swered. Attempts to infect animals with in vitro-generated PrP or PrP* amyloids have failed thus far, indicating that some important components of infectivity cannot be reproduced in the test tube. Moreover, the in vitro cross-seeding barrier between mouse and hamster works in the opposite direction, compared to the whole organisms (see Figure 1, panel C). In nature, mouse prions infect hamsters, but not vice versa (Kocisko et al., 1995). Thus, do not hurry to eat infected beef, unless it is produced in the test tube: BSE prions, although they have presumably originated from sheep scrapie, do not seem to remember their sheep origin well enough to remain harmless for humans. Apparently, other participants, e.g., chaperones, come into play in vivo. Additional assays are required to further decipher mechanisms of the species barrier. The cell-free prion conversion system, previously developed by B. Caughey and coworkers (Kocisko et al., 1995), could still be of use. Despite low efficiency, it seems to reproduce in vivo species-specificity patterns more realistically. Ultimately, in vivo experiments will be required. The extreme reductionist approach, so elegantly demonstrated by Vanik et al. (2004), represents only one of the first steps, although an important one, in the attack on the species barrier.

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Tension Precedes Commitment—Even for a Stem Cell

The differentiation potential of stem cells is influenced by cell density. An article in the April issue of *Developmental Cell* (McBeath et al., 2004) demonstrates that lineage commitment by mesenchymal stem cells is regulated by shape-induced changes in Rho GTPase activity and cytoskeletal tension.

More than 100 years ago, Dr. Julius Wolff proposed that mechanical stresses influence the architecture and physical properties of bone (Wolff, 1892). It is now well established that the application of external distortion forces on various adherent cell types influences several processes, including gene expression, proliferation, differentiation, and apoptosis (Ingber, 2003). However, the molecular basis for the effects of mechanical forces on tissue morphogenesis remains unclear. Although the mechanism by which mechanical force is translated into a biological response is unknown, there is evidence that adhesion molecules, such as integrins, through linkages to the actin cytoskeleton, as well as various signal transduction proteins, play an important role (Alenghat and Ingber, 2002). Adherent cells in contact with extracellular matrix (ECM) components are under "self-imposed" isometric tension that results from the presence of a stiff actin cytoskeleton and is balanced by microtubules and external ECM adhesions (Ingber, 1997). Thus, an external mechanical force could be translated by a balanced change in the preexisting intracellular tension force and a consequent signaling event.

Stress-induced changes in cell shape have been implicated in the remodeling of several tissues, including bone, lungs, and blood vessels, as well as in human disease processes, such as hypertension and osteoporosis. The influence of such mechanical forces on the differentiation properties of stem cells within these tissues is an attractive point of potential regulation. The most thoroughly studied system of lineage commitment and differentiation of adherent cells is the mesenchymal lineage, which yields the cells of connective tissues, including bone, muscle, fat, and cartilage. These cells are derived from a common mesenchymal stem cell (MSC) precursor, and the commitment of MSCs to a particular lineage is influenced by a variety of external cues from the local tissue environment, including secreted growth factors and ECM components (Minguell et al., 2001).

While the effects of differentiation on cell shape are well documented for many cell types, several published reports also suggest that cell shape can influence the differentiation of partially committed precursors of adipocytes, osteoblasts, and chondrocytes (Carvalho et al., 1998; Solursh, 1989; Spiegelman and Ginty, 1983). However, in those studies, the role of cell shape was not separated from effects of cell density or proliferation, nor was the role of cell shape in the lineage commitment of multipotent stem cells examined. In a recent report from McBeath et al. (2004 [April issue of *Developmental Cell*]), the role of cell shape and cytoskeletal tension in the lineage commitment of human bone marrow-derived MSCs has been directly examined. As in previous studies, McBeath et al. (2004) observed that the commitment to a particular mesenchymal lineage is strongly influenced by cell density. They compared the commitment

