HHS Public Access

Author manuscript

Differentiation. Author manuscript; available in PMC 2015 August 22.

Published in final edited form as:

Differentiation. 2013 October ; 86(3): 112–120. doi:10.1016/j.diff.2013.07.004.

From tissue mechanics to transcription factors

Paul A. Janmey[a,*], **Rebecca G. Wells**[b], **Richard K. Assoian**[c], and **Christopher A. McCulloch**[d]

[a]Departments of Physiology and Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA

[b]Departments of Medicine and Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

[c]Departments of Pharmacology and Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA

[d]Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Fitzgerald Building, Toronto, Ontario, Canada M5S 3E2

Abstract

Changes in tissue stiffness are frequently associated with diseases such as cancer, fibrosis, and atherosclerosis. Several recent studies suggest that, in addition to resulting from pathology, mechanical changes may play a role akin to soluble factors in causing the progression of disease, and similar mechanical control might be essential for normal tissue development and homeostasis. Many cell types alter their structure and function in response to exogenous forces or as a function of the mechanical properties of the materials to which they adhere. This review summarizes recent progress in identifying intracellular signaling pathways, and especially transcriptional programs, that are differentially activated when cells adhere to materials with different mechanical properties or when they are subject to tension arising from external forces. Several cytoplasmic or cytoskeletal signaling pathways involving small GTPases, focal adhesion kinase and transforming growth factor beta as well as the transcriptional regulators MRTF-A, NFκB, and Yap/Taz have emerged as important mediators of mechanical signaling.

Keywords

Mechanotransduction; Mechanosensing; Substrate stiffness; Mechanical stress

1. Introduction

Many cell types are highly sensitive to physical as well as chemical stimuli. Transduction of physical stimuli into biochemical signaling pathways has been well characterized in systems such as the response of retinal cells to the absorption of photons, or the activation of ion channels by deflection of actin bundles in the hair cell due to acoustic waves. However, much less is known about how cells respond when the forces they generate internally are opposed by passive, viscoelastic properties of the extracellular matrix (ECM) or by

*Corresponding author. janmey@mail.med.upenn.edu (P.A. Janmey).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript

neighboring cells within tissues. This brief review summarizes recent progress in identifying intracellular signaling pathways, and especially transcriptional programs, that are differentially activated when cells adhere to materials with different mechanical properties or when they are subject to tension arising from externally applied forces.

Mechanical signals can be generally classified as those that arise from the passive mechanical resistance to forces generated by the cell, and those that arise when a cell is actively subjected to forces applied by other cells, fluid flow, or gravity. In all situations, these forces are resisted by the stiffness of the ECM and the glycocalyx that surrounds cells. Changes in tissue stiffness are frequently associated with diseases such as cancer (Levental et al., 2010), fibrosis (Wells, 2008), and atherosclerosis (Duprez and Cohn, 2007; Kothapalli et al., 2012; Mitchell et al., 2010). Some studies suggest that, in addition to *resulting* from pathology, mechanical changes may play a role akin to soluble factors in *causing* the progression of such diseases. For example, measurements of the viscoelasticity of liver in experimentally-induced liver fibrosis in rats showed that the stiffness of liver, as quantified by shear elastic modulus, increased before histologically-detectable increases in ECM deposition or myofibroblast differentiation (Georges et al., 2007) (Perepelyuk et al., 2013). These results suggest that changes in tissue mechanics that can activate liver myofibroblast precursors- hepatic stellate cells (Olsen et al., 2011) and portal fibroblasts (Li et al., 2007) - precede and therefore may cause or at least contribute to development of pathosis. Increased tissue stiffness also appears to contribute to the development and spread of cancer in some models (Levental et al., 2010); the response of cells to abnormal ECM stiffness may then render them resistant to chemotherapeutic agents, possibly because of changes in the cytoskeleton-membrane interface at cell adhesions (Schrader et al., 2011).

Many cell types alter their structure and function in response to the mechanical properties of the materials to which they adhere (Pelham and Wang, 1997) and the type of adhesion receptor by which they bind (Byfield et al., 2009; Chopra et al., 2012; Ganz et al., 2006). Mechanical stimuli can act in concert with or in some cases override or prevent chemical stimulation (Wells and Discher, 2008). In vivo, cells engage their ECM both by mechanosensitive adhesion complexes and by other surface receptors, including those for growth factors and inflammatory mediators, that cannot act as adhesive anchors but that potentially modify the mechanical signals transduced at the cell/ECM interface. The cellular response to substrate stiffness in vitro or to changes in the mechanical properties of tissues during development, injury, or disease can be context-dependent, with different cell types being maximally sensitive to widely different ranges of substrate stiffness (Georges and Janmey, 2005). Substrate stiffness can be sensed by cells within 2 min of their adhesion to substrates with similar surface topologies and adhesion protein densities but different elastic moduli (Yeung et al., 2005). Pioneering studies of substrate stiffness sensing showed that this response does not require protein synthesis (Pelham and Wang, 1997), indicating that the initial response of cells to substrate mechanics requires only signals that are acutely produced in response to tension. There is no obvious universal response to substrate stiffness, but increasing stiffness commonly correlates with increased actomyosin contractility, activation of the small GTPase RhoA, increased tyrosine phosphorylation of numerous proteins, activation of focal adhesion kinase (FAK), and increased Ca^{2+} influx

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

through mechanosensitive channels. How these initial signals integrate with each other and are translated into changes in cytoskeletal structure such as increased synthesis of α-smooth muscle actin (α-SMA) (Hinz et al., 2001), which is a common downstream effect of increased stiffness, and other morphological and functional responses is currently an active area of research.

While attachment of connective tissue cells to the ECM is generally reliant on the formation and remodeling of integrin-mediated, actomyosin-linked adhesions, connective tissue cells can also adhere to each other by intercellular adhesive molecules (e.g. cadherins) that may act as force (Ko et al., 2001) and stiffness (Chopra et al., 2011; Ganz et al., 2006) sensors and regulate gene expression. N-cadherin-mediated adherens junctions are influenced by integrins; fibroblasts may therefore integrate mechanical signals from intercellular and matrix adhesion systems to coordinate gene responses that are involved in differentiation, organogenesis, and wound healing (Linask et al., 2005). Mechanotransduction may not be a single isolated process involving integrins or cadherins (Ko et al., 2001; Potard et al., 1997) but instead may result from a concatenation of processes that require the recruitment of attachment, cytoskeletal, and signaling proteins. Conceivably these proteins then form docking/signaling complexes that are arranged in time and space to regulate transcription.

2. Models of transcriptional response to tissue mechanics

Most studies and models of mechanically triggered transcriptional changes suggest that the transition from physical to biochemical events occurs at the plasma membrane or within the cytoplasm and leads to biochemical changes in transcription factors or regulators that cause their entry into the nucleus to regulate gene expression. While the molecular identity of specific mechanotransducers in mammals has not been clearly defined, analysis of *Caenorhabditis elegans* (Syntichaki and Tavernarakis, 2004) and mammalian cell models (Tsunozaki and Bautista, 2009) has suggested several candidate transduction mechanisms (Fig. 1A). For example, one possible mechanism of mechanotransduction invokes force activation of mechanosensitive plasma membrane channels (Kiselyov and Patterson, 2009; Martinac, 2004), which allows inflow of Ca^{2+} that can act as a second messenger to regulate gene expression. In this context, transient receptor potential channels may play central roles as mechanosensitive channels in mechanosensation (Corey, 2003; Yin and Kuebler, 2010). This may have significant clinical relevance, as illustrated by the discovery of the Chilean rose tarantula toxin as a potential treatment for muscular dystrophy. This toxin, which inactivates stretch-sensitive cation-permeable channels, may offer promise for treatment of this disease for which current therapies are virtually non-existent (Gottlieb et al., 2004). Another common factor in transducing forces exerted at the cell surface to biochemical changes depends on the localization and enzymatic activity of focal adhesion kinase (Klein et al., 2009; Wong et al., 2012; Zebda et al., 2012).

In other models that do not invoke activation of stretch-sensitive membrane channels, mechanotransduction is initiated by a force-dependent step such as activation of a protein kinase or a phosphatase (Kostic et al., 2007), or partial unfolding of proteins to expose cryptic binding sites that are upstream of the activation of the transcription regulating proteins. Once this initial mechanically-stimulated event occurs the process is independent

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

of physical signals and could equivalently, have been produced by an appropriate chemical stimulus.

However, an equally plausible model suggests that mechanical stress, caused either by external force or cell-generated tension, is transmitted directly to transcriptional regulators such as Yap/Taz ((Halder et al., 2012); see Section 3.4.2) (Fig. 1B) or from the cell surface to the nucleus, leading to changes in nuclear shape and altered chromatin organization (Martins et al., 2012; Roca-Cusachs et al., 2008; Versaevel et al., 2012). In this second model, mechanical stresses are transmitted, rather than transduced, either to the transcription regulators or to the nucleus, where the transduction step occurs within chromatin, rather than a protein.

