Epithelial-mesenchymal transitions: insights from development

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Summary
Epithelial-mesenchymal transition (EMT) is a crucial, evolutionarily conserved process that occurs during development and is essential for shaping embryos. Also implicated in cancer, this morphological transition is executed through multiple mechanisms in different contexts, and studies suggest that the molecular programs governing EMT, albeit still enigmatic, are embedded within developmental programs that regulate specification and differentiation. As we review here, knowledge garnered from studies of EMT during gastrulation, neural crest delamination and heart formation have furthered our understanding of tumor progression and metastasis.

Key words: Epithelial-mesenchymal transition, Gastrulation, Neural crest, Heart morphogenesis

Introduction
Epithelial-mesenchymal transition (EMT) is an evolutionarily conserved developmental process that contributes to the formation of the body plan, histogenesis and organogenesis. In the late 19th century, mesenchymal and epithelial cells were recognized as having distinct phenotypes (Duval, 1879) and, although EMT was apparent to embryologists (Platt, 1894), it only became interesting to developmental biologists in the 1960s. Following pioneering work from Elizabeth Hay (Greenburg and Hay, 1982; Hay, 2005), we now know that epithelial cells lose apicobasal polarity and intercellular junctions during EMT. These changes in cell polarity and adhesion disrupt the epithelial basement membrane and allow cellular penetration into an extracellular matrix (ECM)-rich compartment: a process referred to as delamination (see Glossary, Box 1). These newly formed mesenchymal cells transiently express distinct mesenchymal markers, acquire a front-rear polarity and become invasive, favoring cell-ECM rather than cell-cell adhesions.

Interestingly, EMT is not irreversible: cells frequently cycle between epithelial and mesenchymal states via EMT and the reverse process, mesenchymal-epithelial transition (MET). Importantly, EMT has been implicated in pathological conditions, such as organ fibrosis, and in cancer, where it contributes to tumor progression and metastasis (Kalluri and Weinberg, 2009; Thiery et al., 2009). As such, much effort has been devoted to understanding the molecular regulation of EMT during development as an insight into the role and regulation of EMT in pathology.

EMT is context dependent, occurring within the framework of other signaling mechanisms, such as cell fate induction, commitment and differentiation. However, the precise events that drive EMT are not fully understood. Genetic studies in Drosophila originally identified the transcription factors Twist and Snail as potential drivers of EMT during gastrulation (Leptin and Grunewald, 1990). Soon after, a Snail ortholog, Slug (Snai2), was shown to be involved in EMT in chicken embryo gastrulation (Nieto et al., 1994). Since then, several genes encoding transcription factors, cell polarity proteins and effector proteins have been shown to govern EMT in normal and transformed epithelial cells (see Table 1), suggesting that novel mechanisms govern EMT (Peinado et al., 2007; Moustakas and Heldin, 2009; Thiery et al., 2009; Nieto, 2011; Valastyan and Weinberg, 2011). In this Primer (see Box, Development: the big picture), we explore the molecular programs that govern EMT in various developmental contexts and discuss how these developmental studies have provided clues into the control and activation of EMT during cancer.

EMT during development
Four waves of EMT and MET have been described during morphogenesis and organogenesis (see Table 2 for a summary of these different events during development). In mammals, for example, EMT occurs following implantation in the primitive endoderm to form the parietal endoderm (Veltmaat et al., 2000). Subsequently, during implantation, trophoblasts localized at the tip of chorionic villi undergo EMT and invade the endometrium (Kokkinos et al., 2010). EMT, and the reverse process MET, then occur at various stages throughout embryonic development, but we shall focus on the stages of gastrulation, neural crest delamination and heart formation, as they represent three distinct mechanisms of development that are associated with EMT.

Gastrulation: formation of mesoderm and mesendoderm
Different morphogenetic movements during gastrulation (see Glossary, Box 1) apply to different species. Even in the most ancient of species, such as cnidarians, there are no fewer than nine different mechanisms operating during gastrulation (Byrum and Martindale, 2004). In all cases, these complex morphogenetic movements incorporate epithelial cell plasticity, such as when cells invaginate or involute (see Glossary, Box 1) as cell collectives. Interestingly, in the early stages of body plan formation, cells participating in collective migration exchange neighbors through convergence-extension movements (Keller and Shook, 2004). EMT is one of the mechanisms activated during gastrulation that allows cells to ingress (see Glossary, Box 1) into a defined region of the embryo (the primitive streak in amniotes, the vegetal pole in sea urchin and the ventral furrow in Drosophila; see Glossary, Box 1).

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Box 1. Glossary

**Delamination.** The process whereby cells separate from an epithelial layer.

**Endocardial cushion.** A structure formed by endocardial cells that undergo epithelial-mesenchymal transition (EMT) in an hyaluronic acid-rich extracellular matrix region named ‘cardiac jelly’.

**Epicardium.** The outer cell layer of the heart primordium derived from the pro-epicardium, a cluster of cells proximal to the heart and liver.

**Gastrulation.** The embryonic stage corresponding to the formation of the three primary germ layers: ectoderm, mesoderm and endoderm. Gastrulation involves either collective cell migration through invagination or involution (partial EMT), or individual cell migration by ingestion (EMT).

**Heart primordium.** A group of mesodermal cells specified for heart development during gastrulation. These cells assemble into two cardiogenic mesodermal layers, which later migrate anteriorly and fuse into a single heart tube.

**Ingression.** A process that allows single cells to delaminate and migrate into the sub-epiblast territory; it is typical of EMT.

**Invagination.** The process that drives an epithelium to fold inwards or outwards through the coordinated constriction of the apex of cells.

**Involuion.** The mechanism by which a group of cells detaches and migrates into the adjacent territory as cell collectives.

**Neural crest.** A term originally proposed by Marshall in 1879 to define a group of cells in the neural fold of vertebrates that undergoes EMT and migrates extensively before differentiating into cells that give rise to craniofacial structures, most of the peripheral nervous system and melanocytes.

**Primitive streak.** A transient groove, formed at the onset of gastrulation, into which epiblast cells ingress to give rise mesoderm and definitive endoderm in amniotes.

**Somite.** A segmental mass of cells derived from paraxial mesoderm forming in a rostrocaudal sequence on each side of the neural tube posterior to the rhombencephalon. Somites will give rise to the vertebra, dermis and striated muscles.

**Vegetal pole.** A group of cells localized in the ventral side of the embryo from which the primary mesenchymal cells ingress in the blastocoelic cavity.

