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HYPOTHESIS

A common framework for EMT and collective cell migration

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ABSTRACT

During development, cells often switch between static and migratory behaviours. Such transitions are fundamental events in development and are linked to harmful consequences in pathology. It has long been considered that epithelial cells either migrate collectively as epithelial cells, or undergo an epithelial-to-mesenchymal transition and migrate as individual mesenchymal cells. Here, we assess what is currently known about *in vivo* cell migratory phenomena and hypothesise that such migratory behaviours do not fit into alternative and mutually exclusive categories. Rather, we propose that these categories can be viewed as the most extreme cases of a general continuum of morphological variety, with cells harbouring different degrees or combinations of epithelial and mesenchymal features and displaying an array of migratory behaviours.

KEY WORDS: EMT, Collective migration, Epithelial, Mesenchymal

Introduction

The transition of cells from static to migratory behaviour is an important driving force for morphogenesis during embryonic development and during the differentiation of multiple tissues and organs. Cell migration is normally a highly regulated process that, when activated in non-developmental contexts, can lead to a number of pathologies, perhaps the most devastating of which is tumour metastasis. Epithelial cells were long considered to be non-migratory cells, although this assumption had to be dropped as it was found that epithelial cells often display surprisingly motile behaviour, for example during the morphogenesis of early embryos. Indeed, it has now become clear that cells within an epithelium can move relative to one another, while retaining tissue integrity, and thus achieve large overall movements (Bertet et al., 2004; Blankenship et al., 2006; Jaźwińska et al., 2003; Rørth, 2009). Until recently, it was considered that epithelial cells undertake one of two routes to accomplish migration: either to migrate collectively as epithelial cells (Friedl and Gilmour, 2009; Rørth, 2009), or to undergo an epithelial-to-mesenchymal transition (EMT) and migrate as individual mesenchymal cells (Thiery et al., 2009). This classification of two different migratory phenomena was largely driven by the classic understanding of EMT as a binary decision, involving the transition from a full epithelial to a full mesenchymal state (Hay, 2005), and based on the fact that a bona fide mesenchymal cell migrates individually through the extracellular matrix (Acloque et al., 2009; Nieto, 2011).

However, these definitions have been troubling developmental biologists for some time, as the rich variety of migratory events that occur during animal development tend to escape from the simplicity of these definitions. First, not only do many intermediate situations exist, with migrating cells possessing a combination of epithelial and mesenchymal features, but it is evident that these are far more commonly seen *in vivo* than previously thought (Nakaya and Sheng, 2008; Shook and Keller, 2003). Second, it is now clear that mesenchymal cells often migrate exhibiting the coordination and cooperation ascribed to collectively migrating cells (Scarpa and Mayor, 2016; Theveneau and Mayor, 2011). To cope with these observations, distinctions between individual and collective cell migration have evolved from very strict to more inclusive or loose definitions (Rørth, 2012; Theveneau and Mayor, 2011). Accordingly, any attempts to classify migratory events and thus draw parallels between different systems currently requires the use of strict definitions with a great number of exceptions, or the coining of new and not very defined terms such as ‘cells migrate individually together’ (Friedl and Gilmour, 2009; Rørth, 2009) or ‘cells undergo a pseudoEMT’ (Pastor-Pareja et al., 2004).

In this Hypothesis article, we propose a different view. We suggest that collective versus individual, and epithelial versus mesenchymal, appear to be distinct and independent features that combine in variable degrees, not only in different migratory events, but even at different times in a single migratory process. Accordingly, we propose that *in vivo* cell migratory phenomena do not fit into distinct and mutually exclusive morphological categories, but rather should be viewed as a general continuum of morphological variety, which can be achieved by combining diverse and complementary mechanisms.

Below, we review classic examples of EMT and collective migration and describe how they fit into the shared framework we propose for migration. Of note, we focus on what is known regarding cell behaviours, properties and interactions prior to and during migration, rather than on the factors triggering migration and establishing migratory pathways, as these have been extensively reviewed elsewhere (Friedl and Gilmour, 2009; Lamouille et al., 2014; Nieto, 2011; Rørth, 2009; Thiery et al., 2009). Finally, given the spectrum of migratory and invasive cell behaviours observed during oncogenesis (see Friedl and Wolf, 2003), we also discuss our framework in the context of tumour progression.

Cell types and modes of migration

One of the main concepts used to distinguish between single and collective cell migration is based on differences between mesenchymal and epithelial cell behaviours, respectively. However, as remarked previously (Davies and Garrod, 1997; Nakaya and Sheng, 2008), it is not easy to define these cell types based on specific cell biological criteria. A further challenge is that, although mesenchymal cells were originally defined morphologically (Hay, 2005), current investigators often define such cells by their gene expression profiles (reviewed by Shamir and Ewald, 2015). However, many of the mesenchymal markers expressed in human cells are not expressed in lower species (e.g. Brown, 2011; Cho et al., 2016).

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Indeed, epithelial and mesenchymal cells possess a range of distinct features (Fig. 1A). A clear fundamental property of epithelial cells, for example, is that they are polarised along their apico-basal axis and possess a free apical surface, whereas mesenchymal cells do not. Other differences include cell morphologies, the presence or absence of filopodia and front end-back end polarity, migration capacity, cell-cell adhesion, and extracellular matrix interactions (Fig. 1A). However, and as previously noted, there is no single feature that is unique for either an epithelial or a mesenchymal cell type (Nakaya and Sheng, 2008). Furthermore, cells often possess a combination of these features and, even within the categories of epithelial or mesenchymal, a continuum of cell behaviours can be seen. Thus, some highly specialised epithelial cells exhibit elaborate junctions and specialised apical features such as brush borders, cilia or taenidial folds. Other more primitive epithelial cells, such as those in the outer cell layer of *Caenorhabditis elegans*, *Xenopus* and fish blastula, simply have a free apical surface on one side, face embryonic tissue on the basolateral side and contain nascent junctions (Shook and Keller, 2003). Mesenchymal cells similarly display a range of features, with some showing a high degree of front-back polarity, elongated morphology and invasive motility, while other immature mesenchymal cells lack apico-basal polarity and zonula adherens junctions, and are motile.

It is now clear that, rather than being a binary switch from epithelial to mesenchymal behaviour, EMT and the reverse mesenchymal-to-epithelial transition (MET) are graded processes with a range of different outcomes, giving rise to cells that exhibit various combinations of epithelial and mesenchymal features (Fig. 1B). This is abundantly clear when looking at the recent literature, in which a plethora of phrases have been used in an attempt to describe these intermediary phenotypes; cells have been described as having undergone a ‘partial EMT’, ‘intermediate EMT’, ‘intermediate mesenchymal’, ‘incomplete EMT’, ‘semi-mesenchymal EMT’, ‘hybrid epithelial/mesenchymal’, ‘EMT-like’ or ‘metastable’ (reviewed by Grigore et al., 2016). Taken together, we think these observations fit very well with the notion that ‘extreme’ epithelial and mesenchymal cell states should be considered as those states that flank the ends of a continuum of cell features (Fig. 1B).

Apico-basal polarity during the collective migration of epithelial cells

In order for a cell to undergo directional migration, it needs to generate a pulling force at one end and retract its rear; these processes need to be cell-polarised such that there is at least transiently a front and a back (see Rørth, 2009). This is clear in mesenchymal cells, which possess a front-back polarity, and a similar polar organisation often takes place in collectively migrating epithelial groups, with a subset of cells at the front of the group displaying increased protrusions and migratory behaviours with respect to the rest of the cells. These are commonly referred to as ‘leading edge’ cells in an epithelial sheet, or ‘tip’ or ‘leader’ cells in tubes or clusters of epithelial cells, with the remaining cells being called the ‘follower’ or ‘trailing’ cells. In the case of such migrating epithelial cohorts, the majority of cells maintain apico-basal polarity and intact adherens junctions throughout movement, and collective movement is thus achieved through a dynamic physical linkage. However, of great interest for this hypothesis, cells at the leading edge or tip of the cohort showing increased motility always display altered apico-basal polarity and junctional organisation in comparison to the rest of the group. This is clearly seen in the case of migrating epithelial sheets, where cells displaying an increase in actin-rich protrusions and migratory behaviours are found at the leading edge of the sheet, for example during wound healing in mammals and dorsal closure in *Drosophila* (see Box 1).

