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Mechanical Control of Cell Differentiation: Insights from the Early Embryo

Celeste M. Nelson

Departments of Chemical & Biological Engineering and Molecular Biology, Princeton University, Princeton, New Jersey USA; email: celesten@princeton.edu

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Abstract

Differentiation is the process by which a cell activates the expression of tissue-specific genes, downregulates the expression of potency markers, and acquires the phenotypic characteristics of its mature fate. The signals that regulate differentiation include biochemical and mechanical factors within the surrounding microenvironment. We describe recent breakthroughs in our understanding of the mechanical control mechanisms that regulate differentiation, with a specific emphasis on the differentiation events that build the early mouse embryo. Engineering approaches that reproducibly mimic the mechanical regulation of differentiation will permit new insights into early development and applications in regenerative medicine.

Contents

INTRODUCTION

From a single, fertilized egg emerges the approximately 200 or so phenotypically distinct cell types that make up a human or a mouse. These cells are generated through the process of differentiation, a cascade of events that endow cells with identity and function and permit them to construct the tissues and organs necessary for multicellular life (1, 2). Differentiation continues apace in mature tissues, wherein resident stem or progenitor cells divide and differentiate to retain or restore population levels of tissue-specific cell types during homeostasis or after injury (3). The importance of differentiation can also be appreciated by considering a variety of different diseases, including fibrosis and cancer, in which cells within tissues dedifferentiate into a more stem-like state or transdifferentiate to take on new deleterious phenotypes (4).

Our understanding of the molecular signaling pathways and extracellular stimuli that regulate differentiation has deepened over the last decade. These breakthroughs were driven in part by advances in the isolation and culture of embryonic stem (ES) cells (5) and induced pluripotent stem (iPS) cells (6); these advances revealed the transcription factors and other proteins that are essential for maintaining a cell in an undifferentiated state (7, 8), for promoting its self-renewal (9), and for inducing its differentiation down a specific lineage (10). This rich understanding of fundamental mechanisms has permitted the use of stem cells to repair damaged organs in regenerative medicine applications (5). Parallel studies using stem cells isolated from adult tissues, including mesenchymal stem cells (MSCs), showed that the mechanical properties (see the sidebar titled A Brief Primer on Mechanics in Biological Systems) of the microenvironment cooperate with—and can even override—information from biochemical signals to induce differentiation (11–13). For example, in a now-classic study, MSCs that were cultured on hydrogel-based substrata engineered to mimic the stiffness of the brain differentiated down a neurogenic lineage, whereas those cultured on substrata engineered to mimic the stiffness of bone differentiated down an osteogenic route (14). This switch in cell fate depends in part on the physical properties of the nucleus itself; these properties are regulated by scaffolding proteins such as lamin-A, the expression of which is tuned by substratum stiffness (15). Similarly, experiments using Transwell filters with different pore sizes revealed that migration through confined spaces enhances the differentiation of MSCs down the osteogenic lineage (16). The concept of mechanical regulation of stem cell differentiation was thus pioneered through the clever use of engineered culture microenvironments.

ES: embryonic stem

iPS: induced pluripotent stem

Studies focused on the differentiation of individual cells in culture likewise permitted the creation of three-dimensional (3D) in vitro mimetics of tissues and organs known as organoids (17–19). The first stem cell–derived organoids reproduced the 3D organizations of the crypt-villus axis of the intestine (20) and the cortex of the brain (21). In the intervening years, stem cells have been differentiated to form 3D organoids that mimic the architectures of a variety of organs, including different regions of the brain, gastrointestinal tract, liver, pancreas, lung, and kidney (reviewed in

A BRIEF PRIMER ON MECHANICS IN BIOLOGICAL SYSTEMS

Cells and tissues, including those of the developing embryo, must obey Newton's laws of motion. To understand the role of mechanical forces in differentiation, we need to consider the active inputs (the stresses, strains, and flows that impinge upon a cell), the passive inputs (the material properties of cells and their surrounding microenvironment), and the mechanical and biochemical responses of the cell itself.

