a collective manner. In populations of mesenchymal cells that easily make and break cell-cell contacts, collective cell migration occurs when all cells move individually in the direction of a signal. In large cellular structures, static gradients are probably not sufficient for the long-range information required to instruct migration; thus, relaying of the guidance signal (as has been observed in *Dictyostelium*) might be necessary for collective cell migration to occur. Although this has not yet been extensively investigated in other systems, some evidence suggests that it also operates in higher organisms. For example, during chemotactic cancer-cell migration, it has been suggested that macrophages and metastatic fibroblasts signal each other through a paracrine loop involving colony-stimulating factor 1 (CSF-1) and EGF during their migration (Goswami et al., 2005).

In the coordinated migration of epithelial sheets, in which cells are connected by stronger and more specialised cell-cell junctions than those between mesenchymal cells, it also appears that not all cells receive and sense the guiding signal. Rather, movement involves some leader cells at the edge that sense the guiding signal and relay the information to follower cells through cell-contact-dependent or mechanical interactions.

There are many exciting open questions that have yet to be addressed. How are the signals that guide collective migration generated? How do migrating cells modify these signals? It will be essential not only to examine the expression of these signals in situ, but also to visualize the activation of signalling pathways and the actin-myosin cytoskeleton in the migrating cells. Determining how leader cells instruct other cells to follow them, and the role of contact-mediated signalling and mechanosensing in this process, also requires further study. Our understanding of the mechanism of the in vivo migration of epithelial sheets is particularly rudimentary. Do these cells get traction mainly from other cells or from the

extracellular matrix? How are shape changes at the apical side, which is dominated by junctional contacts, coordinated with shape changes at the basal side, which is dominated by cell-matrix interactions? The development of new tools to visualise and quantify intracellular signalling pathways and the nature of the interactions between collectively migrating cells will help to address these questions.

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