The simple fact that these cells are at the edge of the sheet endows them with a distinct polarity: on one side they contact other cells, and on the other they find a free edge. This difference is reflected in their apico-basal polarity; whereas the leading edge cells maintain apico-basal polarity and junctions at the site of cell-cell contact, these are lost from the free edge (Fig. 2), resulting in a cell that is only partially apico-basal polarised. This can be seen by an absence of staining for certain polarity proteins, such as Discs large and Crumbs, or E-cadherin (Arnoux et al., 2005; Bahri et al., 2010). In addition, at their free edge some cells exhibit actin-based protrusions, such as lamellipodia and filopodia, and a contractile purse-string, all of which are used for motility (Jacinto et al., 2002; Wood et al., 2002). Thus, using current terminology, cells at the leading edge would be described as ‘partially’ undergoing a ‘partial’

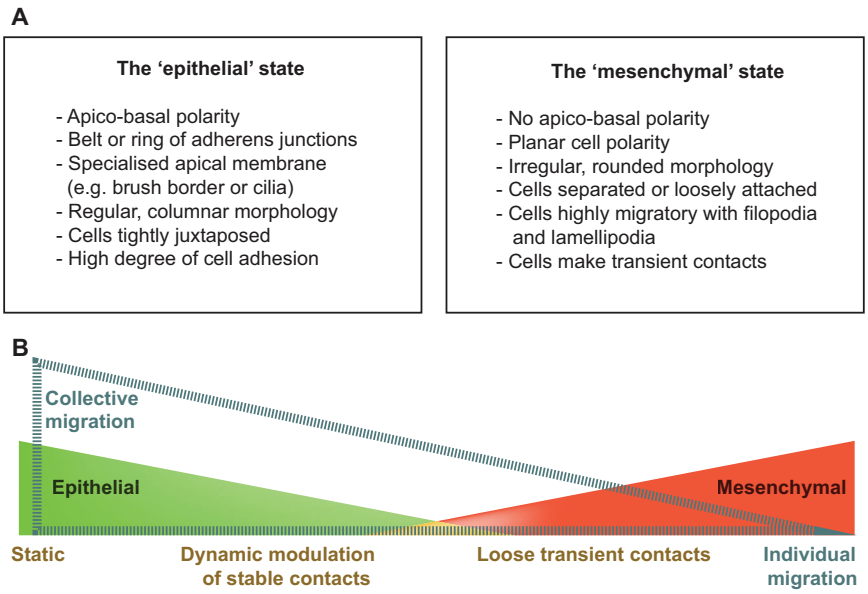

Fig. 1. The ‘spectrum’ model: a common framework for EMT and collective cell migration. (A) Some of the main features that contribute to epithelial or mesenchymal cell states are listed. It should be noted that these characteristics are not present in all cells in one cell state, nor absent in all cells in the other, and none is unique for either cell type. It is the accumulated gain or loss of a number of such features that pushes a cell towards one cell state or another. (B) Epithelial versus mesenchymal cell states and collective versus individual migration are distinct and independent but can combine to variable degrees, resulting in a graded spectrum of cell behaviours that are apparent not only in different migratory events, but even at different times within a single migratory process.

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Box 1. *In vivo* examples of collective migration and/or EMT

Wound healing in mammals. Upon injury, the skin initiates a complex process of events, namely wound healing, that involves inflammation as well as the formation, migration and remodelling of new tissue, and the orchestrated regulation of different cell types.

Dorsal closure in *Drosophila*. Dorsal closure is the process whereby lateral epithelium from the two sides of the embryo is drawn up and over the exposed amnioserosa to form a neat, and subsequently invisible, midline seam where the two segmented epithelial edges meet one another.

***Drosophila* tracheal branch migration.** The *Drosophila* trachea develops from clusters of cells in the ectoderm. The cells of each cluster migrate by responding to a fibroblast growth factor homologue expressed around the tracheal cells at each position at which a new branch will form and grow.

***Drosophila* germband extension.** The germband is a multilayered band of germ layers on the ventral side of the embryo. As gastrulation proceeds, the germband narrows along its dorsal-ventral axis and extends along its anterior-posterior axis via movements driven largely by cell intercalation events.

***Drosophila* border cells.** Border cells are a population of four to six cells found in the ovary that cluster around a pair of polar cells, delaminate from the anterior follicular epithelium, and migrate in between nurse cells to reach the oocyte.

Zebrafish lateral line. The lateral line is a sensory system used to detect changes in water flow. It is initially established by a migratory group of cells that deposit subsets of cells at stereotyped locations along the surface of the fish.

Mammary ductal epithelium. The ductal epithelium of mammary glands originates from a multilayered epithelial placode during embryonic development. During its morphogenesis, the mammary epithelium transitions from a bilayered to a multilayered organisation, with dramatic, reversible changes in epithelial polarity and cell motility.

Mesoderm and endoderm formation. The mesoderm and endoderm are two of the initial three germ cell layers (mesoderm, endoderm and ectoderm) and are formed by the process of gastrulation.

Neural crest cells. These are a group of cells unique to vertebrates that arise from the embryonic ectoderm cell layer, migrate through the embryo and give rise to diverse cell lineages, including melanocytes, craniofacial cartilage and bone, smooth muscle, and peripheral and enteric neurons and glia.

Zebrafish epiblast. The epiblast is the outer of the two layers of the blastoderm that form during gastrulation, corresponding to primitive ectoderm during gastrulation and to the definitive ectoderm after gastrulation.

EMT; however, within our framework, epithelial leading cells can be considered to be acquiring certain mesenchymal features (Fig. 1).

A more subtle modulation of apico-basal polarity is often seen in cells at the distal tip of outgrowing epithelial tubes such as in lungs, mammary glands, nephric ducts and the vasculature. The behaviour of cells at the leading tip of branching tubes has been extensively characterised during the migration of *Drosophila* tracheal branches (see Box 1; Caussinus et al., 2008; Lebreton and Casanova, 2014). Leader cells of tracheal branches adopt a polar organisation with respect to their direction of migration, with the basolateral surface forming the migratory front, and the apical the rear. Furthermore, in these cells the apical membrane is substantially reduced and they display a greater basolateral membrane bearing lamellipodia and filopodia (Fig. 2; Lebreton and Casanova, 2014). Indeed, protrusive activity occurs on the basolateral surface, which colocalises with integrins, so it is likely that this modulation of polarity affords a greater surface area for the migratory machinery. Interestingly, trailing cells elongate their apico-basal membranes in the direction of movement and, in this way, contribute to the overall displacement of the tube, in a similar manner to the polarised remodelling of junctions that underlies extension of the *Drosophila* germband (see Box 1; Blankenship et al., 2006). Notably, the use of planar remodelling (i.e. the extension of lateral sides) versus proximo-distal remodelling (i.e. the modulation of apical sides) reflects the orientation of the cell with respect to the overall direction of movement.

These types of modulations in apico-basal polarity can also be found combined within a single migrating cluster, for example in *Drosophila* border cells (see Box 1). The central cells within these clusters are not thought to be actively motile and, although they maintain full apico-basal polarity throughout migration, their apical domains are highly constricted (Pinheiro and Montell, 2004). The peripheral cells, by contrast, migrate actively and are thus thought to provide the force that propels the cluster forward. These cells have a distinct polarity as a result of their position within the group, i.e. they have one side that contacts other cells and one free edge. The peripheral cells maintain apico-basal polarity and junctions at the site of cell-cell contact but, with remarkable similarity to leading edge cells in an epithelial sheet, they depolarise their remaining surface, as indicated by an overlap of apical and lateral markers (Fig. 2; Niewiadomska et al., 1999; Pinheiro and Montell, 2004).

Another interesting example of collectively migrating epithelial cells are those of the zebrafish lateral line (see Box 1). These cells also migrate collectively, with leading cells displaying increased migratory behaviour in comparison with other cells in the cohort. This difference is again reflected by differences in apico-basal polarity, with cells at the front exhibiting a reduction or delocalisation of apico-basal polarity and adhesion proteins, and a loss of tight junctions (Fig. 2; Revenu and Gilmour, 2009; Revenu et al., 2014). By contrast, cells in the body of the cohort are apico-basally polarised, with mature adherens junctions and desmosomes, but have a much reduced apical membrane and an enlarged basolateral membrane (Hava et al., 2009), similar to the cases mentioned above. Thus, this epithelial state, in which the apical membranes are reduced and the basolateral surface enlarged, is associated with active epithelial migration in a number of different developmental contexts.

