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EMT 2.0: shaping epithelia through collective migration

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Epithelial–mesenchymal transitions (EMTs) drive epithelial remodelling by converting cohesive, stable epithelial layers into individual, motile mesenchymal cells. It is now becoming clear that, from being an all-or-nothing switch, EMT can be applied in a fine-tuned manner to allow the efficient migration of cohesive epithelia that maintain their internal organisation. Recent work suggests that such collective motility involves a complex balance between epithelial and mesenchyme-like cell states that is driven by internal and external cues. Although this cohesive mode requires more complex control than single cell migration, it creates opportunities in term of tissue guidance and shaping that are starting to be unravelled.

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Introduction

Epithelial cells are the building blocks of most complex organs. One defining characteristic of epithelia is stability. Apically localised cell–cell junctions mediate tight physical coupling between epithelial cells to form sheets of almost crystalline order [1,2]. During morphogenetic events such as embryogenesis and wound repair, epithelial sheets are remodelled by a combination of cell proliferation, cell shape changes and local cell rearrangements, all of which are tightly regulated to ensure sheet integrity is maintained [3–5]. One key mode of multicellular morphogenesis that is often considered to be incompatible with epithelial organisation is directed cell migration. In order to actively migrate, it is assumed that epithelial sheets must first downregulate cell–cell junctions and disperse as individual mesenchymal cells, a process known as an epithelial–mesenchymal transition (EMT) [6,7]. EMT drives cells between opposing states, with the motility and disorder of mesenchymal cells providing the flip side to the static order of the archetypal epithelium. However, recent live imaging studies of tissue morphogenesis have revealed that such extreme EMT events may be the exception, with more subtle 'intermediate' transitions being the norm. The packing state of an epithelium can be reduced locally allowing the tissue to become mobile whilst retaining integrity and organisation. Here we review recent work addressing the mechanisms that determine the spatiotemporal pattern of partial EMT events in migrating epithelia *in vivo*. In addition, we discuss how this 'stick'n'move' mode of migration confers epithelia with collective properties such as the guidance of non-motile cells and the mechanical regulation of tissue assembly.

Collective migration, blurring the boundaries of EMT

Embryonic development provides several examples of true EMT where cells organised in sheets or clusters lose their apico-basal polarity and cell contacts, disperse and migrate as single cells towards their assigned destination. Textbook examples of EMT include neural crest cells during emigration [8] and early mesoderm cells during gastrulation (reviewed in [9]). Here, the migrating cells display all features of mesenchyme such as flattened morphology and highly dynamic protrusions. EMT also plays an important role in the early migration of single primordial germ cells (PGC) towards the gonad. In zebrafish, PGC extend dynamic protrusions and need to downregulate E-cadherin to disperse and migrate at normal speed [10]. Interestingly, PGCs in Drosophila switch from a clustered conformation to single cells in order to pass through the midgut by transepithelial migration, a step that involves the relocalisation of E-cadherin by the G-protein-coupled receptor Tre1 [11].