- Active inputs: A force is any input that causes an object, such as a cell or a tissue, to change its shape (deformation) or velocity (speed and direction). In biological systems, forces result from fluid flows, fluid pressures, and tension or compression induced by other cells or tissues. Cells appear capable of sensing both stress (force per unit area, akin to pressure) and strain (deformation per unit length, akin to stretch). Although force and stress are related to each other and often used interchangeably, these parameters are not identical. The application of a constant force can lead to levels of stress that change over space and time as the object (the cell or tissue) deforms.
- Material properties: The mechanical stiffness of any object, including a cell as well as its extracellular microenvironment, affects how that object will respond to the application of force. The stiffer the object, the more force is required to deform it. Compliance and elasticity are commonly used terms to describe stiffness; the softer the object, the more compliant or elastic it is. The Young's (or elastic) modulus refers to the stiffness of a material, independent of its geometry. The higher the modulus, the stiffer the material.
- Cellular responses to mechanical forces: Cells use their cytoskeletons and adhesive contacts to probe the mechanical properties of their surrounding microenvironment. Cytoskeletal tension refers to the pulling forces exerted by the actin, microtubule, and/or intermediate filament networks within a cell. The higher the cytoskeletal tension, the more the cell pulls on its neighbors or extracellular matrix. Cortical tension refers to the contractility of the actomyosin cytoskeleton located at the cell membrane. The higher the cortical tension, the greater the tendency for the cell to round up. Each network responds to changes in the mechanical properties or forces imparted on a cell through processes known as mechanosensing and mechanotransduction.

17 and 22). Stem cell–derived organoids have been used to model human diseases, both for studying basic mechanisms and for screening of potential therapeutics (23, 24). These are exciting applications of a technology borne out of insights into the fundamental processes of differentiation. Furthermore, organoids have served as minimal culture models that mimic aspects of embryonic development and have allowed scientists and engineers to uncover the step-wise ordering of extracellular signals and gene-expression patterns that trigger a pluripotent or multipotent cell to differentiate down distinct lineages outside of the embryo (25, 26).

As with isolated stem cells in two-dimensional (2D) culture, 3D organoid models have also started to reveal the relative roles of mechanical forces in the regulation of differentiation. For example, synthetic matrices that mimic the stiffness of the liver promote the proliferation of liver progenitor cells into organoids (27), whereas matrices that are softer than the liver prevent organoid formation. In contrast, similar experiments with intestinal progenitor cells revealed that the size of the stem cell compartment within intestinal organoids decreases as stiffness increases (28). How stem cells interpret the mechanical properties of their local microenvironment as they form into organoids is therefore likely dependent on their tissue type.

Insights from the above-described culture models, both 2D and 3D, have ignited interest in defining the relative roles of mechanical forces and mechanical signaling in the regulation of differentiation in embryonic and fetal tissues in vivo. It is possible that many of the same regulatory

mechanisms that control differentiation in culture will be found to operate in embryonic and fetal tissues; however, it is just as (if not more) likely that the clever reductionist approaches described above overlook key complexities of the embryonic microenvironment and that an entirely different set of regulatory mechanisms will be discovered from studies in vivo. This review serves as a primer for early embryonic development of the mouse and highlights the mechanical signaling pathways and mechanical forces that have so far been found to instruct each differentiation event that specifies cell fate in the early embryo. We begin with a discussion of the preimplantation embryo, at a stage when each of the eight cells that compose the developing mouse is essentially identical with respect to its differentiation potential. We then describe the mechanical signaling involved in symmetry breaking, segregation of the embryonic and extraembryonic tissues, implantation into the uterus, and differentiation of the three germ layers.

Blastocyst: an early stage of embryonic development in mammals that consists of a hollow ball of cells surrounding an inner cell mass

Inner cell mass (ICM): the cells of the blastocyst that are fated to become the embryo itself along with the primitive endoderm

Trophectoderm (TE): the cells at the outer layer of the blastocyst that are fated to become part of the placenta

Morula: a solid ball of cells generated from early cleavage divisions of a zygote

FROM OOCYTE TO ORGANISM—THE ROLE OF MECHANICS IN THE SYMPHONY OF DIFFERENTIATION EVENTS THAT BUILD THE EARLY MAMMALIAN EMBRYO

Cortical Tension and Pressure: The Mechanical Forces that Sculpt the Blastula

In the preimplantation mammalian (mouse) embryo, the first differentiation event occurs after the 8-cell stage (**Figure 1*a***), when the cells of the blastocyst generate the first lineages that will become the inner cell mass (ICM), which gives rise to the embryo proper, and the outer extraembryonic trophectoderm (TE), which gives rise to the placenta. Mammalian oocytes are spherical and therefore lack any obvious geometric polarity signal that would influence later symmetry-breaking and differentiation decisions (29–31); thus, the cells of the 8-cell-stage embryo are equivalent in their geometry as well as their developmental potential. However, asymmetric division and subsequent cellular rearrangements generate a 16-cell morula that contains a core of nonpolarized cells surrounded by a layer of cells with apicobasal polarity (32). This geometric arrangement appears to require differences in myosin-mediated contraction of the apical actin cortex, such that cells with higher cortical tension become internalized and surrounded by those with lower cortical tension (33–36). It remains unclear how cortical tension is specified within cells at the 8-cell stage, but differences in the distributions of proteins at the apical surfaces of these cells are likely involved (36). Regardless of the underlying molecular details, it is notable that the first symmetry-breaking event in the mammalian embryo is generated by mechanical forces resulting from cortical tension.