The idea that the concept of epithelial cells and tissues should be expanded to include different characteristic states of epithelial function and behaviour has previously been proposed by researchers studying mammary branching morphogenesis (Ewald et al., 2008). It should be noted that apico-basal polarity in the mammary ductal epithelium (see Box 1) is almost completely lost from the leading group of cells in the terminal end buds of the ducts (Ewald et al., 2008). During morphogenesis, the mammary epithelium transitions from a bilayered to a multilayered organisation, with dramatic, reversible changes in epithelial polarity and cell motility. Whereas the main body of mammary ducts consists of a single luminal epithelial bilayer, which is apico-basal polarised with microvilli and contains mature junctional complexes, cells in the growing tip temporarily lose apico-basal polarity, possess few intercellular junctions and show increased motility (reviewed by Shamir and Ewald, 2015). These cells drive the elongation of the growing branch through collective migration in which there are no leading cell extensions or leading actin-rich protrusions; instead, cells display a dense network of interdigitating membrane protrusions (Ewald et al., 2012). Notably, throughout these dynamic rearrangements the cells remain adherent and localise E-cadherin and β-catenin to cell-cell contacts, and are surrounded by a continuous basement membrane. These observations led to the proposition that these cells could represent a ‘morphogenetically

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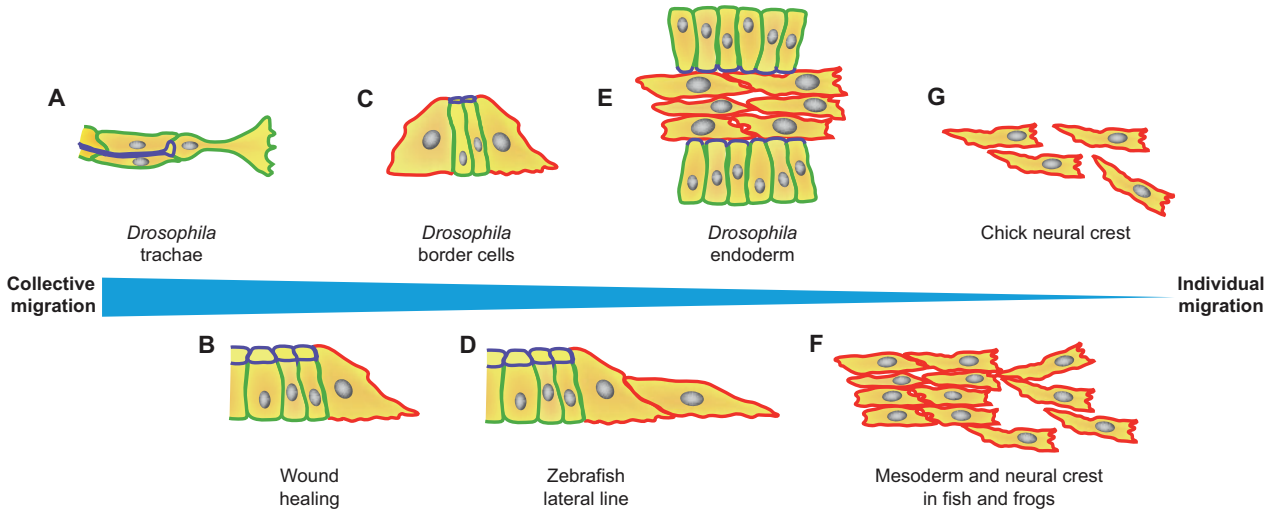

Fig. 2. The spectrum of cell states seen during migratory events *in vivo*. Various cell states observed during *in vivo* migratory events are depicted, with apical membranes outlined in purple, basolateral membranes marked in green, and nonpolarised membranes shown in red. (A) All cells possess apico-basal polarity. Tip or leader cells have smaller apical domains and an extensive basolateral surface, e.g. as seen in migrating branches of the *Drosophila* trachae. (B) All cells possess apico-basal polarity, but cells at the leading edge are partially depolarised, e.g. as seen during wound healing and *Drosophila* dorsal closure. (C) All cells possess apico-basal polarity. Some modulate their apicobasal polarity and another subset of cells are partially depolarised, e.g. as seen during migration of *Drosophila* border cells. (D) Most cells possess apico-basal polarity. Leading cells consist of a group of cells that are either partially or fully depolarised, e.g. as occurs in the zebrafish lateral line. (E) A mixed population of collectively migrating cells. A subset possesses apicobasal polarity whereas the rest have completely lost apico-basal polarity, e.g. as seen for *Drosophila* endoderm cells. (F) All cells have completely lost apico-basal polarity, but the main body of cells migrates collectively, e.g. as seen in mesoderm, endoderm and neural crest populations in fish and frog embryos. (G) All cells have completely lost apico-basal polarity and migrate individually, e.g. as seen for chick neural crest cells.

active epithelial state' (Ewald et al., 2008). However, considering that these cells do not have distinct apical and basolateral domains, according to our proposed framework these cells would be regarded as cells that have acquired mesenchymal features and migrate collectively (Fig. 1B).

In the examples discussed in this section, with the exception of the mammary ductal epithelium, all cells in the migrating group are in constant contact with each other, and even though cells may show reduced apico-basal polarity, polarity is always maintained at sites of cell-cell contact. The ways in which these cells modulate and remodel their apico-basal polarity reflects the high degree of cell plasticity that underlies the continuum of epithelial cell behaviours and states. By contrast, leading cells in mammary ducts go a step further and completely lose apico-basal polarity and thus can be considered to undergo an EMT from an epithelial to a collectively migrating mesenchymal cell state (Fig. 1B).

The array of epithelial phenotypes with respect to EMT

A complete loss of apico-basal polarity during the transition from a stationary to a migratory state is also often seen in entire cohorts of cells during development, for example in the primitive mesoderm and endoderm cells (see Box 1) of all species, and in vertebrate neural crest cells (see Box 1). In these cases, although all of the cells undergo EMT and display a mesenchymal phenotype during migration, there are some interesting differences with respect to their initial epithelial phenotypes that are highly relevant to our spectrum model.

In both flies and fish, the epithelial cells that give rise to the mesoderm are quite primitive; although they exhibit distinct apico-basal polarity and are tightly coupled, they contain immature junctions and have no underlying basement membrane. However, these cells undergo clear changes in morphology and apico-basal polarity as they initiate migration, transitioning from the highly regular shape characteristic of epithelial cells to a typical rounded irregular mesenchymal morphology, and they also extend numerous actin-based protrusions. It has been shown that *Drosophila* mesoderm cells completely lose apico-basal polarity as they initiate migration, with gaps appearing between cells, and polarity proteins such as Crumbs and junctional proteins such as E-cadherin are completely repressed (Leptin, 1991; Sandmann, 2007). Interestingly, and in contrast to mesodermal cells, *Drosophila* endodermal cells undergo EMT at a later stage and initially possess fully formed adherens junctions and apico-basal polarity, which are completely downregulated during the transition to a migratory state (Campbell et al., 2011; Tepass and Hartenstein, 1994). Thus, even when comparing just *Drosophila* endoderm and mesoderm, it is clear that the initial epithelial states prior to EMT differ in what can be considered their degree of 'epithelialness'.

In birds and mammals, the cells that give rise to the mesoderm display even more 'epithelialness'; they contain fully formed junctional complexes and are surrounded by a basement membrane. Indeed, it has been shown in both chick and mouse embryos that the first morphologically apparent change in mesoderm cells within the primitive streak is a breakdown of the basement membrane underlying these cells (Nakaya et al., 2008; Williams et al. 2012). Following this, the cells ingress, and this occurs concurrently with a downregulation of E-cadherin and apico-basal and tight junction proteins.