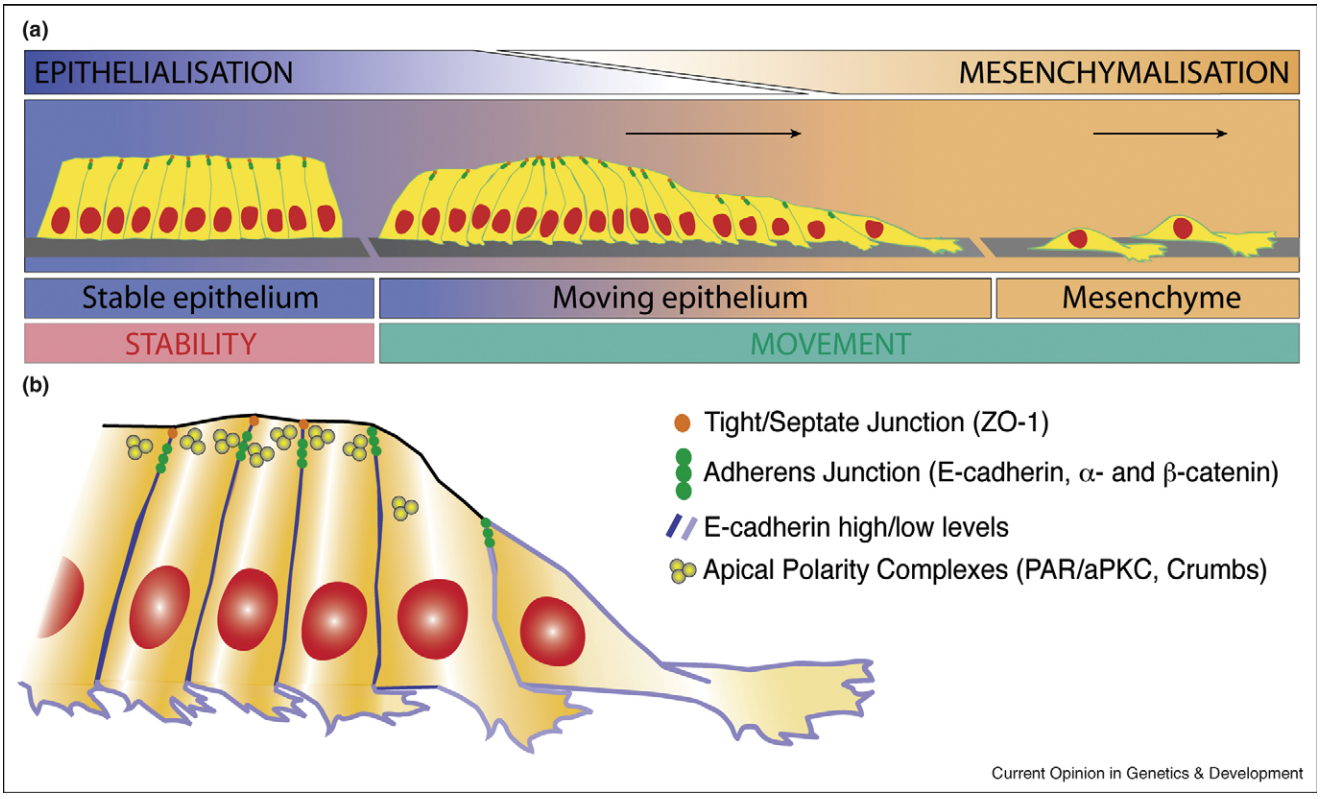
Although EMT is an effective mode to redistribute epithelial cells, it is now becoming apparent that many epithelia undergo efficient migration whilst maintaining integrity [12]. For example, during Drosophila metamorphosis, larval ectodermal tissues are replaced by cells of the imaginal discs and histoblast nests, both of which actively migrate as coherent epithelial sheets that maintain clear cell–cell junctions [13,14••]. Likewise, branching morphogenesis of the Drosophila tracheal system witnesses the directed migration of epithelial tubes, with enriched E-cadherin and ZO-1 proteins at the apical lumen [15]. The zebrafish lateral line primordium is a highly migratory tissue that deposits epithelial rosette-like mechanosensory neuromast organs along the flanks of the embryo [16]. The cells of the primordium express E-cadherin and exhibit foci of the tight junction protein ZO1 and of aPKC at the centre of the tightly packaged rosettes [17••,18]. Whilst these epithelia can migrate

efficiently it does not mean that EMT type mechanisms are not involved in their movement. On the contrary, one common feature of such migrating epithelia is that cells at the edge of these tissues display characteristics that are more typical of mesenchymal cells, such as reduced apico-basal polarity and the presence of highly dynamic actin-rich cellular protrusions. Another characteristic is the loss of tight junctions, septate junctions in the case of insects, from cells at the leading edge [14••,17••,18]. However, unlike the classical definition of EMT, here cells never become completely dissociated but remain coupled to each other and the rest of the epithelium (Figure 1). Furthermore, the number of cells that adopt mesenchymal properties is highly tissue-dependent, suggesting that this transition can be fine-tuned. For example, during tracheogenesis in *Drosophila*, only the tip cell of an elongating tracheal branch protrudes highly dynamic extensions and is actively migrating [19••] whereas in imaginal discs and the lateral line primordium the mesenchyme-like domain extends over several cell diameters. Another interesting case is the Border Cell (BC) cluster that migrates within the egg chamber of *Drosophila*. It is composed of 2 non-migrating polar cells (PC) surrounded by up to 10 outer cells that develop highly dynamic protrusions [20]. The adherens junction (AJ) components E-cadherin and β-catenin as well as the apical polarity markers Crumbs, aPKC, PAR3 and PAR6 are asymmetrically localised such that the highest concentration is at the PC apices, suggesting that they have higher epithelial character than the cells that surround them [21,22,23••,24••,25,26]. Combined, these data support the view that, rather than being all-or-nothing, EMT can be modulated to reduce epithelial organisation locally and promote motility. Furthermore, the heterogeneity in the extent of EMT observed in different contexts implies that this process is tightly regulated within migrating epithelia.