This symmetry-breaking event sets up the 16-cell- and 32-cell-stage murine embryos, both of which contain a single layer of outer cells that have apicobasal polarity around a core of nonpolarized inner cells. In the first lineage-specification event, the nonpolarized inner cells become the ICM while the outer cells become the TE. Mechanical signaling also appears to be critical for driving this fate specification (**Figure 1*b***). In particular, subcellular localization of Yes-associated protein (Yap) distinguishes between the two fates. Yap functions as part of the Hippo signaling pathway (**Figure 2**): When the Hippo pathway is activated, the serine/threonine kinases Lats1 and Lats2 phosphorylate Yap, causing its cytoplasmic retention (37, 38). When Hippo is inactive, Yap shuttles to the nucleus and interacts with Tead-family transcription factors to activate gene expression (39). In parallel, Yap localization and activity are also regulated by mechanical forces (40). Specifically, myosin-mediated contraction of F-actin appears to induce and sustain nuclear localization of Yap and downstream gene expression (41–43). As a consequence, mechanical forces and features of the microenvironment that are generally associated with the formation of actin stress fibers and cell spreading—including shear forces, mechanical compression, and local microenvironmental stiffness—are also associated with the nuclear localization of Yap and activation of gene expression (44, 45).

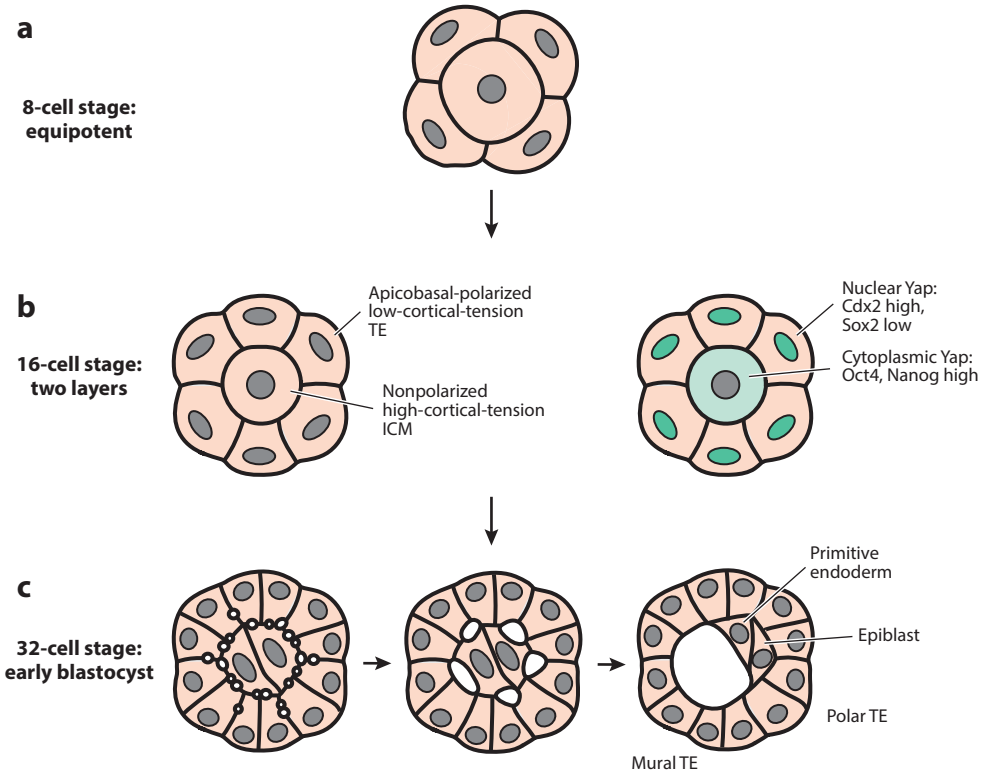

Figure 1

Mechanical forces from cortical tension and luminal fluid pressure influence differentiation in the early mouse embryo. (*a*) At the 8-cell stage, all cells of the embryo are equally capable of differentiating into the different cell types of the embryo proper and the extraembryonic tissues. (*b*) At the 16-cell stage, the embryo contains two layers of cells—an inner layer with high cortical tension and low