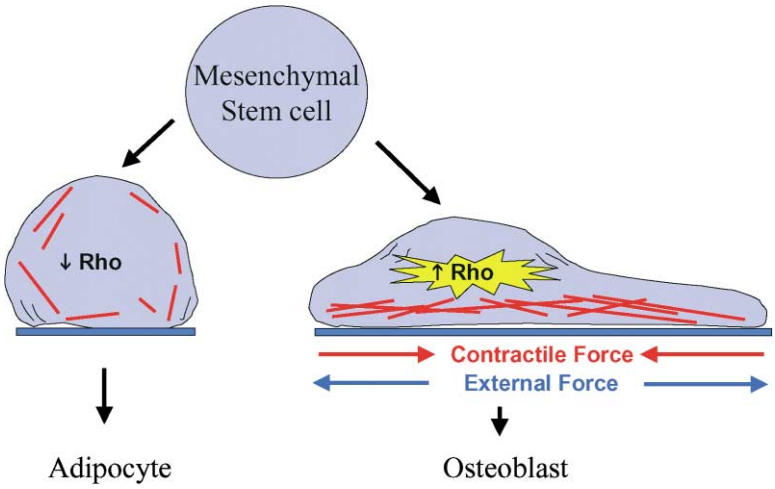

Figure 1. Schematic Illustration of the Role of Cell Shape and Cytoskeletal Tension in the Differentiation of MSCs

When an uncommitted mesenchymal stem cell is plated on either a small (left) or large (right) island of fibronectin (blue lines), the consequent effect on cell shape, Rho GTPase activity, and cytoskeletal tension regulates the commitment to either the adipogenic or osteogenic lineage. The red lines indicate the actin cytoskeleton, which in a "stretched" cell (right) will respond with a force change to compensate for an external force promoted through contact between the cell and the ECM. The mechanism by which the change in cell shape leads to Rho activation is unknown. The ability of activated Rho to regulate cytoskeletal tension requires the Rho effector target, Rho-kinase.

to become adipocytes or osteoblasts, and observed that high density favors adipogenesis whereas low density favors osteogenesis. To separate the potential role of cell density from effects of cell shape, the technique of micropatterning the plating surface was utilized. This involves the "printing" of fibronectin on an otherwise nonadhesive surface in "islands" of varying sizes, such that the ability of cells to spread depends strictly on the island size. Cells will assume either a contracted or spread morphology, depending on the island size, and they can be observed in isolation from surrounding cells. McBeath et al. (2004) found that even when MSCs were exposed to adipogenic media, they would only undergo adipogenesis if they were forced into a contracted morphology, and when they were exposed to osteogenic media, they would only undergo osteogenesis when forced to assume a spread morphology.

This observation suggested a potential role for actomyosin contractility-generated tension in determining lineage commitment, and this was indeed confirmed with the demonstration that pretreatment of MSCs with Y-27632, a pharmacological inhibitor of the Rho GTPase effector target, Rho-kinase (ROCK), that inhibits contractility but not spreading, decreased ostoegenic conversion and increased adipogenic conversion. The Rho GTPase is among the key regulators of cytoskeletal contractility, and so McBeath et al. (2004) also addressed a potential role for Rho in transducing a cell shape cue into a contractile response. Indeed, cells plated at low density, which exhibit a spread morphology, were found to exhibit more Rho activity (and ROCK activity) than contracted cells plated at high density. Moreover, the effect of density was cleanly separated from that of shape with the demonstration that cells plated on small islands (contracted) exhibit less Rho activity than cells plated on large islands (spread). Thus, cell shape and density influence Rho activity, and the apparent role of cell shape in MSC lineage commitment probably reflects a role for ROCK-induced cytoskeletal tension (Figure 1).

The central role of Rho in cytoskeletal regulation prompted the investigators to directly examine Rho's ability to influence lineage commitment. Expression of a constitutively active Rho protein converted MSCs to osteoblasts whereas dominant-negative Rho converted MSCs to adipocytes. Rho-induced osteogenic conversion was blocked by Y-27632, indicating a likely requirement for ROCK-mediated tension. Significantly, these mutant forms of Rho were sufficient to induce lineage commitment in the absence of any exogenous factors, indicating a critical role for Rho downstream of these factors in MSC cell fate choice. Interestingly, when a contracted cell shape was enforced by plating MSCs on small islands, activated Rho could not induce osteogenesis, and similarly, when a spread shape was enforced, dominant-negative Rho was unable to induce adipogenesis. Thus, both cell shape and Rho-induced cytoskeletal tension are required for lineage commitment, but neither one alone is sufficient.

