CHAPTER SEVEN

Mouse Embryo Compaction

M.D. White*, S. Bissiere*, Y.D. Alvarez†, N. Plachta*,[1]

*Institute of Molecular and Cell Biology, A*STAR, Singapore, Singapore
†Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, CONICET, Buenos Aires, Argentina
[1]Corresponding author: e-mail address: plachtan@imcb.a-star.edu.sg

Contents

Abstract

Compaction is a critical first morphological event in the preimplantation development of the mammalian embryo. Characterized by the transformation of the embryo from a loose cluster of spherical cells into a tightly packed mass, compaction is a key step in the establishment of the first tissue-like structures of the embryo. Although early investigation of the mechanisms driving compaction implicated changes in cell–cell adhesion, recent work has identified essential roles for cortical tension and a compaction-specific class of filopodia. During the transition from 8 to 16 cells, as the embryo is compacting, it must also make fundamental decisions regarding cell position, polarity, and fate. Understanding how these and other processes are integrated with compaction requires further investigation. Emerging imaging-based techniques that enable quantitative analysis from the level of cell–cell interactions down to the level of individual regulatory molecules will provide a greater understanding of how compaction shapes the early mammalian embryo.

Current Topics in Developmental Biology, Volume 120
ISSN 0070-2153
http://dx.doi.org/10.1016/bs.ctdb.2016.04.005

1. THE FIRST CHANGE IN CELL SHAPE DURING DEVELOPMENT

Compaction is the first identifiable morphogenetic process during mammalian embryogenesis and it is critical for the divergence of cell lineages and subsequent development. During the transition from 8 to 16 cells, there is a striking morphological change as cells flatten against each other, increasing their contact areas and making the embryo more spherical (Calarco & Brown, 1969; Ducibella, Ukena, Karnovsky, & Anderson, 1977). The change in cell shape is so evident that compacted embryos can be easily distinguished from noncompacted ones using a simple light microscope (Fig. 1).

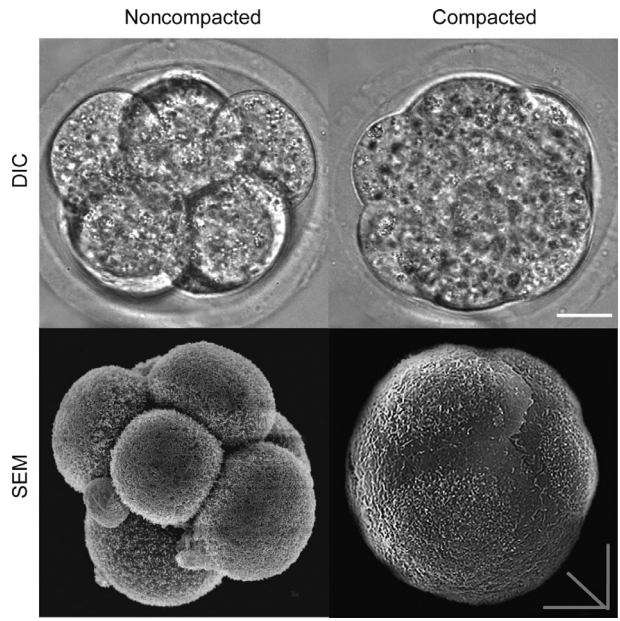

Fig. 1 Morphological changes in preimplantation mouse embryos undergoing compaction. Membranes of individual blastomeres are clearly delineated under DIC optics in a noncompacted 8-cell mouse embryo (*top left panel*). Scanning electron microscopy reveals a uniform distribution of microvilli across all cell surfaces and blastomeres are relatively spherical (*bottom left panel*). Individual cell membranes are no longer discernible by DIC in the compacted embryo (*top right panel*). The blastomeres have flattened and the microvilli are localized to apical zones surrounded by smooth membrane at cell–cell junctions (*bottom right panel*). Scale bars: 10 μm in *x*, *y* and 15 μm in *x*, *y*, *z*.

Embryos that fail to compact will eventually arrest and the degree of compaction is positively correlated with increased success of in vitro fertilization (IVF) treatments (Le Cruguel et al., 2013; Tao et al., 2002). Since the first live birth from a human embryo generated using IVF in 1978 (Steptoe & Edwards, 1978), more than five million babies have been born as a result of this technology. Use of assisted reproductive technologies is increasing worldwide and 80% of today's IVF babies have been born since 2000 (Adamson, Tabangin, Macaluso, & de Mouzon, 2013). Transferring multiple embryos during IVF is associated with adverse pregnancy outcomes (Luke et al., 2015; Practice Committee of American Society for Reproductive Medicine, 2012) so it is crucial to identify and understand the mechanisms that produce the most viable embryo. In addition, beyond the biomedical implications, compaction also provides a unique window into how mammalian cells polarize and interact with each other to form tissue-like structures in vivo.

Mammalian life begins with a newly fertilized zygote, which is round and enclosed within a protective coat of glycoproteins called the zona pellucida. The zygote then undergoes three rounds of cleavage division, from one to two cells, two to four cells, and four to eight cells, producing progressively smaller cells, or blastomeres, after each division. The blastomeres are relatively round and identical in appearance until the late 8-cell stage. Compaction then begins as cell membranes and organelles separate into apical and basolateral domains to establish cell polarity (Fleming & Pickering, 1985; Handyside, 1980; Johnson & McConnell, 2004; Johnson & Ziomek, 1981a; Maro, Johnson, Pickering, & Louvard, 1985; Reeve, 1981; Reeve & Ziomek, 1981). Cell contacts extend outward toward the surface of the embryo minimizing intercellular spaces and placing the cell membranes in close apposition (Goodall & Johnson, 1984; Lo & Gilula, 1979). The blastomeres deform and flatten their apical surfaces forming a tightly grouped mass of cells with indistinct cell boundaries (Fig. 1).

Embryo compaction is critical for blastocyst formation and subsequent development. As the embryo transitions from 8 to 16 cells, the first spatial segregation of cells occurs, with most cells positioned on the exterior of the embryo and a few interior cells completely enclosed. Blastocyst formation is characterized by cavitation and further differentiation of inner and outer cells. The close membrane apposition that arises during compaction likely facilitates the assembly of tight junctions that form a permeability seal between adjacent outer cells. During the 16- to 32-cell stage, the outer cells pump fluid into the intercellular spaces, which coalesce to form a fluid-filled

blastocoel. Blastomeres in the outer layer remain polarized and differentiate into the trophectoderm (TE), eventually giving rise to the fetal part of the placenta. The enclosed blastomeres lose their apical features (Johnson & Ziomek, 1983) and form the pluripotent inner cell mass (ICM), from which the embryo and remaining supportive tissues are derived (Dyce, George, Goodall, & Fleming, 1987).

Due to their similarities in preimplantation development, the mouse has been used as a model for the early human embryo for over 45 years (Cockburn & Rossant, 2010). Although preimplantation development naturally occurs within the oviduct, it can be recapitulated in vitro without adversely affecting the developmental potential of embryos (Summers & Biggers, 2003). Mouse embryos can be easily removed from the maternal oviducts and cultured in simple media conditions. Under these ex utero conditions the embryos develop almost as rapidly as they do in utero and if transferred back to the uterus they can implant and produce viable offspring.

In addition, the relatively large cells of the mouse embryo allow imaging of subcellular processes. Pronuclear microinjection of mRNA or DNA is a well-established technique for expression of exogenous proteins and mouse embryos can endure this process with high efficiency. Furthermore, many genetic tools are available for manipulation of proteins of interest in the mouse. Thousands of genetically modified animals carrying targeted endogenous genes or expressing various transgenic constructs are also now available. This combination of factors makes the mouse an ideal model system for studying compaction in early mammalian development.