Signalling pathways modulate partial EMT *en route*

Some of the first insights into the regulation of EMT within moving epithelia came from genetic studies on

Figure 1



Collective migration of an epithelium with local, graded EMT. **(a)** Schematic highlighting the different morphological cellular states encountered in EMT. On the left, the apico-basally polarised epithelium is highly ordered and static. On the right, two individually migrating cells depict the flattened, labile and dynamic mesenchymal state. In between, a theoretical example of collectively migrating cells is represented. It consists of a group of apico-basally polarised cells exhibiting local melting of the epithelial organisation. This intermediate motile state is controlled by an equilibrium between epithelialising and mesenchymalising cues. The direction of migration is depicted (black arrow). **(b)** Model of the polarised organisation of a collectively migrating group of cells. Numerous cells have the characteristics of a true epithelium. Leading-edge cells undergoing different degrees of EMT develop dynamic membrane protrusions as lamellipodia and filopodia. Depending on the extent of their EMT, they exhibit a loss of tight junctions markers as ZO-1, a reduced level of adhesion proteins as E-cadherin, α-catenin and β-catenin. The apical polarity complexes are also delocalised or reduced.

members of the Jun N-terminal Kinase (JNK) family of MAP Kinases in *Drosophila*. Following the identification of *basket* (DJNK) and *hemipterous* (JNK-Kinase) as regulators of leading edge behaviour during dorsal closure, this pathway has been shown to regulate partial EMT events elsewhere [27]. For example, loss of JNK activity results in reduced numbers of mesenchyme-like peripodial cells at the boundary of imaginal disc epithelium during eversion, whereas JNK hyperactivation, achieved by loss of the negative regulator *puckered*, results in increased mesenchymal character and even loss of cell cohesion [14••]. Interestingly, JNK-signalling has been more recently shown to drive the reverse transition in Drosophila Border Cells, where its loss causes reduced cluster cohesion [23••,24••]. The identification of upstream regulators of JNK-signalling will be an important step towards understanding its spatially controlled effects on EMT [28].

Recent papers using Drosophila trachea and the zebrafish lateral line have shed light on the extracellular signals that spatially control this 'dual state' within these migrating epithelia. In both contexts it is the guidance cues directing migration that are directly responsible for driving a subset of cells towards a mesenchyme-like fate. In the case of Drosophila trachea, discrete FGF spots prefigure the branching pattern. However, the levels of FGFR-activity also directly determine which cell of the branch becomes the leading tip cell [29]. The migrating lateral line primordium is guided by an extrinsic 'stripe' of the chemokine stromal derived factor 1 (SDF1) that runs along the horizontal myoseptum of the zebrafish embryo [30]. Primordia in embryos deficient for Cxcr4b/SDF1 signalling show an expansion of epithelial rosettes at the expense of mesenchyme-like leading edge ([31], DG in preparation). Thus, similar to the dual role of FGF-signalling during tracheogenesis, Cxcr4b/SDF1 interactions are required to both direct tissue migration and drive cells at the leading edge towards a mesenchyme-like fate.

If partial EMT is regulated by extrinsic guidance cues, how is the response of the target tissue restricted to a defined subset of cells? As with any switch, there are active mechanisms that prevent cells with subthreshold signalling levels from responding. As described above, the activation of the FGF receptor by its ligand branchless (*bnl*) leads to the selection of a single mesenchyme-like tip cell, precision not encoded by the extracellular distribution of *bnl* alone. Work by a number of labs has shown that the cell receiving the highest level of ligand actively prevents its neighbours from adopting tip cell fate by expressing the Notch (N) ligand Delta (Dl), a target gene of FGFR-signalling [32]. Increased Dl expression activates N in surrounding cells that, in turn, dampens their ability to respond to FGF [29,32]. Thus, combining FGF-mediated chemotaxis with lateral inhibition provides an elegant method for sharpening the EMT response within the target tissue. Interestingly, it has been subsequently shown that a similar N-Dl lateral inhibition mechanism is involved in restricting the response to VEGF in angiogenic sprouts [33].

