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Living tissues are more than cell clusters: The extracellular matrix as a driving force in morphogenesis

Marta Linde-Medina*, Ralph Marcucio

Department of Orthopaedic Surgery, San Francisco General Hospital, Orthopaedic Trauma Institute, University of California, San Francisco, CA 94110, USA

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ABSTRACT

In the study of morphogenesis, there is a general tendency to look at the extracellular matrix (ECM) as a mechanically passive agent that simply gives support to cells, and consequently, to place all the explanatory burden on cellular behaviors. Here we aimed to show that not only cells, but also the ECM may be an important force of morphogenesis. Understanding the mechanical role of the ECM broadens our view of morphogenesis and stresses the importance of considering embryonic tissues as a composite of cells and ECM.

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1. Cell behaviors as drivers of morphogenesis

The tissue deformations that take place during the generation of body plans and organs are commonly understood in terms of collective cell behaviors (e.g., Firmino et al., 2016; Hopyan et al., 2011; Pearl et al., 2017). Cells can drive morphogenesis directly, by generating forces that deform the tissue, or indirectly, by changing the mechanical properties of the tissue, and thus, its response to an externally applied force (e.g., Davidson et al., 2009; Keller et al., 2008; Lecuit et al., 2011). Cells can actively generate *pulling forces* by contracting their cytoskeleton, a behavior that when coordinated in epithelial sheets can lead, for example, to the formation of tubes, closure of openings or invaginations (for a review see Sawyer et al., 2010). Epithelial cells can also generate pulling forces when undergoing apoptosis. Apoptotic cells remain strongly adhered to neighboring cells during their shrinkage, which produces a pulling force that can contribute to the closure of openings or the formation of epithelial invaginations (Ambrosini et al., 2017). In a similar way, epithelial cells also pull their neighbors when they become rounded during mitosis. Independently from their subsequent division, cells at the tracheal placode that round during mitosis accelerate its invagination (Kondo and Hayashi, 2013). Mesenchymal cells also generate pulling forces when they remain attached to their surrounding extracellular matrix. This tension can be transmitted to the epithelium and contribute to its remodeling (Ingber, 2006). In this manner, tissue invaginations would not only be driven by the activity of epithelial cells, but they could also

* Corresponding author.
E-mail address: linde.m@outlook.com (M. Linde-Medina).

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originate when the epithelium is pulled by the underlying mesenchyme.

Cells do not only pull, but they also generate *pushing forces* when increasing in size, dividing, elongating, migrating or intercalating (e.g., Keller et al., 2008; Kinebrew and Hilfer, 2001). When randomly distributed throughout the tissue, these cell behaviors would mainly lead to changes in size, but when localized or oriented, they may extend the tissue along specific directions, thereby altering its shape. For several decades, it was commonly thought that the pushing forces generated by dividing cells at specific regions (i.e., localized cell proliferation) was the main driving force behind epithelial budding (e.g., Boehm et al., 2010; Ingber, 2006). This view has recently been challenged by studies showing, for example, that epithelial invaginations precedes rather than lags behind peaks of cells proliferation in branching morphogenesis (Nogawa et al., 1998), or that observed proliferation rates do not predict the morphogenetic changes undergone by the limb bud (Boehm et al., 2010).

Nowadays, pushing forces generated by directed cell behaviors in the mesenchyme are thought to be the main driving force of evaginating primordia (Hopyan et al., 2011). However, a recent study has shown that the limb epithelium is capable of contracting in response to the tension exerted upon it by the expanding mesenchyme, which suggests it may play an active role in limb bud morphogenesis (Lau et al., 2015). Pushing forces can also invaginate an epithelium. For example, the expansion of epithelial cells at their basal side, a mechanism called basal relaxation, is involved in the invagination of the optic vesicle in the chicken embryo, and accompanied by apical constriction, it contributes to the gastrulation of the *Drosophila* egg (for a review see Pearl et al., 2017).