In nucleus-based models of mechanotranscription, forces exerted at the interface of the cell and its extracellular environment are transmitted through the mechanically integrated cytoskeleton through the LINC (linker of the nucleoskeleton and cytoskeleton) complex to the lamin network, which underlies the nuclear membrane or to the nuclear matrix, as outlined in Fig. 1. Because lamins and other proteins associated with the nuclear membrane or matrix that bind chromatin can play a role in genomic silencing, altering their physical association with DNA or altering the structure of chromatin by exerting force on it potentially exposes DNA sequences to resident transcriptional machinery and thereby can lead to altered transcription. The complex folding of chromatin within the nucleus and the non-continuous arrangement of coded information within a single gene provide many possible mechanisms by which transcription could be initiated or prevented by altering nuclear and chromatin shape. For example, micropatterning studies reveal a relationship between spreading of cells in the G1 phase of the cell cycle and swelling of the nucleus; this swelling could enhance access of transcription factors to chromatin and thereby enhance proliferation (Roca-Cusachs et al., 2008).

3. Substrate stiffness effects on cell proliferation and differentiation in vitro

One of the major downstream effects of mechanical signaling is altered proliferation. Indeed, the proliferation of many cell types increases with ECM stiffness. This effect is clearly seen by measuring entry into the S phase of the cell cycle or by determining the transcriptional activation of mechanosensitive cell cycle genes as described below. Proximally, these transcriptional changes result from mechanosensitive alterations in the assembly and/or phosphorylation of focal adhesion proteins and the interaction of focal adhesion complexes with actin and the nucleus.

3.1. Mechanical control of cell cycle genes

Cell proliferation is ultimately governed by the cyclin-dependent kinases. These serine/threonine kinases act in the nucleus and mediate progression through distinct stages of the cell cycle. Classical studies by Pardee and colleagues showed that extracellular signals affect cell cycling only within a portion of G1 phase, up to a point termed the restriction point, R; further progression through the mitotic cycle is cell autonomous (Pardee, 1974). Current thinking is that R represents the inactivating phosphorylation of the retinoblastoma protein by the cyclin-dependent kinases. Thus, if mechanosensitive signaling impacts proliferation,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

its effect should be detectable as changes in the activity of the two G1 phase cyclin-dependent kinases, cyclin D-cdk4/6 and cyclin E-cdk2 in mammalian cells. Early studies, which compared cell cycling of fibroblasts in 3D tensioned or relaxed collagen gels, showed that cells in the relaxed gels had higher levels of p27kip1 and lower levels of activated ERK and cyclin D1 (Fringer and Grinnell, 2001; Rosenfeldt and Grinnell, 2000). Similarly, cyclin D1 expression was low in hepatocytes cultured on collagen gels as compared to rigid collagen films (Hansen and Albrecht, 1999). More recently, Klein et al. (2009) used ECM-coated polyacrylamide hydrogels to show that mouse embryonic fibroblasts, MCF10A, and vascular smooth muscle cells exhibit increased expression of the cyclin D1 gene and cell proliferation in response to substratum stiffening. The increase in cyclin D1 gene expression was linked to a mechanosensitive increase in FAK autophosphorylation at Y397, FAK-dependent activation of Rac, and Rac-dependent cyclin D1 transcription. Cyclin D1 gene transcription has been commonly linked to the activation of ERK MAP kinases, and several studies have linked ERK activation to integrin-mediated cell adhesion to substratum. However, Klein et al. reported that ERK activity is not strongly dependent on ECM stiffness, at least not to a degree seen in activation of Rac-GTP, cyclin D1, and S phase entry.

In a complementary approach, Jiang et al. (2009) cultured bladder smooth muscle cells in tense or relaxed 3D collagen gels. As expected, the cells under tension proliferated while the cells in relaxed collagen gels failed to cycle, as determined by BrdU incorporation. The authors did not report effects on cyclin D1, but observed an inverse relationship between intracellular tension and the levels of p27kip1 (a cdk inhibitor for G1 phase cyclin E-cdk2 complexes). p27 mRNA levels were similar in the tensioned and relaxed cells, but the expression of Skp2 (the substrate specificity component of the E3 ligase-degrading p27) was strongly increased in the tensioned cells. This effect was mediated through transcriptional processes and was linked to a mechanosensitive dephosphorylation of the transcription factor NFAT-1c, enabling it to bind to the NFAT site on the Skp2 promoter.

3.2. Mechanical control of immediate-early genes

Wozniak et al. (2012) have studied how the degree of cell adhesion, measured using micropatterning as well as ECM-coated hydrogels, is transduced into mechanosensitive signaling pathways that regulate transcription of immediate-early genes. High-throughput analysis identified serum response factor (SRF) as being particularly mechanosensitive. SRF is a MADS-box transcription factor that regulates genes involved in cell proliferation, migration, cytoskeletal dynamics and myogenesis by binding a conserved DNA sequence [CC(A/T)$_6$GG], known as a CArG box or serum response element (SRE) (Kuwahara et al., 2005). The SRE contains two critical sites, the ternary complex factor (TCF) site and, as discussed below, the CArG box. Although the CArG box had previously been identified as a Rho-regulated target, Wozniak et al. (2012) demonstrated that the response to varying adhesion is conferred by the TCF site rather than the CArG box. Further work showed that two MAP kinase signaling pathways, JNK→Sap and p38→Net, play complementary roles in transducing mechanosensitive signals to SRF and SRE-regulated genes such as fos and egr-1. The authors propose a model in which a highly adhesive environment stimulates JNK-mediated phosphorylation of the transcription factor Sap, followed by Sap binding to

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

the TCF and transcription of SRF-regulated genes. Conversely, a low adhesive environment actively inhibits SRF expression through a p38-dependent phosphorylation of the transcriptional repressor Net, which leads to Net-dependent repression through the TCF site. Wozniak et al. (2012) also found that ERK MAP kinase activity is not strongly mechanosensitive. In contrast, the transcription regulator myocardin related transcription factor (MRTF-A), which stimulates SRF activity through the CArG box rather than the TCF site, has been implicated in the mechanical regulation of myofibroblast differentiation (see below) and JunB expression during epidermal stem cell and (Connelly et al., 2010). Thus, the relative importance of the TCF and CArG sites may differ depending on the mechanosensitive cell read-out.

3.3. A role for cadherins and β-catenin in mechanically regulated cell proliferation

As noted above, mechanotransduction is mediated by integrins and the ECM, and also by cell-cell adhesion through cadherins. Cadherin engagement has the potential to affect intracellular tension through its association with β-catenin/α-catenin complexes because α-catenin has been reported to bind to vinculin, α-actinin, and actin (Kobielak and Fuchs, 2004; Yonemura et al., 2010). Additionally, β-catenin transcriptionally activates the cyclin D1 gene (Tetsu and McCormick, 1999) and a current hypothesis is that the transcriptional activity of β-catenin can be regulated by its recruitment or release from cadherin complexes. Samuel et al. (Samuel et al., 2011) have recently provided strong evidence for a connection between actomyosin-dependent tension, β-catenin-dependent transcription, and proliferation in vivo. In particular, they showed that transgenic expression of conditionally active Rho kinase 2 (ROCK2) in mouse skin leads to tissue stiffening, activation of β-catenin, β-catenin-dependent transcription of cyclin D1, and epidermal cell proliferation. These effects were abrogated by inhibitors of actomyosin contractility. Additionally, the authors reported that β-catenin stabilization by transgenic activated ROCK2 was dependent on a FAK-PI3K/Akt signaling pathway, and that activated ROCK2 leads to the redistribution of β-catenin from cell surface E-cadherin to the cytoplasm and nucleus. These results reveal an in vivo crosstalk between integrin and cadherin signaling complexes during transcriptional regulation of cell proliferation. How Rho/ROCK-stimulated intracellular tension dislodges β-catenin and leads to its nuclear translocation without affecting the cell surface distribution of E-cadherin remains to be determined. It would be particularly interesting to define the localization of α-catenin in these experiments given the controversy surrounding the role of the heterodimeric β-catenin/α-catenin complex in linking surface cadherin to actin (Drees et al., 2005; Yamada et al., 2005)

3.4. Cytoskeletal and tensional control of transcriptional programs

Two distinct transcriptional regulatory pathways have been shown to be involved in the responses of cells to mechanical or spatial signals. Mechanosensing and response generally involves changes in the cytoskeleton and its attachment to the plasma membrane, but the mechanisms by which these transcription regulators interact with the cytoskeleton and cell adhesion sites appear to be fundamentally different, suggesting that there are multiple pathways by which physical signals and transcription are linked.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3.4.1. MRTF-A/Mal/MKL—Myocardin and the related transcription factors MRTF-A and MRTF-B are mechanosensitive proteins expressed in a broad range of cell types (Wang et al., 2002a); they comprise a family of co-activators that mediates the transcriptional regulating activity of SRF (Wang et al., 2001). The SRF CArG box, discussed above (Section 3.2), serves as a docking site for myocardin and MRTFs (Fig. 2). These proteins enhance the transcriptional activity of SRF by forming a ternary complex with SRF on DNA (Wang et al., 2002a). MRTF-A and -B also convey stimulatory signals from the Rho GTPase and the actin cytoskeleton to SRF via their regulated translocation into the nucleus (Miralles et al., 2003) (Fig. 1B). MRTF-A (also known as megakaryocyte acute leukemia protein (MAL) and megakaryoblastic leukemia-1 (MKL-1)) mediates SRF-independent activation of tenascin-C transcription in response to mechanical stress (Asparuhova et al., 2011). MRTF-A can also act as a common mediator of mechanical stress- and neurohumoral stimulation-induced cardiac hypertrophic signaling, which can lead to activation of brain natriuretic peptide gene expression (Kuwahara et al., 2010) and force-induced myofibroblast differentiation (Chan et al., 2010). The ability of SRF to distinguish between different target genes depends on the presence of binding sites for other transcription factors, the number of CArG boxes, and a large number of cofactors.

