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Forms, forces, and stem cell fate

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Cells change their shape and mechanics dramatically during development and tissue healing in response to morphogens, cell–cell contact, adhesion to extracellular matrix, and more. Several regulatory links have been described between cell shape, cytoskeletal tension, matrix adhesiveness and stiffness, and recent studies have begun to uncover how these mechanotransduction pathways can impact transcriptional signaling and cell fate decision. The integrated mechanisms linking cell forces, form and fate are likely critical for driving normal morphogenesis, tissue development, and healing. Dysregulation of these mechanisms may also tip the scale from normal to diseased states. Here, we highlight mechanisms that alter cell shape and mechanics, and the pathways affected by these changes.

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Introduction

The development of complex multicellular organisms, organs, and tissues involves carefully orchestrated rearrangements in the organization of cells resulting from changes in cell shape and polarity, cell migration, as well as cell-generated contractile forces [1]. A critical feature of these multicellular specializations is that the structural and mechanical events are tightly associated with the cellular differentiation programs [2].

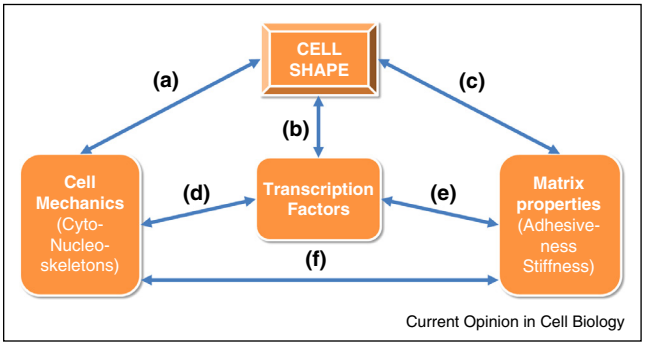
Classically, the progression of differentiation to specific cell types results in the expression of specialized cytoskeletal, adhesive, and extracellular matrix proteins that can change the overall shape, organization, and contractile apparatus of cells (for review on forces in morphogenesis, refer to [3]). In the earliest stages of embryogenesis, for example, the establishment of mesoderm results in a mesenchymal population that invades basally to give rise to new compartments. Differentiation of into specialized cells results in unique shape and structural characteristics associated with their differentiated functions, for example, adipocytes adopt a round morphology critical for lipid storage, requiring decreased adhesion and the disassembly of actin stress fibers during adipocyte differentiation [4]. With the growing body of literature defining scaffolding and polarity proteins that define cellular architecture, we may soon be able to define the molecular basis for how cells organize.

The regulatory link between cell fate and structure, however, is not unidirectional. For example, the degree of cell spreading against an extracellular matrix has been shown to drive changes in cell signaling, proliferation, survival, and stem cell differentiation [5]. Similarly, direct modulation of cellular contractility by non-muscle myosin activity can regulate cell fate [6,7]. Here, we integrate recent literature to describe the current paradigm for how the local physical microenvironment can modulate cell shape and mechanics, and how these changes in cellular form and forces are transduced to drive changes in cellular signaling and fate (Figure 1). These regulatory mechanisms are not limited to development and physiology, and emerging experimental models of altered microenvironments during disease will provide a better understanding of the role of structure-function mechanisms in pathological states.

Cell shape and mechanics as an integrated mechanochemical regulator of cell function

The density of cells in culture has long been recognized as a major regulator of cell proliferation and differentiation [8–10], but how the increase in cell density exerts these effects was largely thought to be via increased juxtacrine and paracrine signaling [11,12]. Folkman and Moscona [13] were the first to suggest an alternative, that the crowding-induced decrease in cell spreading and flattening against the underlying substrate could contribute to growth arrest, and Ingber [14] showed that decreasing matrix ligand availability could phenocopy the decreased spreading and proliferation in the absence of any cell–cell contacts. Using micropatterned substrates to directly control cell shape without the confounding effects of altering matrix density demonstrated that the area of cell spreading could drive changes in cell proliferation and survival [15]. Using bone marrow-derived mesenchymal stem cells as a model for multi-lineage differentiation, we further showed that the degree of cell