Another system that behaves similarly is the neural crest (see Box 1), which is found in fish, birds and mammals. Prior to initiating migration, neural crest cells form a pseudo-stratified epithelium that lacks tight junctions, and they possess adherens junctions composed of N-cadherin but not E-cadherin. Although the cells are surrounded by a basement membrane, in some cases (such as in *Xenopus*) this can become disrupted during neural folding. Thus, prior to initiating EMT, some features of the epithelial state

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have already been lost in the cells that will form the neural crest. Notably, and similar to mesodermal and endodermal cells, neural crest cells always completely lose apico-basal polarity at the onset of migration. Live imaging of chick neural crest delamination using slice cultures shows that this often occurs before neural crest cells withdraw from the apical surface (Ahlstrom and Erickson, 2009). However, a cell can also retract from the tube whilst retaining polarity and fragments of adherens junctions, suggesting that the loss of apico-basal polarity and complete downregulation of adherens junctions are not prerequisites for movement (Ahlstrom and Erickson, 2009).

Considering all these examples in the context of EMT, it becomes clear that not only can a cell transition to variable degrees of the mesenchymal state, it can also transition from various grades of the epithelial state. Moreover, this is achieved by combining diverse and complementary mechanisms, which are very context dependent. Finally, this underlies the importance of considering these processes as a spectrum of cell behaviours rather than a transition from one specifically defined state to another.

Adherens junctions, cell adhesion and apico-basal polarity

The primary mechanism of adhesion in developing epithelia is through E-cadherin-mediated adherens junctions, which are found as a belt or ring at the apico-lateral border of epithelial cells. Adherens junctions mediate adhesion between cells through the trans-dimerisation of E-cadherin on adjacent cell surfaces, which assemble into junctional complexes via the association of the intracellular domain of E-cadherin with β-catenin and α-catenin (reviewed by Baum and Georgiou, 2011).

Recent live-imaging studies have revealed the extent to which E-cadherin is actively turned over at adherens junctions, highlighting the extremely dynamic nature of intercellular adhesion, even in relatively stable epithelia (Kowalczyk and Nanes, 2012; West and Harris, 2016). In cases of collective epithelial cell migration, this continuous modulation of E-cadherin and other junctional components, such as catenins, is key to maintaining tissue integrity throughout cell movements. Additionally, it can provide an active mechanism for tissue morphogenesis, for example driving polarised intracellular cell changes within an epithelial group to promote overall tissue movement, as seen in the *Drosophila* germband (Zallen, 2007) and developing wing (Classen et al., 2005). Moreover, if E-cadherin turnover is polarised across a tissue, this can result in increased cell movements in one part with respect to another. For example, in the *Drosophila* tracheal system, greater E-cadherin exocytosis in the main dorsal trunk makes these cells relatively stable, whereas reduced E-cadherin exocytosis in the smaller branches permits their elongation (Shaye et al., 2008). Thus, similar to apico-basal polarity, the modulation of adherens junctions is a common feature of morphogenetically active epithelia.

Adherens junctions and apico-basal polarity are tightly linked. In all of the cases considered above in which cells modulate their apico-basal polarity, adherens junctions are similarly modified. Furthermore, when cells lose apico-basal polarity, this goes hand in hand with a loss of adherens junctions and vice versa. Accordingly, EMT is often driven by repressing the expression of adherens junction components, such as E-cadherin; owing to the pivotal role of E-cadherin loss, transcription factors that affect EMT, such as Snail, Twist and Zebs, are often referred to as E-cadherin repressors (Galván et al., 2015; Nieto, 2011; Schulte et al., 2012). Intriguingly, it is also possible to affect EMT via the repression of apico-basal polarity proteins without affecting E-cadherin transcription; the loss of apico-basal polarity in turn leads to destabilisation of adhesion junctions (Campbell et al., 2011; Lim and Thiery, 2011). This has been demonstrated both in the *Drosophila* endoderm and in Madin-Darby canine kidney (MDCK) cells, in which a conserved set of GATA factors affects EMT mainly via direct repression of the *crumbs* gene, which in turn impinges on E-cadherin junctions (Campbell et al., 2011).

The intimate link between belt-like adherens junctions and apico-basal polarity is highly conserved and a fundamental feature of epithelial cells. However, this is not exactly the case for cell-cell adhesion as, particularly in primitive epithelia, an absence of a belt of adherens junctions does not equate to a lack of cell-cell adhesion. Significantly, very early *Drosophila* ectodermal epithelial cells possess apico-basal polarity and spots of adherens junctions on their lateral membranes; however, these adherens junctions only later coalesce to form belts of adherens junctions at the apico-basolateral border (Lecuit, 2004; Tepass et al., 2001). Given that the immature ectoderm cells are tightly adherent and maintain integrity across the epithelium, it is likely that the spot adherens junctions initially mediate cell-cell adhesion. Similarly, cells in the zebrafish epiblast (see Box 1) are connected by spots of adhesion over their entire surface, rather than a belt of contact at the apicolateral surface (Shook and Keller, 2003).

Additionally, although E-cadherin is a major component of adherens junctions and is involved in a primary mechanism of cell adhesion, it has to be emphasised that absence of adherens junctions does not mean absence of E-cadherin-mediated adhesion. Indeed, an adhesive function of E-cadherin has been found all around the cell in cases where neither belt nor spot adherens junctions are detected by ultrastructural analyses, such as in the *Drosophila* endoderm (Campbell and Casanova, 2015). These observations have prompted the notion that the molecular components required for the adhesive function of belt adherens junctions provide basic cell-cell adhesive activity independently of their junctional organisation (Niessen and Gottardi, 2008), and that the epithelial specificity for belt adherens junctions could be more related to 'extra-adhesive functions' of E-cadherin, such as apico-basal cell polarisation and intercellular cytoskeleton coupling (Campbell and Casanova, 2015; Niessen and Gottardi, 2008).

Finally, it is important to note that although disruptions to adherens junctions and apico-basal polarity are clearly key steps in EMT, overexpression of E-cadherin alone is not sufficient to block EMT in MDCK cells (Ohkubo and Ozawa, 2004) nor revert the mesenchymal phenotype (Navarro et al., 1993); similarly, Crumbs overexpression is not sufficient to abrogate EMT in the *Drosophila* endoderm (Campbell et al., 2011). This emphasises that, although repression of these key epithelial features is central to EMT, the transition from epithelial to mesenchymal states involves coordinated changes in many additional cell features, such as actin and microtubule organisation, polarised protein trafficking, and migratory and invasive capabilities, none of which is conserved in all EMT processes but each of which can be highly significant in certain contexts of developmental or pathogenic EMT.

The complex relationship between E-cadherin and migratory capacity

Although the impact of E-cadherin on cell behaviour is widely recognised, there is an important debate about its functional role. E-cadherin has long been considered as a protein that assures the static behaviour of epithelial cells and repression of which is necessary for epithelial cells to become mesenchymal and migratory; however, the situation has turned out to be more complicated. Not only can cells adopt many mesenchymal features,

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including migration, while actively transcribing E-cadherin (Campbell and Casanova, 2015; Campbell et al., 2011; Dumortier et al., 2012; Montero et al., 2005; Shamir et al., 2014; Theveneau and Mayor, 2012), there is an increasing number of cases in which the downregulation of E-cadherin in migrating cells leads to a complete block in their migration (Cai et al., 2014; Kardash et al., 2010; Montero et al., 2005; Niewiadomska et al., 1999; Shamir et al., 2014), suggesting that E-cadherin is not simply required for static adhesion but, conversely, that it is also a highly dynamic component actively required for cell migration. In fact, a recent study showed that this is indeed the case in *Drosophila* border cells, where a novel role for E-cadherin as an integrator of mechanical signals during the directional migration of cell clusters was revealed (Cai et al., 2014). These findings have important implications when considering what constitutes an EMT and, indeed, the underlying mechanisms at play.