A direct link between cytoskeletal changes and MRTF-A/Mal/MKL activity (Miralles et al., 2003) is based on the finding that MRTF-A binds and sequesters actin monomers, preventing their assembly into actin filaments. Polymerization of actin and promotion of contraction, which occurs in response to mechanical stress and requires activation of RhoA and ROCK, thus results in the release of MRTF-A, which is able to move into the nucleus and activate SRF. This process in turn activates a program of fibrogenic gene response including binding to CArG boxes in the α-SMA promoter and increasing α-SMA expression (Huang et al., 2012) as shown in Fig. 2.

3.4.2. Yap/Taz/Yorkie—A recently discovered novel link between physical signals and transcription is based on the mammalian transcriptional regulators Yap and Taz and their Drosophila counterpart Yorkie, which were first identified as elements of the growth-controlling Hippo pathway. A systematic study of transcriptional differences between epithelial cells cultured on soft or stiff substrates identified Yap/Taz as involved in stiffness-promoted transcriptional changes. Yap and Taz are largely localized in the nuclei of epithelial and other cells cultured on rigid substrates, but are cytoplasmic or degraded when the same cells are grown on soft substrates (Dupont et al., 2011). The substrate rigidity control of Yap/Taz localization is distinct from its control by kinases involved in Hippo signaling, and appears to involve specific actin structures, internal tension mediated by these structures (Halder et al., 2012) or links to E-cadherin (Kim et al., 2011), but (unlike MRTF-A) not direct binding to actin subunits (Fig. 1B). Changes in substrate rigidity not only alter the nuclear localization of Yap/Taz, but the transcripts they activate contribute to the morphological changes such as formation of stress fibers that typify cell adaptation to rigid substrates. Downregulation of nuclear Yap/Taz prevents aspects of cell response to rigid substrates, and ectopic expression of nuclear-directed Yap stimulates cells on soft substrates to acquire morphologic features of cells on stiff substrates (Dupont et al., 2011). The extent to which Yap, Taz, and Yorkie exert the same cellular effects remains to be full established,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and at least in some cell types (such as trabecular meshwork cells) substrate stiffness affects Yap and Taz differently (Raghunathan et al., 2013; Thomasy et al., 2013).

Although control of MRTF-A and Yap/Taz both involve changes in actin assembly, the mechanisms by which actin controls them appear to be distinct (Fig. 1B). MRTF-A binds directly to actin monomers, which keeps it sequestered in the cytoplasm; Yap/Taz do not appear to bind actin or other cytoskeletal subunits directly, but they have been reported to bind angiomotin, which in turn binds to actin filaments (Chan et al., 2011). Precisely how Yap/Taz trafficking depends on the organization and internal tension within the cytoskeleton is not known, but a largely assembled, perhaps tensed, actin structure might be responsible for inhibiting the kinases that would otherwise enhance degradation of Yap/Taz or promote their cytoplasmic sequestration (Halder et al., 2012).

3.5. Neuronal growth and differentiation in response to ECM softening

As discussed above, ECM stiffening promotes proliferation of most cell types. However, the growth of neurons, neuronal progenitors and neuroblastoma cells shows a strikingly difference, with optimal growth occurring on soft surfaces resembling the stiffness of the brain (Flanagan et al., 2002; Georges et al., 2006). At least in neuroblastoma cells, ECM stiffening leads to a transcriptional downregulation of N-myc, a gene closely linked to cell proliferation (Lam et al., 2010). The growth cones of neurons produce surprisingly little traction stress, despite their abundance of actin and myosin (Betz et al., 2011), and the growth cone retracts when subjected to relatively modest forces that would not dislodge other cell types (Franze et al., 2009). Thus, cells in the neuronal lineage have apparently adapted to their soft microenvironment. Neuronal differentiation (Banerjee et al., 2009; Her et al., 2013; Leipzig and Shoichet, 2009) and neurite extension (Kostic et al., 2007) are also optimal on soft surfaces. A recent study using nanofabricated arrays of pillars with controlled stiffness shows that the differentiation of precursor cells to neuronal as opposed to glial fates depends more strongly on substrate softening than on other features such as spatial patterning (Migliorini et al., 2013).

3.6. Matrix stiffness and endothelial cell differentiation

The formation of capillaries by endothelial cells (Mammoto et al., 2009) and the differentiation of cardiac cell precursors to an endothelial phenotype (Kshitiz et al., 2012) are both tightly regulated by matrix stiffness and employ at least in part similar mechanotransduction pathways. Both processes appear to involve matrix-rigidity dependent changes in the activity or expression of the Rho inhibitor p190RhoGAP, which in turn alters RhoA activation and the balance between the transcription factors TFII-I and GATA2 (Mammoto et al., 2009) (Fig. 1A). In rigidity-dependent cardiac precursor cell differentiation, p190RhoGAP appears to have both RhoA-dependent and RhoA-independent functions (Kshitiz et al., 2012).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

4. Mechanotranscription and differentiation in tissues in response to external stress

Forces affect the metabolic responses and gene expression repertoires of many tissues. Altered gene expression in response to physical signals can manifest as stimulation of bone formation (Martin, 2007; Skerry and Suva, 2003; Turner and Robling, 2004), enhanced turnover of connective tissue matrices (Ozaki et al., 2005), remodeling of the periodontium during orthodontic treatment and dental occlusal trauma (Krishnan and Davidovitch, 2009; Rygh, 1973), induction of cardiac hypertrophy by volume or pressure overload (Catalucci et al., 2008; Tarone and Lembo, 2003) and the generation of ventilator-induced injury in lung (Lionetti et al., 2005; Stenqvist et al., 2008). These reports and many other studies have linked the stimulation of cells and tissues by mechanical forces to regulation of gene expression through a wide variety of mechanotransduction systems. While there is an increasing understanding of how forces activate mechanically-sensitive sensors upstream, there is much less known of how mechanical forces regulate transcriptional responses that underlie the appropriate regulation of gene expression to enable tissue adaptation to applied forces.

4.1. Force transfer systems that mediate mechanotranscriptional activation

Connective tissues provide excellent cell models to study the mechanotransduction systems that regulate gene expression. In connective tissue cells, direct transfer of forces to cells may involve intercellular and/or cell-to-ECM contacts (Chen et al., 2004). The integrin cell surface receptors that bind to ECM molecules are notable because they integrate cell adhesion and cell signaling processes. Integrins can also transfer forces from the ECM to the cytoskeleton (Chiquet et al., 2009; Katsumi et al., 2004). While specific molecular platforms enable cells to respond specifically to mechanical stimuli (Helmke and Schwartz, 2004), the proteins comprising the force sensor and effector systems are not yet defined. Among the cellular elements that are thought to contribute to mechanotransduction and regulation of gene expression, the cytoskeleton is of particular interest in connective tissue cells because it can transmit cellular forces, contributing to the information processing of mechanically-induced signals, especially in structures such as the endothelium (Davies, 2009).

One way by which integrins mediate response to force is by a change in their aggregation state at the plasma membrane. Under certain circumstances integrins can become clustered into aggregates, which, in cultured cells, are termed focal adhesions or focal complexes. These protein complexes are potential sites for transfer of contractile forces to the cytoskeleton in cultured cells and possibly for cells in tissues (Ingber, 2003). Because of the ease of culturing fibroblasts and then applying forces of various levels, frequencies and directions, cultured fibroblasts are now widely used models for exploring mechanosensing and mechanotranscriptional mechanisms in solid tissues. In fibroblasts, force transmission is critically dependent on the attachment of cells to specific ECM molecules such as fibrillar collagen or fibronectin, applied to either the culture substrate (Hinz, 2006) or to beads that attach to cells. With this methodology, mechanical induction of gene expression is experimentally testable: tensile forces applied through ECM proteins like collagen (but not poly-L-lysine, which does not support integrin engagement) promote increased expression

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

of the actin-binding protein filamin A, a process that is dependent on the transcription factor Sp1 (D'Addario et al., 2002).

4.2. Tissue fibrosis requires mechanosensitive gene expression

Mechanical signaling is particularly important in fibrosis. At the tissue level, recent studies show that changes in whole liver stiffness precede the development of histologically-detectable fibrosis (Georges et al., 2007; Perepelyuk et al., 2013) and there is evidence from the study of multiple tissues that stiffness increases markedly with fibrosis (Booth et al., 2012; Georges et al., 2007; Liu et al., 2010). At the cellular level, myofibroblasts contribute to ECM deposition in fibrosis; these cells, which are defined by the *de novo* expression of α-SMA, develop stress fibers and generate contractile force, exerting tension on the surrounding ECM. Myofibroblasts have been shown in multiple tissues including lung, liver, skin, heart, and kidney to be mechanosensitive both for expression of α-SMA and the deposition of fibrillar collagens and other ECM proteins typical of fibrosis (Balestrini et al., 2012; Galie et al., 2011; Li et al., 2007; Olsen et al., 2011; Squier, 1981; Tomasek et al., 2002); increased stiffness results in increased cell-generated tension, which leads to α-SMA expression and ECM deposition, potentially as part of a positive feedback loop. α-SMA expression in myofibroblasts provides an example of mechanical induction of gene expression through mechanically-sensitive transcriptional activators.

