Organizing moving groups during morphogenesis
Virginie Lecaudey and Darren Gilmour

The directed migration of cells drives the formation of many complex organ systems. Although in this morphogenetic context cells display a strong preference for migrating in organized, cohesive groups, little is known about the mechanisms that coordinate their movements. Recent studies on several model systems have begun to dissect the organization of these migrating tissues in vivo and have shown that cell guidance is mediated by a combination of chemical and mechanical cues.

Introduction
Cell migration is essential to many physiological and disease processes such as embryonic morphogenesis, wound healing and cancer metastasis. Studies on single motile cells in culture have lead to a well-established model whereby cells move via the extension and adhesion of a leading edge pointed in the direction of migration and the retraction and loss of adhesion of the trailing edge at the rear. Here, the forces required for the translocation of the cell body are generated at the points of contact with the flat substrate provided by the Petri dish. While these studies have been crucial in understanding the mechanics of cell motility, it is clear that this controlled experimental environment is very different from what cells experience in the three-dimensional context of living tissues. How guides cell groups on their journey? Genetic studies in a wide range of model systems have shown that tissue migration is regulated by the very same extrinsic chemical cues that guide single cells. Examples include members of the epidermal growth factor and fibroblast growth factor families, which are detected through receptor tyrosine kinases present in the plasma membrane. As these are known to guide single cells via a chemo tactic mechanism, it is likely that graded distributions of these factors also determine the directionality of tissue migration in many cases [1**,13]. An important issue regarding cells moving as a cohesive tissue is the extent to which external gradients penetrate multicellular cohorts to control migration behaviour within. It is becoming clear that extrinsic cues drive the movement of tissues not by acting directly on all members of the group but rather by instructing smaller numbers of peripheral leader cells that in turn are responsible for the guidance of naïve followers. This is being guided directly by extrinsic cues [1**]. However, during the morphogenesis of many organ systems it is more common to find cells migrating in some form of adherent group or as tissues. These ‘tissue migrations’ are the focus of this review.

Migrating tissues come in many shapes and sizes and show varying degrees of cohesion and organization (for more details see [2]) (Figure 1). These range from rather loose networks, such as chains of neuronal progenitors in the CNS [3,4] and migrating neural crest cells [5**], to tightly adherent sheets of epithelial cells, where a large number of cells move as a single coherent unit and maintain constant positions throughout [6]. Elsewhere, they can be found as clusters of motile cells, as exemplified by Drosophila border cells (BCs) [7,8] or the migrating primordium of the lateral line (LLP) in amphibian fish [9,10]. Migrating tissues are often employed in sculpting complex three-dimensional forms, including the intricate tubular networks present in the vasculature and the respiratory system. While their forms and functions are diverse, it is clear in all cases that the migratory behaviour of cells within these various tissues must be coordinated to ensure proper movement of the entire group. The aim of this review is to integrate some recent results from several experimental models that shed light on the mechanisms ensuring the concerted movement of tissues during morphogenesis. Because of space limitations, we will not discuss convergent-extension movements during gastrulation, a very important example of collective cell behaviour that has been covered by several excellent recent reviews [11,12].

Getting organized for the journey
What guides cell groups on their journey? Genetic studies in a wide range of model systems have shown that tissue migration is regulated by the very same extrinsic chemical cues that guide single cells. Examples include members of the epidermal growth factor and fibroblast growth factor families, which are detected through receptor tyrosine kinases present in the plasma membrane. As these are known to guide single cells via a chemotactic mechanism, it is likely that graded distributions of these factors also determine the directionality of tissue migration in many cases [1**,13]. An important issue regarding cells moving as a cohesive tissue is the extent to which external gradients penetrate multicellular cohorts to control migration behaviour within. It is becoming clear that extrinsic cues drive the movement of tissues not by acting directly on all members of the group but rather by instructing smaller numbers of peripheral leader cells that in turn are responsible for the guidance of naïve followers. This is...
suggested by the fact that in many contexts only a subset of cells within a tissue display morphological features, such as filopodia and pseudopodia, characteristic of migratory cells [14]. Further support comes from several studies where guidance receptor activation is assayed directly using antibodies that bind specifically to active forms of receptors or downstream signalling components, allowing the identification of responsive cells. This approach was first used with anti-phospho-MAPK (Erk) antibodies to show that FGF signalling becomes restricted to the tips of Drosophila tracheal branches soon after they begin to extend [15]. More recently, anti-phosphotyrosine antibodies have been used as a read-out of guidance receptor activation to show that during normal migration only a subset of BCs responds to the cue secreted by the oocyte [16]. Similarly, during eyelid closure in mouse embryos, the EGF-like growth factor HB-EGF binds to and activates the EGF receptor and the downstream ERK signalling cascade only at the leading edge of the migrating epithelial sheet [17]. The most direct experimental demonstration that not all cells within migrating tissues need to respond to cues in vivo comes from genetic mosaic studies that juxtapose wild-type and migration-defective mutant neighbours. This approach has been particularly informative in the case of Drosophila BC clusters, where wild type cells have been mixed with several different immotile mutants including slbo, slg (E-cad) and sqh (myosin II). Here the wild-type cells can rescue the migration of immobile mutant clusters with an efficiency depending on their proportion [18–20]. These combined findings demonstrate clearly that guidance within tissues can be non-cell-autonomous, and that groups are composed of cells that respond directly to extrinsic cues and cells that do not.