The repression of *E-cadherin* transcription is widely considered to be a crucial step in, and even a landmark for, EMT (Batlle et al., 2000; Cano et al., 2000; reviewed by Huber et al., 2005). However, a more complex relationship between EMT and the transcriptional repression of *E-cadherin* is now emerging. First, as noted above, E-cadherin overexpression alone is not sufficient to block EMT in MDCK cells (Ohkubo and Ozawa, 2004) or revert the mesenchymal phenotype (Navarro et al., 1993). Likewise, in *Drosophila*, when the gene encoding E-cadherin is placed under the control of a ubiquitous promoter, it can fully rescue the lack of the endogenous *E-cadherin* gene (Oda and Tsukita, 2001). This indicates that transcriptional regulation of E-cadherin is not required for epithelial cells to adopt mesenchymal features in their transition to the mesoderm or other tissues. Second, in addition to the transcriptional regulation of E-cadherin, it is clear that changes in protein localisation, trafficking or degradation can modulate the accumulation of E-cadherin at the cell membrane. For example, during EMT in gastrulating mouse embryos, E-cadherin is downregulated at the protein level by p38 MAP kinases, in parallel to transcriptional repression by Snail (Zohn et al., 2006). However, the p38-dependent delocalisation of E-cadherin is not sufficient to drive EMT, as *snail* mutant cells fail to undergo EMT (Barrallo-Gimeno and Nieto, 2005; Carver et al., 2001) and there is still some EMT in mutants lacking p38 activity (Zohn et al., 2006). A similar case can be observed in the *Drosophila* trachea, where E-cadherin downregulation is not sufficient to induce a loss of epithelial cell features (Shaye et al., 2008). Altogether, these results indicate that there is neither a direct relationship between E-cadherin presence and static epithelial cells or between E-cadherin absence and migratory mesenchymal cells, nor a strict relationship between EMT and the transcriptional repression of E-cadherin.

Finally, it can be argued that the crucial step in EMT is a 'cadherin switch' rather than E-cadherin repression. EMT is characterised by a cell altering its cell-cell adhesion molecules relative to those of its tissue of origin, thereby allowing the cell to separate from its neighbours. This reduction in intercellular adhesion is achieved largely by sequential changes in cadherin expression and is known as the 'cadherin switch' (Taneyhill, 2008). However, the functional relevance of the switch from E-cadherin transcription in epithelial cells to N-cadherin transcription as they transit to mesenchymal cells during EMT in organisms such as frog, chick and fly (Hatta and Takeichi, 1986; Nandadasa et al., 2009; Oda et al., 1998) is currently under question, as it has been elegantly proven that it is not required for the segregation or dispersal of the mesodermal germ layer in *Drosophila* (Schafer et al., 2014). Thus, similar to E-cadherin transcriptional repression, it is likely that in some systems a 'cadherin switch' is also dispensable for EMT.

Collective migration and EMT: fundamentally incompatible or mutually beneficial?

Like EMT, collective cell migration was once defined by very strict terms that have evolved over time to include looser definitions. This has ranged from a requirement for stable physical contacts throughout migration, to just a loose or close association throughout the migrating group (Rørth, 2012; Theveneau and Mayor, 2011), again reflecting the diversity of migratory events that exist in nature. However, there is clearly a correlation between the epithelial and mesenchymal state and collective and individual migratory modes, which can also be represented by our spectrum model (Fig. 1B). Let us examine this relationship, based on the most currently used consideration for a migration process to be collective, which is that there is coordination and cooperation between migrating cells (Scarpa and Mayor, 2016).

A highly specialised epithelial cell, with fully developed junctions and elaborated apical and basolateral domains, is relatively static and possesses a low migratory capacity, and is thus not capable of collective or individual migration. However, as discussed previously, any cells possessing a reduced amount of epithelial features (such as fewer or more dynamic junctions and a less structured apico-basal surface) are often able to migrate actively. In addition, although epithelial cells can exchange positions and migrate autonomously (Ewald et al., 2012; Gompel et al., 2001), the physical connection between cells means that the movement of just one cell will influence the behaviour of neighbouring cells. Thus, migratory active epithelial cells are only capable of collective cell migration, and the more cell-cell adhesion and junctions there are between cells, the more 'collective' the migratory process will be (Fig. 1B).

Conversely, at the other extreme of the spectrum, a cell in the utmost mesenchymal state will only undergo individual migration. In many cases, however, mesenchymal cohorts, such as the mesoderm, endoderm and neural crest cells of many species (Campbell and Casanova, 2015; Dumortier et al., 2012; Supatto et al., 2009; Theveneau et al., 2010), clearly exhibit the coordination and cooperation associated with collectively migrating cells (Fig. 2; reviewed by Collins and Nelson, 2015; Haeger et al., 2015; Mayor and Etienne-Manneville, 2016; Theveneau and Mayor, 2013). In fact, with advances in cell labelling and improved *in vivo* imaging, many cell types that were thought to move individually have now been shown to make cell-cell contacts that influence each other's movements (Kulesa and Gammill, 2010; Theveneau et al., 2010). In addition, these types of studies have revealed that the collective migration of mesenchymal cells can also be achieved through mutual chemotaxis between neighbouring cells, and that this is sufficient to maintain collective behaviour in the absence of cell-cell adhesion (reviewed by Haeger et al., 2015; Mayor and Etienne-Manneville, 2016).

Moreover, it has been suggested that to test if a cell cohort is migrating collectively or not, several parameters related to migration (velocity, persistence, polarity, tracks, etc.) as well as the behaviour of an isolated cell, a cell within a group and the average behaviour of the group should be compared (Theveneau and Mayor, 2011). This has simply not been possible in some systems, such as the mouse mesendoderm and neural crest, and thus it remains possible that cells that have been thought to undergo individual mesenchymal migration may in fact be undergoing a collective mesenchymal migration, as seen in more experimentally tractable systems.

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This is the case, for example, for chick mesoderm cells. Live-imaging studies have shown that these cells migrate at high density, in a very directional manner, and are continually in close contact (Chuai et al., 2012). Thus, despite their mesenchymal state, they seem to migrate collectively. Similarly, mouse mesoderm cells appear to be in continual contact during their migration, strongly suggesting that they may also undergo collective migration (Nakatsuji et al., 1986). However, this is currently under debate, as mouse mesoderm cells in explants migrate away from one another and seem to be more independent (Hashimoto et al., 1987), although this raises the question of whether this kind of *in vitro* assay can be used to test for *in vivo* collective behaviour. For example, it is possible that contact inhibition of locomotion, a feature of collectively migrating cells (reviewed by Szabó and Mayor, 2015), may give rise to dispersal *in vitro* whereas environmental cues *in vivo* could counteract contact inhibition and instead promote collective migration. Thus, although current data suggests that similar processes appear to occur in mice, the inability to image these cells *in vivo* makes it difficult to determine how collective their migration is in nature.

In conclusion, cells display varying degrees of interaction as they migrate. Obviously, the extreme ends of the spectrum of epithelial to mesenchymal cell states are fundamentally incompatible with collective migration. However, the medians of these two states are in fact highly compatible, as the features associated with an intermediate epithelial/mesenchymal phenotype and dynamic collective migration overlap, and are perhaps even cooperative and synergistic in their nature. Thus, it is likely that the mixed features of these two processes facilitate each other, and that the great variety of ways in which they can combine gives rise to the rich plethora of cell migration phenomena seen *in vivo*.

Applying the spectrum to metastatic events

Conceptually, it seems obvious that EMT would play a crucial role in the metastatic dissemination of epithelial tumours, conveying invasive migratory properties on tumour cells. However, the pathological importance of EMT has long been under debate for a number of reasons, not least of which is the difficulty of observing tumour dissemination *in vivo*, and added to this is the seemingly irreconcilability between collective cell migration and EMT. As such, discussions about whether these processes occur during tumour metastasis have been obscured by semantics, especially as, similar to the situation in developmental processes, there is a rich variety of migratory events that occur during tumour dissemination that also tend to escape from strict definitions.

This is particularly apparent if one considers that most cancer investigators define EMT on a molecular basis, in terms of a transcriptional programme consisting of a downregulation of E-cadherin, catenins and cytokeratins, and upregulation of the mesenchymal markers N-cadherin, vimentin and fibronectin (Peinado et al., 2007). The drawback to this has been that, just as for developmental morphogenesis, no single gene is specific to all epithelial tissues and no single marker definitively identifies all EMTs in all circumstances. Furthermore, in more and more epithelial-derived cancers, a mix of epithelial and mesenchymal markers is seen, suggesting that the cells are not in a 'pure' epithelial or mesenchymal state (Jolly et al., 2015). Recently, this led prominent cancer researchers to propose that EMT switches cells from a fully epithelial state to one that is partially mesenchymal, with retention of certain key epithelial markers (Ye and Weinberg, 2015), and that this can be represented as an EMT spectrum (Li and Kang, 2016; Nieto et al., 2016).