2. PROPOSED MECHANISMS FOR COMPACTION

2.1 Cell Adhesion

A key driver of tissue morphogenesis is the formation and rearrangement of cell–cell contacts mediated by cell adhesion molecules (CAMs). Pioneering studies demonstrated that when cells from different embryonic germ layers are artificially mixed, they spontaneously sort into separate populations (Townes & Holtfreter, 1955). It was predicted, and later demonstrated (Nose, Nagafuchi, & Takeichi, 1988), that this cell sorting was based on the differential expression of CAMs. These experiments prompted the differential adhesion hypothesis (DAH), in which tissues are equated to immiscible liquids. The main principles of the DAH are that (1) cells adhere to each other, (2) adhesion properties vary between different cells, and (3)

aggregations of cells tend to arrange themselves in a way that minimizes the free energy of the system (Steinberg, 1970). Many developmental morphogenetic processes including epiboly (Kane, McFarland, & Warga, 2005), myotome (Cortes et al., 2003) and rhombomere formation in the zebrafish (Cooke, Kemp, & Moens, 2005), cell sorting in the *Drosophila* imaginal wing disc (Dahmann & Basler, 2000), and patterning of the mouse cerebellum (Gliem et al., 2006) can be explained, at least to some extent, by the DAH. Additionally, the malignant invasion of cancerous cells is consistent with the DAH and alterations in many CAMs have been associated with the development and progression of cancer (Okegawa, Pong, Li, & Hsieh, 2004).

Early studies revealed that calcium-dependent adhesion is critical for embryo compaction (Fleming, Sheth, & Fesenko, 2001). Interfering with adhesion by chelating calcium ions or using antibodies targeting a cell surface glycoprotein decompacted embryos and prevented development to the blastocyst stage (Ducibella & Anderson, 1975; Wales, 1970; Whitten, 1971). Although some members of the calcium-dependent integrin family of CAMs are also expressed throughout early mouse preimplantation development (Sutherland, Calarco, & Damsky, 1993), their function does not appear to be required during compaction (Richa, Damsky, Buck, Knowles, & Solter, 1985). The cell surface glycoprotein essential for compaction was later identified as E-cadherin (uvomorulin) (Hyafil, Babinet, & Jacob, 1981), a member of the multigene cadherin family of homophilic CAMs found in most animal cells (Meng & Takeichi, 2009; Yoshida & Takeichi, 1982). Cadherins function by forming transmembrane macromolecular complexes. They mediate adhesion by ligating their extracellular domain to that of cadherins presented on neighboring cells. Interactions with cytoplasmic and cytoskeletal proteins via their intracellular domain serve to connect the cell membrane to the cortex (Hoffman & Yap, 2015).

Evidence for a leading role for E-cadherin in the DAH is provided by studies demonstrating that manipulating the expression level of E-cadherin directly affects how cells from different tissue layers sort (Foty & Steinberg, 2005; Godt & Tepass, 1998; Schotz et al., 2008). Although named for its expression in epithelial cell layers, E-cadherin is also present from the very early stages of development. It is maternally derived in the oocyte and de novo E-cadherin zygotic synthesis starts at the 2-cell stage (Vestweber, Gossler, Boller, & Kemler, 1987). In blastomeres, E-cadherin is enriched basolaterally, forming intercellular adherens junctions and connecting to the actin cytoskeleton via catenin

proteins (Ozawa, Ringwald, & Kemler, 1990). Embryos lacking zygotic E-cadherin die before implantation. They are able to compact due to residual maternal E-cadherin but fail to form normal blastocysts (Larue, Ohsugi, Hirchenhain, & Kemler, 1994). Embryos lacking both maternal and zygotic E-cadherin cannot compact or form a blastocyst and instead remain as loose clusters of cells (Stephenson, Yamanaka, & Rossant, 2010).

E-cadherin is uniformly distributed in the cell membrane until the 8-cell stage. Then it begins to accumulate in cell–cell junctions and is predominantly localized to basolateral membrane regions by the 16-cell stage (Fig. 2).

Cell adhesion is generally proposed to be proportional to cadherin expression levels (Foty & Steinberg, 2005; Krieg et al., 2008). Hence, if compaction were driven by intercellular differences in adhesion, it would be reasonable to expect variation in the expression of E-cadherin between blastomeres. However, it has recently been demonstrated that there are no marked differences in expression levels or mobility of E-cadherin between cells of the embryo during compaction (Samarage et al., 2015). Furthermore, it is uncertain whether the decrease in surface energy resulting from cadherin ligation could even generate sufficient forces to deform tissues (Maitre et al., 2012). More microscopic differences, or a potential role of other yet unidentified molecules cannot be excluded, but there is currently

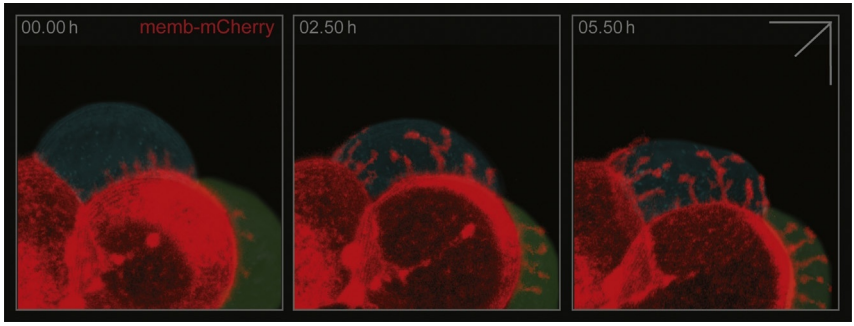

Fig. 2 Filopodia extended by some blastomeres facilitate changes in cell shape that compact the entire embryo. One cell of the 2-cell embryo was injected with membrane-targeted mCherry (memb-mCherry) to label half of the embryo. This allows visualization of memb-mCherry positive filopodia that extend from some blastomeres onto their unlabeled neighbors (DIC image pseudo-colored *blue* and *green*) during compaction. Cells are initially relatively spherical (*left panel*) but both filopodia-extending cells and their neighbors flatten and draw closer together as filopodia are extended (*middle, right panel*). Scale bars: 10 μm in *x, y, z*.

little evidence that compaction is mediated by a change in adhesion at basolateral cell regions.

The redistribution of E-cadherin at the onset of compaction coincides with the assembly of another junctional complex, the gap junction (McLachlin, Caveney, & Kidder, 1983). As the regulation of connexin-based intercellular communication is controlled by E-cadherin in mouse cells in vitro (Jongen et al., 1991), it is plausible that this relocalization of E-cadherin acts as a trigger for the assembly of gap junctions, possibly through calcium signaling. Functional communication between cells at the 8-cell stage might also mark the end of solitary blastomeres and the establishment of a syncytium that may be necessary for the maintenance of compaction. Antibodies targeting the gap junction component connexin 43 (Cx43), block intercellular dye transfer and cause decompaction and extrusion of affected blastomeres from the rest of the embryo (Becker & Davies, 1995). However, the successful progression of Cx43 null embryos through compaction suggests compensatory mechanisms exist, as previously demonstrated by the failure of connexin 36 knockout mice to show the cognitive deficits attributed to acute blockade of neuronal gap junctions (Bissiere et al., 2011). Further investigation is required to dissect the contribution of gap junctions to the process of compaction.

2.2 Cortex Tension

Although widely accepted to explain cell sorting (Grubb, 2006) and elegant in its simplicity, the DAH cannot fully explain many experimental observations and simulations of sorting behavior (Brodland & Chen, 2000). Accounting for the effects of cortical tension prompted development of an alternative model called the differential interfacial tension hypothesis (DITH) (Brodland, 2002). Cortical tension is generated by the contractility of actomyosin networks (Pasternak, Spudich, & Elson, 1989) and is now recognized to be a key determinant of the shape of both individual cells and tissues (Heisenberg & Bellaiche, 2013; Lecuit & Lenne, 2007). A cellular cortex lies under the plasma membrane of most eukaryotic cells and is comprised of a dynamic network of actin filaments connected to the cell membrane. Myosin motor proteins hydrolyse ATP to slide pairs of these actin filaments with respect to each other, generating contractility or expansion of the cortex. The DITH postulates that cells sort to minimize the interfacial tension of the system, which results from both adhesive and cortical tensions. Adhesive tension mediated by cadherins increases contact areas between

cells, but cortical tension generated by actomyosin contractility acts in opposition to decrease intercellular contacts. According to the DITH, cells sort in a manner that maximizes their adhesive tension, while minimizing their cortical tension and the balance of these two forces determines cell shape. Morphogenetic processes such as boundary formation (Monier, Pelissier-Monier, Brand, & Sanson, 2010), cell intercalation (Bertet, Sulak, & Lecuit, 2004), and tissue invagination (Chandrasekaran & Beckendorf, 2005; Lee et al., 2006) can all be described in terms of changes in the balance between adhesive and cortical tensions as defined by the DITH.

