The actin cortex: a bridge between cell shape and function

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Precise control of cell morphogenesis is a key to healthy cell physiology, and cell shape deregulation is at the heart of many pathological disorders. Changes in cell shape strongly correlate with, if not cause, processes such as cell migration, tissue homeostasis, epithelial-amoeboid-mesenchymal transitions and cellular differentiation (Figure 1). In fact, early embryologists defined many cell fate changes based on cell morphology, and used cell shape as a primary identifier of different nascent tissues. Here, we discuss the control of cell shape and mechanics, and the emerging relationship between shape, biochemical signaling and cellular function, highlighting remaining gaps in our understanding and potential directions of future investigations.

Cell shape is defined by cellular mechanical properties and by the cell's physical interactions with its environment. Most cell deformations are driven by changes in the physical properties of the cell surface, which are dominated by the mechanics of the cellular cortex. The cortex is a thin network of actin that lies under and is tethered to the plasma membrane in most animal cells. Cortical actin filaments are organized in a meshwork cross-linked by specific proteins and by myosin motors, which generate contractile stresses in the network (Clark et al., 2014). These stresses give rise to cortical tension, which determines global cell surface mechanics. Gradients in cortical tension result in cortical flows and cellular contractions, such as those driving cleavage furrow ingression, cell body retraction during cell migration and epithelial contractions underlying tissue constriction events (Levayer and Lecuit, 2012).

Over the past decade, an increasing number of biological and biophysical investigations have focused on the actin cortex. Cortex composition has been characterized by mass

spectrometry using isolated cellular blebs to collect sufficient amounts of cortical material (Bovellan et al., 2014). Cortical actin nucleators and various regulators of cortical contractility have been identified (Bovellan et al., 2014; Luo et al., 2013), and tools are available to measure physical characteristics, such as cortical tension and thickness (reviewed in (Clark et al., 2014)). The mechanics of cortical contractions and flows in many morphogenetic events have been dissected, including C. elegans zygote polarization (Mayer et al., 2010), mouse embryo compaction (Maitre et al., 2015) and epithelial constrictions in the Drosophila embryo (Levayer and Lecuit, 2012). However, most past studies have focused on the cortex in itself. In contrast, much less is known about how the cortex is dynamically regulated by specific signaling pathways, how it in turn triggers biochemical signaling events, and how cortical processes are integrated within the cell to drive morphogenesis.

Many morphogenetic processes appear to be driven by transitions between a cortical and a stress fiber dominated organization of intracellular actin networks. Stress fibers are quasi one-dimensional bundles of actin filaments usually connecting two adhesion points, whereas in the cortex, actin forms a roughly isotropic meshwork under the plasma membrane (Figure 2). While the molecular regulation of both the cortex and stress fibers are reasonably well understood, how transitions between these two types of networks are controlled remains elusive. For example, during developmental transitions such as exit from naïve pluripotency or during epithelial to mesenchymal transitions (EMT), actin reorganizes from a mostly cortical arrangement into stress fibers and lamellipodia (Figure 1). How this reorganization, which drives cell spreading, is regulated by the signaling pathways driving cell state changes such as cell differentiation is not understood.

Transitions in actin network organization are also associated with changes in the way the cell interacts with the environment. Stress fibers are usually connected to and promote the formation of integrin-based focal adhesions (Livne and Geiger, 2016), whereas cortical actin is often associated with cadherin-based cell-cell adhesions (Engl et al., 2014). Interestingly, cell-cell contact formation is concomitant with cortical clearing in the contact zone (Maitre et al., 2012) while actin dynamics and tension in turn influence cadherin recruitment (Engl et al., 2014). Thus, the cell's interactions with neighbors and matrix are controlled via a

subtle cross-talk between actin organization, contractility, dynamics and contacts with integrins and/or cadherins. One future challenge will be to fully understand this cross-talk, and how it is modulated during cellular shape changes associated with developmental fate transitions. Extending the already broad libraries of actin binding proteins and identifying connections to adhesions may provide one path towards this goal. These extensions can in turn instruct gain- and loss-of-function studies to shed light on the changes to cell shape and fate that accompany developmental processes.