nuclear Yap that becomes the ICM, and an outer layer with low cortical tension and high nuclear Yap that becomes the TE. (*c*) At the 32-cell stage, the early blastocyst develops a fluid-filled cavity that localizes to one side of the embryo and distinguishes the mural TE from the polar TE, which is adjacent to the ICM. Abbreviations: ICM, inner cell mass; TE, trophectoderm; Yap, Yes-associated protein.

In a manner consistent with the spatial patterns of cellular distention within the early mouse embryo, Yap is phosphorylated and retained in the cytoplasm in the inner cells, whereas Yap is localized to the nucleus in the outer cells (46–48). As soon as spatial differences in Yap localization are detectable, the expression of the TE-associated transcription factor Cdx2 begins to correlate with nuclear Yap (48). Cdx2 represses the pluripotency factors Oct4 and Nanog (47, 49), thus limiting their expression (and pluripotency) to the cells of the ICM. In parallel, Yap represses the expression of the pluripotency marker Sox2 in the outer cells, thus initiating the cascade of events that leads these cells to differentiate into TE (50). Intriguingly, the Yap cofactor Tead4 is also responsible for a key metabolic switch in the two populations of cells at this stage of development: The ICM produces ATP largely through glycolysis (51, 52), while Tead4-induced signaling within the TE promotes the expression of mitochondrial transport chain components and the production of ATP through oxidative phosphorylation (53). Consistently, disrupting Tead4 expression (54, 55) or inhibiting Rho kinase (50) causes the outer layer of cells to express ICM markers and thus prevents their differentiation into the TE.

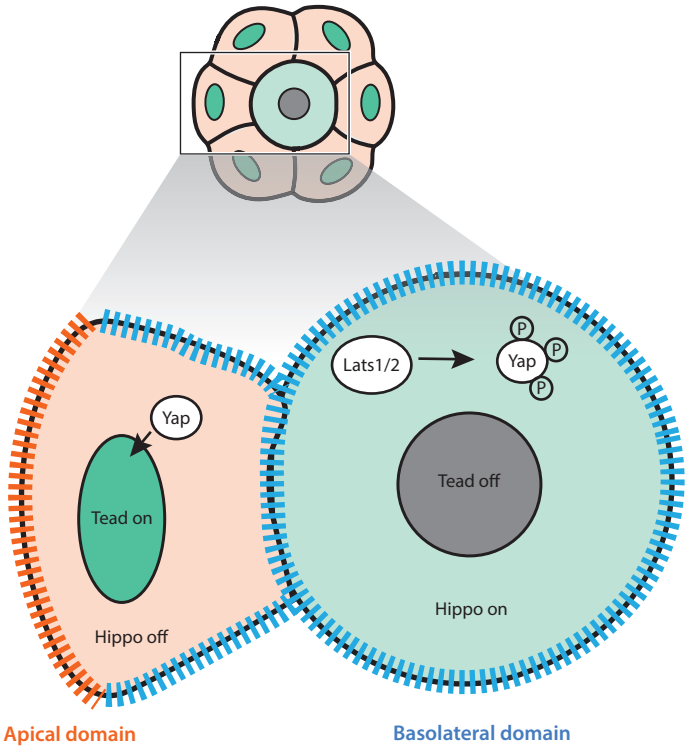

Figure 2

Mechanical signaling through the Hippo/Yap pathway. Activation of the Hippo pathway induces phosphorylation of Lats1/2, which phosphorylate Yap and cause its retention in the cytoplasm.