Recent work examining the function of cortical tension in the preimplantation embryo has demonstrated that it plays a role in driving the compaction process (Maitre, Niwayama, Turlier, Nedelec, & Hiiragi, 2015) and the first spatial segregation of mammalian cells (Samarage et al., 2015). Actomyosin has been shown to clear from cell–cell contacts and accumulate at the surface during compaction (Maitre et al., 2015). This has been proposed to form a contractile shell on the surface of the embryo, to which about 75% of the cell shape changes during compaction are attributed. In this model, E-cadherin does not directly generate forces, but acts to exclude actomyosin from cell–cell contacts, facilitating cortical contractility. E-cadherin is required, however, for the function of a newly discovered class of filopodia proposed to provide an additional mechanism to draw neighboring cells closer together during compaction (Fierro-Gonzalez, White, Silva, & Plachta, 2013).

2.3 Filopodia

Recently, it was demonstrated that the onset of compaction is accompanied by the extension of long membrane protrusions from some cells in the mouse embryo (Fig. 3). These protrusions were identified as filopodia and shown to stretch across the apical membrane of neighboring cells. They differ in both length and molecular composition from previously observed microvilli present at the apical pole of the mouse blastomeres (Calarco & Epstein, 1973; Ducibella et al., 1977). The key in observing the formation of these filopodia during embryonic compaction was to perform pronuclear injection in only one of the cells at the 2-cell stage.

The filopodia contain F-actin and express both E-cadherin and proteins that link it to the actin cytoskeleton, such as α- and β-catenin. Furthermore, these filopodia also express the unconventional myosin protein, myosin-10. This myosin has previously been shown to promote the formation of

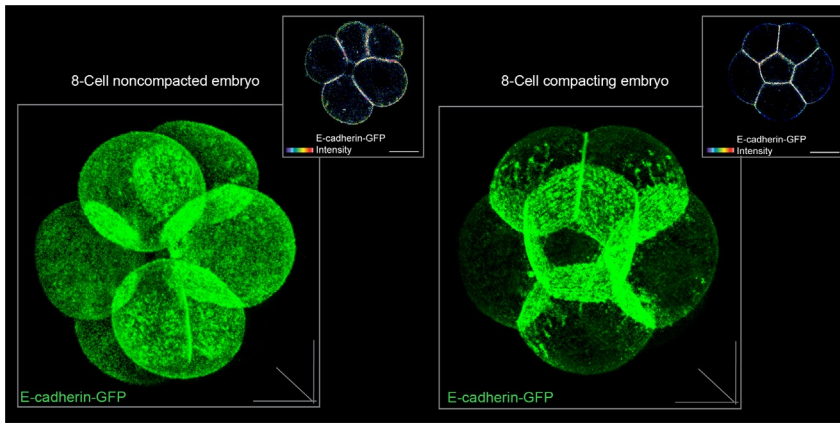

Fig. 3 E-cadherin redistributes to basolateral cell membranes during compaction. E-cadherin-GFP expression in an embryo injected at the 1-cell stage. At the early 8-cell stage, prior to the onset of compaction, E-cadherin-GFP is distributed across the entire cell membrane (*left panel*). As compaction begins, E-cadherin-GFP becomes predominantly localized to the basolateral cell membrane and compaction-specific filopodia (*right panel*). Insets show intensity of E-cadherin-GFP expression in single 2D planes. Scale bars: 10 μm in *x*, *y* and *x*, *y*, *z*.

filopodia-like structures in multiple cultured cell lines (Kerber & Cheney, 2011). In the preimplantation mouse embryo, myosin-10 levels increase during compaction and its expression is both required and sufficient to trigger compaction. These recently discovered filopodia only appear at the 8-cell stage as compaction commences, and disappear around the 16-cell stage when the embryo is fully compacted. Trans interactions between E-cadherin molecules most likely anchor the filopodia to neighboring cell membranes, where they remain for several hours before retracting immediately prior to the division of the filopodia-forming cell. Importantly, disrupting the filopodia using laser-based ablations causes rapid deformation of the cell membrane indicating that filopodia provide structural support for the alterations of cell shape that are required during compaction.

These findings provide a new mechanism controlling compaction relying on the action of long cellular protrusions. However, several open questions remain. For example, it is yet to be determined why only some cells of the embryo extend filopodia while others do not. Indeed, it was observed that only about 60% of cells of the embryo become filopodia-forming cells and that, while they can project filopodia onto up to three other cells simultaneously, they never receive reciprocal filopodia on their own apical membrane. It is also striking how filopodia extend to very similar lengths and their

bases are almost equally spaced. How the number and length of these structures is controlled is currently unknown. This tight regulation of the distribution and timing of filopodia indicates that they may also have an unidentified signaling role. Investigation of these questions will likely reveal further details about how the process of compaction is controlled in the mouse embryo.

3. KEY CELL DECISIONS DURING COMPACTION

Accompanying compaction are several other major morphogenetic processes. The blastomeres develop polarity, reposition to form inner and outer cells, and start to display differential expression of cell fate markers. Understanding how all of these processes are integrated remains an open challenge.

3.1 Cell Position Changes: Division and Internalization

The process of embryo compaction is synchronized with two more key morphogenetic events: cell division and cell internalization.

3.1.1 Cell Division

During compaction, each cell of the 8-cell embryo divides once, thereby producing a 16-cell embryo. Cell division requires the existence of important regulatory mechanisms that must control the timing of each cell division and the positioning of resultant daughter cells within the embryo. Although few mechanistic insights have been elucidated, it is of note that, unlike in many nonmammalian embryos, neighboring cells in the mouse embryo do not divide simultaneously. Instead, a dividing cell typically completes its division minutes to hours before any of its neighbors divide (Fierro-Gonzalez et al., 2013; Samarage et al., 2015). It is plausible that such an orchestration of cell division times ensures that the global embryo architecture is not compromised. As a mouse blastomere undergoes division, it loses its compacted shape and becomes highly spherical, before cleaving into two daughter cells. These daughter cells are also initially spherical and must then flatten and compact to incorporate into the rest of the embryo. Unlike in more tightly packed tissues, if two neighboring cells were to divide at the same time it may become difficult to simultaneously reintegrate the four resulting daughter cells into the embryo. Therefore, elucidating mechanisms that control cell division timing in the embryo and understanding how these might impact on embryo architecture remains an interesting future

challenge. In particular, it will be important to understand how molecular mechanisms regulating the assembly of the mitotic and cytokinesis structures relate to the regulation of cytoskeletal components supporting cell shape and position. It will also be of interest to understand what type of communication exists between a dividing cell and its neighbors in the embryo. For example, can cells receive information regarding the division status of a neighbor cell and change their own division behaviors accordingly? A key goal will be determining how the control of cell division in the early mouse embryo, which is composed of few cells, compares to the control of cell divisions in much more coherent embryonic structures, such as the early *Drosophila* embryo in which mitoses occur in a highly synchronous fashion.

3.1.2 Cell Position

Additionally, during compaction cells must not only divide but also relocate to different positions. Compaction coincides with the spatial separation of embryonic cells into the first two distinct cell lineages of the conceptus. During the 8- to 16-cell stage some cells become internalized to form the pluripotent inner mass of the embryo. By the 16-cell stage, most embryos contain an inner mass composed of three of these pioneer cells. These cells then go on to divide internally to expand the ICM at blastocyst stages, accompanied by a small population of new cells that are internalized during the 16- to 32-cell stage. Understanding how the initial pioneer cells become internalized is of great interest as they form the first separate embryonic compartments. Moreover, repositioning cells to the interior of the embryo generates a unique niche in which for the first time, some cells of the embryo may be exposed to different signals than others.

