It has long been known that tissues and organs grow to a predetermined size even if a fraction of the cell population comprising them is impaired for growth or supercharged for growth. The strong competitors express higher levels of Myc (de la Cova et al., 2004) and send a signal that induces apoptosis in the neighboring, weaker competitors (Moreno et al., 2002). But what is the signal that recognizes the difference between the populations? What maintains this balance in a wild-type tissue? Gibson and colleagues (2006) show that a clone of more rapidly proliferating cells shifts to a polygon profile with a lower average number of sides. Is the correlation between reduced mean number of sides and more rapid proliferation incidental, or might the number of sides be involved in regulating a cell's competitiveness?

Finally, many postmitotic epithelia show a much more regular hexagonal packing pattern than their replicating precursors. The force driving reorganization from the distribution described by Gibson and colleagues (2006) to a more regular hexagonal array is not known. It will be important to determine whether this is a passive physical process or a genetically encoded transition.

A resurgence of mathematical modeling applied to biological problems has provided new insights into a variety of processes. Gibson and colleagues (2006) have enabled us to appreciate a pattern where none was previously apparent, and their result is elegant in its simplicity. It remains to be seen whether we can appreciate the consequences of this pattern.

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Matrix Control of Stem Cell Fate

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A key challenge in stem cell research is to learn how to direct the differentiation of stem cells toward specific fates. In this issue of *Cell*, Engler et al. (2006) identify a new factor regulating stem cell fate: the elasticity of the matrix microenvironment. By changing the stiffness of the substrate, human mesenchymal stem cells could be directed along neuronal, muscle, or bone lineages.

Stem cell maintenance and differentiation are governed by unique local microenvironments (Watt and Hogan, 2000; Fuchs et al., 2004). Identifying specific cues in the microenvironments, such as secreted factors, and understanding how neighboring cells and the extracellular matrix control developmental fate will provide new tools with which to promote the differentiation of stem cells into particular cell types. Many studies have established that complex interactions between soluble and extracellular matrix molecules regulate intracellular signaling and differentiation. Although direct activation of signal transduction by matrix molecules through integrin receptors has been well-studied, the physical properties of the matrix, such as its elasticity or stiffness, are also important (Discher et al., 2005; Vogel and Sheetz, 2006). In this issue, Engler et al. (2006) apply techniques originally used to study the effects of matrix elasticity on the morphology and growth of differentiated cells to provide a new approach to direct stem cell fate.

The importance of sensing the mechanical properties of the extracellular matrix has been established in studies with fibroblasts and tumor

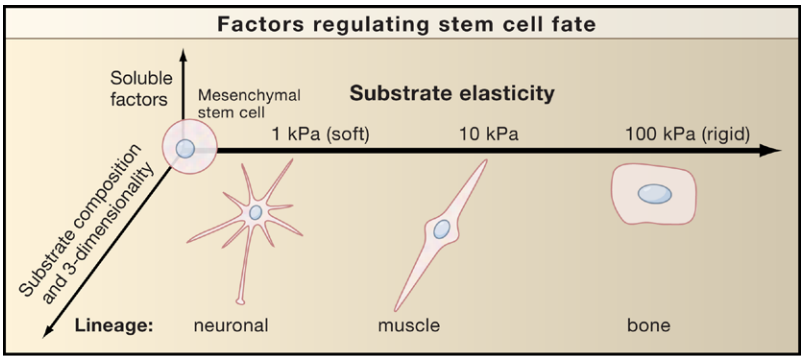

Figure 1. Controlling Stem Cell Fate
Multiple factors can influence the differentiation of stem cells, including secreted soluble factors, the elasticity or compliance of the matrix substrate, and the biochemical composition and dimensionality of the matrix. Engler et al. (2006) examined the effect of matrix elasticity on the differentiation of human mesenchymal stem cells. They showed that soft matrices favored differentiation of mesenchymal stem cells into neuronal-like cells, moderate elasticity promoted myogenic differentiation, and a rigid matrix stimulated osteogenic differentiation.

cells (Discher et al., 2005; Paszek et al., 2005). A current concept is that cells use actomyosin contractility for two-way interactions with the matrix. Cell contraction at integrin-based adhesions is resisted by the matrix, which is followed by the accumulation of additional molecules at these sites. This process leads to a balance of tension forces at the cell-matrix interface. Inhibition of myosin disrupts this cellular response. Evidence also suggests that cellular force-sensing results in intracellular signaling, such as increased Rho GTPase and MAP kinase activity, which can alter gene expression and embryonic development and even promote tumor progression (Paszek et al., 2005; Vogel and Sheetz, 2006; Ingber, 2006). Another emerging concept is that custom-engineered artificial materials can be developed to mimic natural matrices for use in tissue engineering, including induction of stem cell differentiation (Lutolf and Hubbell, 2005).

Engler, Discher, and coworkers (Engler et al., 2006) applied an approach (originally pioneered by Pelham and Wang) to test whether matrix stiffness can modify human mesenchymal stem cell fate. They used an artificial in vitro matrix based on polyacrylamide gels coated with collagen to provide an interface with the stem cells. These matrices ranged from soft to relatively rigid depending on the extent of chemical crosslinking. While keeping the cell culture medium identical, they tested the effects of changing the elasticity on human mesenchymal stem cells, which are known to differentiate into neuron-like, muscle, and bone cells. Their key breakthrough was to discover that on soft substrates mimicking the elasticity or compliance of brain tissue, these stem cells started to show a neuronal phenotype; on substrates of intermediate stiffness resembling striated muscle, the same stem cells developed into the myoblast lineage; and on stiff substrates resembling the matrix precursor to bone (osteoid), the cells began to resemble osteoblasts (Figure 1). This regulation by matrix stiffness proved to be complementary to, and even synergistic with, the regulatory effects of specialized cell culture media previously shown to direct mesenchymal stem cell differentiation into each of these lineages.