**Ventral furrow.** The site of gastrulation in insects appearing as a transient invagination of the blastoderm in the ventral side of the embryo from which mesodermal cells will delaminate.

using shared signal transduction pathways (Thiery et al., 2009). This stresses the importance of evolutionarily conserved genes, such as Snail family members, in driving EMT.

**Gastrulation and EMT in Drosophila embryos**

In the *Drosophila* embryo, the ventral furrow is determined following establishment of the dorsal-ventral gradient (Leptin, 2005). Upon activation and binding of Spätzle to the Toll receptor, following establishment of the dorsal-ventral gradient (Leptin, Drosophila embryo, the ventral furrow is determined from the pro-epicardium, a cluster of cells proximal to the heart and liver.

Gastrulation and EMT in mouse embryos

Gastrulation in the mouse embryo is rapid and thus requires rapid changes in gene expression and function. The downregulation of E-cadherin, for example, is controlled both at the transcriptional level by Snai1 and at the post-translational level by P38 interacting protein (IP)-p38-MAP kinase complex and the FERM protein (EPB4.1L5) (Zohn et al., 2006; Lee et al., 2007; Hirano et al., 2008). In mouse embryos, and in chick, cells specified for gastrulation are not as strictly programmed as those in *Drosophila* or in sea urchin embryos. Nevertheless, embryos are patterned by morphogenetic gradients, and the site of gastrulation is restricted so that only a defined cell population undergoes EMT. Once again, Snail, namely its orthologs, Snai1 and Snai2, are key inducers of EMT in gastrulating mouse embryos (Barrallo-Gimeno and Nieto, 2005). *Snai1* knockout mice show a gastrulation phenotype, similar to that seen in *Drosophila*, suggesting a conserved role for Snai1 in EMT (Carver et al., 2001). By contrast, *Snai2* deletion in mice shows no EMT failure (Jiang et al., 1998). In chicken, Snai2 is expressed in the primitive streak and its perturbation does lead to a gastrulation phenotype (Nieto et al., 1994). The repression of mesendodermal Snai2 by ectodermal Sox3, ensures integrity of the
non-ingressing ectoderm, whereas, reciprocally, Snail2 represses Sox3 to induce EMT in the primitive streak. Similarly, mutual repression between Snai1 and Sox3 is observed in the mouse (Acloque et al., 2011) (Fig. 2A). The integrity of the basement membrane in areas outside the primitive streak is maintained by fibronectin leucine-rich transmembrane protein 3 (Flrt3) expressed in anterior visceral endoderm (AVE) (Egea et al., 2008). In Flrt3 mutant mice, the basement membrane in the AVE is disrupted and the neighboring anterior epiblast cells display EMT characteristics (Egea et al., 2008).

Various other factors have been identified in EMT in mouse gastrulation (Fig. 2B). Members of the thrombospondin type 1 repeat (TSR) superfamily are ECM components important for growth factor activity. Upon O-fucosylation of TSR by the protein O-fucosyltransferase 2 (Pofut2), EMT in the primitive streak is restricted. As such, Pofut2 mutants show elevated expressions of FGF8, Nodal and Wnt3 (Du et al., 2010), crucial signaling proteins in gastrulation (Niswander and Martin, 1992; Crossley and Martin, 1995; Liu et al., 1999; Ciruna and Rossant, 2001; Yamamoto et al., 1999; Ciruna and Rossant, 2001; Yamamoto et al., 1999; Ciruna and Rossant, 2001; Yamamoto et al., 1999). Newly formed mesodermal cells retain some E-cadherin in the mouse epiblast causes failure of cell ingestion from the primitive streak (Kitajima et al., 2000; Arnold et al., 2008). Definition of the endoderm cell lineage requires Eomes/Nodal signaling; by comparison, cardiac fate specification requires low levels of Nodal and occurs independently of Foxh1/Smad4 activation (Costello et al., 2011). This is one of numerous cases where EMT regulators play additional roles in cell fate determination.

Recently, FGFR signaling in chick primitive streak cell movement was studied using the tyrosine kinase inhibitor SU5402. FGFR inhibition altered mesoderm formation, albeit without affecting Snail2 and E-cadherin, suggesting alternate mechanisms for gastrulation in the chick when compared with the mouse (Hardy et al., 2011). These new challenging results, however, should be considered carefully, as there are currently no FGFR-specific inhibitors available and L-CAM (the chicken ortholog of mouse E-cadherin) is downregulated at the primitive streak level (Thiery et al., 1984). Newly formed mesodermal cells retain some E-cadherin expression (Nakaya et al., 2008), which is also seen in the mouse and sea urchin, as E-cadherin proteins cannot be quickly downregulated by transcriptional repression. However, post-translational mechanisms for E-cadherin degradation have been described previously (Wu and McClay, 2007). Small G-proteins also crucially regulate cytoskeletal reorganization and EMT during gastrulation (Fig. 2A,B). Indeed, Rac1 knockout is embryonic lethal, owing to apoptosis in newly formed mesodermal cells (Sugihara et al., 1998). Furthermore, failure to downregulate RhoA causes basement membrane retention and a failure of EMT, suggesting that cell delamination causes the

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**Table 1. Transcription and polarity factors involved in EMT**