The second lineage-specification event occurs during the early blastocyst (32-cell) stage (**Figure 1*c***), when ICM cells begin to express markers of either epiblast or primitive endoderm fate in a stochastic manner (56–58), influenced by their relative position. Specifically, epiblast cells are biased to form adjacent to the TE and are separated from the developing lumen by the primitive endoderm (59–61). At the same time as these fates are specified, secretion of cytoplasmic vesicles causes fluid droplets to accumulate between the inner cells, which eventually coalesce or coarsen to form one large fluid-filled cavity that compresses the ICM to one side of the embryo (62–64). The pressure of this fluid was recently found to increase over the course of embryonic development (65) and to alter the relative numbers of cells in the ICM and the TE in a tension-dependent manner (66). Higher pressure leads to increased expansion of the lumen, and the forces from expansion are transmitted into maturation of tight junctions to maintain blastocyst integrity. Lower pressure appears to result in decreased luminal expansion and an increase in asymmetric divisions, favoring the generation of cells that populate the ICM. In this case, a mechanical signal (pressure) regulates fate decisions by altering the pattern of cell division within the early embryo. Although not shown explicitly, higher pressures would also be expected to increase the strain and stretch within the TE cell layer and thus enhance nuclear Yap and expression of Cdx2. As the lumen expands, the epiblast and primitive endoderm cells appear to undergo differential sorting into their final positions within the ICM (64), with those cells biased toward primitive endoderm fate moving directionally toward the ICM–lumen interface (64). Decreasing luminal pressure mechanically or biochemically decreases specification of primitive endoderm and sorting

Epiblast: the outermost layer of cells in an embryo that will later give rise to ectoderm and mesoderm

Primitive endoderm: a layer of cells in the early embryo that gives rise to the yolk sac

from the epiblast population (64). Whether this sorting is regulated predominantly by chemotaxis, durotaxis, or some other mechanism remains to be determined.

At the mid-blastocyst stage, the cells within the TE have already segregated into two distinct populations with completely different fates—the polar TE, which directly contacts the ICM, and the mural TE, which lacks these heterotypic contacts but surrounds the fluid-filled blastocoel. At the late-blastocyst stage (*E*4.5 in the mouse), the mural TE contacts the endometrium of the uterus and initiates the process of implantation, which leads to drastic changes in the shape and potency of the epiblast. The initially oval, naive epiblast is constricted by its surrounding basement membrane as it grows, forcing it to adopt a circular shape (67, 68). Increased tension and apical constriction within the polar TE exerts force on the epiblast; together, the polar TE and epiblast transform into the postimplantation morphology known as the egg cylinder (67). These changes occur concomitantly with transition of the epiblast from naive to primed pluripotency, but it remains unclear whether the forces of remodeling are correlative or causal in this transition.

After the initiation of implantation, the highly invasive TE cells stop undergoing cytokinesis and commence endoreduplication, which generates highly polyploid primary trophoblast giant cells (TGCs) (69). Primary TGCs promote remodeling of the endometrial tissue by secreting cathepsins and matrix metalloproteinases (MMPs) (70). These cells remove adjacent endometrial epithelial cells via phagocytosis, invade into the uterine stroma, and establish connections with the maternal vasculature (71). Differentiation of TGCs appears to involve an epithelial-mesenchymal transition (EMT), in which cell–cell interactions are destabilized as cells increase their motility and invasiveness and engulf the maternal epithelial and blood cells (71, 72). EMT is then followed by reepithelialization of the postmitotic giant cells to form the vascular structures of the placenta (71, 73). Both EMT and EMT-associated failure of cytokinesis are known to be regulated by mechanical signaling in adult epithelial cells (see the sidebar titled Mechanical Regulation of Epithelial-Mesenchymal Transition and Endoreduplication). It is therefore possible that the mechanical properties of the microenvironment of the endometrium are partly responsible for initiating differentiation of TGCs. Consistent with this possibility, alterations in the mechanical properties of the endometrium were recently found to promote implantation (74).

Endoreduplication: a specialized cell cycle in which the cell replicates its genome without undergoing mitosis, thus resulting in polyploidy

TGC: trophoblast giant cell

MMP: matrix metalloproteinase

EMT: epithelial-mesenchymal transition

MECHANICAL REGULATION OF EPITHELIAL-MESENCHYMAL TRANSITION AND ENDOREDUPLICATION

Epithelial-mesenchymal transition (EMT) is a prototypic example of transdifferentiation that is essential for development of the embryo proper and is also implicated in fibrosis and metastasis (75, 76). EMT is typically stimulated by soluble signals including transforming growth factor-beta (TGFβ) and matrix metalloproteinases (MMPs) and is initiated by changes in the expression of key transcription factors including members of the Snail, Twist, and ZEB families (77, 78). These transcription factors, in turn, lead to the repression of epithelial markers, including E-cadherin, and the expression of mesenchymal cytoskeletal proteins such as vimentin. These molecular changes promote the weakening of cadherin-associated intercellular adhesions and enhance single-cell migration and invasion.