**Coordinating individual movements within moving groups**

**Chemotaxis: tips from a slimy collaborator**

How do these leading cells transmit this extrinsic directional information to the remainder of the group? One paradigm for how cells within motile groups can organize each other’s behaviour comes from Dictyostelium slugs, which are comprised of many thousands of migrating cells that move collectively [21]. Here, a specialized set of cells at the tip of the slug, known as the prestalk cells, form an internal source of the diffusible chemoattractant cAMP that drives periodic waves of migration throughout the entire mass. Responding posterior cells are dependent on this internally generated gradient for their motility; if the tip region is cut off, it continues to migrate while the remainder of the slug is rendered immobile [22]. While tissues moving through embryos are guided by extrinsic cues, it is possible that leading cells adopt a similar strategy to organize the migratory behaviour of neighbours through a relay of guidance molecules. It will be interesting to determine whether the expression of chemoattractants within moving cohorts provides a mechanism for coordinating their behaviour in vivo.
Mechanotaxis: can you feel the force?

In many situations it is more likely that leading cells organize their neighbours by translating extrinsic chemical guidance cues into directional mechanical force. The force in question originates at least in part when leading cells retract their trailing edges, an event that in single cells is known to be dependent on the activity of myosin II [23]. Interestingly, *Dictyostelium* mutants lacking myosin II are able to migrate as single cells but cannot make slugs, suggesting that force generated through the actin–myosin network plays an important role in allowing multicellular movements [24,25]. Likewise, in BC clusters where the activity of myosin II is reduced, the leading cell continues to project long cellular extensions (LCEs) in the direction of migration, suggesting it can respond to guidance cues, but is unable to translocate its cell body or the remainder of the cluster, supporting the idea that contraction of the leading cells generates the force that initiates group migration [18,26].

An open question in the field is how followers detect the mechanical tension generated by leading cells in response to chemical cues. Focal adhesion complexes that form where the activity of myosin II is reduced, the leading cell continues to project long cellular extensions (LCEs) in the direction of migration, suggesting it can respond to guidance cues, but is unable to translocate its cell body or the remainder of the cluster, supporting the idea that contraction of the leading cells generates the force that initiates group migration [18,26].

Recent papers describe an elegant regulatory mechanism whereby migration-induced tension influences the status of the actin cytoskeleton in responding cells via the activity of the serum response factor (SRF). SRF is a transcription factor that controls the expression of several growth-factor-induced genes and is required for cell motility in several contexts [36–38]. A large body of work, mainly from Richard Treisman’s laboratory, has shown that SRF activity is potentiated by situations that stimulate actin polymerization, such as Rho GTPase signalling [39]. The mechanism underlying this regulation remained obscure until their recent demonstration that the SRF co-activator MAL moves from the cytoplasm to the nucleus upon Rho-actin signalling. Once there, it stimulates the expression of SRF target genes, including regulators of actin itself. Somogyi and Rorth have recently described the isolation of mutants in the *Drosophila* homologue of MAL (MAL-D, also known as DMRTF), which show a strong BC migration defect that is apparently due to an inability to organize a robust actin cytoskeleton [40**]. In this multicellular context, the nuclear localization of MAL-D appears to be dynamically regulated and shows a clear correlation with cell shape; MAL-D is observed in the nuclei in a subset of cells in stretched wild-type clusters whereas it remains cytosolic in all cells in rounded *slbo* mutant clusters. Ingenious *in vivo* ‘pulling’ experiments demonstrate that *slbo* mutants can translocate MAL-D to the nucleus when tugged by motile wild-type cells, confirming that this is indeed a direct response to stretching. Given that SRF regulates the transcription of actin regulators, these data suggest a positive feedback loop where cytoskeletal changes induced by pulling forces lead to the nuclear translocation of the mobility of the group. By scratching ‘wounds’ in cultured MDCK epithelial monolayers, Farooqui and Fenteaney have demonstrated that cells several rows behind the wound margin extend ‘cryptic’ lamellipodia basally while maintaining continuous cell–cell contacts apically, suggesting that these cells are actively involved in driving collective migration [34*]. Interfering with either intracellular calcium or gap junctions had no effect on this behaviour, leading to the proposal that it may be stimulated through a direction-sensing mechanotransduction system present at apically located junctions. Using the same culture system it has recently been shown that scratching induces two waves of ERK1/2-type MAP kinase activation propagating from the wound edge to submarginal cells [35]. The second wave is tightly correlated with the motility of the cells; it is no longer present as the wound is closing and inhibition of ERK1/2 activation results in a clear reduction in the rate of migration. Although the mechanism of propagation is not known, it is suggested once again that mechanical tension spreading from the leading edge induces a response in neighbouring cells — ERK activation in this case — that in turn is necessary for sheet migration (Figure 2).
of MAL-D, which results in the concomitant strengthening of the cytoskeleton required for cell migration.