<table>
<thead>
<tr>
<th>Transcription factors</th>
<th>Description</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Snail1 and Snail2</td>
<td>Contain a Snail/Gfi (SNAG) repressor domain at the N terminus and four or five zinc fingers in the C-terminal domain. Bind to E-boxes of the E-cadherin gene promoter and repress its transcription.</td>
<td>Peinado et al., 2007</td>
</tr>
<tr>
<td>Zeb1 and Zeb2</td>
<td>Contain a central homeodomain, four N-terminal zinc fingers and three C-terminal zinc fingers. Zeb factors bind to bipartite E-boxes as in the E-cadherin promoter.</td>
<td>Peinado et al., 2007</td>
</tr>
<tr>
<td>Twist</td>
<td>Contains a basic helix-loop-helix motif that interacts with Bmi1, together with the PR2C complex to repress E-cadherin and p16INK4a through chromatin remodeling. Twist also binds to the E-box of PDGFRα, and this regulation is important for extracellular matrix degradation in tumor metastasis.</td>
<td>Yang et al., 2010; Eckert et al., 2011</td>
</tr>
<tr>
<td>Srp and GATA</td>
<td>Contains two GATA-type zinc fingers that bind to the consensus sequence AGATAAG within regulatory regions.</td>
<td>Brown et al., 2004; Brewer and Pizzey, 2006</td>
</tr>
<tr>
<td>Wt1</td>
<td>Contains four zinc fingers at the C-terminal domain and a proline/glutamine-rich DNA-binding domain at the N terminus.</td>
<td>Hohenstein and Hastie, 2006; Miller-Hodges and Hohenstein, 2012</td>
</tr>
<tr>
<td>Smad</td>
<td>Contains two MAD homology domains. Eight Smad isoforms are functionally classified as TGFβ receptor-regulated Smads (R-Smads are isoforms 1, 2, 3, 5 and 8), common-mediator Smad (Co-Smad is isoform 4) and inhibitory Smads (I-Smads are isoforms 6 and 7).</td>
<td>Moustakas and Heldin, 2009</td>
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<tr>
<th>Polarity genes</th>
<th>Description</th>
<th>Reference</th>
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<tr>
<td>Par3</td>
<td>Contains three PDZ domains and an atypical protein kinase C (PKC)-binding domain.</td>
<td>St Johnston and Ahringer, 2010</td>
</tr>
<tr>
<td>Par6</td>
<td>Contains a PDZ domain and forms the apical protein complex with Par3 and atypical PKCs.</td>
<td>St Johnston and Ahringer, 2010</td>
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<tr>
<td>Crumbs (Crb)</td>
<td>A transmembrane protein, with a large extracellular domain composed of 29 epidermal growth factor-like repeats and four laminin A globular-domain-like repeats. Crb was discovered in Drosophila as an essential protein for maintaining apicobasal polarity and the integrity of embryonic epithelia.</td>
<td>Hurd et al., 2003; St Johnston and Ahringer, 2010; Laprise and Tepass, 2011</td>
</tr>
<tr>
<td>Epb4.11S</td>
<td>Contains a FERM domain at the N terminus, which binds to p12-catenin; the C terminus binds to paxillin.</td>
<td>Hirano et al., 2008</td>
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</table>
basement membrane to break down (Nakaya et al., 2008). In summary, studies of EMT during gastrulation in a variety of model organisms have uncovered a wealth of data about EMT processes and their partial conservation throughout evolution.

The neural crest

Delamination and EMT

Neural crest cells (see Glossary, Box 1) originate in the neural fold or the neural tube, according to a rostrocaudal gradient along the body axis. Neural crest cells then delaminate and migrate as mesenchymal-like cells in defined routes before reaching their target sites, where they differentiate into various derivatives (Sauka-Spengler and Bronner-Fraser, 2008; Minoux and Rijli, 2010; Theveneau and Mayor, 2011). Cranial neural crest, including most vagal crest, must be distinguished from trunk neural crest; in the chick, this has been defined as located anteriorly or posteriorly to somite numbers 3/4, respectively (Ferguson and Graham, 2004). Major morphological differences in these two regions affect crest cell delamination and migration into adjacent territories. The precise origin of the cranial neural crest is still a controversial issue, whereas trunk crest cells originate in the dorsal neural tube. The two regions also differ when overexpressing Snail2, which enhances the production and migration of chick cranial neural crest, but not trunk crest (del Barrio and Nieto, 2002).

Trunk neural crest delamination is spatiotemporally coordinated with the formation of somites (see Glossary, Box 1) from the paraxial mesoderm. Bone morphogenetic protein 4 (BMP4), a member of the TGFβ superfamily, promotes neural crest delamination, and its activation is exquisitely regulated by Noggin (Fig. 3), an antagonist that is itself inhibited by signaling from the newly formed somites (Sela-Donenfeld and Kalcheim, 1999; Sela-Donenfeld and Kalcheim, 2000). Noggin overexpression inhibits BMP4 and Wnt1 expression, whereas in embryos grafted with Noggin-producing cells, β-catenin overexpression rescues delamination and reactivates G1/S transition in the neural crest (Burstyn-Cohen et al., 2004). BMP is modulated by crossveinless 2 (Cv-2), which causes premature neural crest migration when overexpressed (Coles et al., 2004). BMP-Wnt signaling is also triggered by retinoid acid (RA), which forms a rostrocaudal gradient in the paraxial mesoderm. Meanwhile, an opposing

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**Table 2. Successive epithelial-mesenchymal transition during embryonic development**