Whilst the identification of factors driving cells towards a mesenchyme-like state is an important step, it is becoming apparent that this will only provide half of the equation describing epithelial migration. Rather than describing a homogenous default state, the packing of epithelial domains is also dynamically regulated, allowing for further spatiotemporal control of tissue behaviour. For example, *in vivo* imaging of the lateral line primordium in zebrafish revealed that discrete FGF-10 foci induce surrounding cells to adopt a classical columnar epithelial morphology, before assembling into rosettes via concerted apical constriction [17••,34••]. Whilst it is not yet known how the nucleating FGF spots mediate the local increase in epithelial adhesion in this context, differences in epithelial intercalation behaviour in the Drosophila tracheal system can now be explained at the level of E-cadherin expression and trafficking [35••]. Dynamin-mediated endocytosis leads to the reduction of E-cadherin at AJs in cells undergoing intercalation, whereas this downregulation is opposed in the more stable dorsal trunk by rab11-dependent recycling of endocytosed E-cadherin [35••]. Interestingly, embryos expressing rab11-dominant negative constructs also showed defects in cell intercalation, suggesting that a delicate balance of E-cad recycling is required to maintain epithelial stability whilst allowing AJ remodelling during intercalation. Collectively, these papers demonstrate that multiple pathways can modulate cell states within migrating tissues to increase or decrease cell rearrangement as required.

Good reasons for not splitting up

Embryos have therefore evolved highly intricate mechanisms to allow cells to migrate whilst maintaining epithelial organisation. However, epithelial integrity demands that migration is rigorously regimented such that the behaviour of each cell is tightly linked to that of its neighbours, like a three-legged race but with many more individuals taking part. This begs the question of why these epithelia do not go the whole hog and undergo complete dissociation via EMT as many others do. A number of recent investigations have revealed that by remaining mechanically coupled, migrating collectives possess a number of group skills that are not available to migrating individuals.

Cohesion allows collective guidance and displacement

One innovation of migrating collectives is that not all cells need to be able to detect extrinsic guidance cues in order to migrate properly [36]. Small numbers of wild-type cells have been demonstrated to rescue the migration of tracheal

branches [29], border cell clusters [37••] and lateral line primordia [31] that are mutant for their respective guidance receptors. In all cases, it is thought that the wild-type cells guide the migration of mutant followers by mechanical cues propagated via AJs. Indeed, cadherins are known to be able to transduce a mechanical stress [38] and allow the mechanical coupling of cells [39,40]. The idea that cell movements can be mechanically controlled is supported by recent studies demonstrating that Border Cells in clusters with reduced cohesion migrate in different directions [23••,24••]. Delegating the role of navigator to the more motile leading edge cells could enable coupled followers to become assembled into structures that may be incompatible with effective chemotactic movement, such as tubes or rosettes.

Movement as a vehicle of (shape) change

The *raison d'être* of most embryonic migrations events is to generate organised shape. Mechanical coupling potentially allows epithelia to utilise the forces generated by directional migration to control internal organisation. Perhaps the best demonstration of such a mechanism to date comes from recent experiments addressing the effect of tip cell laser ablation on tracheal branch growth [19••]. These experiments revealed that the pulling force generated by the migrating tip cell is required to drive AJ remodelling and cell intercalation within the branch and thus regulate luminal organisation.

Conclusion

The classical view that EMT is a switch that converts static, ordered epithelia into labile, individual mesenchymal cells needs to be re-examined in view of collective migration. Rather than being all-or-nothing, morphogenesis applies EMT in a fine-tuned manner to increase or decrease freedom of movement locally whilst maintaining epithelial characteristics. Such a smooth transition requires a combination of spatially controlled signals, many of which act in direct opposition to change cell state dynamically. Given that these signals ultimately affect the mechanical properties of tissues, understanding EMT during morphogenesis will require a broad, cross-disciplinary approach that ranges from cell and developmental biology to biophysics.

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