Similar to EMT, our understanding of collective migration with respect to tumour progression is evolving rapidly. Collective migration used to be associated with just the invading front of primary tumours, but now it is emerging as a powerful mechanism for the seeding of secondary tumours. Conventional models suggest that metastases are seeded by single cells from the primary tumour; however, there is growing evidence that seeding can also be achieved through the collective action of circulating tumour cells (CTCs) travelling together in clusters (Aceto et al., 2014; reviewed by Cheung and Ewald, 2016). Considering this in light of the spectrum model, it is possible that EMT to the median could actually facilitate the delamination of cell clusters from primary tumours; the cells in such clusters would retain epithelial features that support cluster integrity, and acquire mesenchymal features that drive invasion and migration. In this way, the mechanisms driving EMT would also promote metastasis by enhancing collective cell movement. This notion is supported by studies of breast cancer models showing that CTC clusters both retain and require epithelial gene expression, and can transition between distinct epithelial differentiation states to accomplish the proliferative versus migratory components of metastasis (Cheung and Ewald, 2016). Furthermore, mesenchymal CTCs and CTC clusters exist in human patients, and the relative frequency of epithelial versus mesenchymal phenotype CTCs within a patient can shift with disease progression and cancer therapy (Yu et al., 2013).

One of the major challenges for finding ways to impede tumour dissemination has been the great diversity of mechanisms used by cancer cells to escape, migrate and invade (reviewed by Friedl and Wolf, 2003). Furthermore, cancer cells can modify their migration mechanisms in response to different conditions, which makes it important to understand the broad spectrum of ways in which cells achieve dynamic changes in cell state and migratory mode, and how they interrelate. This underlies the timeliness of incorporating these observations with those seen in developmental contexts into a common framework that enables us to draw parallels across many diverse systems. Rather than disregarding the morphological continuum to fit with pre-established molecular mechanisms and definitions, we should instead strive to unveil the molecular mechanisms that account for the diversity of these morphological observations. Such an approach will help us to understand how cancer cells disseminate in different contexts, and potentially lead to new treatment strategies.

Another key challenge will be to reconcile the use of largely morphological criteria for defining epithelial and mesenchymal cell states in developmental systems with the gene expression criteria that are more commonly used for studying cancer models. A clear example comes when considering E-cadherin, which has been well-established as an important tumour suppressor in a variety of tumour types (Berx and van Roy, 2009; Cavallaro and Christofori, 2004; Hazan et al., 2004; Strumane et al., 2004). However, the idea that adhesion proteins such as E-cadherin principally act as invasion suppressors is incompatible with many experimental and clinical observations (reviewed by Rodriguez et al., 2012). Thus, for example, a recent study showed that, contrary to expectation, the expression of a key EMT-inducer, Twist1, in mammary epithelial cells does not result in a loss of epithelial-specific gene expression; in this case, E-cadherin still localises to cell membranes at every stage of dissemination, and E-cadherin knockdown inhibits the dissemination of Twist1^{+} cells (Shamir et al., 2014). Thus, levels of E-cadherin might be just one feature that determines whether Twist1 induces single cell dissemination or collective epithelial invasion. It thus appears that cellular context, including post-translational

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modifications and protein turnover, might crucially regulate junction dynamics and cell motility and can collaborate with the microenvironment to alter tissue-level phenotypes. It will be important to take all of this into consideration in future studies.

Conclusions

Although categorisation can be useful to establish a common basis for study, the biology of *in vivo* systems frequently escapes strict black and white categories. In particular, when considering migration events in developmental and disease contexts, the use of strict terms (e.g. 'bona fide', 'partial', 'canonical') to define EMT is misleading. Instead, it has become clear that we are dealing with cells transiting between different stages along a continuum. Indeed, considering cell migratory processes in development, only rare exceptions appear to transition from the extreme ends of the spectrum, and it is far more common to find cells shifting in degrees of cell behaviour. Furthermore, it is now indisputable that collective migration overlaps with both epithelial and mesenchymal states, and that this in turn correlates with a spectrum of migratory behaviours. Intriguingly, this diversity implies that cells avail of a wide range of strategies in diverse developmental events, each of which may offer different migratory parameters such as robustness, speed, duration, fluidity and complexity of the underlying mechanism.

We strongly feel that this new approach to EMT and collective migration, derived from studying the array of cell states found *in vivo*, provides a common cellular framework that embraces many observations from both developmental and oncogenic studies. We also note that the idea of an EMT spectrum has very recently been proposed (Li and Kang, 2016; Nieto et al., 2016; Ye and Weinberg, 2015). Here, we go a step further by suggesting that cells transition from different extents of 'epithelialness' to different grades of 'mesenchymalness' in a reversible manner. Furthermore, our model attempts to reconcile two fundamental processes that are clearly interrelated and that, at certain points in the spectrum, go hand in hand. Challenges for future research will be to identify the variety of cell features that underlie these states and how they combine along the spectrum, to understand the mechanisms that drive distinct transitions, and to find corresponding cell markers in order to obtain a more comprehensive view of the prevalence of a given transition in the wide spectrum of cancer types. In doing so, we will be better positioned to advance our understanding of the basic mechanisms at play during tumour progression and will have a better approach to identifying the pathological processes that need to be targeted.

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Competing interests

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References

Aceto, N., Bardia, A., Miyamoto, D. T., Donaldson, M. C., Wittner, B. S., Spencer, J. A., Yu, M., Pely, A., Engstrom, A., Zhu, H. et al. (2014). Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* **158**, 1110-1122.

Acloque, H., Adams, M. S., Fishwick, K., Bronner-Fraser, M. and Nieto, M. A. (2009). Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. *J. Clin. Invest.* **119**, 1438-1449.

Ahlstrom, J. D. and Erickson, C. A. (2009). The neural crest epithelial-mesenchymal transition in 4D: a 'tail' of multiple non-obligatory cellular mechanisms. *Development* **136**, 1801-1812.

Arnoux, V., Côme, C., Kusewitt, D. F., Hudson, L. G. and Savagner, P. (2005). Cutaneous wound reepithelialization. In *Rise and Fall of Epithelial Phenotype: Concepts of Epithelial-Mesenchymal Transition* (ed. P. Savagner), pp. 111-134. Boston, MA: Springer US.

Bahri, S., Wang, S., Conder, R., Choy, J., Vlachos, S., Dong, K., Merino, C., Sigrist, S., Molnar, C., Yang, X. et al. (2010). The leading edge during dorsal closure as a model for epithelial plasticity: Pak is required for recruitment of the Scribble complex and septate junction formation. *Development* **137**, 2023-2032.

Barrallo-Gimeno, A. and Nieto, M. A. (2005). The Snail genes as inducers of cell movement and survival: implications in development and cancer. *Development* **132**, 3151-3161.

Batlle, E., Sancho, E., Francí, C., Domínguez, D., Monfar, M., Baulida, J. and García De Herreros, A. (2000). The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat. Cell Biol.* **2**, 84-89.

Baum, B. and Georgiou, M. (2011). Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. *J. Cell Biol.* **192**, 907-917.

Bertet, C., Sulak, L. and Lecuit, T. (2004). Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* **429**, 667-671.

Berx, G. and van Roy, F. (2009). Involvement of members of the cadherin superfamily in cancer. *Cold Spring Harb. Perspect. Biol.* **1**, a003129.

Blankenship, J. T., Backovic, S. T., Sanny, J. S. P., Weitz, O. and Zallen, J. A. (2006). Multicellular rosette formation links planar cell polarity to tissue morphogenesis. *Dev. Cell* **11**, 459-470.

Brown, N. H. (2011). Extracellular matrix in development: insights from mechanisms conserved between invertebrates and vertebrates. *Cold Spring Harb. Perspect. Biol.* **3**, a005082.

Cai, D., Chen, S.-C., Prasad, M., He, L., Wang, X., Choesmel-Cadamuro, V., Sawyer, J. K., Danuser, G. and Montell, D. J. (2014). Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. *Cell* **157**, 1146-1159.

Campbell, K. and Casanova, J. (2015). A role for E-cadherin in ensuring cohesive migration of a heterogeneous population of non-epithelial cells. *Nat. Commun.* **6**, 7998.

Campbell, K., Whissell, G., Franch-Marro, X., Batlle, E. and Casanova, J. (2011). Specific GATA factors act as conserved inducers of an endodermal-EMT. *Dev. Cell* **21**, 1051-1061.

Cano, A., Pérez-Moreno, M. A., Rodrigo, I., Locascio, A., Blanco, M. J., del Barrio, M. G., Portillo, F. and Nieto, M. A. (2000). The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat. Cell Biol.* **2**, 76-83.