As a transdifferentiation process, EMT is also regulated by the mechanical properties of the surrounding microenvironment. For example, the ability of signals such as TGFβ or MMPs to induce EMT depends on the mechanical stiffness of the underlying matrix. Specifically, exposing cells in stiff microenvironments to EMT inducers leads to elevated expression of Snail and robust EMT (79–83). In contrast, soft microenvironments prevent the induction of Snail, apparently due to decreased signaling through β1 integrin (80, 84). Strikingly, cells that continue to proliferate while undergoing EMT in culture fail to undergo cytokinesis and become multinucleated (85–87), a process that is also promoted by stiff microenvironments and inhibited by soft microenvironments (86).

The polar TE cells that are located adjacent to the ICM continue to undergo cytokinetic divisions and generate the remaining populations of cells that form the placenta (88).

In the human embryo, development and cell-fate specification are likely regulated by different mechanical events than those described above for the mouse. Compaction and cavitation occur later in the human embryo, at the 16-cell and 64-cell stages, respectively (89), and thus the mechanical forces experienced by individual cells at these stages will be different. More strikingly, Yap is localized to the nucleus in both the ICM and TE cells of the preimplantation human embryo (90), suggesting that signaling from cytoskeletal tension and/or Hippo is not responsible for this cell-fate decision in the human. These differences between the early mouse and human embryos suggest caution in generalizing results, both between species as well as between differentiation in culture and in vivo.

The Most Important Stage of Your Life: The Mechanics of Gastrulation

After the cells of the fertilized oocyte are specified as embryonic or extraembryonic, these cells then undergo a complex and highly coordinated series of rearrangements to transform from a single layer of tissue into three apposed layers of endoderm, mesoderm, and ectoderm, known as the primary germ layers. These germ layers give rise to all of the different tissues of the mature organism. As the innermost layer, the endoderm forms the epithelial lining of the gastrointestinal tract, respiratory tract, urogenital system, and endocrine glands. The middle layer, the mesoderm, differentiates into the mesenchyme, mesothelium, striated and smooth musculature, and red blood cells. As the outermost layer, the ectoderm gives rise to the epithelium of the skin and its appendages (including the mammary and sweat glands) as well as neural tissues of the brain and spinal column. The morphogenetic process that generates the primary germ layers is known as gastrulation and varies greatly between different classes of vertebrates (91, 92).

In the mouse, gastrulation begins approximately two days after implantation. Prior to gastrulation, the cup-shaped embryo is composed of a radially symmetric layer of pluripotent cells known as the epiblast (**Figure 3**), which is surrounded laterally by a layer of extraembryonic tissue known as the visceral endoderm (VE) and proximally by the trophoblast or extraembryonic ectoderm (ExE). The ExE forms the fetal portion of the placenta while the VE forms the extraembryonic membranes. The process of implantation provides the embryo with a proximal-distal axis, with the surface of the endometrium serving as the proximal end of the structure. Induction of gastrulation requires the embryo to break its radial symmetry and establish an anterior-posterior axis (which also specifies the head and tail of the organism). At the onset of gastrulation, cells at the posterior pole of the epiblast undergo morphological changes to form the primitive streak. Cells located at the primitive streak undergo an EMT, ingress, and migrate to fill the space between the epiblast and the VE as they form the definitive mesoderm.

Similar to the preimplantation embryo, symmetry breaking of the postimplantation embryo is driven in part by mechanical forces. Prior to the onset of gastrulation, the postimplantation epiblast is surrounded by and constrained within a presumably rigid basement membrane. Small, evenly distributed perforations within the basement membrane, which would weaken the constraint, correlate with growth of the epiblast (93). At the same time as the epiblast is growing, a cluster of extraembryonic cells, known as anterior VE (AVE) cells, emerges at the distal tip of the structure and migrates collectively toward one side (94) where the cells secrete inhibitors of the Wnt and Nodal pathways to inhibit posterior cell fates and thereby establish the anterior pole of the embryo (95). This collective migration is preceded by apical constriction (96) and requires activation of the Rho GTPase Rac1, which permits the AVE cells to extend actin-rich cytoskeletal projections in the direction of migration and to exchange positions with their neighbors