Currently, little is known about how the mechanisms controlling embryo compaction integrate with those regulating the internalization of inner cells. Until recently, even the morphogenetic events founding the inner mass remained poorly defined. Traditional views had assumed that the inner mass formed via highly orientated cell divisions, referred to as asymmetric divisions (Yamanaka, Ralston, Stephenson, & Rossant, 2006; Zernicka-Goetz, Morris, & Bruce, 2009). It was generally accepted that some cells of the early embryo would divide with a highly orientated cleavage plane and push one of their resulting daughter cells into the inner mass as a direct result of the scission. However, these asymmetric cell division events had not been visualized in real time. Most observations, including very recent studies have relied on tracking fluorescently labeled cell nuclei using markers such as histone-GFP (Strnad et al., 2015). However, tracking cell

nuclei is inaccurate because the nuclear spatial coordinates cannot be directly translated into position and morphology of the entire cell. For instance, some cells in the embryo have their nuclei located more basolaterally while others are located more apically (Ajduk, Biswas Shivhare, & Zernicka-Goetz, 2014), so nuclear position does not necessarily inform about the inner/outer status of the entire cell membrane. Instead, tracking cells by their membrane offers substantially more information about the overall position of each cell in the embryo with relation to its neighbors. Membrane segmentation has previously proven useful to investigate morphogenetic processes in *Drosophila* (Gelbart et al., 2012), zebrafish (Xiong et al., 2014), and plant (Yoshida et al., 2014) embryos. Recently, this approach has also been used to follow the process of inner mass formation in living mouse embryos (Samarage et al., 2015). Instead of highly orientated asymmetric cell divisions, most cells founding the inner mass originate from a symmetric division and are subsequently allocated inside the embryo via a process displaying all of the classical features of apical constriction (Sawyer et al., 2010). Subcellular heterogeneities in tensile forces, generated by actomyosin cortical networks were shown to drive a decrease in apical surface area, an increase in basolateral area, and gradual repositioning of cells to the interior of the embryo. Interestingly, on average the first internalization event occurs at the 12-cell stage, exactly as the embryo is compacting. The degree of overlap in the embryo between the processes of compaction and cell internalization remains an important open question.

3.2 Cell Polarity

Unlike cells in nonmammalian embryos, the blastomeres of the early mouse embryo initially show fewer features of cell polarity. At the onset of compaction, cues arising from cell–cell interactions direct the establishment and orientation of polarity. The contact-free surface of each blastomere forms a recognizable apical domain enriched in microvilli, actin, and actin-binding proteins (Ducibella et al., 1977; Louvet, Aghion, Santa-Maria, Mangeat, & Maro, 1996; Reeve & Ziomek, 1981). Cues from the cell membrane induce the asymmetrical localization of apical and basolateral polarity proteins which then reinforce formation of discrete domains through mutually antagonistic interactions. E-cadherin is one of the first proteins to polarize in the mouse embryo, becoming enriched in cell–cell contacts as compaction commences (Fig. 2) (Vestweber et al., 1987). Other classical cell polarity proteins such as Ezrin, Pard6b, and the aPKCs (PKCζ and PKCλ) then

localize to the apical domain while Par-1, Jam-1, and Na/K ATPase accumulate at basolateral cell–cell contacts (Barcroft, Moseley, Lingrel, & Watson, 2004; Louvet et al., 1996; Pauken & Capco, 2000; Thomas et al., 2004; Vinot et al., 2005; Wang, Ojakian, & Nelson, 1990). Asymmetric cell–cell contacts are required and sufficient for blastomeres to polarize (Ziomek & Johnson, 1980), but are not necessary to maintain polarization once it has been established (Johnson & Ziomek, 1981b). Although the precise trigger for the establishment of polarity in the mouse embryo has not been elucidated, it is likely to involve E-cadherin. Embryos lacking both maternal and zygotic *E-cadherin* reveal that it is required to restrict the area of the apical domain and confine basolateral proteins, ensuring appropriate segregation of apical and basolateral domains (Stephenson et al., 2010). However, due to its requirement for cell–cell adhesion, it remains difficult to dissect the exact contribution of E-cadherin to polarity. It is possible that E-cadherin is simply necessary for cells to make sufficient contact for an unidentified cadherin-independent polarity cue to take effect.

The establishment of discrete domains of E-cadherin-mediated adhesion and apical polarity during compaction has important downstream consequences for the acquisition of cell fate. Blastomeres use their specific combination of adhesion and apical polarity to convert information about their position within the embryo into cell fate decisions. This is achieved by regulating the subcellular localization of members of the Hippo signaling pathway through interactions with adhesion complexes or apical polarity domains (Cockburn, Biechele, Garner, & Rossant, 2013; Hirate et al., 2013).

3.3 Cell Fate Decisions

A final, critical event occurring concurrent with compaction is the embarkation of some cells along the pathway to establishing the first cell lineages. Although segregation of most lineage-specific cell markers is not established until the blastocyst stage, the allocation of some cells to the interior of the embryo during compaction is the foundational morphogenetic process forming the future ICM. The internalized cells can be said to have adopted a distinct fate because they will remain inside and only contribute to the expansion of the inner mass (Samarage et al., 2015). This is in contrast to the cells positioned in the outer layer, which still have the potential to contribute to TE, or provide new inner cells during later developmental stages.

Whether blastomeres acquire molecular heterogeneities associated with cell fate before, during, or after compaction is currently still debated

(Burton & Torres-Padilla, 2014; Rossant & Tam, 2009; Zernicka-Goetz et al., 2009). Unlike in some nonmammalian species, identifying molecular heterogeneities that predict cell fate in the early mouse and human embryo has proven challenging (Rossant & Tam, 2009; Zernicka-Goetz et al., 2009). Original observations of morphological uniformity and a lack of evidence for uneven distribution of fate determinants in mouse blastomeres led to a primarily stochastic view of early fate determination (Rossant, 1976; Tarkowski, 1959, 1961; Tarkowski & Wroblewska, 1967). Some studies have concluded that the separation of pluripotent and extraembryonic cell fates does not occur until after the embryo becomes a compacted morula (Dietrich & Hiiragi, 2007; Kurotaki, Hatta, Nakao, Nabeshima, & Fujimori, 2007; Louvet-Vallee, Vinot, & Maro, 2005; Motosugi, Bauer, Polanski, Solter, & Hiiragi, 2005). By contrast, others propose that mammalian cell fates might be predictable as early as the 2- to 8-cell stage (Gardner, 2001, 2002a, 2002b; Piotrowska, Wianny, Pedersen, & Zernicka-Goetz, 2001; Piotrowska-Nitsche, Perea-Gomez, Haraguchi, & Zernicka-Goetz, 2005; Piotrowska-Nitsche & Zernicka-Goetz, 2005; Plachta, Bollenbach, Pease, Fraser, & Pantazis, 2011; Shi et al., 2015). Either way, recent studies have shed further light on how processes occurring during compaction impact on cell fate decisions.

Differentiation of the outer cells of the morula into TE requires the expression of Cdx2 and Gata3, which are driven by the transcription factor Tead4 and its coactivator, Yap1 (Ralston et al., 2010). The Hippo signaling pathway kinase, Lats1/2, phosphorylates Yap1 to control its subcellular localization (Nishioka et al., 2009). Angiomotin (Amot) is required to activate Lats1/2 and switch on the Hippo signaling pathway (Hirate et al., 2013). In outer cells, Amot is sequestered by components of the apical polarity complex and localized to the apical domain (Hirate et al., 2013). Here it is bound to actin and held in an inactive state, preventing activation of the Hippo signaling pathway. Unphosphorylated Yap1 can enter the nucleus and induce transcription of the TE-specific genes Cdx2 and Gata3. In inner cells lacking apical polarity, Amot can interact with Lats1/2 and the E-cadherin adhesion complex at adherens junctions via Nf2 (Hirate et al., 2013). This interaction activates Amot and stabilizes it at adherens junctions where it switches Hippo signaling on. Yap1 is phosphorylated and excluded from the nucleus, promoting the transcription of ICM-specific genes. In this way, the reorganization of proteins into apical and basolateral domains that occurs during compaction directs the subsequent fate of the cell.

Hippo pathway signaling has also been shown in vitro to be responsive to actin rearrangement caused by mechanical cues such as cell shape and geometry (Dupont et al., 2011; Wada, Itoga, Okano, Yonemura, & Sasaki, 2011). It is, therefore, possible that the changes in cell morphology and tensile forces that develop during compaction may act in parallel to regulate the Hippo signaling pathway, although this remains to be demonstrated in the living embryo.

4. OPEN QUESTIONS ABOUT COMPACTION

Although the process of embryo compaction has been studied for decades, important questions remain unresolved regarding how it occurs and how it is regulated. For example, the chain of events that triggers this critical first change in embryo morphology is undetermined. PKC-α-mediated phosphorylation of β-catenin is known to be part of the molecular signal that prompts the onset of compaction (Pauken & Capco, 1999). Inhibiting PKC-α blocks compaction and activating it can induce early compaction (Ohsugi, Ohsawa, & Semba, 1993; Winkel, Ferguson, Takeichi, & Nuccitelli, 1990); however, the upstream event activating PKC-α at this time in the embryo is unknown.