Carver, E. A., Jiang, R., Lan, Y., Oram, K. F. and Gridley, T. (2001). The mouse snail gene encodes a key regulator of the epithelial-mesenchymal transition. *Mol. Cell. Biol.* **21**, 8184-8188.

Caussinus, E., Colombelli, J. and Affolter, M. (2008). Tip-cell migration controls stalk-cell intercalation during Drosophila tracheal tube elongation. *Curr. Biol.* **18**, 1727-1734.

Cavallaro, U. and Christofori, G. (2004). Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat. Rev. Cancer* **4**, 118-132.

Cheung, K. J. and Ewald, A. J. (2016). A collective route to metastasis: seeding by tumor cell clusters. *Science* **352**, 167-169.

Cho, A., Kato, M., Whitwam, T., Kim, J. H. and Montell, D. J. (2016). An atypical tropomyosin in Drosophila with intermediate filament-like properties. *Cell Rep.* **16**, 928-938.

Chuai, M., Hughes, D. and Weijer, C. J. (2012). Collective epithelial and mesenchymal cell migration during gastrulation. *Curr. Genomics* **13**, 267-277.

Classen, A.-K., Anderson, K. I., Marois, E. and Eaton, S. (2005). Hexagonal packing of Drosophila wing epithelial cells by the planar cell polarity pathway. *Dev. Cell* **9**, 805-817.

Collins, C. and Nelson, W. J. (2015). Running with neighbors: coordinating cell migration and cell-cell adhesion. *Curr. Opin. Cell Biol.* **36**, 62-70.

Davies, J. A. and Garrod, D. R. (1997). Molecular aspects of the epithelial phenotype. *BioEssays* **19**, 699-704.

Dumortier, J. G., Martin, S., Meyer, D., Rosa, F. M. and David, N. B. (2012). Collective mesendoderm migration relies on an intrinsic directionality signal transmitted through cell contacts. *Proc. Natl. Acad. Sci. USA* **109**, 16945-16950.

Ewald, A. J., Brenot, A., Duong, M., Chan, B. S. and Werb, Z. (2008). Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. *Dev. Cell* **14**, 570-581.

Ewald, A. J., Huebner, R. J., Palsdottir, H., Lee, J. K., Perez, M. J., Jorgens, D. M., Tauscher, A. N., Cheung, K. J., Werb, Z. and Auer, M. (2012). Mammary collective cell migration involves transient loss of epithelial features and individual cell migration within the epithelium. *J. Cell Sci.* **125**, 2638-2654.

Friedl, P. and Gilmour, D. (2009). Collective cell migration in morphogenesis, regeneration and cancer. *Nat. Rev. Mol. Cell Biol.* **10**, 445-457.

Friedl, P. and Wolf, K. (2003). Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat. Rev. Cancer* **3**, 362-374.

Galván, J. A., Zlobec, I., Wartenberg, M., Lugli, A., Gloor, B., Perren, A. and Karamitopoulou, E. (2015). Expression of E-cadherin repressors SNAIL, ZEB1 and ZEB2 by tumour and stromal cells influences tumour-budding phenotype and suggests heterogeneity of stromal cells in pancreatic cancer. *Br. J. Cancer* **112**, 1944-1950.

Gompel, N., Cubedo, N., Thisse, C., Thisse, B., Dambly-Chaudière, C. and Ghysen, A. (2001). Pattern formation in the lateral line of zebrafish. *Mech. Dev.* **105**, 69-77.

Grigore, A. D., Jolly, M. K., Jia, D., Farach-Carson, M. C. and Levine, H. (2016). Tumor budding: the name is EMT. Partial EMT. *J. Clin. Med.* **5**, 51.

Haeger, A., Wolf, K., Zegers, M. M. and Friedl, P. (2015). Collective cell migration: guidance principles and hierarchies. *Trends Cell Biol.* **25**, 556-566.

Hashimoto, K., Fujimoto, H. and Nakatsuji, N. (1987). An ECM substratum allows mouse mesodermal cells isolated from the primitive streak to exhibit motility similar to that inside the embryo and reveals a deficiency in the T/T mutant cells. *Development* **100**, 587-598.

Hatta, K. and Takeichi, M. (1986). Expression of N-cadherin adhesion molecules associated with early morphogenetic events in chick development. *Nature* **320**, 447-449.

Hava, D., Forster, U., Matsuda, M., Cui, S., Link, B. A., Eichhorst, J., Wiesner, B., Chitnis, A. and Abdelilah-Seyfried, S. (2009). Apical membrane maturation and cellular rosette formation during morphogenesis of the zebrafish lateral line. *J. Cell Sci.* **122**, 687-695.

Hay, E. D. (2005). The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. *Dev. Dyn.* **233**, 706-720.

Hazan, R. B., Qiao, R., Keren, R., Badano, I. and Suyama, K. (2004). Cadherin switch in tumor progression. *Ann. N. Y. Acad. Sci.* **1014**, 155-163.

Huber, M. A., Kraut, N. and Beug, H. (2005). Molecular requirements for epithelial-mesenchymal transition during tumor progression. *Curr. Opin. Cell Biol.* **17**, 548-558.

Jacinto, A., Wood, W., Woolner, S., Hiley, C., Turner, L., Wilson, C., Martinez-Arias, A. and Martin, P. (2002). Dynamic analysis of actin cable function during Drosophila dorsal closure. *Curr. Biol.* **12**, 1245-1250.

Jaźwińska, A., Ribeiro, C. and Affolter, M. (2003). Epithelial tube morphogenesis during Drosophila tracheal development requires Piopio, a luminal ZP protein. *Nat. Cell Biol.* **5**, 895-901.

Jolly, M. K., Boareto, M., Huang, B., Jia, D., Lu, M., Ben-Jacob, E., Onuchic, J. N. and Levine, H. (2015). Implications of the hybrid epithelial/mesenchymal phenotype in metastasis. *Front. Oncol.* **5**, 155.

Kardash, E., Reichman-Fried, M., Maitre, 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; sup pp 41-11.

Kowalczyk, A. P. and Nanes, B. A. (2012). Adherens junction turnover: regulating adhesion through cadherin endocytosis, degradation, and recycling. *Subcell. Biochem.* **60**, 197-222.

Kulesa, P. M. and Gammill, L. S. (2010). Neural crest migration: patterns, phases and signals. *Dev. Biol.* **344**, 566-568.

Lamouille, S., Xu, J. and Derynck, R. (2014). Molecular mechanisms of epithelial-mesenchymal transition. *Nat. Rev. Mol. Cell Biol.* **15**, 178-196.

Lebreton, G. and Casanova, J. (2014). Specification of leading and trailing cell features during collective migration in the Drosophila trachea. *J. Cell Sci.* **127**, 465-474.

Lecuit, T. (2004). Junctions and vesicular trafficking during Drosophila cellularization. *J. Cell Sci.* **117**, 3427-3433.

Leptin, M. (1991). twist and snail as positive and negative regulators during Drosophila mesoderm development. *Genes Dev.* **5**, 1568-1576.

Li, W. and Kang, Y. (2016). Probing the fifty shades of EMT in metastasis. *Trends Cancer* **2**, 65-67.

Lim, J. and Thiery, J. P. (2011). Alternative path to EMT: regulation of apicobasal polarity in Drosophila. *Dev. Cell* **21**, 983-984.

Mayor, R. and Etienne-Manneville, S. (2016). The front and rear of collective cell migration. *Nat. Rev. Mol. Cell Biol.* **17**, 97-109.

Montero, J.-A., Carvalho, L., Wilsch-Brauninger, M., Kilian, B., Mustafa, C. and Heisenberg, C.-P. (2005). Shield formation at the onset of zebrafish gastrulation. *Development* **132**, 1187-1198.

Nakatsuji, N., Snow, M. H. and Wylie, C. C. (1986). Cinemicrographic study of the cell movement in the primitive-streak-stage mouse embryo. *J. Embryol. Exp. Morphol.* **96**, 99-109.

Nakaya, Y. and Sheng, G. (2008). Epithelial to mesenchymal transition during gastrulation: an embryological view. *Dev. Growth Differ.* **50**, 755-766.

Nakaya, Y., Sukowati, E. W., Wu, Y. and Sheng, G. (2008). RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation. *Nat. Cell Biol.* **10**, 765-775.