One of the most important growth factors and transcriptional regulators in fibrosis is transforming growth factor-β (TGF-β), which is required in most contexts for α-SMA expression and myofibroblast differentiation. There is now compelling evidence that the activation of TGF-β is mechanically regulated. TGF-β is secreted as part of a latent complex and stored in the ECM; one component of this latent complex, the latency-associated peptide (LAP), binds directly to certain integrins, linking it to cells. Single molecule analyses have demonstrated that cells exert tension on the LAP through this integrin attachment and that this results in mechanosensitive release of active TGF-β: if the cells and TGF-β complex are attached to a soft ECM, the ECM will deform in response to tension and the complex will remain intact, while if the ECM is stiff, however, resistance to cell-generated tension causes deformation of the LAP, with release of active TGF-β (Balestrini et al., 2012; Wipff et al., 2007). Increased TGF-β leads to increased α-SMA, which in turn leads to increased cell-generated tension, a stiffness-dependent feed-forward loop that may have significant implications for the progression of fibrosis (Wipff et al., 2007). This has been termed "extrinsic" mechanotransduction (Huang et al., 2012).

The effects of TGF-β on α-SMA gene expression are mediated in part by MRTF-A (Crider et al., 2011; Elberg et al., 2008; Sandbo et al., 2011), which was discussed above as a key transcription factor in mechanotransduction pathways. MRTF-A, however, also participates in a TGF-β-independent "intrinsic" mechanotransduction pathway (through RhoA and ROCK) in lung fibroblasts involving changes in the actin polymerization state, leading to α-SMA expression (Huang et al., 2012).

Other mechanotransduction pathways shown to be important in fibrosis include pathways downstream of FAK. This kinase is part of the cellular mechanotransduction apparatus, participating in the general cellular response to mechanical stress (Zebda et al., 2012). At the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

tissue level, it has recently been demonstrated that skin injury results in FAK activation, and that this activation is potentiated by mechanical loading. Increased FAK activation leads, via ERK and the chemokine MCP-1, to increased fibrosis, demonstrating in at least one model the pathway for conversion of mechanical force to fibrosis via FAK (Wong et al., 2012). The canonical Wnt pathway is another pathway demonstrating a stiffness-dependent crosstalk with TGF-β in mediating myofibroblast differentiation. In fibroblasts from cardiac valves, it was shown that TGF-β induced β-catenin nuclear translocation, leading to myofibroblast differentiation, and that this was dependent on ECM stiffness (Chen et al., 2011). Finally, NF-κB, a factor that has an important role linking inflammation and fibrosis (Elsharkawy and Mann, 2007), has been shown to be regulated by mechanical signals in some tissues (Nam et al., 2009; Young et al., 2010). Adipocytes also activate NF-κB and upregulate MCP-1 with increased ECM density (Li et al., 2010)

4.3. Cardiac fibrosis as an example of mechanically induced gene expression in fibrosis

In heart failure, physical and chemical signals lead to the activation of cardiac fibroblasts, which involves their differentiation into myofibroblasts and the excessive accumulation of ECM proteins. Mechanical signaling in cardiac tissues provides an example of a medically important fibrotic response that is strongly associated with abnormal cardiac diastolic function (MacKenna et al., 2000). This fibrotic process is mediated in part by mechanical signals and leads to the *de novo* expression of α-SMA by cardiac fibroblasts. SRF and MRTF-A have been shown to be particularly important in this system.

The α-SMA promoter contains several conserved *cis* elements (Blank et al., 1992; Tomasek et al., 2005) that both positively and negatively affect transcription. In addition to the TCF and CArG elements discussed above, the proximal 400 bases of the α-SMA promoter contain TGF-β control elements (Hautmann et al., 1997) as well as E-box elements that can regulate transcription (Kumar et al., 2003). In fibroblastic cells with low basal levels of actin filaments, stretch-induced activation of α-SMA relies on the CArG B box in the α-SMA promoter (Wang et al., 2002b). In fibroblasts, tensile forces applied through collagen receptors activate the RhoA→Rho-kinase→LIMK→cofilin pathway, which promotes actin filament assembly and nuclear translocation of MRTF-A (Zhao et al., 2007) (Fig. 1). This transcriptional co-activator subsequently activates the α-SMA promoter and thereby enhances the expression of α-SMA. These data provide an example of a mechanical-force-induced transcriptional pathway that may contribute to the development of cardiac hypertrophy.

4.4. Other transcription factors and regulatory systems in tissue mechanotransduction

There are multiple other examples of mechanically-regulated transcription factors and networks of relevance to tissue differentiation and function. In chondrocytes, mechanical stress induces expression of the ECM remodeling enzymes MMP-13 and ADAMTS-5, which are dependent on the RUNX-2 transcription factor (Tetsunaga et al., 2011). In bone, Wnt/β-catenin signaling is a normal physiological response to mechanical loading of skeletal structures (Robinson et al., 2006). In skin, actomyosin-mediated cellular tension enhances tissue stiffness and β-catenin activation, which together contribute to epidermal hyperplasia and tumor growth (Samuel et al., 2011). Cilia function in zebrafish is regulated in response

to epithelial stretch by the foxj1a transcription factor (Hellman et al., 2011), and FOXO transcription factors in respiratory pumps are also mechanosensitive (Pardo et al., 2008). In endothelial cells, fluid shear mediates nuclear translocation of the Nrf2 transcription factor (Hsieh et al., 2009) and mechanical stress can activate serum-, glucocorticoid-regulated kinase 1, which contributes to neointima formation in vein grafts (Cheng et al., 2010). Stretch-induced activation of the transcription factor activator protein-1 helps to regulate monocyte chemoattractant protein-1 (MCP-1) expression during arteriogenesis (Demicheva et al., 2008). These data on vascular biology indicate that transcription factors are part of the cellular circuitry that enables vessel wall adaptations to variations of flow and highlight the broad range of transcription factors involved in mechanotransduction. Research is ongoing to better define similar networks of mechanically-regulated transcription factors in other tissues.

5. Conclusion

The physical environment of a cell is as rich a source of potential signals and information as its chemical environment, and the responses of cells to physical stimuli are also likely to be as complex as their biochemical and genetic signaling pathways. Since many cells are embedded within materials composed of ECM molecules populated by neighboring force-generating cells, the homeostatic state of any single cell depends on inputs from multiple mechanosensitive signaling systems. Perturbations of this state due to changes in ECM composition or cell-generated forces might be central determinants of fibrosis and cancer as well as other pathologies.

A fundamental and still unanswered question in mechanotransduction is whether transcriptional regulators are intrinsic elements of the transduction machinery or rather reactants of biochemical signals originating from the upstream transduction event. For example MRTF-A, which is clearly altered when cells are subjected to stress or cultured on materials with different compliance, responds to changes in the assembly of actin filaments, which lie upstream in the transformation of physical to biochemical signals. Yap/Taz/Yorkie, in contrast, appear to be more directly tied to the initial physical signal, since they do not use the polymerization state of actin as a biochemical readout of the physical state of the cell, and there is evidence for a direct link to cell adhesion sites, perhaps mediated by cell-cell contact points (Kim et al., 2011) or by the cytoskeleton.

As mechanobiology becomes increasingly studied, several signaling pathways, first identified from studies of biochemical stimulation, have been repeatedly implicated in response to mechanical signals. These pathways involve the small GTPases RhoA and Rac, focal adhesion kinase (FAK), LINC proteins and nuclear lamins as cytoskeletal or nuclear membrane protein complexes whose activation is upstream of specific transcriptional events. Farther downstream, the transcription regulators MRTF-A, NF- κB, and Yap/Taz have emerged as particularly important regulators of the mechanoresponse in specific systems. The growing list of mediators of physical stimuli help define how cells respond to physical signals, but do not necessarily shed light on what the initial sensor of the physical stimulus is; cytoskeletal or membrane protein unfolding, ion channel opening, or chromatin structure have all been suggested as potential sensors. Given the complexity of physical signaling,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

there is no reason to expect that one master sensor or responder to forces should exist, nor is it yet clear whether the transcriptional regulators identified thus far are the most important elements of mechanobiology or rather those that were the first discovered or that have the most clearly measurable responses.

The diversity of mechanical response and the recent ability to study mechanobiology with the same precision and predictive nature as has been used to test hypotheses about chemical signaling suggest that there is much to learn in this rapidly advancing field.

References

Balestrini JL, Chaudhry S, Sarrazy V, Koehler A, Hinz B. The mechanical memory of lung myofibroblasts. Integrative Biology. 2012; 4:410–421. [PubMed: 22410748]

Banerjee A, Arha M, Choudhary S, Ashton RS, Bhatia SR, Schaffer DV, Kane RS. The influence of hydrogel modulus on the proliferation and differentiation of encapsulated neural stem cells. Biomaterials. 2009; 30:4695–4699. [PubMed: 19539367]

Betz T, Koch D, Lu YB, Franze K, Kas JA. Growth cones as soft and weak force generators. Proceedings of the National Academy of Sciences of United States America. 2011; 108:13420–13425.