The fact that cell shape can apparently influence Rho activity, while Rho is an established regulator of cell shape, suggests a potentially complex role for Rho in lineage commitment. Certainly, feedback mechanisms in which Rho is both regulating and responding to the state of the cytoskeleton may be involved. Moreover, Rho could influence cell fate choice through shape-independent mechanisms. In a recent related report (Sordella et al., 2003), it was observed that Rho plays an important role in the differentiation of partially committed adipocyte and myocyte precursors, with low Rho activity favoring adipogenic conversion and high Rho activity favoring myogenic conversion. It was determined that an important role for Rho is to modulate the signaling response to IGF-1 (insulin-like growth factor 1), a factor that plays a role in the differentiation of several mesenchymal-derived cell types. Notably, while the ability of Rho to modulate IGF-1 signaling depends on ROCK, it does not appear to involve actomyosin contractility. Taken together with the new findings from McBeath et al. (2004), it seems that Rho may play both cytoskeletal-dependent and -independent roles in the commitment and differentiation of cells within the mesenchymal lineage.

The observation that Rho-induced tension is an important determinant of MSC lineage commitment has important implications in both normal and disease biology. However, many questions remain unanswered. Does Rho mediate all shape change-induced responses during differentiation? Does a soluble factor promote

Rho activation and subsequent shape change-induced lineage commitment? Or, does an external mechanical force initiate the activation of Rho in stem cells? How does cell shape change promote Rho activation? The identification of additional Rho pathway components in this context should help to address these questions, and the identification of small molecular compounds that can influence components of this pathway could eventually yield drugs that are effective in human diseases that involve tissue remodeling as well as in the engineering of tissues in vitro from isolated stem cells.

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HIV-1 Vpu: Putting a Channel to the TASK

Vpu is an HIV-encoded protein that enhances virus release. Previously, this activity was correlated with an intrinsic ion channel activity of Vpu. In this issue of *Molecular Cell*, Hsu et al. (2004) propose an alternative mechanism: they suggest that Vpu functions by inhibiting another ion channel, TASK-1.

Lentiviruses are unique among retroviruses in that they encode a series of proteins not commonly found in other retroviruses. These include the transcriptional activator Tat, the RNA transport modulator Rev, as well as Nef, Vpr, Vif, and Vpu which are generally referred to as accessory proteins. Over the past one-and-a-half decades lentiviral accessory proteins have been the subject of intense research, and much progress has been made in understanding their role in virus replication and pathogenesis. It turns out that all of the HIV accessory proteins are multifunctional. The versatility of these proteins is achieved by a surprisingly simple mechanism that involves protein-protein or protein-nucleic acid interactions. In fact, all of the HIV accessory proteins as well as the regulatory proteins Tat and Rev lack enzymatic activity. Thus, HIV accessory proteins behave like adaptor molecules that connect other viral or cellular proteins or nucleic acids to various preexisting cellular pathways, thereby changing their activity or specificity and thus controlling processes important for viral replication (for review see Strebel, 2003; Bour and Strebel, 2003).

Vpu is a small HIV-1-encoded membrane protein that enhances the release of progeny virions from infected cells and induces the degradation of the HIV receptor molecule CD4. These two functions of Vpu are mechanistically independent, yet both require protein-protein interactions. Indeed, the rapid degradation of the surface receptor CD4 in Vpu-expressing cells that reduces the half-life of CD4 from approximately 6 hr to less than 15 min is caused by the formation of a multiprotein complex consisting of Vpu, CD4, and β-TrCP. TrCP, in turn, is a component of the *S*kpI, *C*ullin, *F*-box protein (SCF^{TrCP}) E3 ubiquitin ligase complex whose normal function is to regulate the ubiquitination and proteasome degradation of cellular proteins such as β-catenin and IκB (Yaron et al., 1998; Spencer et al., 1999; Latres et al., 1999). However, in HIV-infected cells, Vpu—by virtue of its ability to simultaneously bind both TrCP and CD4—redirects the specificity of the SCF^{TrCP} complex to target CD4, which is normally degraded via a lysosomal pathway, into a proteasome-degradation pathway (Margottin et al., 1998).

Another function of Vpu is to increase progeny virus secretion from infected cells. Indeed, this is the activity of Vpu relevant to the current study by Hsu et al. (2004 [this issue of *Molecular Cell*]). In contrast to Vpu-induced degradation of CD4, which is fairly well understood in its mechanistic details, relatively little is known about how Vpu enhances virus release. Vpu—after cotranslational insertion into the host membrane—can self-assemble into homooligomeric complexes that in vitro function as ion-conductive membrane pores (reviewed in Bour and Strebel, 2003). The observation that mutations in Vpu that inhibited ion channel activity also affected the ability of the protein to enhance virus release suggested that these two activities of Vpu are functionally related. However, the question of how an ion channel