Nandadasa, S., Tao, Q., Menon, N. R., Heasman, J. and Wylie, C. (2009). N- and E-cadherins in Xenopus are specifically required in the neural and non-neural ectoderm, respectively, for F-actin assembly and morphogenetic movements. *Development* **136**, 1327-1338.

Navarro, P., Lozano, E. and Cano, A. (1993). Expression of E- or P-cadherin is not sufficient to modify the morphology and the tumorigenic behavior of murine spindle carcinoma cells. Possible involvement of plakoglobin. *J. Cell Sci.* **105**, 923-934.

Niessen, C. M. and Gottardi, C. J. (2008). Molecular components of the adherens junction. *Biochim. Biophy. Acta* **1778**, 562-571.

Nieto, M. A. (2011). The ins and outs of the epithelial to mesenchymal transition in health and disease. *Annu. Rev. Cell Dev. Biol.* **27**, 347-376.

Nieto, M. A., Huang, R. Y.-J., Jackson, R. A. and Thiery, J. P. (2016). Emt: 2016. *Cell* **166**, 21-45.

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

Oda, H. and Tsukita, S. (2001). Real-time imaging of cell-cell adherens junctions reveals that Drosophila mesoderm invagination begins with two phases of apical constriction of cells. *J. Cell Sci.* **114**, 493-501.

Oda, H., Tsukita, S. and Takeichi, M. (1998). Dynamic behavior of the cadherin-based cell-cell adhesion system during Drosophila gastrulation. *Dev. Biol.* **203**, 435-450.

Ohkubo, T. and Ozawa, M. (2004). The transcription factor Snail downregulates the tight junction components independently of E-cadherin downregulation. *J. Cell Sci.* **117**, 1675-1685.

Pastor-Pareja, J. C., Grawe, F., Martín-Blanco, E. and García-Bellido, A. (2004). Invasive cell behavior during Drosophila imaginal disc eversion is mediated by the JNK signaling cascade. *Dev. Cell* **7**, 387-399.

Peinado, H., Olmeda, D. and Cano, A. (2007). Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat. Rev. Cancer* **7**, 415-428.

Pinheiro, E. M. and Montell, D. J. (2004). Requirement for Par-6 and Bazooka in Drosophila border cell migration. *Development* **131**, 5243-5251.

Revenu, C. and Gilmour, D. (2009). EMT 2.0: shaping epithelia through collective migration. *Curr. Opin. Genet. Dev.* **19**, 338-342.

Revenu, C., Streichan, S., Dona, E., Lecaudey, V., Hufnagel, L. and Gilmour, D. (2014). Quantitative cell polarity imaging defines leader-to-follower transitions during collective migration and the key role of microtubule-dependent adherens junction formation. *Development* **141**, 1282-1291.

Rodriguez, F. J., Lewis-Tuffin, L. J. and Anastasiadis, P. Z. (2012). E-cadherin's dark side: possible role in tumor progression. *Biochim. Biophys. Acta* **1826**, 23-31.

Rørth, P. (2009). Collective cell migration. *Annu. Rev. Cell Dev. Biol.* **25**, 407-429.

Rørth, P. (2012). Fellow travellers: emergent properties of collective cell migration. *EMBO Rep.* **13**, 984-991.

Sandmann, T., Girardot, C., Brehme, M., Tongprasit, W., Stolc, V. and Furlong, E. E. (2007). A core transcriptional network for early mesoderm development in Drosophila melanogaster. *Genes Dev.* **21**, 436-449.

Scarpa, E. and Mayor, R. (2016). Collective cell migration in development. *J. Cell Biol.* **212**, 143-155.

Schafer, G., Narasimha, M., Vogelsang, E. and Leptin, M. (2014). Cadherin switching during the formation and differentiation of the Drosophila mesoderm – implications for epithelial-to-mesenchymal transitions. *J. Cell Sci.* **127**, 1511-1522.

Schulte, J., Weidig, M., Balzer, P., Richter, P., Franz, M., Junker, K., Gajda, M., Friedrich, K., Wunderlich, H., Östman, A. et al. (2012). Expression of the E-cadherin repressors Snail, Slug and Zeb1 in urothelial carcinoma of the urinary bladder: relation to stromal fibroblast activation and invasive behaviour of carcinoma cells. *Histochem. Cell Biol.* **138**, 847-860.

Shamir, E. R. and Ewald, A. J. (2015). Adhesion in mammary development: novel roles for E-cadherin in individual and collective cell migration. *Curr. Top. Dev. Biol.* **112**, 353-382.

Shamir, E. R., Pappalardo, E., Jorgens, D. M., Coutinho, K., Tsai, W.-T., Aziz, K., Auer, M., Tran, P. T., Bader, J. S. and Ewald, A. J. (2014). Twist1-induced dissemination preserves epithelial identity and requires E-cadherin. *J. Cell Biol.* **204**, 839-856.

Shaye, D. D., Casanova, J. and Llimargas, M. (2008). Modulation of intracellular trafficking regulates cell intercalation in the Drosophila trachea. *Nat. Cell Biol.* **10**, 964-970.

Shook, D. and Keller, R. (2003). Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development. *Mech. Dev.* **120**, 1351-1383.

Strumane, K., Berx, G. and Van Roy, F. (2004). Cadherins in cancer. *Handbook Exp. Pharmacol.* **165**, 69-103.

Supatto, W., McMahon, A., Fraser, S. E. and Stathopoulos, A. (2009). Quantitative imaging of collective cell migration during Drosophila gastrulation: multiphoton microscopy and computational analysis. *Nat. Protoc.* **4**, 1397-1412.

Szabó, A. and Mayor, R. (2015). Cell traction in collective cell migration and morphogenesis: the chase and run mechanism. *Cell Adh. Migr.* **9**, 380-383.

Taneyhill, L. A. (2008). To adhere or not to adhere: the role of Cadherins in neural crest cell development. *Cell Adh. Migr.* **2**, 223-230.

Tepass, U. and Hartenstein, V. (1994). The development of cellular junctions in the Drosophila embryo. *Dev. Biol.* **161**, 563-596.

Tepass, U., Tanentzapf, G., Ward, R. and Fehon, R. (2001). Epithelial cell polarity and cell junctions in Drosophila. *Annu. Rev. Genet.* **35**, 747-784.

Theveneau, E. and Mayor, R. (2011). Can mesenchymal cells undergo collective cell migration? The case of the neural crest. *Cell Adh. Migr.* **5**, 490-498.

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Theveneau, E. and Mayor, R. (2012). Cadherins in collective cell migration of mesenchymal cells. *Curr. Opin. Cell Biol.* **24**, 677-684.

Theveneau, E. and Mayor, R. (2013). Collective cell migration of epithelial and mesenchymal cells. *Cell. Mol. Life Sci.* **70**, 3481-3492.

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.

Thiery, J. P., Acloque, H., Huang, R. Y. J. and Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871-890.

West, J. J. and Harris, T. J. (2016). Cadherin trafficking for tissue morphogenesis: control and consequences. *Traffic*.

Williams, M., Burdsal, G., Periasamy, A., Lewandoski, M. and Sutherland, A. (2012). Mouse primitive streak forms in situ by initiation of epithelial to mesenchymal transition without migration of a cell population. *Dev. Dyn.* **241**, 270-283.

Wood, W., Jacinto, A., Grose, R., Woolner, S., Gale, J., Wilson, C. and Martin, P. (2002). Wound healing recapitulates morphogenesis in Drosophila embryos. *Nat. Cell Biol.* **4**, 907-912.

Ye, X. and Weinberg, R. A. (2015). Epithelial-mesenchymal plasticity: a central regulator of cancer progression. *Trends Cell Biol.* **25**, 675-686.

Yu, M., Bardia, A., Wittner, B. S., Stott, S. L., Smas, M. E., Ting, D. T., Isakoff, S. J., Ciciliano, J. C., Wells, M. N., Shah, A. M. et al. (2013). Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* **339**, 580-584.

Zallen, J. A. (2007). Planar polarity and tissue morphogenesis. *Cell* **129**, 1051-1063.

Zohn, I. E., Li, Y., Skolnik, E. Y., Anderson, K. V., Han, J. and Niswander, L. (2006). p38 and a p38-interacting protein are critical for downregulation of E-cadherin during mouse gastrulation. *Cell* **125**, 957-969.