The effects of matrix compliance on human mesenchymal stem cells were quantified by microarray analyses. Gene expression of markers for neurons, muscle, or bone was induced 4- to 6-fold on the corresponding substrate. Although these relative increases seem low, the specificity was clear: only stem cells grown on soft substrates with brain-like compliance expressed the phosphorylated form of neurofilament heavy chain; myoD was only expressed by stem cells grown on substrates of intermediate stiffness; and bone CBFα1 was only expressed by stem cells grown on a rigid substrate. In fact, immunofluorescence analysis showed that these three particular lineage-specific markers were present at 50% of the levels that are found in corresponding differentiated cultured cell lines (Engler et al., 2006). There was, however, only limited expression of markers of terminal differentiation. For example, there was no expression of lineage-specific integrins in the myoblast-like cells. Consequently, matrix compliance can initially guide these stem cells into a developmental lineage, but it is not sufficient to complete terminal differentiation.

In a series of incisive experiments, Engler et al. (2006) tested the stability of the commitment to a particular lineage and explored the underlying mechanisms for these effects and compared them to regulation by soluble factors. Although substrate elasticity can specify the initial lineage, treatment with specialized culture medium that promotes a different phenotype could alter the cell lineage if the cells were treated after one week of growth on the artificial matrix. However, the same treatment after 3 weeks did not alter cell lineage. Combining inputs of matrix compliance and soluble cell culture factors that favor the same phenotype resulted in synergistic expression of markers of differentiated cells. This finding will encourage the use of multiple manipulations in parallel to lock cells into a specific lineage.

The authors used the drug blebbistatin to test whether inhibiting myosin II contractility would disrupt cell sensing of matrix stiffness. Myosin II was essential for cell fate determination in response to specific matrix elasticity. Interestingly, sensing of substrate compliance by these stem cells was independent of lineage specification by soluble factors because inhibition of myosin II had no effect on the expression of differentiation markers induced by soluble factors. Inhibition of myosin II only blocked the contribution of matrix compliance when cells received a combination of matrix and soluble factor regulatory inputs. These combinatorial experiments

indicate that the sensing of substrate compliance/elasticity using myosin II contractility is necessary for lineage specification by the matrix whether or not other differentiation signals are present. These findings also demonstrate that after 3 weeks the process becomes resistant to changes by soluble signals, and that matrix and soluble factors can act synergistically to specify cell fate.

These findings raise a number of questions for the future and open up new opportunities for research. Although the approaches in this paper can specify lineages of stem cells in vitro, it is not yet clear how long the effects will last or whether they will be retained in a living organism after implantation. In fact, because disease or injury can often pathologically modify the in vivo recipient site, it may be necessary to coimplant an artificial matrix that can maintain appropriate compliance for the implanted stem cells.

Research with other cell types has established the importance of the composition of the matrix and its three-dimensionality (Cukierman et al., 2001; Griffith and Swartz, 2006). Because this study investigated only one type of matrix molecule (collagen I) and used a two-dimensional rather than a three-dimensional matrix to mimic the in vivo microenvironment, it will be important to test these additional parameters using systems in which matrix compliance can be varied experimentally. Cells in matrices are also known to modify their microenvironment by producing molecules that remodel the matrix in addition to secreting molecules that comprise the matrix. Presumably stem cells are no exception, and clearly the two-way interaction between stem cells and their matrix needs to be explored further. The cells or matrices may need to be modified to retain an appropriate microenvironment. Because the Engler et al. study focused on human mesenchymal stem cells, it will be intriguing to learn whether human embryonic stem cells and other types of adult stem cells are similarly regulated by properties of the matrix. Finally, it will be important to determine the signaling mechanisms by which compliance of the extracellular matrix specifies stem cell lineage and acts synergistically with soluble factors.

The work by Engler et al. provides a potentially powerful new tool for investigating the control of stem cell differentiation and has potential clinical applications. It reminds us that even though specific ligand-receptor interactions of growth factors and matrix molecules are clearly important for regulating cells, the physical properties of the local microenvironment can also play key roles in determining cellular function and fate.

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ZOning out Tight Junctions

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The tight junction is an intricate seal between adjoining epithelial cells that also separates the apical and basolateral membranes within these cells. A paper in this issue of *Cell* by Umeda et al. (2006) demonstrates that loss of the ZO scaffolding proteins prevents the formation of tight junctions but surprisingly does not perturb apico-basal polarity.

Tight junctions act as a barrier to limit solute movement between adjacent epithelial cells. Studies over the last 20 years have begun to elucidate the molecular components that comprise these junctions. In this issue of *Cell*, Umeda et al. (2006) reveal the importance of the Zonula Occludens (ZO) proteins in the formation of this junction. ZO-1 was the first protein component identified in tight junctions. Subsequent studies identified ZO-1 isoforms as well as ZO-2 and ZO-3 as binding partners of