Blank RS, McQuinn TC, Yin KC, Thompson MM, Takeyasu K, Schwartz RJ, Owens GK. Elements of the smooth muscle alpha-actin promoter required in cis for transcriptional activation in smooth muscle. Evidence for cell type-specific regulation. Journal of Biological Chemistry. 1992; 267:984–989. [PubMed: 1730687]

Booth AJ, Hadley R, Cornett AM, Dreffs AA, Matthes SA, Tsui JL, Weiss K, Horowitz JC, Fiore VF, Barker TH, Moore BB, Martinez FJ, Niklason LE, White ES. Acellular normal and fibrotic human lung matrices as a culture system for in vitro investigation. American Journal of Respiratory and Critical Care Medicine. 2012; 186:866–876. [PubMed: 22936357]

Byfield FJ, Wen Q, Levental I, Nordstrom K, Arratia PE, Miller RT, Janmey PA. Absence of filamin A prevents cells from responding to stiffness gradients on gels coated with collagen but not fibronectin. Biophysical Journal. 2009; 96:5095–5102. [PubMed: 19527669]

Catalucci D, Latronico MV, Ellingsen O, Condorelli G. Physiological myocardial hypertrophy: how and why? Frontiers in Bioscience. 2008; 13:312–324. [PubMed: 17981549]

Chan MW, Chaudary F, Lee W, Copeland JW, McCulloch CA. Force-induced myofibroblast differentiation through collagen receptors is dependent on mammalian diaphanous (mDia). Journal of Biological Chemistry. 2010; 285:9273–9281. [PubMed: 20071339]

Chan SW, Lim CJ, Chong YF, Pobbati AV, Huang C, Hong W. Hippo pathway-independent restriction of TAZ and YAP by angiomotin. Journal of Biological Chemistry. 2011; 286:7018–7026. [PubMed: 21224387]

Chen CS, Tan J, Tien J. Mechanotransduction at cell-matrix and cell-cell contacts. Proceedings of the National Academy of Sciences of United States of America. 2004; 6:275–302.

Chen JH, Chen WL, Sider KL, Yip CY, Simmons CA. beta-catenin mediates mechanically regulated, transforming growth factor-beta1-induced myofibroblast differentiation of aortic valve interstitial cells. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011; 31:590–597.

Cheng J, Wang Y, Ma Y, Chan BT, Yang M, Liang A, Zhang L, Li H, Du J. The mechanical stress-activated serum-, glucocorticoid-regulated kinase 1 contributes to neointima formation in vein grafts. Circulation Research. 2010; 107:1265–1274. [PubMed: 20884880]

Chiquet M, Gelman L, Lutz R, Maier S. From mechanotransduction to extracellular matrix gene expression in fibroblasts. Biochimica et Biophysica Acta. 2009; 1793:911–920. [PubMed: 19339214]

Chopra A, Lin V, McCollough A, Atzet S, Prestwich GD, Wechsler AS, Murray ME, Oake SA, Kresh JY, Janmey PA. Reprogramming cardiomyocyte mechanosensing by crosstalk between integrins and hyaluronic acid receptors. Journal of Biomechanics. 2012; 45:824–831. [PubMed: 22196970]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Chopra A, Tabdanov E, Patel H, Janmey PA, Kresh JY. Cardiac myocyte remodeling mediated by N-cadherin-dependent mechanosensing. American Journal of Physiology—Heart and Circulatory Physiology. 2011; 300:H1252–1266. [PubMed: 21257918]

Connelly JT, Gautrot JE, Trappmann B, Tan DW, Donati G, Huck WT, Watt FM. Actin and serum response factor transduce physical cues from the microenvironment to regulate epidermal stem cell fate decisions. Nature Cell Biology. 2010; 12:711–718. [PubMed: 20581838]

Corey DP. New TRP channels in hearing and mechanosensation. Neuron. 2003; 39:585–588. [PubMed: 12925273]

Crider BJ, Risinger GM Jr, Haaksma CJ, Howard EW, Tomasek JJ. Myocardin-related transcription factors A and B are key regulators of TGF-beta1-induced fibroblast to myofibroblast differentiation. Journal of Investigative Dermatology. 2011; 131:2378–2385. [PubMed: 21776010]

D'Addario M, Arora PD, Ellen RP, McCulloch CA. Interaction of p38 and Sp1 in a mechanical force-induced, beta 1 integrin-mediated transcriptional circuit that regulates the actin-binding protein filamin-A. Journal of Biological Chemistry. 2002; 277:47541–47550. [PubMed: 12324467]

Davies PF. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. Nature Clinical Practice Cardiovascular Medicine. 2009; 6:16–26.

Demicheva E, Hecker M, Korff T. Stretch-induced activation of the transcription factor activator protein-1 controls monocyte chemoattractant protein-1 expression during arteriogenesis. Circulation Research. 2008; 103:477–484. [PubMed: 18669921]

Drees F, Pokutta S, Yamada S, Nelson WJ, Weis WI. Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. Cell. 2005; 123:903–915. [PubMed: 16325583]

Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, Elvassore N, Piccolo S. Role of YAP/TAZ in mechanotransduction. Nature. 2011; 474:179–183. [PubMed: 21654799]

Duprez DA, Cohn JN. Arterial stiffness as a risk factor for coronary atherosclerosis. Current Atherosclerosis Reports. 2007; 9:139–144. [PubMed: 17877923]

Elberg G, Chen L, Elberg D, Chan MD, Logan CJ, Turman MA. MKL1 mediates TGF-beta1-induced alpha-smooth muscle actin expression in human renal epithelial cells. American Journal of Physiology. 2008; 294:F1116–1128. [PubMed: 18337547]

Elsharkawy AM, Mann DA. Nuclear factor-kappaB and the hepatic inflammation-fibrosis-cancer axis. Hepatology. 2007; 46:590–597. [PubMed: 17661407]

Flanagan LA, Ju YE, Marg B, Osterfield M, Janmey PA. Neurite branching on deformable substrates. Neuroreport. 2002; 13:2411–2415. [PubMed: 12499839]

Franze K, Gerdelmann J, Weick M, Betz T, Pawlizak S, Lakadamyali M, Bayer J, Rillich K, Gogler M, Lu YB, Reichenbach A, Janmey P, Kas J. Neurite branch retraction is caused by a threshold-dependent mechanical impact. Biophysical Journal. 2009; 97:1883–1890. [PubMed: 19804718]

Fringer J, Grinnell F. Fibroblast quiescence in floating or released collagen matrices: contribution of the ERK signaling pathway and actin cytoskeletal organization. Journal of Biological Chemistry. 2001; 276:31047–31052. [PubMed: 11410588]

Galie PA, Westfall MV, Stegemann JP. Reduced serum content and increased matrix stiffness promote the cardiac myofibroblast transition in 3D collagen matrices. Cardiovascular Pathology. 2011; 20:325–333. [PubMed: 21306921]

Ganz A, Lambert M, Saez A, Silberzan P, Buguin A, Mege RM, Ladoux B. Traction forces exerted through N-cadherin contacts. Biology of the Cell. 2006; 98:721–730. [PubMed: 16895521]

Georges PC, Hui JJ, Gombos Z, McCormick ME, Wang AY, Uemura M, Mick R, Janmey PA, Furth EE, Wells RG. Increased stiffness of the rat liver precedes matrix deposition: implications for fibrosis. American Journal of Physiology. 2007; 293:G1147–1154. [PubMed: 17932231]

Georges PC, Janmey PA. Cell type-specific response to growth on soft materials. Journal of Applied Physiology. 2005; 98:1547–1553. [PubMed: 15772065]

Georges PC, Miller WJ, Meaney DF, Sawyer ES, Janmey PA. Matrices with compliance comparable to that of brain tissue select neuronal over glial growth in mixed cortical cultures. Biophysical Journal. 2006; 90:3012–3018. [PubMed: 16461391]

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Gottlieb PA, Suchyna TM, Ostrow LW, Sachs F. Mechanosensitive ion channels as drug targets. Current Drug Targets. 2004; 3:287–295. [PubMed: 15379605]

Halder G, Dupont S, Piccolo S. Transduction of mechanical and cytoskeletal cues by YAP and TAZ. Nature Reviews Molecular Cell Biology. 2012; 13:591–600.

Hansen LK, Albrecht JH. Regulation of the hepatocyte cell cycle by type I collagen matrix: role of cyclin D1. Journal of Cell Science. 1999; 112:2971–2981. [PubMed: 10444391]

Hautmann MB, Madsen CS, Owens GK. A transforming growth factor beta (TGFbeta) control element drives TGFbeta-induced stimulation of smooth muscle alpha-actin gene expression in concert with two CArG elements. Journal of Biological Chemistry. 1997; 272:10948–10956. [PubMed: 9099754]

Hellman NE, Liu Y, Merkel E, Austin C, Le Corre S, Beier DR, Sun Z, Sharma N, Yoder BK, Drummond IA. The zebrafish foxj1a transcription factor regulates cilia function in response to injury and epithelial stretch. Proceedings of the National Academy of Sciences of United States of America. 2011; 107:18499–18504.