Another key yet poorly understood aspect of the actin cytoskeleton function in development is its role in cellular signaling. Indeed, actin networks not only control cell shape, they also are a center of both mechanical and biochemical signaling. For example, focal adhesion maturation is driven by tension in stress fibers, which upregulates focal adhesion kinase and Src family kinase based signaling. These kinases are upstream mediators of mitogen activated protein kinase (MAPK) signaling, which is essential for a multitude of cellular processes including differentiation. Moreover, there is feedback from MAPK signaling to the actin cytoskeleton: actin organization and contractility is regulated by interactions between Erk and Rho GTPases (Vial et al., 2003). Though less studied, similar interactions are possible between cadherin-based adhesions and Wnt signaling, both canonical and non-canonical (Heuberger and Birchmeier, 2010). It is likely that the connection between actin networks and cellular adhesions drives many other biochemical signaling feedback loops, raising the tantalizing possibility that actin organization and cell shape are more than passive downstream players in cellular transformations such as differentiation.

The possibility that actin actively regulates cell function is buttressed by the fact that there are at least two other connections between actin organization and signaling. First, actin dynamics itself can influence gene expression. Changes in actin organization are likely to modify the intracellular filamentous (F) to monomeric (G) actin ratio both in the cytoplasm and, because actin is actively shuttled into and out of the nucleus, in the nucleus. Increasing levels of G-actin in the nucleus affects transcription, both due to interactions with all three types of RNA polymerase and also because it is involved in the nuclear export of myocardin-related transcription factors (MRTFs). For example, if less G-actin is available in the nucleus

(perhaps because of increased levels of polymerized actin in the cell) then MRTF family members in turn activate the serum response factor (SRF) pathway. SRF activates immediate early genes such as c-fos (Posern and Treisman, 2006), which play major roles in cellular transformations such as differentiation and oncogenesis. Second, the cortex is physically connected to the nucleus via cytoplasmic structural components, and stresses at the cell surface can translate to strain on the nucleus (Pagliara et al., 2014). Increased stress in the actin cytoskeleton can act through the LINC complex on the nuclear membrane and the nuclear lamins to further tune biochemical signaling. For example, increased cytoskeletal stress can stabilize lamin A/C which activates SRF and the retinoic acid pathways, and furthermore acts as a mediator of MAPK signaling (Swift et al., 2013) and activation of immediate early genes. There is also new evidence that stress through the actin cytoskeleton can act directly through emerins (also nuclear membrane proteins) to facilitate polycomb-mediated gene silencing at the nuclear envelope (Le et al., 2016).

It is tempting to give in to despair when considering the myriad of ways in which the actin cytoskeleton affects signaling and vice versa. However, it appears that the nexus of actin-regulated signaling and shape may be actin network organization. Thus, to fully understand the relationship between cell shape and cell function, we must first understand the transitions between different types of actin networks. Then, cytoskeletal investigations must be fully integrated with studies of signaling pathways that drive cell state changes. True comprehension of the interplay between actin network transitions and cell state transitions will require an interdisciplinary push involving biophysicists, molecular and cell biologists studying the cytoskeleton, developmental biologists and stem cell biologists.