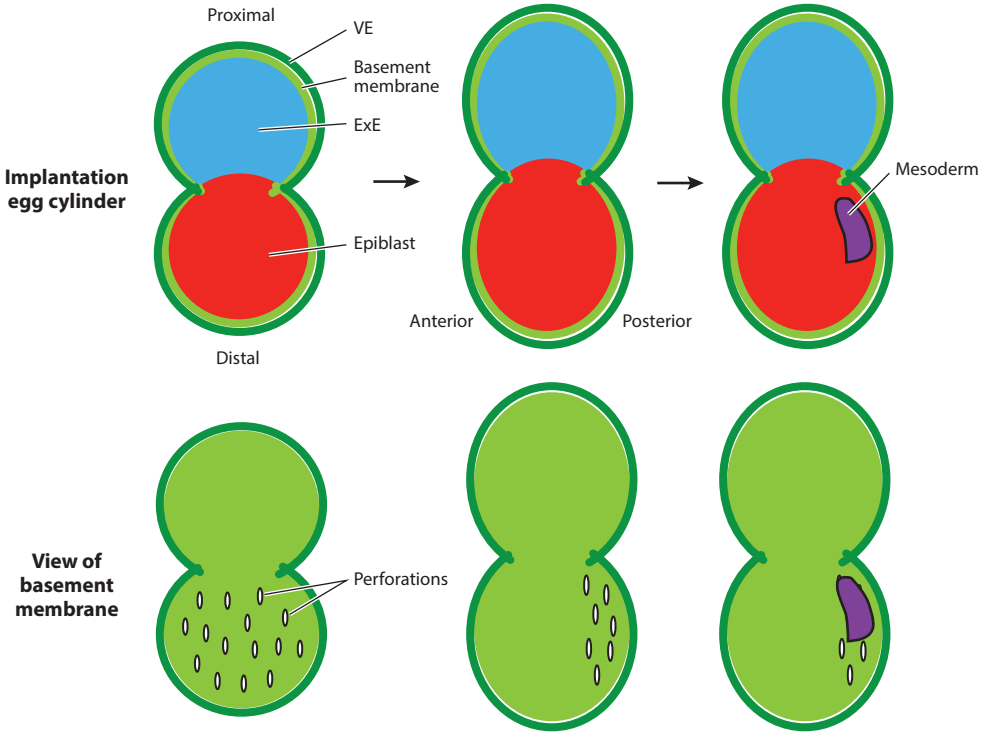

Figure 3

Mechanical signals from the basement membrane along with increases in tissue fluidity promote gastrulation of the mouse embryo. MMP-driven weakening of the basement membrane (indicated by perforations) permits growth of the epiblast (*left*). AVE cells secrete Nodal inhibitors, thus blocking expression of MMPs, leading to an intact basement membrane on the anterior pole of the embryo (*center*). Cells in the posterior side of the embryo undergo EMT and migrate through the weakened basement membrane, establishing the mesoderm (*right*). Abbreviations: AVE, anterior visceral endoderm; EMT, epithelial-mesenchymal transition; ExE, extraembryonic ectoderm; MMP, matrix metalloproteinase; VE, visceral endoderm.

(97)—essentially, this population of cells becomes more fluid-like (see the sidebar titled Solid-Like Versus Fluid-Like Tissues) by altering their packing (98). Particle imaging velocimetry analysis of fluorescently tagged embryos revealed that AVE cells undergo push-pull motions as they migrate collectively, and knockout screens identified the Rho GEF beta-PIX as an essential element in localizing the protrusive activity (99).

Collective migration of the AVE cells correlates with a loss of perforations within the basement membrane, such that the perforations are preferentially skewed toward the posterior side of the epiblast (93). The perforations themselves result from Nodal-dependent activation of the expression of MMP2 and MMP14, which degrade the basement membrane at the posterior side. Since the AVE cells secrete Nodal inhibitors, MMP expression is inhibited at the anterior side and the basement membrane remains intact, thus establishing a mechanical asymmetry concomitantly with the anterior-posterior axis of the embryo. Local weakening of the basement membrane at the posterior side corresponds with formation of the primitive streak—cells within this region of the epiblast undergo an EMT and breach the weakened basement membrane as they ingress to form the mesoderm (93). Generation of the germ layers thus requires changes in the mechanical properties of the microenvironment within the developing embryo.