Helmke BP, Schwartz MA. Putting the squeeze on mechanotransduction. Developmental Cell. 2004; 6:745–746. [PubMed: 15177022]

Her GJ, Wu HC, Chen MH, Chen MY, Chang SC, Wang TW. Control of three-dimensional substrate stiffness to manipulate mesenchymal stem cell fate toward neuronal or glial lineages. Acta Biomaterialia. 2013; 9:5170–5180. [PubMed: 23079022]

Hinz B. Masters and servants of the force: the role of matrix adhesions in myofibroblast force perception and transmission. European Journal of Cell Biology. 2006; 85:175–181. [PubMed: 16546559]

Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Molecular Biology of the Cell. 2001; 12:2730–2741. [PubMed: 11553712]

Hsieh CY, Hsiao HY, Wu WY, Liu CA, Tsai YC, Chao YJ, Wang DL, Hsieh HJ. Regulation of shear-induced nuclear translocation of the Nrf2 transcription factor in endothelial cells. Journal of Biomedical Science. 2009; 16:12. [PubMed: 19272177]

Huang X, Yang N, Fiore VF, Barker TH, Sun Y, Morris SW, Ding Q, Thannickal VJ, Zhou Y. Matrix stiffness-induced myofibroblast differentiation is mediated by intrinsic mechanotransduction. American Journal of Respiratory Cell and Molecular Biology. 2012; 47:340–348. [PubMed: 22461426]

Ingber DE. Tensegrity II. How structural networks influence cellular information processing networks. Journal of Cell Science. 2003; 116:1397–1408. [PubMed: 12640025]

Jiang X, Austin PF, Niederhoff RA, Manson SR, Riehm JJ, Cook BL, Pengue G, Chitaley K, Nakayama K, Nakayama KI, Weintraub SJ. Mechanoregulation of proliferation. Molecular and Cellular Biology. 2009; 29:5104–5114. [PubMed: 19596792]

Katsumi A, Orr AW, Tzima E, Schwartz MA. Integrins in mechanotransduction. Journal of Biological Chemistry. 2004; 279:12001–12004. [PubMed: 14960578]

Kim NG, Koh E, Chen X, Gumbiner BM. E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components. Proceedings of the National Academy of Sciences of United States of America. 2011; 108:11930–11935.

Kiselyov K, Patterson RL. The integrative function of TRPC channels. Frontiers in Bioscience. 2009; 14:45–58.

Klein EA, Yin L, Kothapalli D, Castagnino P, Byfield FJ, Xu T, Levental I, Hawthorne E, Janmey PA, Assoian RK. Cell-cycle control by physiological matrix elasticity and in vivo tissue stiffening. Current Biology. 2009; 19:1511–1518. [PubMed: 19765988]

Ko KS, Arora PD, McCulloch CA. Cadherins mediate intercellular mechanical signaling in fibroblasts by activation of stretch-sensitive calcium-permeable channels. Journal of Biological Chemistry. 2001; 276:35967–35977. [PubMed: 11466312]

Kobielak A, Fuchs E. Alpha-catenin: at the junction of intercellular adhesion and actin dynamics. Nature Reviews Molecular Cell Biology. 2004; 5:614–625. [PubMed: 15366705]

Kostic A, Sap J, Sheetz MP. RPTPalpha is required for rigidity-dependent inhibition of extension and differentiation of hippocampal neurons. Journal of Cell Science. 2007; 120:3895–3904. [PubMed: 17940065]

Kothapalli D, Liu SL, Bae YH, Monslow J, Xu T, Hawthorne EA, Byfield FJ, Castagnino P, Rao S, Rader DJ, Pure E, Phillips MC, Lund-Katz S, Janmey PA, Assoian RK. Cardiovascular protection by ApoE and ApoE-HDL linked to suppression of ECM gene expression and arterial stiffening. Cell Reports. 2012; 2:1259–1271. [PubMed: 23103162]

Krishnan V, Davidovitch Z. On a path to unfolding the biological mechanisms of orthodontic tooth movement. Journal of Dental Research. 2009; 88:597–608. [PubMed: 19641146]

Kshitiz Hubbi ME, Ahn EH, Downey J, Afzal J, Kim DH, Rey S, Chang C, Kundu A, Semenza GL, Abraham RM, Levchenko A. Matrix rigidity controls endothelial differentiation and morphogenesis of cardiac precursors. Science Signaling. 2012; 5:ra41. [PubMed: 22669846]

Kumar MS, Hendrix JA, Johnson AD, Owens GK. Smooth muscle alpha-actin gene requires two E-boxes for proper expression in vivo and is a target of class I basic helix-loop-helix proteins. Circulation Research. 2003; 92:840–847. [PubMed: 12663487]

Kuwahara K, Barrientos T, Pipes GC, Li S, Olson EN. Muscle-specific signaling mechanism that links actin dynamics to serum response factor. Molecular and Cellular Biology. 2005; 25:3173–3181. [PubMed: 15798203]

Kuwahara K, Kinoshita H, Kuwabara Y, Nakagawa Y, Usami S, Minami T, Yamada Y, Fujiwara M, Nakao K. Myocardin-related transcription factor A is a common mediator of mechanical stress- and neurohumoral stimulation-induced cardiac hypertrophic signaling leading to activation of brain natriuretic peptide gene expression. Molecular and Cellular Biology. 2010; 30:4134–4148. [PubMed: 20606005]

Lam WA, Cao L, Umesh V, Keung AJ, Sen S, Kumar S. Extracellular matrix rigidity modulates neuroblastoma cell differentiation and N-myc expression. Molecular Cancer. 2010; 9:35. [PubMed: 20144241]

Leipzig ND, Shoichet MS. The effect of substrate stiffness on adult neural stem cell behavior. Biomaterials. 2009; 30:6867–6878. [PubMed: 19775749]

Levental I, Levental KR, Klein EA, Assoian R, Miller RT, Wells RG, Janmey PA. A simple indentation device for measuring micrometer-scale tissue stiffness. Journal of Physics. 2010; 22:194120. [PubMed: 21386443]

Li Q, Hata A, Kosugi C, Kataoka N, Funaki M. The density of extracellular matrix proteins regulates inflammation and insulin signaling in adipocytes. FEBS Letters. 2010; 584:4145–4150. [PubMed: 20804756]

Li Z, Dranoff JA, Chan EP, Uemura M, Sevigny J, Wells RG. Transforming growth factor-beta and substrate stiffness regulate portal fibroblast activation in culture. Hepatology. 2007; 46:1246–1256. [PubMed: 17625791]

Linask KK, Manisastry S, Han M. Cross talk between cell-cell and cell-matrix adhesion signaling pathways during heart organogenesis: implications for cardiac birth defects. Microscopy and Microanalysis. 2005; 11:200–208. [PubMed: 16060972]

Lionetti V, Recchia FA, Ranieri VM. Overview of ventilator-induced lung injury mechanisms. Current Opinion in Critical Care. 2005; 11:82–86. [PubMed: 15659950]

Liu F, Mih JD, Shea BS, Kho AT, Sharif AS, Tager AM, Tschumperlin DJ. Feedback amplification of fibrosis through matrix stiffening and COX-2 suppression. Journal of Cell Biology. 2010; 190:693–706. [PubMed: 20733059]

MacKenna D, Summerour SR, Villarreal FJ. Role of mechanical factors in modulating cardiac fibroblast function and extracellular matrix synthesis. Cardiovascular Research. 2000; 46:257–263. [PubMed: 10773229]

Mammoto A, Connor KM, Mammoto T, Yung CW, Huh D, Aderman CM, Mostoslavsky G, Smith LE, Ingber DE. A mechanosensitive transcriptional mechanism that controls angiogenesis. Nature. 2009; 457:1103–1108. [PubMed: 19242469]

Martin RB. The importance of mechanical loading in bone biology and medicine. Journal of Musculoskeletal and Neuronal Interactions. 2007; 7:48–53. [PubMed: 17396005]

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Martinac B. Mechanosensitive ion channels: molecules of mechanotransduction. Journal of Cell Science. 2004; 117:2449–2460. [PubMed: 15159450]

Martins RP, Finan JD, Guilak F, Lee DA. Mechanical regulation of nuclear structure and function. Proceedings of the National Academy of Sciences of United States of America. 2012; 14:431–455.

Migliorini E, Ban J, Grenci G, Andolfi L, Pozzato A, Tormen M, Torre V, Lazzarino M. Nanomechanics controls neuronal precursors adhesion and differentiation. Biotechnology and Bioengineering. 2013; 110:2301–2310. [PubMed: 23436578]

Miralles F, Posern G, Zaromytidou AI, Treisman R. Actin dynamics control SRF activity by regulation of its coactivator MAL. Cell. 2003; 113:329–342. [PubMed: 12732141]

Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, Vita JA, Levy D, Benjamin EJ. Arterial stiffness and cardiovascular events: the Framingham heart study. Circulation. 2010; 121:505–511. [PubMed: 20083680]

Nam J, Aguda BD, Rath B, Agarwal S. Biomechanical thresholds regulate inflammation through the NF-kappaB pathway: experiments and modeling. PloS One. 2009; 4:e5262. [PubMed: 19370157]

Olsen AL, Bloomer SA, Chan EP, Gaca MD, Georges PC, Sackey B, Uemura M, Janmey PA, Wells RG. Hepatic stellate cells require a stiff environment for myofibroblastic differentiation. American Journal of Physiology. 2011; 301:G110–118. [PubMed: 21527725]

Ozaki S, Kaneko S, Podyma-Inoue KA, Yanagishita M, Soma K. Modulation of extracellular matrix synthesis and alkaline phosphatase activity of periodontal ligament cells by mechanical stress. Journal of Periodontal Research. 2005; 40:110–117. [PubMed: 15733145]

Pardee AB. A restriction point for control of normal animal cell proliferation. Proceedings of the National Academy of Sciences of United States of America. 1974; 71:1286–1290.

Pardo PS, Lopez MA, Boriek AM. FOXO transcription factors are mechanosensitive and their regulation is altered with aging in the respiratory pump. American Journal of Physiology—Cell Physiology. 2008; 294:C1056–1066. [PubMed: 18272820]

Pelham RJ Jr, Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. Proceedings of the National Academy of Sciences of United States of America. 1997; 94:13661–13665.