<table>
<thead>
<tr>
<th>Targeted cell or tissue</th>
<th>Resulting structures</th>
<th>References</th>
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<tbody>
<tr>
<td>Primary EMT</td>
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<tr>
<td>Primitive endoderm cells</td>
<td>Parietal endoderm cells</td>
<td>Verheijen and Defize, 1999; Veltmaat et al., 2000</td>
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<tr>
<td>Trophoderm</td>
<td>Giant trophoblast</td>
<td>VicoV and Aplin, 1996; Sutherland, 2003; Kokkinos et al., 2010; Abell et al., 2011</td>
</tr>
<tr>
<td>Epiblast at primitive streak</td>
<td>Mesodermal and endodermal cells</td>
<td>See text</td>
</tr>
<tr>
<td>Neural crest</td>
<td>Mesectoderm, peripheral nervous system, enteric nervous system, endocrine cells and melanocytes</td>
<td>See text</td>
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<tr>
<td>Mesodermal cell MET</td>
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<tr>
<td>Axial mesoderm</td>
<td>Notochord</td>
<td>Hay, 2005; Ohta et al., 2010</td>
</tr>
<tr>
<td>Paraxial mesoderm</td>
<td>Somites</td>
<td>Dale et al., 2006; Morales et al., 2007</td>
</tr>
<tr>
<td>Intermediate mesoderm</td>
<td>Precursors of the urogenital system</td>
<td>Perez-Pomares and Munoz-Chapuli, 2002</td>
</tr>
<tr>
<td>Lateral mesoderm</td>
<td>Somatopleure and splanchnopleure</td>
<td>Perez-Pomares and Munoz-Chapuli, 2002</td>
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<tr>
<td>Node precursors from anterior primitive streak</td>
<td>Ciliated rosette-like clusters that inserted into endodermal layer to form the node</td>
<td>Lee et al., 2010</td>
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<tr>
<td>Secondary EMT</td>
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<tr>
<td>Epithelium of palatal halves</td>
<td>Mesenchymal cells in fused palate</td>
<td>Nawshad, 2008; San Miguel et al., 2011</td>
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<tr>
<td>Somites (dorsal part)</td>
<td>Dermal mesenchyme and myoblast</td>
<td>Gros et al., 2005</td>
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<tr>
<td>Somites (ventral part)</td>
<td>Sclerotomal mesenchyme cells</td>
<td>Christ et al., 2007</td>
</tr>
<tr>
<td>Liver diverticulum</td>
<td>Septum transversum mesenchyme</td>
<td>Tanizumi and Miyajima, 2007</td>
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<tr>
<td>Pancreatic bud</td>
<td>Langerhans islets</td>
<td>Johansson and Grapin-Botton, 2002</td>
</tr>
<tr>
<td>Somatopleure</td>
<td>Connective tissue of body wall mesenchyme</td>
<td>Christ et al., 1983</td>
</tr>
<tr>
<td>Splanchnopleure</td>
<td>Endocardial progenitors, angioblast and hematopoietic stem cells</td>
<td>Eichmann et al., 2005; Tavian and Peault, 2005</td>
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<tr>
<td>Secondary MET</td>
<td></td>
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<tr>
<td>Endocardial progenitors</td>
<td>Endocardium</td>
<td>Schluter et al., 2006; Schluter and Brand, 2009; Svensson, 2010; Torlopp et al., 2010</td>
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<tr>
<td>Mesoderm in close proximity to liver bud</td>
<td>Proepicardium (adjacent to sinus venosus endothelium)</td>
<td>Stark et al., 1994; Perantoni et al., 1995; Urban et al., 2006</td>
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<tr>
<td>Presumptive kidney mesenchyme</td>
<td>Pretubular clusters around the Wolfian duct-derived ureteric bud</td>
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<tr>
<td>Tertiary EMT</td>
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<tr>
<td>Endocardium</td>
<td>Endocardial cushion mesenchyme</td>
<td>Carmona et al., 2000; Watt et al., 2004</td>
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<tr>
<td>Proepicardium</td>
<td>Mesothelial cells</td>
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<tr>
<td>Tertiary MET</td>
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<tr>
<td>Mesothelial cells</td>
<td>Epicardium</td>
<td>Watt et al., 2004; Torlopp et al., 2010</td>
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<tr>
<td>Quaternary EMT</td>
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<tr>
<td>Epicardium</td>
<td>Sub-epicardial mesenchymal cells</td>
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EMT, epithelial-mesenchymal transition; MET mesenchymal-epithelial transition.
gradient of FGF signaling prevents neural crest specification and EMT in the trunk by maintaining Noggin expression (Martinez-Morales et al., 2011). It was originally assumed that BMP-Wnt and Snail2 caused cytoskeleton remodeling via RhoB to promote migration (Liu and Jessell, 1998; del Barrio and Nieto, 2002). However, Rho activity is high in the trunk neural fold and prevents EMT through maintenance of N-cadherin; EMT occurs only with diminished Rho activity (Groysman et al., 2008). Delamination of trunk neural crest cells have been examined using live-cell imaging, which revealed that neural crest cell EMT cannot be explained solely by dissolution of adherens junctions. Actomyosin-based contractility in the apical surface can contribute to the neural crest detachment from the neural epithelium, leaving junctional complexes in the ruptured cell tails. In addition, not all neural crest cells detach during mitosis when the plane of cytokinesis is parallel to the basal surface (Ahlstrom and Erickson, 2009b; Ahlstrom and Erickson, 2009a). Thus, the biomechanics of EMT are not fully understood. Similarly, there are uncertainties in the current understanding of the contribution of BMP, Wnt and FGF signals in regulating adhesion and migration machineries.

SoxE protein members Sox8, Sox9 and Sox10 are expressed at different times during neural development (Cheung and Briscoe, 2003). In particular, Sox9 is implicated in crest cell specification and Sox10 in the maintenance of crest cell pluripotency (Kim et al., 2003; Cheung et al., 2005). BMP triggers Sox9-dependent induction of Snail2 (Sakai et al., 2006). Indeed, electroporation of Sox9 into the neural tube causes the expression of several neural crest markers along the entire neural tube (Sox10, Snail2, Cad6B and Foxd3), whereas delamination is dorsally restricted (Cheung and Briscoe, 2003). Snail2 electroporation, together with Sox9, is sufficient to induce EMT in the entire neural tube (Cheung et al., 2005). Of note, the expression of Snail2 and Sox9 can reprogram adult mammary cells into stem cells and promote breast cancer cell metastasis (Guo et al., 2012). Comparatively, electroporation of the winged-helix transcription factor FoxD3 enhances Hnk1 expression, a neural crest cell marker throughout the neural tube; but only those cells located at the dorsal border of the neural tube undergo EMT (Kos et al., 2001). In cooperation with Sox9, the basic leucine-zipper transcription factor Atf4 promotes EMT through induction of FoxD3 (Suzuki et al., 2010). Atf4 is an unstable transcription factor whose transient stability is ensured by the p300 histone acetyltransferase (Suzuki et al., 2010). FoxD3 downregulates N-cadherin expression and induces β1-integrin and laminin, which are required for migration and enhancing Sox10
expression (Cheung et al., 2005). N-cadherin overexpression at the dorsal neural tube prevents neural crest delamination (Nakagawa and Takeichi, 1998), while Snail2 represses cadherin 6B expression to permit EMT (Taneyhill et al., 2007). N-cadherin protein, but not mRNA, is downregulated during neural crest delamination, and the extracellular domain is cleaved by the metalloproteinase Adam10 upon BMP-mediated stimulation (Shoval et al., 2007). Subsequently, the C-terminal fragment of N-cadherin (Ctf1) is further cleaved by γ-secretase, generating a soluble Ctf2, which stimulates β-catenin nuclear translocation and the subsequent induction of cyclin D1 transcription and neural crest migration (Shoval et al., 2007). Epigenetic regulation in neural crest precursors is mediated by the histone-demethylase JumonjiD2A (JmjD2A) (Strobl-Mazzulla et al., 2010). Loss-of-function of JmjD2A results in the depletion of several neural crest specifiers, particularly Snail2 and Sox10, which both have an H3K9me3 epigenetic mark 0.5 kb upstream of the transcriptional start site. However, Snail2 is also associated with an H3K36me3 epigenetic mark for activation. Clearly, JmjD2A, which transiently binds to the Sox10 promoter prior to activation, is modifying the chromatin landscape in both directions, possibly allowing gradual activation of genes following neural crest induction. The delayed activation of Sox10 suggests the need of an additional transcriptional activator for Sox10. Epigenetic mechanisms should be investigated to define their contribution to EMT in a cellular context. It appears that many of the genes have overlapping functions during various stages of NC development, i.e. induction, EMT and migration, and hence it is difficult to identify genes that solely control EMT (Thiery and Sleeman, 2006). Current issues in definitively assessing the role of individual genes in EMT Some genes that control the initial steps of neural crest determination and specification have appeared in evolution prior to the establishment of the vertebrate body plan.
delamination of nerve crest in vertebrates. These genes are expressed in several amphioxus species, in cephalochordates and in Ciona intestinalis, a urochordate species closest to vertebrates; the last may have co-opted existing networks in a novel combination to establish the neural crest. However, none of these genes induces EMT in prochordates (Trainor et al., 2003; Bertrand and Escriva, 2011). Furthermore, in the mouse, the deletion of genes associated with neural crest formation in other models often results in phenotypes that do not affect induction, specification or delamination, but rather differentiation. Zeb2 is so far the only gene whose deletion leads to phenotype that might be associated with an EMT defect; Sox10-expressing cranial neural crest cells form but fail to delaminate in Zeb2-knockout mice (Van de Putte et al., 2003). The lack of an EMT phenotype may result from the possibility that EMT is engaged prior to the activation of Cre in conditional knockouts of potential EMT inducers. Furthermore, the EMT phenotype may only be observed with the deletion of two or several factors, as the chicken trunk neural crest requires the joint action of Sox9/Snail2 or Sox9/Atf4 (Cheung et al., 2005; Sakai et al., 2006; Suzuki et al., 2010).