Figure 1



Cell shape dynamics as a regulator of cell fate. Regulation of cell shape is a complex and dynamic process. Classically, *in vitro* cell shape was thought to be the output of variables such as adhesive ligands or more recently substrate stiffness, while the field of clinical pathology uses cell shape as a histological marker of normal versus diseased cells. During development, morphogenic cues, alignment and tension drive cell shape changes to create new tissues and organs. Using engineering approaches, such as limiting adhesion or altering stiffness, we can modulate cell shape to alter the cell's mechanics (arrows **a** or **c**) for example via Rho mediated tension or actin reorganization, which in turn can regulate transcriptional activity to drive cell fate (**d**). Alternatively, changes to cell's environment during disease or healing changes cell shape (**c**), possibly exacerbating initial pathology (**c**–**e**). It is also possible to imagine that transcriptional changes alter the cell's mechanics (**d**, **f**, **c**), stiffening the local environment, leading to cell shape changes.

spreading could switch their commitment between lineage fates, in which well spread cells undergo osteogenesis while less spread cells undergo adipogenesis [7]. While the area of cell spreading appears to be a major determinant for cell fate signaling, more recent studies have shown that changes in cell aspect ratio, given the same area of cell spreading, can also modulate fate choices [16,17]. Studies re-introducing cell–cell contacts in micropatterned contexts showed that in addition to crowding, the presence of neighboring cells via engagement of cadherins can modulate cell spreading via changes in Rac and Rho GTPase signaling [18–21] (for review on cell–cell contact adhesion signaling refer to [22]). Together, these studies suggested that changes in cell density and cell–cell contact, matrix adhesiveness, and the geometric presentation of matrix could each drive changes in cell shape, and that these cell shape changes were themselves involved in regulating cell signaling and fate.

Cell spreading appears to regulate fate signaling at least in part through its effects on cytoskeletal contractility by activation of non-muscle myosin II. Increasing cell spreading in mesenchymal stem cells upregulates RhoA activity, ROCK activity, myosin phosphorylation, and cell-generated traction forces against underlying matrix leading to osteogenesis and exogenous upregulation of RhoA or ROCK activity triggered osteogenesis while blocking RhoA-mediated contractility induced adipogenesis [7,23,24]. Because RhoA-mediated traction forces are known to be required for the maturation of focal adhesions [21,25], and the degree of focal adhesion assembly directly correlates with the degree of cell spreading [26], it has largely been presumed that the mechanism by which forces are transduced into a fate signal resides within the adhesions. Yet, although some studies suggest the involvement of focal adhesion kinase (FAK) in these proliferation and differentiation responses [27,28], a clear mechanism implicating adhesions remains to be reported.

More recently, substrate stiffness has been shown to also drive changes in cell proliferation and differentiation [6,29–31]. Seeding cells on acrylamide gels of decreasing stiffness led to growth arrest [31], and differentiation changes in a number of different stem cell types, including changes in mesenchymal stem cell lineage commitment [6,32–35]. Interestingly, it was reported that the same ranges of stiffness that altered cell fates also were associated with changes in cell spreading against the substrate [6,36]. By measuring the spread area, traction forces, and focal adhesion assembly of single cells within a population cultured on substrates of different stiffness, we found that traction forces and focal adhesion assembly correlated highly with cell spreading and secondarily with substrate stiffness, suggesting that the effect of substrate stiffness on lineage commitment is driven through stiffness-mediated changes in cell shape, though this sequence has not been directly demonstrated [24]. An important note is that when cell spreading is held constant, cells are still able to alter their mechanics in response to changes in substrate stiffness [36]. Cells are able to undergo 'stiffness matching' in where they reorganize their actin cytoskeleton to essentially match that stiffness of their substrate. Gilbert *et al.* [33] demonstrated this effect in the context of the muscle stem cell niche, maintaining isolated stem cells on a matrix with stiffness matching their *in vivo* niche yielded improved engraftment and healing when implanted. The implication of this result is that cell properties such as shape and cytoskeletal dynamics were unaltered during *ex vivo* culture such that upon implantation the cells could function appropriately. Recent follow-on studies suggest that the approach could be used to heal older muscles, where culturing muscle stem cells from aged mice on soft hydrogels before re-implantation, improves engraftment and regeneration [37••].

As with muscle, many native stem cell niches are soft relative to standard tissue culture plates. Dixon *et al.* [38•] exploited this knowledge to preserve stem-ness of pluripotent stem cells. Using a composite material, cells initially experienced a soft matrix and were poorly attached, remaining stem-like. When the softer material was leached out the cells experienced a stiffer matrix altering their shape to become more spread and began to