During morphogenesis, some regions of the embryo – or the primordium – will actively generate forces, while others will be subjected to them. The deformation of a tissue in presence of an external force depends on its stiffness, which is determined by the mechanical properties and arrangement of its components (e.g., Davidson et al., 2009). Therefore, cells can indirectly alter the shape of a tissue by changing its stiffness. Cell stiffness is mainly regulated by the cytoskeleton and the cytosolic pressure: cells with either a high content of stress fibers or a high cytosolic pressure are stiffer, i.e., more resistant to shape deformation, than cells with low levels of stress fibers or a low cytosolic pressure (e.g., Chan and Ulfendahl, 1997; Gavara and Chadwick, 2016). These two cellular components can directly contribute to tissue stiffness. For example, the inhibition of either F-actin polymerization by latrunculin B, or myosin II activation by Y27632, reduces the stiffness of the embryonic tissues of the frog *Xenopus laevis* up to 70% and 50%, respectively (Zhou et al., 2009). Disruption of actin filaments by blebbistatin decrease the stiffness of embryonic tendons in the chicken embryo by almost 40% (Schiele et al., 2015). However, this direct contribution of the cytoskeleton to tissue stiffness would not be applicable to all its components. For example, microtubules can alter stiffness in some cell lineages, but they do not contribute to tissue stiffness in the frog embryo (Zhou et al., 2010). Regarding intracellular pressure, it has been shown, for example, that cell swelling straightens and increases the stiffness of the notochord of *Xenopus laevis* embryos (Adams et al., 1990).

It is important to stress that cell behaviors can be either the driving forces of a morphogenetic process or the passive response of an externally applied force. For example, as commented previously, cell extrusion generates a pulling force that contracts the tissue. This force may be a driver of both the dorsal closure and the formation of leg invaginations in *Drosophila* (Ambrosini et al., 2017). However, cell extrusion can also be induced when the tissue is externally compressed: if compression becomes too high, the tissue may release the compressive load by extruding cells (Eisenhoffer et al., 2012). This dual role is applicable to other common cell behaviors, (e.g. cell intercalation, cell migration, cell polarity) (e.g., Bénazéraf et al., 2010; Keller et al., 2008). Therefore, one of the main aims in studies of morphogenesis will be to discern which of the observed cell behaviors represent driving forces and which effects.

In the absence of internally generated or externally applied forces, tissues can spontaneously generate forms due to their intrinsic material properties. Tissues behave to some extent like liquids, which adopt the configuration that minimizes their surface free energy. The surface free energy of a cell cluster is minimized when cells pack closely together, maximizing their contact area. In contrast to liquids, the free energy at the cell-cell interfaces or *interfacial tension* is not solely determined by cell adhesion, but also by cortical tension: cell adhesion decreases interfacial tension by increasing the contact area among cells, whereas cortical tension –mainly generated by the cytoskeleton – increases interfacial tension by decreasing the contact area among cells (Lecuit and Lenne, 2007; McMillen and Holley, 2015).

According to the Differential Interfacial Tension hypothesis, an aggregate of cells with different cohesiveness will self-assemble into layers where more cohesive cells are surrounded by less cohesive cells, which minimizes the surface free energy of the tissue (for a review see McMillen and Holley, 2015). Embryonic germ layers differ in their material properties, e.g., the surface ectoderm is less adhesive and stiffer than the deep mesoderm, suggesting that differences in the material properties among embryonic germ layers may contribute to the formation of body plans (Krieg et al., 2008; Maître et al., 2012). Differences in cell adhesion has also been proposed, for example, to explain the separation of the prospective limb mesenchyme from the flank mesenchyme during early limb bud morphogenesis (Damon et al., 2008).

Other morphological motifs that would self-assemble according to the principle of minimization of free energy are the formation of lumens, if the distribution of cell adhesion molecules is not uniform, but localized at a specific region of the cell membrane, and tissue elongation, if cell shape is anisotropic, i.e., cells are longer along one axis (Newman and Bhat, 2008). It is important to stress that the morphogenetic potential derived from the liquid-like behavior was not possible until the appearance of classical cadherins, which conferred fluidity to tissues by allowing the formation of transient cell-cell contacts, and Wnt morphogen, which is involved in cell polarity (Newman, 2016). Liquid tissues is a distinctive feature of metazoans, which represents a major transition in the evolution of multicellularity (Newman, 2016).

2. A look at the extracellular matrix

Cells can both generate forces and change the mechanical properties of the tissue *indirectly*, by altering their surrounding ECM. This implies that the ECM has the potential of driving morphogenesis (for other roles of the ECM see Daley and Yamada, 2013; Rozario and DeSimone, 2010). The ECM can generate *pulling forces*. For example, collagen fibers contract in response to the traction forces exerted upon them by migrating cells (Harris et al., 1981). In some branching organs, collagen fibers align parallel to the epithelial surface, where a flux of migrating mesenchymal cells has been observed. The contraction of these polarized collagen fibers by mesenchymal cells could compress and fold the epithelial layer, contributing to the formation of clefts (i.e., the split of the tip of a primordium into two parts) (Hieda and Nakanishi, 1997; Wan et al., 2008) (Fig. 1a). This idea is supported by the observation that degradation of collagen inhibits the formation of clefts (for a review see Hieda and Nakanishi, 1997).

By increasing its volume through matrix deposition and/or

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