Furthermore, an independent study has shown a requirement for MAL-D in regulating the migration of both tracheal and mesodermal cells [41]. During tracheogenesis, loss of MAL-D function causes a truncation of terminal branches, a phenotype that has been previously described in Drosophila SRF mutants [37,38], whereas expression of a hyperactive form of MAL-D that is constitutively nuclear causes their overgrowth. While the MAL-D loss-of-function allele has no obvious mesoderm phenotype, expression of the constitutively nuclear form appears to enhance the migration of mesodermal cells such that they end up located more dorsally than normal. In both cases, forced nuclear localization of MAL-D apparently leads to increased migratory behaviour, in accordance with the finding that it strengthens the cytoskeleton. As both the trachea and mesoderm move as adherent groups of cells, it will be interesting to know whether MAL-D activity is modulated by cell–cell stretching in these and other contexts.
Conclusions

It is becoming clear that group migration is regulated by the combined influence of extrinsic chemical guidance cues and local mechanical interactions between cells. A complete understanding of this complex problem will therefore require a multi-disciplinary approach. Several recent papers have combined the use of genetics, imaging and physical ‘tools’ to study the forces involved in embryonic movements with exciting results. For example, atomic force microscopy has been used to measure the adhesive forces generated by primary zebrafish embryonic cells plated on cadherin substrates, and these quantitative findings have been correlated with the cells’ in vivo behaviour [42]. Similarly, a combination of laser microsurgery and quantitative modelling has been used to dissect the forces driving morphogenetic movement during dorsal closure in Drosophila [43]. On the imaging side, it will be essential to develop probes that allow the mechanical activation of biochemical pathways to be monitored and quantified within living embryos. Once again, many recent developments show great promise in this regard, such as FRET probes that can monitor the dynamics of SRC activation upon mechanical stimulation [44]. Finally, the identification of biochemical systems that convert mechanical tension into different types of migratory behaviour will be a high priority for the future.

Acknowledgements

We are grateful to Pernille Rorth, Francesca Peri, Petra Haas and Stefan Eimer for critical reading of the manuscript. We thank Ferenc Jankovic, Damian Brunner, Stefan Luschnig, Arturo Alvarez-Buylla and Pernille Rorth for images. V.L. was supported by a fellowship from la Fondation pour la Recherche Médicale.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

● of special interest
●● of outstanding interest


29. Tzima E, Iraji-Tehrani M, Kisses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA: A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature 2005, 437:428-431. As endothelial cells show several coordinated responses to shear stress, such as aligning in the direction of flow, they provide a very nice model system for studying how mechanical force can affect cell behaviour. These authors identify a complex containing three transmembrane
proteins that is present at adherens junctions and address the relative contributions of all three in mediating mechanosensation in this context.


Using a combination of confocal and electron microscopy in a wound closure model, it is shown that submarginal cells several rows from the leading edge extend cryptic basal lamellipodia under cells in front of them and crawl actively while maintaining apical cell-cell contacts.


This study uses the experimental strengths of the border cell (BC) model to address both the function and the regulation of MAL/SRF during cell migration in vivo. Cell lacking MAL-D show reduced F-actin accumulation, which leads to a strong defect in the invasive migration of BCs. BCs mutant for mal-d rupture to produce fragments that are able to migrate in a directional manner, supporting the suggestion that these cells have a weakened cytoskeleton. The authors then exploit the fact that BCs move in a cohesive cluster to show that the nuclear localization of MAL-D is regulated by cell stretching.


This paper shows that the SRF/MAL-D transcriptional complex is required during trachea development and mesodermal migration. Together with the previous paper, these data suggest MAL-D as a very good candidate to transduce mechanical forces in tissues undergoing extensive cytoplasmic extension or migration.