In fact, little is known about how the timing of compaction is controlled. Why do mouse embryos begin to compact at the 8-cell stage and not earlier or later? The timing of compaction is independent of cell number so must be regulated through another mechanism (Fernandez & Izquierdo, 1980). The total cytoplasmic volume of the embryo remains constant during cleavage division of the 1-cell embryo to the 8-cell embryo. This results in an exponential increase in nucleocytoplasmic ratio with the largest change occurring during the first three to four cleavage divisions (Aiken, Swoboda, Skepper, & Johnson, 2004). Artificially increasing the nucleocytoplasmic ratio by extracting cytoplasmic material from the 1-cell embryo induces early compaction at the 4-cell stage (Lee, Lee, Yoon, Roh, & Kim, 2001). Although this may indicate a direct link between the onset of compaction and the cell's nucleocytoplasmic ratio, the answer is more likely to lie in the cytoplasmic composition. Restoring the volume of a cytoplasmically depleted 1-cell embryo has varying effects on the timing of compaction depending on the stage of the embryo that donor cytoplasm is derived from. It is probable that degradation or dilution of an inhibitory cytoplasmic factor present in the 1-cell embryo regulates the timing of compaction, but the identity of this factor remains unknown.

And finally, how is compaction integrated with other processes occurring during this critical developmental window, such as changes in cell polarity, the start of cell differentiation, and the morphological reorganization of the entire embryo?

5. EMERGING TECHNIQUES AND FUTURE WORK

New techniques based on imaging will enable the discovery of further mechanisms regulating compaction. The field of developmental biology has benefited greatly from the establishment of live-imaging methods. Yet, a key future requirement will be the utilization of more quantitative analytical tools to study compaction at multiple levels, ranging from macroscopic cell–cell interactions to the dynamics of regulatory molecules (Fig. 4).

Although it is now possible to label cells in the embryo with various fluorescently tagged proteins, new studies should focus on the development of computational image segmentation approaches to quantify the main changes in cell shape and position during compaction (Fig. 4A). A greater challenge is the development of techniques to track the dynamics of subcellular features such as the cytoskeleton, including actin, microtubules, and intermediate filaments, as well as various intracellular organelles, including the nucleus, endoplasmic reticulum, and mitochondria and filopodia (Fig. 4A). Revealing the dynamic behaviors of these structures may facilitate the discovery of other cell processes involved in compaction. Computational image segmentation approaches can also provide quantitative information about the subcellular forces acting in the embryo (Fig. 4B). Measuring the contact angles between cells reveals the local balance of adhesion and tension and changes in the distribution of these forces can be tracked throughout compaction.

In addition to techniques for studying whole cells or intracellular structures, methods for studying the dynamics of the key regulatory proteins controlling compaction also need to be applied. For example, compaction provides an excellent system to probe the dynamics of actomyosin networks. Previous work has performed measurements of adhesion forces and cortex tension using dual pipette aspiration assays. However, this approach is very invasive and cannot reveal the subcellular organization of tensile forces acting during compaction. It will be important to study actomyosin dynamics at a more microscopic level to understand the forces driving compaction. Laser ablations offer a good entry point to probe the relative magnitude and directionality of tensile forces, as they can be targeted to defined subcellular regions and their noninvasiveness allows repeat measurements during development.

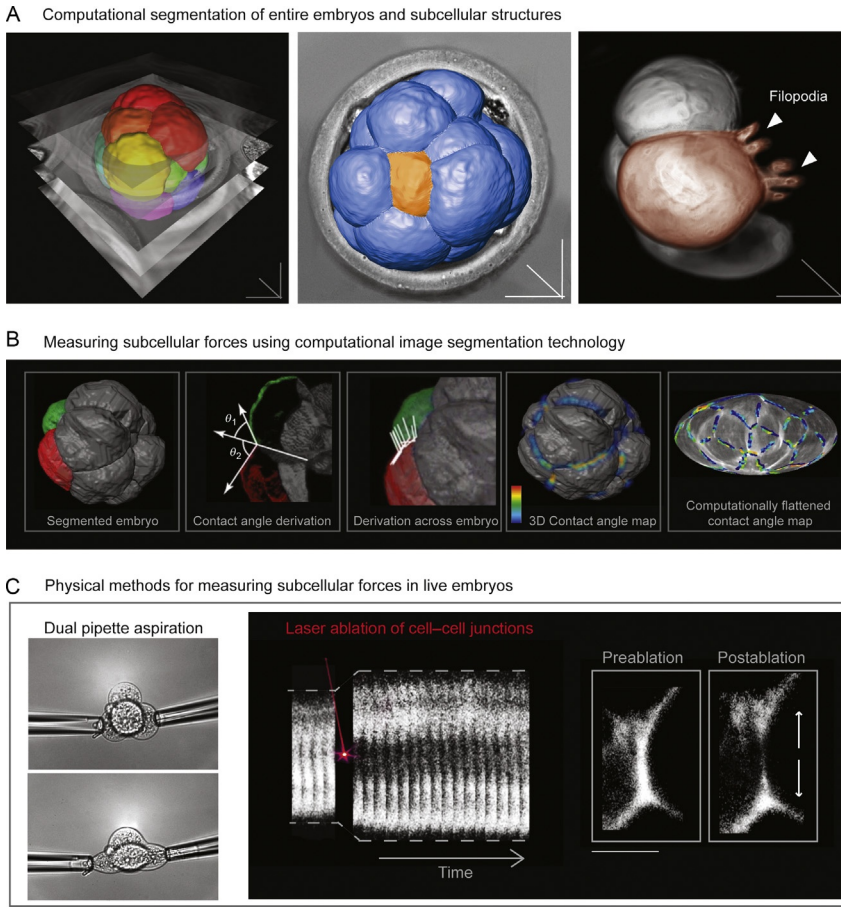

Fig. 4 Emerging technologies for quantitative investigations of mechanisms controlling compaction. New quantitative imaging-based technologies are facilitating the investigation of compaction. (A) Computational segmentation of entire embryos (*left*, *middle panel*) or selected cells and filopodia (*arrowhead*, *right panel*). Embryos expressing fluorescently labeled proteins are imaged in 4D using two-photon microscopy, and individual features are computationally segmented. (B) Image segmentation technologies enable calculation of the subcellular distribution of biomechanical forces. Contact angles at cell–cell junctions result from the balance of subcellular forces and can be measured at multiple points along the junction. This provides a 3D map of contact angles, which can be projected to a 2D map of the apical surface of the embryo. (C) Dual pipette aspiration is a physical method for measuring subcellular forces in the embryo. An embryo is held between two micropipettes (*top left panel*) and aspiration is increased (*bottom left panel*) until the cells separate. Although this method provides useful information about the force of cell–cell adhesion, it is not compatible with ongoing development of the embryo. A less invasive physical technique for measuring subcellular forces is laser ablation. Targeting high laser energy into a localized region of cell cortex ablates cortical structure causing rapid recoil about the ablation that reflects the size and directionality of subcellular forces in that region. When cell–cell junctions are laser ablated (*right panel*) the degree by which adjacent vertices separate (*white arrows*, *right panel*) reveals the residual tensile forces. Scale bars: 5 μm in *x*, *y* and 10 μm in *x*, *y*, *z*.

The mobility of E-cadherin and many of its intracellular interacting proteins could be probed by combining the use of fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS), and image correlation techniques such as raster image correlation spectroscopy (RICS) (Digman & Gratton, 2011; Kaur et al., 2013). Moreover, new photoactivatable and photoconvertible proteins are excellent tools for labeling defined subpopulations of regulatory proteins and following their behaviors with high temporal resolution imaging.

The engineering of more light-activatable regulatory proteins would be a very valuable tool to manipulate compaction at the subcellular level. So far, most of these tools have been used to study cell migration in culture conditions, yet the accessibility of the mouse embryo during compaction should make future studies in this direction possible.

Finally, the effects of many molecular and mechanical perturbations can now be tested at the level of gene expression even in single cells. Recently, the mouse embryo has been analyzed at the single-cell level using RNAseq (Shi et al., 2015; Tang et al., 2011), thus these methods could be rapidly applied to investigate gene expression changes occurring during normal or manipulated compaction.