SOLID-LIKE VERSUS FLUID-LIKE TISSUES

By definition, epithelial tissues comprise sheets of cells that form adhesive contacts with their neighbors. The shapes of the individual cells affect how they pack together to form their resident tissues and can be described using similar conceptual parameters as the ones that physicists use to describe the packing of foams (100). However, cells and tissues are not passive objects, and recent insights into their mechanical properties have been gleaned by considering them as forms of active, granular matter. One metric that is commonly used to predict the mechanical properties and behaviors of epithelial sheets is the cell-shape index, which is given as the ratio of a cell's perimeter to the square root of its area (101–103). The cell-shape index is smallest for a cell with a circular cross section and largest for an elongated cell. Therefore, for a given cross-sectional area, an epithelial cell within a stereotypical cobblestone monolayer has minimized its connections with its neighbors and its cell-shape index. These conditions are typically associated with static epithelial tissues in which the resident cells have limited motility and maintain their neighbors, a so-called jammed or frozen, solid-like state. In contrast, an epithelial cell with an elongated morphology has increased its connections with its neighbors and its cell-shape index, conditions that are associated with migratory epithelial tissues in which the resident cells undergo neighbor exchanges in an unjammed or fluid-like state. These two extremes, solid-like and fluid-like, have different mechanical properties: The jammed tissue is more rigid and mechanically stable than the unjammed tissue, which is more plastic and malleable.

The transition between jammed and unjammed tissues is similar to a glassy phase transition and has been implicated in studies of epithelial sheets in culture as well as in developing embryos (reviewed in 104). In *Drosophila*, the unjammed state is associated with an increase in fluidity that permits the movements necessary for formation of the ventral furrow during gastrulation (105), convergent-extension during morphogenesis of the germ band (106), and cellular rearrangements in the wing disc epithelium (107). In the zebrafish, the unjammed state represents a phase transition that decreases the rigidity of the tissue and promotes movement of the blastoderm over the yolk sac during epiboly (108, 109) and elongation of the body axis (110) and tail bud (111, 112). Similarly, the unjammed state corresponds to an increase in fluidity during gastrulation in *Xenopus* embryos (113). In avian embryos, an increase in tissue fluidity promotes formation of the primitive streak in quail (114), elongation of the body axis in chicks (115), and extension of the airway epithelium in the chick lung (116).

LESSONS FROM THE EARLY EMBRYO

We often anthropomorphize the differentiation of a pluripotent cell by referring to this event as a cell-fate decision, implicitly suggesting that the cell in question is presented with a variety of options and deliberately chooses its fate according to its developmental aspirations or simple expedience. In the embryo, these cell-fate decisions often accompany a symmetry-breaking event—a population of genetically identical cells becomes heterogeneous because of changes in morphology, positioning, local mechanical properties, or differential acquisition of apicobasal polarity. Although these alterations have been historically classified by corresponding differences in the expression or localization of proteins, it is important to note that each would also be expected to lead to heterogeneities in the mechanical microenvironment: A cell with apicobasal polarity experiences different mechanical forces than a cell completely surrounded by neighbors in all three dimensions. As described above, these differences in mechanical forces can themselves alter signaling, in particular through the subcellular localization of mechanosensors such as Yap, and thereby differentially direct differentiation.

The mechanical signaling networks that regulate the symphony of differentiation events in the early embryo are also important for regulating pluripotency and differentiation of stem cells in culture. ES cells are derived from the ICM and have the potential to differentiate into all cells present in the mature organism (117). Pluripotency appears to be defined specifically by the

expression of four different transcription factors, Oct4, Sox2, Klf4, and Myc (117–119), which are also expressed in the early embryo. Differentiated adult cells can be reprogrammed into iPS cells by expressing these four factors (120). Yap is highly expressed in ES cells (121), inactivated during their differentiation (122), and enhanced during the reprogramming of iPS cells, suggesting that Yap itself (and possibly mechanical signaling) is involved in the switch to and from pluripotency.

The mechanical signals that instruct cell-fate decisions in the early embryo also appear to play instructive roles during later differentiation events, both in vivo and in culture. Symmetry breaking in intestinal organoids (which are initially spherical, just like the preimplantation embryo) is accompanied by differences in the subcellular localization of Yap, which is required to enable the Paneth cell lineage by activating Notch signaling via Dll1 (123). Regulation of luminal fluid volume (and presumably fluid pressure) is essential for differentiation of enterocytes and morphogenesis of the crypt-villus axis in intestinal organoids (124, 125). Similarly, the pressure of the luminal fluid within the embryonic mouse lung regulates the rate of morphogenesis, differentiation of resident cell types, and maturation of the organ (126, 127). For most developing systems, the mechanical signaling pathways downstream of fluid pressure remain to be uncovered, but it will be interesting to define the overlap between organogenesis in vivo, organoid morphogenesis ex vivo, and mechanotransduction in the early embryo.

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