Perepelyuk M, Terajima M, Wang AY, Georges PC, Janmey PA, Yamauchi M, Wells RG. Hepatic stellate cells and portal fibroblasts are the major cellular sources of collagens and lysyl oxidases in normal liver and early after injury. American Journal of Physiology—Gastrointestinal and Liver Physiology. 2013; 304:G605–614. [PubMed: 23328207]

Potard US, Butler JP, Wang N. Cytoskeletal mechanics in confluent epithelial cells probed through integrins and E-cadherins. American Journal of Physiology. 1997; 272:C1654–1663. [PubMed: 9176157]

Raghunathan VK, Morgan JT, Dreier B, Reilly CM, Thomasy SM, Wood JA, Ly I, Tuyen BC, Hughbanks M, Murphy CJ, Russell P. Role of substratum stiffness in modulating genes associated with extracellular matrix and mechanotransducers YAP and TAZ. Investigative Ophthalmology and Visual Science. 2013; 54:378–386. [PubMed: 23258147]

Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C, Kharode Y, Sauter L, Babij P, Brown EL, Hill AA, Akhter MP, Johnson ML, Recker RR, Komm BS, Bex FJ. Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. Journal of Biological Chemistry. 2006; 281:31720–31728. [PubMed: 16908522]

Roca-Cusachs P, Alcaraz J, Sunyer R, Samitier J, Farre R, Navajas D. Micropatterning of single endothelial cell shape reveals a tight coupling between nuclear volume in G1 and proliferation. Biophysical Journal. 2008; 94:4984–4995. [PubMed: 18326659]

Rosenfeldt H, Grinnell F. Fibroblast quiescence and the disruption of ERK signaling in mechanically unloaded collagen matrices. Journal of Biological Chemistry. 2000; 275:3088–3092. [PubMed: 10652290]

Rygh P. Ultrastructural changes in pressure zones of human periodontium incident to orthodontic tooth movement. Acta Odontologica Scandinavica. 1973; 31:109–122. [PubMed: 4516276]

Samuel MS, Lopez JI, McGhee EJ, Croft DR, Strachan D, Timpson P, Munro J, Schroder E, Zhou J, Brunton VG, Barker N, Clevers H, Sansom OJ, Anderson KI, Weaver VM, Olson MF. Actomyosin-mediated cellular tension drives increased tissue stiffness and beta-catenin activation

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

to induce epidermal hyperplasia and tumor growth. Cancer Cell. 2011; 19:776–791. [PubMed: 21665151]

Sandbo N, Lau A, Kach J, Ngam C, Yau D, Dulin NO. Delayed stress fiber formation mediates pulmonary myofibroblast differentiation in response to TGF-beta. American Journal of Physiology. 2011; 301:L656–666. [PubMed: 21856814]

Schrader J, Gordon-Walker TT, Aucott RL, van Deemter M, Quaas A, Walsh S, Benten D, Forbes SJ, Wells RG, Iredale JP. Matrix stiffness modulates proliferation, chemotherapeutic response, and dormancy in hepatocellular carcinoma cells. Hepatology. 2011; 53:1192–1205. [PubMed: 21442631]

Skerry TM, Suva LJ. Investigation of the regulation of bone mass by mechanical loading: from quantitative cytochemistry to gene array. Cell Biochemistry and Function. 2003; 21:223–229. [PubMed: 12910474]

Squier CA. The effect of stretching on formation of myofibroblasts in mouse skin. Cell and Tissue Research. 1981; 220:325–335. [PubMed: 7197588]

Stenqvist O, Odenstedt H, Lundin S. Dynamic respiratory mechanics in acute lung injury/acute respiratory distress syndrome: research or clinical tool? Current Opinion in Critical Care. 2008; 14:87–93. [PubMed: 18195632]

Syntichaki P, Tavernarakis N. Genetic models of mechanotransduction: the nematode *Caenorhabditis elegans*. Physiological Reviews. 2004; 84:1097–1153. [PubMed: 15383649]

Tarone G, Lembo G. Molecular interplay between mechanical and humoral signalling in cardiac hypertrophy. Trends in Molecular Medicine. 2003; 9:376–382. [PubMed: 13129703]

Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature. 1999; 398:422–426. [PubMed: 10201372]

Tetsunaga T, Nishida K, Furumatsu T, Naruse K, Hirohata S, Yoshida A, Saito T, Ozaki T. Regulation of mechanical stress-induced MMP-13 and ADAMTS-5 expression by RUNX-2 transcriptional factor in SW1353 chondrocyte-like cells. Osteoarthritis and Cartilage. 2011; 19:222–232. [PubMed: 21094261]

Thomasy SM, Morgan JT, Wood JA, Murphy CJ, Russell P. Substratum stiffness and latrunculin B modulate the gene expression of the mechanotransducers YAP and TAZ in human trabecular meshwork cells. Experimental Eye Research. 2013; 113C:66–73. [PubMed: 23727052]

Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nature Reviews Molecular Cell Biology. 2002; 3:349–363. [PubMed: 11988769]

Tomasek JJ, McRae J, Owens GK, Haaksma CJ. Regulation of alpha-smooth muscle actin expression in granulation tissue myofibroblasts is dependent on the intronic CArG element and the transforming growth factor-beta1 control element. American Journal of Pathology. 2005; 166:1343–1351. [PubMed: 15855636]

Tsunozaki M, Bautista DM. Mammalian somatosensory mechanotransduction. Current Opinion in Neurobiology. 2009; 19:362–369. [PubMed: 19683913]

Turner CH, Robling AG. Exercise as an anabolic stimulus for bone. Current Pharmaceutical Design. 2004; 10:2629–2641. [PubMed: 15320750]

Versaevel M, Grevesse T, Gabriele S. Spatial coordination between cell and nuclear shape within micropatterned endothelial cells. Nature Communications. 2012; 3:671.

Wang D, Chang PS, Wang Z, Sutherland L, Richardson JA, Small E, Krieg PA, Olson EN. Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. Cell. 2001; 105:851–862. [PubMed: 11439182]

Wang DZ, Li S, Hockemeyer D, Sutherland L, Wang Z, Schratt G, Richardson JA, Nordheim A, Olson EN. Potentiation of serum response factor activity by a family of myocardin-related transcription factors. Proceedings of the National Academy of Sciences of United States of America. 2002a; 99:14855–14860.

Wang J, Su M, Fan J, Seth A, McCulloch CA. Transcriptional regulation of a contractile gene by mechanical forces applied through integrins in osteoblasts. Journal of Biological Chemistry. 2002b; 277:22889–22895. [PubMed: 11953441]

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Wells RG. Cellular sources of extracellular matrix in hepatic fibrosis. Clinics In Liver Disease. 2008; 12:759–768. [PubMed: 18984465]

Wells RG, Discher DE. Matrix elasticity, cytoskeletal tension, and TGF-beta: the insoluble and soluble meet. Science Signaling. 2008; 1:pe13. [PubMed: 18334714]

Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. Journal of Cell Biology. 2007; 179:1311–1323. [PubMed: 18086923]

Wong VW, Rustad KC, Akaishi S, Sorkin M, Glotzbach JP, Januszyk M, Nelson ER, Levi K, Paterno J, Vial IN, Kuang AA, Longaker MT, Gurtner GC. Focal adhesion kinase links mechanical force to skin fibrosis via inflammatory signaling. Nature Medicine. 2012; 18:148–152.

Wozniak MA, Cheng CQ, Shen CJ, Gao L, Olarerin-George AO, Won KJ, Hogenesch JB, Chen CS. Adhesion regulates MAP kinase/ternary complex factor exchange to control a proliferative transcriptional switch. Current Biology. 2012; 22:2017–2026. [PubMed: 23063436]

Yamada S, Pokutta S, Drees F, Weis WI, Nelson WJ. Deconstructing the cadherin-catenin-actin complex. Cell. 2005; 123:889–901. [PubMed: 16325582]

Yeung T, Georges PC, Flanagan LA, Marg B, Ortiz M, Funaki M, Zahir N, Ming W, Weaver V, Janmey PA. Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. Cell Motility and the Cytoskeleton. 2005; 60:24–34. [PubMed: 15573414]

Yin J, Kuebler WM. Mechanotransduction by TRP channels: general concepts and specific role in the vasculature. Cell Biochemistry and Biophysics. 2010; 56:1–18. [PubMed: 19842065]

Yonemura S, Wada Y, Watanabe T, Nagafuchi A, Shibata M. alpha-Catenin as a tension transducer that induces adherens junction development. Nature Cell Biology. 2010; 12:533–542. [PubMed: 20453849]

Young SR, Gerard-O'Riley R, Harrington M, Pavalko FM. Activation of NF-kappaB by fluid shear stress, but not TNF-alpha, requires focal adhesion kinase in osteoblasts. Bone. 2010; 47:74–82. [PubMed: 20353839]

Zebda N, Dubrovskyi O, Birukov KG. Focal adhesion kinase regulation of mechanotransduction and its impact on endothelial cell functions. Microvascular Research. 2012; 83:71–81. [PubMed: 21741394]

Zhao XH, Laschinger C, Arora P, Szaszi K, Kapus A, McCulloch CA. Force activates smooth muscle alpha-actin promoter activity through the Rho signaling pathway. Journal of Cell Science. 2007; 120:1801–1809. [PubMed: 17456553]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