6. CONCLUSIONS

Compaction offers an excellent experimental system to study how cells interact with each other in a whole embryo to form the first tissue-like structures during mammalian development. Yet, most studies so far have relied on the use of static analysis and our understanding of this morphogenetic process is limited to a few cellular components. A key future challenge is to perform more integrative dynamic analyses to reveal how various cellular and molecular processes regulate these early changes in cell shape. As is the case for other fields, new cross-disciplinary collaborations among labs experienced in embryology, imaging, genetics, and mechanobiology will be of great benefit to our understanding of this critical process in mammalian development.

REFERENCES

Adamson, G. D., Tabangin, M., Macaluso, M., & de Mouzon, J. (2013). The number of babies born globally after treatment with the assisted reproductive technologies (ART). *Fertility and Sterility*, *100*, S42.

Aiken, C. E., Swoboda, P. P., Skepper, J. N., & Johnson, M. H. (2004). The direct measurement of embryogenic volume and nucleo-cytoplasmic ratio during mouse preimplantation development. *Reproduction*, *128*, 527–535.

Ajduk, A., Biswas Shivhare, S., & Zernicka-Goetz, M. (2014). The basal position of nuclei is one pre-requisite for asymmetric cell divisions in the early mouse embryo. *Developmental Biology*, *392*, 133–140.

Barcroft, L. C., Moseley, A. E., Lingrel, J. B., & Watson, A. J. (2004). Deletion of the Na/K-ATPase alpha1-subunit gene (Atp1a1) does not prevent cavitation of the preimplantation mouse embryo. *Mechanisms of Development*, *121*, 417–426.

Becker, D. L., & Davies, C. S. (1995). Role of gap junctions in the development of the preimplantation mouse embryo. *Microscopy Research and Technique*, *31*, 364–374.

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

Bissiere, S., Zelikowsky, M., Ponnusamy, R., Jacobs, N. S., Blair, H. T., & Fanselow, M. S. (2011). Electrical synapses control hippocampal contributions to fear learning and memory. *Science*, *331*, 87–91.

Brodland, G. W. (2002). The Differential Interfacial Tension Hypothesis (DITH): A comprehensive theory for the self-rearrangement of embryonic cells and tissues. *Journal of Biomechanical Engineering*, *124*, 188–197.

Brodland, G. W., & Chen, H. H. (2000). The mechanics of cell sorting and envelopment. *Journal of Biomechanics*, *33*, 845–851.

Burton, A., & Torres-Padilla, M. E. (2014). Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis. *Nature Reviews. Molecular Cell Biology*, *15*, 723–735.

Calarco, P. G., & Brown, E. H. (1969). An ultrastructural and cytological study of preimplantation development of the mouse. *The Journal of Experimental Zoology*, *171*, 253–283.

Calarco, P. G., & Epstein, C. J. (1973). Cell surface changes during preimplantation development in the mouse. *Developmental Biology*, *32*, 208–213.

Chandrasekaran, V., & Beckendorf, S. K. (2005). Tec29 controls actin remodeling and endoreplication during invagination of the Drosophila embryonic salivary glands. *Development*, *132*, 3515–3524.

Cockburn, K., Biechele, S., Garner, J., & Rossant, J. (2013). The Hippo pathway member Nf2 is required for inner cell mass specification. *Current Biology*, *23*, 1195–1201.

Cockburn, K., & Rossant, J. (2010). Making the blastocyst: Lessons from the mouse. *The Journal of Clinical Investigation*, *120*, 995–1003.

Cooke, J. E., Kemp, H. A., & Moens, C. B. (2005). EphA4 is required for cell adhesion and rhombomere-boundary formation in the zebrafish. *Current Biology*, *15*, 536–542.

Cortes, F., Daggett, D., Bryson-Richardson, R. J., Neyt, C., Maule, J., Gautier, P., et al. (2003). Cadherin-mediated differential cell adhesion controls slow muscle cell migration in the developing zebrafish myotome. *Developmental Cell*, *5*, 865–876.

Dahmann, C., & Basler, K. (2000). Opposing transcriptional outputs of Hedgehog signaling and engrailed control compartmental cell sorting at the Drosophila A/P boundary. *Cell*, *100*, 411–422.

Dietrich, J. E., & Hiiragi, T. (2007). Stochastic patterning in the mouse pre-implantation embryo. *Development*, *134*, 4219–4231.

Digman, M. A., & Gratton, E. (2011). Lessons in fluctuation correlation spectroscopy. *Annual Review of Physical Chemistry*, *62*, 645–668.

Ducibella, T., & Anderson, E. (1975). Cell shape and membrane changes in the eight-cell mouse embryo: Prerequisites for morphogenesis of the blastocyst. *Developmental Biology*, *47*, 45–58.

Ducibella, T., Ukena, T., Karnovsky, M., & Anderson, E. (1977). Changes in cell surface and cortical cytoplasmic organization during early embryogenesis in the preimplantation mouse embryo. *The Journal of Cell Biology*, *74*, 153–167.

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., et al. (2011). Role of YAP/TAZ in mechanotransduction. *Nature*, *474*, 179–183.

Dyce, J., George, M., Goodall, H., & Fleming, T. P. (1987). Do trophectoderm and inner cell mass cells in the mouse blastocyst maintain discrete lineages? *Development*, *100*, 685–698.
Fernandez, M. S., & Izquierdo, L. (1980). Blastocoel formation in half and double mouse embryos. *Anatomy Embryology*, *160*, 77–81.
Fierro-Gonzalez, J. C., White, M. D., Silva, J. C., & Plachta, N. (2013). Cadherin-dependent filopodia control preimplantation embryo compaction. *Nature Cell Biology*, *15*, 1424–1433.
Fleming, T. P., & Pickering, S. J. (1985). Maturation and polarization of the endocytotic system in outside blastomeres during mouse preimplantation development. *Journal of Embryology and Experimental Morphology*, *89*, 175–208.
Fleming, T. P., Sheth, B., & Fesenko, I. (2001). Cell adhesion in the preimplantation mammalian embryo and its role in trophectoderm differentiation and blastocyst morphogenesis. *Frontiers in Bioscience*, *6*, D1000–D1007.
Foty, R. A., & Steinberg, M. S. (2005). The differential adhesion hypothesis: A direct evaluation. *Developmental Biology*, *278*, 255–263.
Gardner, R. L. (2001). Specification of embryonic axes begins before cleavage in normal mouse development. *Development*, *128*, 839–847.
Gardner, R. L. (2002a). Experimental analysis of second cleavage in the mouse. *Human Reproduction*, *17*, 3178–3189.
Gardner, R. L. (2002b). Patterning is initiated before cleavage in the mouse. *Annals of Anatomy*, *184*, 577–581.
Gelbart, M. A., He, B., Martin, A. C., Thiberge, S. Y., Wieschaus, E. F., & Kaschube, M. (2012). Volume conservation principle involved in cell lengthening and nucleus movement during tissue morphogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 19298–19303.
Gliem, M., Weisheit, G., Mertz, K. D., Endl, E., Oberdick, J., & Schilling, K. (2006). Expression of classical cadherins in the cerebellar anlage: Quantitative and functional aspects. *Molecular and Cellular Neurosciences*, *33*, 447–458.
Godt, D., & Tepass, U. (1998). Drosophila oocyte localization is mediated by differential cadherin-based adhesion. *Nature*, *395*, 387–391.
Goodall, H., & Johnson, M. H. (1984). The nature of intercellular coupling within the preimplantation mouse embryo. *Journal of Embryology and Experimental Morphology*, *79*, 53–76.
Grubb, B. J. (2006). Developmental Biology, Eighth Edition. Scott F. Gilbert, editor. *Integrative and Comparative Biology*, *46*, 652–653.
Handyside, A. H. (1980). Distribution of antibody- and lectin-binding sites on dissociated blastomeres from mouse morulae: Evidence for polarization at compaction. *Journal of Embryology and Experimental Morphology*, *60*, 99–116.
Heisenberg, C. P., & Bellaiche, Y. (2013). Forces in tissue morphogenesis and patterning. *Cell*, *153*, 948–962.
Hirate, Y., Hirahara, S., Inoue, K., Suzuki, A., Alarcon, V. B., Akimoto, K., et al. (2013). Polarity-dependent distribution of angiomotin localizes Hippo signaling in preimplantation embryos. *Current Biology*, *23*, 1181–1194.
Hoffman, B. D., & Yap, A. S. (2015). Towards a dynamic understanding of cadherin-based mechanobiology. *Trends in Cell Biology*, *25*, 803–814.
Hyafil, F., Babinet, C., & Jacob, F. (1981). Cell-cell interactions in early embryogenesis: A molecular approach to the role of calcium. *Cell*, *26*, 447–454.
Johnson, M. H., & McConnell, J. M. L. (2004). Lineage allocation and cell polarity during mouse embryogenesis. *Seminars in Cell & Developmental Biology*, *15*, 583–597.
Johnson, M. H., & Ziomek, C. A. (1981a). The foundation of two distinct cell lineages within the mouse morula. *Cell*, *24*, 71–80.