Overall, the genetic regulatory network that controls EMT in the neural crest is not fully understood, especially in terms of how extracellular signals control epigenetic mechanisms, cell cycle, polarity, adhesion, migration and cytoskeletal remodeling. It is likely that neural crest cells progressively engage into EMT, with the delamination phase activated only when the local environment become permissible.

### Heart morphogenesis: cardiac cushion, heart valve and epicardium development

Heart morphogenesis involves the complex cycling between EMT and MET that is initiated during gastrulation. First, cardiogenic specified cells undergo EMT, migrate rostrally and form two cardiogenic territories through MET. This leads to the formation of the heart primordium (see Glossary, Box 1). A second cycle of EMT/MET creates the endothelial cell lining of the heart, followed by a third cycle that forms the endocardial cushion (see Glossary, Box 1) and its derivatives (Fig. 4A). This third cycle of EMT during cardiac morphogenesis involves endothelial cells from the atrioventricular canal (AVC) and the outflow tract (OFT); thus, this should in fact be designated as an endothelial-mesenchymal transition (EndMT) (see Box 2). MET drives the formation of the epicardium (see Glossary, Box 1) from a cluster of mesothelial-derived cells that develop beneath the sinus venosus in the E9.0-9.5 mouse embryo, which are themselves formed by EMT of the pro-epicardium. Subsequently, in the fourth cycle of EMT, a group of epicardial cells delaminates and gives rise to epicardial-derived cells (EPDCs), the mesenchymal cells that populate the sub-epicardium and form coronary smooth muscle, endothelial cells and cardiac fibroblasts (Fig. 4B) (Chua et al., 2011).

The first cycle of EMT at gastrulation involves induction of Snail1/2, which contributes to the formation of mesenchymal cells; part of this cell population rapidly acquires myogenic properties in response to a complex signaling pathway mediated by TGFβ family members (Ladd et al., 1998). The formation of the endocardium in the second cycle also involves TGFβ family members (Sugi and Markwald, 2003), although the detailed mechanisms remain to be explored. Most studies have focused on the third (involving EndMT; Box 2) and fourth (Fig. 4) cycles of EMT, partly owing to the availability of suitable experimental approaches, but also in order to understand the origin of major congenital heart defects (Bruneau, 2008; Perez-Pomares and de la Pompa, 2011). EMT in the fourth cycle differs quite substantially from the classical mechanisms of EMT driven by Snail, as in EndMT or as seen during gastrulation and neural crest delamination.

The zinc-finger transcription factor Wilms tumor gene 1 (Wt1), is inactivated in a significant fraction of pediatric kidney cancers, designated as Wilms tumors (Call et al., 1990; Gessler et al., 1990; Huang et al., 1990). Wt1 is expressed in pro-epicardium, in the developing epicardium and in EPDCs (Moore et al., 1999). Wt1 knockout mice show impaired formation of an intact epicardium (Moore et al., 1999), while Wt1 Gata5-Cre conditional knockout mice have a reduced number of EPDCs (Martinez-Estrada et al.,...
It is debatable whether Wt1 causes epicardial EMT by activating Snail1 and repressing E-cadherin by directly binding to their promoters (Martinez-Estrada et al., 2010) or by promoting canonical and non-canonical Wnt pathways (von Gise et al., 2011); the latter is supported by the impaired EPDC formation observed following the conditional knockout of β-catenin in pro-epicardium (Zamora et al., 2007). An interesting attempt to unravel Wt1 function showed that the Wnt4 gene is activated in kidney mesenchyme undergoing MET, but is repressed in epicardium undergoing EMT, with both processes directly mediated by Wt1 binding to the same transcriptional regulatory site of Wnt4 (Essafi et al., 2011). In the kidney mesenchyme, the CBP co-activator is recruited to the Wt1-bound Wnt4 locus, whereas, in the epicardium, the brain-abundant membrane-attached signal protein 1 (Basp1) co-repressor is recruited. The boundary of the Wnt4 locus is marked by CCCTC-binding factor (CTCF) and by cohesin, and loss of Wt1