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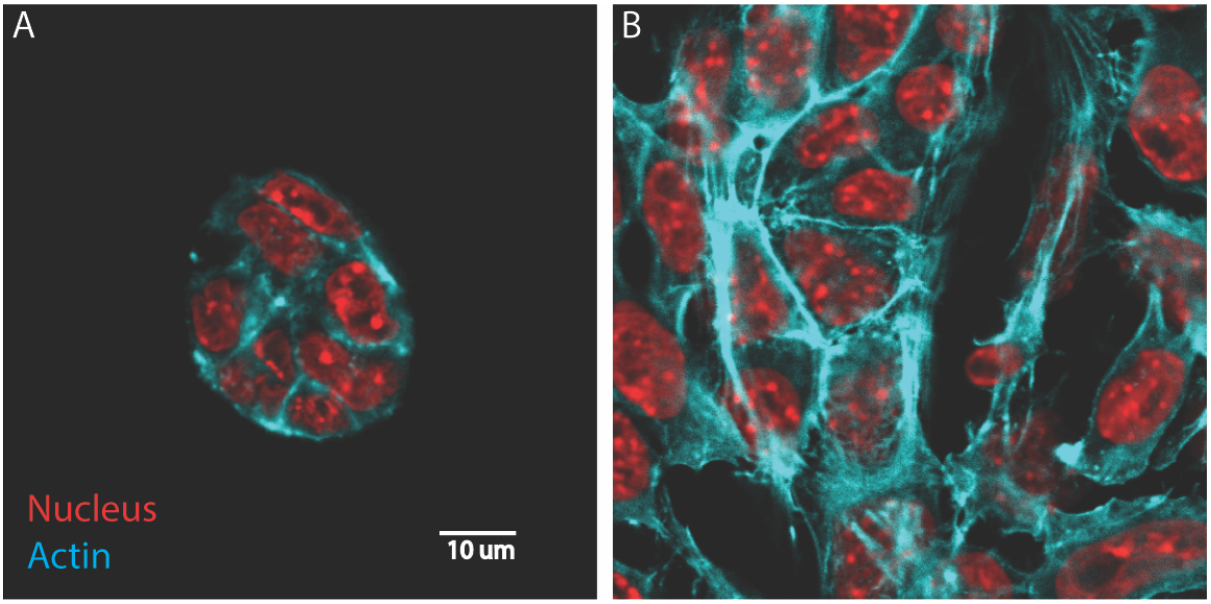

Figure 1: Embryonic stem cells in a naïve phase of pluripotency (A) and after exit from naïve pluripotency (B). During this time, actin (in cyan) transitions from cortical actin to stress fibers as the cells spread. Nuclei are labeled in red.

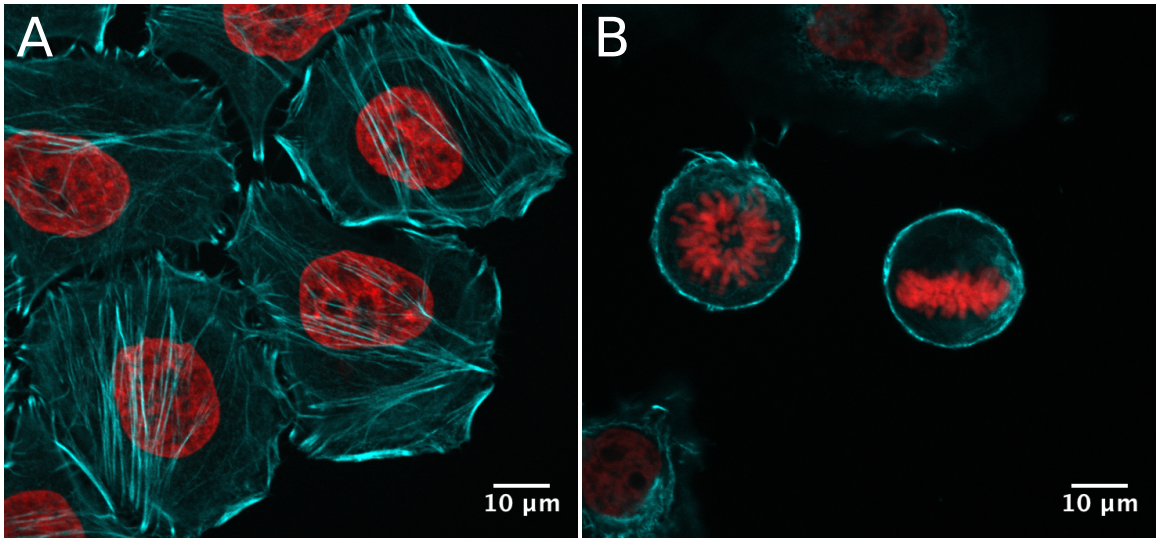

Figure 2: Different types of actin networks in interphase (A) and mitotic (B) HeLa cells stained with DAPI to detect DNA (red) and phalloidin to mark F-actin (cyan). Interphase cells are spread and actin is primarily organized in stress fibers. Mitotic cells are rounded and actin is predominantly cortical.

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