Johnson, M. H., & Ziomek, C. A. (1981b). Induction of polarity in mouse 8-cell blastomeres: Specificity, geometry, and stability. *The Journal of Cell Biology*, *91*, 303–308.
Johnson, M. H., & Ziomek, C. A. (1983). Cell interactions influence the fate of mouse blastomeres undergoing the transition from the 16- to the 32-cell stage. *Developmental Biology*, *95*, 211–218.
Jongen, W. M., Fitzgerald, D. J., Asamoto, M., Piccoli, C., Slaga, T. J., Gros, D., et al. (1991). Regulation of connexin 43-mediated gap junctional intercellular communication by Ca2+ in mouse epidermal cells is controlled by E-cadherin. *The Journal of Cell Biology*, *114*, 545–555.
Kane, D. A., McFarland, K. N., & Warga, R. M. (2005). Mutations in half baked/E-cadherin block cell behaviors that are necessary for teleost epiboly. *Development*, *132*, 1105–1116.
Kaur, G., Costa, M. W., Nefzger, C. M., Silva, J., Fierro-Gonzalez, J. C., Polo, J. M., et al. (2013). Probing transcription factor diffusion dynamics in the living mammalian embryo with photoactivatable fluorescence correlation spectroscopy. *Nature Communications*, *4*, 1637.
Kerber, M. L., & Cheney, R. E. (2011). Myosin-X: A MyTH-FERM myosin at the tips of filopodia. *Journal of Cell Science*, *124*, 3733–3741.
Krieg, M., Arboleda-Estudillo, Y., Puech, P. H., Kafer, J., Graner, F., Muller, D. J., et al. (2008). Tensile forces govern germ-layer organization in zebrafish. *Nature Cell Biology*, *10*, 429–436.
Kurotaki, Y., Hatta, K., Nakao, K., Nabeshima, Y., & Fujimori, T. (2007). Blastocyst axis is specified independently of early cell lineage but aligns with the ZP shape. *Science*, *316*, 719–723.
Larue, L., Ohsugi, M., Hirchenhain, J., & Kemler, R. (1994). E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 8263–8267.
Le Cruguel, S., Ferre-L'Hotellier, V., Moriniere, C., Lemerle, S., Reynier, P., Descamps, P., et al. (2013). Early compaction at day 3 may be a useful additional criterion for embryo transfer. *Journal of Assisted Reproduction and Genetics*, *30*, 683–690.
Lecuit, T., & Lenne, P. F. (2007). Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nature Reviews. Molecular Cell Biology*, *8*, 633–644.
Lee, D. R., Lee, J. E., Yoon, H. S., Roh, S. I., & Kim, M. K. (2001). Compaction in preimplantation mouse embryos is regulated by a cytoplasmic regulatory factor that alters between 1- and 2-cell stages in a concentration-dependent manner. *The Journal of Experimental Zoology*, *290*, 61–71.
Lee, J. Y., Marston, D. J., Walston, T., Hardin, J., Halberstadt, A., & Goldstein, B. (2006). Wnt/Frizzled signaling controls C. elegans gastrulation by activating actomyosin contractility. *Current Biology*, *16*, 1986–1997.
Lo, C. W., & Gilula, N. B. (1979). Gap junctional communication in the preimplantation mouse embryo. *Cell*, *18*, 399–409.
Louvet, S., Aghion, J., Santa-Maria, A., Mangeat, P., & Maro, B. (1996). Ezrin becomes restricted to outer cells following asymmetrical division in the preimplantation mouse embryo. *Developmental Biology*, *177*, 568–579.
Louvet-Vallee, S., Vinot, S., & Maro, B. (2005). Mitotic spindles and cleavage planes are oriented randomly in the two-cell mouse embryo. *Current Biology*, *15*, 464–469.
Luke, B., Stern, J. E., Kotelchuck, M., Declercq, E. R., Hornstein, M. D., Gopal, D., et al. (2015). Adverse pregnancy outcomes after in vitro fertilization: Effect of number of embryos transferred and plurality at conception. *Fertility and Sterility*, *104*, 79–86.
Maitre, J. L., Berthoumieux, H., Krens, S. F., Salbreux, G., Julicher, F., Paluch, E., et al. (2012). Adhesion functions in cell sorting by mechanically coupling the cortices of adhering cells. *Science*, *338*, 253–256.

Maitre, J. L., Niwayama, R., Turlier, H., Nedelec, F., & Hiiragi, T. (2015). Pulsatile cell-autonomous contractility drives compaction in the mouse embryo. *Nature Cell Biology*, *17*, 849–855.

Maro, B., Johnson, M. H., Pickering, S. J., & Louvard, D. (1985). Changes in the distribution of membranous organelles during mouse early development. *Journal of Embryology and Experimental Morphology*, *90*, 287–309.

McLachlin, J. R., Caveney, S., & Kidder, G. M. (1983). Control of gap junction formation in early mouse embryos. *Developmental Biology*, *98*, 155–164.

Meng, W., & Takeichi, M. (2009). Adherens junction: Molecular architecture and regulation. *Cold Spring Harbor Perspectives in Biology*, *1*, a002899.

Monier, B., Pelissier-Monier, A., Brand, A. H., & Sanson, B. (2010). An actomyosin-based barrier inhibits cell mixing at compartmental boundaries in Drosophila embryos. *Nature Cell Biology*, *12*, 60–65 (Suppl. pp. 1–9).

Motosugi, N., Bauer, T., Polanski, Z., Solter, D., & Hiiragi, T. (2005). Polarity of the mouse embryo is established at blastocyst and is not prepatterned. *Genes & Development*, *19*, 1081–1092.

Nishioka, N., Inoue, K., Adachi, K., Kiyonari, H., Ota, M., Ralston, A., et al. (2009). The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. *Developmental Cell*, *16*, 398–410.

Nose, A., Nagafuchi, A., & Takeichi, M. (1988). Expressed recombinant cadherins mediate cell sorting in model systems. *Cell*, *54*, 993–1001.

Ohsugi, M., Ohsawa, T., & Semba, R. (1993). Similar responses to pharmacological agents of 1,2-OAG-induced compaction-like adhesion of two-cell mouse embryo to physiological compaction. *The Journal of Experimental Zoology*, *265*, 604–608.

Okegawa, T., Pong, R. C., Li, Y., & Hsieh, J. T. (2004). The role of cell adhesion molecule in cancer progression and its application in cancer therapy. *Acta Biochimica Polonica*, *51*, 445–457.

Ozawa, M., Ringwald, M., & Kemler, R. (1990). Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 4246–4250.

Pasternak, C., Spudich, J. A., & Elson, E. L. (1989). Capping of surface receptors and concomitant cortical tension are generated by conventional myosin. *Nature*, *341*, 549–551.

Pauken, C. M., & Capco, D. G. (1999). Regulation of cell adhesion during embryonic compaction of mammalian embryos: Roles for PKC and beta-catenin. *Molecular Reproduction and Development*, *54*, 135–144.

Pauken, C. M., & Capco, D. G. (2000). The expression and stage-specific localization of protein kinase C isotypes during mouse preimplantation development. *Developmental Biology*, *223*, 411–421.

Piotrowska, K., Wianny, F., Pedersen, R. A., & Zernicka-Goetz, M. (2001). Blastomeres arising from the first cleavage division have distinguishable fates in normal mouse development. *Development*, *128*, 3739–3748.

Piotrowska-Nitsche, K., Perea-Gomez, A., Haraguchi, S., & Zernicka-Goetz, M. (2005). Four-cell stage mouse blastomeres have different developmental properties. *Development*, *132*, 479–490.

Piotrowska-Nitsche, K., & Zernicka-Goetz, M. (2005). Spatial arrangement of individual 4-cell stage blastomeres and the order in which they are generated correlate with blastocyst pattern in the mouse embryo. *Mechanisms of Development*, *122*, 487–500.