Fig. 4. Epithelial-mesenchymal transition and mesenchymal-epithelial transition during heart formation in chick and mouse embryos. (A) The cartoon on the left depicts formation of the cardiac cushion located at the atrio-ventricular region. The detail (middle) illustrates how myocardial BMP2, endocardial Notch1 and TGFβ receptors determine the site for endothelial-mesenchymal transition (EndMT), which leads to the formation of the cardiac cushion. Transcription factors, such as Snail1, the Smad proteins, the Hey proteins, Gata4 and Fog proteins, are important for this process. Depiction of the network that connects BMP, Notch and Erbb3 signaling, leading to EndMT, is shown on the right. (B) Cross-sectional representation of the external part of the embryonic heart at 11 days (left). Gray, epicardial cells; blue, subepicardial cells; red, myocardial cells; pink, coronary endothelial cells; green, coronary smooth muscle cells; yellow, epicardial cells undergoing asymmetrical cell division; gray turning blue, cells undergoing epithelial-mesenchymal transition (EMT). The series of molecular events occurring in delaminating epicardial cells is shown alongside: cells (yellow) undergo EMT when they exhibit a microtubule spindle orientation perpendicular to the basement membrane. The orientation of the spindle is dependent on the maintenance of adherens junctions mediated by β-catenin and Numb localization. Platelet-derived growth factor receptor β (PDGFRβ) activates Sox9 and PI3-kinase for actin remodeling and motility. The gap junctional protein connexin 43 connects to microtubules linked to the organizing center for its alignment with the direction of cell migration. Wt1 activates Snail, which in turn represses E-cadherin; this mechanism is still debated (Martinez-Estrada et al., 2010; von Gise et al., 2011). Wt1 also activates canonical and non-canonical Wnt signaling, and directly stimulates the transcription of Raldh2, which in turn facilitates the synthesis of retinoic acid (RA). Notch signaling is also important for Raldh2 expression and acts upstream of TGFβ and PDGFRβ in driving migration of epicardial-derived cells.
switches the Wnt4 locus chromatin domain into either a repressed or an activated state. Loss of Ctcf expands the on/off switch to the flanking region, affecting neighboring genes; however, it does not affect Wt1-mediated regulation of the chromatin structure. Epicardium- and myocardium-derived signals mediate epicardial cell delamination, migration in the sub-epicardial space and invasion into myocardium (Perez-Pomares and de la Pompa, 2011). However, it is unclear which signals primarily induce EMT. In vitro, TGFβ promotes EMT in epicardial explants (Compton et al., 2006), but TGFβ is produced mostly by the epicardium, suggesting an autocrine mechanism. Defective EMT and migration is observed following epicardial-specific deletion of platelet-derived growth factor receptor β (PDGFRβ), which signals through the activation of phosphoinositide 3-kinase (PI3K) and Sox9 (Mellgren et al., 2008; Smith et al., 2011). Notch signaling may also play a role in epicardial EMT, as a constitutively active Notch1 intracellular domain promotes EMT in epicardial cells; this is probably mediated by Raldh2/RA signaling, as Notch1 deletion results in reduced Raldh2 expression (del Monte et al., 2011; Grieskamp et al., 2011). Wt1 also controls RA signaling via the activation of Raldh2 (Guadix et al., 2011; von Gise et al., 2011) (Fig. 4B).

Evidence suggests that overexpression of TGFβ receptor 3 (TGFBRIII) in a Tgfb3 knockout background rescues epicardial cell invasion. However, this rescue is diminished by siRNA targeting of the partitioning-defective protein Par6 (Pard6a – Mouse Genome Informatics) and the E3-ubiquitin ligase Smurf1 (Sanchez and Barnett, 2011), suggesting that the Par6-Smurf1-RhoA pathway, and hence regulation of cell polarity, is also important in epicardial EMT. In addition to epithelial polarity, the microtubule spindle during cell division plays an intriguing role in epicardial EMT; only epicardial cells with a microtubule spindle positioned perpendicular to the basement membrane enter into the sub-epicardium. Perturbation of β-catenin disrupts adherens junctions, causing an abnormal distribution of Numb and randomized spindle orientation (Wu et al., 2010). The gap junction protein connexin 43 (Cx43; Gja1 – Mouse Genome Informatics) regulates the microtubule-organizing center of epicardial cells through tubulin binding (Rhee et al., 2009). Indeed, Cx43-knockout mice show a coronary vascular developmental defect due to failed alignment between the microtubule-organizing center and the direction of epicardial cell migration. Epicardial EMT has received much attention recently, but no clear-cut delineation has been made between the mechanisms promoting EMT and migration, and those regulating the subsequent differentiation into several distinct lineages. The promoters used in vivo for conditional deletion are already expressed in the pro-epicardium rather than uniquely in the epicardium.

**Diversity of mechanisms potentially driving EMT**

The aforementioned studies demonstrate that distinct paracrine or autocrine signals can trigger EMT and that EMT is regulated by numerous ECM components and soluble growth factors that activate downstream signaling cascades and other signaling pathways (Fig. 5) (Tucker et al., 1990; Miettinen et al., 1994; Valles et al., 1996; Zoltan-Jones et al., 2003; Shintani et al., 2008). These signaling pathways elicit a mesenchymal phenotype by promoting the disassembly of junctional complexes and inducing actin cytoskeleton re-organization, favoring the activation of transcriptional regulators, such as Snail1 and Snail2, to prioritize EMT over other mechanisms, such as proliferation or differentiation (Blanco et al., 2007; Franco et al., 2010).

**Box 2. Heart EndMT**

The acronym EndMT (endothelial-mesenchymal transition) is used to distinguish endothelial cell delamination from the more common epithelial cell delamination. EndMT occurs in endothelial cells invading the cardiac jelly to form a cardiac cushion, which subsequently establishes the atrioventricular valves (Nakajima et al., 2000). EndMT is controlled by three distinct signaling pathways: TGFβ, Notch and ErbB3.

In chick embryos, TGFβ induces epithelial to mesenchymal transition (EMT) and cell invasion into the extracellular matrix via activation of TGFβR1 and TGFβRII, an essential co-receptor (Townsend et al., 2012). TGFβ signaling is linked to actin remodeling, possibly in part through zyxin, a focal adhesion-associated LIM protein implicated in EndMT under TGFβ-Twist-1-zyxin regulation (Mori et al., 2009). In mouse embryos, BMP2 signaling drives EndMT, as shown by the absence of a cardiac cushion in endothelial Smad4 knockout mice (Moskovitz et al., 2011). Deletion of the long form of latent TGFβ binding protein 1 (Ltbp1) generates a hypoplastic endocardial cushion and hyperplastic valves, revealing both its promotion and attenuation roles in EMT in different contexts (Todorovic et al., 2011).

In the second pathway, Notch promotes TGFβ2-induced EMT and induces the transcription of Snail1 (Timmerman et al., 2004). Mutations of NOTCH1 or its ligand JAG1 in humans cause heart valve defects (Li et al., 1997; Garg et al., 2005). Knockout of the Notch target Hey2 or a double knockout of Hey1/Heyl leads to defects involving ventricular septal and atrioventricular pulmonary valves (Fischer et al., 2007). The valve presumptive territory expands if the balance of myocardial BMP2 or endocardial Notch1 is perturbed. Mice with constitutive endocardial Notch1 activity exhibit ectopic levels of Hey1 and Heyl. Furthermore, invasive behavior is triggered following BMP2 treatment and can be inhibited by reducing Snail1, TGFβ2 or Notch1 levels. Notch1 activation in myocardium represses BMP2 and impairs EndMT, whereas Notch1 deletion in the endocardium abolishes endocardial Hey1, Hey2 and Heyl expression, and extends BMP2 expression to the ventricular endocardium (Luna-Zurita et al., 2010).