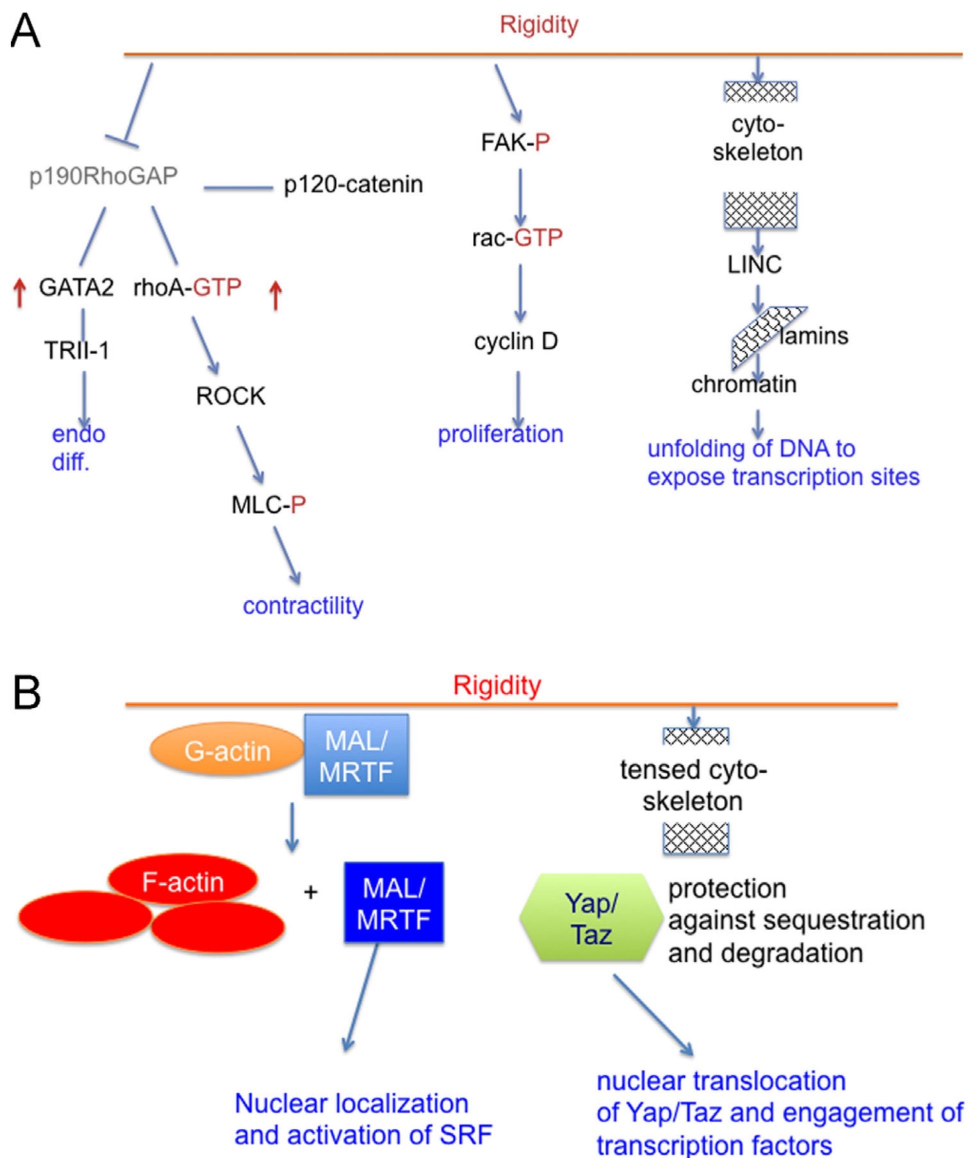

Fig. 1.

Signaling pathways that are regulated by matrix stiffness and alter internal tension, proliferation, differentiation, or other transcriptional programs. (A) Rigid substrates lead to inhibition of p120-RhoGAP, and therefore increased activity of signals that depend on GTP-Rho. Substrate rigidity also increases FAK phosphorylation, Rac activity and processes such as proliferation that lie downstream of these signals. Cytoskeletal contractility due to increased Rho and Rac activity can transmit tension from the rigid substrate to the nuclear membrane or chromatin to directly alter gene accessibility or reaction with transcriptional complexes. (B) Two different proposed mechanisms linking substrate mechanics to transcription. Transcription activated by MAL/MRTF and SRF depends on changes in actin assembly that are regulated by substrate stiffness, and not necessarily to the physical stimuli directly. Yap/Taz/Yorkie, in contrast, appear to respond directly to intracellular tension,

perhaps mediated by attachment to the cytoskeleton or its membrane linkages but not to biochemical changes in the actin network.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

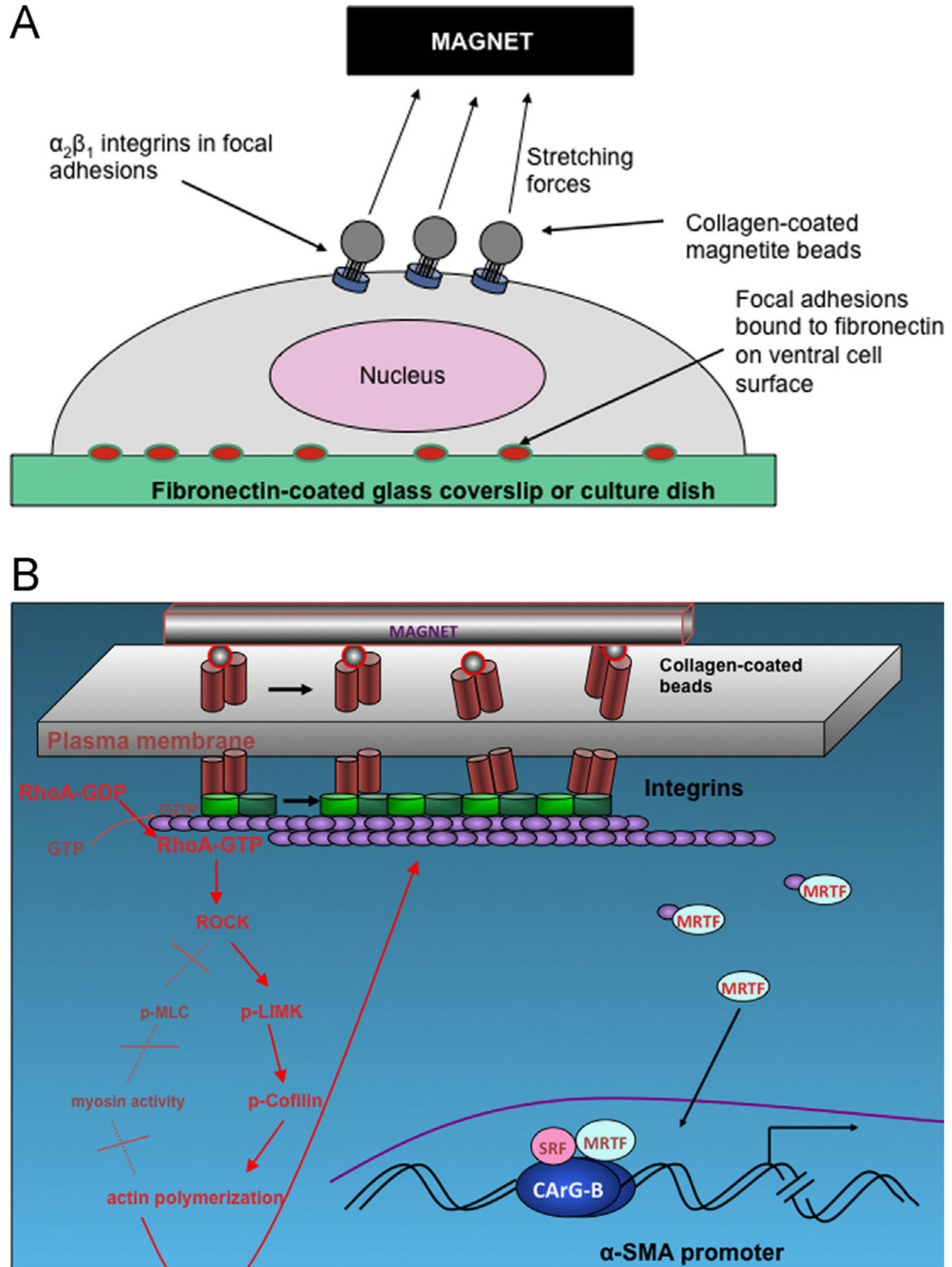

Fig. 2.

Signaling pathways activated by external force. (A) Cell stretching model for analyzing mechanotranscriptional signaling pathways. Focal adhesions are depicted on the ventral surface but adhesions also form in response to collagen beads attached to the dorsal cell surface. Collagen beads are attached to the dorsal surfaces of cells by β1 integrins. Stretching forces are applied (0.5–2 pN/mm^2 surface area of cell). The stretching forces can be adjusted in terms of amplitude, direction and duration, to suit the desired experimental condition. (B) RhoA and MRTF-A as actin-related regulators of cellular response to external mechanical stress. When forces are imposed on integrins bound to collagen coated bead

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

substrates, actin assembly regulation is initiated by changes in RhoA activity. In parallel, changes in the state of actin assembly alter the degree to which the transcription regulator MRTF-A is sequestered in the cytoplasm by its interaction with G-actin. Liberation of MRTF-A when actin polymerization increases leads to SRF activation and expression of gene products such as α-SMA that are characteristic of mesenchymal cell adhesion to rigid substrates and a more contractile phenotype.