Plachta, N., Bollenbach, T., Pease, S., Fraser, S. E., & Pantazis, P. (2011). Oct4 kinetics predict cell lineage patterning in the early mammalian embryo. *Nature Cell Biology*, *13*, 117–123.

Practice Committee of American Society for Reproductive Medicine. (2012). Multiple gestation associated with infertility therapy: An American Society for Reproductive Medicine Practice Committee opinion. *Fertility and Sterility*, *97*, 825–834.
Ralston, A., Cox, B. J., Nishioka, N., Sasaki, H., Chea, E., Rugg-Gunn, P., et al. (2010). Gata3 regulates trophoblast development downstream of Tead4 and in parallel to Cdx2. *Development*, *137*, 395–403.
Reeve, W. J. (1981). Cytoplasmic polarity develops at compaction in rat and mouse embryos. *Journal of Embryology and Experimental Morphology*, *62*, 351–367.
Reeve, W. J., & Ziomek, C. A. (1981). Distribution of microvilli on dissociated blastomeres from mouse embryos: Evidence for surface polarization at compaction. *Journal of Embryology and Experimental Morphology*, *62*, 339–350.
Richa, J., Damsky, C. H., Buck, C. A., Knowles, B. B., & Solter, D. (1985). Cell surface glycoproteins mediate compaction, trophoblast attachment, and endoderm formation during early mouse development. *Developmental Biology*, *108*, 513–521.
Rossant, J. (1976). Postimplantation development of blastomeres isolated from 4- and 8-cell mouse eggs. *Journal of Embryology and Experimental Morphology*, *36*, 283–290.
Rossant, J., & Tam, P. P. (2009). Blastocyst lineage formation, early embryonic asymmetries and axis patterning in the mouse. *Development*, *136*, 701–713.
Samarage, C. R., White, M. D., Alvarez, Y. D., Fierro-Gonzalez, J. C., Henon, Y., Jesudason, E. C., et al. (2015). Cortical tension allocates the first inner cells of the mammalian embryo. *Developmental Cell*, *34*, 435–447.
Sawyer, J. M., Harrell, J. R., Shemer, G., Sullivan-Brown, J., Roh-Johnson, M., & Goldstein, B. (2010). Apical constriction: A cell shape change that can drive morphogenesis. *Developmental Biology*, *341*, 5–19.
Schotz, E. M., Burdine, R. D., Julicher, F., Steinberg, M. S., Heisenberg, C. P., & Foty, R. A. (2008). Quantitative differences in tissue surface tension influence zebrafish germ layer positioning. *Human Frontier Science Program*, *2*, 42–56.
Shi, J., Chen, Q., Li, X., Zheng, X., Zhang, Y., Qiao, J., et al. (2015). Dynamic transcriptional symmetry-breaking in pre-implantation mammalian embryo development revealed by single-cell RNA-seq. *Development*, *142*, 3468–3477.
Steinberg, M. S. (1970). Does differential adhesion govern self-assembly processes in histogenesis? Equilibrium configurations and the emergence of a hierarchy among populations of embryonic cells. *The Journal of Experimental Zoology*, *173*, 395–433.
Stephenson, R. O., Yamanaka, Y., & Rossant, J. (2010). Disorganized epithelial polarity and excess trophectoderm cell fate in preimplantation embryos lacking E-cadherin. *Development*, *137*, 3383–3391.
Steptoe, P. C., & Edwards, R. G. (1978). Birth after the reimplantation of a human embryo. *Lancet*, *2*, 366.
Strnad, P., Gunther, S., Reichmann, J., Krzic, U., Balazs, B., de Medeiros, G., et al. (2015). Inverted light-sheet microscope for imaging mouse pre-implantation development. *Nature Methods*, *13*, 139–142.
Summers, M. C., & Biggers, J. D. (2003). Chemically defined media and the culture of mammalian preimplantation embryos: Historical perspective and current issues. *Human Reproduction Update*, *9*, 557–582.
Sutherland, A. E., Calarco, P. G., & Damsky, C. H. (1993). Developmental regulation of integrin expression at the time of implantation in the mouse embryo. *Development*, *119*, 1175–1186.
Tang, F., Barbacioru, C., Nordman, E., Bao, S., Lee, C., Wang, X., et al. (2011). Deterministic and stochastic allele specific gene expression in single mouse blastomeres. *PLoS One*, *6*, e21208.
Tao, J., Tamis, R., Fink, K., Williams, B., Nelson-White, T., & Craig, R. (2002). The neglected morula/compact stage embryo transfer. *Human Reproduction*, *17*, 1513–1518.

Tarkowski, A. K. (1959). Experiments on the development of isolated blastomers of mouse eggs. *Nature*, *184*, 1286–1287.
Tarkowski, A. K. (1961). Mouse chimaeras developed from fused eggs. *Nature*, *190*, 857–860.
Tarkowski, A. K., & Wroblewska, J. (1967). Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. *Journal of Embryology and Experimental Morphology*, *18*, 155–180.
Thomas, F. C., Sheth, B., Eckert, J. J., Bazzoni, G., Dejana, E., & Fleming, T. P. (2004). Contribution of JAM-1 to epithelial differentiation and tight-junction biogenesis in the mouse preimplantation embryo. *Journal of Cell Science*, *117*, 5599–5608.
Townes, P. L., & Holtfreter, J. (1955). Directed movements and selective adhesion of embryonic amphibian cells. *Journal of Experimental Zoology*, *128*, 53–120.
Vestweber, D., Gossler, A., Boller, K., & Kemler, R. (1987). Expression and distribution of cell adhesion molecule uvomorulin in mouse preimplantation embryos. *Developmental Biology*, *124*, 451–456.
Vinot, S., Le, T., Ohno, S., Pawson, T., Maro, B., & Louvet-Vallee, S. (2005). Asymmetric distribution of PAR proteins in the mouse embryo begins at the 8-cell stage during compaction. *Developmental Biology*, *282*, 307–319.
Wada, K., Itoga, K., Okano, T., Yonemura, S., & Sasaki, H. (2011). Hippo pathway regulation by cell morphology and stress fibers. *Development*, *138*, 3907–3914.
Wales, R. G. (1970). Effects of ions on the development of the preimplantation mouse embryo in vitro. *Australian Journal of Biological Sciences*, *23*, 421–429.
Wang, A. Z., Ojakian, G. K., & Nelson, W. J. (1990). Steps in the morphogenesis of a polarized epithelium. I. Uncoupling the roles of cell-cell and cell-substratum contact in establishing plasma membrane polarity in multicellular epithelial (MDCK) cysts. *Journal of Cell Science*, *95*(Pt. 1), 137–151.
Whitten, W. K. (1971). Nutritional requirements for the culture of preimplantation embryos in vitro. *Advances in the Biosciences*, *6*, 129–139.
Winkel, G. K., Ferguson, J. E., Takeichi, M., & Nuccitelli, R. (1990). Activation of protein kinase C triggers premature compaction in the four-cell stage mouse embryo. *Developmental Biology*, *138*, 1–15.
Xiong, F., Ma, W., Hiscock, T. W., Mosaliganti, K. R., Tentner, A. R., Brakke, K. A., et al. (2014). Interplay of cell shape and division orientation promotes robust morphogenesis of developing epithelia. *Cell*, *159*, 415–427.
Yamanaka, Y., Ralston, A., Stephenson, R. O., & Rossant, J. (2006). Cell and molecular regulation of the mouse blastocyst. *Developmental Dynamics*, *235*, 2301–2314.
Yoshida, S., Barbier de Reuille, P., Lane, B., Bassel, G. W., Prusinkiewicz, P., Smith, R. S., et al. (2014). Genetic control of plant development by overriding a geometric division rule. *Developmental Cell*, *29*, 75–87.
Yoshida, C., & Takeichi, M. (1982). Teratocarcinoma cell adhesion: Identification of a cell-surface protein involved in calcium-dependent cell aggregation. *Cell*, *28*, 217–224.
Zernicka-Goetz, M., Morris, S. A., & Bruce, A. W. (2009). Making a firm decision: Multifaceted regulation of cell fate in the early mouse embryo. *Nature Reviews. Genetics*, *10*, 467–477.
Ziomek, C. A., & Johnson, M. H. (1980). Cell surface interaction induces polarization of mouse 8-cell blastomeres at compaction. *Cell*, *21*, 935–942.