The third pathway, which is mediated by ErbB3 signaling, is regulated by Gata4 upstream of ErbB3, a member of the epidermal growth factor receptor (EGFR) tyrosine kinase family (Rivera-Feliciano et al., 2006). A Tie-2 Cre-specific deletion of Gata4 causes a failure of EndMT and cardiac cushion formation. Increased EMT is found in the Fg2 (Zfp52l2)-knockout explant culture of outflow tract and atrioventricular canal cushions in a collagen gel invasion assay, suggesting that Fg2, a GATA interaction partner, attenuates Gata4 function in EndMT (Flagg et al., 2007). In addition, Gata4 interacts with Smad4 and cooperatively activates Id2 in atrioventricular septal development (Moskovitz et al., 2011).

Importantly, EMT involves the disruption of epithelial polarity. Snail1 represses the transcription of Crumbs3 (Crb3 – Mouse Genome Informatics), thus abolishing the junctional complexes of Par and Crumbs, proteins that are involved in establishing cell polarity (Whiteman et al., 2008). Similarly, Zeb1 promotes metastasis and loss of cell polarity (Spaderna et al., 2008). In Drosophila, the posterior midgut joins the anterior midgut in a partial EMT. During this process, Serpent, an ortholog of GATA repressor, represses Crumbs transcription (Campbell et al., 2011). In addition, signaling regulatory molecules act directly on polarity proteins, bypassing transcription factors, such as Snail and Zeb1. For example, TGFβRII binds to the tight junction protein occludin and locally assembles into a complex containing Par6 (Barrios-Rodiles et al., 2005). Activated TGFβRII phosphorylates Par6, which binds to Smurfl and causes RhoA ubiquitylation and the
Carcinomas initially remain confined within a basement mem-
brane, but eventually infiltrate into the surrounding stroma
and intravasate into the blood and lymph vessels where they are
circulating. Carcinomas, i.e. derived from epithelia, can
collectively infiltrate and invade the stroma resulting in
cancer progression. Vice versa, knowledge acquired in studying
EMT in development offers a unique opportunity to investigate
whether similar pathways are re-activated during carcinoma
progression and invasion. Recurrent observations prompted the
hypothesis that carcinomas may have co-opted these mechanisms
for invasion and distant dissemination (Thiery, 2002; Kalluri and
Weinberg, 2009). The role of EMT in tumor progression has
been repeatedly emphasized, including the transient acquisition
of stemness (Polyak and Weinberg, 2009; Thiery et al., 2009;
Valastyan and Weinberg, 2011). Although still debated, there is
increasing evidence that some carcinoma move as solitary cells;
but collective invasive migration also seems to play a major role
(Thiery, 2009). Deciphering the complex pathways that drive
EMT in development offers a unique opportunity to investigate
whether similar pathways are re-activated during carcinoma
progression. Vice versa, knowledge acquired in studying
invasion and metastatic cascades may provide new leads for
developmental studies. Such an example is the discovery of the
roles of alternative splicing and miRNAs in EMT. Alternative
splicing of FGFR2 in association with induced EMT was
initially documented in the rat carcinoma cell-line NBT-II
dissolution of junctions (Ozdamar et al., 2005). Dynamic changes
in other small G-proteins, such as Rac1 and Cdc42, cause actin
cytoskeleton remodeling, potentially leading to the loss of
intercellular adhesion and cell movement through the formation of
filopodia and lamellipodia (Braga et al., 1997; Tapon and Hall,
1997; Chu et al., 2011).

Theoretically, in every case, extracellular stimulation,
transcription factor-linked signaling, cell polarity changes and
cytoskeletal remodeling have to be accomplished for the
manifestation of EMT. However, cell polarity and cytoskeletal
remodeling have not been carefully analyzed. Studies using
cultured cells independently of developmental context have
therefore contributed breakthrough discoveries for unraveling
EMT.

Understanding EMT in cancer: insights from
development
Most tumors are carcinomas, i.e. derived from epithelia. Carcinomas initially remain confined within a basement
membrane, but eventually infiltrate into the surrounding stroma
and intravasate into the blood and lymph vessels where they are
passively transported to distant sites. Circulating carcinoma cells
can extravasate and localize in the parenchyma of distant organs
where they will resume growth to form metastatic tumors.
Numerous features in the metastatic cascade resemble embryonic cells undergoing EMT
and invasive migration. Recurrent observations prompted the
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Fig. 5. Major signaling proteins and transcription factors involved in epithelial-mesenchymal transition. The first cell (left) represents a cell
with intact junctional complexes and epithelial polarity. The second two cells are progressively engaging in epithelial-mesenchymal transition (EMT).
Growth factors activate membrane receptors, leading to changes in actin cytoskeleton remodeling and the loss of apico-basal polarity. The DDR1
complex activates RhoE, which weakens actomyosin contractility at points of cell-cell contact (Hidalgo-Carcedo et al., 2011). TGFβ receptors localized in
tight junctions trigger non-canonical pathways, leading to RhoA ubiquitylation and degradation, and the destabilization of cortical actin microfilament-
associated tight junctions. The activation of transcriptional repressors, such as Snail and Serpent (Srp/GATA), downregulates genes encoding junctional
proteins, including E-cadherin, claudins and occludin, thus compromising epithelial integrity (Spaderna et al., 2008; Whiteman et al., 2008; Campbell et
al., 2011). EPB4.1L5 sequesters p120-catenin, which in turn weakens adherens junctions binding to paxillin and promotes β1-integrin-based cell-matrix
adhesion. β1-Integrin signaling complex presumably contains FAK and Src. In Drosophila, partial EMT in the posterior midgut and is controlled by Srp
(Campbell et al., 2011). Srp represses an apical polarity gene, Crb, resulting in the re-distribution of E-cadherin (not shown). Likewise Snail represses
Crumbs3 and Zeb1 represses Crumbs3 and Lgl2. Partial EMT is also evident in trophoblast stem cells, in which the loss of CBP or downregulation of
H2A/H2B acetylation promotes a CD44high/CD24low phenotype; this can be reproduced by Snail overexpression (Abell et al., 2011). CBP activity is
controlled by MAP3K4/JNK. Full-blown EMT can be executed by Snail. Snail is phosphorylated by Pak1 (Pak – FlyBase) and glycogen synthase kinase 3β
(GSK3β) for activation and downregulation, respectively (Zhou et al., 2004; Yang et al., 2005). Matrix metalloproteinases (MMPs) are also activated by
Snail.
Fig. 6. Micro-RNA network regulating epithelial-mesenchymal transition. Major signaling pathways involved in the regulation of transcription factors, such as Snail and Zeb proteins, repress the transcription of E-cadherin and cause epithelial-mesenchymal transition (EMT). Fosl1 is a leucine zipper protein that forms the transcription factor complex AP1. Fosl1 causes miR-221 and miR-222 to target Trps1, which in turn inhibits Zeb2 (Stinson et al., 2011). Zeb1 and Zeb2 are also targeted by the miR-200 family (Christoffersen et al., 2007; Burk et al., 2008; Gregory et al., 2008; Korpal et al., 2008), which is regulated by the ratio of the levels of Akt1 to Akt2 (Iliopoulos et al., 2009). The reciprocal inhibitory relationship of miR-200 with Gata3, which is stimulated by Jag2 and Notch signals are also important for EMT, and TGFβ-triggered Gata3 induces EMT via Snail1 (Yang et al., 2011). Snail1 is targeted by miR-30a (Kumarswamy et al., 2011). However, Snail1 activates miR-661 (Vetter et al., 2010). MicroRNA important for cancer metastasis also include miR-10b, which targets homeobox D10 (Hoxd10) and activates RhoC (Ma et al., 2007). It is uncertain whether these regulatory micro-RNAs and EMT factors encompass a regulatory network. Micro-RNAs that attenuate Zeb or Snail transcripts are shown in blue. Micro-RNAs that play a role in promoting EMT are shown in pink.

(Savagner et al., 1994). This is regulated through the mutual exclusion of exons IIIb and IIIc by epithelial splicing regulatory proteins (Esrr1 and Esrr2) (Warzeca et al., 2009). The ESRP-regulated splicing network revealed a high-affinity ESRP-binding motif in transcripts of genes encoding multiple regulators of cell polarity, adhesion and migration, as well as the vesicular transport system (Warzeca et al., 2010). An alternative splicing signature for EMT has also been generated through RNA deep-sequencing analysis in a breast cancer cell line. The results revealed possible functions of RBFOX, muscleblind-like (MBNL), CEFL, hnRNP and ESRP classes of splicing factors in the regulation of EMT-dependent splicing (Shapiro et al., 2011). The identification of MBNL1 is particularly interesting, as it exhibits a restricted expression pattern in canal region endocardium and ventricular myocardium during endocardial cushion development in chick embryos. Furthermore, MBNL1 knockdown in atrioventricular explants increases the TGFβ-dependent EMT in endocardial cells (Vajda et al., 2009). Thus, it will be interesting to determine the phenotypes of ESRP-deficient mice.

New studies using cancer lines have shown that some microRNAs can be incorporated into the EMT networks (Fig. 6). Importantly, independent studies on microRNAs in cultured cells modeling EMT have led to the discoveries of miR-200 families that downregulate Zeb protein transcriptional repressors of E-cadherin (Christoffersen et al., 2007; Burk et al., 2008; Gregory et al., 2008; Korpal et al., 2008; Brabletz and Brabletz, 2010). Interestingly, Zeb2 is also regulated by the trichorhinophalangeal syndrome type 1 protein (Trps1) of the GATA family of transcriptional repressors. Trps1 is targeted by miR-221/222, which are themselves induced by the basal-like transcription factor Fosl1 (Fra1) via mitogen-activated protein kinase kinase (MEK) activation (Stinson et al., 2011). Perturbation of the miRNA processing machinery protein Dicer (Dicer1 – Mouse Genome Informatics) has revealed the possible role of miRNAs in development. The conditional deletion of Dicer using Wnt1-Cre did not perturb neural crest delamination, but affected neurogenesis and the craniofacial skeleton (Huang et al., 2010; Zehir et al., 2010). Moreover, transgenic mice, in which Dicer1 was deleted in the pro-epicardium, die immediately after birth with profound cardiac defects, including impaired coronary vessel development; this implicates micro-RNA processing as having a role in epicardial EMT (Singh et al., 2011).

Conclusion
In most species, EMT contributes to a number of different developmental events and is essential for proper morphogenesis and organogenesis. Diverse mechanisms control the execution of EMT and these are embedded into other programs that control cell fate induction, commitment and differentiation. Genetic analyses in Drosophila set the basis for understanding epistasis in the EMT program in gastrulation. These and subsequent studies demonstrated that Snail and Twist play major roles in modulating cell shape in the early phase of gastrulation in invertebrates, whereas only Snail is used in vertebrates. However, FGF signaling is shared between Drosophila and mouse gastrulation. The detailed gene regulatory network that governs gastrulation and EMT in each species is not yet available, but much progress has been made with the sea urchin model.

The neural crest exhibits another striking example of EMT, although detailed molecular pathways have also not been established. Undoubtedly, Snail is, once again, crucial in neural crest EMT, although other transcriptional regulators contribute indirectly to the delamination and early differentiation programs. Although Zeb2 involvement is clear, the inactivation of numerous other genes expressed in the neural territory has not yielded phenotypes compatible with EMT functions. Recent findings for MmJ2A2 in chicken and Wt1 in mouse have increased the repertoire of the possible role of miRNAs in development. The conditional deletion of Dicer using Wnt1-Cre did not perturb neural crest delamination, but affected neurogenesis and the craniofacial skeleton (Huang et al., 2010; Zehir et al., 2010). Moreover, transgenic mice, in which Dicer1 was deleted in the pro-epicardium, die immediately after birth with profound cardiac defects, including impaired coronary vessel development; this implicates micro-RNA processing as having a role in epicardial EMT (Singh et al., 2011).

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of candidate genes that should be investigated for their role in cancer invasion and metastasis. Heart morphogenesis also offers an attractive model for examining the molecular control of EMT. In the mouse, several new pathways controlling EMT have been uncovered, with Snail acting as an important effector and Wt1 mediating a crucial initiation step in epicardial EMT (Fig. 4).

In vitro studies with normal epithelial cells and carcinoma cells have complemented the developmental studies. These cell-based studies have thus generated a wealth of information for deciphering the molecular mechanisms of EMT, and have also identified novel players, such as regulatory proteins, for alternative splicing and microRNAs. Together, these findings deepen our understanding of EMT in both development and disease, and will undoubtedly help to provide new therapeutic strategies for interferring with cancer invasion and metastasis.

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